Statewide Symposium in Regenerative Medicine Poster Abstracts

Friday May 17, 2019

5:30 p.m. – 7 p.m. **Reception and Poster Presentations**

Poster Abstracts

Research Poster Abstracts

Jennifer Barrila

Three-Dimensional Tissue Culture Models: Next Generation Predictive Preclinical Platforms for Human Health and Disease

Jennifer Barrila[1], JiseonYang[1], Rebecca J. Forsyth[1], Sandhya Gangaraju[1], C. Mark Ott[2], Cheryl A. Nickerson[1,3]
[1]Center for Immunotherapy, Vaccines, and Virotherapy, Biodesign Institute, ASU; [2]NASA Johnson Space Center; [3]School of Life Sciences, ASU

Tissues and organs provide the structural and biochemical landscapes upon which microbial pathogens and commensals function to regulate health and disease. While flat two-dimensional (2-D) monolayers have provided important insight into understanding host-pathogen interactions and infectious disease mechanisms, these reductionist models lack many essential features present in the native host microenvironment that regulate infection, including three-dimensional (3-D) architecture, multicellular complexity, commensal microbiota, gas exchange and nutrient gradients, and physiologically relevant biomechanical forces (e.g., fluid shear, stretch, compression). A major challenge in tissue engineering for infectious disease and microbiome research is recreating this dynamic 3-D microenvironment (biological, chemical, physical) to more accurately model the initiation/progression of host-pathogen interactions in the lab. Our research focuses on bioengineering and application of 3-D tissue culture models of human intestinal and lung mucosa which represent two major portals of entry for pathogens, including those that are multidrug resistant. We apply these models - engineered to contain immune cells - as predictive human surrogates to advance understanding of disease pathogenesis in the context of host-pathogen and host-microbiome interactions to drive basic research discoveries and clinical applications. We have also generated *ex vivo* functional lung tissue for transplantation efforts by using cadaveric decellularized lung scaffolds recellularized with stem cells. Our establishment and characterization of 3-D models using dynamic Rotating Wall Vessel/RWV bioreactor technology and their practical application as predictive platforms for disease research, drug/therapeutic development, synthetic biology, and regenerative medicine provide specific examples of how these fields can be advanced by using appropriate, biologically meaningful 3-D models. Our 3-D models respond to challenge with microbial pathogens, toxins, patient microbiota, and drugs/therapeutics in key ways that reflect the in vivo scenario, which cannot be recapitulated by traditional in vitro cell culture models. Use of these pre-clinical models offers a promising option to facilitate faster and more cost-effective drug development.

George Bjorklund

Regenerative rehabilitation for traumatic brain injury

Bjorklund, GR[1], Mousa, G[1], Addington, CP[1], Anderson, TR[2], Stabenfeldt, SE[1], Kleim, JA[1], [1]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ; [2]University of Arizona, College of Medicine-Phoenix, Phoenix, AZ

Approximately 1.7 million Americans sustain a traumatic brain injury (TBI) annually, leading to a $7 billion economic burden within the US. Current options for therapy are limited to supportive care that primarily serve to alleviate the symptoms of the initial, mechanical injury. However, following mechanical injury there is an expansive biochemical, secondary injury that is largely responsible for many of the long-term deficits associated with TBI. Efforts to curb the deleterious effects of the secondary injury using preclinical stem cell transplantation have had moderate success; yet, their mechanisms of benefit are yet to be fully understood. We have found preclinical neural progenitor/stem cell (NPSC) transplants to promote neuroplasticity within the motor cortex following a rodent model of TBI; albeit, transplant-mediated neuroplasticity is unstructured and does not mimic native motor cortex organization. Thus, combinatorial NPSC transplant and motor rehabilitation therapy may provide greater functional benefit than either therapy individually. To this end, we have coupled NPSC transplants with a motor rehabilitation regimen following TBI in rodents to investigate the therapeutic benefit of a combinatorial regenerative rehabilitation approach to TBI. Adult male Long-Evans rats from Harlan (n=9-10 per group) were trained on a skilled forelimb reaching task for 5 weeks using the Vulintus training cage and randomly assigned treatment groups such that baseline average reaching scores were the same across groups. Rats were then subjected to either sham

surgery or a moderate controlled cortical impact (CCI). Two days after CCI, reaching success rate was assessed prior to administering of saline vehicle or NPSCs (1.8 x 10^5cells). Five days after CCI, reaching success rate was assessed and rats receiving rehabilitation began the rehab regimen; rats that were not receiving rehabilitation were assessed once weekly. We observed the largest functional gains in skilled reaching among the rats receiving both NPSC transplants and motor rehabilitation compared to all other conditions. Rats receiving NPSC transplants and rehab demonstrated a nearly 12-fold increase in reaching success rate over their baseline impairment by week 3 of rehab and significantly greater functional gains than rats receiving only NPSC transplants ($p=0.0233$ at week 3) or rehabilitation ($p\leq0.03$ at weeks 2,3). CCI was effective in inducing impairment in reaching success rate ($p\leq0.0007$, pre- v. post-CCI). These data implicate a role for motor rehabilitation in improving the efficacy of NPSC transplants in promoting functional recovery after TBI and vice versa. Given that NPSC transplants often suffer low rates of survival in the injury microenvironment we put forth that the behavioral cues provided by motor rehabilitation may create a more permissive environment for transplants and/or direct NPSC transplant-mediated neuroplasticity within the injury environment.

Gregory Branigan

Patient-derived Cell Resource Development at The Center for Innovation in Brain Science

University of Arizona

In the 21st century there is not a single *cure* for a single neurodegenerative disease[1]. While therapies exist, they provide symptom relief while the degenerative process marches ever forward. Globally, neurological disorders rank as the leading cause of disability-adjusted life-years (250 million life-years lost), and the second-leading cause of deaths[2,3]. Worldwide, the burden of neurological disorders has accelerated over the past 25 years[2,3]. Driven by a global expansion in the aging population, the prevalence and burden of age-associated neurodegenerative diseases has dramatically increased[2,3].The Center for Innovation in Brain Science (CIBS) at the University of Arizona has acquired and banked human iPSC and fibroblast lines from male and female with sporadic age-associated neurodegenerative diseases (n=12/disease) and age-matched healthy controls. From these lines, we will generate indirectly and directly reprogrammed NSCs and neuronal cell types relevant to AD (cortical neurons), PD (midbrain dopaminergic neurons), ALS (motor neurons) and MS (oligodendrocyte lines). These cells will be phenotypically profiled both genetically (bioenergetic, protein, immune, electrophysiological/MEA) and molecularly (transcriptome, metabolome) to determine inherited, age- and sex-related mechanisms in neurodegeneration. Using this approach, CIBS will work to establish the transcriptomic and metabolomic profiles for neurodegeneration, generate and characterize reprogrammed patient-derived neural cells and determine the molecular pathways driving early age-associated neurodegenerative diseases vulnerability. Preliminary results of deep phenotyping show common protein aggregations in CNS and peripheral fibroblast indicating possible development of novel biomarkers and therapies that will arise from these studies.

Costanza Lo Cascio

Radiation-Induced Phenotypic Plasticity in Human Glioma Stem Cells

Barrow Neurological Institute

Glioblastoma (GBM) is the most common and lethal primary malignant brain tumor in adults with a median survival of just 14 months. The current standard-of-care treatment is ineffective and fails to significantly prolong survival. Moreover, these invasive tumors display extensive intratumoural heterogeneity and resistance to radio- and chemotherapy,posing a major clinical challenge due to inevitable tumor recurrence.Following exposure to aggressive multimodal treatment, GBMs frequently shift their biological features upon recurrence, acquiring a more resistant phenotype. However, the temporal dynamics and molecular mechanisms that facilitate GBM recurrence are poorly understood. The objective of our study was to determine how molecularly distinct patient-derived glioma stem cells (GSCs) temporally adjust their expression profile and phenotype in response to ionizing radiation *in vitro* and *in vivo*. We find that human GSCs undergo dramatic molecular and phenotypic changes in response to a single dose of ionizing radiation. The observed treatment responses differ depending on

the genetic background of the patient-derived GSCs. We find that ionizing radiation causes a transient decrease in the expression of key stemness genes followed by drastic morphological changes and a concomitant increase in the levels of cell fate markers. These treatment-induced cellular alterations commence immediately following treatment (within 24-48 hours) and become more pronounced over the course of one week. If irradiated GSCs are allowed to recover for two weeks, we observe the presence of heterogeneous cell populations that were absent prior to treatment. We also performed single-cell RNA sequencing and mass cytometry at multiple different timepoints post-treatment to discover and characterize novel cell subpopulations that emerge from different human GSCs after irradiation. Moreover, cell viability experiments reveal that human GSCs previously exposed to radiation are more radioresistant upon re-treatment compared to their naïve, untreated counterparts – suggesting that the aforementioned phenotypic shifts promote treatment resistance. Our results suggest that GSC responses to radiation are dynamic, and that surviving cells are capable of adopting novel cellular states over a matter of hours.

Devon M. Coleman

REPAIRING THE CELL-TRANSPLANT PARADIGM FOR PARKINSON DISEASE: SCIENCE AND ETHICS

Jason S. Robert and Devon M. Coleman, Biology and Society, Arizona State University, Tempe, AZ 85287

Parkinson Disease has proven to be both very difficult to address through cell transplantation and yet still beckons to researchers as putatively low-hanging fruit in the cell transplantation world. Fetal ventral mesencephalic (FVM) cell transplants were successful in reducing symptoms in a small number of cases, but only in the hands of a small number of clinician-scientists whose results have been difficult to replicate. Possible explanations include variation in the quality (for clinical purposes) of the fetal tissues used, variation in mode, site, and mechanism of delivery, and the number of cells / size of sample delivered; additionally, there were concerns about the methods for measuring success, questions about the mode of action, and issues associated with inclusion and exclusion criteria for participants. Concerns on many of these fronts needed to be alleviated in order to proceed with the cell transplant paradigm for treating PD. FVM research dwindled, but the cell transplant paradigm was rescued twenty years ago when there was much excitement about cultivating human pluripotent stem cells (hPSCs) for transplantation. Using cells derived from hPSC research would address the first of the possible explanations of the demise of FVM transplants: the clinical quality of the cells to be transplanted. No longer would surgeons be transplanting gross fetal tissue that might vary from fetus to fetus (as from surgery to surgery); instead, hPSC scientists imagined creating clinical grade cells for transplantation. Were everyone to use the same calls, or well-characterized variants thereof, rapid clinical progress might be achieved on the path to cell-based treatments for PD.
And yet another two decades have passed without a successful cell-transplant treatment for PD. One possible explanation is that research has been hampered by ethical restrictions on deriving and studying hPSCs from human embryos. A more probable explanation is that the almost-exclusive reductionistic focus on the cellular material to be transplanted has led to the neglect of all of the other issues originally identified in FVM research. As we move into a new round of attempts in the cell-transplantation paradigm, it is imperative that we step back and systematically explore more system-level issues to make sure we do not repeat past mistakes.

Mandy Corenblum

Title: A PATIENT iPSC-BASED PLATFORM FOR INVESTIGATING IDIOPATHIC PARKINSON'S DISEASE

Corenblum MJ[1,2], Annadurai A[1,5], Shrestha K[4], Madhavan L[1,2,3],[1] Department of Neurology, [2] Center for Innovation in Brain Science, [3] Evelyn F McKnight Brain Institute, [4] Graduate Interdisciplinary Program in Applied Biosciences, [5] Pharmacology and Toxicology, University of Arizona, Tucson, AZ 85724

Human induced pluripotent stem cells (iPSCs) are proving to be a valuable source of patient cells for generating neural phenotypes relevant to Parkinson's disease. Here we compared iPSC-derived midbrain dopamine (DA) neurons derived from the skin fibroblasts of late-onset idiopathic Parkinson's disease (PD) subjects and age-matched controls (AMCs). Specifically, we comparatively analyzed several neurodegeneration relevant aspects including DA neuron survival, differentiation,

morphology, mitochondrial function, oxidative stress, and autophagy. Our data indicate that the iPSCs from PD subjects had lower viability rates, and a reduced capacity to generate neurons when induced to differentiate via a floorplate dual SMAD inhibition method. At day 42 post-differentiation, the efficiency of tyrosine hydroxylase positive (TH^+) DA neuron generation did not differ between the PD and AMC cultures. However, the morphology of the DA neurons from PD subjects appeared altered in that the cells displayed a smaller soma size, reduced number of neurites, and shorter neurite lengths, compared to AMC cultures. Moreover, PD DA neurons expressed higher levels of reactive oxygen species, and compromised mitochondrial function. In addition, it was found that autophagy was dysregulated in the PD neurons, and was associated with increased protein levels of alpha-synuclein, as compared to AMC cells. Our current studies are further extending these findings by examining the activity profile (electrophysiological and others) of the iPSC-derived DA neurons. In summary, our study develops an iPSC-based neuronal model that captures a phenotype relevant to the study of idiopathic PD, as well as biomarker and therapeutic testing.

Joshua Cutts

Investigating a Multi-State Model of WNT Signaling

Arizona State University

The WNT signaling pathway plays a critical role in many developmental processes as well as the maintenance of tissue homeostasis in adults. In addition, dysfunction in WNT signaling results in numerous human diseases. Canonical WNT signaling is classically described by the 'two-state' model. This model posits that in the 'off' state in the absence of a WNT ligand, cytoplasmic β-catenin is continuously degraded by the action of the APC/Axin/GSK-3β destruction complex. In the 'on' state in the presence of WNT ligands, this protein destruction complex is disrupted, allowing β-catenin to translocate into the nucleus where it interacts with the DNA-bound TCF/LEF proteins to regulate target gene expression. However, this 'two-state' model does not adequately explain the mechanisms by which WNT signaling can elicit distinct patterns of target gene expression and cell responses at specific signaling thresholds. For example, in the development and patterning of many tissues, the WNT pathway attains different levels of activity through gradients of WNT signaling activity. In turn, the positional information supplied by these WNT signaling gradients produces the appropriate spatial pattern of cellular differentiation. Elucidating the mechanisms of how a graded WNT signal leads to precise changes in transcriptional responses has been difficult because the lack of an in vitro model where WNT signaling molecules cause distinct cellular phenotypes at different concentrations. To that end, we have developed an in vitro human pluripotent stem cell (hPSC)-based model that recapitulates the same in vivo developmental effects of the WNT signaling gradient on the anterior-posterior (A/P) patterning of the neural tube during early development (Stem Cell Reports. 2014 Dec 9;3(6):1015-28). Using this model along with genome-wide expression analysis (RNA-seq) and DNA binding analysis (ChIP-seq), we are uncovering the mechanisms by which specific levels of WNT activity are translated into precise transcriptional responses and cell identities. Overall, the new insights gained from this research will lead to the better understanding of how various WNT pathway activity levels lead to cancer or other pathological conditions.

Tsogtbaatar Enkhtuul

Metabolic remodeling during nuclear reprogramming is critical for acquisition and maintenance of pluripotency

Enkhtuul Tsogtbaatar[1], Ryounghoon Jeon[2], D. Kent Arrell[2], Rhianna Harm[2], Andre Terzic[2] and Clifford Folmes[1].
[1] Departments of Cardiovascular Medicine and Biochemistry and Molecular Biology, Mayo Clinic Arizona, Scottsdale, Arizona 85259, [2] Center for Regenerative Medicine, and Departments of Cardiovascular Medicine and Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, Minnesota 55905

Energy metabolism is traditionally considered a reactive homeostatic system that prioritizes specific pathways to match stage-specific cellular energy demands. There is, however, growing appreciation of metabolic pathways as active regulators of vital cell functions. Case in point, the stem cell lifecycle – from maintenance and acquisition of stemness to lineage commitment and specification – is increasingly recognized as a metabolism-dependent process. We previously demonstrated

that somatic cell nuclear reprogramming for the generation of induced pluripotent stem cells (iPSCs) requires remodeling of the metaboproteome and mitochondrial infrastructure in support of a metabolic transition from oxidative metabolism to glycolysis. To date, specific metabolic pathways critical for acquisition and maintenance of pluripotency remain unknown. To address this knowledge gap, liquid chromatography tandem mass spectrometry (LC-MS/MS) based metabolomics was used to profile metabolic differences between iPSCs, their parental mouse fibroblasts (MEFs) and embryonic stem cells (ESCs). This analysis confirmed that iPSCs and ESCs rely on glucose as a main energy source and identified a number of metabolic pathways modulated during nuclear reprogramming, including: i) accumulation of the oxidative pentose phosphate pathway metabolites 6-phosphogluconate, sedoheptulose-7-phosphate and histidine; ii) increased levels of a-ketoglutarate, serine and NADH associated with the high proliferative potential of iPSCs; and iii) reorganization of the tricarboxylic acid cycle, leading to elevated levels of a-ketoglutarate, citrate and acetyl-CoA. Beyond their metabolic pathway- specific function, a number of these metabolites also have significant impact on cellular epigenetic status, including a-ketoglutarate that regulates pluripotency by suppressing the accumulation of repressive histone modification and DNA methylation, and acetyl-CoA that serves as a protein acetylation substrate. Therefore, quantitative label-free proteomics was used to investigate the impact of acetyl-CoA accumulation on the global acetylation profile during nuclear reprogramming, and demonstrated distinct profiles in pluripotent stem cells compared to MEFs. Future studies will apply 13C-labeling analysis to trace carbon fate through the tricarboxylic acid cycle and its contribution to post-translational protein modifications, and to validate specific acetylated targets vital for acquisition and maintenance of pluripotency. In summary, our data indicates that metabolic remodeling during nuclear reprogramming serves a fundamental role in providing substrates for protein post-translational modifications that regulate stem cell fate.

Deepanjan Ghosh

Bioactive silk dressing for accelerated wound healing

Deepanjan Ghosh[a], Sudhakar Godeshala[b], Michael Bejarano[c], Harini Muralikrishnan[b], Shraddha Patil[c], David DiCaudo[d], Jacquelyn Kilbourne[e], and Kaushal Rege[a,b,c#] [a]Biological Design, [b]Chemical Engineering, [c]Biomedical Engineering, [e]Department of Animal Care Technologies Arizona State University, Tempe, AZ 85287, USA, [d]Mayo Clinic, Scottsdale, AZ 85259, USA

Wound healing typically follows a programmed sequence which involves hemostasis, inflammation, proliferation, and remodeling. In this work, we show local delivery of histamine, an immune modulator in combination with silk fibroin (SF) film resulting in faster closure of acute and slow healing diabetic wounds when compared to conventional wound dressing (Tegaderm). Histamine was applied topically on 5 mm full-thickness wounds and covered with either Tegaderm or a SF-gold nanorods (GNRs) film and irradiated with 800 nm near-infrared laser (NIR) laser. Significant reduction in the wound area and improved tissue biomechanical properties were observed in histamine treated wounds. SF-GNRs film-histamine treated wounds showed complete wound closure (p-value <0.0001, n=10) and higher tissue strength (p-value <0.01, n=6) compared to Tegaderm-histamine treated wounds at day 7 post-wounding in acute wounds and 11 days in diabetic wounds. Immunohistological analyses also showed that SF-GNRs film-histamine treatment promoted angiogenesis ($CD31^+$ cells, p-value <0.05, n=4) and myofibroblast mediated wound contraction ($aSMA^+$, p-value <0.05, n=4). This also resulted in the increased epidermal thickness (Pan-cytokeratin, p-value <0.01, n=4) and reduced proliferation ($Ki67^+$ cells, p-value <0.01, n=4) compared to Tegaderm-histamine treated wounds. IL-1beta and IL-6 levels in serum samples showed significant increased at day 1 post-wounding and reduced to basal level at day 3 in SF-GNRs-histamine treated wounds showing a robust early inflammatory response to set the stage for subsequent proliferation and remodeling. We conclude that this silk dressing was effective in promoting wound healing and in combination with single histamine dose it outperformed clinically approved polyurethane wound dressing.

Hanah Goetz

Multiple Partial States Accelerate Epithelial-to-Mesenchymal Transition

Hanah Goetz, Juan Melendez-Alvarez, and Xiaojun Tian, Biodesign Institute, Arizona State University, Tempe, Az

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Epithelial-to-mesenchymal transition (EMT) is a fundamental cellular process and plays important roles in development, tissue regeneration, and cancer metastasis. Interestingly, EMT is not a binary process but proceeds with multiple partial intermediate states. However, the functions of these partial states are not fully understood. Here, we will focus on a general question about how the number of partial EMT states affects cell transformation. We used a general hidden Markov model to describe the transition among the epithelial state, partial EMT states, and mesenchymal state. Our systematic analysis makes several unanticipated predictions. First, several microstates exist in one microstate, which makes the EMT a non-Markov process. Second, increasing the number of partial EMT states could accelerate the EMT transition. This work advances our understanding of the dynamics and functions of the cell plasticity during EMT.

David Gonzales

Stem Cells Are Necessary to Bridge a Critical Size Gap when a Biomimetic Scaffold is used to Regenerate a Large Segmental Femoral Defect

University of Arizona, Tucson Az

Introduction: Critical size long bone defects can result from severe traumatic injuries or large bone resections secondary to cancer treatment. While surgical options exist for this problem, there are drawbacks to each method and no current surgical treatment ensures successful healing. Our lab has shown that polybutylene terephthalate (PBT) scaffolds induce rapid bone regeneration in rodent and canine models. These scaffolds support the growth of adipose derived mesenchymal stem cells (MSCs) and allow bone regeneration in a sheep model. In this study a biomimetic PBT scaffold without MSCs was placed to determine whether MSCs are necessary for successful bone bridging.

Methods: All studies were approved by the IACUC. Biomimetic scaffolds were designed using 12µm resolution µCT images of sheep femoral head trabecular bone. The CT image was used to produce a STL file with the same diameter as a sheep femur that also incorporated space for an intramedullary rod and support struts to increase scaffold strength and facilitate strain gauge attachment. PBT scaffolds 42mm in length were produced using a Stratasys 1650 fused deposition modeler and were coated in b-tricalcium phosphate. Three scaffolds were characterized using µCT, and mechanical testing was performed following attachment of rosette strain gauges. Scaffolds were sterilized in ethylene oxide prior to surgery. Three male sheep were used in this study. During surgery a 42mm mid-diaphyseal femoral defect was created using a sagittal saw. In one sheep the defect was left empty and the space maintained with a locked intramedullary nail (control). One sheep received a PBT scaffold without MSCs prior to placement of the nail (PBT). In the third sheep a PBT scaffold with MSCs was placed in the defect (PBT/MSC). Adipose derived MSCs were harvested from the tail fat pad one week prior to scaffold placement. Sheep were held for 6 months. Activity was monitored with 24hr video. Bone formation was analyzed with monthly radiographs. Post-sacrifice CT was performed on explanted experimental and contralateral control femora.

Results: CT analysis of scaffolds demonstrated a porosity of 71.6±0.2%. Scaffolds demonstrated a linear elastic response with a compressive stiffness of 2.8GPa and strength of 9.44±0.94MPa.

Sheep had normal gait within one month, spent 25% of the time standing and walking and the remaining time resting. Following removal of the intramedullary nail the control and PBT sheep demonstrated gross mechanical instability while the PBT/MSC sheep demonstrated bony union. CT imaging showed average bone growth within scaffold pores of 0.60cm^2 in PBT and 0.47cm^2 in PBT/MSC sheep. Cortical bone formation averaged 1.88cm^2 in control, 0.46cm^2 around the PBT scaffold, and 2.52cm^2 around the PBT/MSC scaffold. The PBT/MSC sheep demonstrated bone bridging on radiographs at 3 months. Unoperated contralateral femora had 2.68±0.9$cm^2$of cortical bone.

Discussion: Biomimetic PBT scaffolds produced in this study have sufficient strength to support physiologic loading during healing. PBT scaffolds with MSCs have been shown to bridge this defect within 3 months. This study demonstrated that scaffolds without MSCs have significant bone formation within scaffold pores, but decreased cortical bone formation and nonunion at 6 months. Additional quantitative CT and histological measurements are being performed on sheep used in this study. Future experiments using strain gauged scaffolds will confirm the benefit of seeding scaffolds with MSCs and allow for correlation of *in vivo* strain measurements and healing.

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Kassondra Hickey

Redesigning SDF-1 delivery strategies with in vitro, in vivo, and in silico analyses

K. Hickey, S. M. Grassi, M. R. Caplan, S. E. Stabenfeldt, School of Biological and Health Systems Engineering, Arizona State University, Tempe AZ 85281

Statement of Purpose: Upon brain injury, stromal cell-derived factor-1(SDF-1)/chemokine receptor 4(CXCR4) signaling axis incites transient directed migration of CXCR4+ neural progenitor cells to the site of injury, away from the normal migratory path to the olfactory bulb. We, and others, are interested in exploiting this signaling cascade via controlled release biomaterial devices to modulate/tune stem cell recruitment for maximal regenerative capacity. Previously, we explored SDF-1 loaded poly-lactic-co-glycolic acid nanoparticles (PLGA NPs) as a means to deliver exogenous SDF-1. Notably, SDF-1 delivered intracortically in PLGA NPs stimulated widespread and increased activation of CXCR4 and SDF-1 across the cortex (mouse) compared to bolus SDF-1 injection. Assessing potential mechanisms for the widespread signal propagation in a short time frame suggests critical autocrine/paracrine signaling may be at play. This study focused on integrating *in vitro* signaling assays with *in silico* modeling to better understand and model the kinetics of SDF-1 propagation following exogenous SDF-1 delivery. Moreover, the *in silico* model will be used to tailor drug delivery approaches that maximizes autocrine/paracrine signals for enhanced neural regeneration.

Methods: To probe the dynamics of SDF-1/CXCR4 autocrine/paracrine signaling, we used *in vitro* cell cultures to measure gene expression of SDF-1 and CXCR4 following administration of SDF-1: endothelial (bEnd3 cells; ATCC CRL-2299), primary microglial and primary astrocytes. Primary mixed glial cell cultures were isolated from CXCR4-eGFP+ P0-P2 (ASU IACUC approved). Mixed cultures were sorted for microglia (CD11b) or astrocytes (Glast-1; Miltenyi). Cell cultures were exposed to recombinant murine SDF-1α (Peprotech; 400ng/mL) for 30min intervals over 120min; control receiving no SDF-1 supplement. Extracted RNA (Qiagen) was converted to cDNA (Invitrogen) for reverse transcription quantitative polymerase chain reaction (RT-qPCR) with validated primers for GAPDH, SDF-1, and CXCR4 (Thermo Scientific). Reverse transcriptase negative and no template controls were included; relative gene expression ratios were determined via the Pfaffl method. Based on previous *in vivo* results, we developed two COMSOL SDF-1 delivery models.[3] The first model assumed pure diffusion of SDF by limiting the reaction such that SDF-1 and CXCR4 may bind but not induce downstream expression of either cytokine or receptor. The second model incorporated both diffusion and an autocrine response in the SDF-1/CXCR4 signaling axis. Each simulation was run for 24 hours to view predicted concentration gradients.

Results: Evaluation of the SDF-1/CXCR4 signaling dynamics of key cell types revealed that both primary mouse microglia and astrocytes increased SDF-1 gene expression within 30 minutes after exposure to SDF-1 while bEnd3 cultures increased after 60 minutes. Similarly, astrocytes and microglia demonstrated a concomitant increase of CXCR4 expression at 30 minutes while bEnd3 cultures exhibited delayed CXCR4 expression at 90 minutes. These results confirm that glial and endothelial cells actively engage autocrine/paracrine response to exogenous SDF-1. I*n silico* simulations revealed that SDF-1 gradient formation observed in our initial *in vivo* studies was not recapitulated with pure diffusion. At 24 hours, SDF-1 modeled with diffusion only showed no sustained gradient in contrast to the spatial gradient formed by SDF-1 modeled with an autocrine response. These results demonstrate that autocrine signaling is necessary to induce the gradients visualized *in vivo*.

Conclusions: The results of our *in vivo* study demonstrated the potential sustained exogenous SDF-1 therapy holds in eliciting an endogenous SDF-1 response. We utilized gene expression to elucidate SDF-1/CXCR4 signal dynamics with endothelial and glial cell types where we identified the dynamics of SDF-1 and CXCR4 expression following exposure to exogenous SDF-1. We utilized in silico modeling to validate our findings and design future SDF delivery strategies.

Asha Karthik

Can you tell the difference between CIRM and For-Profit Clinics?

Asha Karthik and Dr. Emma Frow, Arizona State University, School of Innovation in Society Tempe, AZ 85281

In 2004, Proposition 71 was passed to fund The California Institute for Regenerative Medicine (CIRM) with $3 Billion to "accelerate stem cell treatments to patients with unmet medical needs". 14 years later, their 3 billion dollars are dwindling, and they have not yet brought a stem cell therapy to market. They are expected to request for more fund in the 2020 election.

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California has nearly 80 clinics that are selling stem cell treatments direct to consumers to cure conditions ranging from MS to cosmetic factors. Both are public entities, CIRM is seen as more legitimate through its classification as a governmental agency, the clinics less so. They both, however, are marketing heavily to their potential audiences.
This poster analyzes the manner in which and these for profit clinics are marketing themselves to the public. It is driven on the research questions: What types of evidence are CIRM and for profit clinics mobilizing to support their practices? How are these forms of evidence similar or different in content? The research was performed online and based upon the evidence provided by 24 for-profit clinic in clinics. In the end, it was discovered that CIRM is consistently pushing rhetoric about their impact in the field of the cell treatment, reasonably similar to for-profit clinics. However, CIRM is able to gain credibility by discussing the money structure and promoting the research that is being conducted in an academic and relevant manner.

Maeve Kennedy

Design of Electrospun Scaffolds for Tissue Regenerative Response

Maeve Kennedy[1], Michael McPhail[1], Manisha Shah[1], David G. Lott[1,2] , [1]Head and Neck Regenerative Medicine Laboratory, Center for Regenerative Medicine, [2]Department of Otorhinolaryngology, Mayo Clinic, Scottsdale, AZ 85259

Electrospun scaffolds provide an artificial substrate that mimics the natural extracellular matrix (ECM) and can be tuned to elicit specific cellular responses. The long-term goal of this work is to design a multi-scale, implantable, 3D porous substrate to induce tissue regeneration. It is important to characterize the properties of electrospun scaffolds to elicit optimal cell adhesion, infiltration, and proliferation when implanted *in vivo*. In this work, electrospun scaffolds were generated from polycaprolactone (PCL) and characterized using scanning electron microscopy (SEM). A mixture of 10% w/v PCL and 3:1 chloroform to methanol was electrospun using a Spraybase system. Scaffolds were generated with varied solution flow rates, polymer concentrations, and voltages. Electrospinning was performed under uniform ambient conditions and for the same amount of time for each of the runs. The fiber diameter, fiber alignment, and porosity of the electrospun mats were then characterized with SEM. The Image-J plugin Diameter-J was used to characterize the diameter of electrospun fibers and the porosity of electrospun mats. The Image-J plugin Orientation-J was used to characterize the alignment, or orientation, of the fibers. The electrospinning parameters were found to have minimal effects on fiber diameter and alignment. However, voltage directly affects porosity, with higher voltage resulting in decreased scaffold porosity. Future work will consist of a cell study to determine the cell viability, count, and integration when cultured on electrospun scaffolds.

Warner Kostes

Investigating the Role of SP5 in WNT Signaling Using an in vitro Model of Neural Patterning

Warner Kostes, Josh Cutts, and Dr. David Brafman. Arizona State University, Tempe, AZ 85287

The WNT signaling pathway, a highly conserved evolutionary pathway, coordinates an array of complex biological processes during development and is responsible for the proper maintenance of tissue homeostasis and healthy cells in adults. Deregulation of this pathway causes severe congenital defects, underlies multiple diseases and disorders, and frequently drives oncogenic transformation. Traditionally, the canonical WNT pathway is thought to switch between an ‘on’ and ‘off’ state — commonly depicted as a ‘two-state’ model. In the ‘off’ state, a protein destruction complex is stabilized which causes the proteolytic degradation of β-catenin. Conversely, in the ‘on’ state, the presence of WNT ligand leads the co-receptor LRP5/6 to form a complex with the WNT-bound Frizzled (FZ) receptors. In turn, this leads to destabilization of the destruction complex and the rate of β-catenin degradation is slowed. Subsequently, β-catenin translocates to the nucleus where it interacts with DNA binding proteins encoded by the TCF/LEF genes to regulate target gene expression.
Although the ‘two-state’ model of signaling has served as a foundation for understanding WNT signaling, this model alone is not accurate in accounting for how WNT signaling orchestrates complex biological processes to induce cellular responses at distinct signaling thresholds. For example, several studies have shown that a low, but not high level, of WNT signaling activity can cause malignancies.
In relation to my research, a number of studies have shown that an endogenous gradient of WNT signaling controls cell fate

along the anterior posterior (A/P) axis of the neural tube. As before, the 'two-state' model of WNT signaling cannot account for the distinct levels of pathway activity responsible for the precise regional identities along the developing neural tube. This 'two-state' model of WNT signaling is not sufficient to address questions fundamental to the underlying mechanisms by which A/P patterning of the neural tube is achieved at various signaling thresholds. Previous studies within the Brafman laboratory led to the development of an in vitro human pluripotent stem cell (hPSC) model that recapitulates the same effects of the WNT signaling gradient on the early A/P patterning of the neural tube observed during in vivo development. Prior work has identified the downstream WNT target gene and transcriptional repressor, SP5, as a potential negative regulator of the WNT signaling pathway. To this end, my research seeks to establish SP5 as a mediator of WNT signaling in specifying the A/P regional identity of hPSC-derived neural cells.
Former studies have revealed SP5 as a highly up-regulated target of WNT signaling in the A/P specification of hPSC-derived neural cells. This project aims to examine the effects of SP5 knockdown on determining hPSC-derived neural cell identity. Using an hPSC line containing loss-of-function SP5 alleles, I will determine the role of SP5 in mediating the WNT-induced neural posteriorization of differentiating hPSCs. Additionally, I will measure and relate the level of SP5 expression to A/P regional identity through the use of an hPSC line in which YFP has been knocked-in at the SP5 locus forming an SP5-YFP fusion protein. Lastly, I will employ ChIP-seq technology to observe SP5's transcriptional targets during the specification of particular A/P positional identities throughout the genome.

Danielle Larson

Treatment of Scaphoid Fractures and Nonunions with Significant Bone Loss Using Patient Specific 3D Polymer Scaffolds

David S. Margolis, M.D., Ph.D., Danielle Larson, and Efren Barron, Orthopedics Research Lab, Tucson AZ 85721

Background: Scaphoid fracture is the most common carpal fracture and can result in significant patient morbidity. Acute displaced fractures and nonunions commonly present with deformity and significant bone loss. Both vascular and nonvascularized bone grafts are used to treat scaphoid fractures with significant bone loss, but these procedures are not always successful at healing the fracture. In addition, there can be significant donor site morbidity. The current study aims to produce patient specific scaffolds that can address this problem without the use of autograft. The scaffold material being used has been shown to facilitate growth of engineered bone and hyaline cartilage tissue using stem cells in a large animal model. The overall goal of this project is to regenerate bone tissue that can be used to treat fractures and nonunions with significant bone loss as well as replace necrotic tissue.
Methods: High resolution computed tomography (CT) was used to scan a cadaver scaphoid. The CT image produces a DICOM file, which was inverted to produce an image that is representative of the contralateral scaphoid. The DICOM file was subsequently converted to a STL file. SolidWorks was used to manipulate the STL file to incorporate a screw track along the central axis of the scaphoid as well as introduce variations in scaffold porosity to allow for tissue engineered bone growth in the central portion and hyaline cartilage production on the surface. The portion of the scaphoid that needs to be replaced to restore anatomic alignment is selected. This modified STL file is then exported to a Stratasys fused deposition modeler and a patient specific scaffold is produced using polybutylene terephthalate (PBT).
Results: Patient specific scaffolds can be rapidly produced using this technique. Scaffolds addressing both central bone loss and the entire proximal pole of the scaphoid were produced using a CT scan. As proof of principle that scaffolds could be used to treat scaphoid bone loss, a scaffold was placed into a cadaver following creation of a central bone defect. Placement of a scaffold and fixation with the cannulated screw allowed for restoration of the carpal alignment during the surgical procedure.

Ileana Lorenzini

Human *in vitro* culture systems of C9orf72-ALS/FTD patient-derived iPSC cortical neurons and microglial cells to study mechanisms of synaptopathy.

Ileana Lorenzini, Jennifer Levy, Camelia Burciu, Divya Bhatia, Benjamin Rabichow, Mo Roberts, Shiv Shah, Rita Sattler
Barrow Neurological Institute, Phoenix, Arizona.

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The discovery of a hexanucleotide repeat expansion in the *C9ORF72*gene (C9) as the cause of chromosome 9-linked amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) provides an opportunity to investigate common pathobiological mechanisms underlying motor and cognitive dysfunction seen in both diseases.Cognitive dysfunction observed during normal ageing parallels the selective loss of synapses, decreased spine density and altered neuronal morphology. Furthermore, published research in Alzheimer's disease and FTD, suggests that synaptic pruning mediated by microglial cells can be re-activated during neurodegeneration, leading to synapse loss and dysfunction. Interestingly, C9orf72 knockout mice display altered immune responses in microglia, with age-related neuroinflammation exhibiting similarities seen in C9 patient tissue. These findings suggest that inappropriate neural-immune interactions may contribute to synaptic impairment and ultimately cognitive decline in C9-ALS/FTD. *We hypothesize that synaptic defects in C9 dementia are mediated by microglial cells and the neural-immune complement pathway.* To test our hypothesis, we generated patient-derived human stem cell differentiated cortical neurons (hiPSC-CNs) and microglial cells (hiPSC-MGs) from three C9 patient population clinically diagnosed as: C9-ALS, C9-ALS/FTD and C9-FTD. We evaluated neuronal structure and activity by using three dimensional (3D) imaging tools and a multi-electrode array system, respectively. HiPSC-CNs exhibited changes in dendritic branching, dendritic length, spine density, alterations in the expression pattern of synaptic proteins as well as in neuronal excitability. Furthermore, we examined hiPSC-MGs mono-cultures for gene expression changes, specific brain markers and brain function such as phagocytosis. Our results show that synaptic deficits are present in all C9 patient groups with varying degree of severity, which likely contributes to cognitive impairment and neuronal cell death found in C9orf72 patients. Our goal is to establish an *in vitro* co-culture system of hiPSC-derived cortical neuron-microglial cells to determine the role of microglial cells in neuronal synaptic dysfunction in C9 associated diseases. This human *in vitro* co-culture model not only allows for the studies of C9 disease pathogenesis, but any other neurodegenerative disorder characterized by synapse loss and synaptic dysfunction, including other subtypes of FTD, Alzheimer's disease and Down's Syndrome.

Helen Magee

A de novo mutation in RHOB disrupts neuronal morphology via alteration of GTPase Kinetics

Phoenix Children's Hospital, Phoenix Az

Cerebral palsy is a developmental movement disorder caused by disruption of normal neuromotor development. In addition to well-recognized causes such as prematurity or hypoxic-ischemic injury, single gene mutations are being increasingly recognized as important causes of cerebral palsy. We have recently identified *de novo* variants in the small GTPase *RHOB* in CP patients. Canonically, RHOB pairs with its effector ROCK to activate LIM kinase. RHOB controls actin dynamics, in turn influencing neuritogenesis, neuronal migration, and cellular differentiation and proliferation. We studied the effects of the RHOB p.S73F variant on RHOB function. We found evidence for hyperactivation of RHOB p.S73F by *in vitro* GST-Rhotekin RBD pulldown assay, as well as altered responses to GAP/GEF regulation. S73F patient-derived induced pluripotent stem cell-neurons (iPSC-neurons) showed excessive neuritic sprouting relative to control iPSC-neurons. Taken together, our results indicate an important role for *RHOB* in developmental neuritogenesis and add to the growing body of literature indicating that a subset of CP patients harbor mutations in genes controlling axon/dendrite morphogenesis during early neurodevelopment.

Joslyn Mangal

Metabolite-Based Modulation of Dendritic Cells for Developing Effective Immunotherapy

Joslyn Mangal[1+], Sahil Inamdar[2+], Subhadeep Dutta[3], Deepanjan Ghosh[1], Kaushal Rege[1,2,3] and Abhinav P. Acharya[1,2(*)],
(1)Biodesign Institute, (2)Chemical Engineering, (3)School of Molecular Sciences, Arizona State University, Tempe, AZ

Slow wound healing is characterized by prolonged inflammation and postponed anti-inflammatory responses. Tissue regeneration and wound healing is a combination of complex processes that are intricately coordinated by mononuclear phagocytic cells and lymphocytes. Notably, studies have utilized scaffolds to deliver biologics-based chemoattractants, which

then induce chemotaxis of immune cells into the wound bed, leading to faster wound healing. One major problem with such strategies is that these biologics are soluble in nature, which suggests that they need to be administered several times. Moreover, US FDA approval of biologics requires stringent controls and is generally expensive to translate into clinic. Therefore, if there were a method to provide sustained delivery of synthetic immunomodulators that after a one-time application, can provide local wound healing cues, then this can transform wound healing dramatically in aging and diabetic populations. Notably, metabolic pathways control immune cell functions, and modulating these pathways can determine the overall immune responses. Interestingly, we demonstrate that sustained delivery of central-carbon metabolites are sufficient to modulate the function of immune cells. This was accomplished by first generating novel polymers with central-carbon metabolite monomers. Microparticles, ~1 μm in diameter (as observed by DLS and SEM) were generated from these polymers using oil in water emulsions and these microparticles degrade hydrolytically to release the metabolites (as observed by NMR). Notably, dendritic cells (DCs - antigen presenting cell, forms a bridge between innate and adaptive immunity) were capable of phagocytosing (engulfing) these microparticles, and thus may provide intracellular delivery of the metabolites. Notably, these metabolite-based polymeric microparticles were able to differentially modulate bone marrow-derived DC function (activation and suppression), as observed by flow cytometry quantification of CD11c, CD86, and MHC surface markers, and IL-10 and IL-12 cytokine production in cell culture media. These microparticles were also added to syngeneic mixed lymphocyte reaction of bone marrow derived DCs and $CD3^+$ T-cells derived from spleen of C57BL/6j mice. Upon phagocytosis of these microparticles by DCs, T-cell suppression or activation was observed *in vitro* by staining for surface markers (CD4, CD8, CD25, CD11c) and intracellular markers (Tbet, RORγT, FOXp3, Ki67, and GATA3), as well as, anti- and pro-inflammatory cytokines (IL-10 and IL-12). In summary, the metabolite-based polymers were capable of controlling the pro- or anti-inflammatory responses of immune cells *in vitro* and potentially may control the immune responses *in vivo* as well. These results suggest that metabolite-based polymers may have potential applications in different areas of regenerative medicine as a whole, and wound healing in particular. (*in vivo* experiments currently being conducted)

Brianna I. Martinez

Probing the injury microenvironment: Phage-based biomarker discovery of complex neural injury pathologies

B.I. Martinez, A. Witten, G. Mousa, S.S. Margulies, S.E. Stabenfeldt, [1]School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ 85287

Introduction: Current diagnostic tools for traumatic brain injury (TBI) have limited utility due to low sensitivity to heterogeneous injury pathophysiology. Thus, there is critical need for a unique panel of biomarkers for development of more efficient diagnostic tools. Here, we combined antibody fragment phage display with the potency of next generation sequencing (NGS) analysis as an *in vivo* and *ex vivo*biomarker discovery tool for rodent and porcine models of brain injury respectively. Methods: Animal studies were conducted in accordance to protocols approved by ASU and University of Pennsylvania IACUC. For *in vivo* biopanning, adult C57Bl/6 mice were anesthetized and subjected to either controlled cortical impact or sham surgery. Immediately prior to sacrifice, domain antibody (dAb) phage were intravenously injected (1, 7, or 21 days post-injury). Phage were eluted from injured tissue and amplified for a second biopanning round. For *ex vivo* biopanning, fresh-frozen tissue acquired from the porcine rotational acceleration brain injury model was incubated with dAb phage; eluted phage from injured tissue were amplified for two biopanning rounds. Following completion of the biopanning rounds, the diversity of the enriched phage libraries was evaluated with NGS via MiSeq 2x300. **Results:** Analysis revealed that each tissue and control library sample yielded 7000-12,000 dAb sequences. For each injury group (*in vivo* and *ex vivo*), the biopanning process enriched several dAb sequences at levels ranging from 246-fold to 8000-fold. Sequence overlap among injury groups across all experiments was at most 4%, comparable to overlap with the control library. This divergence of dAb sequences across groups reveals biopanning sensitivity to injury conditions and timepoints. Moreover, sequence motifs for the top 30 enriched sequences for each library revealed structural variability among CDR3s from injured vs control groups. From these enriched sequences, we selected unique dAbs not present in control libraries to characterize in future studies. Discussion:This phage display biopanning and analysis pipeline are sensitive to the heterogeneous and diverse effects of neural injury, thus demonstrating its feasibility as a biomarker discovery tool. Both approaches revealed unique dAbs for further exploration and characterization as targeting tools for biomarkers (mass spectrometry and immunohistochemistry).

Poster Abstracts

Alec McCall

Development of 3D printed molds to generate complex hydrogel geometries for islet macroencapsulation

Alec McCall, Jessica D. Weaver, School of Biological and Health Systems Engineering, Arizona State University, Tempe Az

Islet cell transplantation via macroencapsulation devices is a potential therapy to treat type I diabetes in the absence of chronic systemic immunosuppression. One of the greatest limiting factors of this technology is the size of the encapsulating device. Large device geometries result in decreased transport efficiency of nutrients and insulin though the hydrogel barrier. By designing devices with geometries which minimize diffusion distances (e.g. a spiral or crimped sheet geometry), more effective nutrient transport can improve encapsulated cell viability and function. A potential method to fabricate complex three-dimensional hydrogel geometries is injection molding. We demonstrate here the design of three-dimensional printed injection molds for the fabrication of complex hydrogel device geometries. Advantages of this strategy include rapid mold prototyping and facile implementation in a surgical setting.

David Medina

Retinoid activating nanoparticles increase lifespan and reduces neurodegeneration in the SOD1G93A mouse model of ALS

Barrow Neurological Institute, Phoenix Az

Retinoic acid (RA) has been established to have important roles in both neuronal development and normal nervous system function. Recent evidence has shown that changes in the RA signaling pathway are correlated with ALS pathology and other neurodegenerative disorders. In this study, we sought to examine the neuroprotective role of retinoic acid (RA) signaling in amyotrophic lateral sclerosis (ALS). We hypothesized that activation of retinoid signaling will reduce disease progression in $SOD1^{G93A}$ transgenic mouse model of ALS. Specifically, we investigated the therapeutic value of targeted retinoid activation via the RA receptor β (RARβ). To address this question, we utilized adapalene, an FDA approved RARβ agonist, which our lab had previously found to be neuroprotective in cultured motor neurons. Currently adapalene is used clinically as a topical treatment for dermatological disorders, however, due to its hydrophobicity, and rapid clearance rates, delivery of this drug to the nervous system is a major challenge for assessing its therapeutic value. To address this, we engineered adapalene loaded polymeric nanoparticles (Adap-NPs) composed of poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) to achieve delivery to the CNS. Adap-NPs were administered to $SOD1^{G93A}$transgenic mice via lateral tail vein injections 3x a week starting at 61 days of age. Motor function was measured weekly using the motor tasks, (e.g. rotarod) during treatment to determine treatment effect on disease progression. Treatment effect on survival, disease onset and progression were measured. Markers of neurodegeneration and inflammation in the spinal cord were also measured to determine the effect of Adap-NPs on ALS-like pathology. We found that chronic administration of Adap-NPs resulted in a significant increase in average lifespan in transgenic mice and delayed disease progression (Gehan-Breslow-Wilcoxon test $p=0.03$; $p=0.04$, respectively). This increase was associated with significantly improved motor performance in multiple motor tasks, and reduction of roughly 50% in spinal motor neuron loss in transgenic mice ($p<0.05$). These data provide supporting evidence for targeting the retinoic acid signaling pathway as a therapeutic approach for ALS. In addition, this study also highlights value of utilizing nanomedicine approaches to improve the delivery of drug candidates. Retinoic acid (RA) has been established to have important roles in both neuronal development and normal nervous system function. Recent evidence has shown that changes in the RA signaling pathway are correlated with ALS pathology and other neurodegenerative disorders. In this study, we sought to examine the neuroprotective role of retinoic acid (RA) signaling in amyotrophic lateral sclerosis (ALS). We hypothesized that activation of retinoid signaling will reduce disease progression in $SOD1^{G93A}$ transgenic mouse model of ALS. Specifically, we investigated the therapeutic value of targeted retinoid activation via the RA receptor β (RARβ). To address this question, we utilized adapalene, an FDA approved RARβ agonist, which our lab had previously found to be neuroprotective in cultured motor neurons. Currently adapalene is used clinically as a topical treatment for dermatological disorders, however, due to its hydrophobicity, and rapid clearance rates, delivery of this drug to the nervous system is a major challenge for assessing its therapeutic value. To address this, we engineered adapalene loaded polymeric nanoparticles (Adap-NPs) composed of poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) to achieve delivery to the CNS. Adap-NPs were administered to

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Juan Melendez-Alvarez

Restore the Memory of Gene Circuit by Uncoupling Growth-Mediated Feedback

Biodesign Institute, Arizona State University, Tempe Az

The synthetic gene circuits are inevitably coupled with the growth feedback, in which the gene circuit affect the cell growth and the cell growth affect the expression of the genes in the circuits [1, 2]. Growth-mediated feedback between the synthetic gene circuit and host can lead to emerged complex behaviors. For example, a non-cooperative positive autoregulatory system, when coupled with growth-mediated feedback, gained significantly increased effective cooperativity and thus resulted in bistability [3, 4]. However, its adverse impact on gene circuits remains unexplored. Here, we found that the growth feedback disguises true behaviors of memory gene circuits. Specifically, the memory of gene circuit is lost due to the fast growth of the host cell. Decoupling of growth feedback reveals a broad range of hysteresis. The underlying principle is demonstrated with a mathematical model by integrating cell growth and the dynamics of gene circuit. Our results suggest that circuit-host interaction negatively affect desired functions of gene circuits and thus should be well controlled.

Katherine Minter-Dykhouse

Ploidy and Proliferation during in vitro Cardiogenesis

Katherine Minter-Dykhouse[1,2,3], Alicia Saarinen1, Mohammed Daramy[1], Timothy Nelson[2,5,6], and Clifford Folmes.[1,4,7], [1]Stem Cell and Regenerative Metabolism Laboratory, [2]Wanek Family Program for Hypoplastic Left Heart Syndrome, Mayo Clinic, Rochester, MN 55905, [3]Department of Molecular Pharmacology and Experimental Therapeutics, [4]Department of Biochemistry and Molecular Biology, [5]Department of Pediatric and Adolescent Medicine, [6]General Internal Medicine, [7]Department of Cardiovascular Disease, Mayo Clinic

Despite its essential role the heart has limited regenerative capacity, which combined with the increasing prevalence of heart disease has highlighted a substantial need for cardiac regenerative therapies. While our understanding of cardiogenesis in animal models, notably mouse and zebrafish is detailed, an accessible human model has until recently been lacking. The advent of human induced Pluripotent Stem Cells (hiPSCs) and protocols for their differentiation to cardiac lineages has provided investigators with a highly relevant platform for the modeling of human cardiac development in both health and disease, as well as a means to generate patient-derived cardiomyocytes in vitro for regenerative therapies. An area of particular relevance to the development of cardiac regenerative strategies is the elucidation of the mechanisms governing cardiomyocyte cell cycle, both in the context of stimulating DNA replication and cardiomyocyte cell cycle. Using the directed differentiation of human iPSCs to cardiomyocytes as an in vitro model of cardiogenesis, we have assessed the proliferative capacity of developing cardiomyocytes, identifying the timeframe during which cardiomyocytes lose their proliferative potential, which is associated with the emergence of multinucleated and/or polyploid cells. While multi-nucleation is a common and normal phenomenon in mammalian heart development in vivo, polyploidy is thought to be associated with pathologies such as hypertrophic and dilated cardiomyopathies. Further analysis of the multinucleated and/or polyploid cardiomyocytes revealed elevated levels of markers associated with cellular stress (GADD45A and p53-pSer15) and both

quiescence (p21/CDKN1A) and senescence (p16ink4a/CDKN2A). Our findings suggest that the in vitro directed differentiation of human iPSCs to cardiomyocytes exerts both premature and excess stress on these immature cardiac cells resulting in a phenotype more associated with pathology than regeneration. This highlights the need to further optimize the methods used to generate and evaluate cardiomyocytes produced in vitro, whether they are being used to model human development or as a regenerative therapy.

Gergey Mousa

HDACi-Loaded Nanoparticles Enhance Functional Outcomes Following Traumatic Brain Injury

Gergey Mousa[1], Connor Copeland[1], Briana I. Martinez[1,2], Kamawela Leka[1], George R. Bjorklund[1], Kyle T. Householder[3], Jason M. Newbern[2], Rachael W. Sirianni[3], Sarah E. Stabenfeldt[1] [1]School of Biological and Health Systems Engineering, [2]School of Life Sciences, Arizona State University, Tempe, AZ 85287, [3]Vivian L. Smith Department of Neurosurgery, University of Texas Medical School, Houston, TX 77030

Traumatic brain injury (TBI) is a major cause of disability, with approximately 1.7 million incidents reported annually. Following a TBI, patients are likely to sustain sensorimotor and cognitive impairments and are at an increased risk of developing neurodegenerative diseases later in life. Despite TBI's prevalence and enduring effects, robust therapies that treat TBI neuropathology are not available in the clinic. One emerging therapeutic approach is to target epigenetic mediators that modulate a variety of molecular regulatory events acutely following injury. Specifically, previous studies demonstrated that histone deacetylase inhibitor (HDACi) administration following TBI reduced inflammation, enhanced functional outcomes, and was neuroprotective. Here, we evaluated a novel quisinostat-loaded PLA-PEG nanoparticle (QNP) therapy in treating TBI as modeled by a controlled cortical impact in adult C57BL/6 mice (CCI; 2 mm tip diameter, 6.0 m/s over the frontoparietal cortex); all experiments were approved by ASU IACUC. Briefly, we evaluated initial pharmacodynamics within the injured cortex via histone acetylation levels following administration of QNPs. A second cohort of mice underwent a battery of behavioral assessment over the course of a month following CCI and QNP intervention (rotarod, open field, gridwalk, and Morris water maze). We observed that QNP administration acutely following injury increased histone acetylation specifically within the injury penumbra, as detected by Western blot analysis. Initial behavioral results indicate that QNP treatment dampened motor deficits as measured by increased rotarod latency to fall relative to blank nanoparticle- and saline-treated controls. Additionally, open field results show that QNP treatment altered locomotion following injury. These results suggest that HDACi therapies are a beneficial therapeutic strategy following neural injury and demonstrate the utility for nanoparticle formulations as a mode for HDACi delivery following TBI.

Luis Novelo

Comparative Analysis of the Regulatory Framework for Experimental Stem Cell Treatments in the US and Mexico

Arizona State University, Tempe Az

Experimental stem cells treatments are currently overseen by contrasting federal regulations in the United States and Mexico. While the US continues to debate on the efficacy of these treatments and the appropriate amount of regulation, Mexico has adopted a model that only prioritizes patient safety. As a result, stem cell treatments are openly administered in Mexico and are actively promoted by the federal government. The implications that this has on patients remains to be seen, but patients are currently expected to assume more responsibility and risk in researching their desired treatment in Mexico than in the US.

Joanna Palade

Molecular analysis of muscle progenitor cells on extracellular matrix coatings and hydrogels

Joanna Palade, Caroline Addington, Alan Rawls, Sarah Stabenfeldt, Jeanne Wilson-Rawls, Arizona State University, Tempe, Az

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Satellite cells are adult muscle progenitors that could be used as an autologous source of stem cells to treat the severe muscle damage that might result from traumatic injury or disease. However, attempts to use these cells therapeutically have been largely unsuccessful, as *ex vivo*expansion of satellite cells leads to poor re-engraftment and loss of myogenic potential. Further, injection of satellite cells into damaged muscle directly results in extremely low survival of injected cells. To overcome these challenges, several groups have explored innovative bioengineering techniques to design scaffolds for satellite cells and muscle repair, with varying degrees of success. We wanted to examine the complex biology of myogenic progenitor cells in response to culture on biomimetic hydrogels and coatings comprised of extracellular matrix proteins present in the muscle stem cell niche, like collagen I, laminin, and fibronectin. We also wanted to compare the behavior of primary satellite cells to C2C12 cells, an immortalized myoblast cell line often used in these bioengineering studies. Viability, proliferation, differentiation, and myogenic gene expression were assayed for both cell types. On the hydrogels, satellite cells neither proliferated, nor differentiated into multinucleated myotubes, remaining instead as single cells. Gene expression analysis using quantitative RT-PCR at days 3 and 6 revealed a dynamic expression profile that suggested the satellite cells initially attempt to differentiate and exit the cell cycle but eventually enter a quiescent state. Additionally, the composition and relative stiffness of the hydrogel did not significantly alter satellite cell behavior. C2C12 cells, however, were able to both proliferate, and differentiate into multinucleated myotubes on the hydrogels. They upregulated myogenic transcription factors, like MyoD and myogenin, indicating they have entered the myogenic program. When cultured on plates coated with the same ECM proteins, satellite cells both proliferated and differentiated well, albeit not as robustly as they did on Matrigel coated plates. C2C12 cells proliferated exceptionally well on ECM coated plates and did not express the muscle growth inhibitor myostatin. Indeed, when siRNA constructs targeting myostatin were used in satellite cells, proliferation improved on both coated plates and hydrogels, demonstrating that at least in part, the proliferation defect is myostatin-dependent. These findings suggest that substrate stiffness alters satellite cell biology more than protein composition, and that depending on context, C2C12 cells behave differently than satellite cells, indicating they may not be a suitable model for these studies.

Swechchha Pradhan

Isolation of inner ear stem cells and their differentiation into auditory hair cells in vitro using CRISPR

John Quinn

DUAL Control: A novel genetic tool to study and manipulate tissue regeneration

John Quinn and Robin Harris, School of Life Sciences, Arizona State University, Tempe, Az 85287

Regeneration is a complex biological phenomenon that is limited to certain organisms and tissues. The process of regeneration has been studied using numerous different model organisms in the hope of ultimately applying these findings to human biology. However, many of these models are limited by the lack of genetic tools, poor accessibility, and long experimental duration. Drosophila melanogaster, the common fruit fly, is a well established and powerful model for exploring the genetics of development and disease, for which abundant genetic tools exist. Drosophila have larval tissues called imaginal discs that are capable of significant regeneration early in larval life. Our lab studies regeneration in the wing imaginal disc due to its dispensability for survival. Historically, regeneration of these tissues was demonstrated by dissecting the imaginal disc from larvae, physically wounding the organ and transplanting it into the abdomen of an adult fly for ex-vivo culture. The organ was then recovered to assay regeneration. These experiments are laborious, low throughput and require excessive manipulations. To overcome these limitations, our lab has developed a novel genetic tool to study regeneration of Drosophila larval tissues in vivo. This tool utilizes two parallel yet independent expression systems; one that directs expression of a pro-apoptotic gene to the wing imaginal disc, which induces damage and stimulates regeneration, and another that allows the subsequently regenerating tissue to be targeted for genetic manipulations, such as RNAi and ectopic gene expression. This combinatorial arrangement permits unprecedented spatial and temporal control over regeneration, and thus we have called it Duration and Location Control, or DUAL Control. Importantly, this new system can take advantage of genome-wide screening libraries available to the Drosophila community, allowing rapid identification of genes involved in regrowth and patterning, and investigation of manipulations that can augment regeneration. Using this platform, we have

already identified several epigenetic silencing factors that play a key role in limiting tissue regeneration with developmental maturity, revealing that epigenetic control over regeneration is an important mechanism. The conservation of these factors across species, including humans, ensures these findings will be broadly applicable.

Inad Rabadi

Development of Organ-On-Chip and Circulating Tumor Cell Collection Devices for Modeling Lung Metastasis of Osteosarcoma

Inad Rabadi [1], Adam Akkad [1], Shelby Rheinschmidt [1], Aparna Sertil [2], Pooja Hingaroni [3], Jian Gu [1,2]
[1]Center for Applied Nanobioscience and Medicine, and [2]Department of Basic Medical Sciences, UA College of Medicine, Phoenix, AZ 85004 [3]Center for Cancer and Blood Disorders, Phoenix Children's Hospital, Phoenix, AZ 85016

Overall survival for metastatic osteosarcoma (OS) is dismal at less than 20% with no new advancements in therapy over the past several decades. The most common site of OS metastasis is in the lungs. It is hypothesized that the resistance of pulmonary metastasis to currently available treatment is in part due to the "protective" role the lung microenvironment has on metastatic cells. It is also well recognized by the OS community that targeting (micro)metastasis and its progression, i.e. tumor lesions undetectable in clinic, can be critical in terms of breaking the survival plateau of OS patients observed over past few decades. Therefore, a predictive model that can capture the complex interactions between cancer cells and their physicochemical microenvironment*in vivo*can be critical for drug and chemotherapeutic strategy development. 2D and even 3D spheroid cell cultures have been used, but are not adequate in this aspect. Likewise, animal models can be expensive and may fail to recapitulate the human condition. Recently, microfluidic engineered organ-on-chip models have emerged as promising humanized *in vitro*platforms for drug screening while mimicking unique tissue structures in 3D. In this project, we demonstrate our progress in building organ-on-chip devices that model the Lung for study of OS metastasis. Two models of our "Lung-on-chip" are presented: the first focuses on conditioning OS spheroid growth media, and the second implements a hydrogel model of pulmonary interstitial space. We will also show results of using a filter device to collect OS cells by size with an initial collection efficiency of > 80%—a device which could be used for circulating tumor cell collection. With these models, we hope to mimic the lung microenvironment and therefore provide a viable platform to aid in the development of metastatic OS treatment.

Sreedevi Raman

INVESTIGATING APOE EFFECTS IN ALZHEIMER'S DISEASE USING HIPSC DERIVED ASTROCYTES

Arizona State University, Tempe Az, 85287

Alzheimer's disease (AD) is the sixth leading cause of death in the United States and poses an increasing burden on the healthcare system. Following the first report of AD in 1907, numerous clinical trials addressing the classic amyloid hypothesis have failed to identify a cure. It is essential to understand the role of genetic risk factors such as the cholesterol transport protein apolipoprotein E (ApoE) in the more prevalent sporadic form of the disease. ApoE is highly expressed by astrocytes in the human brain, where it is found as the E2, E3 and E4 isoforms, with ApoE3/3 being the most common genotype. The less common E4 allele greatly increases the risk of developing sporadic AD and reduces the mean age of onset from 84 to 68 years, whereas the E2 allele has a protective effect. The mechanism of this effect remains unknown and ApoE has been implicated in amyloid peptide aggregation and clearance defects in animal models. Although the mouse model of AD has provided invaluable mechanistic insight and led to the identification of early biomarkers of the disease, translation into human therapy has been unsuccessful. With the advent of iPSC technology, it is possible to model AD using reprogrammed patient cells and study disease progression as it relates to the complex human genetic landscape. We differentiated six (two non-demented control, familial and sporadic AD) patient derived iPSC lines into neural progenitor cells (NPC) and subsequently into astrocytes on laminin and VDP, a synthetic substrate that supports the maintenance of cell types of the CNS. Our robust protocol generated mature astrocytes that secreted ApoE, exhibited calcium transience and were responsive to inflammatory stimuli. These astrocytes took up fluorescently labeled Ab-42, indicative of their role in Ab peptide clearance.

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They retained their astrocytic identity and functionality following cryopreservation. Additionally, the differentiation protocol was scaled up to a bioreactor system to generate mature functional astrocytes. We are currently studying the isoform specific effects of ApoE in astrocytes and neurons generated using isogenic lines derived from a familial AD patient. We plan to study the mechanism ApoE isoform specific effects in co-culture systems of astrocytes and neurons derived from patient iPSCs.

Shelby Rheinschmidt

Development of a Heart Disease Tissue Model using Cardiomyocytes from Patient hiPSCs

Shelby Rheinschmidt[1,3], Onur Oncer B.S.[1,2,3], Zoey Nunn B.S.[1,2,3], Jared Churko Ph.D.[1,2,3] , [1]Sarver Heart Center, [2]iPSC Core, University of Arizona Health Sciences, [3]Departmet of Cellular and Molecular Medicine, University of Arizona Health Sciences, Tucson, AZ, 85724, USA

Engineered Heart Tissues (EHTs) are 3D cell constructs used to model the heart myocardium. These tissues have many similar qualities to the heart such as being able to beat in a 3D sheet thus allowing for the analysis of arrhythmias. EHTs are most commonly made with animal cardiomyocytes however, using human cardiomyocytes would be favorable to enable accurate heart disease studies. To test if human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) can effectively be used to form EHTs, hiPSCs were differentiated into cardiomyocytes and tissues were made with a modified protocol developed for rat EHTs. An agarose gel and spacers were used to form a mold and master mix of fibrinogen, thrombin, DMEM, matrigel, and hiPSC-CMs was placed into this mold. Flexible posts were also placed in the molds that were used to measure the force of hiPSC-CM contraction. Seven days after the tissues were generated, eight EHTs were left unpaced and eight tissues were paced (using an electrical pulse) at 0.5 Hz for seven days. Subsequently, the pacing frequency was increased by 0.33 Hz until 3.14 Hz was reached. The peak frequency is the equivalent to an exercised heart beating at 188.4 beats per minute. The data shows that the tissues being paced had a 1.32-fold increase in contraction force compared to the unpaced tissues. This method of 3D modeling will later be used to characterize heart diseases.

Adham Y Saleh

An *in vitro* comparison of 3D printed scaffolds for bone tissue engineering

Adham Y Saleh MD[1], Michael J McPhailPhD[1], Manisha K ShahPhD[1], David G LottMD[1,2] , [1]Head and Neck Regenerative Medicine Laboratory, Mayo Clinic, Scottsdale, AZ 85259, [2]Dept of Otorhinolaryngology, Mayo Clinic, Phoenix, AZ 85054

Introduction

The goal of this work is to develop a three-dimensional (3D) scaffold for bone tissue engineering of head and neck sites. Current reconstructive approaches for bone defects, such as mandibular defects or semicircular canal dehiscence (SCD), require harvesting autologous tissues. This approach can result in extended surgical time, increased time to recovery and donor site morbidity, and the harvested tissues lack the desired geometry. Tissue engineered constructs, composed of a scaffold and stem cells, can be used to repair the bone defect. A tissue engineering scaffold can be generated in any desired shape with 3D printing, to match the bone defect. In this work, we evaluate two materials in 3D printed scaffolds for their osteogenic potential.

Methods

The 3D printed scaffolds were made from 45,000 mw polycaprolactone (PCL) or PCL mixed 20% by weight with hydroxyapatite (PCL/HA) with an Envisiontec Bioplotter. Scaffolds were printed in a cylindrical shape with a 6 mm diameter and two layers for a total height of 640 µm. All scaffolds were printed at 80°C with a 22-gauge needle. Due to varying material viscosities, PCL scaffolds were printed at a speed of 2.8 mm/s and the PCL/HA scaffolds were printed at 2 mm/s. Strand diameters were nominally 400 µm with a spacing of 550 µm between strand centers. Bone marrow derived stem cells were seeded onto the scaffolds (50,000 cells per scaffold) in triplicate in a 96 well plate in serum free osteogenic medium or serum containing growth medium (control). After three weeks, osteogenic differentiation was assessed by immunofluorescence for osteocalcin and quantification of calcium deposits using Alizarin Red.

Results and Discussion

Poster Abstracts

No significant difference was observed between the osteogenically-induced PCL and PCL/HA scaffolds. However, the induced samples had greater calcium deposition compared to their corresponding control scaffolds. Both osteogenically-induced PCL and PCL/HA scaffolds stained positive for osteocalcin, a protein involved in bone mineralization and calcium deposition. Due to the similarities in hydroxyapatite to mineralized bone, we plan to continue further cell viability, proliferation, and differentiation studies with the PCL/HA scaffold instead of the PCL-only scaffold for bone tissue regeneration.

Kristina Sin

Modulation of VEGF Signaling in Atrial-like induced pluripotent stem cell derived Cardiomyocyte Differentiation

Kristina Sin[1,4], Zoey Nunn B.S.[2,3,4], Jared Churko, Ph.D.[2,3,4], [1]Department of Biochemistry, Molecular and Cellular Biology, College of Science, [2]Sarver Heart Center, [3]iPS Cell Core, University of Arizona Health Sciences, [4]Department of Cellular and Molecular Medicine, University of Arizona Health Sciences, Tucson, AZ, 85724, USA

Atrial fibrillation (AF) is the most common form of arrhythmia, impacting nearly 1 in 10 adults over the age of 65 in the US and is projected to increase as our population ages. AF is a highly complex condition that evolves over time and displays variability from patient-to-patient, which makes the studying of this disease incredibly difficult. Though animal models have previously been employed for preclinical drug trials, these models are limited by interspecies differences in phenotype, physiology, and pathology to accurately represent AF. The advent of human induced pluripotent stem cells (hiPSCs) and ability to differentiate hiPSCs into cardiomyocytes (hiPSC-CMs) offers a promising and inexhaustible source of cells for the creation of *in vitro* cardiac models that may faithfully replicate human phenotypes and the cellular responses exhibited *in vivo*. Current cardiomyocyte differentiation protocols display cell type heterogeneity between ventricular, atrial, and nodal cell types, thereby impeding for the creation of cost-efficient, homogenous models. It is critical to understand the spatiotemporal environment for atrial differentiation in the embryo so as to effectively recapitulate atrial differentiation *in vitro*. We have recently identified two subpopulations of hiPSC-CMs that are regulated by the atrial marker *NR2F2* and atrial identity suppressor *HEY2*. Both markers are opposingly regulated by VEGF-Notch signaling as well as strongly implicated in governing the atrial versus ventricular gene signature programming. Active VEGFR2/VEGF signaling inhibits *HEY2*expression while promoting the expression of *NR2F2*. It is hypothesized that by inhibiting the VEGF decoy receptor VEGFR1, atrial differentiation may be promoted by way of enacting the downstream effects of *NR2F2* to thus suppress the ventricular program. Defining novel and more efficient protocols for the generation of atrial cardiomyocytes will provide the means necessary for cost-efficient and patient-specific modeling of atrial-specific disorders such as AF.

Gayathri Srinivasan

A biomanufacturing platform for the large-scale neuronal differentiation of human pluripotent stem cell-derived neural progenitor cells

Srinivasan G, Henson T, Morgan D, Varun D, Brookhouser N & Brafman D, School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ 85287

Human pluripotent stem cell derived neural progenitor cells (hNPCs) have the unique properties of long-term *in vitro* expansion as well as differentiation into the various neurons and supporting cell types of the central nervous system (CNS). Because of these characteristics, hNPCs have tremendous potential in the modeling and treatment of various CNS diseases. However, large-scale neuronal differentiation of hNPCs and cryopreservation of their neuronal derivatives are major challenges in utilizing hNPCs for basic and translational applications. Here, we used a fully defined peptide substrate- a vitronectin derived peptide (VDP) as the basis for a microcarrier (MC)-based suspension culture system that enables the highly efficient neuronal differentiation of several hNPC lines. We further used this MC-based system in conjunction with a low shear rotating wall vessel (RWV) bioreactor for the large-scale cortical neuronal differentiation of hNPCs from patient-specific hiPSCs. Specifically, using a 55 mL bioreactor vessel, we were able to reproducibly generate over 125 million hNPC-derived neurons. Neurons generated in this bioreactor system could be dissociated, cryopreserved and replated onto VDP coated 2-D surfaces with high levels of cell viability, which will be important for downstream high-content phenotypic drug

screening assays where the culture of neurons in 2-D will be required. Cryopreserved neurons also maintained the expression of cortical neuronal markers and exhibited spontaneous calcium spikes. In the future, this fully defined and scalable biomanufacturing system will provide a platform for the generation and cryopreservation of hNPC-derived neurons under GMP/GLP standards in numbers (>10^9) necessary for many downstream drug screening and regenerative medicine applications.

Zack Strong

Renal Cell Carcinoma (RCC) Fibronectin Fibrillogenesis After Increasing Cytosolic Calcium Levels

Northern Arizona University, Flagstaff, Az

Renal cell carcinoma (RCC) accounts for about 90% of all renal cancers. Fibronectin (FN) fibrillogenesis is the production of fibrils in the extracellular matrix (ECM) and required to prevent renal cancer metastasis. The Von Hippel-Lindau (VHL) gene is a tumor suppressor that is lost or mutated in 95% RCC cases. Interestingly, VHL is required for the formation of a FN ECM. However, the mechanism relating the VHL gene to fibrillogenesis is unknown. It is thought that intracellular calcium (Ca2+) is required for FN fibrillogenesis, and promyelocytic leukemia protein (PML) has been linked to the release of Ca2+ from the endoplasmic reticulum into the cytosol. We observed from mass spectrometry data that PML interacts with FN, leading us to believe that there exists a functional link between PML and FN. Work carried out by others have shown that cytosolic PML and Ca2+ play critical roles in increasing cancer cell survival mechanisms contributing to treatment resistance. Understanding calcium signaling and its relation to FN fibrillogenesis (which is anti-tumorigenic in renal cancers) could possibly shed light on mechanisms that regulate fibrillogenesis, as well as determine whether treatment resistance is associated with unregulated intracellular Ca2+. This project focused on two isogenic renal cancer cell lines RCC– (VHL null; tumorigenic), and RCC+ (VHL complemented; non-tumorigenic). We examined changes in protein composition after treatment with three calcium inhibitors at varied time intervals. BAPTA-AM is a calcium chelator that decreases intracellular calcium levels. Thapsigargin increases cytosolic calcium by preventing calcium uptake into the ER. U73122 inhibits the phospholipase C pathway, decreasing cytosolic calcium. We hypothesized that increasing the cytosolic calcium and PML-FN interaction would increase FN fibrillogenesis. To establish this hypothesis we performed immunofluorescence, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and Western Blotting techniques to examine the protein composition of the RCC cells after treatment.

Sambhavi Subramanian

Necroptosis inhibition of vaccinia virus pathogenesis in mice

Biodesign Institute, Arizona State University, Tempe, Az

Vaccinia virus encodes the multifunctional protein, E3 that functions in evading the interferon induced antiviral response. The E3 protein contains two highly conserved functional domains. The well-characterized carboxy – terminus contains a double stranded RNA binding domain that aids in interferon (IFN) resistance. The less-well characterized amino terminus contains a Z-DNA binding domain. Deletion of N-terminus domain in E3 (VACVΔ83N) results in attenuation of the virus in mouse model and the pathogenesis is restored in an IFN knockout mouse model. Necroptosis is a pro-inflammatory, caspase independent mechanism of programmed cell death. Necroptosis has been implicated in the pathogenesis of many diseases ranging from cancer, inflammatory diseases to neurodegenerative diseases such as Alzheimer's and also in viral infections. In IFN treated L929 cells, VACVΔ83N induces necroptosis and death is dependent on host RIPK3 protein and on DAI, a sensor of virus infection. In this study, we characterized the role of IFN sensitivity of VACVΔ83N in necroptosis during pathogenesis in a mouse model. Our results demonstrate that in a C57BL/6 model, IFN-treatment significantly reduced pathogenesis during Δ83N infection while there was no significant difference during WT infection. Additionally, in a DAI or MLKL knockout model, there is no significant difference in pathogenesis between non-IFN treated and IFN-treated animals during Δ83N infection and the pathogenesis is higher than in a C57BL/6 mice. Infection with point mutant of key predicted ZNA interacting residue (VACV_P63A) resulted in reduced pathogenesis in a C57Bl/6 model while this pathogenesis was restored in the DAI or MLKL

knockout mouse model. In summary, this data suggests that virus-induced necroptosis is important for inhibiting vaccinia virus pathogenesis in mice.

Yuka Sugamura
Multiomics Analysis of Ovarian Cancer Progression
School of Biological and Health Systems Engineering, Arizona State University

School of Biological and Health Systems Engineering, Arizona State University
Ovarian cancer is a fatal gynecological malignancy and the fifth leading cause of cancer death in women in the United States. Poor prognosis can be attributed to several factors, including: 1) advanced stage diagnosis accompanied by metastasis into the peritoneal cavity, 2) propensity of ovarian cancer to develop chemoresistance, and 3) high rate of recurrence. Although advances have been made in the field of cancer research, many important questions remain unanswered within ovarian cancer. For instance, a deeper understanding of the mechanisms related to tumorigenesis, metastasis, and chemoresistance would provide essential insight into early stage disease progression and treatment strategies. In this research, we aim to develop a system to monitor cellular processes in real-time through technical multiomics approach. Tissue engineering techniques using stem cell- or cancer stem cell-derived organoids and spheroids will be explored in order to recapitulate the tumor microenvironment and heterogeneity of ovarian cancer. This work has the potential to aid in the development of effective measures for prevention, early detection, and treatment of this devastating disease.

R. Kevin Tindell
Fabricating Scaffolds with Gradients in Fiber Alignment and Chemistry for Interfacial Tissue Repair
Arizona State University, Tempe, Az

Statement of Purpose: Although there have been significant advances within tissue engineering, translation has proven difficultwith ongoing challenges regenerating complex tissues with a heterogeneous structure and multiple cell types. For example, the tendon to bone interfacial tissue gradually transitions from highly aligned fibrous tissue to calcified bone [1]. For transitions between musculoskeletal tissues (e.g., ligament or tendon to bone, muscle to tendon, cartilage to bone, etc.), the interfacial region is critical for transferring mechanical load from one tissue type to another. Developing new biomaterial fabrication strategies capable of mimicking the heterogeneous nature of interfacial tissues, as well as other complex tissues, is vital for spatially controlling cellular behavior and resulting in functional interfacial tissue regeneration. Toward this aim, we produced electrospun scaffolds with gradients in fiber alignment and chemistry for interfacial tissue repair
Methods: For fabricating fiber orientation gradients, a 14.3 wt% polycaprolactone (PCL) solution was made with a 1:1 ratio of tetrahydrofuran and dimethylformamide. Magnetically-assisted electrospinning was performed by placing magnetic strip(s) in different configurations onto the fiber collector. Optical micrographs of the scaffolds were performed and analyzed with ImageJ to evaluate fiber alignment percentage as a function of scaffold location. To fabricate scaffolds with spatially controllable biochemical gradients, a 3.25 wt% norbornene-modified hyaluronic acid (NorHA) with a functionality of 40% was prepared in deionized water with a 0.05% I2959 as a photointiator and a stoichiometric ratio of norbornene/dithiothreitol (DTT) of 0.5. Fibrous scaffolds were crosslinked under 10 mW/cm^{2}UV-light for 5 min. After electrospinning, the scaffold was swollen in 25µM rhodamine-methacrylate or fluorescein-methacrylate with 0.05% I2959. For biomolecular photoconjugation, a sliding photomask with a velocity of 30 µm/s was used to spatially tune scaffold exposure to the 10 mW/cm^2 UV-light and create a scaffold chemistry gradient. Unconjugated molecules were removed via washing for 1-2 days prior to analysis. Fluorescence microscopy was used to image conjugated rhodamine or fluorescein dye on the scaffold surface and ImageJ was used to quantify fluorescence intensity with scaffold distance.
Results: Magnetically-assisted electrospinning results in fiber alignment in the direction of the applied magnetic field. Eectrospun fibers were highly aligned when spun near or onto a magnet (~80%, region A) and transitioned to unaligned fibers away from the magnet (~30%, region C). Importantly, this transition occurs within a millimeter and is similar to the scale

observed in native tendon-bone junctions. Mesenchymal stromal cells (MSCs) seeded onto gradient scaffolds showed higher aspect ratios on the aligned fibers compared to unaligned fibers. To spatially control dual biochemical gradients on fibrous scaffolds, NorHA electrospun fibers were photopatterned with rhodamine-methacrylate and fluorescein-methacrylate via a sliding mask technique. Regions where the sliding mask prevented UV-light from penetrating the scaffold resulted with no conjugation of dye. Rhodamine was first patterned onto the scaffold, followed by 1-2 days of washing; the scaffold was then flipped 180^{o} and photopatterned with fluorescein to formulate a dual chemical gradient. Gradient lengths were within the millimeter scale, thus producing high resolution chemical gradients with similar lengths as the tendon-bone junction.
Conclusions: In this work, we fabricated a novel electrospun scaffold through magnetically-assisted electrospinning to mimic the native structure of the tendon-bone junction. Moreover, photopatterning biomolecular gradients via a sliding mask produced high resolution chemical gradients. Future work will entail combining magnetically-assisted electrospinning with biomolecular photopatterning to create simultaneous gradients in fiber alignment and chemistry for improving interfacial tissue repair.

Stephanie Valenzuela
Cyclic Shear Loading of Fat Extracted Mesenchymal Stem Cells on Bone Cores Promotes Hyaline-like Cartilage Formation
University of Arizona: Dept. of Orthopedic Surgery, Tucson Az

Osteoarthritis is a degenerative joint disease that is characterized by cartilage damage and wear. Tissue regeneration using adipose-derived stem cells (ASC) offers a less invasive treatment option than total joint replacement. ASC have been observed to differentiate into chondrocytes, forming a hyaline-like cartilage under the right conditions. Seeding human patellarcartilage-coveredd bone cores from osteoarthritis patients with endogenous ASCs and then loading the core surfaces induced cell proliferation and hyaline-like cartilage formation. Endogenous ASC were extracted from peri-patellar fat following a total knee surgery. Cells were fed Stem Cell Expansion Media (SCEM) every other day and expanded into flasks where cell confluency reached about 85%. Using joint discards from the same patient that fat was collected from, 9 mm cores were punched using a surgical bone core punch. Punches perpendicular to the surface were produced in areas that had cartilage so that loading could be carried out perpendicular to the surface simulating loads that would be expected in a patient's joints. Cores with unleveled surfaces were used as unloaded controls. This project focused primarily on cyclic shear loading and its stimulatory effects that are a loading characteristic of walking. The loading system applied a cyclic, shear load onto the surface of the cores with silicon covered surface loading pins. Cores were held for 48 hours to allow cells to adhere to the core surfaces in the new environment. Adhesion proteins and growth factors were utilized in this in-vitro study to facilitate cartilage formation on the core surfaces. Loaded cores showed 55% more living cells within the cartilage tissue surfaces compared to controls but only slightly thicker tissue layers. The combination of seeding, loading and proteins create an environment that encourages hyaline-like cartilage formation and regeneration in patients.

Ian Vicino
The Use of a Modified Campenot Trichamber System to Investigate Axonal Exocytosis of Herpes Simplex Virus
Ian Vicino[1,2], Ian B. Hogue[1] , [1] Center for Immunotherapy, Vaccines, and Virotherapy, Biodesign Institute, [2] Interdisciplinary Graduate Program in Neuroscience, Arizona State University, Tempe, AZ, USA

Herpes simplex virus (HSV) is a neuroinvasive alphaherpesvirus that infects approximately two-thirds of the world's population. After initial infection of epithelial cells, HSV enters the axons of sensory and autonomic neurons, travels retrograde to the neuronal cell body, and establishes a life-long latent infection in peripheral ganglia. Occasionally, virus replication reactivates, newly assembled virus particles are capable of anterograde axonal sorting and transport, and virus spreads back to epithelial cells, where the virus can spread to new hosts. HSV particles are also capable of spreading via the central processes of pseudounipolar sensory neurons into the CNS. Herpesvirus infections of the CNS can be deadly or debilitating as in the case of Herpes Simplex Encephalitis (HSE), but it is increasingly apparent that HSV also spreads into the CNS asymptomatically, and may contribute to neuroinflammation and neurodegenerative disease. Our laboratory is

interested in the basic molecular and cell biology of HSV infection and spread in the nervous system. To investigate the exocytosis and spread of HSV particles from the axons of peripheral neurons, we have adopted a modified Campenot trichamber system to fluidically separate neuronal cell bodies from their axons. These devices consist of a 20mm diameter circular Teflon ring transected by two walls that divide the cell culture volume into three fluidically-isolated chambers. These Teflon rings are mounted on a standard 35mm tissue culture dish using silicone vacuum grease and cell culture media thickened with methylcellulose to form aqueous micro-channels for axon penetration. We will dissect the superior cervical ganglion (SCG) from rat embryos (E17-18), dissociate them to form a single-cell suspension, and seed approximately 200,000 cells into the left soma chamber. After 2-3 week in vitro, axons penetrate under the chamber walls, though the middle chamber, and into the right neurite chamber. The fluidic separation allows us to initiate infection by adding HSV to the cell bodies, incubate approximately 24h to allow viral replication and anterograde axonal transport, and then measure the concentration of virus in the axon compartment. To identify cellular mechanisms of virus particle exocytosis from axons, we plan to inhibit cellular factors known to be involved in exocytosis from axons, infect cell bodies with HSV, and measure virus spread from axons in the neurite chamber. This study may help reveal therapeutic strategies to prevent recurrent herpes labialis.

Efren Barron Villalobos

Dynamic Bioreactor for Engineered Cartilage Tissue

Efren Barron Villalobos[1,2], Dallas E. Altamirano[2], Samuel S. Freitas[2], David A. Gonzales[1], Xinyi Gu[4], Danielle Larson[1,3], Trinny Tat[2], David S. Margolis[1,2] , [1]Orthopaedic Research Laboratory, Orthopeadic Surgery, College of Medicine, [2] Biomedical Engineering, College of Engineering, [3] Biosystems Engineering, College of Engineering, [4] Electrical and Computer Engineering, College of Engineering, The University of Arizona, Tucson, Arizona 85724

Introduction: Articular cartilage within joints does not heal spontaneously following sports injuries and other traumatic events. Cartilage damage leads to pain, decreased mobility, and can progress to diffuse cartilage degeneration and osteoarthritis. There are no current medical or surgical treatments that restore osteoarthritic joints to their native condition, and patients will commonly require joint replacement. In order to develop new treatments to regenerate cartilage, autologous adult stem cells have been used to produce cartilage-like tissues. To date these engineered tissues have not had the same histological or mechanical properties as native tissues. One approach to improving the quality of tissue engineered cartilage is to apply a load to the engineered tissues in tissue culture to facilitate alignment of cells and extracellular matrix. We are able to apply simple tensile and compressive loads in tissue culture, but are unable to mimic the complex loading patterns our lab has measured from scaffolds implanted within knee joints. The goal of this project was to design a dynamic tissue culture bioreactor that can produce controlled mechanical forces in compression and shear based on load measurements collected *in vivo*. This will allow further testing to determine whether application of biomimetic loading patterns lead to better quality tissue engineered cartilage compared to use of simple sinusoidal loading patterns.

Methods: The bioreactor was built with ¼" polycarbonate sheets, with ports for gas exchange. Two force applicators (Nema 23 stepper motors) controlled with an Arduino board were fixed in the vertical and horizontal direction and designed to apply up to 20% axial and 10% shear strains. The system was tested by applying loads to 3D printed scaffolds containing axial and shear rosette strain gauges. The ability to apply the same load magnitudes and patterns as those measured *in vivo* were confirmed using a load cell as the bioreactor loaded scaffolds. System sterility was confirmed by testing medium placed around scaffolds loaded over a three-day period.

Results: The bioreactor system is able to load up to 6 scaffolds while recording strain measurements from two rosette strain gauges in each scaffold and maintain sterility through multiple loading sessions for three days. Measurements collected from strain gauges within the scaffolds and the load cell were able to accurately reproduce the load pattern (Figure2) using a desired magnitude up to 20% axial strain.

Discussion: This study demonstrated a bioreactor could be designed to culture up to 6 scaffolds containing multiple rosette strain gauges. The system has demonstrated accurate reproduction of axial loading patters based on *in vivo* data and maintains sterility through multiple loading sessions over three days. Currently confirmatory testing of the shear component and long-term sterility experiments are being performed. Following complete characterization of this system it will be used

to test whether application of biomimetic loading patterns produced a higher quality tissue engineered cartilage tissue compared to tissue grown with simple sinusoidal loading patterns.

Lucas Vu

Biomarkers of Glia Activation in Amyotrophic Lateral Sclerosis (ALS)

Barrow Neurological Institute and Saint Joseph's Hospital and Medical Center, Phoenix, Az

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by the degeneration of motor neurons in the brain and spinal cord. There are only two FDA approved drugs that are used to treat ALS, however, patient survival is usually only extended by a few months. Therefore, there is a critical need for further drug development. A significant majority of clinical trials for ALS have failed at different stages presumably due to the highly complex etiology resulting in a heterogeneous patient population. Enriching these patients into subsets that exhibit a particular pathogenic mechanism would aid in identifying those that could benefit from specific therapeutic approaches. However, one challenge is the absence of biomarkers for each pathogenic mechanism of disease. A number of recent ALS clinical trials have tested drugs that targeted inflammation (NP001 and Actemra). Therefore, additional biomarkers of neuroinflammation would be quite useful for stratification. Chitinases such as chitotriosidase (Chit-1), chitinase 3 like protein 1 (CHI3L1) and chitinase 3 like protein 2 (CHI3L2) have recently been explored as candidate biomarkers for ALS. These biomarkers are expressed by activated glial cells suggesting their involvement in the pathogenesis of ALS. While these chitinases have been explored, there are very few longitudinal studies and none have assessed their levels in both cerebrospinal fluid (CSF) and plasma obtained from the same patient. In this study, we measured levels of Chit-1 and CHI3L1 in CSF and plasma samples obtained from the same patient to assess if these chitinases were viable neuroinflammatory biomarkers for ALS. From a cross-sectional analysis, increased levels of CSF Chit-1 were observed in ALS samples as compared to both neurologic disease controls (DCs) and healthy controls (HCs). There were no differences in levels of plasma chit-1 between ALS and the control groups. CSF CHI3L1 exhibited no significant differences between ALS and either control group but differences were observed between DCs and HCs. There were no differences in levels of plasma CHI3L1 between ALS and the control groups. CSF but not plasma Chit-1 levels correlated with ALS functional rating scale revised (ALSFRS-r) scores. CSF CHI3L1 correlated with disease duration. Longitudinal analyses of these chitinases in CSF showed steady levels over time highlighting stability of these proteins in the CSF. Additionally, absolute levels of CSF Chit-1 and CSF CHI3L1 were able to segregate patients based on disease progression rates (fast vs. slow progressing ALS) indicating utility as a segregation biomarker. We also demonstrated, via immunostaining, that CHI3L1 co-localizes with GFAP in post mortem spinal cord tissue from ALS patients suggesting that CHI3L1 is a marker for activated astrocytes. Taken together, these results suggest potential use of CSF Chit-1 and CHI3L1 as neuroinflammatory biomarkers for ALS that could be used to segregate the patient population based on disease progression rate.

Cindy Xu

Transcriptional analysis of scar-free wound healing and early stages of tail regeneration in the lizard,*Anolis carolinensis*

Cindy Xu[1], Elizabeth Hutchins[1,2], Minami A. Tokuyama[1], Jeanne Wilson-Rawls[1], Kenro Kusumi[1,2] , [1]School of Life Sciences, Arizona State University, Tempe, AZ, USA, [2]Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

Among amniote vertebrates, lizards display the greatest ability for appendage regeneration. They are able to autotomize, or self-amputate, their tails and regrow the appendage. While the regrown tail differs in structure from the original, there is regeneration and patterning of multiple tissues including spinal cord, cartilage, muscle, vasculature and skin. In contrast, tail amputation in mammals leads to fibrosis and scar formation. The earliest stage of tail regeneration in the green anole lizard, *Anolis carolinensis*, is characterized by scar-free wound healing and onset of growth prior to 10 days post autotomy (DPA). During this period, cellular debris is removed from the stump, a wound epithelium is formed, and vasculogenesis is observed. To identify genes involved in early tail regeneration, we performed whole transcriptome sequencing of the regenerating tail at 0.5, 1, 2, 3, 4, and 5 DPA. A total of 5,315 genes were identified as differentially expressed between these

time points, with a marked shift in gene expression patterns starting at 2 DPA. Differentially expressed transcripts prior to 2 DPA were enriched for gene ontology categories related to the immune system, T cell receptor signaling, and the p38/MAPK signaling pathway. Genes expressed at 3-5 DPA were enriched for cell proliferation, developmental growth, and Wnt/Hippo signaling pathways. The identification of a specific immunomodulatory profile at early stages of regeneration may help in the development of approaches for future regenerative medical therapies.

Liqiang Zhang

Promotion of accelerated healing in a mouse wound model with a viral immunomodulator Serp-1/chitosan gel

Liqiang Zhang, Jordan R. Yaron, Sarah Wallace, Michelle Burgin, Amanda Tafoya, Alexandra R. Lucas, Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ 85287

Statement of Purpose: Skin wounds are now considered as one of the major threats to public health and the economy. Based on understanding of the mechanisms of wound healing, numerous therapeutic methods have been developed to promote accelerated wound healing, including slow-release hydrogels for small molecules or growth factors delivery. In this study, we applied the viral immunomodulatory protein Serp-1, a serine proteinaseinhibitor (serpin), to promote accelerated wound healing. Serp-1 is a purified, secreted 55kDa glycoprotein derived from *Myxomavirus* that interferes with the inflammation processes of its host, the European rabbit (Oryctolagus cuniculus) [1]. Our systematic studies on Serp-1 protein demonstrate highly potent anti-inflammatory functions in a wide range of animal models of vascular disease and transplant. Serp-1 also significantly reduced markers of heart damage in a small Phase 2 clinical trial. Serp-1 proved safe in animal models and in Phase I and Phase 2a clinical trials in humans [2]. We examined local delivery of Serp-1 in a hydrogel and assessed wound healing with topical Serp-1 treatment. Topical delivery methods for Serp-1 wound treatments were compared to determine the optimal application approach.

Methods: Serp-1/chitosan hydrogel was prepared using a modified methods published for the spinal cord injury injection [3]. 10 mg of 85% de-acetylated chitosan (low molecular weight, Sigma-Aldrich) was swelled in 10 ml sterilized di-water over-night with gentle rotation at 4 °C. After freeze-thaw, 30 mg of purified Serp-1 (1.8 mg/mL saline solution) was mixed with the chitosan and stored at 4 °C for 6 hours. The Serp-1/chitosan mixture was then lyophilized and the powder stored at -20 °C or dissolved into type I collagen solution (Sigma-Aldrich) until use. A 300 µL, 1 µg Serp-1 per 10 µL, hydrogel was prepared before surgery. 30 µL of Serp-1/chitosan hydrogel was applied onto each wound. Chitosan-collagen without Serp-1 was used as a negative control.

Serp-1 treatment was assessed in a mouse excisional wound splinting model for wound healing. We created full-thickness skin wounds and applied Serp-1 protein topically either in saline (1µg/20 µl/wound) with a second bolus on day 3 or as a one time application of 3 µg/30 µl chitosan hydrogel applied immediately after surgery (day 0). Wound size was measured daily for 6 days and tissue collected on day 7, followed by IHC staining to characterize the healing progress.

Results: Compared to control, i.e. wounds treated with saline or chitosan only, wounds treated with Serp-1 had significantly faster wound closure by 20% in day 6 (Figure 1, $P < 0.01$)). A single application of 3.0 µg Serp-1 in chitosan-collagen hydrogel improved wound healing similar to Serp-1 in saline solution. No benefit to wound healing was observed from chitosan-collagen gel alone.

Serp-1 treatment promoted angiogenesis during new tissue growth during wound healing. CD31 immunostaining demonstrated a significant increase in angiogenesis (Figure 2A). There is about 3.5 fold increase in capillary density at wound edges with Serp-1 treatment when compared to controls ($p<0.01$) (Figure 2B).

Conclusions: A unique anti-inflammatory serpin, Serp-1, has been extensively tested as a systemic therapy in animal models of inflammation and a small human clinical trial. Serp-1 has not been tested as a topical treatment for wound healing. Serp-1 has a short half-life when given intravenously and we postulated that slow release of Serp-1 is necessary to maximize the activity. This current study demonstrates that Serp-1 applied to wounds in a mouse model can significantly promote accelerated wound healing when given in a hydrogel or saline. In ongoing work, we are exploring the molecular mechanism of action.

Poster Abstracts

Sponsor Research Poster Abstracts

Bio-Techne

Stephanie Pappas

Single-Cell Western Analysis of iPSC-derived Neural Progenitors Confirms High Differentiation Efficiency and Population Distribution Dynamics

S. Pappas, K. Patel, K. Flynn, D. Galitz, J. Sabat, S. Luhowskyj, S. Stoesz, E. Jabart, J. Cooper, and J. Aho

Human pluripotent stem cells, including embryonic (ES) and induced pluripotent stem (iPS) cells, offer an essentially unlimited source of neural cells that can be used to investigate mechanisms of human neurological disease and neural regeneration. A critical step during the derivation of neurons, astrocytes, or oligodendrocytes from pluripotent stem cells is generating a robust and homogeneous neural progenitor cell population, which ultimately impacts the efficiency of downstream differentiation protocols and helps control experimental reproducibility. Understanding population heterogeneity is an important step in the optimization of differentiation protocols, which is challenging with existing methods. This study demonstrates how Single-Cell Western blot analysis can complement traditional verification approaches by providing insights into protein expression both at the population and single-cell level. In this study, human iPS cell lines were differentiated into neural progenitor cells using the standardized protocol and reagents in the StemXVivo® Neural Progenitor Differentiation Kit. Combining our standardized differentiation protocol for neural progenitor cells with Milo™ Single-Cell Western technology (ProteinSimple), we were able to assess population protein expression dynamics during the differentiation of iPS cells into neural progenitor cells. Using Oct-3/4 as a marker for pluripotent stem cells and Pax6 as a marker for neural progenitor cells we show, at the single-cell level, that during differentiation the cells shift from an exclusively Oct-3/4 expressing population to one that is robustly Pax6-positive. We were also able to identify subpopulations of neural progenitor cells that express Pax6 at high or low levels. Additionally, single-cell analysis also used to characterize the population distribution of terminally-differentiated neural cells following growth factor withdrawal-induced differentiation of iPS-derived neural progenitor cells.

STEMCELL Technologies

Brett Peterson

Rescue of Drug-Induced Long QT Syndrome Type 2 Using a hERG Channel Activator in Human Pluripotent Stem Cell-Derived Cardiomyocytes

Vincenzo Macri[1], Jessica Norberg[1], Mark Hills[1], Terry Thomas[1], Allen Eaves[1,2], Stephen Szilvassy[1], Sharon Louis[1]
[1]STEMCELL Technologies, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada

An important step in drug development is the evaluation of cardiac toxicity. Candidate drugs must have minimal effects on the hERG current that is essential for cardiac repolarization. Drugs that block the hERG channel can prolong the QT interval, leading to lethal ventricular arrhythmias. The hERG channel is prone to promiscuous interactions with drugs due to easy access to the channel pore. hERG channel activators can shorten repolarization in human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). One such activator, ginsenoside Rg_3, was recently shown to interact with the voltage-sensing domain of the hERG channel to stabilize the activated state. Ginsenoside Rg_3 may change the conformation of the open channel and potentially limit subsequent drug blockade. Here we show that Ginsenoside Rg_3 can rescue drug-induced LQTS2 in hPSC-CMs, suggesting that hERG activators that target the voltage-sensing domain may be used to offset cardiac safety issues of promising candidate drugs.

Poster Abstracts

ASU Biosciences Core Lab Posters

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