POSTER CONTEST & SESSION

Engineering Cells and Tissues for Discovery and Therapy

12th Annual Wisconsin Stem Cell Symposium

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NOTE: Each submitter's name is in bold and italicized.

POSTER CONTEST

(1) Protein Synthesis-dependent and Independent Modulation of the Growth Cone Cytoskeleton in Human Neurons Downstream of Axon Guidance Cues

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Local protein synthesis (PS) within growth cones is necessary for cue-directed axon guidance, as demonstrated in many developing animal model systems, but not as yet in human neurons. However, one complicating factor is that guidance cues that modulate PS often also directly regulate signals that influence the cytoskeleton, such as the Rho GTPases. To begin to test the roles and necessity of local PS downstream of axon guidance cues in human neurons, we are studying human neurons differentiated from induced pluripotent stem cells (iPSCs). Using iPSC-derived neurons allows us to study PS-dependent and direct cytoskeletal modulation downstream of axon guidance cues in a variety of human neuronal cell types. We show that early human forebrain neurons derived from iPSCs and embryonic stem cells respond to canonical axon guidance cues via growth promotion, inhibition, and turning. Moreover, utilizing an iPSC disease model of Tuberous Sclerosis Complex (TSC) with a mutation in TSC2, a key negative regulator of PS within growth cones, we show a role for TSC2-dependent cytoskeletal modulation of RhoA downstream of EphrinA1. Our results suggest that neural network connectivity defects in TSC, an autism spectrum disorder, may result from defects in both direct RhoA regulation of the cytoskeleton, as well as PS-dependent signaling downstream of axon guidance cues.

(2) Derivation and Transplantation of hPSC-derived, Region-specific Motor Neuron Populations into Developing Chick Embryos

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Motor neurons (MNs) represent the only efferent signaling pathway by which the central nervous system (CNS) connects to peripheral tissues. They display diverse phenotypical heterogeneity, and specific subtypes are patterned during human development to distinct regions throughout the posterior CNS. Here, we describe protocols for differentiating human pluripotent stem cells (hPSCs) into MNs progenitors of cervical spinal cord identity, and further differentiating those progenitors into early-stage and mature spinal MNs. The differentiation protocol uses a chemically defined system that modulates Wnt/β- catenin and fibroblast growth factor (FGF) signaling to induce colinear HOX activation within neuromesodermal progenitors to achieve a cervical identity. Then, the addition of RA and SHH fixes the cervical identity by halting HOX activation, transitioning the cultures into definitive neuroectorderm, and inducing a ventral, PAX6+/OLIG2+, cervical MN progenitor phenotype. To induce a motor neuron phenotype, the progenitors are reseeded at a lower density and treated with a notch inhibitor to generate early-stage MNs, and kept in culture in the presence of neurotrophic factors for extended duration to generate mature MNs. In order to test the engraftment potential of these cells and determine the optimal stage for transplantation, we used a novel and reproducible injection method to transplant cells into the neural tubes of developing chick embryos and assess their engraftment ability 48 hours post-transplantation. The results of these experiments were quantified using a defined scale ranging from no cells in the neural tube, to hPSC-derived MNs projecting axons through the ventral horn of the chick's spinal cord. This study highlights the importance of transplanting cell types at the correct developmental stage to promote engraftment and accurately assess functionality of hPSC-derived cells in animal models.

(3) GATA2 is Dispensable for Generation of Hemogenic Endothelium But Required for Endothelial-to Hematopoietic Transition

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Understanding molecular mechanisms leading to formation of hemogenic endothelium (HE) and blood through endothelial-to-hematopoietic transition (EHT) is critical for advancing technologies for de novo production of hematopoietic stem cells (HSCs). Whereas GATA2 is recognized as a critical factor required for blood and HSC formation during HE stage of development in the embryo, it remains unclear whether GATA2 affects HE development or EHT process. Here, we conducted loss-of-function and functional restoration approaches with engineered GATA2-/- human embryonic stem cell lines with conditional GATA2 expression and analyzed their differentiation potential. We demonstrated that GATA2-/- cells were capable to produce HE, but failed to undergo EHT, which was restored following conditional GATA2 expression. In addition, we found GATA2-deficinet cells were still capable of producing very limited numbers of GATA2-independent hematopoietic progenitors (HPs) lacking granulocyte and granulocyte-macrophage (GM) progenitors, but retaining lymphoid potential. Overall, our results revealed that GATA2 is essential factor involved in regulation of EHT and specification of bipotential GM progenitors.

(4) Self-assembly of a Perfusable Vascular Network via Human iPSC-derived Endothelial Cells in Dynamic, Synthetic Microenvironments

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Researchers have attempted to create engineered tissues in hydrogels, which are generally thicker than 100µm. Due to their biocompatibility and resemblance to extracellular matrix components, hydrogels are used to mimic natural microenvironments. However, the lack of nutrition within the core of thick engineered tissues have limited their applications. Here, we have used a degradable poly(ethylene glycol) (PEG) hydrogel, induced pluripotent stem cell (iPSC) derived endothelial cells, and a custom made bioreactor to create a perfusable three dimensional vascular network for testing of potential vascular disrupting compounds. We have created a stable vascular network using iPS cell-derived endothelial cells and primary human pericytes, cultured in MMP-degradable PEG hydrogels derivatized with the cell adhesion ligand Arg-Gly-Asp (RGD). Our preliminary studies indicated that PEG hydrogels incorporating synthetic peptides had the potential to support a vascular network with perfusable characteristics. We

hypothesized that dynamic fluid flow would induce capillary tube formation, thus creating more stable vascular tissue organization. We used two specialized bioreactors (in collaboration with MIT/DARPA and CN BIO), which created fluid flow underneath the hydrogels in the range of 1-3µL/sec. Culture in bioreactors at a flow rate of 1µL/sec resulted in a thicker vascular network when compared to static culture, and endothelial cells (CD31+) were co-localized with pericyte-like cells (PDGFR-β+). Using two-photon fluorescent lifetime imaging microscopy (FLIM), we also quantified vascular network formation and metabolic dynamics and showed that the network was more stable, perfusable and organized in dynamic flow conditions. We propose that vascular network formation, under the influence of dynamic flow, can potentially create advanced, perfusable organoids, which will provide more physiologically relevant human organoid models.

(5) Metabolic Shift during Cardiomyocyte Differentiation Promotes Cell Survival during NAMPT Inhibition: Applications for Eliminating Unwanted Cells

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Human pluripotent stem cells (hPSC) are a renewable source for the generation of cardiomyocytes (hPSC-CM) that can be utilized for drug toxicity testing and modeling human disease. However, the widespread clinical application of hPSC-CM is limited by technical challenges including cell purity, maturation, and potential for teratoma tumor formation from remnant hPSC. We have recently shown that inhibition of nicotinamide phosphoribosyltransferase (NAMPT) selectively eliminates hPSC from hPSC-CM *in vitro*. NAMPT, the rate limiting enzyme in a salvage pathway for NAD synthesis, is important for maintaining sufficient NAD levels to support pluripotency. In hPSC, NAMPT inhibition leads to a loss in ATP and cell death. As we previously determined that hPSC-CM are resistant to NAMPT inhibition, this study tested how *in vitro* differentiation and maturation influence the mechanisms that control susceptibility to NAMPT inhibition. We found that resistance to NAMPT inhibition increases as hPSC differentiate and, by day 28 of differentiation, hPSC-CM survive extended periods of NAMPT inhibition and maintain their ability to spontaneously contract. Cell survival during NAMPT inhibition correlates with utilization of glycolysis and mitochondrial respiration to maintain ATP, despite a significant loss in NAD levels. Furthermore, we find that glycolytic flux and mitochondrial respiration are differentially maintained in a cell type dependent manner during NAD depletion. These findings identify an important role for the NAMPT-dependent NAD salvage pathway in hPSC and provide evidence to support a novel strategy by which inhibition of NAMPT can be used to effectively eliminate unwanted cells from hPSC-derived cardiomyocyte cultures.

(6) Endothelin Receptor Type Directs Multilineage Differentiation of Tissue-Derived Stem Cells in Response to Endothelin-1 Induction

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Blood vessels composed of endothelial cells come in direct contact with mesenchymal stem cells (MSCs) in different tissues, suggesting possible interaction between MSCs and endothelial cells. In this study, we hypothesized that endothelin-1 (ET1) can differentially regulate the fate of pre-differentiated adipose-derived stem cells (ASCs) and bone marrow-derived MSCs (BMSCs). Pre-differentiated ASCs and BMSCs were treated with ET1 for two cell passages and then induced for multilineage differentiation. We further analyzed the expression level of ET1 receptors, endothelin receptor type A (ETAR) and endothelin receptor type B (ETBR), using flow cytometry. Our results showed that adipogenesis of ET1-pretreated ASCs and osteogenesis of ET1-pretreaetd BMSCs were enhanced compared to control cells. Bone marrow-derived MSCs expressed a greater amount of ETAR and ETBR than ASCs with ETBR about three times more abundant than ETAR in both cell types. We then treated the cells with an ETBR antagonist, BQ788, prior to

ET1 induction to determine whether ETBR plays a role in differentially regulating the fate of ASCs and BMSCs. The effect of ET1 on enhancing adipogenesis of ASCs and osteogenesis of BMSCs was attenuated by blocking ETBR. In addition, western blot analysis indicated that the regulation by ET1 was mediated through ETBR activation of the AKT and MAPK signaling pathways. We further found that the ETBR+ subpopulations of ASCs and BMSCs pretreated with ET1 were prone to becoming adipocytes and osteoblasts, respectively. Our findings provide insight into the regulation of MSC fate by endothelin-1 within its niche for developing viable regenerative medicine approaches.

(7) MDM2 Inhibition Rescues Neurogenic and Cognitive Deficits in a Mouse Model of Fragile X Syndrome

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Fragile X syndrome, the most common form of inherited intellectual disability, is caused by loss of the fragile X mental retardation protein (FMRP). However, the mechanism remains unclear, and effective treatment is lacking. We show that loss of FMRP leads to activation of adult mouse neural stem cells (NSCs) and a subsequent reduction in the production of neurons. We identified the ubiquitin ligase mouse double minute 2 homolog (MDM2) as a target of FMRP. FMRP regulates Mdm2 mRNA stability, and loss of FMRP resulted in elevated MDM2 mRNA and protein. Further, we found that increased MDM2 expression led to reduced P53 expression in adult mouse NSCs, leading to alterations in NSC proliferation and differentiation. Treatment with Nutlin-3, a small molecule undergoing clinical trials for treating cancer, specifically inhibited the interaction of MDM2 with P53, and rescued neurogenic and cognitive deficits in FMRP-deficient mice. Our data reveal a potential regulatory role for FMRP in the balance between adult NSC activation and quiescence, and identify a potential new treatment for fragile X syndrome.

(8) Engineering Skeletal Muscle from Human iPS Cells To Study Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neuromuscular disease featuring motor neuron cell death, skeletal muscle atrophy, and ultimately death from respiratory failure. Recent studies show that the earliest signs of ALS pathology occur in skeletal muscles, specifically at the neuromuscular junctions. While these studies support the idea that skeletal muscle is an active contributor to the disease process in ALS, the disease mechanisms within skeletal muscle are still largely uncharacterized. To investigate these mechanisms, we prepared myogenic progenitors and skeletal myocytes from ALS patient-derived iPS cells using a free-floating spherical culture protocol that was recently established in our laboratory. The myogenic progenitors were terminally differentiated into mature skeletal myotubes for 2-12 weeks. We successfully created skeletal muscle cells in culture from a variety of patient cell lines of different ALS backgrounds in both familial (SOD1, TARDBP, FUS, C9ORF72) and sporadic forms. Our current study has specifically focused on the C9ORF72 repeat expansion, a newly discovered mutation that is responsible for the majority of familial ALS cases. Skeletal myocytes with the C9ORF72 mutation exhibited specific pathology associated with the genetic mutation, abnormal protein aggregations, and increased susceptibility to oxidative stress. These results support the capabilities of ALS patient-derived iPS cells for in vitro disease modeling. In addition to standard two-dimensional culture techniques for cell differentiation, we are testing alternative platforms to engineer skeletal muscle tissue. Our current approaches include 1) micropatterned surfaces to control directionality of myotube formation, 2) three-dimensional muscle constructs to mimic muscle bundles, and 3) co-cultures of motor neurons and muscles to simulate the formation and pathology of neuromuscular connections. We believe that these in vitro disease models with patient-derived iPS cells can be used to discover new insights into the mechanisms behind this devastating neuromuscular disease.

(9) Mathematical Modeling to Predict CAR T Cell Cytotoxicity

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Chimeric antigen receptor (CAR) T-cells are genetically engineered T-cells that have been used to target cancer-associated antigens. The profound success of CAR T-cells in treating hematological malignancies (e.g. leukemia by targeting CD19) has lead many companies to attempt to scale up and manufacture these engineered cell therapies [1]–[3]. Even once companies have devised ways to mass produce these therapies, there must be a specific set of characteristics that will define their product. These characteristics can include the percentage of CAR+ cells, expression levels of the CAR, activation levels of the T-cells prior to implantation, and exhaustion levels of the CAR T-cells. In order to manufacture efficient and predictable CAR T-cells, the functional influence of different sources of heterogeneity in CAR T-cells needs to be taken into account.

To address this issue, we developed a microscale co-culture system to observe interactions between gene-edited anti-GD2 CAR T-cells and M21 GD2+ melanoma cancer cells. This system allowed us to overcome the technical challenges involved with studying functional effects among small subpopulations of edited T-cells and cancer cells in vitro. By using rate constants measured on our microwell co-culture system, we developed mathematical models to track CAR T-cell and tumor cell interactions. These models use inputs such as number of CAR T-cells and number of target cells while predicting the decline in tumor cell number. This information will be of high clinical relevance to better define CAR T-cell mixtures to minimize the amount of off-target cytotoxicity and rate of exhaustion of the CAR T-cells before tumor regression can be achieved.

References: [1] M. Sadelain et al., Nat. Rev. Cancer, 2003. [2] G. Welstead et al., Editas Medicine /Juno Therapeutics, 2016. [3] L. Poirot et al., Cancer Res., 2015.
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(10) The Roles of Retinoic Acid Nuclear Hormone Receptors in Earlier Maturation and Barrier Enhancement in an hPSC-derived Model of the Blood-brain Barrier

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The blood brain barrier (BBB) regulates central nervous system (CNS) health by restricting ion and molecular flux across CNS blood vessels. Brain microvascular endothelial cells (BMECs), which line CNS capillaries, form the physical BBB, yet their dysfunction is implicated in many CNS diseases. Understanding signaling pathways implicated BMEC development and maintenance may provide new therapeutic targets to treat *human* CNS diseases. Human pluripotent stem cells (hPSCs) offer the unique opportunity to examine signaling pathways implicated in *human* BMEC development and maintenance *in vitro* as hPSCs transition from pluripotent cells to BMECs. The aim of this study was to untangle retinoic acid's (RA) previously established role in BMEC maturation and increased barrier tightness. Small molecule activation of RA receptors RARα and RXRα at key time points were sufficient to elevate barrier tightness and lead to earlier expression of VE-cadherin, a mature endothelial cell marker. Dual activation of both receptors led to additive effects on VE-cadherin expression, transendothelial electrical resistance (TEER), and increased occludin tight junction junctional distribution. At subactivating doses, dual activation led to a substantial increase in TEER, indicating both receptors act synergistically to enhance BBB fidelity. These results point to RARα and RXRα receptors as potential therapeutic targets for improving BBB fidelity.

GENERAL POSTER SESSION

(11) Metabolomics Identifies Metabolic Markers of Maturation in Human Pluripotent Stem Cell-Derived Cardiomyocytes

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Cardiovascular disease is a leading cause of death worldwide. Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) hold immense clinical potential and recent studies have enabled generation of virtually pure hPSC-CMs with high efficiency in chemically defined and xeno-free conditions. Despite these advances, hPSC-CMs exhibit an immature phenotype and are arrhythmogenic in vivo, necessitating development of strategies to mature these cells. hPSC-CMs undergo significant metabolic alterations during differentiation and maturation. A detailed analysis of the metabolic changes accompanying maturation of hPSC-CMs may prove useful in identifying new strategies to expedite hPSC-CM maturation and also may provide biomarkers for testing or validating hPSC-CM maturation. In this study we identified global metabolic changes which take place during long-term culture and maturation of hPSC-CMs derived from three different hPSC lines. We have identified several metabolic pathways, including phospholipid metabolism and pantothenate and Coenzyme A metabolism, which showed significant enrichment upon maturation in addition to fatty acid oxidation and metabolism. We also identified an increase in glycerophosphocholine and the glycerophosphocholine:phosphocholine ratio as potential metabolic biomarkers of maturation. These biomarkers were also affected in a similar manner during murine heart development in vivo. These results support that hPSC-CM maturation is associated with extensive metabolic rewiring and understanding the roles of these metabolic changes has the potential to develop novel approaches to monitor and expedite hPSC-CM maturation.

(12) Activated Cardiac Progenitors from Human Induced Pluripotent Stem Cells with Enhanced Capacity to Generate Mature Cardiomyocytes in Conductive Microtissue

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Cardiomyocytes from human iPS cells have promise in disease modeling, drug discovery, and therapy, but the challenge remains to create mature and organized cardiomyocytes like those of the native heart. While groups have increased maturity of iPS-derived cardiomycytes by extended culture and electrical, metabolic, and biomechanical stimulation, we hypothesized that epigenetic activation during the formation of cardiac progenitors could enhance their capacity to form mature cells. We found that administration of the innate immune agonist PolyIC during formation of cardiac progenitors in defined small molecule differentiation increased histone acetylation and decreased HDAC expression without increasing cell death. To test if epigenetic activation could restore signaling factors present in development, we measured the expression of endogenous Wnt ligands and inhibitors in cardiac differentiation lacking the small molecule inhibitor of Wnt and observed that PolyIC rescued endogenous Wnt inhibition and cardiomyocyte differentiation by augmenting decreased expression of Wnt 3 ligand. Compared to normal cardiac progenitors, activated progenitors had enhanced proliferation, future cardiomyocyte yield, and a dominant increase in cell proliferation pathways in RNAseq. This was lead by genes such as Jagged1 of the Notch pathway expressed in the developing cardiac crescent and heart tube, and Notch inhibition blocked progenitor proliferation. Activated progenitors differentiated into more mature cardiomyocytes based on larger size, optical upstroke velocity, oxidative metabolism, and expression of markers of CM maturation including cTnI, cardiac actin, and αMHC, which were blocked by Notch inhibition. Singularized activated progenitors could self-assemble and differentiate into organized, synchronized, beating cardiomyocyte sheets containing a network of HCN4+ conduction system cells that were sensitive to the nodal current blocker ivrabradine in contrast to the poorly coupled cardiomyocytes

from nonactivated progenitors. Impact: Activated cardiac progenitors with the capacity to give rise to cardiomyocytes with enhanced maturation and organization hold promise for improving disease modeling, drug screening and therapy.

(13) A Novel Dissociation Buffer Enables 3D Expansion of Human Pluripotent Stem Cells Without ROCK Inhibitors for cGMP Stem Cell Manufacturing

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3-D bioreactors hold promise to allow biomanufacturing of clinical grade human pluripotent stem cells (hPSCs) derived products in a scalable fashion. However, passaging of hPSCs using single cell-passaging with standard enzymatic-passaging methods of hPSCs can be associated with genomic instability and apoptosis. We hypothesize that hPSC dissociation in larger aggregates using non-enzymatic solution may facilitate 3D cell expansion of hPSCs in current good manufacturing practices (cGMP). Human PSCs rely on E-cadherins for cell-to-cell adhesion and intracellular signaling. E-cadherin protein dimerization benefits from the extracellular calcium ions (Ca+2) which maintains the rigidity of cadherin dimers to bind to neighboring E-cadherins of other hPSCs. Strong chelators, like EDTA, chelate Ca+2 from cadherins of hPSCs and dislodge them in very small cell clusters. The potency of EDTA, though useful in laboratory research, makes it difficult to control cell aggregate size and the operational window in 3D culture. We postulate that the integrity of cadherin dimers must be maintained to dislodge hPSCs in large aggregates. To address the manufacturing bottlenecks, a passaging solution needs to also provide a longer window to operate without generating small cell aggregates. To date, no reports have investigated the mild chelation of divalent cations in an osmotic solution to ease hPSC detachment in large aggregates. Here, we show that a novel dissociation buffer (DB) in an optimized osmotic solution enables hPSC detachment in larger aggregates than EDTA and facilitates 3D culture of hPSCs without the use of a ROCK inhibitor (RI). RI is not approved by the Federal Drug Administration (FDA).

(14) Development of an Isogenic Stem Cell-Derived Blood Brain Barrier Model: Applications in Alexander's Disease.

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The Blood Brain Barrier (BBB) is critical in maintaining a physical, metabolic, transport barrier between the blood and the brain. The BBB consists of brain microvascular endothelial cells (BMECs) that form the brain micro-vasculature and are supported by astrocytes, neurons, pericytes, and neural stem cells, that altogether form the neurovascular unit (NVU). We have successfully differentiated BMECs, astrocytes, and neurons from human induced pluripotent stem cells (iPSCs). Compared to other in vitro BBB models, these iPSC-derived BMECS exhibit more physiologic BBB phenotypes, such as a high transendothelial electrical resistance (TEER), low permeability, polarized efflux transport, and expression of tight junctional markers. The addition of co-culture with neurons and astrocytes further elevated TEER, reduced permeability, and improved tight junction continuity in the endothelial cell population. Varying combinations of neurons and astrocytes to roughly similar estimates of the human brain (1 neuron: 3 astrocytes) was found to be the most BBB enhancing. The ability to derive an isogenic NVU consisting of astrocytes, neurons, and BMECs from the same original stem cell source has immense implications in further understanding healthy and pathological BBB states. One application of the BBB model has been in collaboration with the Su-Chun Zhang laboratory to develop an Alexander's Disease (AxD) BBB model. AxD is a neurological disorder caused by a single gene mutation of the glial fibrillary acidic protein (GFAP) in astrocytes. Our initial studies have indicated that the co-culture of AxD-derived astrocytes with healthy BMECs significantly decreased barrier tightness compared to BMECs co-cultured with healthy astrocytes. Interestingly, when the GFAP mutations were corrected via CRISPR/CAS9, there was a restored BBB-tightening comparable to control levels. The ability to derive the entire neurovascular unit from AxD patients or combine AxD and healthy neurovascular components will allow for one to begin dissecting the role of astrocytes in BBB integrity of AxD patients.

(15) Mathematical Modeling and Analysis of CAR T-cells Used in Adoptive Cell Therapy for Cancer

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T-cell immunotherapy shows significant clinical success for CD19 lymphomas, but for few other tumors. This lack of success has been attributed to the nature of the engineered CAR T-cells used in the therapy of these tumors. Utilizing patient data from clinical trials targeting CD19 lymphomas and GD2 presenting neuroblastomas along with a modified mathematical model of immunotherapy, we intend to understand how anti-CD19 CAR T-cells are outperforming CAR T-cells targeting GD2. The major factors affecting CAR T-cell therapy patient outcomes is the potency of the engineered CAR, the degree of exhaustion of the T-cell and the in vivo proliferation and stimulation capabilities of the engineered T-cells. The degree and location of metastases also affect the T-cell therapy outcome. Our model considers the proliferation of CAR T-cells, the exhaustion of CAR T-cells, dosing and estimated bone marrow tumor burden and growth rate estimates based on patient data and clinical intuition. We intend to find differences that can be tested in to aid development of better CAR T-cell therapies targeting solid tumors.

(16) Synthetic Hydrogel Microcarrier Coatings for Stem Cell Expansion

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Microcarriers are scalable support surfaces for cell growth that enable high levels of expansion, and are particularly relevant for expansion of human mesenchymal stem cells (hMSCs). The goal of this study was to develop a poly(ethylene glycol) (PEG)-based microcarrier coating for hMSC expansion. Commercially available microcarriers do not offer customizability of microcarrier surface properties, including elastic modulus and surface cell adhesion ligands. Our lab has previously demonstrated that tuning these material properties on PEG-based hydrogels can modulate important cellular growth characteristics, such as cell attachment and expansion, which are important in microcarrier-based culture. Eosin-Y was adsorbed to polystyrene microcarriers and used as a photoinitiator for thiol-ene polymerization under visible light. Resultant PEG coatings were over 100 μm thick and localized to microcarrier surfaces. This thickness is relevant for cells to react to mechanical properties of the hydrogel coating, and coated microcarriers supported hMSC attachment and expansion. hMSC expansion was highly favorable on coated microcarriers with cyclic RGD peptide added to the prepolymer in serum-free media, with doubling times under 25 hours in the growth phase, and retained osteogenic and adipogenic differentiation capacity after culture on microcarriers. Microcarriers with a customizable shell are a new, modular system enabling scalable cell expansion and delivery. These microcarriers with defined, synthetic coatings enable tailorable surfaces for cell expansion that may be suitable for a variety of biomanufacturing applications.

(17) Maturation of hPSC-derived Cardiomyocytes via Coculture with Endothelial Cells

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Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) hold great potential for regenerating heart tissue. This is due to the ability to expand hPSCs in culture before differentiation to allow production of sufficient numbers of CMs for implantation. Unfortunately, these hPSC-derived CMs currently have been shown to cause arrhythmias in primate hearts likely caused by the immature phenotype of the implanted CMs. This immature phenotype is characterized by a difference in marker expression, electrical and mechanical functionality, and morphology in comparison to adult CMs. One possible cause for this CM immaturity is the lack of intercellular interactions that are normally present in human development. We can obtain both EC and CM progenitors efficiently from hPSCs by temporally modulating the Wnt pathway. Through co-culturing these progenitors, we can incorporate the signaling interactions occurring in the embryo as the progenitors are further specified to ECs and CMs and matured. With the use of a transwell system and direct co-culture, we will demonstrate the importance of EC interactions with CMs

in their ability to induce CM maturation. Primarily, analysis of the CMs and ECs is done via flow cytometry and immunocytochemistry to measure protein expression and organization. Elucidation of intercellular communication mechanisms between ECs and CMs will provide insight into mechanisms of heart development and will facilitate efforts to generate more mature models of human heart tissue from hPSC sources.

(18) A Novel Peptide Tag Enables Simple and Sensitive Bioluminescent Quantification of Tagged Proteins

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Dysregulation of protein expression is a key mechanism of tumorigenesis. The most commonly used approach to monitor changes in expression is to perform SDS-PAGE, followed by immunoblotting, a labor-intensive process that requires high-quality antibodies to detect proteins at endogenous levels of expression. We have developed a novel protein tag utilizing NanoLuc Binary Technology (NanoBiT), a binary complementation system based on NanoLuc luciferase. The tag, designated High BiT (HiBiT), is only 11 amino acids in length, which minimizes potential interference with protein function. The amount of HiBiT-tagged protein is measured using a lytic detection reagent containing Large BiT (LgBiT), which binds with high affinity to HiBiT (KD~1 nM) to reconstitute a bright, luminescent enzyme. HiBiT-tagged proteins can be quantified in cell lysates over 7 orders of magnitude of linear dynamic range with a limit of detection of less than 10-19 moles (3 fg of 30 kDa protein). The simple add-mix-read assay protocol can be completed in minutes, providing an assay that is compatible with high-throughput applications. The sensitivity of the assay allows quantification at endogenous levels of expression, and the small tag size is ideal for CRISPR-mediated genome editing. HiBiT-tagged proteins separated by SDS-PAGE can be detected on blots at sub-picogram levels with a detection reagent containing LgBiT. By eliminating the multiple steps of blocking, binding, and washing of traditional blotting techniques, the protocol takes minutes instead of hours. Additionally, the cell surface expression, internalization, or secretion of HiBiT-tagged proteins can be measured in minutes using a non-lytic detection reagent containing cell-impermeable LgBiT and furimazine. HiBiT represents a next-generation protein tag that allows simple quantification of proteins of interest in their cellular context and following SDS-PAGE.

(19) Rapid Phenotypic and Functional Maturation of iPSC-Derived Human Neurons for Drug Discovery

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Neurons derived from human induced pluripotent stem cells (iPSCs) represent a tremendous opportunity to create platforms for drug discovery with a high relevance to human psychiatric and neurological diseases. iPSCs can be generated from both healthy individuals and from patients with a diagnosed CNS disorder. Indeed, for patients harboring a known genetic mutation, isogenic control iPSCs can also be created using gene-editing technologies such as CRISPR. A range of neuronal subtypes can then be generated from these iPSC lines. However, implementation of this approach has been slowed due to long maturation times for cultured human neurons. Here, we demonstrate the rapid maturation of four subtypes of neurons derived from human iPSCs: (1) spinal motor neurons, (2) midbrain dopaminergic neurons, (3) cortical glutamatergic neurons, and (4) cortical GABAergic neurons. These neurons mature within 7-14 days after plating as evidenced by diverse measures. They display a complex morphology, including extensive neurite branching. The neurons express classical subtype-specific markers as well as pre- and post-synaptic proteins. Finally, they show functional activity as demonstrated by robust spiking in multi-electrode array (MEA) studies and calcium imaging. As a proof-of-concept for high throughput screening, we knocked a nanoluciferase reporter into the survival motor neuron gene in iPSCs derived from spinal muscular atrophy patients. Motor neurons from the reporter SMA iPSCs respond to positive compounds in a dose-dependent manner in a 384-well format. Given that these neurons can be produced in high purity (>70-90%) and mature in approximately one week, they provide a powerful system for CNS drug discovery and development.

(20) Biomaterials-mediated Delivery of mRNA for Therapeutic Applications

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Gene delivery is a fundamental strategy to regulate gene expression across therapeutic and research applications in regenerative medicine. Classic gene delivery strategies utilize plasmid DNA (pDNA) to deliver the gene of interest. However, these methods are not ideal for in vivo applications, because of risks of insertional mutagenesis and low non-viral transfer efficiency of pDNA to non-mitotic populations. Non-viral delivery of in vitro-transcribed mRNA is safe and has high transfection efficiency, but is limited by short-lived timeframes of desired gene upregulation - on the order of hours. Here we present a biomaterials-based approach, whereby biomimetic mineral coatings on microparticles (MCMs) are designed for both efficient non-viral transfection and stable protein delivery. MCMs delivering mRNA encoding for basic fibroblast growth factor (bFGF) resulted in a two-fold increase in cell proliferation and 50% increase in expansion of primary human dermal fibroblasts compared to delivery of mRNA without MCMs. Additionally, the proliferation increase persisted beyond 48 hours, significantly longer than the period of bFGF expression. Lastly, the increase in cell expansion and proliferation with MCM-mediated delivery of mRNA was higher or equivalent to pDNA delivery, despite the overall lower expression of bFGF. Our materials-based mRNA delivery strategy leverages the advantages of higher non-viral efficiency in non-mitotic cell populations with an improved safety profile compared to viral delivery, and potentiates a longer and more robust biological response to a therapeutically relevant cytokine.

(21) Utilizing Stem-cell Derived Blood-brain Barrier Models to Examine Bacterial and Viral Infections

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The blood-brain barrier (BBB) is a highly selective cellular barrier that functions to maintain homeostasis in the central nervous system (CNS). The BBB is primarily comprised of specialized brain endothelial cells that are supported by other cell types of the neurovascular unit (NVU). Together, these cells create an exclusive environment to promote brain function, while excluding toxins and pathogens. While the BBB is a formidable barrier for most potential pathogens, some possess the ability to cross and cause inflammation leading to meningitis and encephalitis. Little is known about how meningeal and encephalitic pathogens are able to cross the BBB and cause life-threatening inflammation in the CNS. Utilizing a stem-cell derived NVU model, we examine bacterial and viral pathogens and their interactions with the BBB. Furthermore we examine pro-inflammatory pathways that may contribute to the NVU response to these clinically important pathogens. Data gathered from these projects will provide insights into novel mechanisms of disease, and may shed light on novel therapeutic intervention.

(22) Towards De Novo Generation of Human Neural Tube Slice Cultures

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Animal models do not adequately recapitulate the cell phenotype diversity and tissue cytoarchitecture of human brain and spinal cord tissues. Thus, there is a pressing need for scalable, human-derived, and organotypic in vitro models of central nervous system (CNS) tissues for disease modeling and drug and toxin screening. Recent studies demonstrate that neurally differentiating human pluripotent stem cells (hPSCs) possess the ability to self-organize into structures reminiscent of the fetal brain. In 2D culture, this phenomenon results in formation of polarized areas of neural stem cells (NSCs), known as neural rosettes, which resemble cross-sectional slices of the developing neural tube. The neural tube is the source of all primary CNS tissue in vivo, therefore neural rosettes could serve as an excellent starting point for generating tissue models of all CNS tissues. However, neural rosettes are a transient phenotype in culture

and form in random locations with no consistent size or shape. To harness the morphogenetic potential of NSCs, we have engineered micropatterned tissue-culture substrates capable of efficiently and reproducibly generating arrays of neural rosettes of standard size and shape. Combining this technology with differentiation protocols developed in our lab, we have produced arrays of standardized neural rosettes with NSC populations corresponding to both the brain and spinal cord. Further, we have engineered the surface chemistry of these substrates to allow for in situ control of tissue growth kinetics, facilitating radial expansion of arrayed neural rosettes while maintaining a polarized neuroepithelial zone. Here, we also present preliminary data on establishing dorso-ventral axis patterning via morphogen gradients using microfluidic gradient generators. Upon completion, we will have established a system capable of generating human neural tube slice cultures de novo that begin to recapitulate embryonic tissue cytoarchitecture with the potential to create organotypic levels of fetal CNS cell phenotype diversity.

(23) Engineering 3D Neural Organoid Morphology Using PVOH-Ca Sacrificial Templates

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Human pluripotent stem cells (hPSCs) provide unlimited potential for engineering a wide variety of in vitro tissue models to investigate human physiology and disease. Much progress has been achieved in using embryoid body culture approaches to generate 3D mimetics of brain, kidney, liver and prostate tissues, a.k.a. organoids. However, the morphogenesis that occurs within these organoid cultures is uncontrolled and spontaneous, thereby producing tissues with unnatural and highly variable cyto-architectures and morphologies. To progress towards controlled morphogenesis within 3D organoids, we developed a versatile method for controlling the microscale morphology of neural organoids derived from hPSCs. Here, we demonstrate reproducible engineering of 3-D, cylindrical neural organoids using sacrificial templates composed of a polyvinyl alcohol-calcium composite. The PVOH-Ca templates are mass-produced using injection molding, and can be used to cast internal voids of <10% deviation in dimension in alginate, acrylamide, and polyethylene glycol hydrogels. Using these PVOH-Ca templates, we developed a method to reproducibly engineer 3-D cylindrical neural organoids with 500µm diameters, which approximates the morphology of the germinal neural tube in the developing human embryo. Immunostaining of cryosectioned organoid slices revealed the ability of the differentiated cells to polarize as evidenced by the presence of Pax6, Laminin, and N-Cadherin throughout the length of the organoids. We hypothesize that this enhanced control of organoid morphology will yield a more biomimetic organoid morphogenesis process and thereby more physiologically relevant 3-D tissue models. This represents an important proof-of-principle of a versatile system for generating 3-D neural organoid of biomimetic morphology.

(24) Micropatterned Substrates to Promote and Dissect Reprogramming of Human Somatic Cells

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Cellular reprogramming of human somatic cells to generate induced pluripotent stem cells (iPSCs) generates valuable precursors for disease modeling, cell therapy, tissue engineering, toxicology and drug discovery. However, the reprogramming process can be inefficient, noisy and generate many partially reprogrammed cells in addition to fully reprogrammed iPSCs. To address these shortcomings, we developed a micropatterned substrate that allows for dynamic live-cell microscopy of thousands of cell subpopulations undergoing reprogramming. On this substrate, we were able to both watch and physically confine cells into discrete islands during reprogramming while preserving many of the biophysical and biochemical cues within the cells' microenvironment. Cells within 300 µm diameter islands reprogram in two phases: first erasing somatic cell identity, and then establishing pluripotency. During the first 12 days of reprogramming, micropatterning facilitated a change in shape, size and clustering of nuclei to promote somatic identity erasure. Increased proliferation, cell density and decreased intercellular YAP signaling

accompanied these nuclear changes. Reprogramming progression could be visually tracked using the nuclear characteristics of reprogramming cells within islands. A combination of eight nuclear characteristics could distinguish partially reprogrammed cells from those that were fully reprogrammed. A regression model using these nuclear characteristics predicted full reprogramming with high accuracy, and predictions were confirmed by isolation of several high-quality iPSC lines from patient cells. Micropatterned culture substrates constitute a new tool for facile iPSC production and can be used in high-throughput to probe and understand the subcellular changes that accompany human cell fate transitions.

(25) Micropatterned Soft Substrates Improve hESC-derived Cardiomyocyte Maturation

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The field of cardiac engineering has made great strides in generating cardiomyocytes from pluripotent stem cells (hPSC-CMs); however, it is currently lacking in its ability to mature these cells from fetal to an adult-like phenotype. The microenvironment of the native myocardium allows for cardiomyocytes to undergo a series of functional changes ultimately leading to their mature adult state. By recapitulating some of these biomimetic cues in vitro it may be possible to create a more mature engineered cardiac construct. Previously our lab showed the cytoskeletal structure of cardiomyocytes derived from hPSC-CMs resembles a more mature, adult phenotype when seeded onto micropatterned lanes on a glass substrate. We sought to further improve maturation of these cells by combining surface topology with a substrate stiffness near that of the healthy myocardium. To overcome pattern distortions caused by tacky substrates, a trans-print method was employed using a sacrificial polyvinyl alcohol (PVA) film that resulted in the precise pattern transfer of proteins from the film to the soft substrate. The hPSC-CMs grown on micropatterned soft substrates for 5 days displayed enhanced maturity markers compared to those grown on micropatterned glass substrates for 30 days. These results demonstrate environmental cues are not mutually exclusive, highlighting the need for pro-maturation strategies involving multiple signals to better recapitulate the native human heart.

(26) Enhancement of Arterial Specification in Human Pluripotent Stem Cell Cultures through ETS1 Overexpression Promotes Definitive Hematoendothelial Program with Broad Myelolymphoid Potential

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Identification of the regulators that lead to hemogenic endothelium (HE) formation with definitive hematopoietic potential should help to design strategies to recapitulate HSC development from human pluripotent stem cells (hPSCs). Here, using ETS1 conditional H1 hESC line, we found that ETS1 induction at the mesodermal stage of differentiation dramatically enhances the arterial specification in hPSC cultures and formation of CD144+DLL4+CXCR4+CD43-CD73- arterial type HE with lymphoid potential and the capacity to produce red blood cells with high expression of BCL11a and □-globin. ETS1 effect was mediated through upregulation of NOTCH signaling. Together, these findings demonstrated that promotion of arterial specification in cultures could aid in generation of definitive hematopoiesis from hPSCs.

(27) Directed Differentiation of Human Pluripotent Stem Cells to Blood-brain Barrier Endothelial Cells in a Defined System

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The blood-brain barrier (BBB) is comprised of specialized endothelial cells that are critical to neurological health. A key tool for understanding human BBB development and its role in neurological disease is a reliable and scalable source of functional brain microvascular endothelial cells (BMECs). Human pluripotent stem cells (hPSCs) can theoretically generate unlimited quantities of any cell lineage in vitro, including BMECs, for disease modeling, drug screening and cell-based therapies. Here, we demonstrate a chemically-defined method to differentiate hPSCs into BMECs in a developmentally relevant progression via small molecule activation of developmental signaling pathways. hPSCs are first induced to mesoderm commitment by activating canonical Wnt signaling. Next, these mesoderm precursors progress to endothelial progenitors, and treatment with retinoic acid (RA) leads to acquisition of BBB-specific markers and phenotypes. hPSC-derived BMECs generated via this protocol exhibit endothelial properties, including tube formation and LDL uptake, as well as BMEC-specific efflux transporter activities. Notably, these cells exhibit high transendothelial electrical resistance (TEER), above 3000 Ω·cm2. These hPSC-derived BMECs serve as a facile and robust human in vitro BBB model that can be used to study brain disease and inform therapeutic development.

(28) Novel Platform for Transplantation of Pluripotent Stem Cell-derived Beta Cells

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Human pluripotent stem cell (hPSC) therapy has been envisioned for use in regenerative medicine as a surrogate source of beta cells for diabetic patients. An ideal beta cell replacement therapy strives towards both generating an abundant supply of functional beta cells and identifying an ideal minimally invasive, well-vascularized, retrievable site for transplantation that is clinically applicable. Our hypothesis is that natural pancreatic matrix hydrogel (P-ECM) will provide a microenvironment that promotes engraftment and function of hPSC-derived islet-like clusters (hPSC-ILCs) after transplantation in a prevascularized subcutaneous (PV-SQ) site. To create the PV-SQ site a 5-Fr nylon catheter was implanted in immunodeficient mice for 4-6 weeks. To determine if the prevascularization is beneficial to the survival of the transplanted cells (ILCs or islets in combinations with P-ECM, ECs and MSCs), mice received cells in the vascularized and SQ spaces. Additionally, hPSC-derived endothelial cells (hPSC-ECs) and mesenchymal stem cells (MSCs) will be added to determine if a vascularized construct can be made without the PV-SQ step. C-peptide levels are measured and immunohistochemistry performed after graft retrieval. Catheter retrieval after 3 weeks showed neovascularization in the voided space (CD31+ cells). Graft histology at 5 weeks post ILCs transplantation in the PV-SQ space, showed neovascularization (CD31+) and mature markers of islet cells (e.g. insulin, glucagon). Mice transplanted with human islets±P-ECM (2,000 IEQ) per site are in progress. C-peptide measurements show release of human c-peptide up to 5 weeks in both groups. Following experimental end the graft will be retrieved and analyzed (casp-3, islet markers, vasculature). If successful, by using a pluripotent stem cell source of functional beta cells and by providing a safe and healthy environment for transplanting cells, this unique transplant approach may overcome many of the limitations which exist in clinical beta cell replacement therapies.

(29) A CRISPR Approach to Monitoring Hypoxia-Inducible Proteins in Real-Time

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Hypoxia-inducible factor 1A (HIF1A) regulates expression of genes implicated in various aspects of oncogenesis, including angiogenesis, cell survival, metastasis, and glucose metabolism. Overexpression or hypoxia-induced stabilization of HIF1A has been associated with poor prognosis in cancer patients, making HIF1A and its associated pathway a high-profile target for anticancer therapies. We sought to develop a live-cell assay to monitor abundance of endogenous HIF1A and HIF1A-inducible proteins that could be used to identify potent and specific inhibitors of the hypoxia signaling pathway. To accomplish this goal, mammalian cell lines were edited by CRISPR using a Cas9:crRNA ribonucleoprotein complex with a single-stranded oligonucleotide donor DNA to introduce the HiBiT tag at the C-terminus of HIF1A and a number of known hypoxia-inducible proteins, including BNIP3, ANKRD37, HILDPA and KLF10 . The 11 amino acid HiBiT peptide and its complementing 18 kDa polypeptide, known as LgBiT, spontaneously reconstitute into an active luciferase derived from the NanoLuc enzyme. Co-expression of LgBiT in edited cells, followed by addition of the cell-permeable luciferase substrate, leads to generation of a bright, steady luminescent signal that directly correlates with abundance of the HiBiT fusion. The edited cells were treated with several known modulators of the HIF1A signaling pathway, and changes in the abundance of the protein fusions were followed in real-time by monitoring luminescence. The HiBiT tag was also used to validate size and subcellular localization of the fusion proteins using bioluminescence imaging and antibody-free blotting. As expected, all tested compounds induced HIF1A accumulation. However, the downstream targets of HIF1A generated differing response to the chemical modulators, warranting further investigation into the modes by which these compounds act. By coupling the speed and efficiency of CRISPR-mediated editing with the small size and brightness of HiBiT, it was possible to generate a live-cell assay to monitor abundance of proteins along the HIF1A pathway. This assay could easily be adapted to screen for compound-induced effects on protein levels of HIF1A as well as HIF1A- induced changes in expression patterns.

(30) Scarless Gene Editing of Human Pluripotent Stem Cells via Transient Puromycin Selection

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Precise, genome edited human pluripotent stem cells have broad applications in drug discovery, toxicology, disease modeling and regenerative medicine. Here, we present a facile method for rapid introduction or correction of disease-associated variants in both human embryonic and induced pluripotent stem cells with high efficiency using the CRISPR/Cas9 system. By utilizing plasmid vectors that express a puromycin N-acetyl-transferase (PAC) gene whose expression and translation is linked to that of Cas9, we transiently select for transfected cells based on their early levels of Cas9 protein. This selection method is scarless in that it enables precise changes defined by the donor oligonucleotide template without integration of additional vector sequences. Isolation of clonal cell populations containing both heterozygous and homozygous gene edits could be readily accomplished without cell sorting or high-throughput sequencing in as little as two weeks. Genome edited stem cells isolated using this method did not contain any detectable off-target mutations and display expected functional phenotypes after directed differentiation. Further, the robustness of this method is demonstrated by its ability to produce precision genome edits at a variety of genomic loci in several patient derived cell lines using both feeder and feeder-free culture conditions.

(31) Human Pancreas ECM-derived Platform for Enhancement of Beta Cell Differentiation and Transplantation

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Stem cell-based therapies, such as the differentiation of beta cells from human pluripotent stem cells (hPSC), hold great potential for the treatment of diabetes. Despite recent progress of in vitro differentiation, producing consistent physiologic function in vitro is needed to extend this therapy to patients. Extracellular matrix (ECM), a tissue-specific and essential component of the tissue microenvironment, plays a developmental role through structural and chemical stimulation. No studies to date have explored the potential of using human pancreatic ECM (hP-ECM) to enhance the differentiation of hPSCs into functional beta cells. We first seek to derive and characterize an hP-ECM hydrogel platform with cell culture and transplantation applications.

Human pancreas was homogenized and decellularized with deoxycholate. The hP-ECM was examined for ECM protein and GAG retention, and the removal of lipids, DNA and other cell material. hP-ECM was solubilized with pepsin and made into hydrogel. We tested hP-ECM hydrogel in vitro and in vivo for biocompatibility in short term experiments with islets and ILCs, and assessed immune responses in vivo in humanized mice.

Our results indicate that hP-ECM hydrogel is amenable to culture of islets and differentiated islet-like cells (ILCs). Human islets and ILCs cultured on and within hP-ECM hydrogel adhere, survive and continue to express islet-specific genes. In ILCs embedded in hydrogel for up to 7 days, Pdx1+ cells continue to proliferate, express Ki-67, and are negative for apoptosis markers. When injected subcutaneously into a humanized, immunocompetent mouse, the hydrogel does not show signs of human T cell or B cell infiltration, indicating hypoimmunogenicity.

Using this platform in combination with ILCs, we intend to test for enhanced beta cell differentiation and function in vitro, and improved engraftment and function in vivo. These materials may also enable other bioengineering strategies for treating diabetes.

(32) Investigating the Utility and Function of a Novel Human Cardiomyocyte Cell Surface Protein

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) are invaluable for the study of human development, disease modeling, drug testing, and regenerative medicine. However, the use of hPSC-CM for these research and clinical applications is currently limited by our inability to identify and select homogeneous populations of functionally defined hPSC-CM from heterogeneous cultures. Though immunophenotyping may overcome these current challenges, there remains a paucity of suitable cell surface markers that can be used as cell type, subtype, and maturation stage specific markers for hPSC-CM. To address this need, Cell Surface Capture (CSC) Technology, a chemoproteomic approach for identifying extracellular domains of cell surface proteins, was applied to hPSC-CM. Applying the CSC to 19 time points across the first 100 days of hPSC-CM differentiation has identified >1300 cell surface proteins. From these data, we have selected one previously undocumented cardiomyocyte cell surface transmembrane protein that is unique to cardiomyocytes, LSMEM2. Targeted quantitation by mass spectrometry has confirmed the presence of this protein emerges during in vitro differentiation once the cells have committed to the cardiomyocyte fate and it is present in human heart tissue. Novel monoclonal antibodies have been developed to this new protein and antibody specificity has been validated at the epitope and cell type level and efforts to validate at the protein-level are ongoing. Further characterization of the protein sequence and post-translational modifications is being achieved through top-down MS to measure intact proteoform(s). The function of the protein is being studied through use of a CRISPR/Cas9 generated knockout and functional analyses of cells sorted by this marker are ongoing. As phylogenetic analyses of this novel protein reveals high sequence conservation and that it emerged in species with functionally four-chambered hearts, we expect that LSMEM2 will be relevant to cardiomyocyte function and will be useful as a marker of cardiomyocyte identity.

(33) Potential of Bone Morphogenetic Protein-6 to Increase Bone Formation in Patients with Type 1 Diabetes

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Patients with type 1 diabetes mellitus frequently experience bone-related problems such as osteopenia and osteoporosis. These patients exhibit decreased bone mineral density and mass, leading to long term risks of fracture. Studies involving type 1 diabetic murine models have shown reduced osteoblast activity in long bones, causing impairment of bone formation. Little is known, however, about the biological mechanism underlying this link in humans. Our preliminary results have revealed a decreased capacity for osteogenic differentiation of bone marrow-derived mesenchymal stem cells (MSCs) from type 1 diabetic donors compared to that of the cell from non-diabetic donors. Furthermore, the MSCs from diabetic donors have reduced production of bone morphogenetic protein 6 (BMP6). When BMP6 was supplemented to MSC cultures, osteogenic differentiation of cells from diabetic donors were upregulated as shown by increased expression of *CBFA1* and *OC*; increased matrix calcification; and increased alkaline phosphatase production. Conversely, removing extracellular BMP6 from the culture medium using antibodies resulted in decreased bone formation for non-diabetic MSCs. Finally, injection of BMP6 into STZ-induced diabetic mouse improved the bone mineral density and trabecular bone growth compared to the non-treated controls as measured by DEXA, MicroCT, and qXRI. Interestingly, BMP6 has no effect on osteogenesis and bone formation in control mice. We hypothesized that the deficiency of BMP6 may partially contribute to the bone loss associated with type 1 diabetes. Additionally, our results may also provide insight into the development of BMP6 as a pharmacological agent for patients suffering from degenerative bone diseases resulting from hyperglycemia.
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