BioPharmaceutical Technology Center
I N S T I T U T E

12th Annual Wisconsin Stem Cell Symposium
Engineering Cells and Tissues for Discovery and Therapy
SPEAKER ABSTRACTS

Integrated, multi-scale & spatial-temporal cell biology – a next step in the post genomic era
Rick Horwitz, Ph.D.

A major goal of cell science is to understand and predict cellular behaviors, promising major insights into homeostasis, disease, development and regeneration as well as new approaches to manipulate them. Most cellular behaviors result from the organization of cellular components into discrete functional units, i.e., molecular machines and regulatory complexes, that operate locally and transiently. The localized organization and activity, in turn, arise from the subsets of genes expressed, posttranslational modifications, and the cellular environment, often resulting from the repurposing and specialization of canonical cellular components.

The mission of the Allen Institute for Cell Science is to understand and predict cellular behaviors, taking an integrative approach. The initial project is developing dynamic, visual data on cell organization and activities at multiple spatial and temporal scales, beginning with the mesoscale, using fluorescently tagged, genome-edited, human induced pluripotent stem cells. We quantify the relative locations and dynamics of the major cellular functional units and activities as the stem cells execute characteristic activities, including the cell cycle and differentiation, and in response to perturbations. e.g., drugs and agonists. The goal is a visual database of dynamic cellular organization and activity at multiple spatial and temporal scales. The focus is on the integrative changes across the cell, rather than a deep focus on a single process. The organizational and dynamic image data are being combined with genomic and other information to model cellular organization and its transitions, with an initial goal of predicting cell type, state or behavior, and pathology from organization.

The quantitative image data are being integrated into a visual database, an “animated cell”, which also serves as an output for computational modeling. The animated cell is multi-scale, including existing structural data, and useful for fine grained models. All data, models, reagents, and tools generated by the Institute are being made publically available.

Human pluripotent stem cells in understanding genetic cardiovascular disease and effects of drugs
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Derivation of many different cell types from human pluripotent stem cells (embryonic stem cells or HESCs and induced pluripotent stem cells or hiPS cells) is an area of growing interest both for potential cell therapy and as a platform for drug discovery and toxicity. Most particularly, the recent availability of methods to introduce specific disease mutations into human pluripotent stem cells and/or to derive these cells as hiPS cells by reprogramming from any patient of choice, are creating unprecedented opportunities to create disease models “in a dish” and study ways to treat it or slow down its rate of development. Understanding the underlying developmental mechanisms that control differentiation of pluripotent cells to their derivatives and mimicking these in defined culture conditions in vitro is now essential for moving the field forward. We have used these methods to produce isogenic pairs of hiPSC lines to compare diseased and corresponding control cardiomyocytes and vascular endothelial cells and identify disease related phenotypes and mechanisms. The use of isogenic pairs has proved crucial since variability between “healthy

control" hiPSC lines is often greater than the difference between a diseased cells and its isogenic control. We have also examined drug responses of hESC-derived cardiomyocytes to a variety of cardiac and non-cardiac drugs and shown that iPSC derived cardiomyocytes with mutations in ion channel genes can accurately predict changes in cardiac electrical properties and reveal drug sensitivities also observed in patients. Similar studies will be described using vascular endothelial cells from hPSC. Relevant in all cases is the development of appropriate bioassays in which to measure disease phenotypes which may be highly cell type specific dependent. For heart cells, this might be electrical activity or contractions force; for vascular cells, responses to fluid flow flow and inflammation. Various approaches to this will be presented.

The Promise of T-Cell Engineering
Michel Sadelain, Ph.D.

Chimeric antigen receptors (CARs) are synthetic receptors that redirect and reprogram T cells to mediate tumor rejection. The most successful CARs used to date are those targeting CD19, which offer the prospect of complete remissions in patients with chemorefractory/relapsed B cell malignancies. CAR therapy is based on the genetic engineering of autologous T lymphocytes, imposing patient-specific T cell manufacturing. Advances in genetic engineering and stem cell biology may alter this paradigm. CARs are typically transduced using γ-retroviral or other randomly integrating vectors, which may result in variegated CAR expression and transcriptional silencing. Using CRISPR/Cas9 to edit human T cells, we have established conditions yielding efficient target gene disruption and CAR insertion in a single step. We have found that directing a CD19 CAR to the human T cell receptor (TCR) alpha chain (TRAC) locus not only results in efficient and uniform CAR expression in human peripheral blood T cells, but, remarkably, also enhances T cell potency, vastly outperforming that of conventionally generated CAR T cells. We further show that CAR gene expression under the control of the TCR alpha promoter minimizes tonic signaling and allows effective regulation of CAR expression. These findings highlight the potential of genome editing to advance immunotherapies. We previously described that CAR T cells could be generated from T cell-derived induced pluripotent stem cells (TiPSC). TiPSC-derived T cells demonstrated anti-tumor activity in mice, but also displayed an innate-like phenotype and function, resembling γδ T cells despite expression of their endogenous αβ TCR. Through further genetic engineering of TiPSC and optimization of T cell differentiation, we are now able to induce human CD8ab T cells. Significant challenges nonetheless remain to reliably and efficiently produce therapeutic CAR T cells from pluripotent stem cells.

Precise Gene Editing in Human Pluripotent Stem and T Cells
Krishanu Saha, Ph.D.

Gene-edited pluripotent stem and T cells are important resources for drug discovery, toxicology, disease modeling, tissue engineering, immunotherapy and regenerative medicine. Recently, we developed new CRISPR-Cas9 strategies to correct pathogenic point mutations and introduce transgenes precisely using homology-directed DNA repair. These strategies reduce and, in some cases, eliminate undesired allelic modifications associated with non-homologous end joining.

Blood-Brain Barrier Modeling Using Human Pluripotent Stem Cells
Eric V. Shusta, Ph.D.

The blood-brain barrier (BBB) plays an important role in maintaining brain health and is often compromised in disease. Moreover, as a result of its significant barrier properties, this endothelial interface restricts uptake of neurotherapeutics. A renewable cell source for human BBB modeling could prove enabling for brain research and pharmaceutical development. We recently

demonstrated that endothelial cells generated from human pluripotent stem cells (hPSCs) can be specified to possess many BBB attributes, including well-organized tight junctions, polarized efflux transport, and nutrient transporter expression. Importantly, hPSC-derived BBB endothelial cells respond to cues provided by other cells of the neurovascular unit such as human pericytes, astrocytes and neurons to generate very tight barrier properties as measured by transendothelial electrical resistance (~5000 ohmxcm^2), while exhibiting molecular permeability that correlates well with *in vivo* brain uptake. In this talk, we will demonstrate that the process of hPSC differentiation to BBB cells is also compatible with disease modeling using patient-derived induced pluripotent stem cell lines, can be used in the isogenic modeling of the neurovascular unit, and the can be employed for the evaluation of experimental drug permeability attributes.

Human Pluripotent stem cell-derived organoids as new models to study development and disease of the digestive tract

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Successful efforts to direct the differentiation of human embryonic and induced pluripotent stem cells (PSCs) into specific organ cell types *in vitro* have largely been guided by studies in embryonic development. We have used principles of developmental biology to generate complex, three-dimensional organ tissues with improved functionality from human PSCs *in vitro*. We identified that by modulating FGF, Wnt and BMP signaling pathways, we can direct anterior-posterior patterning PSC-derived definitive endoderm as well as gut tube morphogenesis *in vitro*. The resulting three-dimensional gut tube tissues resembled either foregut or mid/hindgut. These gut tube tissues could be further directed into specific organ tissue types by additional manipulation of embryonic signaling pathways. For example using a temporal series of growth factor manipulations that mimic embryonic intestinal development we generated three-dimensional human small intestinal and colonic organoids (HIOs and HCOs). We have also generated foregut-derived organoids including fundic and antral gastric organoids. Organoids contain epithelial structures diverse cell types that are unique to their representative organ. Moreover, we are able to manipulate specific cell lineages using genetic gain- and loss-of-function approaches. We have also engineered additional complexity into organoids, for example we have incorporated a functional enteric nervous system into HIOs and generated intestinal tissue that is capable of peristaltic-like motility. Lastly, we are using organoids to model diseases caused by genetic or infectious agents. For example we are investigating the gastric epithelial response to *Helicobacter pylori*, epithelial repair mechanisms, and modeling genetic forms of digestive disease such as Hirschsprung's disease.

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Engineering Multicellular Organization
Peter Zandstra, Ph.D.
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