Meeting Report for the 2017 National Institutes of Health National Heart, Lung, and Blood Institute Progenitor Cell Biology Consortium

Cardiovascular Bioengineering Workshop and Symposium

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The 2017 Cardiovascular Tissue Engineering Workshop and Symposium—sponsored, in part, by the National Heart, Lung, and Blood Institute's Progenitor Cell Biology Translational Consortium—was held on March 2 to 3, 2017, in the Hill Student Center and the Alumni House of the University of Alabama, Birmingham (Figure). The 2017 Workshop and Symposium built on the success of last 2 years' annual meeting[1] and was hosted by Dr Jianyi (Jay) Zhang, Professor and Chair of Biomedical Engineering at University of Alabama, Birmingham. The Workshop was attended by Progenitor Cell Biology Consortium investigators and scientists in the field to focus on their bioengineering works with pluripotent stem cells and engineered heart tissue. The Symposium was open to a broader audience (faculty members, research fellows, medical residents, and PhD students) and featured presentations by international leaders in the field of cardiovascular bioengineering.

Opening Statements

The meeting opened with remarks from **Dr Jianyi (Jay) Zhang**, Chair of the Progenitor Cell Biology Consortium Cardiovascular Bioengineering Interest Group; **Dr Denis Buxton**, Director of Cardiovascular Sciences at the National Heart, Lung, and Blood Institute; **Dr Victor J. Dzau**, President of the National Academy of Medicine and Professor of Medicine and Pathology at Duke University School of Medicine; and **Dr Selwyn M. Vickers**, Senior Vice President for Medicine and Dean of the School of Medicine at University of Alabama, Birmingham.

Cardiovascular Stem Cells and Regenerative Medicine Session

Dr Daniel J. Garry (University of Minnesota) discussed several key signaling pathways that promote cardiomyocyte and vascular proliferation after injury, many of which also direct cardiogenesis during embryogenesis. His laboratory has previously shown that Ets variant 2 is an upstream regulator of microRNA 130a, which controls endothelial lineage specification but has no effect on the hematopoietic lineage.[2] He has continued to explore the key roles of Ets variant 2 and microRNA 130a in specifying and promoting the endothelial lineage by conducting both overexpression and loss-of-function experiments. **Dr Sean Wu** (Stanford University) discussed how knowledge gained from studies of cardiac development can be incorporated into the creation of functional cardiac tissue. The mature heart is composed of a variety of cell types, which are distributed throughout the 4 heart chambers, and Dr Wu's laboratory has performed genome-wide single-cell RNA-sequencing analyses to identify a set of genes that determine the anatomic location of each cell type in fetal hearts.[3] **Dr John P. Cooke** (Houston Methodist Research Institute) described his group's progress in the transdifferentiation of human fibroblasts into induced endothelial cells. Their approach is composed of 2 steps: (1) the activation of endogenous immune signaling pathways, which increases epigenetic plasticity, and then (2) the addition of endothelial differentiation factors, such as vascular endothelial growth factor, fibroblast growth factor, bone morphogenic protein 4, 8-bromoguanosine 3′,5′-cyclic monophosphate, and an inhibitor of the transforming growth factor beta receptor. **Dr Victor J. Dzau** (Duke University) presented his laboratory's work on the paracrine mechanisms induced by stem-cell therapy.

Mechanisms of Action Session

Dr Raj Kishore (Temple University) presented his work on how exosomes mediate the role of stem cells in myocardial repair. Exosomes participate in cell–cell communication by transferring their contents to specific cells that are targeted by the exosome's microRNA and protein signature; however, cellular stress can lead to differences in exosome content and function, even when the exosomes are generated from the same cell source. **Dr Michael E. Davis** (Georgia Tech College of Engineering and Emory University School of Medicine) presented his laboratory's work with technologies for investigating the roles of exosomes and other factors in stem-cell–mediated cardiac repair. **Dr Gangjian (GQ) Qin** (University of Alabama, Birmingham) presented his group's work on enhancing stem cell metabolism for cardiac repair. Dr Qin showed that E2F1 (E2F transcription factor 1), a classic cell-cycle regulator, is a potent inhibitor of mitochondrial oxidative metabolism in bone marrow endothelial progenitor cells. Genetic deletion of E2F1 results in

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Figure. 2017 Progenitor Cell Translational Consortium (PCTC) Faculty and Consortium Organization Committee (from left to right). First row: Kelly Hillard, Joel L. Berry. Second row: Mark Sussman, Roberto Bolli, Victor J. Dzau, Jianyi (Jay) Zhang, Raj Kishore; Third row: Sean Wu, Elizabeth A. Lipke, Brenda Ogle, Yabing Chen, Michael Terrin, Michael E. Davis, Leigh Griffiths, John P. Cooke. Fourth row: Daniel J. Garry, Kevin Healy, Prasanna Krishnamurthy, Jack M. Rogers, Bjorn C. Knollmann, Fifth row: Gangjian (GQ) Qin, Gregory P. Walcott, Kevin (Chunxiang) Zhang, Timothy J. Kamp, Sumanth D. Prabhu, Stefan Janssens, Denis Buxton. Sixth row: João Silva Soares, William Holman, Joseph C. Wu, Philippe Menasche, Shijun Hu, Lei Ye.

a metabolic switch from glycolysis to oxidative phosphorylation, which favors endothelial progenitor cell differentiation and neovascularization in the ischemic cardiac tissue. **Dr Sumanth D. Prabhu** (University of Alabama, Birmingham) discussed how immune cells participate in cardiac remodeling and heart failure. **Dr Prasanna Krishnamurthy** (University of Alabama, Birmingham) presented his laboratory's work on the role of efferocytosis in cardiac repair. **Dr Robert S. Balaban** (National Heart, Lung, and Blood Institute) described his laboratory's investigations of how the mitochondrial reticulum supports metabolic homeostasis in cardiac cells. The mechanisms by which the energy produced during mitochondrial oxidative phosphorylation is distributed within a muscle cell has been an active area of research. By using state-of-the-art, 3-dimensional electron microscopy and super-resolution optical imaging, Dr Balaban's group showed that large regions of the cell are permeated by a mitochondrial reticulum and that this reticulum conducts the mitochondrial membrane potential (ie, the primary source of energy for mitochondrial ATP production) to mitochondria that are located in regions of high ATP demand. This exceptionally rapid, conductive mechanism of energy distribution works in parallel with the slower, facilitated-diffusion mechanisms to balance energy production and consumption in different parts of the cell.

Tissue Engineering Session: Vessels, Valves, Tissue Patches, and Organs

Dr Palaniappan Sethu (University of Alabama, Birmingham) presented his laboratory's work on the use of electromechanical stimulation for differentiating human induced pluripotent stem cells (iPSCs) into cardiomyocytes. **Dr João Silva Soares** (University of Texas, Austin) described his work with Michael S. Sacks, PhD, at the Center for Cardiovascular Simulation. Drs Soares and Sacks have developed new techniques in modeling, experimentation, and simulation that can enable researchers to predict how matrix production, quality, and stiffness, as well as cell growth, in engineered tissues will respond to changes in a variety of controllable stimuli. Predictive tools such as these can serve as a valuable and cost-effective guide for the design and clinical development of all engineered tissues. **Dr Ho-Wook Jun** (University of Alabama, Birmingham) presented his group's work on the development of an endothelium-mimicking nanomatrix that promotes healing. **Dr Adam Engler** (University of California, San Diego) discussed how polymorphisms in noncoding RNAs elevate the risk of coronary artery disease and infarction. These polymorphisms cannot be studied in animals because they have only recently appeared in the human genome; thus, his laboratory and collaborators have edited the genomes of patient-derived vascular smooth muscle cells, endothelial cells, and cardiomyocytes and then used a combination of microfluidic experimental techniques and genomic analyses to show that ANRIL (antisense noncoding RNA in the INK4 locus; a long, noncoding RNA) impairs vascular cell function and alters the adhesion and contractile transcriptome of differentiated iPSCs. **Dr Joel L. Berry** (University of Alabama, Birmingham) discussed his group's work on the use of an in vitro, 3-dimensional bioreactor for drug development.

Dr Nenad Bursac (Duke University) presented data demonstrating the successful development of functional human cardiac-tissue patches with clinically relevant dimensions (4 cm×4 cm), fast and uniform action potential propagation, and contractile activity that approaches the strength observed in adult myocardium. **Dr Jack M. Rogers** (University of Alabama, Birmingham) discussed several optical and electric

mapping technologies that are available for investigating arrhythmogenesis in the hearts of large animals. These methods will be essential for detecting and correcting any arrhythmogenic complications that may occur in response to regenerative cardiac therapy. **Dr Vladimir G. Fast** (University of Alabama, Birmingham) described his work on new methods for imaging membrane potentials and intracellular calcium handling in cardiac tissue. His presentation included the results from recent imaging studies of electric activation and calcium transients in engineered cardiac tissue patches that had been created from 3 human iPSC-derived cardiac cell types: cardiomyocytes, endothelial cells, and smooth muscle cells. **Dr Kevin Healy** (University of California, Berkeley) presented his laboratory's work on the development of tissue chip technology that effectively models human heart tissue and on bio-inspired tunable hyaluronic acid–based hydrogels for matrix-assisted cell transplantation. Compared with 2-dimensional cultures of iPSCs or human iPSC-derived cardiomyocytes, the cardiac organ chips have drug IC_{50} (half maximal inhibitory concentration) values that more accurately reflect clinical data and correctly predict the effect of drugs that typically produce false positives.

Human PSC and Cardiovascular Cell Therapy Session

Dr Philippe Menasché (Hôpital Européen Georges-Pompidou) discussed the clinical use of embryonic stem cells and their cardiac-specified progeny. Because cardiovascular cell therapy seems likely to be most effective if the cells are phenotypically matched to the tissue/organ being treated, Dr Menasché's group has developed a process for upscaling embryonic stem cells and then committing them to the cardiac lineage, thereby, generating a population of cardiac progenitor cells (CPCs), which are primarily characterized by the expression of stage-specific embryonic antigen-1. Dr Menasché presented data from the first in-human safety and feasibility study of this technology, the ESCORT trial (Transplantation of Human Embryonic Stem Cell–derived Progenitors in Severe Heart Failure; NCT02057900), which has been conducted in 6 patients with ischemic heart disease. **Dr Joseph C. Wu** (Stanford University) discussed how human iPSCs can be used to correlate a patient's genotype with his or her phenotype (ie, clinical history). These correlations are often difficult to determine because most common diseases are associated with hundreds of genetic risk variants, any one of which produces only a small effect, and because DNA sequencing analysis is largely blind to epigenomic, transcriptomic, proteomic, metabolomic, and other -omic factors. However, modern genome editing techniques can be used to insert genetic variants into a specific human iPSC-derived cell type, which can then be evaluated in a series of assays to determine whether the variant alters cellular function in a manner that is consistent with the disease phenotype. **Dr Roberto Bolli** (University of Louisville) presented an entirely new strategy to improve the effectiveness of cell therapy—the administration of repeated doses of cells. This is a veritable paradigm shift that may well change the way cell therapy will be performed in the future. He proposed that a major reason for the modest, borderline, or negative results obtained to date in clinical trials is the use of inadequate treatment protocols, based on one dose. He pointed out that neither adult nor embryonic stem/progenitor cells survive long term in the recipient heart; because their beneficial effects are mediated by transient release of paracrine factors, there is no reason to assume that one administration will achieve maximal therapeutic benefit. He suggested that because the engraftment rate of transplanted cells is exceptionally low for virtually all stem/progenitor cell types, it is unreasonable to expect a single dose of cells (which is the dosing regimen used in nearly all preclinical and clinical studies conducted to date) to be sufficient to impart full benefits. He also presented data in 2 rodent models (rats and mice), indicating that repeated cell dosing produces a cumulative effect on left ventricular function and structure that is superior to the benefits observed after a single dose. Collectively, these results demonstrate that the efficacy of cell-based therapies cannot be adequately evaluated after 1 treatment; just like pharmaceutical products, which are eliminated via metabolic processes, cells are cleared from the body after administration and must be readministered. Repeated cell dosing is a disruptive concept that will fundamentally transform the design of both preclinical and clinical investigations. This concept could revolutionize the field by dramatically improving the effectiveness of cell therapy. **Dr Timothy J. Kamp** (University of Wisconsin, Madison) described his laboratory's work with induced CPCs. Previously, his group generated induced CPCs by reprogramming the fibroblasts of a genetically modified mouse line that expressed enhanced yellow fluorescent protein from the Nkx2.5 promoter as a reporter of early CPC identity.[4] Dr Kamp's new work builds on this success by using the same 5 reprogramming factors (Mesp1, Tbx5, Gata4, Nkx2.5, and Baf60c) to generate induced CPCs from the lung and cardiac fibroblasts of wild-type C57/Bl6 mice which, because they are genetically unmodified, are a more relevant model for future clinical studies of human-induced CPC technology. **Dr Bjorn C. Knollmann** (Vanderbilt University) described his laboratory's work on the maturation of iPSC-derived cardiomyocytes in vitro for disease modeling and cell therapy. **Dr Roger Hajjar** (Icahn School of Medicine, Mount Sinai) presented his group's work on gene therapy and genome engineering in various cardiovascular diseases. The safety of adeno-associated vectors in patients with heart failure has expanded their use in various cardiovascular diseases.[5] The early trials with adeno-associated vectors have revealed that higher doses and improved delivery are critical for efficacious gene delivery in patients.[5]

Cardiovascular Sciences Session

Dr Doris A. Taylor (Texas Heart Institute) described her group's work toward building a beating heart in the laboratory, as well as their experiences with simpler structures, such as cardiac patches. **Dr Yabing Chen** (University of Alabama, Birmingham) presented her work on the osteogenic differentiation of vascular smooth muscle cells into bone-like cells, which is regulated by the transcription factor Runx2 and involves both oxidative stress signals and post-translational modifications by O-linked N-acetylglucosamine. **Dr Kevin (Chunxiang) Zhang** (University of Alabama, Birmingham) described his group's work on the involvement

of mitochondrial long noncoding RNAs in cardiovascular biology and disease. **Dr Joseph Tector** (University of Alabama, Birmingham) discussed the progress of pig xenotransplantation technology toward clinical use. **Dr Mark Sussman** (San Diego State University) presented his laboratory's work with endogenous cardiac stem cells. **Dr Stefan Janssens** (KU Leuven, Belgium) discussed his laboratory's work on the therapeutic use of nitric oxide in the heart. He focused on translational studies using blood outgrowth endothelial progenitor cells (the major endogenous nitric oxide source) in ischemic cardiomyopathy and on inhalation strategies with nitric oxide gas to reduce ischemic damage and limit maladaptive left ventricular remodeling. He also summarized novel insights from a first-in-man randomized controlled trial in 250 ST-segment–elevation myocardial infarction patients using inhaled nitric oxide for cardioprotection.

Closing Statements

Closing remarks were provided by **Dr J. Iwan D. Alexander**, Dean of the School of Engineering at the University of Alabama, Birmingham.

Summary

The main goal of the 2017 Cardiovascular Tissue Engineering meeting was to promote research that will lead to the clinical trials of engineered cardiovascular tissues. The event's organizers would like to take this opportunity to express their appreciation and gratitude to the invited speakers and audience members for their time, participation, and enthusiasm. The Third Annual Cardiovascular Tissue Engineering Workshop and Symposium, Cardiovascular Bioengineering 2018 (CVBE 2018), will be held on March 1 to 2, 2018.

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Disclosures

None.

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