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Convergences of Life Sciences and Engineering in Understanding and Treating Heart Failure

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Abstract

On March 1 and 2, 2018, the National Institutes of Health 2018 Progenitor Cell Translational Consortium (PCTC) and Cardiovascular Bioengineering Symposium (CVBE) was held at the University of Alabama at Birmingham. Convergence of life sciences and engineering to advance the understanding and treatment of heart failure was the theme of the meeting. Over 150 attendees were present for more than 40 scientists presenting their latest works on engineering human functional myocardium for disease modeling, drug development, and heart failure research. The scientists, engineers and physicians in the field of cardiovascular sciences, met and discussed on the most recent advances in their works and propose future strategies in overcoming the major roadblocks of cardiovascular bioengineering and therapy. Particular emphasis was given for manipulation and using of stem/progenitor cells, biomaterials, and methods to provide molecular, chemical and mechanical cues to cells in order to influence their identity and fate *in vitro* and *in vivo.* Collectively, these works are profoundly impacting and progressing toward deciphering the mechanisms and developing novel treatments for left ventricular dysfunction of failing hearts. Here we present some important perspectives that emerged from this meeting.

Keywords

Heart Failure; Tissue Engineering; Stem cells; Biomaterial

The convergence of life sciences and engineering continues to offer hope for revolutionary treatments for some of the most devastating diseases. Illuminating the path forward are advances in the manipulation and use of stem/progenitor cells and the scaffolds supporting their viability, differentiation, and function. Methods to provide chemical and mechanical cues to cells and control their fate *in vitro* and *in vivo* are profoundly impacting the progress in disease modeling, drug development, and cell therapy. In particular, addressing the daunting challenge of heart disease will require collective engagement of scientists, engineers, and clinicians. In a recent meeting of the National Institutes of Health 2018 Progenitor Cell Translational Consortium (PCTC) and Cardiovascular Tissue Engineering Symposium (CVBE) at the University of Alabama, Birmingham, on March 1, 2018, scientists, engineers and physicians have met and discussed the most recent advances in their work and to propose future strategies in cardiovascular bioengineering and therapy. Here we present some important perspectives that emerged from this meeting.

Disorders of ventricular function and structure, including ischemic injury, valvular disease, hypertrophy, congenital abnormalities or cardiomyopathies reduce cardiac output and/or impair diastolic relaxation of the heart, and eventually lead to heart failure (HF). A common cause of systolic dysfunction is ischemic injury to the heart. Whereas percutaneous revascularization and coronary artery bypass surgery have transformed treatment of coronary disease, these therapies do not address the root cause of HF due to ischemic injury, i.e., the loss of cardiomyocytes and their replacement by a noncontractile fibrous scar. Regenerative medicine approaches aim to address this loss of functional tissue.

While the first era of cardiac “regenerative” medicine has focused on the exclusive use of cells (from various origins), it soon became evident that clinically relevant benefits were unlikely to be achieved without the restoration of an appropriate cell-matrix cross-talk. The recognition of the importance of this cross-talk has accelerated the development of cardiovascular bioengineering strategies with the goal to regenerate the damaged myocardium by providing both cellular and extracellular cues. While cell- and biomaterial-based therapies for ischemic cardiomyopathy are still in their infancy, results from laboratory studies and clinical trials have yielded hugely valuable information. The challenge of complete myocardial regeneration will require our ability to control the reparative processes at various spatial scales from molecule to organ [1]. The journey to meet this challenge may be long, yet the knowledge gained along the way will not only improve modeling and understanding of the disease, but also foster the discovery of new and more efficient therapies [2]. Recently, a common denominator for many of these efforts has been the utilization of human pluripotent stem cells (hPSC) and their derivatives.

Disease Modeling

Cell and tissue-level models of cardiac disease

Cardiovascular disease (CVD) modeling at the cellular level can reveal much about the mechanisms of the disease. Whether the obtained knowledge can be clinically translated is highly dependent on how well the models recapitulate human CVD and mimic patients’ responses to therapy [3]. More than 20 years ago, the first human “disease gene” for familial hypertrophic cardiomyopathy was identified. However, in the following 2 decades, it has

been difficult to study human CVD, and HF in particular, due to the limited ability to culture human cardiomyocytes. With the generation of human induced pluripotent stem cells (iPSC) in 2007 by Dr. Shinya Yamanaka (2012 Nobel Prize winner in Medicine & Physiology) [4] and the increased efficiency of differentiating iPSC into cardiomyocytes (iPSC-CMs) and endothelial cells (iPSC-ECs) [5], this landscape has now changed dramatically. For the first time, it is now possible to create patient- and disease-specific cells to improve our understanding of the molecular mechanisms underlying many CVDs.

Dilated cardiomyopathy (DCM) [6], hypertrophic cardiomyopathy (HCM) [7], long QT syndrome (LQTS) [8], and congenital heart disease (CHD) have been the subject of impressive modeling studies using human iPSC technology [9]. Collectively, they suggest that iPSC can provide new opportunities for studying the molecular mechanisms of cardiac diseases in humans. By obtaining the genetic (e.g., DNA-seq) and phenotypic (e.g., clinical history) profiles of large populations of CVD patients and normal controls, we start to understand the differential responses of common CVD drugs in these populations. A recent example is provided by the finding that the response of iPSC-derived cardiomyocytes to anthracyclines was different in patients who developed a chemotherapy-induced clinical cardiotoxicity versus those who did not, thereby supporting the interest of these cells as a prediction tool in cardio-oncology [10]. Other multi-disciplinary approaches seek to demonstrate that a diverse biobank of patient- and disease-specific iPSC can be used for implementing “precision medicine” and “clinical trial in a dish” concepts [11]. These studies will likely have broad scientific and clinical impact toward understanding the molecular basis of CVD and design of better drugs.

At the tissue level, hiPSCs-derived cardiovascular cells can be used within engineered microphysiological systems for modeling of disease, testing drug therapies, and development of new regenerative strategies. As with single cell systems, fateful emulation of human physiology and pathology using “organs on a chip” microtissue systems is dependent upon their structural and functional maturity. Recent studies show that adult-like human heart muscle micro-tissues can be grown from early-stage iPSC-derived cardiomyocytes if cells are encapsulated in fibrin gel and subjected to physical conditioning of an increasing intensity (Figure 1). After only 4 weeks of culture, these tissues display adult-like gene expression profiles, remarkably organized ultrastructure with physiologic sarcomere length and density of mitochondria, and a dense network of transverse tubules (t-tubules). The training regimen causes switch to oxidative metabolism and results in positive force-frequency relationship and functional calcium handling. While this approach did not result in adult levels of functionality, it enabled physiologic responses to drugs and recapitulating disease phenotypes [12].

One important consideration for development of improved disease models pertains to the role of the extracellular matrix (ECM) in spurring cardiomyocyte maturation and cardiac tissue organization [13]. Although the soluble factors necessary for differentiation of stem and progenitor cells to cardiac cell types are well-studied, our understanding of how the cells interpret signals from the insoluble substrate – mainly composed of extracellular ECM proteins – is incomplete. Knowing the mechanistic contribution of the ECM to the dynamics of stem cell state is relevant for *in vitro* platforms for drug screening, toxicity testing, and

disease modeling and is critical for *in vivo* therapeutic strategies involving tissue and whole organ regeneration where ECM exposure is inevitable. Characterizing the pathways linking integrin engagement and activation of transcription factors associated with different stages of cardiac development, from fetal to adult, should enable better control of engineered tissues containing ECM to enhance functional maturation and augment tissue repair.

Another important consideration that is often forgotten by cardiovascular bioengineers is that the heart is not merely a collection of cardiomyocytes, but contains other cell types that are important for proper cardiac function. Chief among these are the vascular cells that compose the coronary arteries and microvasculature. In addition to providing the nutrition and oxygenation for cardiac function and cardiac repair, the vasculature communicates directly with cardiomyocytes to affect their function. For example, endothelial-derived nitric oxide reduces cardiac contractility by affecting cardiac metabolism and actin-myosin coupling [14]. Improved models of organs-on-a-chip should incorporate endothelial cells, which may also be derived from iPSCs [15].

In the post-genomic era, proteomics is the next frontier allowing an in-depth understanding and modeling of the function of cellular systems in HF and the development of personalized treatments. A comprehensive analysis of all “proteoforms” that arise from genetic variations and post-translational modifications (PTMs) is essential for gaining a transformative understanding of disease mechanisms, validating modeled disease phenotypes, and identifying new therapeutic targets. Top-down mass spectrometry (MS)-based proteomics is arguably the most powerful method to comprehensively characterize proteoforms for better understanding the underlying causes of cardiac diseases. It directly analyzes intact proteins providing a “bird’s eye view” to observe all types of modifications including PTMs (phosphorylation, acetylation, etc.) and sequence variants (mutants, alternatively spliced isoforms, amino acid polymorphisms) simultaneously in one spectrum [16, 17]. This approach has been utilized to link HF contractile dysfunction to altered sarcomeric PTMs in animal models and human clinical samples as well as to perform comprehensive assessment of hiPSC-CM maturation *in vitro* and reveal the molecular mechanism of improved cardiac function from transplanted hiPSC-CM tissue patches *in vivo*. For example, cardiac transplantation of hiPSC-derived cardiomyocytes, endothelial cells and smooth muscle cells successfully reversed the up-regulation of proteins related to fibrosis and apoptosis occurring after a myocardial infarction, thereby providing mechanistic insights into the effects of this trilineage cell transplantation [17, 18]. Parallel to the recent advancement in omics data acquisition technologies, major efforts have been made to develop computational strategies for deep-mining omics datasets. For example, a biotin switch-based proteomics approach enabled the identification of 1,655 cardiac proteins carrying oxidative stress sensitive PTMs on 3,324 Cysteine residues in a mouse model of cardiac hypertrophy [19]; moreover, computational algorithms (e.g., cubic spline-based temporal clustering) were implemented to decipher complex datasets on layers of PTM features. This approach revealed insights on how global oxidative proteomic signatures are correlated with the progression of cardiac hypertrophy. These platforms (e.g., Reactome pathway database) and workflows (e.g., machine-learning-empowered molecular signature extraction) offer supports on omics phenotyping to better understand cardiovascular disease, including iPSC biology and pathology.

Challenges to iPSC applications

The use of iPSC in disease modeling and drug development still faces important challenges. First, iPSC generation remains technically demanding, and requires the activation of an entire network of pluripotency genes, and the parallel suppression of genes enforcing the lineage of the somatic cell that is being reprogrammed. Global changes in the transcriptional profile occur in parallel with genome-wide alterations in histone proteins, noncoding RNAs, and DNA methylation. These genome wide alterations are associated with profound changes in signaling pathways, nuclear structure, metabolism and morphology of the cell that are incompletely characterized [20]. New insights into the mechanisms of nuclear reprogramming to pluripotency may enhance the efficiency and fidelity of the generation of iPSC. Recent work has shown that cell-autonomous innate immune signaling is required for nuclear reprogramming to pluripotency, for complete reprogramming of a somatic cell to another lineage, or for transformation of cell to a substantively new phenotype (e.g. senescent to juvenile state) [21]. When pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) are detected by pattern recognition receptors (PRRs), an inflammatory signaling cascade is set into motion that results in the generation of inflammatory cytokines. This same signaling cascade, mediated by transcriptional factors, such as NF_Kb, IRF3 and IRF7, also sets in motion a series of events that increase epigenetic plasticity including altered expression and activity of key epigenetic enzymes and complexes, in part stimulated by a switch from oxidative to glycolytic metabolism. The result defined three major epigenetic processes that are activated by innate immune signaling, which include changes in the expression of key epigenetic enzymes are observed including an increase in the expression of histone acetyltransferases (HATs), and a reduction in histone deacetylases (HDACs). Importantly, there is a “Goldilock’s zone” for innate immune signaling whereby too little, or too much, activation of innate immunity, reduces DNA accessibility and impairs cellular reprogramming. These findings provide a mechanistic foundation for therapeutic manipulation of innate immune signaling toward improved reprogramming and regenerative therapies [22].

A second challenge, specific for human PSC-derived cardiomyocytes (hPSC-CMs), is that they do not faithfully recapitulate all the structural and functional attributes of their native adult counterparts. While new small molecules, identified by high-throughput screening, and mechanical/electrical stimulation and 3D cell culture can enhance maturation of the differentiated cardiomyocytes, the efficiency of this process would likely benefit from a better understanding of early fetal and neonatal human heart development. In this regard, some important areas of research involve understanding of: (1) Stress-kinase signaling in left vs. right ventricle asymmetric remodeling in neonatal heart, (2) RNA processing, including targeted alternative splicing and degradation in acquiring morphological and functional maturation, and (3) The potentially important roles of long non coding (lnc) RNAs in neonatal cardiomyocyte maturation exemplified by the finding that transcriptomic analyses of neonatal hearts have identified the ratio between one of these lncRNA and its partner gene as a marker for segregating distinct phenotypes of congenital heart defects [23]. Furthermore, studying transcriptional regulation pathways that orchestrate heart development and cardiomyocyte maturation can also yield unanticipated links with some of the key processes which underlie HF, such as neurohormonal signaling. This is illustrated by the finding that

G-protein receptor kinases (GRKs) which mediate desensitization and downregulation of β-adrenergic receptors implicated in adult HF, also redundantly modulate Smoothened-GATA transcriptional crosstalk in fetal mouse hearts with a relationship between GRK gene ablation and atrio-ventricular canal abnormalities [24, 25].

Thus far, much has been already learned about cells intended for cardiac repair. However, to promote clinical applications of hPSCs, additional studies are required to (1) Fully characterize the phenotype and secretome of derived cells and establish appropriate quality controls, and (2) Understand the precise mechanisms underlying the therapeutic effects of hPSC derivatives and their secretome. Furthermore, animal studies are still necessary prior to clinical evaluation of cardiovascular cell therapies. Small animal models are commonly used in cardiovascular research because of their many advantages over large animal models including a short life span that allows the investigators to follow the natural history of the disease at an accelerated pace. Also, the use of genetically modified animals allows for rigorous mechanistic studies that can be further validated in larger animal models [26]. These *in vivo* studies are not only mandatory to test the safety and efficacy of stem cells but also to assess how bioengineering can optimize outcomes through improvement of cell survival and engraftment and/or long-lasting release of cell-secreted factors (e.g. exosomes).

Cell Therapy of Ischemic Heart Disease

One advantage of using human pluripotent stem cells for cardiac repair is the ability to produce theoretically unlimited quantities of cells (of any lineage) and generate both cardiovascular progenitor cells (hPSC-CVPC) and more mature cardiomyocytes. Other cell sources for cardiac repair, derived from adult tissues, are also being explored in addition to hPSC. Among them, human mesenchymal stem cells (hMSCs) from the bone marrow and adipose tissue are the most commonly studied. However, despite intensive research, supplying therapeutically competent hMSCs for clinical applications remains a challenge. Originally isolated and expanded as plastic adherent cells, hMSCs can undergo *in vitro* self-assembly into three-dimensional (3D) aggregates. Recent studies have shown that hMSC 3D aggregation improves a range of biological properties, including stemness and multilineage potential, secretion of therapeutic factors, and resistance against ischemic condition [27]. The metabolic reconfiguration towards aerobic glycolysis underpins 3D aggregation-mediated hMSC functional activation [28]. When transplanted *in vivo*, aggregate-derived hMSC have extended survival that is accompanied by improvement of LV function [29–31].

As with hPSC-CVPC, key attribute of the hMSC regenerative potential is their secretion of trophic factors (including extracellular vesicles, EV) that forms a pro-regenerative “milieu” through modulation of immune responses and promotion of angiogenesis and tissue regeneration. Potential use of MSC- or CVPC-derived EV in clinics will require their characterization for the most functionally effective fraction (exosomes, microparticles), if any; the best method for purifying this fraction under Good Manufacturing Production (GMP) conditions; the development of potency assays usable as quality controls for ensuring the reproducibility of batches and last, but not least, the optimal modality for vesicle delivery. It is unlikely that a “flush-and-go” type of approach can achieve sustained therapeutic benefits because of leakage of the injectate through the venous and lymphatic

systems and/or the pericardial cavity (in case of direct intramyocardial administrations). Here again, tissue engineering merges with cell biology in that biomaterials could be used as effective controlled-release carriers. The concept is illustrated by the capacity of an engineered hydrogel patch to slowly release EV secreted by hiPSC-CMs in a rat model of acute MI, resulting in reduced infarct size, cardiomyocyte apoptosis and hypertrophy, arrhythmic burden, and improved cardiac function 4 weeks post-infarction [32]. The hiPSC-CM secreted EVs were shown to be enriched with cardiac-specific miRNAs, which might account for these beneficial outcomes.

Interestingly, the first cell type which has entered the clinical arena, i.e., skeletal myoblast, is also currently the only one which has gained approval from the Japanese regulatory authorities for marketing under the form of the Heartsheet® product and this treatment is now covered by Japanese health insurance. The product is made of autologous myoblasts cultured onto temperature-sensitive polymers which, upon lowering of the temperature, detach as a scaffold-free sheet of cells whose cohesiveness is ensured by their self-secreted matrix and which can then be delivered (eventually after stacking several sheets one on top of the other) onto the epicardium. The approval was based on the results of a phase I clinical trial entailing implantation of myoblast cell sheets in HF patients with ischemic or idiopathic dilated cardiomyopathy. This prospective, single arm and non-randomized study demonstrated that, in the patients with ischemic cardiomyopathy, their cardiac function, symptoms and tolerance for exercise were significantly improved [33]. Several preclinical studies have shown that the mechanism and advantages of this treatment are to maximize paracrine effects such as cytokine-mediated angiogenesis and anti-fibrosis. Interestingly, while the early myoblast experience had been clouded by the occurrence of arrhythmias, such was not the case in the above mentioned clinical trial, possibly because intramyocardial injections were replaced by the cell sheet delivery mode. Nevertheless, the arrhythmic risk remains as an ongoing concern for cardiac cell therapies [34]. As a final glimpse into a near future, the cell-sheet technique has also been utilized for regenerative therapy with iPS-derived cardiomyocytes in a porcine heart failure model [35], thereby laying the grounds for an upcoming clinical trial.

The persisting challenge of low cell engraftment

A major roadblock that still hampers the efficacy of cell therapies is the low rate of cell retention which occurs with virtually all types of cells studied heretofore [36–38]. Such an engraftment, however, is mandatory even if one assumes a predominant paracrine mechanism of action since cells have at least to be transiently present to have enough time for releasing the factors underpinning their benefits. This issue has been tackled for a long time [39] and, broadly speaking, cell survival and retention enhancing strategies have primarily involved cell preconditioning, genetic cell engineering, and cell scaffolding. Several recent approaches to address this challenge are outlined below.

Enhanced proliferation of transplanted cells

Lentiviral overexpression of human CCND2 (gene encoding Cyclin D2, a cell-cycle activator that regulates G1-S transition) in hiPSC-CMs, was recently shown [40] to increase the graft size and improve myocardial recovery in a mouse model of myocardial infarction

by increasing the proliferation of grafted cells (Figure 2A). In vitro, markers for cell-cycle activation and proliferation were ~3–7 fold higher in CCND2-overexpressing hiPSC-CMs (hiPSC-CCND2^{OE}CMs) than in hiPSC-CMs with normal levels of CCND2 (hiPSC-CCND2^{WT}CMs). In the mouse MI model, cardiac function, infarct size, and the number of engrafted hiPSC-CCND2^{OE}CMs and hiPSC-CCND2^{WT}CMs were similar one week after treatment; however, at 4 weeks of treatment, CCD2 overexpression yielded a three-fold increase in cell engraftment and enhanced improvement in cardiac function and infarct size. No tumor formation was observed in any hearts. Thus, CCND2 overexpression prevented the cell-cycle exit in implanted hiPSC-CMs leading to a more efficient myocardial repair as evidenced by enhanced remuscularization of injured myocardium, increased angiogenesis in border zone, and improved LV chamber function. This proof-of-concept study warrants future developments to precisely control the increase in graft size and mitigate potential arrhythmogenic risks.

Manipulation of the immune system

The harsh inflammatory milieu intrinsic to acutely infarcted or chronically failing hearts may present a formidable roadblock to cell-based cardiac repair. Fundamentally, the tissue immune cell profile is a prime determinant of the local inflammatory response. Hence, a better understanding of the immune cell activity in the infarcted and failing heart is of critical importance for understanding whether and how immunomodulatory strategies can promote cardiac repair. Murine hearts with ischemic cardiomyopathy 8 weeks after large myocardial infarction exhibit robust expansion of: 1) Pro-inflammatory ‘M1’ type macrophages, 2) Classical and plasmacytoid dendritic cells (DCs), and 3) $CD8^+$ and $CD4^+$ T-cells. In addition, mice with ischemic HF exhibit increased circulating pro-inflammatory monocytes, classical DCs, and $CD8^+$ and $CD4^+$ T-cells; profound splenic remodeling indicative of heightened antigen processing; and expanded splenic antigen-experienced effector and memory $CD4^+$ T cells. A series of studies in HF mice, incorporating splenectomy, adoptive transfer of unselected splenocytes and splenic $CD4^+$ T-cells, and antibody-mediated $CD4^+$ T-cell depletion, indicated that splenic immune cells: 1) Underlie the chronic inflammatory response in HF, 2) Traffic and home to the failing heart, and 3) Exhibit immune memory and are primed to induce tissue injury that promotes pathological cardiac remodeling. These findings suggest that ischemic cardiomyopathy is in part an immune-mediated disease, with a central role for the spleen. Furthermore, recent work has suggested that the reparative effects of intravenously administered hMSC were related primarily to immunomodulatory effects, independent of cell engraftment in the heart [41]. Hence, targeting immune cell populations, particularly in the spleen, may allow immunomodulation in HF to aid the effectiveness of cardiac cell therapies [42].

Control of cell rejection

In addition to inflammation, another contributor to low cell survival and graft attrition is immune rejection when transplanted cells are of an allogeneic origin, an increasing trend given their advantages regarding product consistency and streamlining of transplantation logistics. Immune rejection of transplanted cells could be addressed using immunosuppressive drugs, the side effects of which are well documented, and possibly, in the future, the use of Human Leukocyte Antigen (HLA)-haplotyped iPSC lines or even

universal iPSC lines[43] where the side-effect of β2-globulin knock-down to eliminate surface expression of class I antigens (Figure 2B[43]), i.e., an increased susceptibility to damage by Natural Killer cells, is handled by a forced expression of HLA-E [44]. There are some significant progress recently in efforts to engineer "universal" hiPSC cell lines[43] to avoid the need for concomitant immunosuppressive therapy. In line with the idea of inducing a state of immune tolerance, an alternative strategy to allogeneic human cell transplantation is xenotransplantation. The general objective is to humanize the organ of interest in pig so it will be an acceptable donor graft for the patient [45]. As one of the challenges associated with xenotransplantation is organ rejection triggered by donor endothelial cells, a novel strategy is being pursued to generate pigs with humanized vascular cells. This approach could be used as a universal platform for exogenic organ production by reducing immunological rejection [46]. So far, the only clinical application of xenotransplantation in the field of cardiac repair is the endoventricular injection of a decellularized extracellular porcine myocardial matrix. The outcomes of this trial (NCT02305602) will reveal if this strategy is worth a further pursuit considering that decellularized porcine pulmonary arteries were previously shown to induce a strong immune cell response *in vitro* [47].

Modulating Cardiac Metabolism

Recent advances in metabolomics have provided evidence for accumulation of cardiac metabolites that may activate signal transduction pathways that impact cellular proliferation and survival [48, 49]. Many of these metabolites can be released from the failing myocardium and could potentially impact the viability of implanted cells or the extracellular matrix. Although some studies have examined the potential effects of metabolic modulation on the recovery of the failing myocardium, analysis of the relationship between metabolic modulation and engraftment of cardiac progenitor cells remains an important future direction.

Tackling the "reductive stress"

Over the last 6 decades, numerous studies have reported that enhanced oxygen-derived free radicals trigger injury in several human diseases, including cardiovascular complications supporting the theory of oxidative stress. However, this view may have to be somewhat revisited in light of the discovery of the role of "Reductive Stress (RS)" [50] whereby sustained activation of pathways that facilitate the constant generation of reducing equivalents (i.e. GSH, NADPH), hence resulting in RS, impair the basal cellular signaling mechanisms operating through harmless pro-oxidative events. This, in turn, may disrupt single and/or a combination of key processes such as cell growth, maturation, differentiation, and survival. While the possible clinical relevance of these findings is suggested by the fact that RS may be implicated in the pathogenesis of HF in a subset of cardiac patients [51], manipulation of the RS for improving survival of transplanted cells still remains elusive at this stage.

Use of biomaterials for cell delivery

Biomaterials have also been used to overcome the low engraftment of injected cells by addressing physical damage to cells during injections, lack of cell-matrix attachment, and hypovascularization of the target areas. For example, cell encapsulation in an injectable

thermoresponsive hydrogel served to boost cell retention and attenuate immune reaction [52]. Platelet binding molecules or whole platelet membranes have been used to enhance adherence of vascularly injected cells to the injured endothelium [53]. Furthermore, cell mimicking microparticles or synthetic stem cells have been developed to overcome some of the drawbacks associated with natural stem cells, including poor storage/shipping stability [54]. To address hypovascularization at the site of cell transplantation, human iPSCs were differentiated into endothelial cells (ECs) within a three-dimensional (3D) fibrin scaffold [54]. By modulating both p38MAPK and MEKERK1/2 signaling, EC differentiation efficiency could be dramatically increased to >85%. Similarly, Etv2 has been identified as a master regulator of the endothelial lineage [55] with Etv2-miR130a-Jarid2 cascade regulating vasculogenesis and vascular patterning without impacting the hematopoietic lineages [56]. Forced overexpression of Etv2 promoted reprogramming of differentiated cell populations to an endothelial fate yielding the functional improvement of injured organs. Notably, the use of biomaterials to organize and deliver cells in a form of an *in vitro* engineered cardiac tissue patch holds promise to further localize delivered cells and concentrate their paracrine action to the site of injury. Recent studies have demonstrated generation of highly functional hiPSC-derived cardiac tissue patches with a clinically relevant size (up to 4cmx4cm). With absolute and specific forces of ~20mN and ~25mN/mm^2, respectively, and velocity of action potential propagation of ~30cm/s, these tissues have started to approach functional metrics of the native human heart muscle [57]. In an independent study, paracrine actions of large patches implanted in a porcine MI model yielded improved left ventricular function, wall stress, and infarct size by reducing apoptosis and normalizing phosphorylation of sarcomeric regulatory proteins in host cardiomyocytes. Still, the lack of electromechanical integration between the patch and recipient heart remains an important challenge to overcome in the field [57, 58].

Repeated cell delivery

While the single administration of any drug is unlikely to achieve sustained therapeutic benefits, cell therapies have been performed as a one-shot delivery modality in almost all preclinical and clinical studies heretofore. It is thus reasonable to assume that the therapeutic benefit of transplanted cells could be optimized by repeated cell administrations. Indeed, using a rat [59] and a mouse [60] model of chronic ischemic cardiomyopathy and two different cell types (c-kit$^+$ cardiac progenitor cells and cardiac mesenchymal cells (MSCs), it has been found that repeated cell doses are markedly more effective than a single dose. The mechanisms involved are again thought to be paracrine, potentially including anti-fibrotic and anti-inflammatory actions of transplanted cells [59, 60]. It could be argued that the superiority of multiple doses [59, 60] was due to the greater total number of cells given rather than repeated treatments. However, the cumulative effects of repeated cell doses on LV function, myocardial fibrosis, and myocardial infiltration by inflammatory cells were not recapitulated by a single equivalent dose [61], suggesting that the duration of myocardial exposure to the transplanted cells is more important than the intensity of such exposure. This concept of repeated dosing should likely be considered in the design of future clinical protocols.

In conclusion, the field of cardiovascular bioengineering has made great strides in use of iPSC technology for modeling congenital heart disease and transplanting functional cardiac muscle to the injured heart. A recurrent question posed at the symposium was, “What next?” and presentations from attendees suggested development of *in vitro* methodologies to: 1) accelerate functional maturation of iPSC-derived cardiomyocytes, 2) design realistic heart microtissues containing most, if not all, cells present in the native myocardium, including a functional vasculature, and 3) mimic cardiac injury and scarring, as the important next steps to enable *in vitro* modeling of acquired adult heart diseases such as MI and HF. Furthermore, increase in transplanted tissue volume, functional integration with recipient heart, and minimally invasive methods to deliver engineered tissues on the heart surface were considered to be critical future steps in making cardiac patch therapies a clinical reality. Importantly, symposium attendees agreed that understanding the effects of transplanted cells or cell derivatives on the host immune system and the possibility to manipulate immune cells to enhance regenerative, cardioprotective, and functional benefits of therapy has the potential to improve all cell-based strategies for treatment of ischemic heart disease. Finally, moving products of cardiovascular bioengineering to the clinical and commercial sector will require the development of scientifically robust, reproducible, and cost effective technologies, a feat that is only achievable through organic collaboration of basic scientists, engineers, and clinicians.

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Abbreviations

HF	Heart failure
hPSCs	Human pluripotent stem cells
hiPSCs	Human induced pluripotent stem cells
hPSC-CMs	Human pluripotent stem cells-derived cardiomyocytes
hPSC-ECs	Human pluripotent stem cells-derived endothelial cells
hPSC-SMCs	Human pluripotent stem cells-derived smooth muscle cells
hPSC-CVPCs	Human pluripotent stem cells-derived cardiovascular progenitor cells
hMSCs	Human mesenchymal stem cells
CVD	Cardiovascular disease
ECM	Extracellular matrix
MS	Mass spectrometry

HLA Human Leukocyte Antigen

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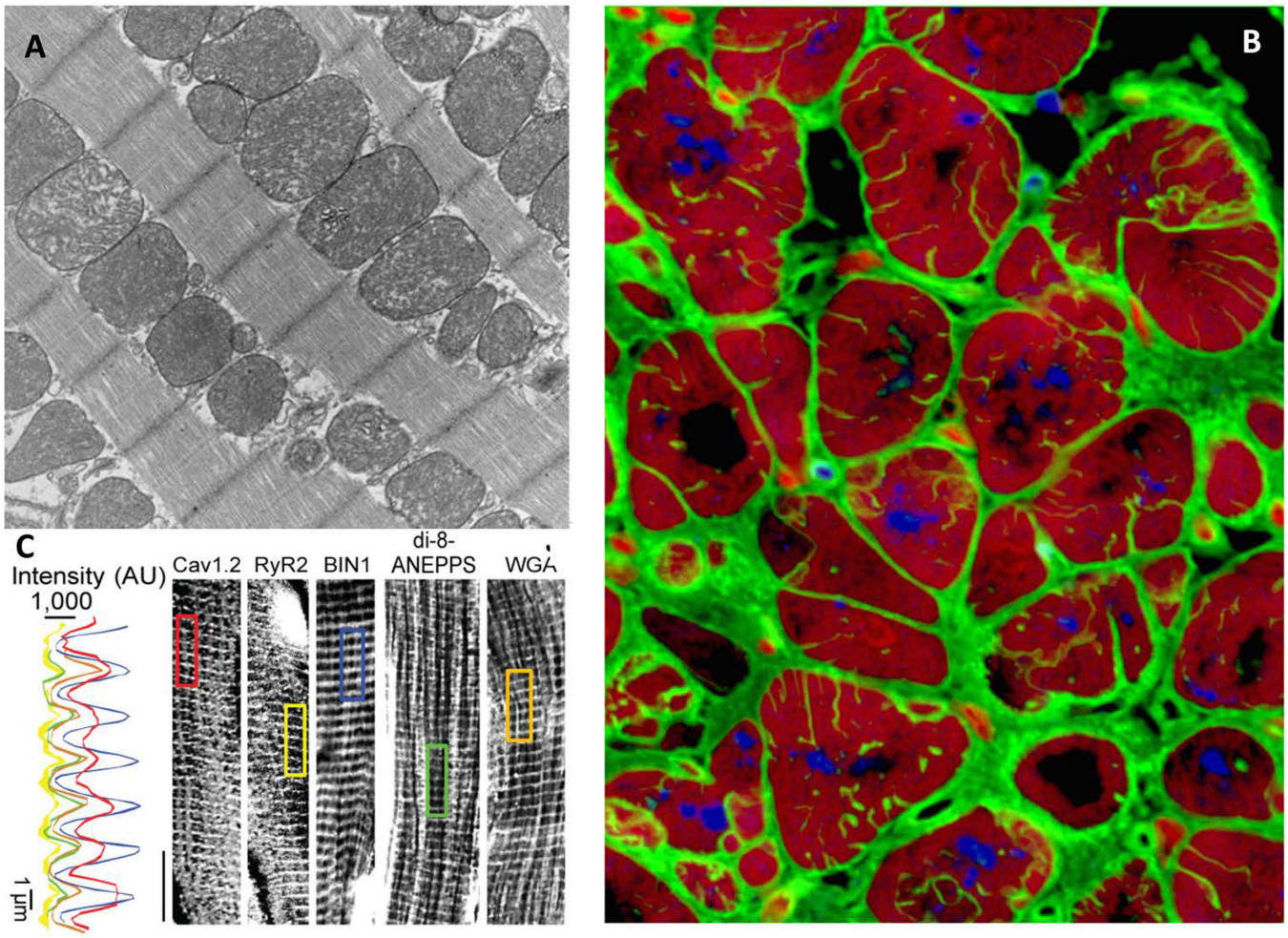

Figure 1. Adult-like human heart muscle formed from iPS cells.

(A) Transmission electron microscopy shows registers of sarcomeres and abundant mitochondria (measured to be present at physiologic density). **(B)** Muscle cross-section shows dense networks of transverse tubules (T-tubules, green, WGA; red, cardiac troponin T; blue, nuclei). **(C)** T-tubules (measured using WGA and di-8-ANEPPS were co-localized with the bridging integrator 1 (BIN1), ryanodine receptor 2 (RYR2), and L-type calcium channels (CaV1.2, encoded by CACNA1C) with spacing optimized for calcium handling. The presence of ultrastructural machinery for contraction–relaxation was confirmed by the positioning of T-tubules in proximity to the cardiac calcium pump SERCA2A and the sodium–calcium exchanger NCX1.

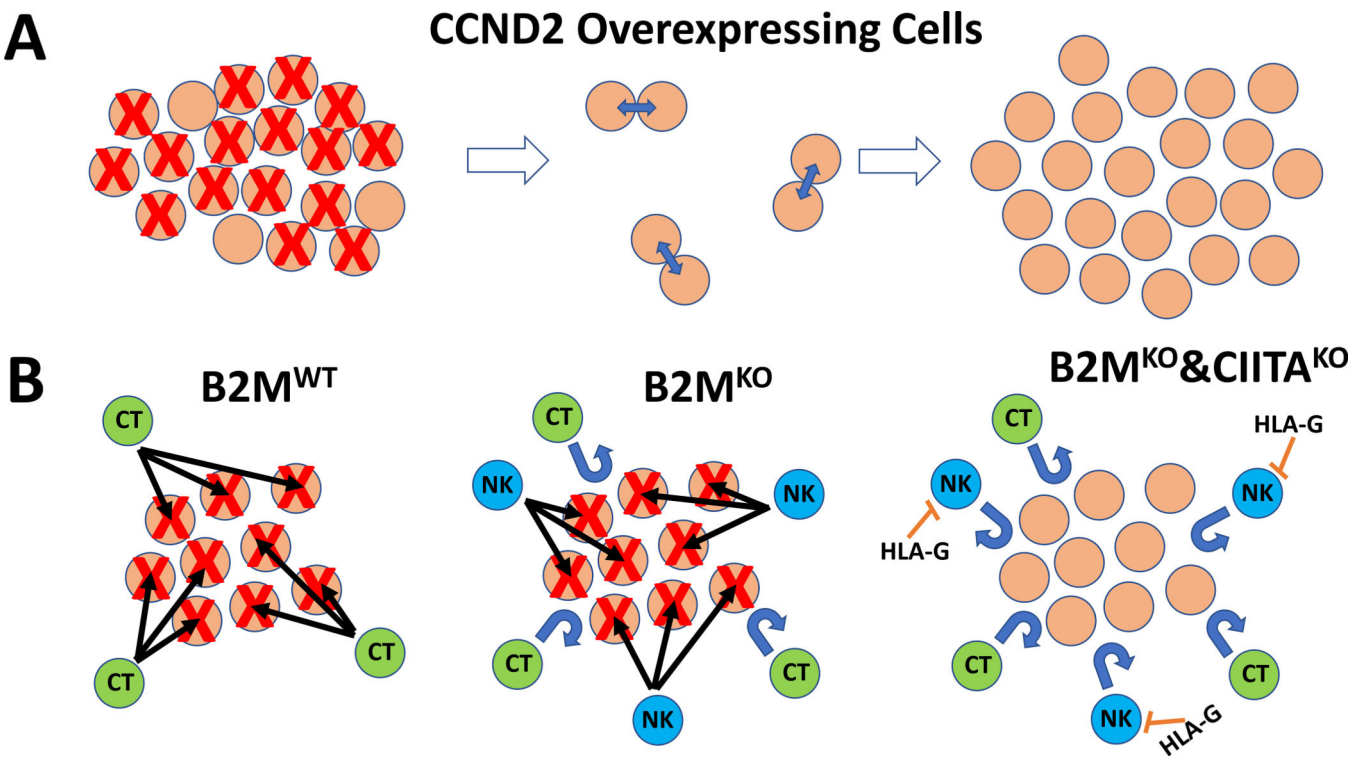

Figure 2. Novel strategies to enhance graft size.

(A) Recent experiments in a mouse MI model indicate that both graft sizes and the reparative potency of transplanted human iPSC-CMs can be significantly improved via the lentiviral overexpression of α-MHC driven human CCND2. Although the initial engraftment of CCND2-overexpressing iPSC-CMs may have been low, the surviving cells proliferated and likely replaced at least some of the lost myocardial tissue. These observations support the feasibility of this strategy for remuscularizing infarcted hearts and the development of techniques for controlling graft size and mitigating the potential risk of arrhythmia**. (B)** Efforts to engineer "universal" hiPSC cell lines[43], and to avoid the need for concomitant immunosuppressive therapy include the development of human leukocyte antigen (HLA)-haplotyped iPSCs or universal iPSC lines coupled with the forced expression of HLA-E, which reduces susceptibility to natural killer cells by overcoming the "missing self" response (a side-effect of β2-globulin knock-down).