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Induced Pluripotent Stem Cell Cardiomyocytes Provide an In Vitro Model of Heart Disease and a Potential Source of Cell Replacement

Ischemic heart disease remains a major cause of death and disability worldwide (Moran Andrew E. et al., 2014). While interventions for heart disease have come a long way, restoring functionality to damaged myocardium remains a challenge. Both mechanical and electrical repair of the host myocardium are essential to salvage the damaged tissue. This is especially difficult following a myocardial infarction (MI) as the infarct area is subject to quick and extensive cell death via necrosis then apoptosis (Prabhu & Frangogiannis, 2016). Stem cells have emerged as a candidate for potential replacement therapies. Adult stem cells (ASCs) are taken from bone marrow or adipose tissue. Induced Pluripotent Stem Cells (iPSCs) are cells that have been reverse engineered into a pluripotent state. Both ASCs and iPSCs have proven preclinical usefulness in treating heart disease (Gao et al., 2007; Guo et al., 2020). It is theorized that ASCs act primarily through paracrine signaling while iPSCs may have a greater capacity to differentiate into cardiac myocytes (CMs) *in vivo* (Guo et al., 2020). Additionally, iPSCs can differentiate into CMs (iPSC-CMs) *in vitro*, making them a useful cellular model for heart disease (Karakikes et al., 2015; Smith et al., 2017). This profile will highlight the uses of iPSCs as they pertain to modeling and treating MI. Attention will be paid to stem coupling, electrophysiology, and experimental designs that investigate the role of gap junctions in stem cell therapies.

Background

Myocardial Infarction

MI occurs when a coronary artery becomes occluded and leads to tissue stress and possibly death. MI is especially deadly in humans as there is little redundancy in cardiac arterial

networks. The region of myocardium supplied by the affected coronary artery becomes ischemic, loosing essential access to oxygen and nutrients. The CMs immediately affected become necrotized and irreversibly damaged. In response to this stress, the surrounding cardiac myocytes release pro-apoptotic factors leading to massive but possibly preventable cell death and dysfunction. Calcium ions (Ca^{2+}) are a potential apoptotic factor mediating this preventable cell death. Under normal conditions, Ca^{2+} ions aid in the synchronization of cardiac action potentials. However, excess intracellular accumulation of Ca^{2+} ions has been shown to trigger both mechanical and electrical dysfunction of CMs. These Ca^{2+} ions travel primarily through Connexin-43 (Cx43) based gap junctions (Sridhar et al., 2020). This implicates Cx43 as a potential therapeutic target in the prevention of deadly post MI pathologies such as heart failure and arrythmias.

Stem Cell Therapy

Stem cell therapies attempt to fully replace dead or dysfunctional tissue. Originally therapies sought to generate functionally identical patches of replacement tissue from exogenous cell sources. However new paradigms, specifically in cardiology, emphasize the role of paracrine factors in aiding the natural remodeling process of the affect heart. Bone marrow derived mesenchymal stem cells (MSCs) have been shown to aid in the repair of damaged myocardium through the release of protective paracrine factors such as insulin-like growth factor-1 (IGF-1) and VEGF (Lin et al., 2020; Zisa et al., 2009). Additionally MSCs have been shown to express Cx43 and potentially protect against arrythmias (Mureli et al., 2013). This suggests early cell communication between MSCs and CMs is essential to developing an effective therapy. CMs like many primary cell lines, have an incredibly limited lifespan in culture. This makes *in vitro* research especially difficult. iPSCs were originally developed as potential source of cells for

direct replacement. However, they have also proven to successfully model CMs *in vitro* (Smith et al., 2017).

Modeling MI and Therapies with Stem Cells

In Vitro Physiology

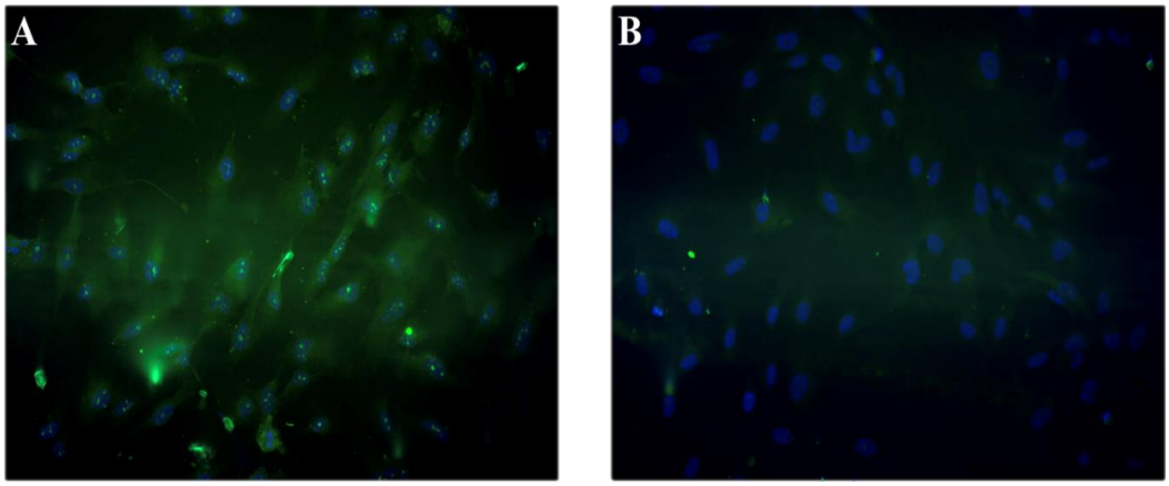
iPSCs were originally developed as an alternative to embryonic stem cells. The first iPSCs were made by reprogramming skin fibroblasts using transcription factors (Glicksman, 2018). They are pluripotent, able to differentiate into any cell in the human body but not totipotent, embryonic in origin. iPSC-CMs are created when a stromal cell is reprogrammed to a pluripotent state and then driven down the developmental pathway of CMs. This is accomplished via selective induction of gene expression. One method to drive a reprogrammed fibroblast down a CM pathway is to subject it to growth factors such as activin-A and bone morphogenetic protein 4 (BMP-4). This mimics the effects of the Activin/Nodal and BMP4 signaling pathways that embryonic cells would experience when differentiating into mesodermal tissues such as myocardium (Kattman et al., 2011). Other pathways that have been exploited to create iPSC-CMs include the transforming growth factor-β (TGF-β) and Wnt pathways (Karakikes et al., 2015). When fully differentiated iPSC-CMs demonstrate high homology with CMs, expressing genes (MYL2, MYL7, MYH6, and TNNT2) and all functional ion channels (sodium, potassium, and calcium) associated with terminal cardiac differentiation (Karakikes et al., 2015). In addition, fully differentiated iPSC-CMs display transverse tubules, sarcomeres, and similar mitochondrial densities characteristic of adult CMs. These findings are functionally supported by iPSC-CMs ability to spontaneously contract *in vitro* (Ronaldson-Bouchard et al., 2018).

Gap Junctions

Of particular interest in models of MI are gap junction channels. Gap junctions are responsible for synchronous contraction via rapid ion transfer in the myocardium. Gap junctions are composed of various proteins known as Connexins (Cx). Connexin 43 (Cx43) is the dominate protein in ventricular CMs, and is implicated in both proper and pathological functioning of the ventricles (Schulz et al., 2015). For this reason, Cx43 dysregulation, is likely implicated in decreased cardiac out following heart failure and arrythmia following MI. iPSC-CMs have been shown to express Cx43 and form Cx43 gap junctions with other cells, further validating their use as a model for MI (Biendarra-Tiegs et al., 2020). MSCs have also been shown to express Cx43 (Mureli et al., 2013). Taken together these findings suggest that co-cultures of iPSC-CMs and MSCs can form cardiac gap junctions. This could be used as a model to investigate early stem cell coupling and electrophysiological integration.

Figure 1

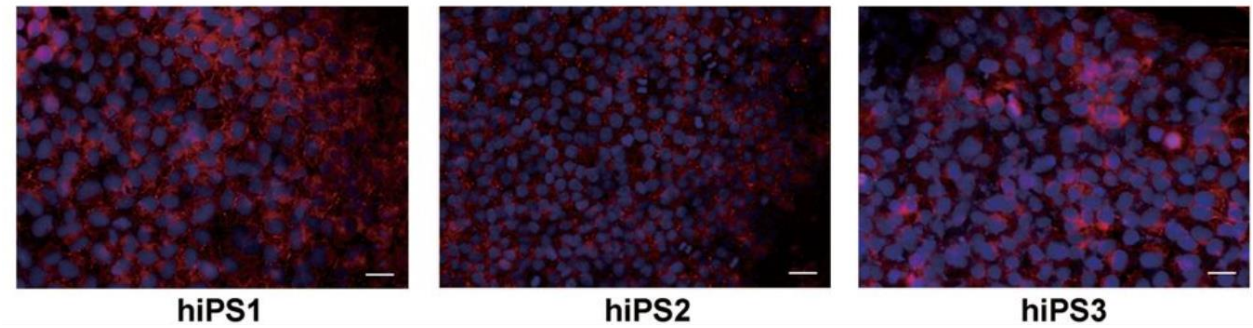
Human MSCs Express Cx43



Note. (A) Cx43 expression (green) in the hBM-MSC, (B) Negative controls. DAPI nuclear stain is represented in blue for both A and B (Tietema & Geenen, 2019).

Figure 2

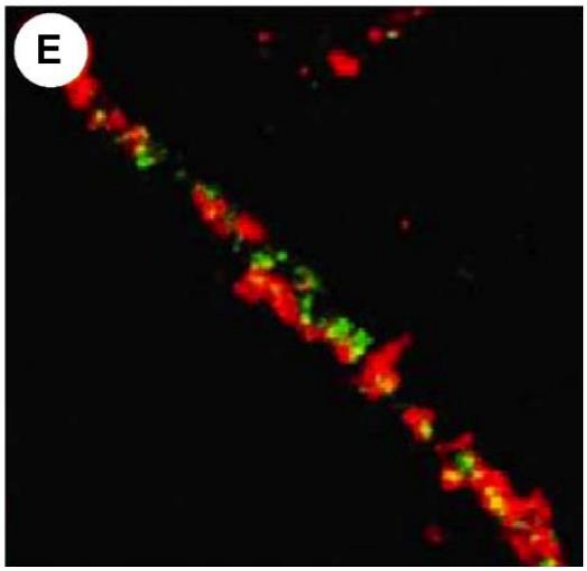
Human iPSCs Express Cx43



Note. Cx43 expression (red) in iPSCs derived from embryonic fibroblasts. Nuclei are shown with blue DAPI staining (Ke et al., 2013).

Figure 3

Human CMs express Cx43



Note. Human left ventricular CMs expressing Cx43 (red) gap junctions localized along an intercalated disc. $P2X_1$ receptors stained green (Jiang et al., 2005).

Genetic Manipulation

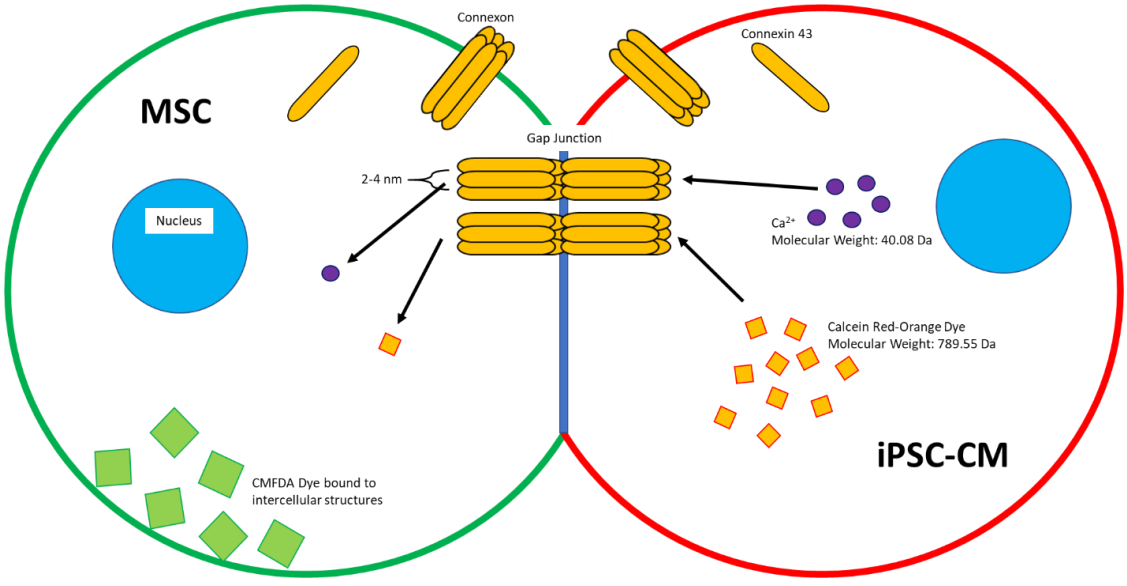
Genetic manipulation is a powerful tool to study gain or loss of function in cell models of diseases. iPSC-CM alone are capable of directly modeling arrythmia, particularly genetically acquired ion channel deficiencies (Smith et al., 2017). iPSC-CM have been shown to be sensitive

to genetic manipulation through lentiviral transfection leading to overexpression of a desired gene (Gorabi et al., 2019). In the case of MSCs, similar lentiviral techniques have proven effective in upregulating transcription of target genes (Fan et al., 2019). It is theoretically possible to upregulate Cx43 through similar manners in order to further study the role of gap junctions in stem cell therapies for MI. Other methods of upregulating Cx43 in non-CMs have been reported, including exposure to eicosapentaenoic acids, coleusin factor, and vascular endothelial growth factor (VEGF) (Geng et al., 2007; Li et al., 2017; Yang et al., 2019). Further investigation of these factors could lead to the development of a chemical cocktail that promotes increased engraftment and electrical integration between MSCs and MI modeling iPSC-CMs. VEGF in particular, is an appealing target in the treatment of MI as it is has been shown to increase vascularity, improved left ventricular ejection fraction, and aid in stem cell retention in pre-clinical treatments of MI (Matsumoto Ryo et al., 2005; Pons et al., 2008).

Experiments of Interest

Figure 4

Intercellular Traffic Through Cx43 Gap Junctions



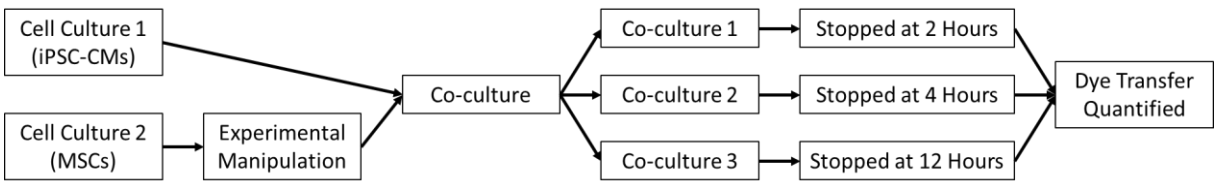
Note. Gap junctions, allow specific classes of molecules to transfer between cells. This enables impermeable dyes to label cells and permeable dyes to mimic Ca^{2+}transfer.

Coupling of iPSC-CMs and MSCs

One potential way to investigate the properties of stem cells is through co-culture experiments. Co-cultures can be accomplished by combining two cell cultures from the same cell line (i.e. MSCs and MSCs) or two cell cultures from different lines (i.e. iPSC-CMs and MSCs). The iPSC-CMs, MSC co-culture model is especially interesting because it would allow researchers to investigate early cell coupling following the introduction of MSCs into a myocardial environment. Transfer of potentially pro-apoptotic factors such as Ca^{2+} ions through Cx43 gap junctions could be measured using dye-transfer experiments. In a dye-transfer experiment, the cell culture modeling the host, in this case the iPSC-CM, is represented in at a greater density and marked with a transferable dye. The culture modeling transplanted cells, in this case the MSCs, would be identified with an untransferable marker and represented at a lesser density. This untransferable marker could either be a membrane impermeable dye or a fluorescent cell surface tag. The cells cultures would then be introduced to one another and dye transfer into the transplanted cells would be measured over time. Other outcomes of interest include impact on spontaneous contractions, transplanted cell retention, and potential transfer of membranes or other structures between cells.

Figure 5

Cell Coupling Experimental Diagram

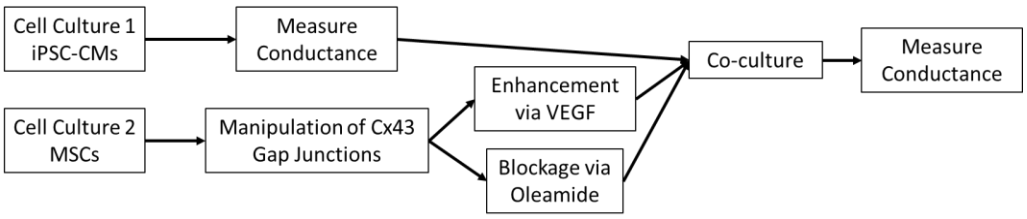


Electrical Conduction Through Co-cultures

Electrical conduction in transplanted stem cell models is of particular interest due to their potential to correct or create arrythmias. Conduction through cultured myocytes can be measured through optical mapping of fluorescent voltage sensitive dyes. This technique has been able to measure transmembrane action potentials in neonatal rat ventricular myocytes. This is especially relevant as it demonstrated upregulation of Cx43 gap junctions was directly implicated in an increase in conduction velocity (Darrow Bruce J. et al., 1996). In theory this could be used to measure action potential length and conduction velocity in an iPSC-CM culture before and after co-culture with MSCs. Other experimental conditions of interest include co-culture of MSCs exposed to either gap junction inhibitors, such as oleamide, or a gap junction promoter, such as VEGF. These experiments would provide valuable insight into how and when stem cells electrically integrate into the heart and affect arrythmias. Proper electrical integration is essential for functional cell replacement, therefore having the ability to slow down or speed up the ultimate conduction velocity through transplanted stem cells is necessary to maximize therapeutic benefit. There are also gaps in our understanding of the relationship between VEGF and Cx43 gap junctions. While there is strong evidence that VEGF increases the expression of Cx43, theoretically an abundance of Cx43 gap junctions may decrease VEGF through negative feedback. Little information on this topic exists, especially in the context of stem cells, justifying the need for these types of experiments.

Figure 6

Electrical Conductance Experimental Diagram



Conclusion

iPSC-CMs have clear usefulness in the *in vitro* study of MI. They reliably replicate the physiology of adult CMs. Their controlled differentiations make them a well-characterized model for studying genetic manipulation. The presence of functional Cx43 gap junctions in their membrane makes them uniquely qualified to investigate the electrophysiological properties of potential CM replacement candidates, including MSCs. Investigation of iPSC-CMs and MSCs through the proposed experiments could offer a greater understanding of the cellular environment transplanted stem cells experience leading to the optimization of MSC therapies.

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