

Cell Therapy and Methods of Stem Cell Delivery for Regeneration of Heart Tissue Following Myocardial Infarction

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Abstract

Myocardial infarction (MI) results in irreversible loss of cardiomyocytes (CMs) and can often lead to heart failure. Due to the minimal regeneration capacity of the myocardium, novel therapeutic techniques are needed. Cell therapy has emerged as a promising treatment for MI and involves the introduction of stem cells into the infarct area where they can proliferate and regenerate functional heart tissue. These cells can be delivered by various methods, including direct injection, scaffold formation, and cell sheet preparation. Of these, cell sheet technology appears to hold the most promise as it allows for maximal cell engraftment and retention without significant harmful side effects. Furthermore, the best composition of the cell sheet has since been debated. Cell sheets composed of skeletal myoblasts (SMs), mesenchymal cells (MSCs), and pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have proven to be of the greatest interest for engraftment into infarcted myocardium. Due to their vast differentiation and proliferation potential, pluripotent stem cells show a unique ability to readily regenerate functional myocardium following transplantation. iPSCs express the same pluripotency and induce similar effects *in vivo* as human ESCs, while circumventing certain sourcing problems and controversies, making them a favorable alternative. Identification of an effective combination cell delivery method that allows for prolonged improvement in heart function while decreasing rates of cell death would represent a major advancement in stem cell therapy and the clinical treatment of MI.

Keywords

Stem cell; Delivery method; Cell sheet technology; Myocardial infarction

Introduction

Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide and is the main cause of heart failure. MI can be caused by obstruction of coronary arteries leading to a reduced supply of blood flow to the heart muscle. Ischemia of heart tissue results in cardiomyocyte (CM) death, representing an irreversible injury. The formation of fibrous scar tissue in the damaged area and associated dilation of the left ventricular (LV) cavity disrupts the normal functioning of the heart and can increase risk of rupture, conduction abnormalities, or sudden cardiac death. Standard therapies are largely palliative measures, aiming to reduce remodeling and mitigate the risk of further complications [1]. The human heart has little to no regenerative capacity and the only current procedure able to address the problem of CM loss is heart transplantation. However, the worldwide donor shortage has presented a need for alternative therapies. Cell transplantation, otherwise known as cardiomyoplasty, is an emerging technology with the potential to regenerate functioning contractile tissue. The goals of cardiomyoplasty are to regenerate myocytes at the site of injury, stimulate revascularization to reverse ischemia, and prevent harmful pathological remodeling [2]. However, numerous obstacles to cell transplantation have been revealed. Researchers have experienced problems with limited cell retention and early cell death, most likely as a result of insufficient oxygen and nutrient supply to the damaged area [3, 4]. Various combinations of stem cell types and implantation methods have been studied in an effort to solve these problems and provide an efficient and effective cell transplantation method. Study techniques have included intravenous (IV), intracoronary (IC) artery, and intramyocardial (IM) injection of stem cells. More recently, cells have been incorporated into bio-scaffolds and single- or multi-layered sheets to be surgically implanted directly onto the heart muscle [3]. Of these, cell sheet technology seems to be the most promising technique for long-term myocardial regeneration as it has the ability to circumvent problems related to tissue targeting, cell adhesion, and mechanical coupling while also avoiding the harmful side effects of scaffold degradation.

Intravenous (IV) Injection

Intravenous infusion of stem cells has been studied as a potential technique for treating post-MI patients and is appreciated for its simplicity and non-invasive nature. Studies have shown that stem cells intravenously introduced into the blood stream are attracted to the injured tissue and are capable of engraftment into the ischemic myocardium. In studies using mesenchymal stem cells (MSCs) and infarct rat models, myogenesis and angiogenesis was induced in the infarct area. Moderate improvement in LV function as well as a decrease in infarct size has been observed, though the effects were transient with a cell survival time of only 6 months [2]. However, no muscle thickening of the infarct wall was observed, indicating the absence of true muscle regeneration. This led researchers to believe that the improvement in heart function was due primarily to paracrine effects stimulating surrounding viable

muscle cells and increasing endogenous levels of vascular epithelial growth factors (VEGFs) rather than proliferation and regeneration of the injected cells themselves [2, 5].

Since cells are introduced to the body at a site distant from the injury, IV delivery relies on the homing of the cells to the infarct area and their subsequent incorporation into the myocardial tissue. Studies have shown low percentages of cell migration and adhesion to the heart due to a lack of directional homing which could potentially result in entrapment of donor cells by other organs, mainly the lungs [6]. However, the safety of IV infusion was confirmed by a clinical trial which showed an absence of unintended cell engraftment 12 months after treatment. Furthermore, improvements in cardiac arrhythmias and LV function were seen in patients at the 12-month follow-up [7]. While IV injection of stem cells has proven to be both safe and relatively simple, the effects are short-lived and non-specific. These issues could potentially be addressed by determining the optimal concentration of cells that should be injected to maximize engraftment and retention, as research suggests that the lowest effective dose may be 1 million MSCs/kg [8]. Further research needs to be done to address the homing and adhesion mechanisms of cells before IV injection can be considered for therapeutic use.

Intracoronary (IC) Artery Injection

Intracoronary artery infusion provides a more direct method of delivery of cells into the myocardial region than allowed by IV injection. Cells are delivered to the heart via the coronary artery using an over-the-wire balloon catheter. Cells can either be administered in a nonocclusive manner which maintains coronary flow, or in an occlusive manner involving transient balloon inflation. The latter maximizes the concentration of cells delivered to the site of injury by increasing the contact time of cells with the artery wall [9]. IC artery injection allows for the global diffusion of cells into the myocardium and a significant increase in engraftment when compared to IV administration [10]. It is also relatively non-invasive, resulting in little to no damage upon engraftment. The first application of IC injection by Ken Suzuki *et al.*, used normal rat hearts as recipients to demonstrate that this delivery method did not damage the myocardium or disrupt cardiac function. The cells were delivered at a concentration of 2 million cells/mL [11]. IC administration of MSCs into a porcine MI experimental model showed improved perfusion and LV function but seemed to have no effect on LV remodeling [12]. These mixed results were mimicked in a double-blind human clinical trial after a 2-year follow-up of patients receiving IC administration of bone marrow-derived progenitor cells. The trials showed that there are no delayed threats following treatment and the positive effects of improved contractility and reduced infarct size were maintained beyond the first months. However, there was no significant improvement in ejection fraction [13]. The inconsistency of trial results makes it unclear whether or not IC administration would be a feasible technique for myocardial regeneration. It is again necessary to determine the optimal size and dose of cells to be delivered as there is risk of embolization in the relatively small coronary arteries could result in decreased blood flow and greater myocardial injury [10].

Intramyocardial (IM) Injection

Intramyocardial injection is the most direct, precise, and accurate approach to stem cell introduction into infarcted myocardium. This method does not require homing or mobilization mechanisms and is

more site specific, resulting in less systemic engraftment. Cells can be introduced directly into the myocardium by surgical administration or catheter-based administration for surgically high-risk patients. In a study by Luciano C. Amado *et. al.*, MSCs were safely and effectively delivered to a region of damaged myocardium in a pig model. This resulted in improved ejection fraction and a dramatic reduction of infarct size. The authors also denied the presence of arrhythmias, which continue to be one of the major concerns surrounding cellular transplantation [14]. In a more recent clinical study, follow-ups with patients were conducted every few months over the course of 5 years. Improved LV function and decreased remodeling were similarly reported. However, they did note the presence of arrhythmic storms. There was a decrease in beneficial effects at long-term, indicating a potential loss of efficacy over time, most likely due to high rates of cell death [15]. The cells also tend to settle in cavities surrounding the fibrous scar tissue which limits the dissemination of cells throughout the myocardium when compared to the IV and IC injection methods [16].

One of the major disadvantages of IM injection is that it is a relatively invasive procedure and could potentially result in myocardial perforation at the site of injection, causing acute inflammation [10,17]. This can also result in leakage of cells from the injection site leading to a decrease in overall engraftment [17]. Before this technique can be further applied therapeutically, it is necessary to assess potential means of mitigating subsequent damage to the myocardium. The issues regarding cell adhesion and cell retention must also be addressed.

In general, injection methods have proven successful at mending small, damaged areas and preventing further remodeling of the injured myocardium, however, the shape, size, and location of cell adhesion can be difficult to control [3]. In addition, the poor retention of graft cells and overall decline of beneficial effects over time seems to render stem cell injection an insufficient therapeutic technique as it fails to improve cardiac function in the long-term. It is necessary to explore alternative targeted delivery methods that can improve cell adhesion and survival.

Biomaterial Scaffolds

Engineered tissue transplantation is a novel solution to these problems that aims to enhance cell adherence by providing a physical scaffold for the cells. A recent review by Sui *et. al.*, reported findings on 5 natural materials (such as gelatin, Matrigel, and collagen) and 7 synthetic materials that can be used for engineering of biological scaffolds, though the optimal scaffold has yet to be identified [18]. The implantation of MSC-seeded cellulose patches directly onto the surface of the affected heart region demonstrated improved cellular retention and survival, allowing for potential restoration of the dilated left ventricle and improved cardiac function in rat models [19]. However, when the scaffold degrades, the spaces previously occupied by the biodegradable polymers are often replaced by large quantities of extracellular matrix (ECM), forming a tissue with a low cell density [20]. Additionally, after long-term exposure to scaffold transplantation, dramatic inflammation and foreign body reactions can occur. Upon biodegradation of scaffolds, cytotoxic elements could be released into the surrounding environment, resulting in further inflammation and irritation [16]. Paul V. Kochupura *et. al.*, posed the idea that acellular porcine urinary bladder ECM could be used in place of a fibrous scaffold [21] which would address these problems by allowing for the formation of a denser tissue that does not induce a dramatic

immune response. While bio-scaffolds are advantageous in that they provide physical support to enhance cell adhesion, further research must be conducted to determine the safest and most effective scaffolding material. Until this can be determined, bio-scaffolds do not appear to be a reliable myocardial regeneration technique for clinical application.

Cell Sheet Technology

To address issues associated with biodegradable scaffolds, scaffold-free cell sheet technology has emerged. The first cell sheet was designed in 1990 by Noriko Yamada *et. al.*, Cells were cultured with the temperature-responsive polymer poly-*N*-isopropylacrylamide (PIPAAm). This polymer exists in a hydrophobic state at 32°C and in a hydrophilic state below 32°C. At low temperatures, the polymer expands and detaches from the surface, allowing for collection of a viable monolayered cell sheet [22]. This method of cell harvest without the use of enzymatic proteolytic treatments, such as trypsinization, allows for the preservation of cell-to-cell adhesions [23] (Figure 1). Additionally, the retainment of biological ECM on the basal side of the cell sheet can act as an adhesive agent, allowing the culture surfaces to be directly attached to the host tissue without the use of fibrin glue or a suture. This unique characteristic has shown to enhance cell engraftment and retention [20, 25].

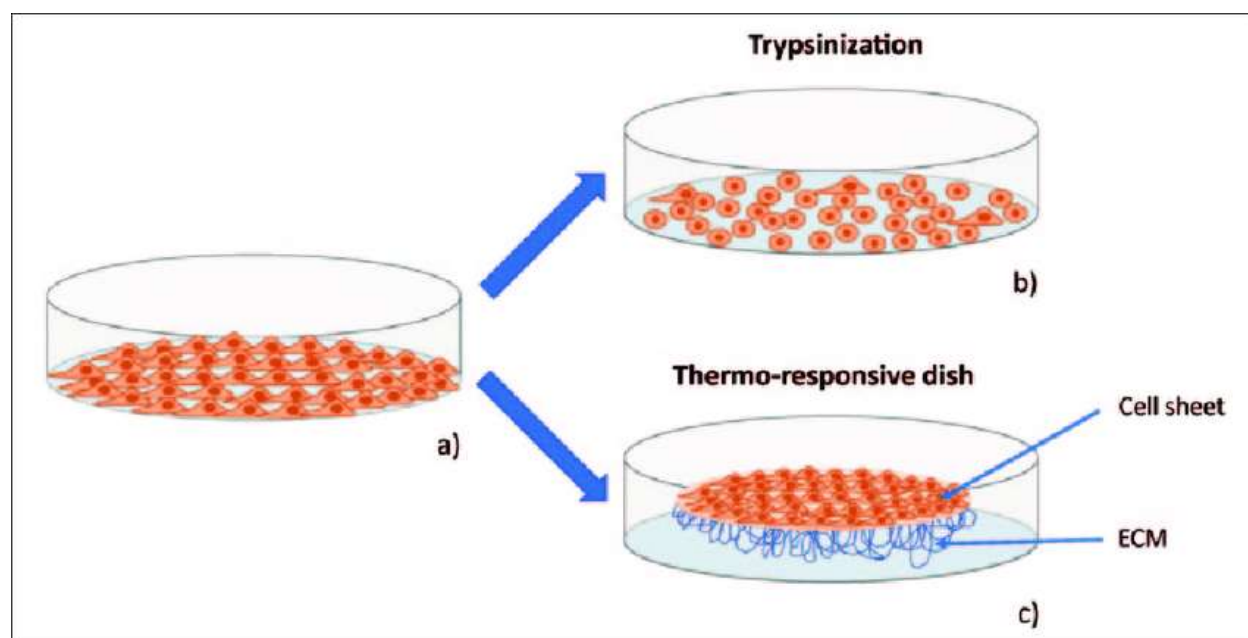


Figure 1: Cell sheet formation and detachment from a thermo-responsive dish. (a) Cells adhere to the hydrophobic surface of the media and form cell-to-cell junctions. (b) detachment of cells from the media through enzymatic digestion disrupts cell connections and cells separate. (c) Cells harvested on a thermo-responsive surface can be harvested as a contiguous cell sheet, preserving cellular junctions following a decrease in temperature [24].

Numerous studies have shown that cell sheet technology can improve LV function, increase neovascularization, and decrease fibrosis and remodeling in MI models [4, 16, 25, 26]. Cell sheet

technology appears to be one of the most promising novel therapeutic techniques as it has the potential to overcome the major obstacles presented with cell injection and bioscaffold engineering. The stem cell composition of the cell sheet is still a heavily researched and debated topic. According to a review by Rui Guo *et al.*, possible cell sources for sheet technology in cardiac transplantation include fibroblasts, endothelial cells, CMs, skeletal myoblasts (SMs), bone marrow or adipose-derived MSCs, adipose-derived stem cells, and pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) [27]. The cells of most interest which hold the greatest potential for therapeutic application are SMs, MSCs, and ESC/iPSC-derived CMs.

Skeletal Myoblast-Derived Cell Sheets

Skeletal myoblasts (SMs) were the first cell source to be incorporated into cell sheet technology for the treatment of ischemic heart disease [27]. SM-derived cell sheets have since been studied in both small and large animal models [16,28]. Autologous SMs are widely used in studies in order to avert ethical and cell source issues. Following preparation of SM-derived cell sheets and implantation into the infarct area, the SMs are able to differentiate into myotubes and retain skeletal muscle properties [9,27]. Across all trials, this resulted in significant wall thickening, reduced fibrosis, and improved LV systolic and diastolic function. SMs have also shown ischemia resistance, great proliferative potential, and enhanced neovascularization, deeming them a feasible option for incorporation into cell sheet therapy [16,17,28]. In one study involving implantation of SM-derived cell sheets in ischemic rat hearts, improvement of cardiac performance was seen up until 8 weeks after transplantation accompanied by immense cellularity and significant production of widespread capillaries. The great cellularity was in part attributed to the release of growth factors by transplanted cells which promoted cell migration, adhesion, and proliferation. High levels of stromal-derived factor 1 (SDF-1), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF) were detected. SDF-1 aids in the recruitment of hematopoietic stem cells while HGF and VEGF promote angiogenesis and antifibrosis [16]. Another study using a 3-layered myoblast cell sheet in a canine model yielded similar results regarding functional improvement, attenuation of remodeling, and secretion of growth factors. This trial represented the first successful application of myoblast cell sheets in a large animal model [17]. However, SMs do not have the ability to differentiate into CMs *in vivo*, electrically isolating them from each other and from the native cells of the heart. These cells are unable to form communicative gap junctions with neighboring cells as they do not express the cell adhesion molecules N-cadherin or connexin 43, resulting in an increased arrhythmogenic potential [28]. SM sheets could potentially be coupled with synchronized contraction devices to promote rhythmic beating of layered cell sheets. However, since electrical coupling is vital to the proper functioning of the heart, it is unlikely SMs alone will be able to truly regenerate myocardium.

Mesenchymal Stem Cell-Derived Cell Sheets

Mesenchymal stem cells (MSCs) are multipotent cells that have the ability to differentiate into CMs at low rates *in vivo*, as well as osteoblasts, chondrocytes, and adipocytes. The two primary sources of MSCs are bone marrow and adipose tissue. In a study directly comparing MSCs isolated from both sources, it was found that overall, adipose-derived MSCs (ADSCs) were superior to bone marrow-derived MSCs

(BM-MSCs) in the treatment of heart failure, though they both represent viable options [29]. Studies involving MSC-derived cell sheets has resulted in enhanced regional wall motion, prevented remodeling, reversal of wall thinning, accelerated angiogenesis, and high proliferation and survival rates [1, 4, 26, 30]. As described by Dongsheng Zhang *et. al.*, these effects can be enhanced by genetically engineering the cell sheet graft to prolong cell survival and promote integration of new blood vessel networks with native coronary circulation. This study demonstrated that maintaining high levels of SDF-1 greatly increased the number of MSCs penetrating the ischemic myocardium from the cell graft. This was accomplished by treating the cells with diprotin A which inhibits the enzymatic breakdown of SDF-1 [1]. Furthermore, following differentiation of the MSCs into CMs upon implantation, the cells were able to form gap junctions with host CMs *in vivo* resulting in electrical coupling with no significant arrhythmias reported [28]. However, it is suspected that due to the low differentiation rate of MSCs, paracrine effects serve as the primary therapeutic pathway rather than the replacement of damaged cells by donor cells. For example, BM-MSCs are able to secrete a wide array of factors that promote stem cell recruitment and angiogenesis. These factors include VEGF, platelet-derived growth factor, matrix metalloproteinases, and SDF-1 [4]. ADSCs also secrete numerous angiogenic and antiapoptotic factors that likely account for their positive effects on function [31]. The isolation of autologous MSCs through procedures such as liposuction or aspiration from bone marrow has been an attractive means of acquiring these cells due to the mitigated risk of immune rejection and ethical issues. However, autologous therapy has significant timing restrictions as cell sheets can take multiple weeks to prepare, making this method inappropriate for patients with acute heart failure. Further research needs to be conducted to assess MSC differentiation and the exact mechanisms by which MSCs implantation improves cardiac function. Cell source issues also need to be addressed by either developing quicker methods of cell sheet formation or exploring allogeneic transplant options.

Pluripotent Stem Cell-Derived Cell Sheets

Pluripotent stem cells include both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs are isolated from the inner cell mass of the blastocyst, typically at 5 days after fertilization [26]. Due to their vast differentiation potential and proliferation capacity, they are of keen interest in regenerative therapy for treating MI and heart failure. ESCs can be differentiated into CMs *in vitro* by various chemical and coculturing techniques [32]. Cell sheets composed of ESC-derived CMs (ES-CMs) have been successfully engrafted into injured myocardium in animal models and result in overall improvement of heart function. ES-CMs were observed to express connexin 43 and beat spontaneously and synchronously with neighboring CMs. Furthermore, unidirectional action potential propagation was observed, suggesting the presence of gap junctions [33]. Additional studies have been done in which ES-CMs were co-cultured with adipose tissue-derived stroma cells and endothelial cells which allowed for improvement in contractile function and reduction in remodeling while also increasing angiogenesis [34, 35]. It is also believed that the CMs themselves are capable of inducing neovascularization through paracrine release of VEGF which further supports cardiac function [36].

However, while ESCs are of great interest in regenerative therapy due to their ability to efficiently differentiate into CMs and readily couple with native cardiac cells, their application is limited by several

factors. The vast differentiation and proliferation capacity of ESCs raises concerns of tumorigenicity, though this problem seems to arise primarily following implantation of undifferentiated ESCs [27, 37]. Fully differentiated ES-CMs showed no evidence of teratoma formation five months after transplant in a primate model, suggesting differentiation of ESCs and extensive purification of the sample could evade this problem [33]. Furthermore, the allogeneic sourcing of ESCs poses the problem of immune rejection. While certain studies report ESC derivatives to be immune privileged based on their prolonged survival time [33-36], a more recent study showed that these cells and their derivatives faced vigorous immune rejection when implanted in a host heart [37]. Therefore, it is still uncertain whether allogeneic transplantation of ESCs is safe for human clinical trials. The sourcing of ESCs also raises intense ethical controversy, representing an unavoidable obstacle to the use of these cells in clinical trials.

The recent emergence of iPSC technology offers a promising mechanism of tissue regeneration that circumvents the ethical and immunological concerns associated with ESCs. In 2006, Kazutoshi Takahashi and Shinya Yamanaka first discovered that pluripotency could be induced in differentiated mouse somatic cells by retroviral transduction of four specific transcription factors (KLF4, Sox2, Oct4, Myc) [38]. A later study showed that iPSCs could similarly be generated from human dermal fibroblasts and other somatic cells by targeting of the same four transcription factors. In theory, a patient's own somatic cells could be reprogrammed into a pluripotent state, allowing for the creation of patient and disease specific stem cells, similar in morphology and proliferation to ESCs [39]. An analysis of iPSC and ESC-derived CMs showed impressive similarity between the two pluripotent stem cell lines. Firstly, the iPSCs showed a similar time course for differentiation and demonstrated a capacity, like ESCs, to differentiate into nodal, atrial, and ventricular-like phenotypes. Both cell lines also showed comparable cardiac gene expression patterns and nearly identical sarcomeric organizations. Furthermore, both iPSC and ESC-derived CMs were able to respond to β -adrenergic stimulation, represented by a decrease in action potential duration and an increase in contraction rate [40]. Similar to ESCs, human iPSC-derived CMs (iPS-CMs), when incorporated into a cell sheet, showed spontaneous and synchronous beating due to the formation of electrical connections [41]. Additionally, transplantation of human iPS-CM sheets improved cardiac function in a porcine ischemic model by attenuating LV remodeling and increasing neovascularization. The iPS-CMs were detectable 8 weeks after transplantation, but very few survived in the long term. This could either be attributed to immunological rejection due to the allogeneic transplantation of human iPS-CMs into a nonhuman model, or to high rates of cell death due to insufficient blood supply [42]. Further research is needed to find means of improving cell survival before this technology can be implemented in clinical trials.

Another obstacle is presented by the low reprogramming efficiency of human fibroblasts, resulting in few transduced cells actually acquiring an iPSC identity. The differentiation of somatic cells into iPSCs may be influenced by epigenetic factors retained from their tissues of origin. [32, 39]. To address this issue, Liying Zhang, *et al.*, proposed that iPSC-derived cells may be more abundant and effective if they were engineered from cardiac-lineage cells, rather than dermal fibroblasts. Their study showed significant improvements in cardiac function, increased vascularization, and a reduction in cell death [43]. Similar to ESCs, there is a risk of undifferentiated iPSCs leading to tumorigenesis, but this issue can be addressed by exploring ways to improve induction, differentiation, and purification methods. Lastly,

like all autologous transplantation methods, timing could pose a potential hurdle to clinical application of iPSCs for treatment of acute heart failure. It takes a considerable amount of time to isolate a patient's somatic stem cells, induce pluripotency, initiate differentiation into CMs, and construct a viable cell sheet for implantation. It is important to determine at what time heart failure is irreversible to assess the feasibility of this technique.

Overall, pluripotent stem cells show the most potential for regeneration of true myocardial tissue that can exist and function compatibly with the native host tissue. Due to the immunogenic, tumorigenic, and ethical hurdles faced by the use of ESCs in human clinical trials, iPSCs seem to be a more favorable alternative. While the safety of human iPSC-CM sheets has been confirmed in animals [42], this technology has yet to be tested in human models.

Decreasing Risk of Graft Cell Death

Injured myocardium represents a harsh environment for transplanted cells, characteristic of hypoxia, nutrient deprivation, and inflammation. This greatly hinders cell survival, especially when the introduced cells are not prepared to withstand such a harsh environment. Resisting the pro-death environment in damaged myocardial tissue remains a significant obstacle to the application of cell therapy in clinical trials [44].

The low survival rates of engrafted cells are most commonly due to insufficient blood supply to the injured site resulting in inadequate nutrition of the cells and an accumulation of waste products [23]. Co-transplantation of endothelial cells with differentiated CMs has been reported to improve angiogenesis, resulting in the formation of capillary networks *in vivo* [20, 35]. Additionally, the direct administration or upregulation of supplemental growth factors, most notably VEGF, has been shown to play a large role in the acceleration of angiogenesis and subsequent improvement in cardiac function [16, 36, 44]. A study by Yuya Tanaka, *et al.*, found that hypoxic preconditioning of cell sheets resulted in a significant increase in endogenous VEGF levels. This promoted the formation of new blood vessels *in vivo* and greatly enhanced therapeutic efficacy when compared to non-treated cells [4]. Supplementing stem cell delivery methods with an angiogenic treatment, like the techniques described above, could result in an increase in oxygen and nutrient supply to the cells and a subsequent decrease in associated cell death.

Cell death can also be prevented by directly blocking specific pathways involved in apoptosis. Michael Laflamme, *et al.*, identified a cocktail of pro-survival factors that targeted multiple parallel processes thought to contribute to graft cell death. The cocktail sought to inhibit apoptotic pathways related to ischemia, anoikis, and inflammation. Administration of the pro-survival cocktail in conjunction with the stem cells resulted in a significant increase in graft size [45]. On the other hand, overexpression of certain molecules involved in survival signaling pathways can block apoptotic cell death and thus improve cardiac function. Numerous survival signaling pathways have been identified that can be directly targeted to attenuate cell death, including signaling by AKT and several MAP kinases. Furthermore, culturing of cells in a microenvironment which closely mimics the harsh environment of injured heart tissue allows the cells to initiate production of certain survival signals prior to

implantation, resulting in an increased likelihood of survival [44]. Apoptosis is a complex, multifactorial process with which we are still widely unfamiliar. Therefore, further research must be done to investigate instigators of cell death, apoptotic pathways, and survival signaling cascades before cell therapy can be reliably used for treatment of MI in humans.

Discussion

The use of stem cell therapy has sparked great enthusiasm in the world of regenerative medicine and appears to be a feasible option for treatment of MI and other ischemic heart conditions. The first delivery method to be explored was the transplantation of stem cells by injection. However, as new research emerges, this technique has since been proven to be relatively inefficient. The IV, IC, and IM injection methods have been appreciated for their relatively simple approach, but pose problems related to inadequate specificity and low engraftment rates. These issues are accompanied by an observed decrease in efficacy over time, therefore deeming injection a suboptimal approach. Biodegradable scaffolds seeded with donor stem cells were engineered in response to these problems. However, while these scaffolds were able to offer a greater degree of control and promote cellular retention, their immunogenicity and cytotoxicity caused inflammation and irritation, further perpetuating the injury. Stem cell sheet technology represent the newest advancement in regenerative cell therapy and holds the most promise for future clinical application. Polymerized stem cell sheets harvested from temperature-responsive culture surfaces can be easily retrieved and attached to the host tissue without disrupting important cell-to-cell connections. This targeted approach greatly increases cell adhesion and retention, allowing for further cell proliferation and association with the host CMs. Exploration of various stem cell compositions of cells sheets has suggested SMs, MSCs, and pluripotent stem cells to be the most effective. SMs were the first cells to be incorporated into cell sheet technology and were initially of interest due to their myogenic capacity. However, transplanted SMs are unable to differentiate into CMs *in vivo* and are therefore electrically isolated from each other and the neighboring host cells. Without the ability to synchronously contract, SMs will never be able to truly mimic functioning myocardium. On the other hand, MSCs demonstrated CM differentiation which allowed for the formation of electrical gap junctions with the surrounding cardiac cells. However, MSCs show limited rates of differentiation into CMs *in vivo*. Nevertheless, the use of MSC cell sheets is both safe and effective and can offer a feasible therapeutic technique. Pluripotent stem cells are able to overcome limitations in differentiation potential and proliferate indefinitely when properly induced. ESCs are the most widely studied type of pluripotent stem cell and are hailed for their ability to develop into any adult human cell type, including CMs. ES-CM sheets show advanced electrical coupling and spontaneous contraction, indicating an ability to truly regenerate functioning myocardium. However, they are met with numerous obstacles such as immune rejection, teratoma formation, and ethical controversy. While their immunogenicity and tumorigenicity could potentially be addressed and solved for in future modified studies, the ethical dilemma still remains. These problems inspired the development of iPSCs, which allow for autologous isolation and administration. Human iPSCs almost indistinguishably mimic human ESCs in both morphology and functionality, making them a useful alternative to ESCs. Cell sheets composed of iPS-CMs similarly induced synchronous beating and resulted in improved cardiac function and attenuation of remodeling. Due to their ability to truly regenerate

myocardium while avoiding issues presented by other cell types, iPSC-CM sheets show unmatched potential for regeneration of human myocardium in stem cell therapy. However, even iPSCs are not invulnerable to the high death rates that plague all other cell types and delivery methods. Stem cells administered in conjunction with angiogenetic or pro-survival treatments could significantly increase cell survival rates and ensure lasting efficacy. In sum, prior to application of cell therapy for treatment of MI in clinical trials and eventual clinical practices, we must first determine the most effective cell type and delivery method while also addressing all associated risks and obstacles in order to obtain long-term positive effects on cardiac function.

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