Biomaterial Approaches for Stem Cell-Based Myocardial Tissue Engineering

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ABSTRACT: Adult and pluripotent stem cells represent a ready supply of cellular raw materials that can be used to generate the functionally mature cells needed to replace damaged or diseased heart tissue. However, the use of stem cells for cardiac regenerative therapies is limited by the low efficiency by which stem cells are differentiated *in vitro* to cardiac lineages as well as the inability to effectively deliver stem cells and their derivatives to regions of damaged myocardium. In this review, we discuss the various biomaterial-based approaches that are being implemented to direct stem cell fate both *in vitro* and *in vivo*. First, we discuss the stem cell types available for cardiac repair and the engineering of naturally and synthetically derived biomaterials to direct their *in vitro* differentiation to the cell types that comprise heart tissue. Next, we describe biomaterial-based approaches that are being implemented to enhance the *in vivo* integration and differentiation of stem cells delivered to areas of cardiac damage. Finally, we present emerging trends of using stem cell-based biomaterial approaches to deliver pro-survival factors and fully vascularized tissue to the damaged and diseased cardiac tissue.

KEYWORDS: stem cell, pluripotent stem cell, biomaterials, cardiac regeneration

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Introduction

Cardiovascular diseases are the leading causes of morbidity and mortality in the United States. Every 43 seconds an American experiences a myocardial infarction (MI), which can lead to the death of up to 1 billion cardiomyocytes in the left ventricle, translating to approximately 50 g of muscle mass.[1–3] Unlike in some model organisms such as zebrafish, the mammalian heart has limited regenerative capacity.[4] As a result, cardiac injury triggers a pathologic adaptive cascade resulting in tissue remodeling, myocyte hypertrophy, and eventual catastrophic heart failure.[5,6] Current therapeutic strategies such as surgical, endovascular, and pharmacological interventions[6–9] are merely palliative in nature and do not adequately address the true cause of heart failure – the loss of functional myocytes and supporting cardiac tissue.[10] As such, heart transplantation is the only effective treatment option to replace damaged or diseased myocardium. However, the limited number of available donors and complications from immune rejection of transplanted organs make cardiac transplants impractical for the vast number of people affected by heart failure and disease.

Over the past several years, the integration of stem cell biology with biomaterials science has resulted in the development of several promising strategies for the regeneration of various tissues and organs.[11] In this review, we discuss the extent to which biomaterial-based approaches are aiding myocardial regenerative medicine efforts in the following ways: (i) improving the *in vitro* differentiation of stem cells to cardiomyocytes and (ii) guiding the delivery and integration of transplanted stem cells. We then speculate on the future of biomaterial-based approaches for stem cell myocardial tissue engineering.

Stem Cell Types for Cardiac Repair

Although a variety of mature cell types isolated from primary and fetal tissue sources have been used to repair the damaged cardiac tissue in animal models and clinical trials,[12,13] this review focuses on the development of stem cell-based biomaterial approaches for myocardium regenerative purposes. Broadly speaking, stem cells are defined by two common characteristics: (i) the ability to self-renew or proliferate indefinitely and (ii) the potential to differentiate into one or more specialized cell types. As such, stem cells can be categorized into two types, which have differing differentiation potentials: (i) pluripotent stem cells [PSCs; including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)], which can give rise to hundreds of cell types that comprise the adult body, and (ii) adult stem cells, which can only differentiate into a small subset of specialized mature cells. The characteristics, advantages, and limitations of each of these

cell sources for cardiac regenerative medicine purposes are summarized in Table 1.

Pluripotent stem cells. PSCs, which include ESCs and iPSCs, have the potential to differentiate into hundreds of specialized cell types that comprise the fully mature adult body. Although there are some slight genetic and epigenetic differences between ESCs and iPSCs,[14,15] both the cells have the ability to provide the raw material that is necessary for cardiac tissue engineering. There are a wide variety of protocols used to generate cardiomyocytes from PSC through the temporal addition of growth factors that mimic *in vivo* cardiac development.[16–26]

Embryonic stem cells. ESCs are derived from the inner cell mass of a preimplantation embryo. The first ESCs were isolated from mouse embryos by two independent groups in the early 1980s.[27,28] In 1998, Thomson led a group of researchers who developed for the first time methods to isolate and propagate human ESCs (hESCs).[29] This seminal discovery ushered in a new era of regenerative medicine where hESCs could be used for the generation of functionally mature human cells, including cardiac tissue.

Several groups have reported the differentiation of mouse ESCs (mESCs)[30–32] and hESCs[33–36] to cardiomyocytes that express well-organized sarcomeric proteins and display synchronous contractile activity. Further genetic and molecular analyses of *in vitro* derived cardiomyocytes have revealed that these cells display properties similar to early-stage, fetal cardiomyocytes, thereby potentially limiting their therapeutic potential.[37] In fact, several studies have evaluated the potential of ESC-derived cardiomyocytes in repairing the damaged cardiac tissue in animal models of MI. As such, these studies have shown that transplanted cardiomyocytes derived from both mESCs[38,39] and hESCs[23,40–42] integrate with host tissue and can lead to the improvement of cardiac function. However, there remains considerable debate as to whether these transplanted cells suppress[43] or induce[44,45] cardiac arrhythmias in injured hearts. Finally, additional hurdles such as complications associated with immune rejection and ethical issues may limit the clinical application of cardiomyocytes derived from hESCs.[46] Despite these challenges, there are ongoing clinical trials assessing the feasibility and safety of a transplantation of hESC-derived cardiac-committed progenitor cells derived in patients with severe heart failure (ClinicalTrials.gov Identifier: NCT02057900).

Induced pluripotent stem cells. IPSCs are PSCs generated through the reprograming of somatic cells into a pluripotent state. IPSCs were first generated by Yamanaka's group in 2006 from mouse fibroblasts[47] and then in 2007 from human fibroblasts.[48] Because generation of human induced pluripotent stem cells (hiPSCs) does not involve the destruction of human embryos, they are not subject to the same ethical considerations as hESCs. HiPSCs have an additional advantage that they do not generate an immune response in the recipient from which they were derived, although recently this has been subject to a considerable debate.[49,50] Additionally, cardiomyocytes generated from patient-specific cells can be used to provide important insights in disease pathology, progression, and mechanism, as well as an unlimited source of cells, and to enable the development of compounds and the screening of potential drugs.[51–53]

Adult stem cells. Tissue-specific adult stem cells are more limited in their differentiation potential compared to PSCs. Additionally, unlike PSCs, which can be propagated in culture indefinitely, adult stem cells are difficult to maintain and expand *in vitro.* Within the body, adult stem cells are located in complex microenvironments, called niches, which tightly regulate their self-renewal and differentiation.[54] Adult stem cells have been isolated from a variety of tissues, including the mammary glands (mammary stem cells),[55] the base of the crypt of the intestinal epithelium (intestinal stem cells),[56] basal layer of the epidermis (epidermal stem cells),[57] Subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus in the central nervous system (neural stem cells),[58] the bulge region of the epithelial stem cells in the hair follicle,[59] the basal layer of the seminiferous tubules (germline stem cells),[60] under the basal lamina of myofibers (muscle satellite cells),[61] and the bone marrow (hematopoietic stem cells).[62] The following three additional adult stem cell populations have been widely used in cardiac tissue engineering applications: (i) mesenchymal stem cells (MSCs), (ii) adipose-derived stem cells (ADSCs), and (iii) cardiac progenitor cells (CPCs).

Bone marrow-derived MSCs. MSCs are derived from the nonhematopoietic stromal component of the bone marrow.[63,64] Most commonly, MSCs are isolated using fluorescence- or magnetic-activated cell sorting with a combination of positive (eg, CD13, CD29, CD44, CD73, CD90, CD105, STRO-1) and negative (eg, CD3, CD14, CD15, CD28, CD33, CD34, CD45, HLA-DR) selection markers.[65,66] Several groups have shown that MSCs have the potential to differentiate into a variety of nonmarrow cells such as bone, cartilage, connective tissue fat, and endothelial cells.[67] However, the existence and differentiation potential of MSCs has been somewhat controversial as some studies suggest that MSCs and fibroblasts are identical.[68] Nonetheless, several groups have reported the *in vitro* directed differentiation of MSCs to cardiomyocyte-like cells through a variety of approaches, including incubation with media that has been conditioned on primary ventricular cardiomyocytes[69] and addition of chemical factors such as the DNA methylation inhibitor 5-azacytidine.[70,71] Although cells generated using these methods express early cardiomyocyte markers such as cardiac myosin heavy chain (MHC), cardiac troponin T (cTnT), and connexin 43, in-depth electrophysiological and functional analyses of such resultant populations have yet to be reported.

Despite the lack of *in vitro* analysis of the cardiac differentiation potential of MSCs, several studies have reported

Table 1. Stem cell populations utilized for cardiac tissue engineering applications.

STEM CELL	ADVANTAGES	DISADVANTAGES	CLINICAL TRIALS
Embryonic stem cells (ESCs)	Robust *in vitro* expansion Broad differentiation potential	Potential tumor formation upon *in vivo* transplantation Potential for immune rejection Ethical issues associated with derivation	NCT02057900: Transplantation of human embryonic stem cell-derived progenitors in severe heart failure
Induced pluripotent stem cells (iPSC)	Robust *in vitro* expansion Broad differentiation potential Limited ethical issues Ability to generate patient-specific therapies	Potential tumor formation upon *in vivo* transplantation Use of oncogenes for derivation Genetic and epigenetic instability	None reported
Bone-marrow derived mesenchymal stem cells (MSCs)	Limited ethical issues Ability to generate autologous therapies	Limited *in vitro* expansion Difficult to isolate Limited cardiac differentiation potential	NCT00279175: REPAIR-AMI: intracoronary progenitor cells in acute myocardial infarction NCT00684021: Use of adult autologous stem cells in treating people who have had a heart attack (the TIME study) NCT00877903: Prochymal® (human adult stem cells) intravenous infusion following acutemyocardial infarction (AMI)
Adipose derived stem cells (ADSCs)	Easy to isolate Robust *in vitro* expansion Limited ethical issues Ability to generate autologous therapies	Limited cardiac differentiation potential	NCT01556022: Safety and feasibility trial of adipose-derived regenerative cells in the treatment of chronic myocardial ischemia (ATHENA) NCT02052427: Safety and efficacy of adipose-derived regenerative cells in the treatment of chronic myocardial ischemia (ATHENA II) NCT01449032: MesenchYmal STROMAL CELL therapy in patients with chronic myocardial ischemia (MyStromalCell Trial)
Cardiac progenitor cells (CPCs)	Robust *in vitro* expansion Broad cardiac differentiation potential Limited ethical issues Ability to generate autologous therapies	Difficult to isolate Lack of consensus on purification methods	NCT00474461: Cardiac stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO) NCT00893360: CArdiosphere-derived aUtologous stem CElls to reverse ventricUlar dySfunction (CADUCEUS)

the transplantation of MSCs or MSC-derived cardiac cells in animal models of cardiac damage.[72–77] For example, in a study performed by Orlic et al, isolated MSCs were injected into the ventricular portion of an infarcted heart.[78] The engrafted MSCs generated de novo myocardium and ameliorated the outcome of coronary artery disease in the treated animals. Along similar lines, transplantation of MSC-derived cardiac cells into a cryoinjury-derived scar in the left ventricle resulted in the repair of scar tissue and improved cardiac function.[70]

Because of the promising results observed in preclinical animal models of cardiac injury and disease, numerous clinical trials have been performed to examine the ability of MSCs to ameliorate or reverse the effects of tissue damage caused by MI. Overall, the results of these clinical trials have been met with mixed success.[79] Some trials have demonstrated that autologous MSC transplantation leads to improved ventricular function and survival in patients several years after transplantation.[80] On the other hand, recent studies have not shown significant improvement of ventricular function after either intracoronary[81] or transendocardial[82] delivery of autologous MSCs in patients with acute MI[81] or ischemic cardiomyopathy.[82]

Adipose-derived stem cells. ADSCs have been isolated using cell sorting approaches from a variety of sources, including human white and brown adipose tissues.[83,84] Similar to MSCs, ADSCs have the ability to undergo osteogenesis, chondrogenesis, and adipogenesis. However, ADSCs may be advantageous over MSCs as a source of material for cell-based therapies because of relative ease of their isolation and ability for their long-term *in vitro* expansion. Several groups have examined the *in vitro* ability of ADSCs to differentiate into cardiomyocytes.[85–87] For example, Planat-Bénard et al demonstrated that the addition of 5-azacytidine to ADSC cultures resulted in cells that expressed cardiac-specific markers such as GATA4, NKX2.5, ANP, MLC2v, and MLC2a.[85] Additionally, ultrastructural and electrophysiological analyses revealed the presence of functional atrial, ventricular, and nodal cardiomyocytes. Similarly, other groups have shown that modulation of soluble signaling pathways such as Wnt/β-catenin[87] and vascular endothelial growth factor[88] enhances the cardiac differentiation of ADSCs. On the other hand, some argue that ADSCs lack inherent cardiac differentiation potential[89] and that only through direct fusion with primary cardiomyocytes can ADSCs display cardiomyocyte-like phenotypes.[90]

In subsequent studies in animal models of cardiac damage, delivery of ADSCs through direct intramyocardial injection[91] or indirectly through intravenous[92] or intracoronary[93] injections has resulted in the repair of damaged myocardial tissue and improved cardiac function. A clinical trial examining the effects of transendocardial injections of ADSCs in patients with nonrevascularizable ischemic myocardium demonstrated that ADSC-treated patients showed significant improvements in total left ventricular mass and reductions in inducible ischemia. Additionally, these studies revealed that ADSCs preserved ventricular function, myocardial perfusion, and exercise capacity in ischemic patients.[94] Additional ongoing clinical trials are examining the safety and efficacy of ADSCs in patients with chronic myocardial ischemia (ClinicalTrials.gov Identifiers: NCT02052427, NCT01556022, and NCT01449032).

Cardiac progenitor cells. Several studies over the past decade have demonstrated the existence of a CPC population that can contribute to cardiac tissue homeostasis and repair.[46,95–98] CPCs can be isolated from functionally mature cardiac tissue using a variety of cell surface markers, including c-Kit,[95] Sca-1,[96] CD31,[97] Flk-1,[99] Flt1+/Flt4+,[98] or on the ability to efflux Hoechst dye.[100] Although there is a lack of consensus of the specific markers that should be used to isolate CPCs from primary tissue,[101] CPCs share the following common characteristics: (i) express early cardiac markers (eg, GATA4, NKX2.5), (ii) can be expanded *in vitro* through modulation of various pathways such as Wnt/β-catenin[102,103] and FGF[104] signaling, and (iii) are capable of generating the three major cell types that comprise the myocardium – cardiomyocytes, smooth muscle, and endothelial cells. As it relates to *in vitro* generation of cardiomyocytes, treatment of CPCs with 5-azacytidine or other signaling molecules such as TGF-β results in cells that express cardiomyocyte-related sarcomeric proteins (eg, β-MHC, a-actinin), contract spontaneously, and display action potentials that resemble those of mature cardiomyocytes.[97,99,105] Although CPCs robustly differentiate into cardiomyocytes *in vitro,* there is considerable debate to the extent to which endogenous CPCs contribute to cardiomyocytes in the heart.[106–110]

The therapeutic potential of CPCs has been extensively studied in animal models of cardiac damage and disease. For example, Dawn et al demonstrated that intravascular injection of CPCs results in increased cardiac mass and ventricular function during hypertrophy or ischemia.[111] Along similar lines, it has been reported that in aortic stenosis[112] and ischemic heart failure,[113] the activation of endogenous CPCs results in myocyte formation and myocardial regeneration.

There are several early clinical trials that are examining the ability of CPCs to ameliorate the effects of cardiac injury and disease. In one such trial, autologous CPCs were delivered through intracoronary injections in patients with postinfarction left ventricular dysfunction.[114] Patients examined one year after treatment showed a significant increase in ventricular function and decrease in infarct size. In a similar study, autologous CPCs isolated from endomyocardial biopsies were infused into the infarct-related artery of patients who suffered an MI.[115]

Classes of Biomaterials for Stem Cell Cardiac Muscle Repair

A variety of biomaterial scaffolds have been used for the *in vitro* generation of stem cell-derived cardiac tissue and the *in vivo* delivery of stem cells to damaged myocardium. These biomaterials can be classified into the following categories (Table 2).

Extracellular matrix protein-based biomaterials. Extracellular matrix protein (ECMP)-based biomaterials are attractive scaffolds for cardiac tissue engineering and regeneration because they retain their inherent biological activity to support cell adhesion, survival, and differentiation. These biomaterials include those isolated from animal sources, such as Matrigel™ and Geltrex™, and those from purified or recombinant sources, such as collagen, laminin, fibronectin, and vitronectin.[116–118] ECMP-based biomaterials are biocompatible and can be proteolytically degraded into nontoxic by-products. In addition, the degradation rate of ECMP-based materials is highly variable and dependent upon several factors such as implantation location and extent of material cross-linking.[119] As an example, biomaterials composed of gelatin, a denatured derivative of collagen, have a higher degradation rate than collagen itself.[119]

Decellularized matrices. Although ECMP-based materials can be used as stem cell substrates, they do not readily mimic the complexity and architecture of native tissue. On the other hand, it has been demonstrated that decellularized matrices, which can be readily obtained through the detergent treatment of intact cardiac tissue,[120] retain the complex mixture of collagens, elastin, and glycosaminoglycans that comprise *in vivo* tissue.[121] As such, these decellularized matrices, which maintain the composition and structure of *in vivo* tissue, have gained a wide use in cardiac regenerative medicine purposes.[121] Similar to ECMP-based biomaterials, decellularized matrices are degraded *in vivo* into safe by-products.[122]

Natural biomaterial scaffolds. Several naturally occurring biomaterials have been used for *in vitro* and *in vivo* cardiac regenerative medicine purposes. These naturally occurring materials are advantageous because they contain the proteins, polysaccharides, and other cell adhesive domains that are found in native tissue. Naturally occurring biomaterials that have been used in cardiac tissue engineering include silk fibroin (biodegradable polypeptide secreted from worms and insects), chitosan (polysaccharide-based material isolated from crustacean shells), fibrin (generated through the polymerization of the protein fibrinogen isolated from blood plasma), alginate (polysaccharide-based material obtained from brown algae), and agarose (polysaccharide-based material obtained from red algae).[123–130] The physicochemical properties of these natural biomaterials can be manipulated, so that they can be naturally degraded within days to weeks after implantation.[119,124,131] As such, when implanted *in vivo,* these materials will persist long enough to promote integration with the native tissue but degrade quickly enough not to disrupt mechanical coupling that is critical to myocardial function.[132]

Synthetic polymer-based materials. Several synthetic polymer-based materials have been used for cardiac regenerative medicine purposes.[133] Compared to ECMPs and decellularized scaffolds, polymer-based materials are easily fabricated and tunable, thereby allowing iterative engineering of materials for specific stem cell responses. Polymers that have been used for stem cell-based cardiac tissue engineering include poly(ethylene glycol) (PEG), poly(lactic acid) (PLA), poly(caprolactone) (PCL), poly(l-lactide-*co*-caprolactone) (PLCL), poly(glycolide-*co*-caprolactone) (PGCL), poly(glycerol sebacate) (PGS), and polyurethane (PU).[134–140] While some polymer-based biomaterials such as PGS,[141,142] PLCL,[143] and PGCL[144] biodegrade into nontoxic natural metabolites over the course of several weeks or months,[143] other polymer-based materials can release potentially harmful by-products of degradation. For example, it has been demonstrated that PU-based biomaterials can oxidize, thereby leading to post-implantation complications.[145] To that end, modifications, such as coating PU-based materials with an antioxidant layer, have been shown to reduce adverse degradation effects *in vivo*.[145]

Application of Biomaterials to Aid *in Vitro* Differentiation of Stem Cells to Cardiac Tissue

The development of reproducible and efficient methods for differentiating stem cells to functionally mature cardiomyocytes *in vitro* is a necessary step for the application of these cells for disease modeling, drug screening, and regenerative medicine purposes. In this section, we will review the current biomaterial-based approaches that are being implemented to guide the differentiation of adult stem cells and PSCs toward cardiomyocytes.

ECMP-based biomaterials. ECMP-based materials have been used as matrices for the cardiac differentiation of a variety of stem cell types. Cardiogel, a naturally occurring extracellular matrix (ECM) containing a complex mixture of laminin and fibronectin isolated from cardiac fibroblasts,[146] has been used to direct the differentiation of MSCs to cardiomyocytes.[147] ECMPs from both purified and recombinant sources have also been used as natural biomaterials for the generation of cardiomyocytes from stem cell populations. For example, Santiago et al examined the effect of individual ECMPs, including collagens type I, III, IV, laminin, and fibronectin, on the cardiac commitment of MSCs.[148] The authors found that collagen can be remodeled to form fibrils that guide the differentiation of MSCs into cells representative of cardiac muscle.[148] Along similar lines, Miskon et al reported that differentiation of MSCs on collagen type I matrix elevated expression of cardiomyocyte-related genes in the resultant populations.[149] Likewise, Tan et al reported that MSCs differentiated on collagen V matrices had higher expression of cardiac-related genes such as GATA4, NKX2.5, and cTnT compared to cells differentiated on collagen I matrices.[150] In fact, cardiac cells generated on collagen V matrices prevented chamber dilation and improved contractile function when injected into the injured myocardium of animals subject to an MI.[150] On the other hand, other nonfibrillar ECMPs such as laminin have been shown to facilitate the differentiation of ADSCs toward cardiomyocytes.[151]

Table 2. Classes of biomaterials used for stem cell-based cardiac muscle repair.

BIOMATERIAL CLASSIFICATION	KEY APPLICATIONS			
	IN VITRO		*IN VIVO*	
	ADULT STEM CELLS	PLURIPOTENT STEM CELLS	ADULT STEM CELLS	PLURIPOTENT STEM CELLS
Extracellular matrix protein (ECMP)	Van Dijk et al (2008): Laminin facilitated the CM differentiation of ADSCs Santiago et al (2009): Identified collagen type I as optimal matrix for cardiac commitment of MSCs	Baharvard et al (2005): Cardiogel enchanced the differentiation of ESCs to CMs Zhang et al (2012): Matrigel™ sandwich promotes CM preparations of high purity and yield	Maureira et al (2012): Repair of chronic MI with autologous MSCs seeded in collagen scaffolds Araña et al (2014): Epicardial delivery of collagen patches seeded with ADSCs in model of chronic MI	Kofidis et al (2004): Matrigel™–based scaffold to deliver ESCs to the damaged ventricular areas of post-MI heart
Decellularized matrices	French et al (2012): Decellularized ventricular ECM enhance CPC maintenance, expansions, and differentiation	De Quach et al (2010): Decellularized matrix promotes cardiac differentiation of ESCs Duan et al (2011): Composite hydrogel comprised of collagen type I and decellularized heart matrix differentiates ESCs to CMs	N/A	Lesman et al (2010): Decellularized matrices seeded with ESC-derived CMs integrated with host coronary vasculature upon transplantation to the heart
Natural materials	Di Felice et al (2013): Silk scaffold enchances cardiac commitment of CPCs Liu et al (2013): Chitosan substrates enchanced the cardiomyogenic potential of CPCs	Schaaf et al (2011): Fibrin scaffold used to generate highly functionalized heart tissue from ESCs Zhang et al (2013): 3-D fibrin scaffolds enhance the functional maturation of ESC-derived CMs	Guo et al (2011): Transplantation of MSCs in fibrin improves cardiac function after MI Sun et al (2014): Embedded ADSCs in fibrin scaffolds led to improved ventricular function in model of acute MI	Lü et al (2010): Injection of temperature-responsive chitosan hydrogel improve myocardial performance in MI hearts Habib et al (2011): Transplantation of ESC-derived CMs in silk matrix increased ventricular performance in a MI model
Synthetic polymer-based materials	Crowder et al (2013): PCL carbon nanotube composite scaffolds were to enhance cardiac differentiation of MSCs Tran et al (2013): Emulsion electrospun PLCL scaffolds enhanced cardiomyogenic differentiation of MSCs	Gupta et al (2011): Combinatorial identification of 4% PEF-86% PCL- 10% PCL as optimal substrate for cardiac differentiation of PSCs Lee et al (2014): Graphene enhances the cardiomyogenic differentiation of ESCs	Fukuhara et al (2005): MSC-seeded PGA scaffolds enhanced angiogenesis and improved function of the infarcted heart Jin et al (2009): Transplantation of MSCs with PLCL scaffolds reduced scar size and improved cardiac function in animal model of MI	Chen et al (2010): Elastomeric patch derived from PGS for delivery of ESC to the heart

The differentiation of hESCs toward cardiomyocytes has been achieved by culture on gelatin,[21,152,153] cardiogel,[154] and Matrigel™,[155] a gelatinous protein mixture secreted by mouse sarcoma cells, which consists mainly of collagen, laminin, and entactin.[156] In another line of investigation, Burridge and colleagues investigated the use of defined ECMP-based matrices for the cardiac differentiation of hiPSCs.[157] While there was no difference in cardiomyocyte differentiation efficiency between recombinant laminin, fibronectin, and vitronectin matrices, recombinant laminin substrates did aid in the adhesion and survival of iPSC-derived cardiomyocytes.

Three-dimensional (3-D) architecture has been incorporated into ECMP-based materials to improve the generation of cardiomyocytes from PSCs. In one such study, hESCs and hiPSCs were differentiated between a Matrigel matrix sandwich. Differentiation of cells in this system resulted in cardiomyocyte preparations of high purity (up to 98%) and yield (up to 11 cardiomyocytes for each input PSC).[19] Additionally, the cardiomyocyte populations were functionally mature and displayed action potentials typical of nodal, atrial, and ventricular cardiomyocytes. More recently, polydimethylsiloxane (PDMS) templates were used to engineer collagen-based 3-D, self-assembled scaffolds, termed biowires, for the generation of cardiomyocytes from hESCs and hiPSCs.[158] Differentiation of PSCs in these scaffolds resulted in aligned cardiac tissue with a high degree of ultrastructural organization, enhanced conduction velocity, and improved calcium handling and electrophysiological characteristics when compared to cardiomyocytes generated using conventional approaches.

Decellularized matrices. Decellularized matrices have been implemented to enhance the cardiac differentiation of MSCs, ADSCs, and CPCs. For example, decellularized ventricular ECMs have been used to enhance CPC maintenance, expansion, and differentiation.[9] In another study, decellularized full thickness ventricular matrices were repopulated with MSCs and human umbilical vein endothelial cells to engineer fully vascularized cardiac tissue.[159]

Likewise, decellularized heart ECMs have been used for *in vitro* generation of cardiomyocytes from hESCs and mESCs.[120,160] In fact, these native tissue matrices increased the sarcomeric organization and enhanced the maturation of hESC-derived cardiomyocytes when compared to conventional cell culture coatings such as gelatin or collagen.[120] Hybrid materials consisting of ECMPs and decellularized cardiac ECM have also been used to direct the differentiation of hESCs to cardiomyocytes.[161] For example, composite hydrogels composed of collagen type I and decellularized matrix from porcine heart were used to efficiently differentiate hESCs to cardiomyocytes.[161] Interestingly, decellularized hydrogels with a high collagen content promoted the function and contractile activities of cardiomyocytes compared with low collagen content or pure collagen gels.[161]

Natural biomaterial scaffolds. Silk fibroin substrates have been used extensively for the cardiac differentiation of adult stem cell populations. In one such study, silk fibroin nanometric nets were fabricated and seeded with CPCs.[162] After three weeks of culture, CPCs differentiated into cells that expressed high levels of cardiac- and sarcomeric-related proteins.[162] In fact, these scaffolds not only induced alignment of the cardiomyocyte populations but also synthesis of titin, a protein critical to sarcomere assembly.

Polysaccharide-based scaffolds have also been implemented for cardiac differentiation of stem cell populations.[163–165] For example, Liu et al demonstrated that chitosan substrates enhanced the cardiomyogenic potential of ADSCs when compared to cells cultured on standard tissue culture polystyrene substrates.[163] In a related study, chitosan elevated intracellular calcium levels in differentiating ADSCs, thereby significantly upregulating the expression of cardiac marker genes GATA4, NKX2.5, and MYH6.[164] Along similar lines, another polysaccharide-based material, alginate, preserved the cardiac differentiation potential of CPCs.[166]

Composite biomaterials have also been investigated for their effect on the cardiac differentiation potential of MSCs.[167,168] To that end, Yang et al found that MSCs more efficiently differentiated to cardiac cells when cultured on hybrid substrates consisting of silk fibroin and hyaluronic acid when compared to cells differentiated only on silk fibroin matrices.[168] The same group found that by incorporating the polysaccharide chitosan into these silk fibroin/hyaluronic acid scaffolds significantly elevated the cardiomyogenic differentiation of MSCs.

Fibrin-based scaffolds have been widely used for the cardiac differentiation of PSCs.[169–171] As an example, highly functionalized heart tissue was engineered by differentiating hESCs in a fibrin substrate.[170] Specifically, differentiated cells displayed highly organized and oriented networks of sarcomeres, as well as electrophysiological properties indicative of mature cardiomyocytes. Additional studies have demonstrated that the effect of fibrin on the cardiac differentiation of mESCs and hESCs is improved in a 3-D culture system.[169,171] For instance, Zhang et al investigated the effects of dimensionality of fibrin constructs on the structural and functional maturation of hESC-derived cardiomyocytes.[171] Compared to cardiomyocytes generated in two-dimensional fibrin substrates, cardiomyocytes generated in 3-D scaffolds exhibited significantly higher conduction velocities, longer sarcomeres, and elevated expression of genes involved in cardiac contractile function.[171]

Synthetic polymer-based materials. A variety of polymer-based materials have been engineered for the differentiation of adult stem populations toward the cardiac lineage. In a recent work, PU, 3-hydroxybutryate-*co*-4-hydroxybutryate [P(3HB-*co*-4HB)], and polypropylene carbonate (PPC) substrates were studied for their ability to support the adhesion and cardiac differentiation of MSCs.[172] The authors

found that substrates composed of PU and P(3HB-*co*-4HB) permitted optimal cell growth and cardiac differentiation. In another study, PCL carbon nanotube composite scaffolds were used to enhance cardiac differentiation of MSCs.[173] Moreover, MSCs cultured on the composite scaffolds inherently assumed an elongated morphology allowing the cells to be more receptive to cardiac inducing factors. Additional PCL-based copolymer scaffolds have been used as an effective means to direct the cardiac differentiation of MSCs. For example, differentiation of MSCs on PLA-*co*-PCL scaffolds resulted in elevated expression of cardiac-related genes, *alpha actinin*, and *MHC*.[174] Finally, composite scaffolds consisting of polymers and ECMPs have been used for the cardiogenic differentiation of MSCs.[175] MSCs differentiated on PGS-collagen hybrid scaffolds more efficiently differentiated to cardiomyocyte-like cells than cells differentiated on substrates that only contained collagen.[175]

Synthetic polymer-based materials have also been used for the derivation of mature cardiac cells from CPCs. PGS has been used to develop biomimetic substrates that guide the adhesion, growth, and differentiation of CPCs.[176] Along similar lines, PEG has been used to generate *in vitro* CPC niches to control their function and fate.[177] These highly anisotropic substrates augmented CPC adhesion, migration, and proliferation. In turn, these substrates enhanced the differentiation of CPCs to mature cardiomyocytes through a nanotopography response mediated via p190RhoGAP.

Along similar lines, synthetic polymer-based substrates have been used for the cardiac differentiation of PSCs. As an example, the culture of hESCs on graphene-based polymer scaffolds enhanced their cardiomyogenic differentiation compared with differentiation on Matrigel substrates.[178] In an effort to precisely tune the polymer physicochemical properties required for cardiac differentiation of mESCs, Gupta et al prepared a combinatorial polymer library by copolymerizing PEG, PCL, and carboxylated PCL (CPCL) and used electrospinning to develop scaffolds to mimic the ECM network.[179] Through measurement of a-myosin heavy chain (a-MHC) expression and calcium (Ca^{2+}) signaling dynamics, the authors observed that the most compliant substrate tested, 4%PEG–86%PCL–10%CPCL, allowed for the most efficient cardiac differentiation of mESCs. Additionally, by altering the elastic modulus of the 4%PEG–86%PCL–10%CPCL substrates, the authors were able to further promote maturation of mESC-derived cardiomyocytes.

Hybrid scaffolds consisting of polymers, ECMPs, and other materials have been implemented as an effective means to direct the fate of hESCs toward cardiomyocytes. For example, poly(lactic-*co*-glycolic acid) (PLGA) and collagen scaffolds were fabricated using electrospinning methods to precisely control the fiber diameters to mimic the ECM of *in vivo* cardiac tissue.[180] Differentiation of hESCs to cardiomyocytes on the composite PLGA/collagen scaffolds was found to be more efficient than on the substrates composed solely of PLGA or collagen.

Biomaterial-Based Methods for the *In Vivo* Delivery of Stem Cell Populations to Repair Cardiac Tissue

Current methods of stem cell delivery for cardiac regenerative purposes are inadequate as the integration and survival of transplanted cells is low, thereby reducing their therapeutic potential.[181,182] As such, biomaterial scaffolds have emerged as a promising approach to effectively deliver stem cells to damaged cardiac tissue. The following design considerations must be taken into account when implementing biomaterial-based approaches for the delivery of stem cells to repair cardiac tissue: (i) provide the appropriate strength and elasticity to withstand contraction and relaxation (or cyclic stretch) of the myocardium, (ii) capable of biodegrading without the generation of any toxic products once new tissue is formed, and (iii) conducive to support the contraction, proliferation, and differentiation of stem cells and their derivatives. In this section, we will review the various biomaterial-based approaches that are being used to deliver stem cells to repair injured or diseased myocardium.

ECMP-based biomaterials. Collagen-based scaffolds have been widely implemented as an efficient means to deliver stem cells to damaged myocardial tissue.[183–186] In fact, it has been reported that the use of collagen as a stem cell delivery vehicle significantly reduced the localization and engraftment of transplanted cells to other organs and uninjured myocardium.[183] Collagen-based matrices have been used to deliver MSCs to damaged cardiac tissue in animal models of MI. For example, a patch consisting of autologous MSCs seeded in a collagen scaffold that was engrafted into the epicardial surface of a chronic MI scar led to enhanced angiogenesis and significantly improved cardiac function.[184] In a related study, Simpson et al demonstrated that the delivery of MSCs using such methods led to elevated ventricular remodeling and function compared to MSCs delivered through direct injection.[185] Addition of glycosaminoglycans to these MSC seeded collagen scaffolds has led to improved cell retention, neovascularization, and tissue repair.[187]

The use of collagen scaffolds for cell delivery has not been limited to use with only MSCs. Araña et al examined the effect of collagen patches seeded with ADSCs on cardiac function in models of chronic MI.[188] The delivery of ADSCs in collagen substrates led to increased cell engraftment as well as improvement in cardiac function, myocardial remodeling, and revascularization. Moreover, the level of fibrosis, a factor that critically impairs cardiac recovery in chronic MI, was significantly reduced in animals that received ADSC seeded collagen patches.

Matrigel™ has been used as a substrate to deliver PSCs and their derivatives to sites of cardiac damage. Kofidis et al used Matrigel™ scaffolds to deliver undifferentiated mESCs to the damaged ventricular areas of a postinfarcted heart.[189] Overall, the mESC seeded scaffold engrafted within the injured area and prevented ventricular wall thinning. Importantly, no signs of teratoma formation were reported, and the

engrafted cells remained viable and expressed high levels of the cardiomyocyte-related proteins, connexin 43 and alpha-sarcomeric actin.

Decellularized matrices. Because of the ability to match the biochemical properties of native heart tissue, decellularized matrices are emerging as a promising approach to deliver stem cells to regions of cardiac damage. Additionally, it has been shown that when delivered to the *in vivo* heart tissue, these scaffolds have the potential to promote stem cell differentiation, cardiac regeneration, and angiogenesis.[190] Lesman and colleagues used decellularized matrices and hESC-derived cardiomyocytes as the basis for the engineering of 3-D tissue-engineered cardiac muscle.[191] Upon transplantation to the heart, the engineered muscle formed cardiac tissue grafts and integrated with the host coronary vasculature. In the future, such engineered tissue could be used to ameliorate or reverse the effects of cardiac damage.

Natural biomaterial scaffolds. Fibrin-based scaffolds have been effectively used to improve adult stem cell engraftment and survival in cardiac regenerative medicine applications. Embedding ADSCs in fibrin scaffolds leads to improved ventricular function and remodeling in a model of acute MI when compared to direct ADSC implantation.[192] Along similar lines, Guo et al demonstrated that the delivery of CPCs in fibrin matrices promoted their survival, engraftment, and cardiomyogenic differentiation in an animal model of MI.[193] In turn, improved myocardial tissue repair and cardiac function were observed in animals that received CPCs delivered in fibrin scaffolds compared to the animals that received CPCs or fibrin alone.

Polysaccharide containing matrices have been broadly used as adult stem cell delivery vehicles. Encapsulation of MSCs[194–196] or ADSCs[197] in alginate enhanced retention and survival of MSCs in several animal models of MI. As such, alginate encapsulation facilitated paracrine effects, such as increased angiogenesis and decreased scaring, and improved cardiac function.[195] Similarly, Wang and colleagues demonstrated that delivery of ADSCs in chitosan hydrogels to regions of the heart that had been damaged by MI enhanced cell survival and increased differentiation to cardiomyocytes.[165] Moreover, cell delivery in chitosan prevented adverse matrix remodeling, elevated angiogenesis, and preserved cardiac function. Interestingly, a direct comparison of alginate and chitosan matrices revealed that the delivery of ADSCs in alginate scaffolds improved cell retention in the infarcted heart when compared to chitosan scaffolds.[198] In order to leverage the beneficial effects of both alginate and chitosan, Ceccaldi et al examined the efficacy of MSCs seeded in composite scaffolds that comprised various alginate/chitosan ratios in ameliorating the effects of acute MI.[199] The authors found that an alginate/chitosan ratio of 40/60 led to the highest improvement in cardiac function and attenuation of fibrosis.

The application of PSCs and their derivatives for *in vivo* cardiac repair have benefited from the use of natural biomaterial scaffolds as delivery vehicles. For example, the transplantation of hESC-derived cardiomyocytes in photocrosslinkable PEGylated-fibrinogen matrices led to increased ventricular performance in a MI model.[200] Along similar lines, several studies have demonstrated that fibrin scaffolds loaded with hESC-derived cardiac cells and mESC-derived cardiac cells can reverse the fibrotic effects of MI and lead to improved ventricular function when delivered to regions of cardiac damage.[201–203] Finally, the coinjection of mESCs and hESCs in polysaccharide-based scaffolds such as alginate and chitosan in infarcted heart tissue has led to the generation of new myocardium and preservation of cardiac function.[204,205]

Synthetic polymer-based materials. Owing to the ability to tailor their physicochemical properties, synthetic polymer-based materials have been widely implemented as scaffolds for the *in vivo* delivery of stem cells for cardiac tissue engineering purposes. For instance, bioengineered polyglycolic acid (PGA) cloths seeded with MSCs have been used to induce angiogenesis and improve function in an infarcted heart.[206] In a related study, transplantation of MSCs within PLCL scaffolds reduced scar size and improved cardiac function in an animal model of MI.[207] Similarly, MSCs seeded in PLCL scaffolds that were injected into infarcted cardiac tissue migrated to damaged myocardium, augmented neovascularization, and improved ventricular function.[208]

Several injectable biodegradable hybrid materials have been engineered for cardiac tissue engineering applications. For example, Xu et al developed a hydrogel that comprised thiolated collagen and oligo(acryloyl carbonate)-*b*-poly(ethylene glycol)-*b*-oligo(acryloyl carbonate) (OAC-PEG-OAC) for the encapsulation of MSCs to be used for cardiac regeneration purposes.[209] As such, these composite hydrogels combined the intrinsic biological activity of collagen and the structural integrity of the OAC-PEG-OAC polymers. When used in an infarction model, these hybrid hydrogels reduced infarct size, increased ventricular wall thickness, and improved cardiac function. In a similar study, the intramyocardial delivery of MSCs in silanized poly(hydroxypropyl) methylcellulose hydrogels attenuated ventricular remodeling and rescued cardiac function in a model of MI.[210]

Polymer-based scaffolds have also been used for the delivery of PSCs and their derivatives to regions of damaged myocardial tissue. In one such study, a heart patch was engineered from the synthetic elastomer PGS that was seeded with hESC-derived cardiomyocytes.[211] Upon suture over the left ventricle, these patches remained intact over a two-week period without any negative impacts on ventricular function. In the future, such patches could be used for stem cell-based cardiac regeneration strategies.

Future Trends and Techniques in Biomaterial-Based Approaches for Stem Cell Myocardial Tissue Engineering

One of the main challenges that need to be addressed to move stem cell-based approaches for cardiac regeneration

from bench-to-bedside is enhancing the survival, engraftment, and differentiation of cells in the ischemic or fibrotic host tissue.[212] One emerging approach to overcome this hurdle is to engineer biomaterial-based systems for the dual delivery of pro-survival soluble signaling cocktails and stem cells to regions of damaged myocardium. For example, thermosensitive *N*-isopropylacrylamide (NIPAAm) hydrogels have been engineered to release bFGF for the enhanced differentiation of MSCs into cardiomyocyte-like cells under ischemic conditions.[213] Karam and colleagues developed a PLGA-based system that could be used to encapsulate ADSCs along with two cardiac inducing growth factors, hepatocyte growth factor and insulin-like growth factor (IGF-1).[213] The authors demonstrated that sustained release of hepatocyte growth factor and IGF-1 enhanced the cardiac differentiation of encapsulated ADSCs. Similarly, encapsulation of CPCs in an alginate hydrogel containing superoxide dismutase, a reactive oxygen species scavenger, prevented doxorubicin-induced apoptosis.[214] Finally, biomaterial-based scaffolds loaded with prosurvival and proangiogenic factors such IGF-1 and thymosin β4 have been used to deliver hiPSCs and their derivatives to infarcted cardiac tissue.[215] In fact, the use of such scaffolds led to reduced infarct size and the formation new vasculature in the host tissue. In the future, engineered biomaterials to deliver stem cells along with pro-survival drugs may enable sustained tissue preservation and potentially promote regeneration of ischemic cardiac tissue.[134]

Another emerging approach for the stem cell-based repair of ischemic tissue is the use of biomaterials to develop pre-vascularized tissues that can be delivered via surgery to sites of cardiac injury. To that end, Pagliari et al developed a multistep procedure to engineer pre-vascularized 3-D cardiac bio-substitutes.[216] Specifically, MSCs and CPCs were seeded in a highly porous biocompatible gelatin scaffold. Exposure of the scaffold to fluid flow within a modular bioreactor stimulated the formation of VCAM-1-positive vascular cells forming tube-like structures around the scaffold and pores, which contact cTnT expressing cardiomyocytes. One could imagine that in the future such vascularized constructs could interconnect with host vasculature and be used to stimulate repair of damaged *in vivo* cardiac tissue.

In the future, the ability to effectively move biomaterial approaches for stem cell-based myocardial tissue engineering from bench-to-bedside will require limiting the potential for complications in patients. For example, while recent progress has been made in the directed differentiation of human pluripotent stem cells (hPSCs) to immature myocardial cell types,[26] these protocols yield a heterogeneous cell population consisting of nodal-, atrial-, and ventricular-like CM subtypes.[36,217] As such, these heterogeneous populations have displayed a high degree of arrhythmogenic properties, thereby potentially limiting their clinical application.[44,45] Before such cell types can be used in patients, reproducible methods for the generation of homogenous, subtype-specific CMs need to be developed. Another challenge of stem cell-based cardiac therapies is the high potential for allogenic immune rejection by recipients.[218] To that end, continued advances in directed genome modification may allow for the generation of stem cell-derived cardiac tissue that evades allogenic immune responses.[219]

Author Contributions

Contributed to the writing of the manuscript: JC, MN, DAB. Made critical revisions and approved final version: JC, MN, DAB. All authors reviewed and approved of the final manuscript.

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