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Patching the Heart: Cardiac Repair from Within and Outside

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Abstract

Transplantation of engineered tissue patches containing either progenitor cells or cardiomyocytes for cardiac repair is emerging as an exciting treatment option for patients with postinfarction left ventricular (LV) remodeling. The beneficial effects may evolve directly from remuscularization or indirectly through paracrine mechanisms that mobilize and/or activate endogenous progenitor cells to promote neovascularization and remuscularization, inhibit apoptosis, and attenuate LV dilatation and disease progression. Despite encouraging results, further improvements are necessary to enhance current tissue-engineering concepts and techniques and to achieve clinical impact. Herein, we review several strategies for cardiac remuscularization and paracrine support that can induce cardiac repair and attenuate LV dysfunction from both within and outside the myocardium.

Keywords

Cell Therapy; Heart Failure; Tissue Engineering; Paracrine

INTRODUCTION

Heart failure often develops as a consequence of the limited endogenous capacity for cardiac regeneration after acute myocardial infarction (MI). [1] Cardiac progenitor cells (CPCs) that have been transplanted into hearts with MI can repair the damaged myocardium to some degree, despite their low engraftment rates and limited evidence of transdifferentiation into myocardial cells. [2-14] However, as progenitor- and stem cell-based tissue engineering technologies continue to evolve, it now appears possible to deposit and retain large numbers of therapeutic cells in close proximity to the region of myocardial tissue damage, and even to replace scar tissue, thus preventing scar bulging and LV dilation, reducing wall stress at the border zone (BZ) of the infarct, improving myocardial bioenergetics, and, finally, remuscularizing the heart. [15-18]

Tissue-engineered cardiomyoplasty, using engineered myocardium or cell sheets, was first introduced at the beginning of the millennium[19-25]. Recently, the paracrine activity of the engineered tissue has been enhanced by using fibrin patches as the cell carrier. [16-18, 26] Importantly, evidence of myocardial salvage has been obtained with both the direct-remuscularization and paracrine approaches in rodent and porcine allograft models. [17, 23, 25] Paracrine factors led to significant increases in BZ vascular density and in the regeneration

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None

of myocytes from endogenous CPCs. Although these reports suggest that tissue-engineered patches could have therapeutic potential for the repair of myocardial injury, the overall engraftment rate and biophysical integration of grafts needs further optimization.

Engineering cardiac patches for heart repair is a challenging biotechnological objective. This review summarizes the progress in cardiac patch engineering (Table 1) and critically analyzes the problems and considerations that have to be incorporated into the design of an optimal engineered cardiac patch for heart-failure therapy. Despite the relatively straightforward biological rationale, the mechanisms responsible for the observed therapeutic effects are, in most cases, incompletely understood. Here, we review the available data on tissue-engineered heart repair and highlight evidence of the specific mechanisms of action associated with tissue-engineered patches.

PATCHING THE HEART WITH TISSUE-ENGINEERED MYOCARDIUM

Generating functional bioengineered human cardiac tissue for the treatment of end-stage heart disease has been a tremendous challenge for researchers and physicians alike. Tissue engineering was first proposed as a means to rebuild organs for therapeutic applications in the early 1990s[85] and later extended to the cardiac field.[20-22, 86] Generally, bioengineered cardiac tissue constructs can be divided into two categories: scaffold-based and scaffold-free. A number of three-dimensional (3D) scaffolds have been fabricated from natural biological materials, such as collagen, fibrin, and alginate, from naturally occurring extacellular matrix mixtures (Matrigel™) or decellularized heart matrix, and from synthetic polymers.

SCAFFOLD-BASED APPROACHES

Collagen

Collagen is the most prevalent extracellular component of the myocardium and has been used as a matrix for studying myocardial electrophysiology and contraction since the early 1990s[27, 28]. It is self-polymerizing at 37°C and physiological pH, can be molded into a variety of shapes, and when seeded with heart cells, the resulting matrix possesses many of the physiological characteristics of cardiac tissue[29, 30, 32, 37, 41, 87]. A key property of collagen hydrogel-based engineered heart muscle (EHM) is its contractile performance, which reproduces many, but not all, aspects of contractile performance in native myocardium[29, 30, 37]. The first EHM produced from mammalian (in this case, neonatal rat heart) cells was generated with a combination of collagen and Matrigel™ [29]. Mechanical loading was subsequently identified as an important factor for tissue maturation[29, 38], and direct comparisons with the development of native heart tissue identified organotypic maturation in collagen-based EHM that possessed the capacity to support hypertrophic growth[32]. Implantation of collagen-based EHM promoted further maturation, which was paralleled by vascularization and innervation[20, 23].

One week after transplantation onto uninjured rat hearts, collagen scaffolds containing cardiomyocytes that had been generated through the controlled differentiation of human embryonic or induced-pluripotent stem cells (hESC-CMs or hiPSC-CMs, respectively), endothelial cells (ECs), and stromal cells contained vascular structures derived from the transplanted human cells and were perfused by the host animal's coronary circulation.[41] In a rat MI model, transplantation of a collagen scaffold containing mesenchymal stem cells (MSCs) was associated with improvements in infarct size, ventricular wall thickness, angiogenesis, perfusion, and contractile function, and with increases in the growth of myofibroblast-like tissue[44]. Collagen scaffolds have also been used to deliver vascular endothelial growth factor (VEGF) both directly, by modifying the protein to include a

collagen-binding domain[88], or indirectly, by seeding the scaffold with cells that have been genetically engineered to express VEGF[89], thereby extending the duration of protein delivery and promoting vascular growth in infarcted myocardial tissue[88].

The fusion of individual EHM rings into multi-loop constructs enables the creation of engineered tissues with, in principle, unlimited dimensionality (Figure 1)[31]. Implantation of a multi-loop patch two weeks after MI in rats led to sustained therapeutic benefits with enhanced regional contractility 4 weeks after engraftment[23]. Furthermore, an alternative pattern of EHM ring assembly can be used to generate cardiac-tissue pouches that provide restraint and contractile support to failing hearts (Figure 1)[33], and in-vitro studies demonstrate that EHM rings can serve as a test bed for receptor pharmacology [29], growth factor signaling [30, 34], and target validation through the use of adenoviral transduction [35] and hypertrophic signaling [32, 90]. hESC- and hiPSC-CMs can also be used to generate collagen-based EHM[41, 87, 91], and parthenogenetic stem cells have recently been introduced as another source of stem cells for cardiomyocytes and myocardial tissue engineering [40]. Further advances in collagen-based myocardial tissue will likely involve biomimetic culture platforms that incorporate electrical[36] and mechanical stimulation[29].

Fibrin

Fibrinogen is cleaved and cross-linked by exposure to thrombin and clotting factor XIII to form an insoluble fibrin mesh that captures blood cells to form a blood clot. Fibrin meshes can also be used to capture any target cell for tissue engineering. In vivo, fibrin attracts leukocytes and, in particular, macrophages, and is slowly lysed by endogenous proteases. Anisotropic alignment of rat neonatal cardiac cells in fibrin scaffolds was associated with increases in twitch force, despite declines in total collagen and protein content[47]. Fibrinogen can also be conjugated with polyethylene glycol (PEG) to generate scaffolds that covalently bind growth factors and other proteins[51, 52], and drug screening platforms have recently been established with fibrin-based engineered heart tissue[53, 92]. In a mouse MI model, treatment with a PEGylated fibrin patch containing stromal cell-derived factor-1 (SDF-1) was associated with increases in CPC recruitment and improvements in infarct size and cardiac function; the patch was placed over the site of infarction, where it was associated with increases in SDF-1 levels for up to 28 days[51].

Fibrin patches with defined biophysical properties can be created by mixing solutions of fibrinogen and thrombin, and because the mixture typically solidifies in less than one minute, the patch can be created in situ by injecting the two solutions into a mold placed over the infarct site. This method has been used to deliver autologous MSCs to swine[26], and hESC-derived vascular cells (hESC-VCs) (i.e., smooth-muscle cells [SMCs] and ECs) to both swine and mice[17, 18, 24], after experimentally induced MI. The transplanted MSCs differentiated into myocyte-like cells[8] and were associated with greater thickening of the infarcted wall during contraction, while the hESC-VC treatments were associated with improvements in myocardial function, perfusion, and energy metabolism, and with declines in left-ventricular wall stress and remodeling.[93] Furthermore, the proportion of cells retained in the ischemic region was significantly higher when the cells were administered via a patch than when injected directly into the myocardium.[93] Thus, a fibrin patch can be used as a unique platform for cell delivery.

Other 3D Biological Scaffolds

Other 3D biomaterials that have been used to engineer myocardial tissue equivalents include alginates[54, 56] and silks[62]. However, preformed matrices do not appear to be ideally suited for the assembly of cardiomyocytes into a functional 3D cardiac syncytium of contracting cells because the rigid structures/pores of the matrix isolate the cardiomyocytes from each

other, thereby preventing direct intracellular contact. This caveat also applies to preformed collagen/gelatin sponges; however, gold nanowires have been used to improve the synchronous excitation of engineered cardiac tissue by bridging the electrically resistant pore walls of alginate.[57] In addition, it seems essential that the exogenous matrix be rapidly replaced to prevent interference with tissue formation. Vascularization of the matrix occurs quickly in conjunction with the immune response to the presence of foreign materials.

Decellularized Matrix

Recellularization of a previously decellularized rat heart was successfully attempted in 2008, which suggests that whole organs can be reengineered by using a natural ECM blueprint[70]. Natural scaffolds made from decellularized porcine small-intestinal submucosa (SIS)[75] and bladders[72] and from human myocardial tissues[71] have been used to generate 3D cardiac patches. For example, decellularized SIS has been layered with multiple sheets of neonatal rat cardiomyocytes to produce patches of up to 800-μm thickness, resulting in cardiac muscle constructs that contracted synchronously for up to 10 days[75]. Cell sheets broke apart when applied to decellularized bladder matrix, presumably because of the roughness of the matrix surface, but remained intact and proliferative when the voids of the matrix were filled with hyaluronan hydrogel; the hydrogel also enabled a greater number of cells to be seeded. Hydrogels made of fibrin and containing suspended human mesenchymal progenitor cells have been applied to decellularized sheets of human myocardium[71] to create cardiac patches that were subsequently evaluated in a nude-rat model of MI. The patches dramatically increased both the recruitment of mesenchymal progenitor cells and vascular growth in the infarcted region, and measurements of contractility and left ventricular (LV) dimensions were similar to those obtained in uninjured hearts; integration of the patch with the myocardium of the recipient heart was not examined.

Synthetic Polymers

Polygycolic acid (PGA)[68], poly ε-caprolactone-co-l-lactide (PCLA)[69], poly-glycerolsebacate (PGS)[66], and polydimethylsiloxane (PDMS)[94] have been extensively used as tissue engineering substrates. These materials typically must be coated with extracellular matrix proteins to enable cardiomyocyte attachment and have many of the same limitations associated with biological matrices (as described above) for heart-muscle engineering on a macroscopic scale. However, they may be well suited for depositing cells with paracrine activity onto the heart and for providing structural support to prevent further ventricular dilatation.

Injectable Systems

Injectable scaffolds have been used to prevent LV wall thinning and bulging of the ischemic myocardium, to inhibit LV remodeling[59] and to promote angiogenesis by recruiting endogenous CPCs[60, 61]. The injection of a cell-free, in situ–forming, alginate hydrogel into recent (7 days) and old infarcts (60 days) provided a temporary scaffold that attenuated adverse cardiac remodeling and improved contractility, and the benefits were comparable to those obtained with the intramyocardial injection of neonatal cardiomyocytes[59]. Angiogenesis can be enhanced by integrating RGD motifs into an alginate hydrogel[60], and similar results were obtained with alginate scaffolds that had been loaded with insulin-like growth factor 1 (IGF-1) and hepatocyte growth factor (HGF)[61]. In a rat model of acute MI, intramyocardial injections of the affinity-bound IGF-1/HGF alginate biomaterial increased angiogenesis and mature blood vessel formation at the infarct, preserved scar thickness, attenuated infarct expansion, and reduced scar fibrosis after 4 weeks. In an alternative approach, cardiomyocyte retention was improved when the cells were co-injected with nanofibers made of poly-glycerolsebacate (PGS)[67].

SCAFFOLD-FREE APPROACHES

The development of scaffold-free cell sheets first became practical after the invention of a temperature-responsive polymer substrate, poly-(*N*-isopropylacrylamide) (PIPAAm)[95]. Cardiomyocyte cell sheets are grown on the polymer, released by a drop in temperature, and then stacked to yield multilayered tissues[19, 79, 96]. Electrical connections between cardiomyocyte sheets form quickly, probably because, in contrast to enzymatic sheet release, the temperature responsive plates leave all surface protein structures intact, thus facilitating gap junction formation[73]. Capillary growth can be induced by creating stacks composed of both cardiomyocyte and ECs[77,74], and engrafted cell sheets survived and remained contractile for up to 1 year after implantation[76]. Other scaffold-free tissue engineering approaches include the construction of cardiac microtissues by using a hanging drop method or spontaneous aggregation.[81, 97] Both cell-sheet and cell-aggregation technologies have been successfully used with cardiomyocytes derived from pluripotent stem cells[25, 80, 81, 83, 84], and recently, composite cell sheets consisting of monkey adipose-derived stromal cells and monkey ESC-derived SSEA-1^+ cells were tested in vivo with encouraging results[78]. Thus, cell sheet-based cardiac tissue engineering may prove to be a useful strategy for cardiovascular tissue repair that suppresses LV dilatation, increases wall thickness, and improves LV chamber function.

MYOCYTE TURNOVER

The concept of cardiomyocyte turnover in the adult heart is relatively new, and whether the limited regenerative capacity of the adult heart is mediated by the proliferation of pre-existing cardiomyocytes or through the activity and derivatives of endogenous CPCs remains a matter of intense debate. [98-108] Several markers can be used to identify endogenous CPCs with cardiogenic potential, including c-Kit[2, 3], Sca-1[14, 109], Abcg2[110] and Isl1 [111, 112]. Once the types of CPCs are fully characterized, and their pathways of activation are deciphered, biomaterial patches can be designed to promote CPC activation and cardiomyocyte turnover, and then placed over the site of myocardial injury, leading to the more comprehensive activation of mechanisms for myocyte regeneration both within and outside the myocardium.

PATCH-ASSOCIATED PARACRINE SUPPORT in MYOCARDIAL PROTECTION and the ACTIVATION OF ENDOGENOUS REPAIR MECHANISMS

Cell Patch- and Paracrine Support-induced Myocardial Repair from Endogenous Cardiac Progenitor Cells

Although CPCs that have been transplanted into hearts after MI can repair the damaged myocardium to some degree, this activity is limited because the engraftment rate is low and trans-differentiation of the engrafted cells occurs even less frequently.[4-10] Thus, myocyte regeneration from the transplanted cells has been suboptimal[11-14], and additional benefits might be obtained by applying a fabricated cell patch over the periscar BZ to mobilize endogenous repair mechanisms, stabilize the chronically evolving infarct scar, reduce BZ wall stress, and improve myocardial bioenergetics. The Zhang group used a fibrin patch to enhance delivery of hESC-VCs for myocardial repair. [17] The hESC-VCs that were loaded into the patch released a variety of cytokines that promote angiogenesis and survival, and reduce apoptosis. [17, 93] Both *in vitro* and *in vivo* experiments demonstrated that the hESC-VCs effectively inhibited myocyte apoptosis, and the patch-enhanced delivery of hESC-VCs alleviated abnormalities in BZ myocardial perfusion, contractile dysfunction, and LV wall stress. These results were also accompanied by the pronounced recruitment of endogenous

c-kit^{+} cells to the injury site, and were directly associated with a remarkable improvement in myocardial energetics, as measured by a novel *in vivo* ^{31}P magnetic resonance spectroscopy method. Similar findings were obtained with fibrin patches containing VCs derived from human induced pluripotent stem cells (hiPSCs) [18]. Four weeks after MI and treatment, LV structural and functional abnormalities were less severe in hearts that received the patch-enhanced celltherapy, and these improvements were accompanied by significant reductions in infarct size[18]. hiPSC-VC transplantation also mobilized endogenous progenitor cells into the BZ, attenuated regional wall stress, stimulated neovascularization, and improved BZ perfusion, which led to marked increases in BZ contractile function and ATP turnover rate[18].

Cytokine-associated Myocardial Protection and Increased Density of Myocardial Resistant Vessels

It is a rather consistent finding in the literature that,1) only a very small percentage of transplanted cells show long term engraftment in the recipient myocardium[8, 113, 114], and 2) that an even smaller fraction of the engrafted CPCs trans-differentiate into cardiomyocytes or VCs[8, 113, 114]. These findings have led to the belief that early paracrine interactions between the transplanted cells and native cardiomyocytes, VCs, and/or (possibly) cardiac and vascular progenitor cells provide much of the benefit associated with cell transplantation[115, 116]. Indeed, many reports indicate that cell transplantation performed shortly after an ischemia-reperfusion (I-R) event is associated with early declines in apoptosis of the injured cardiomyocytes. This sparing of native cardiomyocytes that would otherwise have died after the initial I-R insult likely reduced the size of the infarct and led to the subsequent declines in LV remodeling and dysfunction that were observed in cell-treated animals[8, 10]. Furthermore, evidence reported by Yamashita's group indicates that cardiomyocytes may be the main source of VEGF in cardiac-tissue sheets[80]; they found that the omission of cardiomyocytes from transplanted sheets led to the disappearance of neovascularization and functional improvement, suggesting that the beneficial effects of the sheet were induced by cardiomyocyte-secreted cytokines.

Although these findings support the view that paracrine effects initiated by the engrafted cells contribute to declines in infarct size by decreasing apoptosis when the therapy closely follows the ischemic event, cardiomyocyte apoptosis may also be reduced at later time points, and if so, this decline may result from the somewhat delayed increase in BZ capillary density after cell-patch transplantation[8, 10, 117]. The patch-enhanced delivery of hESC-VCs and hiPSC-VCs was accompanied by significant improvements in myocardial perfusion in both the infarct and the BZ as well as a significant increase in the number of resistant vessels in the myocardium, which suggests that vessels generated by the transplanted, stem-cell derived VCs were functional[17, 18]. Furthermore, these vessels are the smallest muscular arterioles (50 ~150 μm) that regulate myocardial perfusion, and each arteriole supports capillaries where the exchange of oxygen and carbon dioxide occurs. Thus, since it has been previously reported that LV hypertrophy and failure is associated with subendocardial ischemia during elevated cardiac work states[118, 119], this increase in resistant-vessel density could provide the structural basis for the increases in myocardial blood flow, declines in apoptosis, and improvements in LV contractile performance that have been observed in preclinical and clinical studies[17, 120-122].

CONCLUSION and FUTURE WORKS

Acute myocardial damage is exacerbated by chronic myocardial overload and LV dilatation, which often leads to heart failure. Consequently, the goal for future regenerative/reparative cardiac therapies includes at least two components: 1) minimizing oxidative myocardial damage and remodeling secondary to LV chamber dilatation, and 2) rebuilding the muscle

of infarcted and chronically failing hearts to improve contractile performance. Although the paracrine mechanisms induced by cytokine administration may be well suited for delaying and preventing pathological remodeling and disease progression, the repair of significant ventricular scar bulging will likely require the generation of new cardiac muscle. Thus, strategies that combine the administration of paracrine factors with the creation and integration of engineered cardiac tissue are particularly attractive for the treatment of LV dilatation in hearts with postinfarction LV remodeling.

The beneficial effects of engineered cardiac tissue patches have been clearly demonstrated in animal models, and the clinical feasibility of this approach is supported by the successful transplantation of collagen sponges that lack cardiomyocytes[123, 124] and of cell sheets that contain skeletal myoblasts[125]. Nevertheless, cardiac patches have yet to be extensively investigated in clinical studies, primarily because suitable sources for human cardiomyocytes are lacking. The availability of human stem cells, including hiPSCs, and highly efficient protocols for directing differentiation[15, 126, 127] could alleviate this scarcity and may enable patients to be treated with patches engineered from their own cells,[15, 127] thereby minimizing the potential complications that could evolve from the induction of the immune and inflammatory responses. However, the time required to generate hiPSC-CMs precludes their use in patients who need prompt treatment; thus, many patients who are candidates for cardiac-patch therapy will benefit from the continued development of patches that contain allogenic cell lines.

Cardiac patch therapy can only be successful if the patch quickly becomes integrated and perfused by the recipient’s coronary circulation. Although current tissue engineering platforms are rapidly vascularized, and many interventions can successfully promote neovascularization in the myocardium over time, the vessels may not develop quickly enough to prevent a significant fraction of the cardiomyocytes in the patch from initiating necrotic processes and apoptosis, which can occur within 30 minutes of exposure to no flow ischemia. Furthermore, the engineered tissue is less mature than the recipient myocardium, so endogenous mechanisms for sensing and responding to hypoxic conditions within the patch may not be activated by the absence of immediate vascular support. Thus, the integrity and function of the transplanted patch may be improved by the addition of factors that stimulate vessel growth, impede apoptosis, and promote graft maturation.

Engineered cardiac-tissue patches possessing both contractile and paracrine activity have been successfully applied in acute and subacute models of myocardial injury, and patches designed to promote CPC activity and/or cardiomyocyte turnover could be placed over the injury site to stimulate cardiomyocyte regeneration both at the myocardial surface and within the tissue (Figure 2). This strategy can also be extended to include the creation of engineered cardiac-tissue pouches that provide paracrine and contractile support over the entire ventricular surface (Figure 1). However, the clinical acceptance of any patch-based therapy will require the development of a practical and minimally invasive delivery method; for example, an endoscope/catheter-based system could be used to access the pericardial sac through the abdomen and diaphragm, and then solutions could be injected through the catheter to form a hydrogel-based patch in situ. Collectively, these advancements could lead to the development of a new generation of patch-based cellular therapies that combine paracrine support and remuscularization to promote cardiac repair both from within and from outside the myocardial tissue.

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References

1. McMurray JJ, Pfeffer MA. Heart failure. Lancet. 2005; 365:1877–1889. [PubMed: 15924986]
2. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. Nature. 2001; 410:701–705. [PubMed: 11287958]
3. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003; 114:763–776. [PubMed: 14505575]
4. Muller-Ehmsen J, Whittaker P, Kloner RA, Dow JS, Sakoda T, Long TI, Laird PW, Kedes L. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. Journal of molecular and cellular cardiology. 2002; 34:107–116. [PubMed: 11851351]
5. Murry CE, Whitney ML, Laflamme MA, Reinecke H, Field LJ. Cellular therapies for myocardial infarct repair. Cold Spring Harb Symp Quant Biol. 2002; 67:519–526. [PubMed: 12858578]
6. Reinecke H, Murry CE. Taking the death toll after cardiomyocyte grafting: A reminder of the importance of quantitative biology. Journal of molecular and cellular cardiology. 2002; 34:251–253. [PubMed: 11945017]
7. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation. 2002; 105:93–98. [PubMed: 11772882]
8. Zeng L, Hu Q, Wang X, Mansoor A, Lee J, Feygin J, Zhang G, Suntharalingam P, Boozer S, Mhashilkar A, Panetta CJ, Swingen C, Deans R, From AH, Bache RJ, Verfaillie CM, Zhang J. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. Circulation. 2007; 115:1866–1875. [PubMed: 17389266]
9. Tang XL, Rokosh G, Sanganalmath SK, Yuan F, Sato H, Mu J, Dai S, Li C, Chen N, Peng Y, Dawn B, Hunt G, Leri A, Kajstura J, Tiwari S, Shirk G, Anversa P, Bolli R. Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. Circulation. 2010; 121:293–305. [PubMed: 20048209]
10. Wang X, Jameel MN, Li Q, Mansoor A, Qiang X, Swingen C, Panetta C, Zhang J. Stem cells for myocardial repair with use of a transarterial catheter. Circulation. 2009; 120:S238–246. [PubMed: 19752374]
11. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE, Dulce R, Pattany PM, Valdes D, Revilla C, Heldman AW, McNiece I, Hare JM. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circulation research. 2010; 107:913–922. [PubMed: 20671238]
12. Mouquet F, Pfister O, Jain M, Oikonomopoulos A, Ngoy S, Summer R, Fine A, Liao R. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. Circulation research. 2005; 97:1090–1092. [PubMed: 16269652]
13. Pfister O, Mouquet F, Jain M, Summer R, Helmes M, Fine A, Colucci WS, Liao R. Cd31- but not cd31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. Circulation research. 2005; 97:52–61. [PubMed: 15947249]
14. Wang X, Hu Q, Nakamura Y, Lee J, Zhang G, From AH, Zhang J. The role of the sca-1+/cd31- cardiac progenitor cell population in postinfarction left ventricular remodeling. Stem Cells. 2006; 24:1779–1788. [PubMed: 16614004]

15. Ye L, Zhang S, Greder L, Dutton J, Keirstead SA, Lepley M, Zhang L, Kaufman D, Zhang J. Effective cardiac myocyte differentiation of human induced pluripotent stem cells requires vegf. PloS one. 2013; 8:e53764. [PubMed: 23326500]
16. Ye L, Xiong Q, Zhang P, Lepley M, Swingen C, Zhang J, Kamp TJ, Kaufman D, Zhang J. Transplantation of human induced pluripotent stem cells derived cardiac cells for cardiac repair [abstract]. Circulation. 2012; 126:A16457.
17. Xiong Q, Ye L, Zhang P, Lepley M, Swingen C, Zhang L, Kaufman DS, Zhang J. Bioenergetic and functional consequences of cellular therapy: Activation of endogenous cardiovascular progenitor cells. Circulation research. 2012; 111:455–468. [PubMed: 22723295]
18. Xiong Q, Ye L, Zhang P, Lepley M, Tian J, Li J, Zhang L, Swingen C, Vaughan JT, Kaufman DS, Zhang J. Functional consequences of human induced pluripotent stem cell therapy: Myocardial atp turnover rate in the in vivo swine heart with postinfarction remodeling. Circulation. 2013; 127:997–1008. [PubMed: 23371930]
19. Shimizu T, Yamato M, Isoi Y, Akutsu T, Setomaru T, Abe K, Kikuchi A, Umezu M, Okano T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circulation research. 2002; 90:e40. [PubMed: 11861428]
20. Zimmermann WH, Didie M, Wasmeier GH, Nixdorff U, Hess A, Melnychenko I, Boy O, Neuhuber WL, Weyand M, Eschenhagen T. Cardiac grafting of engineered heart tissue in syngenic rats. Circulation. 2002; 106:I151–157. [PubMed: 12354725]
21. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. Circulation. 1999; 100:II63–69. [PubMed: 10567280]
22. Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, Granot Y, Cohen S. Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? Circulation. 2000; 102:III56–61. [PubMed: 11082363]
23. Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. Nature medicine. 2006; 12:452–458.
24. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, Hao H, Ishino K, Ishida H, Shimizu T, Kangawa K, Sano S, Okano T, Kitamura S, Mori H. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nature medicine. 2006; 12:459–465.
25. Kawamura M, Miyagawa S, Miki K, Saito A, Fukushima S, Higuchi T, Kawamura T, Kuratani T, Daimon T, Shimizu T, Okano T, Sawa Y. Feasibility, safety, and therapeutic efficacy of human induced pluripotent stem cell-derived cardiomyocyte sheets in a porcine ischemic cardiomyopathy model. Circulation. 2012; 126:S29–37. [PubMed: 22965990]
26. Liu J, Hu Q, Wang Z, Xu C, Wang X, Gong G, Mansoor A, Lee J, Hou M, Zeng L, Zhang JR, Jerosch-Herold M, Guo T, Bache RJ, Zhang J. Autologous stem cell transplantation for myocardial repair. American journal of physiology. Heart and circulatory physiology. 2004; 287:H501–511. [PubMed: 15277194]
27. Souren JE, Schneijdenberg C, Verkleij AJ, Van Wijk R. Factors controlling the rhythmic contraction of collagen gels by neonatal heart cells. In vitro cellular & developmental biology : journal of the Tissue Culture Association. 1992; 28A:199–204. [PubMed: 1582995]
28. Souren JE, Peters RC, Van Wijk R. Collagen gels populated with rat neonatal heart cells can be used for optical recording of rhythmic contractions which also show ecg-like potentials. Experientia. 1994; 50:712–716. [PubMed: 8070530]
29. Zimmermann WH, Fink C, Kralisch D, Remmers U, Weil J, Eschenhagen T. Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. Biotechnology and bioengineering. 2000; 68:106–114. [PubMed: 10699878]
30. Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. Circ Res. 2002; 90:223–230. [PubMed: 11834716]

31. Naito H, Melnychenko I, Didie M, Schneiderbanger K, Schubert P, Rosenkranz S, Eschenhagen T, Zimmermann WH. Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle. Circulation. 2006; 114:I72–78. [PubMed: 16820649]
32. Tiburcy M, Didie M, Boy O, Christalla P, Doker S, Naito H, Karikkineth BC, El-Armouche A, Grimm M, Nose M, Eschenhagen T, Zieseniss A, Katschinksi DM, Hamdani N, Linke WA, Yin X, Mayr M, Zimmermann WH. Terminal differentiation, advanced organotypic maturation, and modeling of hypertrophic growth in engineered heart tissue. Circulation research. 2011; 109:1105–1114. [PubMed: 21921264]
33. Yildirim Y, Naito H, Didie M, Karikkineth BC, Biermann D, Eschenhagen T, Zimmermann WH. Development of a biological ventricular assist device: Preliminary data from a small animal model. Circulation. 2007; 116:I16–23. [PubMed: 17846298]
34. Vantler M, Karikkineth BC, Naito H, Tiburcy M, Didie M, Nose M, Rosenkranz S, Zimmermann WH. Pdgf-bb protects cardiomyocytes from apoptosis and improves contractile function of engineered heart tissue. Journal of molecular and cellular cardiology. 2010; 48:1316–1323. [PubMed: 20307544]
35. El-Armouche A, Singh J, Naito H, Wittkopper K, Didie M, Laatsch A, Zimmermann WH, Eschenhagen T. Adenovirus-delivered short hairpin rna targeting pkcalpha improves contractile function in reconstituted heart tissue. Journal of molecular and cellular cardiology. 2007; 43:371–376. [PubMed: 17628588]
36. Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, Freed LE, Vunjak-Novakovic G. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101:18129–18134. [PubMed: 15604141]
37. Eschenhagen T, Fink C, Remmers U, Scholz H, Wattchow J, Weil J, Zimmermann W, Dohmen HH, Schafer H, Bishopric N, Wakatsuki T, Elson EL. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: A new heart muscle model system. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1997; 11:683–694. [PubMed: 9240969]
38. Fink C, Ergun S, Kralisch D, Remmers U, Weil J, Eschenhagen T. Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2000; 14:669–679. [PubMed: 10744624]
39. Guo XM, Zhao YS, Chang HX, Wang CY, E LL, Zhang XA, Duan CM, Dong LZ, Jiang H, Li J, Song Y, Yang XJ. Creation of engineered cardiac tissue in vitro from mouse embryonic stem cells. Circulation. 2006; 113:2229–2237. [PubMed: 16651472]
40. Didie M, Christalla P, Rubart M, Muppala V, Doker S, Unsold B, El-Armouche A, Rau T, Eschenhagen T, Schwoerer AP, Ehmke H, Schumacher U, Fuchs S, Lange C, Becker A, Tao W, Scherschel JA, Soonpaa MH, Yang T, Lin Q, Zenke M, Han DW, Scholer HR, Rudolph C, Steinemann D, Schlegelberger B, Kattman S, Witty A, Keller G, Field LJ, Zimmermann WH. Parthenogenetic stem cells for tissue-engineered heart repair. J Clin Invest. 2013; 123:1285–1298. [PubMed: 23434590]
41. Tulloch NL, Muskheli V, Razumova MV, Korte FS, Regnier M, Hauch KD, Pabon L, Reinecke H, Murry CE. Growth of engineered human myocardium with mechanical loading and vascular coculture. Circulation research. 2011; 109:47–59. [PubMed: 21597009]
42. Soong PL, Tiburcy M, Zimmermann WH. Cardiac differentiation of human embryonic stem cells and their assembly into engineered heart muscle. Curr Protoc Cell Biol. Chapter 23(Unit23):28.
43. Streckfuss-Bomeke K, Wolf F, Azizian A, Stauske M, Tiburcy M, Wagner S, Hubscher D, Dressel R, Chen S, Jende J, Wulf G, Lorenz V, Schon MP, Maier LS, Zimmermann WH, Hasenfuss G, Guan K. Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts. Eur Heart J.
44. Maureira P, Marie PY, Yu F, Poussier S, Liu J, Groubatch F, Falanga A, Tran N. Repairing chronic myocardial infarction with autologous mesenchymal stem cells engineered tissue in rat promotes angiogenesis and limits ventricular remodeling. Journal of biomedical science. 2012; 19:93. [PubMed: 23146158]

45. Ross JJ, Tranquillo RT. Ecm gene expression correlates with in vitro tissue growth and development in fibrin gel remodeled by neonatal smooth muscle cells. Matrix biology : journal of the International Society for Matrix Biology. 2003; 22:477–490. [PubMed: 14667840]
46. Weinbaum JS, Qi J, Tranquillo RT. Monitoring collagen transcription by vascular smooth muscle cells in fibrin-based tissue constructs. Tissue Eng Part C Methods. 2010; 16:459–467. [PubMed: 19635030]
47. Black LD 3rd, Meyers JD, Weinbaum JS, Shvelidze YA, Tranquillo RT. Cell-induced alignment augments twitch force in fibrin gel-based engineered myocardium via gap junction modification. Tissue engineering Part A. 2009; 15:3099–3108. [PubMed: 19338433]
48. Long JL, Tranquillo RT. Elastic fiber production in cardiovascular tissue-equivalents. Matrix Biol. 2003; 22:339–350. [PubMed: 12935818]
49. Grassl ED, Oegema TR, Tranquillo RT. Fibrin as an alternative biopolymer to type-i collagen for the fabrication of a media equivalent. Journal of biomedical materials research. 2002; 60:607–612. [PubMed: 11948519]
50. Liau B, Christoforou N, Leong KW, Bursac N. Pluripotent stem cell-derived cardiac tissue patch with advanced structure and function. Biomaterials. 2011; 32:9180–9187. [PubMed: 21906802]
51. Zhang G, Nakamura Y, Wang X, Hu Q, Suggs LJ, Zhang J. Controlled release of stromal cell-derived factor-1 alpha in situ increases c-kit+ cell homing to the infarcted heart. Tissue engineering. 2007; 13:2063–2071. [PubMed: 17518719]
52. Zhang G, Hu Q, Braunlin EA, Suggs LJ, Zhang J. Enhancing efficacy of stem cell transplantation to the heart with a pegylated fibrin biomatrix. Tissue Eng Part A. 2008; 14:1025–1036. [PubMed: 18476809]
53. Hansen A, Eder A, Bonstrup M, Flato M, Mewe M, Schaaf S, Aksehirlioglu B, Schwoerer AP, Uebeler J, Eschenhagen T. Development of a drug screening platform based on engineered heart tissue. Circulation research. 2010; 107:35–44. [PubMed: 20448218]
54. Dar A, Shachar M, Leor J, Cohen S. Optimization of cardiac cell seeding and distribution in 3d porous alginate scaffolds. Biotechnol Bioeng. 2002; 80:305–312. [PubMed: 12226863]
55. Dvir T, Benishti N, Shachar M, Cohen S. A novel perfusion bioreactor providing a homogenous milieu for tissue regeneration. Tissue engineering. 2006; 12:2843–2852. [PubMed: 17518653]
56. Shapiro L, Cohen S. Novel alginate sponges for cell culture and transplantation. Biomaterials. 1997; 18:583–590. [PubMed: 9134157]
57. Dvir T, Timko BP, Brigham MD, Naik SR, Karajanagi SS, Levy O, Jin H, Parker KK, Langer R, Kohane DS. Nanowired three-dimensional cardiac patches. Nature nanotechnology. 2011; 6:720–725.
58. Dvir T, Kedem A, Ruvinov E, Levy O, Freeman I, Landa N, Holbova R, Feinberg MS, Dror S, Etzion Y, Leor J, Cohen S. Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:14990–14995. [PubMed: 19706385]
59. Landa N, Miller L, Feinberg MS, Holbova R, Shachar M, Freeman I, Cohen S, Leor J. Effect of injectable alginate implant on cardiac remodeling and function after recent and old infarcts in rat. Circulation. 2008; 117:1388–1396. [PubMed: 18316487]
60. Yu J, Gu Y, Du KT, Mihardja S, Sievers RE, Lee RJ. The effect of injected rgd modified alginate on angiogenesis and left ventricular function in a chronic rat infarct model. Biomaterials. 2009; 30:751–756. [PubMed: 19010528]
61. Ruvinov E, Leor J, Cohen S. The promotion of myocardial repair by the sequential delivery of igf-1 and hgf from an injectable alginate biomaterial in a model of acute myocardial infarction. Biomaterials. 2011; 32:565–578. [PubMed: 20889201]
62. Patra C, Talukdar S, Novoyatleva T, Velagala SR, Muhlfeld C, Kundu B, Kundu SC, Engel FB. Silk protein fibroin from antheraea mylitta for cardiac tissue engineering. Biomaterials. 2012; 33:2673–2680. [PubMed: 22240510]
63. Carrier RL, Papadaki M, Rupnick M, Schoen FJ, Bursac N, Langer R, Freed LE, Vunjak-Novakovic G. Cardiac tissue engineering: Cell seeding, cultivation parameters, and tissue construct characterization. Biotechnology and bioengineering. 1999; 64:580–589. [PubMed: 10404238]

64. Bursac N, Papadaki M, Cohen RJ, Schoen FJ, Eisenberg SR, Carrier R, Vunjak-Novakovic G, Freed LE. Cardiac muscle tissue engineering: Toward an in vitro model for electrophysiological studies. The American journal of physiology. 1999; 277:H433–444. [PubMed: 10444466]
65. Radisic M, Deen W, Langer R, Vunjak-Novakovic G. Mathematical model of oxygen distribution in engineered cardiac tissue with parallel channel array perfused with culture medium containing oxygen carriers. Am J Physiol Heart Circ Physiol. 2005; 288:H1278–1289. [PubMed: 15539422]
66. Engelmayr GC Jr, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. Nat Mater. 2008; 7:1003–1010. [PubMed: 18978786]
67. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Sridhar R, Ramakrishna S. Minimally invasive injectable short nanofibers of poly(glycerol sebacate) for cardiac tissue engineering. Nanotechnology. 2012; 23:385102. [PubMed: 22947662]
68. Fukuhara S, Tomita S, Nakatani T, Fujisato T, Ohtsu Y, Ishida M, Yutani C, Kitamura S. Bone marrow cell-seeded biodegradable polymeric scaffold enhances angiogenesis and improves function of the infarcted heart. Circ J. 2005; 69:850–857. [PubMed: 15988112]
69. Matsubayashi K, Fedak PW, Mickle DA, Weisel RD, Ozawa T, Li RK. Improved left ventricular aneurysm repair with bioengineered vascular smooth muscle grafts. Circulation. 2003; 108(Suppl 1):II219–225. [PubMed: 12970236]
70. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: Using nature's platform to engineer a bioartificial heart. Nature medicine. 2008; 14:213–221.
71. Godier-Furnemont AF, Martens TP, Koeckert MS, Wan L, Parks J, Arai K, Zhang G, Hudson B, Homma S, Vunjak-Novakovic G. Composite scaffold provides a cell delivery platform for cardiovascular repair. Proc Natl Acad Sci U S A. 2011; 108:7974–7979. [PubMed: 21508321]
72. Turner WS, Wang X, Johnson S, Medberry C, Mendez J, Badylak SF, McCord MG, McCloskey KE. Cardiac tissue development for delivery of embryonic stem cell-derived endothelial and cardiac cells in natural matrices. Journal of biomedical materials research. Part B, Applied biomaterials. 2012; 100:2060–2072.
73. Haraguchi Y, Shimizu T, Yamato M, Kikuchi A, Okano T. Electrical coupling of cardiomyocyte sheets occurs rapidly via functional gap junction formation. Biomaterials. 2006; 27:4765–4774. [PubMed: 16737736]
74. Sekiya S, Shimizu T, Yamato M, Kikuchi A, Okano T. Bioengineered cardiac cell sheet grafts have intrinsic angiogenic potential. Biochemical and biophysical research communications. 2006; 341:573–582. [PubMed: 16434025]
75. Hata H, Bar A, Dorfman S, Vukadinovic Z, Sawa Y, Haverich A, Hilfiker A. Engineering a novel three-dimensional contractile myocardial patch with cell sheets and decellularised matrix. Eur J Cardiothorac Surg. 2010; 38:450–455. [PubMed: 20335044]
76. Shimizu T, Sekine H, Isoi Y, Yamato M, Kikuchi A, Okano T. Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. Tissue engineering. 2006; 12:499–507. [PubMed: 16579683]
77. Sekine H, Shimizu T, Hobo K, Sekiya S, Yang J, Yamato M, Kurosawa H, Kobayashi E, Okano T. Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation. 2008; 118:S145–152. [PubMed: 18824746]
78. Bel A, Planat-Bernard V, Saito A, Bonnevie L, Bellamy V, Sabbah L, Bellabas L, Brinon B, Vanneaux V, Pradeau P, Peyrard S, Larghero J, Pouly J, Binder P, Garcia S, Shimizu T, Sawa Y, Okano T, Bruneval P, Desnos M, Hagege AA, Casteilla L, Puceat M, Menasche P. Composite cell sheets: A further step toward safe and effective myocardial regeneration by cardiac progenitors derived from embryonic stem cells. Circulation. 2010; 122:S118–123. [PubMed: 20837902]
79. Kushida A, Yamato M, Konno C, Kikuchi A, Sakurai Y, Okano T. Decrease in culture temperature releases monolayer endothelial cell sheets together with deposited fibronectin matrix from temperature-responsive culture surfaces. J Biomed Mater Res. 1999; 45:355–362. [PubMed: 10321708]

80. Masumoto H, Matsuo T, Yamamizu K, Uosaki H, Narazaki G, Katayama S, Marui A, Shimizu T, Ikeda T, Okano T, Sakata R, Yamashita JK. Pluripotent stem cell-engineered cell sheets reassembled with defined cardiovascular populations ameliorate reduction in infarct heart function through cardiomyocyte-mediated neovascularization. Stem Cells. 2012; 30:1196–1205. [PubMed: 22438013]
81. Stevens KR, Pabon L, Muskheli V, Murry CE. Scaffold-free human cardiac tissue patch created from embryonic stem cells. Tissue Eng Part A. 2009; 15:1211–1222. [PubMed: 19063661]
82. Stevens KR, Kreutziger KL, Dupras SK, Korte FS, Regnier M, Muskheli V, Nourse MB, Bendixen K, Reinecke H, Murry CE. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:16568–16573. [PubMed: 19805339]
83. Matsuura K, Wada M, Shimizu T, Haraguchi Y, Sato F, Sugiyama K, Konishi K, Shiba Y, Ichikawa H, Tachibana A, Ikeda U, Yamato M, Hagiwara N, Okano T. Creation of human cardiac cell sheets using pluripotent stem cells. Biochemical and biophysical research communications. 2012; 425:321–327. [PubMed: 22842572]
84. Lee P, Klos M, Bollensdorff C, Hou L, Ewart P, Kamp TJ, Zhang J, Bizy A, Guerrero-Serna G, Kohl P, Jalife J, Herron TJ. Simultaneous voltage and calcium mapping of genetically purified human induced pluripotent stem cell-derived cardiac myocyte monolayers. Circulation research. 2012; 110:1556–1563. [PubMed: 22570367]
85. Langer R, Vacanti JP. Tissue engineering. Science. 1993; 260:920–926. [PubMed: 8493529]
86. Zimmermann WH, Eschenhagen T. Cardiac tissue engineering for replacement therapy. Heart failure reviews. 2003; 8:259–269. [PubMed: 12878835]
87. Soong, PL.; Tiburcy, M.; Zimmermann, WH. Cardiac differentiation of human embryonic stem cells and their assembly into engineered heart muscle. In: Bonifacino, Juan S., et al., editors. Current protocols in cell biology/editorial board. Vol. Chapter 23. 2012. p. 28
88. Gao J, Liu J, Gao Y, Wang C, Zhao Y, Chen B, Xiao Z, Miao Q, Dai J. A myocardial patch made of collagen membranes loaded with collagen-binding human vascular endothelial growth factor accelerates healing of the injured rabbit heart. Tissue engineering. Part A. 2011; 17:2739–2747. [PubMed: 21682575]
89. Lu Y, Shansky J, Del Tatto M, Ferland P, Wang X, Vandenburgh H. Recombinant vascular endothelial growth factor secreted from tissue-engineered bioartificial muscles promotes localized angiogenesis. Circulation. 2001; 104:594–599. [PubMed: 11479259]
90. Kehat I, Davis J, Tiburcy M, Accornero F, Saba-El-Leil MK, Maillet M, York AJ, Lorenz JN, Zimmermann WH, Meloche S, Molkentin JD. Extracellular signal-regulated kinases 1 and 2 regulate the balance between eccentric and concentric cardiac growth. Circulation research. 2011; 108:176–183. [PubMed: 21127295]
91. Streckfuss-Bomeke K, Wolf F, Azizian A, Stauske M, Tiburcy M, Wagner S, Hubscher D, Dressel R, Chen S, Jende J, Wulf G, Lorenz V, Schon MP, Maier LS, Zimmermann WH, Hasenfuss G, Guan K. Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts. European heart journal. 2012
92. Schaaf S, Shibamiya A, Mewe M, Eder A, Stohr A, Hirt MN, Rau T, Zimmermann WH, Conradi L, Eschenhagen T, Hansen A. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. PloS one. 2011; 6:e26397. [PubMed: 22028871]
93. Xiong Q, Hill KL, Li Q, Suntharalingam P, Mansoor A, Wang X, Jameel MN, Zhang P, Swingen C, Kaufman DS, Zhang J. A fibrin patch-based enhanced delivery of human embryonic stem cell-derived vascular cell transplantation in a porcine model of postinfarction left ventricular remodeling. Stem Cells. 2011; 29:367–375. [PubMed: 21732493]
94. Feinberg AW, Feigel A, Shevkoplyas SS, Sheehy S, Whitesides GM, Parker KK. Muscular thin films for building actuators and powering devices. Science. 2007; 317:1366–1370. [PubMed: 17823347]
95. Okano T, Yamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(n-isopropylacrylamide). Journal of biomedical materials research. 1993; 27:1243–1251. [PubMed: 8245039]

96. Kushida A, Yamato M, Konno C, Kikuchi A, Sakurai Y, Okano T. Temperature-responsive culture dishes allow nonenzymatic harvest of differentiated madindarby canine kidney (mdck) cell sheets. Journal of biomedical materials research. 2000; 51:216–223. [PubMed: 10825221]

97. Kelm JM, Lorber V, Snedeker JG, Schmidt D, Broggini-Tenzer A, Weisstanner M, Odermatt B, Mol A, Zund G, Hoerstrup SP. A novel concept for scaffold-free vessel tissue engineering: Self-assembly of microtissue building blocks. Journal of biotechnology. 2010; 148:46–55. [PubMed: 20223267]

98. Kajstura J, Urbanek K, Perl S, Hosoda T, Zheng H, Ogorek B, Ferreira-Martins J, Goichberg P, Rondon-Clavo C, Sanada F, D'Amario D, Rota M, Del Monte F, Orlic D, Tisdale J, Leri A, Anversa P. Cardiomyogenesis in the adult human heart. Circulation research. 2010; 107:305–315. [PubMed: 20522802]

99. Leri A, Kajstura J, Anversa P. Role of cardiac stem cells in cardiac pathophysiology: A paradigm shift in human myocardial biology. Circulation research. 2011; 109:941–961. [PubMed: 21960726]

100. Huntgeburth M, Tiemann K, Shahverdyan R, Schluter KD, Schreckenberg R, Gross ML, Modersheim S, Caglayan E, Muller-Ehmsen J, Ghanem A, Vantler M, Zimmermann WH, Bohm M, Rosenkranz S. Transforming growth factor beta(1) oppositely regulates the hypertrophic and contractile response to beta-adrenergic stimulation in the heart. PloS one. 2011; 6:e26628. [PubMed: 22125598]

101. Singh BN, Koyano-Nakagawa N, Garry JP, Weaver CV. Heart of newt: A recipe for regeneration. Journal of cardiovascular translational research. 2010; 3:397–409. [PubMed: 20559775]

102. Kikuchi K, Holdway JE, Werdich AA, Anderson RM, Fang Y, Egnaczyk GF, Evans T, Macrae CA, Stainier DY, Poss KD. Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. Nature. 2010; 464:601–605. [PubMed: 20336144]

103. Kikuchi K, Holdway JE, Major RJ, Blum N, Dahn RD, Begemann G, Poss KD. Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. Developmental cell. 2011; 20:397–404. [PubMed: 21397850]

104. Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK, Cai L, Wang M, Wu TD, Guerquin-Kern JL, Lechene CP, Lee RT. Mammalian heart renewal by pre-existing cardiomyocytes. Nature. 2013; 493:433–436. [PubMed: 23222518]

105. Gupta V, Poss KD. Clonally dominant cardiomyocytes direct heart morphogenesis. Nature. 2012; 484:479–484. [PubMed: 22538609]

106. Jopling C, Sleep E, Raya M, Marti M, Raya A, Izpisua Belmonte JC. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature. 2010; 464:606–609. [PubMed: 20336145]

107. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. Science. 2011; 331:1078–1080. [PubMed: 21350179]

108. Thompson SA, Burridge PW, Lipke EA, Shamblott M, Zambidis ET, Tung L. Engraftment of human embryonic stem cell derived cardiomyocytes improves conduction in an arrhythmogenic in vitro model. Journal of molecular and cellular cardiology. 2012; 53:15–23. [PubMed: 22713758]

109. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schneider MD. Cardiac progenitor cells from adult myocardium: Homing, differentiation, and fusion after infarction. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100:12313–12318. [PubMed: 14530411]

110. Martin CM, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S, Goetsch SC, Gallardo TD, Garry DJ. Persistent expression of the atp-binding cassette transporter, abcg2, identifies cardiac sp cells in the developing and adult heart. Developmental biology. 2004; 265:262–275. [PubMed: 14697368]

111. Mordwinkin NM, Burridge PW, Wu JC. A review of human pluripotent stem cell-derived cardiomyocytes for high-throughput drug discovery, cardiotoxicity screening, and publication standards. Journal of cardiovascular translational research. 2013; 6:22–30. [PubMed: 23229562]

112. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin LZ, Cai CL, Lu MM, Reth M, Platoshyn O, Yuan JX, Evans S, Chien KR. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature. 2005; 433:647–653. [PubMed: 15703750]
113. Iso Y, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, Sobel BE, Delafontaine P, Prockop DJ. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem Biophys Res Commun. 2007; 354:700–706. [PubMed: 17257581]
114. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Deb A, Dzau VJ, Pratt RE. Mesenchymal stem cells overexpressing akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. Mol Ther. 2006; 14:840–850. [PubMed: 16965940]
115. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by akt-modified mesenchymal stem cells. Nat Med. 2005; 11:367–368. [PubMed: 15812508]
116. Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006; 20:661–669. [PubMed: 16581974]
117. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with akt prevent remodeling and restore performance of infarcted hearts. Nat Med. 2003; 9:1195–1201. [PubMed: 12910262]
118. Murakami Y, Zhang Y, Cho YK, Mansoor AM, Chung JK, Chu C, Francis G, Ugurbil K, Bache RJ, From AH, Jerosch-Herold M, Wilke N, Zhang J. Myocardial oxygenation during high work states in hearts with postinfarction remodeling. Circulation. 1999; 99:942–948. [PubMed: 10027819]
119. Zhang J, Merkle H, Hendrich K, Garwood M, From AH, Ugurbil K, Bache RJ. Bioenergetic abnormalities associated with severe left ventricular hypertrophy. The Journal of clinical investigation. 1993; 92:993–1003. [PubMed: 8349829]
120. Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J, Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (scipio): Initial results of a randomised phase 1 trial. Lancet. 2011; 378:1847–1857. [PubMed: 22088800]
121. Tang XL, Rokosh G, Sanganalmath SK, Yuan F, Sato H, Mu J, Dai S, Li C, Chen N, Peng Y, Dawn B, Hunt G, Leri A, Kajstura J, Tiwari S, Shirk G, Anversa P, Bolli R. Intracoronary administration of cardiac progenitor cells alleviates left ventricular dysfunction in rats with a 30-day-old infarction. Circulation. 2010; 121:293–305. [PubMed: 20048209]
122. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (caduceus): A prospective, randomised phase 1 trial. Lancet. 2012; 379:895–904. [PubMed: 22336189]
123. Chachques JC, Trainini JC, Lago N, Masoli OH, Barisani JL, Cortes-Morichetti M, Schussler O, Carpentier A. Myocardial assistance by grafting a new bioartificial upgraded myocardium (magnum clinical trial): One year follow-up. Cell Transplant. 2007; 16:927–934. [PubMed: 18293891]
124. Chachques JC, Trainini JC, Lago N, Cortes-Morichetti M, Schussler O, Carpentier A. Myocardial assistance by grafting a new bioartificial upgraded myocardium (magnum trial): Clinical feasibility study. The Annals of thoracic surgery. 2008; 85:901–908. [PubMed: 18291168]
125. Sawa Y. myocardial regeneration for heart failure. Nihon Rinsho. 2010; 68:719–725. [PubMed: 20387567]
126. Burridge PW, Keller G, Gold JD, Wu JC. Production of de novo cardiomyocytes: Human pluripotent stem cell differentiation and direct reprogramming. Cell stem cell. 2012; 10:16–28. [PubMed: 22226352]

127. Zhang J, Klos M, Wilson GF, Herman AM, Lian X, Raval KK, Barron MR, Hou L, Soerens AG, Yu J, Palecek SP, Lyons GE, Thomson JA, Herron TJ, Jalife J, Kamp TJ. Extracellular matrix promotes highly efficient cardiac differentiation of human pluripotent stem cells: The matrix sandwich method. Circulation research. 2012; 111:1125–1136. [PubMed: 22912385]

Non-standard Abbreviations and Acronyms

3D	three dimension
Abcg2	ATP-binding cassette sub-family G member 2
BLI	bioluminescent imaging
BrdU	bromodeoxyuridine
BZ	border zone
CHF	congestive heart failure
CPC	cardiac progenitor cell
c-Kit	stem cell growth factor receptor
EC	endothelial cell
ECM	extracellular matrix
EHM	engineered heart muscle
ESC	embryonic stem cell
GFP	green fluorescent protein
HEP	high energy phosphates
hESC	human embryonic stem cell
hESC-CM	human embryonic stem cells derived cardiomyocyte
hESC-VC	human embryonic stem cells derived vascular cell
HGF	hepatocyte growth factor
hiPSC-CM	human induced pluripotent stem cells derived cardiomyocyte
IGF	insulin-like growth factor 1
I/R	ischemia reperfusion
I-R	ischemia-reperfusion
IZ	infarct zone
Luc	firefly luciferase
LV	left ventricular
MI	myocardial infarction
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MSC	mesenchymal stem cell
PCL	poly ε-caprolactone
PCLA	poly ε-caprolactone-co-l-lactide
PDGF-BB	platelet derived growth factor-BB

PDMS	polydimethylsiloxane
PEG	polyethylene glycol
PGA	polygycolic acid
PGF	poly(glycerol sebacate)
PGS	poly-glycerol-sebacate
PIPAAM	poly-(*N*-isopropylacrylamide)
RGD	arginine-glycine-asparagine
RZ	remote zone
Sca-1	stem cell antigen-1
SDF-1	stromal cell-derived factor-1
SIS	small-intestinal submucosa
SkM	skeletal myoblasts
SMC	smooth muscle cell
SSEA-1	stage-specific embryonic antigen-1
VC	vascular cell
VEGF	vascular endothelial growth factor

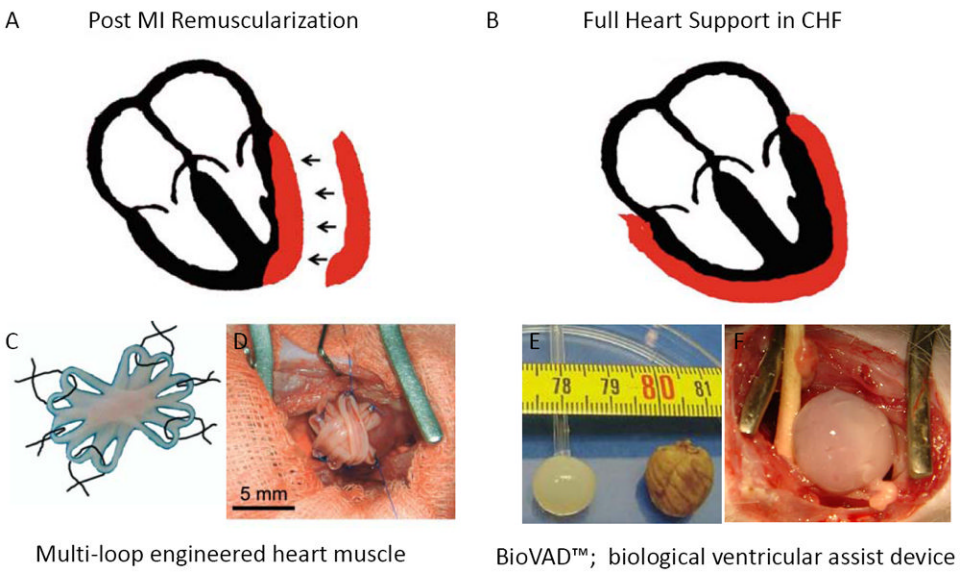

Figure 1.

Applications of engineered heart muscle (EHM). Schematic presentation of (A) an EHM patch and (B) an EHM pouch (red). (C) Illustration of a multi-loop EHM with five loops fused together to form an asterisk-shaped stack with a solid center surrounded by 10 loops that are used for surgical fixation of the EHM graft. (D) The patch was fixed over the site of infarction in rats with six single-knot sutures. (E) The dimensions of an EHM pouch and an explanted rat heart are shown. (F) An EHM pouch was implanted over a healthy rat heart to simulate its use as a biological ventricular assist device. Panels C and D are modified from Zimmermann et al. Nat Med. 2006; 12(4): 452-8[23]; panels E and F are modified from Yildirim et al. Circulation 2007;116(11 Suppl): I16-23[33].

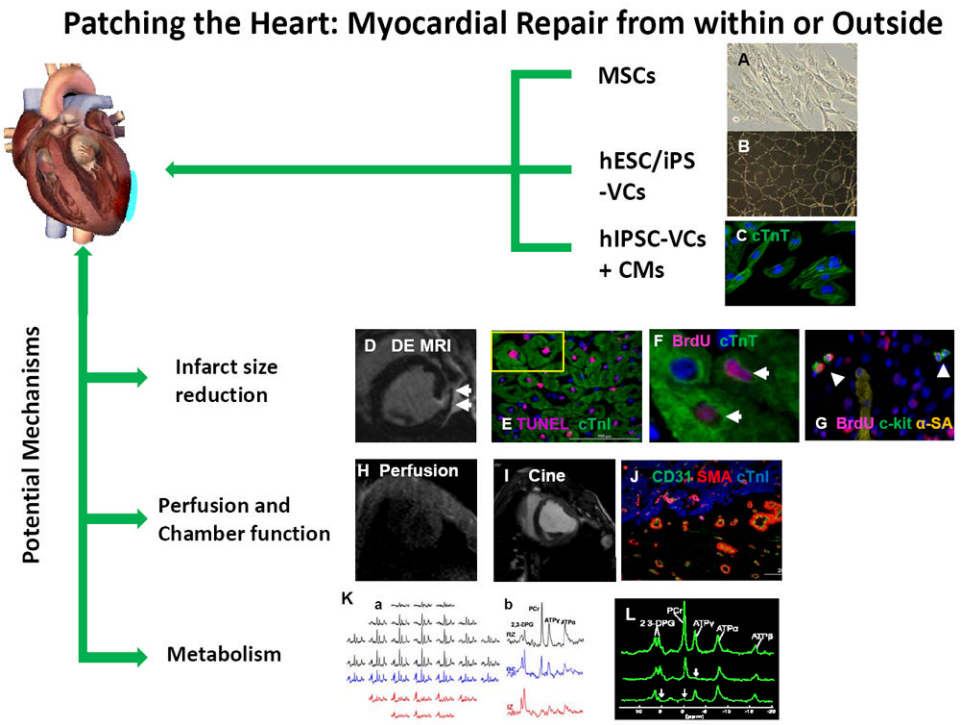

Figure 2.

The benefits associated with our in vivo method for cardiac patch application. A circular 3D porous biodegradable cardiac patch (blue) is created over the infarcted region by mixing thrombin and fibrinogen solutions that contain different progenitor cell types, such as mesenchymal stem cells (MSCs), vascular cells (VCs) generated through the controlled differentiation of either human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs), or both hiPSC-VCs and cardiomyocytes (hiPSC-CMs). The fibrinogen can be modified to bind peptides for different purposes, such as guiding differentiation or impeding apoptosis. The solution typically solidifies in less than one minute to form a 3D porous cardiac patch that provides structural support as well as a platform for the transplanted progenitor cells, and ultimately increases the cell engraftment rate. The transplanted cells release growth factors and other cytokines that reduce apoptosis, promote angiogenesis, and activate endogenous mechanisms for cardiomyocyte renewal, which leads to declines in infarct size and to improvements in myocardial perfusion, metabolism, and contractile function. Measurements of in vivo myocardial bioenergetics and the ATP turnover rate (via ^{31}P magnetization saturation transfer) suggest that the patch also protects against adverse changes in cardiomyocyte energy metabolism, perhaps by reducing wall stress and bulging at the site of the infarction. Collectively, these benefits improve cardiac contractile function and impede the progression of LV dilatation. (Panels B and K courtesy of Xiong Q et al Circ Res. 2012 ;111(4):455-68; Panels F, G, I, J and L, courtesy of Xiong Q et al Circulation 2013; 127(9): 997-1008).

Table 1

Summary of Studies

		Cell Lines and Other Components	Reference	Summary/Observations
Natural Scaffolds	Collagen	Neonatal rat heart cells	Souren[27,28]	Floating collagen layers were used as a matrix for CM seeding and studies of myocyte contractile behavior
			Zimmermann[29]	First introduction of EHTs from neonatal rat CMs in collagen/Matrigel matrix mixtures and demonstration of the importance of mechanical loading
			Zimmermann[30]	3D EHTs show morphological, ultra-structural, and function properties of native myocardium and show distinct responses to growth factor stimulation
			Naito[31]	The non-myocytes and in particular fibroblasts are important for optimal EHT function and capillarization; Matrigel can be replaced by insulin and T3; fusion of individual EHTs can be employed to generate large tissue patches
			Tiburcy[32]	Immature CMs terminally differentiate and reach a postnatal degree of mature in EHTs
			Zimmermann[20]	EHTs are quickly capillarized and innervated after implantation
			Zimmermann[23]	Multi-loop EHTs can be used to enhance contractile performance of infarcted rat hearts
		Fetal rat heart cells	Li[21]	Cardiomyocytes can be seeded on gelatin sponge (Gelfoam); implanted grafts remain viable
		Chick embryo heart cells	Eschenhagen [37]	First introduction of cardiomyocyte populated matrices (CMPMs) from embryonic chick cardiomyocytes in collagen gels
			Fink[38]	Stretching of EHTs induces hypertrophy and functional improvement
		Mouse embryonic stem cells	Guo[39]	Use of mouse embryonic stem cells as cardiomyocyte source for EHT construction demonstrated
		Mouse parthenogenetic stem cells	Didié40	Use of mouse parthenogenetic stem cells (PSCs) as cardiomyocyte source for engineered heart muscle (EHM) construction demonstrated; proof-of-concept for the utility of PSC-EHM in heart repair
		Human embryonic and induced pluripotent stem cells	Tulloch[41]	Human EHTs generated and cardiomyocyte proliferation observed
			Soong[42]	Human EHTs generated under serum-reduced conditions
			Streckfuss-Bömeke[43]	Different iPSC sources for cardiomyocyte derivation and EHT construction
		Rat MSCs	Maureira[44]	Contractility↑, perfusion↑, infarct size↓

		Cell Lines and Other Components	Reference	Summary/Observations
	Fibrin	Neonatal rat vascular SMCs	Ross[45]	ECM gene expression correlates with tissue growth and development in fibrin gel
			Weinbaum[46]	Evaluates a method for monitoring collagen transcription in EHTs
		Neonatal rat cardiac cells	Black[47]	Cell alignment improves twitch force
		Human fibroblasts or rat SMCs	Long[48]	Elastogenesis can be achieved in tissue engineering
			Grassl[49]	Fibrin stimulates collagen production by SMCs
		miPSC-CMs	Liau[50]	Cardiac tissue derived from pluripotent stem cells has advanced structure and function
		Porcine MSCs	Liu[26]	Neovascularization↑, wall thickening fraction↑
		SDF-1α	Zhang[51]	Contractility↑, c-Kit+ cell homing↑
		HGF	Zhang[52]	Contractility↑, neovascularization↑, LV dilation↓, scar size↓
		hESC-ECs and hESC-SMCs	Xiong[17]	Contractility↑, neovascularization↑, bioenergetics↑, c-Kit+ cell homing↑, hypertrophy↓, apoptosis↓
		hiPSC-EC and hiPSC-SMC	Xiong[18]	Contractility↑. neovascularization↑, ATP turnover↑, c-Kit+ cell homing↑, hypertrophy↓, apoptosis↓
	Fibrin and matrigel	Neonatal rat cardiac cells	Hansen[53]	Introduces a simple system for constructing and evaluating EHTs
	Alginate	Neonatal rat cardiac cells	Dar[54]	Evaluates methods for optimizing cell seeding and distribution in 3D scaffolds
			Dvir[55]	Evaluation of a novel perfusion bioreactor that provides a homogenous milieu for tissue regeneration
		Fibroblasts	Shapiro[56]	Alginate may provide an excellent support for cell transplantation
		Fetal rat cardiac cells	Leor[22]	Contractility↑, angiogenesis↑, LV dilation↓
		Neonatal rat cardiac cells and gold nanowires	Dvir[57]	Gold nanowires promote conductivity
	Alginate and omentum	Neonatal rat cardiac cells	Dvir[58]	Contractility↑, angiogenesis↑, scar thickness↑, LV dilation↓
	Injectable alginate	Calcium-crosslinked alginate	Landa[59]	Contractility↑, LV dilation↓
		Peptide modified (without cells)	Yu[60]	Angiogenesis↑, LV function↑
		IGF-1 and HGF	Ruvinov[61]	Angiogenesis↑, infarct↓
	Gelatin gelfoam	Fetal rat cardiac cells	Li[21]	Cardiac graft survived and formed junctions with host heart cells
	Silk protein fibroin*	Neonatal rat cardiac cells	Patra[62]	Silk fibroin is suitable for cardiac-patch engineering

		Cell Lines and Other Components	Reference	Summary/Observations
Synthetic Scaffolds	PGA	Neonatal rat cardiac cells	Carrier[63]	PGA introduced as synthetic material for myocardial tissue engineering
			Bursac[64]	Use of PGA seeded with cardiomyocytes as a model for cardiac electrophysiology
	Polyglycerol sebacate	Neonatal rat cardiac cells	Radisic[65]	Mathematical modeling of oxygen consumption
			Engelmayr[66]	Polyglycerol sebacate forms an accordion-like honeycomb scaffold that promotes heart-cell alignment and mechanical properties
	Injectable polyglycerol sebacate	Rabbit CMs	Ravichandran[67]	Evaluates a minimally invasive technique that uses injectable polyglycerol sebacate fibers for scaffold creation
	Poly ε-caprolactone	Mouse bone marrow cells	Fukuhara[68]	Angiogenesis↑, LV function↑
Decellularized hearts	PCLA	Rat SMCs	Matsubayashi[69]	Contractility↑, wall thickness↑, LV dilation↓
	Decellularized rat heart	Neonatal rat cardiac cells	Ott[70]	Evaluates the use of a decellularized matrix for engineering a bioartificial heart
	Decellularized heart	Human MPCs	Godier-Furnemont[71]	Contractility↑, LV dilation↓
	Decellularized porcine bladder	hESC-ECs, HUVECs, mouse CMs	Turner[72]	Evaluates the use of a decellularized matrix for delivering ESC-derived cardiac cells
Cell Sheets		Neonatal rat ventricular CMs	Haraguchi[73]	Electrical coupling of CM sheets occurs rapidly
			Sekiya[74]	Bioengineered cardiac cell sheets have intrinsic angiogenic potential
			Hata[75]	Evaluates a 3D cardiac patch made of cell sheets and decellularised matrix
			Shimizu[19]	Spontaneous, macroscopic beating is observed in subcutaneously implanted sheets
			Shimizu[76]	Subcutaneously implanted sheets survive for at least one year
		Neonatal rat CMs and rat ECs	Sekine[77]	Contractility↑, angiogenesis↑, fibrosis↓
		Rat adipose-derived MSCs	Miyahara[24]	Contractility↑, scar size↓, LV dilation, mortality↓
		Monkey stromal cells and monkey SSEA-1 and CPCs	Bel[78]	Angiogenesis↑, cell engraftment rate↑
		Bovine aortic endothelial cells	Kushida[79]	Evaluates a temperature-responsive surface for culturing cell sheets
		mESC-derived cardiac cells	Masumoto[80]	Contractility↑, wall thickness↑, angiogenesis↑, fibrosis↓

	Cell Lines and Other Components	Reference	Summary/Observations
	hESC-CMs	Stevens[81]	Evaluates a novel scaffold-free human myocardial patch made of hESC-CMs
	hESC-CMs and ECs	Stevens[82]	Cell engraftment↑
	hiPSC-CMs	Matsuura[83]	hiPSC-CMs can be used to create thick cardiac tissues
		Lee[84]	Evaluates a multiparametric imaging system that simultaneously measures action potentials and intracellular calcium-wave dynamics of cardiac cell sheets
		Kawamura[25]	Contractility↑, angiogenesis↑, LV dilation↓

3D: three-dimensional; CM: cardiomyocyte; CPC: cardiac progenitor cell; EC: endothelial cell; ECM: extracellular matrix; EHT: engineered heart tissue; hESC: human embryonic stem cell; HGF: hepatocyte growth factor; hiPSC: human induced-pluripotent stem cell; HUVEC: human umbilical-vein endothelial cell; IGF-1: insulin-like growth factor 1; LV: left ventricuar; miPSC: murine induced-pluripotent stem cell; MPC: mesenchymal progenitor cell; MSC: mesenchymal stem cell; PCLA: ε-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide; PDGF-BB: platelet-derived growth factor BB; SDF-1: stromal cell-derived factor 1; shRNA: small-hairpin RNA; SkM: skeletal myoblast; SMC: smooth muscle cell; SSEA-1: stage-specific embryonic antigen 1; VEGF: vascular endothelial growth factor.