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*REVIEW ARTICLE*

# Tissue Engineering Strategies for Myocardial Regeneration: Acellular Versus Cellular Scaffolds?

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Heart disease remains one of the leading causes of death in industrialized nations with myocardial infarction (MI) contributing to at least one fifth of the reported deaths. The hypoxic environment eventually leads to cellular death and scar tissue formation. The scar tissue that forms is not mechanically functional and often leads to myocardial remodeling and eventual heart failure. Tissue engineering and regenerative medicine principles provide an alternative approach to restoring myocardial function by designing constructs that will restore the mechanical function of the heart. In this review, we will describe the cellular events that take place after an MI and describe current treatments. We will also describe how biomaterials, alone or in combination with a cellular component, have been used to engineer suitable myocardium replacement constructs and how new advanced culture systems will be required to achieve clinical success.

**Keywords:** cardiac tissue engineering, cardiac patch, acellular scaffolds, extracellular matrix scaffolds, myocardial infarction, heart repair

## Introduction

Heart disease remains one of the leading causes of death in industrialized nations with myocardial infarction (MI) contributing to at least ~20% of the reported deaths.[1] An acute MI occurs when a coronary artery that feeds oxygenated blood to the right and left ventricles gets occluded, thus resulting in areas of hypoxia. The hypoxic environment, if maintained for sufficient amount of time, eventually leads to cellular death due to lack of oxygen and nutrients triggering an inflammatory response. The inflammatory environment is responsible for the clearing of dead cells and stimulating neighboring cells to increase matrix production ultimately leading to scar tissue formation.

The scar tissue that forms after an MI is unable to contract and, due to the high stresses present during the normal pumping action of the heart, the infarct area deforms over time leading to myocardial remodeling and reduced cardiac output. Although reperfusion and pharmacological treatments have shown some improvements in patients after an MI, the scar tissue is not completely removed and cardiac output is not restored to pre-MI levels.[2] If significant damage is sustained, a heart transplant is a potential treatment option. However, heart transplantation remains limited by low availability and the need for life immunosuppression of the transplant recipient.

Tissue engineering and regenerative medicine principles provide an alternative approach to restoring myocardial function after an MI. By combining the expertise of multiple fields, such as engineering, biology, medicine, biochemistry, and pharmacology, tissue engineers try to create suitable tissue replacements capable of restoring function and improving quality of life. This review will first describe the cellular events that take place after an MI with emphasis on the host tissue response dominated by inflammatory cells such as macrophages. The review then will describe how biomaterials, alone or in combination with a cellular component, have been used to engineer suitable myocardium replacement constructs. Given the complexity of the myocardial tissue, we will also discuss ideas for new advanced culture systems that can help assemble and test the new generation of engineered cardiac devices.

## Myocardial Infarction

A heart attack or MI is caused by the stenosis and/or occlusion of a coronary artery leading to improper delivery of oxygenated blood to regions of the heart. This condition

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is classified based on the extent of occlusion into ST-Elevation myocardial infarction (STEMI) when occlusion is completely blocked, or non-ST-Elevation myocardial infarction (NSTEMI) when occlusion is not complete. The physiological and clinical description of MI is described in details elsewhere[2]; in brief, the occlusion can affect the major coronary arteries such as the left anterior descending, left circumflex, and the right coronary artery. Once the artery is sufficiently occluded, oxygenation and nutrient deficit downstream of the occlusion result in the gradual death of the myocardial tissue (Fig. 1). The infarct site refers to the portion of the necrotic myocardium that is damaged or in the process of being damaged by the hypoxic conditions. Most infarcts involve the death of the full thickness of the ventricular wall (transmural infarction), although in some cases perfusion from neighboring vessels can help delay and/or minimize injury. At the early stages of the infarct there is a decrease in aerobic glycolysis, an increase in anaerobic glycolysis (accumulation of lactic acid), production of high-energy phosphates, and reduced contractility.[2]

Necrosis and apoptosis begin to occur, leading to the activation of the inflammatory response through the recruitment of neutrophils and subsequently monocytes from peripheral blood. Recent studies have shown the active recruitment of circulating peripheral blood monocytes and a monocyte population resident in the spleen following an MI.[3] In addition, studies have shown waves of pro- and anti-inflammatory monocytes circulating at different times after the infarct with classically activated monocytes found soon after the ischemic injury and then followed by alternatively activated monocytes.[4] Once recruited to the infarct, monocytes differentiate toward macrophages and begin the labor of removing cellular debris.[5,6]

During the initial phase of the inflammatory response after the recruitment of the monocytes, there is a strong presence of classically activated macrophages or M1 macrophages within the myocardial tissue. These are macrophages associated with the removal of pathogens and cellular debris and express proinflammatory cytokines such as IL-1β, TNF-α, and IL-6. These macrophages are present at high numbers during the first week after an infarct in mice with a gradual decrease over time.[4,5,7,8] Following this initial proinflammatory phase, there is a gradual shift in the type of macrophage toward more alternatively activated macrophages (M2), which are characterized by the secretion of CCL-17, TIMP-1, and IL-10. These macrophages are typically associated with wound healing responses and are thought to activate fibroblasts, smooth muscle, and endothelial cells.[5,6,9–11]

Myocardial regeneration is the process by which the injured myocardium is restored to its original structure and function. As described above, the normal healing process for postinfarction cardiac tissue involves the generation of a fibrous scar, which provides mechanical support but is devoid of functional cardiomyocytes. Most treatment strategies focus on improving the performance of an already damaged tissue

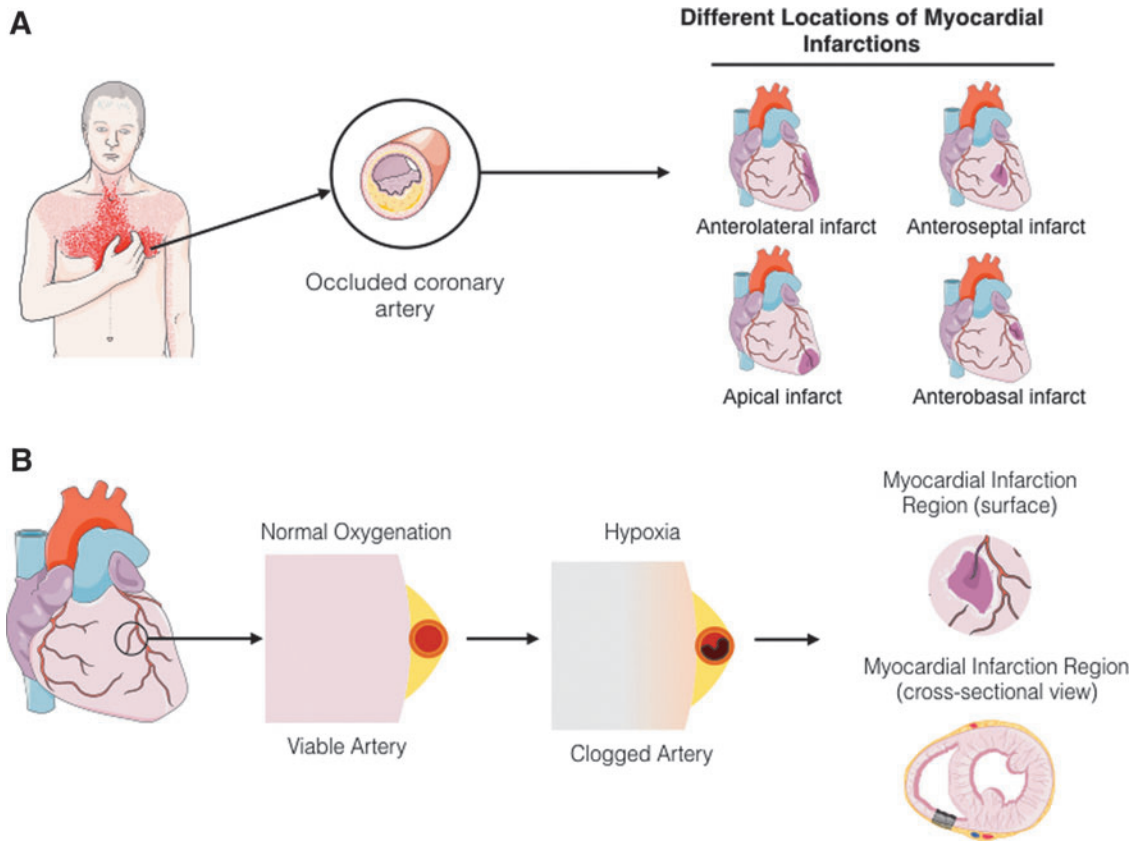


**FIG. 1.** Diagrammatic representation of an MI. **(A)** When symptoms of a heart attack are felt, it represents the occlusion of one or multiple coronary arteries supplying oxygen and nutrients to the heart. **(B)** Once the artery is occluded, there is hypoxia due to the limited diffusion of oxygen, resulting in the death of myocardial tissue. Artwork provided by Servier Medical Arts. MI, myocardial infarction. Color images available online at www.liebertpub.com/teb

and not in regenerating the myocardium to restore or establish normal function. More recently, new therapeutic approaches based on progenitor cells have been developed with the goal of regenerating tissue to its normal structure and function.

## Current Cardiac Treatments

Advancements in medical interventions have improved the prognosis post-MI considerably, but the incidence of heart failure is still increasing, likely as a result of the increasing number of patients who now survive the initial attack. Currently, the only approved treatments for end-stage heart failure post-MI are left ventricular assist devices and heart transplantation. The first is plagued by the complications of a chronic external assist device, which include bleeding (30%), right ventricular failure (20–30%), thromboembolism (3–35%), primary device failure (6% –6 months, 64% –2 years), and infection (18–59%).[12] The second is a limited resource in which proper matching of the donor organ to the patient is a great challenge, limiting even further its use. A number of surgical approaches have been developed as preventative measures to improve patient survival and their quality of life, and which can be an option for patients excluded from cardiac transplantation lists. The most common include angioplasty, left ventricular reconstruction, and cellular cardiomyoplasty.

### *Ischemic tissue revascularization*

There is agreement that initial treatment for STEMI is restoring blood flow to the ischemic tissue *via* tissue reperfusion. The main alternatives for reperfusion can be classified into pharmacologic, surgical, or mechanical. The pharmacological breakdown of blood clots (thrombus) in stenotic coronary arteries is known as thrombolysis. The mechanical alternative to reperfusion is known as primary percutaneous surgical alternative coronary intervention or primary coronary angioplasty, where the occlusion is mechanically expanded to allow blood flow to resume. The surgical alternative is known as coronary artery bypass graft (CABG) surgery, which when compared with angioplasty is highly invasive (requiring open heart surgery) and requires extra surgery to obtain the vein graft.

The use of primary angioplasty for the treatment of STEMI was first described as a rescue treatment in the case of failed intracoronary thrombolysis and was studied extensively as an adjunctive therapy. In general terms, the procedure consists of feeding a deflated balloon or other device (e.g., stent) on a catheter from the inguinal femoral artery or radial artery up through blood vessels until they reach the site of blockage in the heart. At the blockage, the balloon is inflated to open the artery, allowing blood to flow. Primary angioplasty has been shown to be more effective to thrombolysis for treatment of patients with acute STEMI in randomized trials.[13–16] The use of angioplasty requires the procedure to be performed preferably within 90 min of the patient presenting to the emergency room, which most hospitals cannot provide.

There is strong evidence that with increasing duration and severity of ischemia, more cardiac tissue damage can develop, allowing a variety of reperfusion-associated pathologies, known as reperfusion injury. This condition results in cardiac tissue damage through myocardial stunning, microvascular and endothelial injury, and irreversible cell damage, necrosis, apoptosis, autophagy, or necroptosis.[17,18] Reperfusion injury has been observed in each of the cardiac tissue revascularization strategies mentioned above and under certain conditions can be lethal. There are various pharmacological and nonpharmacological interventions used to reduce reperfusion injury. In the case of pharmacological interventions, the use of drugs such as cyclosporine-A, metoprolol, and glucose modulators has shown some promising results, but a long list of failed examples makes them a weak alternative. In contrast, nonpharmacological interventions have focused on limiting the infarct size as means to reduce reperfusion injury.

### *Left ventricular reconstruction*

After MI, the formation of scar tissue leads to changes in left ventricular (LV) size, shape, structure, and physiology through a process known as myocardial remodeling.[19] During this process, there is thinning of the LV walls, with the elliptical LV becoming more spherical and dilated.[20] A number of different surgical techniques and modifications have been developed to restore LV shape and reduce its volume to improve LV function and are collectively known as LV reconstruction.[21–24] This is a specific surgical procedure developed for the management of heart failure with LV remodeling caused by coronary artery disease.[25] Despite its success, these procedures have not found general acceptance in the medical community. Possible reasons include a lack of robust prospective randomized data showing the mortality benefit of this technique in patients with ischemic cardiomyopathy and dilated ventricles that were referred for CABG. To address these concerns, the Surgical Treatment for Ischemic Heart Failure (STICH) trial was developed to evaluate the role of cardiac surgery in the treatment of patients with coronary artery disease and LV systolic dysfunction.[26] A major question addressed by this study was if left ventricular reconstruction improved patient outcome when combined with CABG. The results of this clinical trial showed no significant difference between performing CABG alone or when combined with LV reconstruction.[26] These surgical techniques, and the use of nonbioactive materials as tissue replacements, helped spark the interest in exploring innovative use of biomaterials and tissue engineering constructs.

### *Cellular cardiomyoplasty*

Cell transplantation is an area of growing interest in clinical cardiology, as a potential means of treating patients after acute MI. Cellular cardiomyoplasty is a therapeutic strategy in which progenitor cells are used to repair regions of damaged or necrotic myocardium. The ability of transplanted progenitor cells to improve function within the failing heart has been shown in experimental animal models and in some human clinical trials.[27] The progenitor cells involved in these new therapeutic approaches include bone marrow or adipose tissue-derived mesenchymal stem cells (MSCs), hematopoietic precursor cells, endothelial progenitor cells, endogenous cardiac stem cells, and skeletal muscle-derived cells.[28,29]

Three mechanisms have been proposed to describe how cardiomyoplasty improves myocardial function: (1) transdifferentiation of the administered stem cells into cardiomyocytes, endothelial cells, and smooth muscle cells,[30,31] (2) fusion

between the stem cell and endogenous cardiac myocytes,[32] and (3) release of paracrine factors that stimulate endogenous cardiac repair mechanisms.[33,34] Conflicting results showing a lack of transdifferentiation have put into question its role in cardiomyoplasty and motivated the search of alternative hypotheses like fusion and paracrine signaling.[30,35] Further studies suggested that the lack of transdifferentiation shown was related to differences in experimental procedures, but the exact mechanism remains unknown.[36] In addition, it has been consistently reported that the level of fusion between stem cells and cardiomyocytes remains low,[32,37] suggesting that additional mechanisms may be involved. Current results support paracrine signaling as the principal mechanism for the improvement of myocardial function. In it, stem cells release cytokines and chemokines to stimulate other cells into the regenerative process. In fact, MSCs have been shown to stimulate host myocardial precursor cells to amplify and differentiate into cardiomyocytes *in vivo*.[38]

The clinical application of cellular cardiomyoplasty for the treatment of the ischemic tissues after acute MI involves tissue revascularization, isolation of autologous stem cells from the patient, and implementation through repeat cardiac catheterization or intramyocardial injection.[39] A major limitation for the application of cellular cardiomyoplasty as a treatment option is stem cell retention and engraftment after intramyocardial implantation.[40] A significant proportion of the transplanted cells leak out through the needle track that is made by the puncturing needle or enter systemic circulation.[41,42] Cell retention is normally less than 10%, regardless of the delivery route within the first 24 h.[43] Although it was initially thought that cell death by apoptosis was the reason for low engraftment,[44,45] it has been demonstrated that venous drainage and the contraction of the beating heart are the main reasons for cell loss.[40,46] Short-term cell retention is necessary for subsequent long-term engraftment and cardiac tissue functional improvement after acute MI. Other unresolved issues include cell delivery method and route, cell distribution, time transplantation, cell type, cell number, and viability. There are new therapeutic approaches involving engineering culture systems, the use of novel biomaterials for mechanical support of the cells and for controlled release of therapeutics, and tissue engineering (Fig. 2).

## Engineered Cardiac Patch

Engineered cardiac patch is fabricated to mimic the native extracellular matrix (ECM) and offer mechanical support and cell delivery into the region of infarction. Its application helps to limit LV remodeling, prevent dilatation and thinning of the infarct zone, enhance mechanical properties of ventricle, and reduce cardiomyocyte apoptosis. In addition, it aids to retain viable transplanted stem cells, which stimulate the formation of vasculature, myofibroblasts, and cardiomyocytes. Hence, the optimal properties of a scaffold involve high porosity, microenvironment similar to ECM, good mechanical properties, biodegradability, and biocompatibility. Natural polymers (i.e., collagen, fibrin, chitosan, alginate, natural ECM, peptides) and synthetic polymers (i.e., polycaprolactone [PCL], polyglycerol sebacate [PGS], and polyurethanes) are a choice of materials to fabricate scaffolds[47,48] (Fig. 3). The Tables 1 and 2 summarize some characteristics of natural and synthetic polymers used for cardiac patch, respectively.

### *Acellular biomaterial approach to cardiac repair*

Cardiac tissue has limited self-renewal capacity, which limits its ability to regenerate and repair itself after injury. Due to the challenges and limitations on the use of biomaterials with exogenous cells, acellular injectable biomaterials and patches have been evaluated as mechanical supports for MI. Acellular scaffolds have several advantages compared to cellular scaffolds such as (1) their off-the-shelf availability for immediate implantation (e.g., SynerGraft®, AlloDerm®, DermaMatrix®), (2) their limited immune reaction,[49] and (3) low cost and extended shelf life.[50] Cardiac tissue scaffolds should exhibit elasticity matching the myocardium, host cell integration and vascularization, mechanical stability, and nonimmunogenicity to support tissue function and regeneration. The following sections briefly discuss the advantages and limitations of major biomaterials used as acellular constructs for myocardial repair and regeneration with emphasis given to naturally occurring biomaterials.

Collagen. Due to its abundance in connective tissue, collagen type I scaffolds are increasingly being used in tissue repair applications. Collagen type I provides a tissue-like environment for cell attachment and growth, which facilitates host cell integration. Its main attributes include biocompatibility, biodegradability, and fibrous contractile structure.[51,52] Collagen type I comprises about 80% of the collagen matrix in cardiac tissue,[53] making it the choice of preference for cardiac tissue scaffolds. Gaballa *et al.*[54] grafted a three-dimensional (3D) collagen type I scaffold onto infarcted myocardium in rats and found that the scaffold induced neovessel formation and reduced LV remodeling 3 weeks after implantation. However, solid porous collagen has a lower elastic modulus,[55] which can limit its mechanical integration to the cardiac tissue. Serpooshan *et al.* optimized the elastic modulus of collagen type I gel to improve myocardial contractility in the injured heart.[56] Collagen type I was molded using a plastic compression technique to generate dense tissue scaffolds with a high elastic modulus. Four weeks post-MI in mice, collagen type I patches showed host cell infiltration and new blood vessel formation. Echocardiography showed significant improvement in cardiac function, diminished fibrosis, and inhibition of LV dilation and wall thinning.

Collagen in combination with other biomaterials such as chitosan has shown an increase in compression modulus, which makes it more suitable for the stabilization of the ventricular wall.[57] Incorporation of angiogenic factors, such as thymosin β4, in composite collagen–chitosan hydrogels has been shown to induce cell migration and improve angiogenesis *in vivo*.[58] Other forms of collagen such as gelatin have shown good cardiac cell attachment and viability, but the tensile strength and degradation rates are inferior to collagen, making it a less attractive option for cardiac tissue implants. These results indicate that collagen scaffolds can exert beneficial effects on cardiac remodeling and function

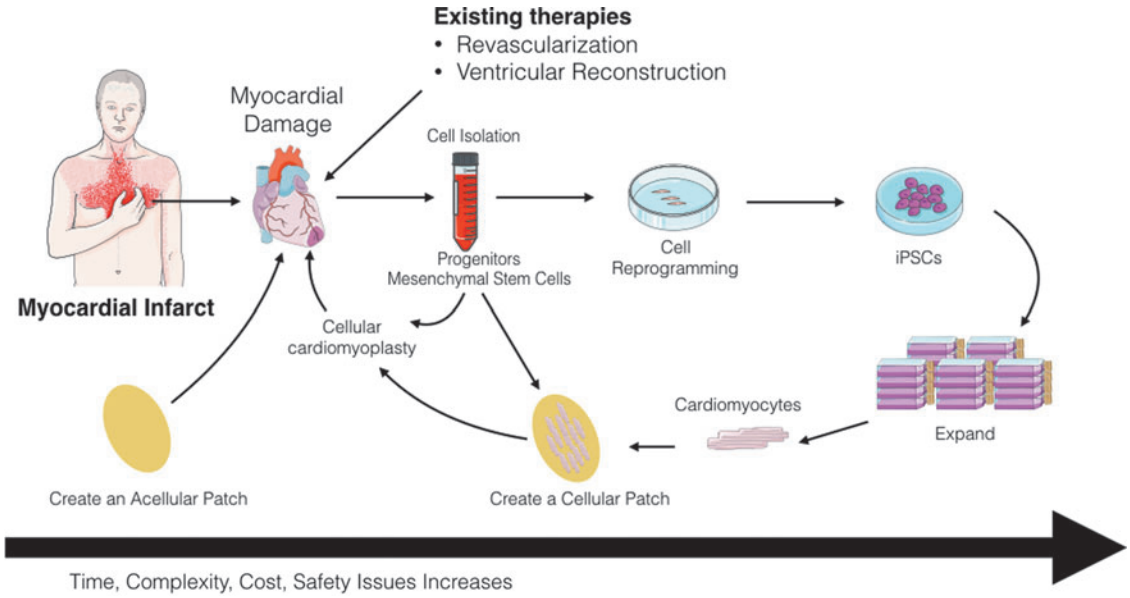


**FIG. 2.** Diagrammatic representation of the different approaches that can be used to repair infarcted myocardial tissue. An acellular patch can be used as an off-the-shelf product that can be implanted soon after myocardial infarction. Alternatively, cells can be harvested (i.e., progenitor cells) and injected back into the patient. Another approach can be the isolation of somatic cells (i.e., blood cells), reprogrammed, expanded, differentiated, and assembled into a bioengineered cardiac tissue that can then be implanted back into the patient as an autologous patch. These approaches have different timing and expenses associated with them that can have potential impact on their clinical use. Artwork provided by Servier Medical Arts. Color images available online at www.liebertpub.com/teb

after injury. Cardiac cell integration and function should be further evaluated to determine its long-term clinical potential.

Hyaluronic acid. Hyaluronic acid (HA), also known as hyaluronan, is a natural linear polysaccharide abundantly found in the ECM of several tissues. Its structure and ligand binding properties have been linked to angiogenesis[59] and tissue repair.[60] Thus, HA has become an important component in scaffolds used for tissue repair and regeneration. In cardiac tissue, HA has shown modest results for cardiac function recovery postinfarction. Yoon *et al.* were one of the first groups that demonstrated the regenerative potential of HA for heart tissue. A significant decrease in both infarcted area and apoptotic index as well as an increase in local

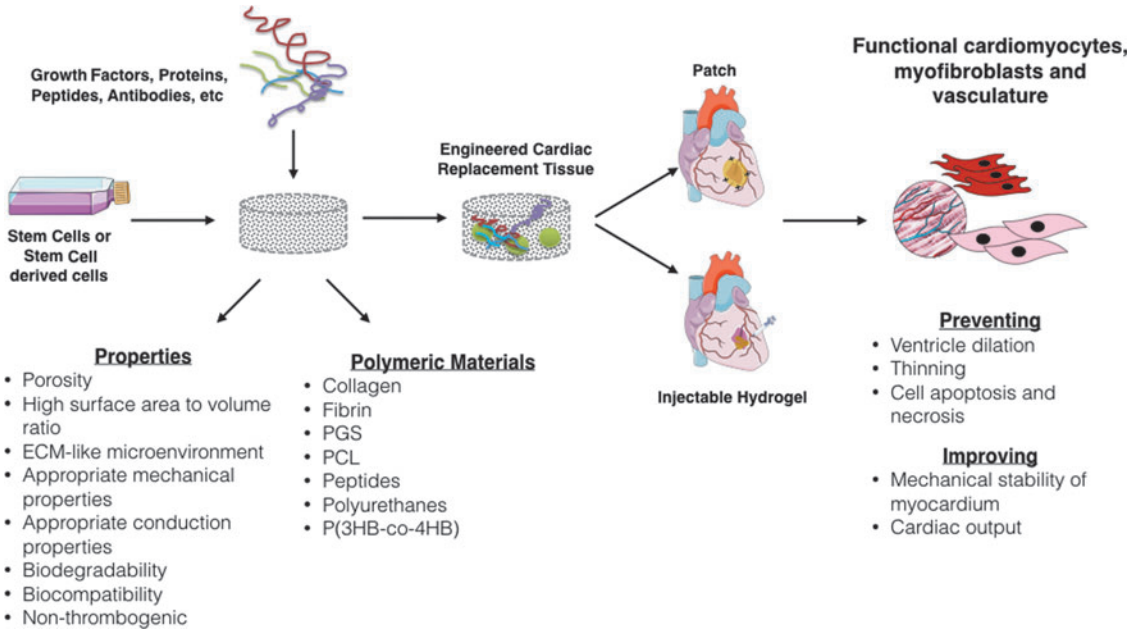


**FIG. 3.** Diagrammatic representation of tissue engineering strategies using cellular and acellular scaffolds. The goal is to find the optimal configuration that restores myocardial function to preinfarct levels. Color images available online at www.liebertpub.com/teb

Table 1. Some Natural Polymers for Cardiac Patch

| *Material* | *Stem cells* | *Porosity and/or pore size* | In vivo *model* | *Signaling* | *Ref.* |
|---|---|---|---|---|---|
| Collagen | hMSCs | — | Male CDF rats | α-SMA | 118 |
| | Autologous stem cells | 400–600 μm | Male C57/BL6 mouse | Anti-sarcomeric actin antibody and Anti-vWF | 121 |
| | hMSCs<br>hESC-MC | — | Male athymic RNU nude rats | CD105, CD73, Angiogenin, PDGF-B, VEGF, and CXCL1 | 105 |
| | Autologous mesenchymal stem cells | — | Wistar rats | $CD44^+$, $CD90^+$, $CD45^-$, $CD34^-$, Desmine, and α-smooth muscle actinin | 119 |
| | hESC-derived cardiovascular progenitors<br>Autologous r-ADSCs | — | Female Wistar rats<br>Rowett nude female rats | Tbx5 and Nkx 2.5 | 116 |
| Natural ECM | BM-MSCs | 91.2% ± 1.3%; 130.5 ± 25.3 mm | Male syngeneic Lewis rats | α-SMA, bFGF, vWF, PDGF-B, IGF-1, HGF, MEF2D, MYH6, Type I collagen, SMMHC, CD68, and IL-6 | 139 |
| | hMSCs | — | Adult mongrel dogs | Sarcomeric α-actinin, Atrial natriuretic peptide Cardiotin, Subunit of the Cav 1.2, and cardiac troponin-T | 137 |
| | Cardiac progenitor cells | — | — | α-MHC, Troponin T, Troponin C, GATA-4, nkx 2.5, α-SMA, Smooth muscle 22α, Fibroblast-specific protein 1, vWF, tie2 | 135 |
| | BMMCs | 19.5 ± 17.9 μm | — | Sarcomeric α-actinin, Myosin heavy chain Cardiac troponin T, and vWF | 134 |

ADSC, adipose tissue-derived stem cell; ECM, extracellular matrix; hMSC, human mesenchymal stem cell; hESC-MC, human embryonic stem cell-derived mesenchymal cells; α-SMA, α-smooth muscle actin.

vasculature were observed in rats injected with an acrylated HA hydrogel into the epicardium of the infarcted region.[61] Similar results have been observed by Abdalla *et al.* by evaluating the recovery of cardiac function in the infarcted heart of rats postinjection of HA gel into the peri-infarct region.[62] Transthoracic echocardiography revealed a significant increase of about 18% in ejection fraction in HA-injected groups compared to the control group. Decreased collagen deposition and increased levels of VEGF were also observed supporting reduced scarring and new vasculature formation in response to HA.

The molecular weight of HA has been shown to affect its regenerative potential in the myocardium. Evaluation of different molecular weights of HA-based hydrogels (50, 130, and 170 kDa) showed that the lowest molecular weight had the most significant regeneration and function recovery of the infarcted myocardium.[63] The regenerative potential of HA-based hydrogel was markedly reduced in chronic models of MI, indicating that the injection time is a major determinant for cardiac repair. The compressive modulus of HA gels is also an important variable for stabilization of the infarcted myocardium. Use of hydrogels with high compression modulus (43 kPa) significantly reduced LV remodeling and improved function when compared with lower modulus hydrogel and control groups in an ovine model.[64] Due to the increase in wall stress during systole, a high compression modulus may be more suitable to reduce myocardial stress distribution. Thus, two major factors, the molecular weight of HA and injection time, are main determinants for HA-mediated repair in the infarcted myocardium. Overall, several studies support the use of HA-based hydrogels as a promising novel therapy to reduce scarring and promote vasculature formation in the infarcted myocardium.

Alginate. Alginate is an anionic polysaccharide present in brown seaweed. It is biocompatible and has been used for food and pharmaceutical applications.[65] Its capacity for *in situ* gelation and nonthrombogenic properties makes it attractive for cardiac tissue applications. In heart tissues, intramyocardial injections of alginate hydrogels have shown significant clinical potential for the improvement of LV function and reduced remodeling potentially by providing mechanical support to the damaged ECM. Yu *et al.* showed that acellular alginate hydrogels could improve cardiac function, reduce remodeling, and increase neovascularization

Table 2. Other Polymeric Material for Cardiac Patch

| *Stem cell* | *Polymer* | *Porosity/pore size* | *Elastic modulus (MPa) Tensile stress Tensile strain (MPa) Tensile strain at break* | In vivo *model* | *Signaling* | *Ref.* |
|---|---|---|---|---|---|---|
| Cardiomyocytes differentiated from hESC | Poly(glycerol sebacate) | — | — | Adult male Sprague Dawley rats | — | 144 |
| cSca-1, BMMCs, and ADSCs | Nanopeptides (cell–PuraMatrix™) | — | — | Wild mice (C57Bl/6J) | Anti-vWF), anti-SMA, anti-α-sarcomeric actinin anti-CD31,VEGF, bFGF, and PDGF-bb | 152 |
| BM-MSCs | PPC, PU, and [P(3HB-co-4HB)] | — | — | — | CD34, CD45, CD90, CD73, CX43, and cTn T | 153 |
| Neonatal cardiomyocytes | PGS/collagen | — | 2.06 MPa (TS) 57.87% (TSA) 83.65% (TSB) 4.24 (EM). | — | Cardiac-specific marker proteins α-actinin, Troponin, β-MHC, and cx43 | 146 |
| BM-MSC | PCL/Gelatin | 83.6% ± 0.8%/ 0.83 ± 0.15 μm | — | Female Sprague Dawley rats | CD31, cTnT, and Cx43 | 47 |
| Cardiac progenitor cells | PCL/CNT | — | 11 MPa (EM) 1.3 MPa (TS), 131% (TSB) | — | sca-1, CD34, GATA-4, CD44, CD29, and CD31 | 150 |
| BMMSCs | PGS/Fibrinogen | — | — | Farm pigs (Sus scrofa) Yorkshire Swine | α-sarcomeric actinin, troponin T, CD68, and CD31 | 117 |
| hMSCs | PEG/Alginate | — | — | Male nude rats (Crl:NIH-Foxn1$^{rnu}$) | anti-vWF, anti-BRDU | 154 |
| Neonatal rat cardiac cell | Fibrin | — | 86.0 ± 3.8 (EM) 75.7 ± 11.5 (UTS) | Female Fisher F344 syngeneic immune-competent rats | SMA +, CD31, Type I collagen and Type IV collagen | 48 |

PCL, poly(caprolactone); PEG, poly(ethylene glycol); PGS, polyglycerol sebacate; TS, tensile stress; TSA, tensile strain; TSB, tensile strain at break; EM, elastic modulus.

in a chronic rodent model of ischemic cardiomyopathy.[66] The angiogenic effect of alginate can be further enhanced by incorporating RGD peptides into the biopolymer, but the structural changes reduce the therapeutic effects of the hydrogel.[67] Landa *et al.* evaluated the effect of alginate hydrogel in the recovery of cardiac function of rats post-MI.[68] There was an increase in scar thickness, and reduced LV systolic and diastolic dilatation was typically observed even after injection 60 days postinfarction. These results were comparable to those achieved by neonatal cardiomyocyte transplantation.

The use of alginate hydrogels has also been shown to improve cardiac function in large animal models. Intracoronary injection of alginate hydrogel prevented LV remodeling and increased scar thickness in a swine model of MI.[69] The alginate hydrogel was replaced by myofibroblasts, which support local tissue restoration while limiting general myocardial remodeling. Implantation of alginate hydrogel in dogs with heart failure produced by intracoronary microembolizations (LV ejection fraction <30%) significantly improved ventricular wall stability and function.[70] Injection of alginate hydrogel expanded the LV wall and improved the LV systolic and diastolic functions at levels comparable to those observed in dogs in long-term therapy with beta-blockers.[71] These observations motivated evaluation in patients with ischemic ($n=4$) and nonischemic ($n=2$) dilated cardiomyopathy. Patients that received alginate implants showed improvements in LV size and function as early as 3 days. Reductions in LV volumes and an increase in ejection fractions were sustained for over 3 months. Due to their promising results, alginate hydrogels are to date the only and first injectable biomaterial in clinical trials for treating MI. However, lack of integration between alginate and cardiac cells might be the major limitation for tissue regenerative applications.

Chitosan. Chitosan is a cationic hydrophilic polysaccharide derivate from chitin commonly found in crustacean shells. It has been extensively used in biomedical applications, including wound healing,[72,73] drug delivery systems,[74] and surgical adhesives.[75] The porosity of chitosan can be controlled as a function of freeze-drying,[76] which is important for host cell migration and tissue integration. However, chitosan alone is noncell adhesive and has a high compressive modulus, which requires its chemical modification and/or mixing with other biomaterials to obtain optimal mechanical and physiological properties for cardiac tissue. Pok *et al.* evaluated a multilayered scaffold formed by a gelatin–chitosan hydrogel around a self-assembled PCL core for use as a cardiac patch.[77] Gelatin and chitosan ratios of 50:50 and 25:75 significantly improved cell adhesion while retaining the mechanical strength of PCL. Mixtures of chitosan and collagen have also shown potential to improve cardiac function. Deng *et al.* combined chitosan with collagen to increase the compressive modulus of collagen as a potential implant for stabilization of the ventricular wall.[57] Ahmadi *et al.* investigated the effects of a collagen chitosan matrix on cardiac remodeling.[78] Mice received local injection of collagen–chitosan matrix 2 weeks post-MI. LV ejection fraction was improved only in collagen–chitosan-treated mice over a 3-week follow-up period. Thus, combination of porous chitosan with lower compressive moduli and cell-adherent biomaterials may have the potential to increase tissue integration and therefore increase the mechanical stability of the ventricle postinfarction.

Fibrin. Fibrin-based scaffolds are biopolymer gels formed from fibrinogen, a glycoprotein that contains two Arginine–Glycine–Aspartic acid (RGD) sequences in each amino acid chain and is converted by thrombin into fibrin during blood clot formation simulating the last step of the blood coagulation cascade.[79,80] Fibrin polymerizes *in situ* upon the combination of fibrinogen and thrombin. Fibrin glue has been tested as an injectable scaffold for cardiac tissue repair. Christman *et al.* examined the effects of injectable fibrin glue as a scaffold and wall support in the ischemic myocardium in rats. Fibrin glue alone or with skeletal myoblasts was injected into the LV 1–2 days after left coronary artery occlusion. Five weeks after injection, fibrin glue alone or with cells preserved infarct wall thickness, reduced infarct size, increased blood flow, and improved cardiac ejection function.[81,82] Huang *et al.* performed a comparative study to determine the therapeutic potential of fibrin, collagen type I, and Matrigel as injectable biomaterials for MI repair. Injection of each individual biomaterial into the infarct zone significantly increased vascularization compared to the saline solution control group at 5 weeks post-treatment in rats. The angiogenic potential was similar for all three polymers probably due the shared common binding sites for $a_vb_3$ integrin, which is associated to angiogenesis. The major disadvantage of fibrin gels is poor mechanical properties, not able to support the stresses generated during myocardial contraction.[83] Therefore, fibrin should be combined with other high-compression moduli components such as chitosan or collagen to increase mechanical strength and reduce degradation rates.

Decellularized extracellular matrices. Decellularized matrices are derived from biological tissues in which cells have been removed, but the architecture and components of the ECM are preserved. The main advantages of decellularized matrices are preserved structure, size, and components of native ECM without the presence of cellular antigens that could induce an immune reaction.[84] In cardiac tissue studies, decellularized matrices have shown to promote endothelial cell and cardiac cell infiltration. Decellularized urinary bladder ECM (UB-ECM) has been evaluated as an epicardial patch for repairing the infarcted LV.[85] At 6–8 weeks postinfarction, pigs received either a UB-ECM or expanded polytetrafluoroethylene (ePTFE) patch in the LV. At 3 months, the decellularized matrix was resorbed showing a highly vascularized tissue enriched in collagen and myofibroblasts. At the same time point, ePTFE had a foreign body response and calcification. Similarly, Robinson *et al.*[85] used a urinary bladder matrix (UBM) scaffold for repairing the infarcted LV in pigs. Results showed that after 3 months the constructs had a significant increase in cardiac marker expression (i.e., α-smooth muscle actin $(SMA)^+$ myofibroblasts, α-sarcomeric actin, myosin-HC, tropomyosin, and connexin 43) and the number of contractile cells (i.e., expressing α-SMA). It has been well established that UBM scaffolds are superior to synthetic Dacron in regenerating myocardial tissue. This fact is due to their potential capacity to promote cardiomyocyte differentiation and/or migration, allowing the ventricular wall to

approach its normal thickness, and finally resulting in improved regional mechanical function.[86,87] Moreover, natural matrices from small intestine submucosa (SIS)[88] and porcine sternum[89] used in rats models have showed to be a good alternative to promote angiogenesis, enhance cardiac function, and decrease apoptosis, through the recruitment of c-kit$^+$ cells, myofibroblasts, and macrophages after MI. Tan *et al.* used a decellularized SIS patch with MSCs on a MI rabbit model[90] and showed significant improvements in LV function, wall thickness, and vasculature. Decellularized ventricular and pericardial matrices are two additional options for MI repair.[91–93] Singelyn *et al.* have shown that decellularized porcine ventricular tissue can be solubilized and self-assembled *in situ* upon injection into myocardial tissue. Smooth muscle cells and endothelial cells were able to infiltrate the decellularized matrix both *in vitro* and *in vivo* with a significant increase in blood vessel density. These studies were performed in the healthy rat myocardium, which have better recovery rates than in MI.

Synthetic materials. Synthetic materials have been widely used in tissue engineering applications due to improved mechanical properties, material uniformity, and low risk of infection compared to natural biomaterials. Synthetic polymers can be modified with high precision to meet tissue-specific properties such as appropriate degradation rates, porosity, and mechanical strength. Several synthetic polymers have been evaluated for cardiac tissue implants, including poly(ethylene glycol)(PEG), polyvinyl alcohol, poly(caprolactone)(PCL), polypropylene, polyester, and poly(N-isopropylacrylamide) (PNIPAM).[94,95] Meshes made of polyester and poly(propylene) have been successfully used as LV restraints to prevent LV remodeling and dilation in animal models and human patients.[96–100] Injection of a thermosensitive hydrogel containing PCL and PNIPAM into the myocardium 4 days postinfarction in rabbits was found effective to prevent ventricular wall thinning and reduce systolic and diastolic dilatation after 30 days of treatment.[95] Similarly, PEG hydrogels have prevented LV remodeling and dilation, but lack vascularization unless codelivered with cells.[101] While the mechanical properties and stability of synthetic polymers are superior to natural polymers, cell integration is a major limitation. Blends of synthetic and natural polymers with or without growth factors are often preferred to support cell migration and tissue replacement of the implant.[102,103]

### *Engineered cellular constructs for cardiac repair*

Although acellular scaffolds have many advantages over cellular scaffolds, the use of cells has also been shown to improve healing and tissue regeneration. In many cases, the addition of a cellular component showed improvement over the material alone.[104] The use of an engineered cellular construct is a therapeutic strategy to regenerate the myocardium lost after an MI by supporting the function of the myocardium *via* a mechanically suitable material and the surviving cells using an appropriate exogenous repair cell. The cardiac patch is a 3D carrier for cell delivery fabricated *in vitro* and implanted over the infarcted tissue[47] with the goals of improving repair cell retention and engraftment, limiting LV remodeling, preventing LV dilatation and thinning, enhancing the mechanical properties of ventricle, and reducing cardiomyocyte apoptosis.[48] In addition, it can also provide the means to stimulate angiogenesis, release of cytokines, and myocardial perfusion.[48,105] All these properties depend on the choice of scaffold material and repair cells that will ultimately couple with the native cells.

One of the first patches developed for delivering cells to an MI site consisted of a cardiomyocyte-enriched extract from fetal rat ventricular muscle dispersed in a commercially available gelatin scaffold (i.e., Gelfoam, Merck, Co.).[106] Although this cardiac patch showed improved cell survival and retention, it was not able to demonstrate any improvement in cardiac function. In contrast, a similar type of patch based on alginate and fetal rat cardiomyocytes showed improvement on both cell survival and cardiac function in a rat MI model.[107] This variability in results is common and needs to be considered. These are mainly due to differences in degree of injury to the heart due to the infarct procedure used.[108] Additional studies in the development of cardiac patches have shown a limited use of cardiomyocytes. This is because these cell types are terminally differentiated, thus limiting their proliferative capacity.[109]

As an alternative, many researchers are studying a recently detected small population cardiac progenitor cells (CPCs) negative for blood lineage markers (Lin$^-$) and positive for stem cell surface markers (i.e., c-kit, Sca-1, and MDR-1), which have the potential for myocardial regeneration.[110] A potential treatment option could be to attract this cell population to the infarct site and provide them with the appropriate environment to stimulate myocardial regeneration. This environment should promote not only the migration of CPCs but also of any other cell types that might promote myocardial regeneration (e.g., macrophages, MSCs).

It is important to first differentiate between the term repair, which defines the natural healing process that replaces the damaged tissue with a scar, and regeneration (our therapeutic objective), in which the damaged tissue rebuilds itself to its normal structure and function.[111] The cardiac repair process involves an inflammatory response, where macrophages remove dead cardiomyocytes and secrete angiogenic and profibrotic cytokines, chemokines, and proteases.[112] This is followed by fibrosis, in which macrophages and myofibroblasts work together in reinforcing the ventricular wall through the secretion of connective tissue, eventually forming the scar tissue. This structure is so dense that it prevents the migration of CPCs, essential for cardiac regeneration, making fibrosis an important hurdle in mammals' capacity to regenerate myocardium after MI.[113] In addition, it has been found that acute inflammation is necessary to activate the regenerative response in the neonatal mouse heart.[114]

Another type of progenitor cells that have been tested for myocardial regeneration are MSCs loaded into scaffolds made of natural and synthetic polymers.[115] Collagen, fibrin, PGS, PCL, nanopeptides, polyurethane (PU), and 3-hydroxybutyrate-co-4hydroxybutyrate [P(3HB-co-4HB)] are examples of these scaffold biomaterials. The use of these patches has shown increased vascularization and improvement in cardiac function. They are also an improvement over cellular cardiomyoplasty, which is limited because in this technique cell survival involves enzymatic cell dissociation before injection and because of the poor vascularization in

the infarct site.[116] Cardiac patches could overcome these limitations by retaining viable transplanted stem cells in a scaffold that would facilitate cell migration and cell adhesion toward the infarcted area.[48] In addition, these transplanted cells could stimulate formation of vasculature and cardiomyocytes. As with most of the cell-bearing scaffolds, optimal properties of these include high porosity, high surface area to volume ratio, microenvironment similar to natural ECM, good mechanical properties, biodegradability, and biocompatibility.[47,117] These parameters can be modulated with the choice of materials used and the synthesis conditions chosen for its fabrication.

Collagen. Collagen matrices loaded with MSCs have proven to be a good alternative biomaterial for cardiac patches. Simpson *et al.* used collagen matrices seeded with human MSCs (hMSCs) to treat MI in the male CDF rat model.[118] The results showed that the application of this patch promoted myocardial regeneration and induced the expression of α-SMA marker, which was associated with an increase in myofibroblasts within the infarct region. Likewise, Maureira *et al.* developed a cardiac patch based on collagen and autologous MSCs, which was implanted in an MI rat model.[119] The results of this study showed reverse remodeling of the infarcted area, improvement of perfusion, a reduced infarction, an increase of ventricular wall thickness, enhanced angiogenesis, and formation of myofibroblast-like tissue.

Similarly, the effectiveness of collagen seeded with adipose tissue-derived stem cell epicardial patches to preserve LV function, decrease fibrosis, and increase vessels in the infarct area was confirmed in reported studies.[116] Furthermore, studies have evaluated the response of collagen matrices seeded with hMSCs and human ESC-derived mesenchymal cells (hESC-MC) to repair the infarcted myocardium of athymic nude rats.[105] This *in vitro* study showed similar responses with regard to stem cell potency, viability, and cell proliferation for both cell types, while the results from the *in vivo* study demonstrated maintenance of the diastolic function and attenuated adverse LV remodeling. Interestingly, even with the observed differences in response to secreted paracrine factors, local angiogenic effects were seen in both cell types. Shi *et al.* demonstrated that collagen scaffolds covalently conjugated with the Sca-1 antibody (present in adult murine hematopoietic stem cells[120]) can attract native autologous stem cells to encourage cardiomyocyte regeneration after MI.[121] In another study, collagen I patches seeded with human bone marrow $CD133^+$ cells were used on cryoinjured rat hearts to assess their *in vitro* and *in vivo* cardiomyocyte differentiation potential.[122] Results showed a capacity to induce angiogenesis, but not to induce cell differentiation. In a similar type of study, a collagen type I patch seeded with human umbilical cord blood mononuclear cells was used in MI mouse model to assess their cardiomyocyte differentiation potential.[123] These patches showed improvements in vascularization and cardiac function, as measured by an increase in ejection fraction. The same group also conducted the Myocardial Assistance by Grafting a New Bioartificial Upgraded Myocardium (MAGNUM) phase I clinical trial using the same autologous cell-seeded patches.[124] Ten patients presenting LV postischemic myocardial scars that were undergoing CABG had the seeded patches fixed onto the ischemic tissue. Results showed that the patch increased the LV wall with viable tissue and helped normalize cardiac wall stress in injured regions, improving diastolic function.

Fibrin. Fibrin is a hydrogel that contains adhesion molecules and has been shown to be biocompatible. However, fibrin-based scaffolds must be stabilized with other materials to compensate for their rapid reabsorption.[125,126] Studies demonstrate the feasibility of applying injectable fibrin gels into the infarcted myocardium.[127,128] Recently, Bago *et al.* observed cardiomyogenic differentiation of adipose tissue-derived progenitor cells using fibrin scaffolds in a mouse model.[129] This system promoted endothelial lineage, increased vessel density, improved cardiac function, and diminished scar size after MI. Wendel *et al.* reported infarct size reduction, elimination of LV wall thinning, vascularization, and the restoration of cardiac function in rats 4 weeks after transplantation of the cardiac patch, which consisted of fibrin scaffolds seeded with neonatal rat cardiac cells.[48] Geuss *et al.* showed PEGylated fibrin gels as an alternative to promote proliferation and cardiomyogenic differentiation.[126] However, by comparing two-dimensional (2D) versus 3D cell cultures, contractile activity was maintained when cells were cultured on layers of PEGylated fibrin and significantly reduced when cultured as aggregates. The authors argued that the lack of contractility was related to low cardiomyocyte proliferation and not to the influence of mechanical properties (i.e., elastic modulus).

To develop cardiac patches that more closely mimic ECM, current tissue engineering approaches to myocardial regeneration are implementing the use of therapeutic genes to enhance paracrine action. An example, insulin-like growth factor-1 (IGF-1), which is a hormone that has been shown to enhance cardiac differentiation, reduces apoptosis and enhances neovascularization.[79,130,131] Recently, Li *et al.* published the first study showing cardiac repair in a large animal model using fibrin patch seeded with IGF-1-modified MSCs.[79] The results of this study were interesting because, despite the absence of cardiomyogenic differentiation *in vivo*, it promoted cardiac repair through other pathways. Similarly, Ye and colleagues used three cell types: human iPSC (hiPSC)-derived cardiomyocytes, endothelial cells, and smooth muscle cells in combination with fibrin patches seeded with IGF-1-encapsulated microspheres to treat MI in a porcine model.[132] The results demonstrated cardiac repair without developing ventricular arrhythmias. In addition, Xiong *et al.* seeded fibrin patches with hESC-derived endothelial and smooth muscle cells and implanted on a porcine MI model.[133] Results after 7 days showed a significant improvement in LV ejection fraction, which was maintained for over 4 weeks.

ECM scaffolds. An innovative alternative that has proven very effective is the use of natural matrices as scaffolds. Wang *et al.* demonstrated that a cardiac patch consisting of decellularized porcine myocardium seeded with porcine bone marrow MSCs can support cardiomyogenic differentiation and angiogenesis *in vitro* (Fig. 4A).[134] In addition, decellularized human myocardium was seeded with human mesenchymal progenitor cells suspended in a fibrin hydrogel and fixed on the MI tissue in a nude mice model.[104]

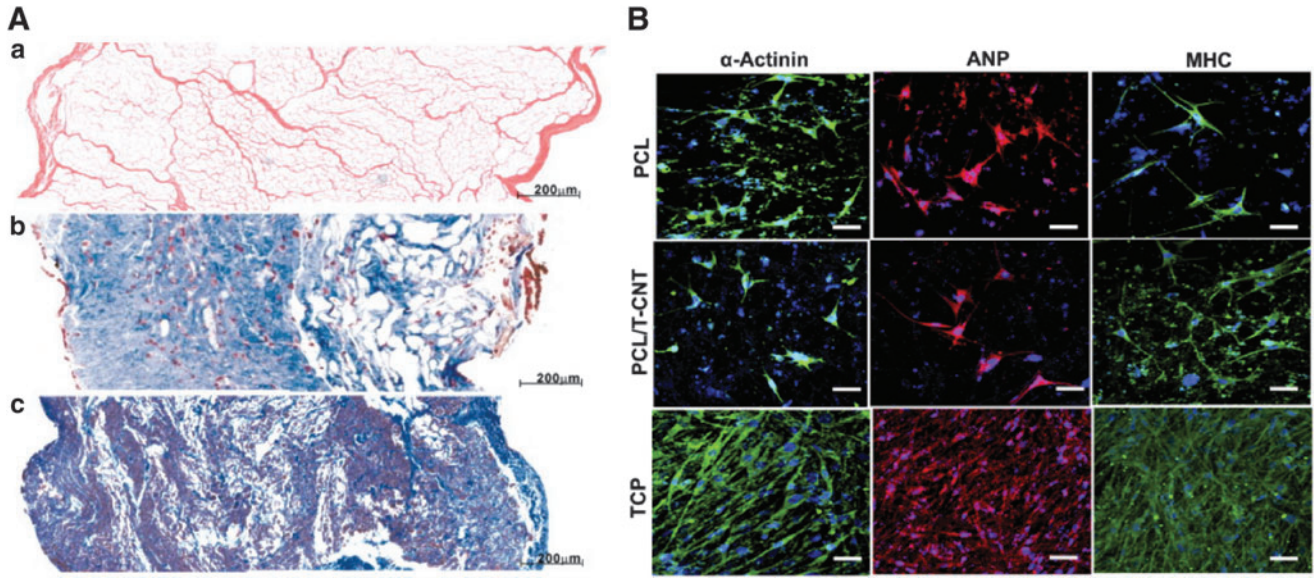


**FIG. 4.** **(A)** Decellularized porcine myocardium can support cardiomyogenic and angiogenic differentiation. **(a)** Large pores evenly distributed across the 2 mm thick acellular scaffold (H&E). **(b)** Edge to edge view of thorough recellularization at 2 weeks in which cells were found to infiltrate and distribute within the myocardial scaffold. **(c)** Tissue remodeling was observed in the 4-week recellularization tissue construct; cells were still observed, and cell density was found higher than of the 2 week's construct. Reprinted with permission from Wang *et al.*[134] **(B)** Immunofluorescence images showing the expression of cardiomyocyte markers, α-actinin, ANP, and MHC in CPCs that have been induced to differentiate 21 days after seeding onto PCL or PCL–TCNT meshes, or TCP. Scale bar, 50 μm. Reprinted with permission from Wickham *et al.*[150] ANP, atrial natriuretic peptide; CPC, cardiac progenitor cell; MHC, myosin heavy chain; TCP, tissue culture plastic. Color images available online at www.liebertpub.com/teb

Results showed a significant improvement in cardiac function and vascularization. French *et al.* indicated that CPCs seeded on naturally derived cardiac ECM show enhanced adhesion, maturation, proliferation, and survival compared with a collagen matrix.[135] Singelyn *et al.* used porcine myocardial tissue as an injectable myocardial matrix into rat myocardium.[91] The results demonstrated that this matrix not only allowed the formation of nanofibrous structures containing glycosaminoglycans but also the migration of endothelial and smooth muscle cells. The feasibility of this

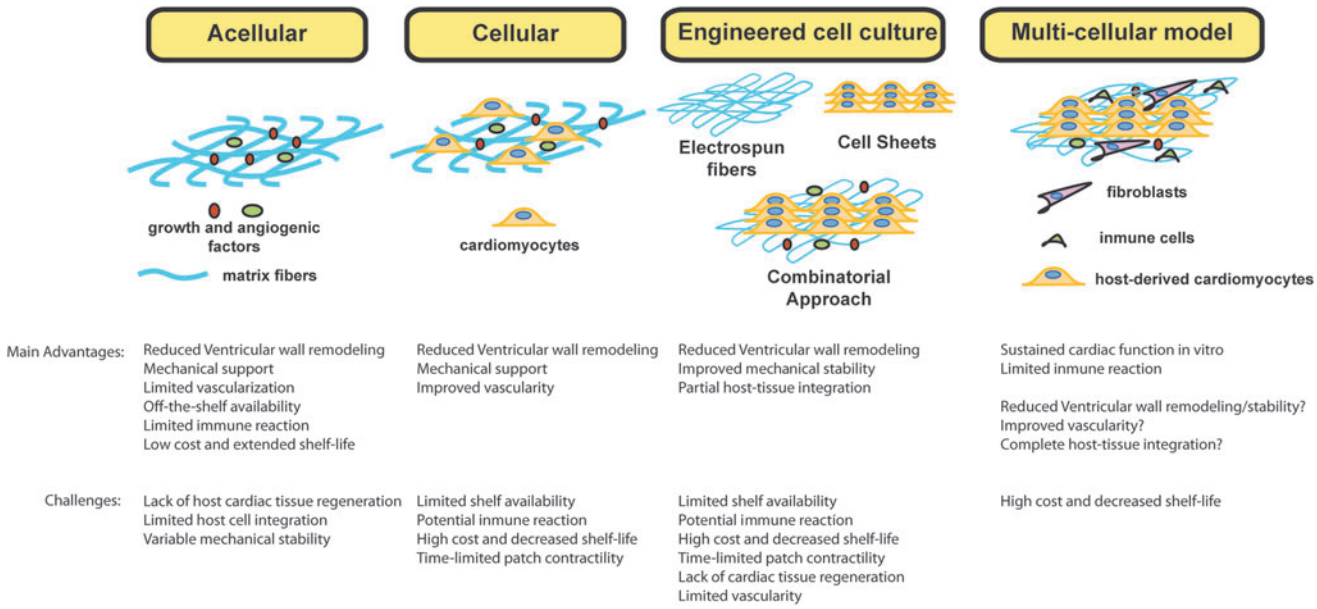


**FIG. 5.** Summary of engineered approaches to cardiac tissue regeneration. Extracellular matrix materials such as collagen, alginate, hyaluronic acid, and chitosan are used alone or combined as a cardiac patch to provide mechanical strength and stabilize the ventricular wall (acellular). Addition of cells to extracellular matrix constructs (cellular) or engineered matrices improves cardiac function and vascularization compared to acellular models. The question mark (?) implies it remains to be determined in future studies. Color images available online at www.liebertpub.com/teb

approach was demonstrated in a large animal model through a transendocardial catheter injection.[136]

Extracellular matrices derived from porcine UBM can also serve as an inductive scaffold for myocardial repair. Potapova *et al.* found formation of myocytes and improved mechanical function in canine heart using UBM seeded with partially differentiated hMSCs.[137] Shah *et al.* also probed the ability of this natural matrix to support and promote the growth of mature cardiomyocytes.[138] In addition, Wei *et al.* used decellularized bovine pericardial tissue as scaffolds seeded with multilayered MSCs on a male syngeneic Lewis rats MI model.[139] The advantage of this study was the development of vasculogenesis, angiogenesis, and differentiation of MSCs toward myofibroblast, although no mature cardiomyocytes were found within the patch.

Poly (glycerol sebacate). In 2002, Langer and coworkers reported the synthesis of PGS, a biodegradable elastomer composed of glycerol and sebacic acid with desirable chemical and physical properties for tissue engineering applications.[140] In addition, this polyester is considered a good material for cardiac patches because of its biodegradability, excellent mechanical properties, and low cost.[141–143] Chen *et al.* used PGS patches to deliver differentiated cardiomyocytes from hESCs to treat MI in adult male Sprague Dawley rats.[144] The results showed that this system not only maintained active cardiomyocytes beating for an extended time but it also supported cells during *in vivo* implantation. In addition, the PGS/fibrinogen cardiac patch has demonstrated excellent mechanical properties, good cell–scaffold interactions, and capacity to stimulate the expression of cardiac-specific markers (i.e., α-actinin, troponin, ß-myosin heavy chain, and connexin 43).[145] In addition, PGS/fibrinogen/VEGF scaffolds loaded with MCSs have been shown to promote both the expression of cardiac and endothelial cell markers while preventing negative ventricular remodeling *in vivo* in a porcine MI model.[117] Likewise, it was demonstrated that PGS/collagen patches can provide a good local microenvironment similar to the natural interactions existing between cells and the native ECM, stimulating cardiogenic differentiation of MSCs.[146]

Polycaprolactone. PCL is a FDA-approved biodegradable synthetic polymer used as a scaffold material for cardiac tissue regeneration due to its mechanical properties and biodegradability. Nevertheless, PCL must be combined with other polymers (e.g., dextran, poly-vinylalcohol, and polyethylene oxide) to improve its hydrophobicity through the presence of ester and keto groups in its structure, which inhibit cell binding.[147] Therefore, electrospun nanofibrous scaffolds fabricated from this material can simulate the ECM microenvironment, allowing diffusion of nutrients, cell adhesion, cell growth, migration, proliferation, and differentiation.[148] Studies have reported that the use of a PCL/oligomer hydrogel can simulate ECM.[149] The incorporation of thiophene-conjugated carbon nanotubes into PCL scaffolds enhances the mechanical properties, cell adhesion, proliferation, and differentiation of CPCs (Fig. 4B).[150] Similarly, *in vivo* studies in rats have demonstrated that PCL/gelatin scaffolds loaded with MSCs can restrain LV remodeling, improve cardiac function, and promote both angiogenesis and cardiomyogenesis in the infarcted area.[47] In addition, scaffolds of the PCL derivative poly(glycolide-co-caprolactone) were seeded with bone marrow mononuclear cells and fixed in a rat MI model.[151] Results after 4 weeks showed significant improvements in ejection fraction, ventricular diameter, and hemodynamics.

Others. Research in the development of cardiac patches has also explored other polymeric materials. For instance, a commercial biological matrix (cell-PuraMatrix™) formed of self-assembled peptides can serve as a biological scaffold to evaluate the feasibility of different types of cells involved in myocardial repair in mice [(i.e., clonal stem cell antigen-1-positive cardiac progenitors (cSca-1), bone marrow mononuclear cells, skeletal myoblasts, and adipose tissue-derived MSCs)]. Results indicate that cSca-1 is better in preventing cardiac remodeling and systolic dysfunction compared to the other cell types.[152] *In vitro* and *in vivo* studies have demonstrated that [P(3HB-co-4HB)] and PU can support stem cell growth,[153] while a cardiac patch consisting of hMSCs encapsulated in alginate and seeded in polyethylene glycol hydrogels stimulates healing after a heart attack.[154] In addition, Miyagi *et al.* developed a hybrid scaffold using a gelatin scaffold (i.e., Gelfoam, Merck, Co.) coated with PCL (for reinforcement) and seeded with bone marrow MSCs and the cytokine stem cell factor and stromal cell-derived factor-1alpha.[155] Results in a small animal ventriculotomy model showed a significant improvement in cardiac function when compared to controls (i.e., animals and unmodified gelatin sponge). This improvement was observed in all groups with the scaffold alone, with or without cytokines or cells.

## Advanced Culture Systems

Appropriate cell function and tissue integration depend on cell–cell and ECM interactions. In the myocardium, electrical stimuli are propagated through interconnected cardiomyocytes highlighting the importance of gap junctions and cell polarization for appropriate function. Intercellular communication between cardiomyocytes is essential for the coordinated and synchronized contraction of the cardiac muscle. This communication occurs through gap junctions, which are membrane channels formed by the connexin family proteins which allow the propagation of rapid anisotropic impulses. These connexins have been shown to play a crucial role in determining impulse conduction and the heart morphogenesis, as well as in several cardiomyopathies, such as myocardial ischemia.[156] *In vitro*, cell polarization and cell–cell interconnectivity can be achieved by modulating cell adhesion to the culture surface. In this section, we discussed current engineered techniques used to develop optimal ECM architecture and improve cardiac cell–cell adhesion and function.

### *Electrospinning nanofibers*

Electrospinning is a technique that uses an electrical charge to produce nanofibers from polymer solutions. This technique has gained a lot of popularity for tissue engineering applications due to its ability to produce a network of interconnected nanofibers with similar fibrous architecture to natural ECM found in soft tissues. Several biocompatible and biodegradable polymers have been used to develop nanofiber networks using the electrospinning

technique for cardiac tissue regeneration applications. Zong *et al.* used electrospinning to fabricate biodegradable nonwoven poly(lactide)- and poly(glycolide)-based (PLGA) scaffolds.[157] In these polymeric fibers, primary cardiomyocytes developed mature contractile machinery (sarcomeres) and showed electrical activity using voltage-sensitive dyes over 7 days. Similar cardiomyocyte contractility and survival have been observed with other biodegradable polymers used in electrospinning, such as polyurethane,[158] poly(lactic-co-glycolic) acid (PLGA),[159] and PCL.[160] However, these *in vitro* studies were carried out in the absence of fibroblasts, which can modulate cardiomyocyte function and structure[161] and thereby impact long-term survival and tissue integration. Hussain's group addressed the fibroblast gap in 3D cardiac tissue constructs by fabricating a 3D chitosan nanofiber scaffold using an electrospinning technique to maintain cardiomyocyte function with fibroblast cocultures.[162] A mat of chitosan fibers ($\sim 150\,\mu m$ thick) was coated with fibronectin to enhance cell adhesion of neonatal cardiomyocytes isolated from rats in coculture with 3t3-J2 fibroblasts. Cardiomyocyte cocultures maintained cell polarization and expressed high levels of Connexin43, a gap junction protein required to propagate electrical stimuli, over 3 weeks. Image analysis of intracellular calcium ion staining showed that cardiomyocytes maintained an elongated morphology and beating frequency of $17 \pm 3$ contractions per minute only in 3D cocultures, but not in 2D or 3D monocultures highlighting the importance of fibroblasts for long-term function in cardiac tissue constructs. Overall electrospinning techniques have shown great potential for heart tissue scaffold. However, *in vivo* studies are needed to evaluate heart function and recovery over time following MI.

### *Engineered cell sheets*

Cells sheets have improved cell survival compared to single cells injected in tissues[163] and have been used clinically for the repair of several tissues such as eye cornea,[164] cartilage,[165] and heart.[166] Cell sheets are formed by detachment of 2D cell monolayers using surface detachable polymers or magnetic particles. Okano and colleagues develop a method to harvest cell sheets by lowering the culture temperature, using the temperature-responsive polymer poly(N-isopropylacrylamide) (PIPAAm).[167] When covalently grafted on the surface of culture dishes, cells adhere *via* serum and cell membrane proteins, due to PIPAAm hydrophobicity at the standard culture temperature of 37°C. Below its critical temperature of 32°C, PIPAAm becomes very hydrophilic and protein nonadhesive; thus, at lower temperatures, cell sheets detach without damaging cell–cell junctions or ECM proteins underneath the cell sheets. Using this method, Okano and colleagues fabricated a 3D sheet of pulsatile neonatal rat cardiomyocytes.[167] The multilayer cardiac construct was macroscopically observed to pulse spontaneously and showed a diffuse pattern of Connexin 43 gap junctions. Long-term survival was observed up to 1 year, and the cell sheet construct showed well-differentiated sarcomeres and abundant mitochondria.[168] Usually thick cell sheets (>300 μm) are preferred for tissue transplant, but limited due to lack of sufficient vascularization. To overcome this limitation, Okano's research group explored the possibility of adding endothelial cells to the 3D contractile constructs.[169] Cell sheets were stacked and *transplanted* into the dorsal subcutaneous tissue of rats. One week after transplantation, fluorescence-labeled endothelial cells in the stack formed continuous tubular blood vessel networks in the construct and integrated with the underlying host tissues. The myocardial endothelial tissue grafts showed improved vascularization compared to myocardial cell sheets that can potentially overcome the limits of mass transport to create functional integrated tissues. However, these studies were carried out in subcutaneous tissue rather than in damaged heart tissue; thus, results may not fully reflect their potential for myocardial repair.

Sawa's group evaluated the use of myoblast sheets for treatment of MI. Two weeks after MI, cell sheets were directly implanted over the scar area and heart function was monitored. Myoblast sheet MI-treated group demonstrated uniform repair in the anterior wall and improved function probably due to remodeling of the geometry of the LV chamber. In addition, myoblast sheets showed higher secretion of proangiogenic factors HGF and VEGF compared to single cell injection and controls.[168] In another study, clinical implantation of autologous myoblast sheets in a patient who had been supported with a LV assist system for dilated cardiomyopathy showed significant improvement.[170]

Another strategy termed magnetite force-based tissue engineering has been successfully used to generate cell sheets for tissue engineering applications.[171,172] This method uses a magnetic field to generate sheets of magnetized cells. Liposomes are loaded with magnetite to display a positive charge that electrostatically fuses with the cell membrane. Cells are grouped to form sheets by placing a magnet at the bottom of a cell culture well. Ishii *et al.* used this technique to implant a multilayered sheet of adipose-derived regenerative cells (ADRC) in the infarcted myocardium.[173] After 28 days of implantation, echocardiographic measurements revealed that the decreases in LVFS and LVEF following MI had significantly improved in ADRC sheets compared to collagen gel and ADRC controls. One of the main advantages of this cell sheet technique is the improvement in angiogenesis more effectively than direct injection of cell suspensions, which can explain the observed significant decrease in cardiac fibrosis and increased myocardial capillaries, compared with the control groups. One major drawback in this study is that ADRC sheets were placed on the surface on the infarcted myocardium after 1 min of coronary occlusion, which could have contributed to the decrease of adverse remodeling as necrotic tissue is minimal. Cell death can occur as quickly as 20 min after coronary artery occlusion, which is within the time frame of MI, and can negatively impact the regenerative potential of the tissue. Thus, additional studies with various times of sheet implantation are required to determine the potential of these cell sheets for cardiac regeneration.

### *Multicellular constructs and advanced culture systems*

As our understanding of the architecture and composition of myocardial tissue increases, it becomes apparent that a single cell type-based tissue engineering approach may not

be sufficient to repair the myocardium. Two culture and triculture systems might be required to provide the appropriate combination able to improve cell coupling with the host's cells and ultimately improve function. This will also require a new 3D culture system to recapitulate the ultrastructural composition and orientation of the myocardium while allowing for proper diffusion of nutrients and cell survival. The only cell sources that can reproducibly provide functional cardiomyocytes *in vitro* are pluripotent stem cells (ESCs and iPSCs). Although these cells provide functional myocytes, when assembled into 3D tissues an optimal ratio of supporting cells (such as fibroblast) is needed for the formation of myocardial-like tissue.[174] As shown in previous sections, the infiltration of these supporting cells is a requirement for a viable scaffold. For instance, the combination of cardiomyocytes with endothelial cells and mesenchymal cells has been shown to improve contractility *in vitro*, further supporting the role of multiple cells during the formation of engineered tissues.[175] Another level of complexity that will need to be added is the incorporation of biophysical stimulation. This has been reviewed elsewhere,[176,177] but will be needed to improve the function and maturity of cardiac constructs *in vitro*.

Another interesting approach has been to decellularize whole organs to provide the best 3D environment for cells to attach and grow in their appropriate *in vivo* compartments.[178,179] The work by Wang *et al.* is a good example, in which a 2-mm section of porcine myocardium was reseeded with differentiated bone marrow mononuclear cells.[134] This scaffold maintained the cardiomyocyte-like phenotype of the seeded cells and showed angiogenesis potential and recovery of the native tissue's mechanical properties after remodeling. Knowing the extent of recapitulation of the native structure or the correct combination of cells needed to provide physiologically relevant bioengineered tissues still remains to be determined. It is clear that although some cardiomyocyte 2D culture systems have proved adequate for gathering cardiac physiological data in applications such as drug discovery,[180] these are not adequate for tissue regeneration applications, where the *in vivo* tissue complexity needs to be recapitulated. Future culture systems should advance toward 3D cultures providing appropriate biophysical conditions and oxygen tension to support native-like bioengineered cardiac tissues for implantation.

In addition to creating tissues in the laboratory, it is also important to test such constructs under physiologically relevant conditions. The traditional approach has been to test cardiac patches in animal models (often in small animal models). This approach provides information regarding survival of the cardiac repair cells, but remains a poor model when testing human-derived cells given the physiological differences between rodents and humans and the need for immune-compromised animals. The development of *in vitro* culture systems that use relevant cells during the host tissue response such as macrophages to test bioengineered cardiac tissues has been proposed.[9–11] As explained in previous sections, after an MI there is a dynamic response driven by different subtypes of macrophage (proinflammatory vs. prohealing), which play a major role during the remodeling process. However, the interactions between inflammatory cells and engineered cardiac patches have been widely ignored during *in vitro* screenings. A recent study by Pallotta *et al.* showed that there is potential cross talk between the macrophage subtype and cardiomyocytes derived from human ESCs further supporting the need to understand these interactions.[181] Advanced culture systems that incorporate the host tissue response and repair cells will allow for the manufacture and screening of bioengineered cardiac tissues that can be more readily translated to clinical use. The main advantages and challenges of the different approaches to cardiac tissue regeneration are summarized in Figure 5.

## Conclusions and Future Directions

Engineering approaches toward the repair of myocardial tissue have shown promise in the past, but full restoration of myocardial function remains elusive. As our understanding of the heart's physiology and function grows, tissue engineering principles can be applied toward the design of an engineered cardiac patch that ultimately restores function to the heart and prevents myocardial wall remodeling. Among the potential approaches are (1) acellular scaffolds that provide biological activity and biophysical support to the heart and (2) cellular scaffolds that provide the minimum combination of cells and biomaterial composition needed for increased biological activity and biophysical support. It is also clear that advanced culture systems (i.e., triculture systems) will be needed to create more advance engineered cardiac tissues and to create better screening tools to test bioengineered cardiac patches *in vitro*.

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## Disclosure Statement

No competing financial interests exist.

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