Published in final edited form as:

J Cardiovasc Transl Res. 2011 October ; 4(5): 575–591. doi:10.1007/s12265-011-9303-1.

Strategies for Tissue Engineering Cardiac Constructs to Affect Functional Repair Following Myocardial Infarction

Kathy Yuan Ye and

Department of Biomedical Engineering, Tufts University, Medford, Massachusetts,
kathy.ye@tufts.edu

Lauren D. Black III

Department of Biomedical Engineering, Tufts University, Medford, Massachusetts,
lauren.black@tufts.edu

Abstract

Tissue engineered cardiac constructs are a high potential therapy for treating myocardial infarction. These therapies have the ability to regenerate or recreate functional myocardium following the infarction, restoring some of the lost function of the heart and thereby preventing congestive heart failure. Three key factors to consider when developing engineered myocardial tissue include the cell source, the choice of scaffold, and the use of biomimetic culture conditions. This review details the various biomaterials and scaffold types that have been used to generate engineered myocardial tissues, as well as a number of different methods used for fabrication and culture of these constructs. Specific bioreactor design considerations for creating myocardial tissue equivalents *in vitro*, such as oxygen and nutrient delivery as well as physical stimulation are also discussed. Lastly, a brief overview of some of the *in vivo* studies that have been conducted to date and their assessment of the functional benefit in repairing the injured heart with engineered myocardial tissue is provided.

Keywords

Tissue engineering; myocardial infarction; biomaterials; bioreactor; heart failure; polymer scaffolds

Introduction

Cardiovascular disease is currently the leading cause of death in the United States [1] and is rapidly becoming one of the leading causes of death in the world [2]. The buildup of atherosclerotic plaques in the coronary arteries leads to ischemia, and, if left untreated, the complete occlusion of the artery results in myocardial infarction (MI). While recent studies have demonstrated that there is a limited regeneration potential in the adult heart [3], the level of cellular turnover is far below what would be needed to restore the contractile function lost in response to a significant MI. In the absence of a regenerative repair of the injured portion of myocardium, the subsequent remodeling of the infarcted area often leads to a collagenous scar in place of normal tissue [4]. Ultimately, it is the loss of this portion of the contractile myocardium that begins a cascade of compensatory mechanisms that, while initially beneficial, over time, lead to congestive heart failure [5]. Though many therapies, including various pharmaceuticals, can be used to slow down the progression to heart

failure, these treatments do not restore contractile function to the affected tissue [6]. Ultimately, heart transplantation is the gold standard for treating congestive heart failure, allowing for the best patient outcomes [7], and complete restoration of pumping function of the heart. However, the need for replacement organs continues to grow while the number of transplantable organs is on the decline [8]. Furthermore, given the associated complications of this treatment, including those related to the need for lifelong immunosuppressant therapy [9], there exist a need for new and innovative strategies for restoring the contractile and electrical function to the injured heart following MI.

One such strategy is cellular cardiomyoplasty, whereby cardiomyoblasts or other types of stem cells are directly injected into the injured heart of the patient in hopes of generating new cardiomyocytes and restoring some of the loss of heart function caused by MI [10]. So called “cell therapy” has been studied in clinical trials investigating its efficacy in restoring heart function following injury [11,12] and while these studies demonstrate statistically significant improvement in myocardial function, this improvement is often not clinically relevant and the functional benefit of the cell injection is, for the most part, transient [13,14]. Investigations into this transiency have determined that the technique often suffers from limited cell implantation (~5-10% of injected) [15,16], and a lack of appropriate cell functionality of those cells that do engraft [17]. Indeed, some cell sources had a negative effect in response to the myocardial environment, resulting in arrhythmias due to inappropriate electrical integration [18].

Acellular cardiac constructs delivering bioactive molecules have also be used as another method of remodeling damaged scar tissue [19]. The bioactive molecules target cells towards the infarcted regions, while the construct reinforces the heart mechanically to prevent further left ventricular dilation [20]. The addition of cells in cardiac constructs can increase the signaling molecules produced and reduce negative remodeling to preserve cardiac function [21]. The use of tissue engineering techniques to generate 3D engineered myocardium has the potential to result in improved cell retention over cell therapy and offers the ability to generate morphology and function similar to the native tissue through control of the *in vitro* culture conditions. In order to increase the viability and function of the implanted cells, one would need to create an environment that best recapitulates the structure and composition of the healthy myocardial tissue, while also retaining the biochemical and electromechanical properties [22]. Furthermore, the ideal biomaterial scaffold for engineered myocardium would be biodegradable and biocompatible, with mechanical properties that are similar to the native myocardium. This is especially important since the engineered tissue construct must be able to support the constant loads that the heart undergoes during physiological function [23] and in the case of acute infarctions, help prevent the deterioration of the heart tissue [24]. Though constructs have been shown to improve heart function after chronic infarctions [25], earlier interventions after MI can help prevent heart failure [26-28].

Many methods of culturing constructs have been established. Each of the culturing strategies, *in vitro*, *in situ*, and *in vivo*, comprises of unique benefits and challenges. For example, *in vitro* culture conditions can be used to closely mimic the native biophysical environment that would lead to enhanced tissue function upon implantation *in vivo* [29]. However, complete integration between the construct and native tissue needs to be considered [30]. Additionally, creating constructs *in situ* allows for the earlier repair of MI, with the biomaterial acting as a supporting matrix while the cells simultaneously remodel the infarction [31]. However, *in situ* constructs are affected by the harsh microenvironment of the infarcted region [32]. Lastly, *in vivo* systems allow the patient's body to vascularize and remodel the construct [33]. However, the secondary implant creates a donor surgical site with its own morbidity issues that are likely to create additional pain and possible infections

[34]. No matter which method is used to generate the engineered myocardium, three key factors to consider when developing engineered myocardial tissue include the cell source, the choice of scaffold, and the culture conditions.

Many different cell types including cardiomyocytes [35-37], embryonic stem cells [38], bone marrow-derived mesenchymal stem cells [39], and even adipose-derived adult stem cells [40], have been used to generate engineered cardiac constructs. Each has its advantages and disadvantages. While the cell source used in generating engineered cardiac tissues is an important component of the construct, we will not address this topic in the present review. A thorough discussion of the potential cell sources for cardiac tissue engineering, and their benefits and drawbacks, is addressed in another review in this issue by Radisic *et al.* In this review, we will focus on the creation of engineered myocardium tissue constructs as a therapeutic option for treating myocardial infarction. In particular, we will detail the various scaffold types used to generate these tissues, as well as a number of different methods that have been used for fabrication and culture of engineered myocardial constructs (see Figure 1 for a Summary). Lastly, we will summarize some of the *in vivo* studies to date and their assessment of the functional benefit in repairing the injured heart with engineered myocardial tissue with an eye towards potential clinical application.

Scaffolds

An important consideration in all areas of tissue engineering is the scaffold on which the cells could be seeded. Indeed, the properties of the substrate can influence cell functions including proliferation [41], migration [17], contraction force [42], calcium transients [43] and differentiation [44]. Scaffold types can be classified by both the material used (Natural vs. Synthetic) and the structure (Preformed vs. Hydrogel). The various scaffolds used in cardiac tissue engineering and their individual benefits and drawbacks are discussed below.

Biomaterial Type

Natural Biomaterials

Natural biomaterials more closely mimic the native extracellular matrix (ECM), which helps facilitate cell growth and proliferation during *in vitro* culture. ECM proteins collagen and fibrin contain peptide sequences, to which cells will adhere, that interact with integrins on the cell surface [45]. Integrins are heterodimer cell surface receptors that serve to anchor cells to the ECM, and their activation via both binding and substrate stiffness has been implicated in a number of cell functions including proliferation and differentiation [46]. Collagen I is a predominant ECM protein that helps to create 3D structures that are very similar to native environments, while also allowing host remodeling via normal protease degradation [47]. Based on the observation that collagen is one of the main ECM components of the myocardium, it has been used for both hydrogel [35] and spongelike [48,49] engineered myocardial constructs. Furthermore, collagen I has been FDA approved for therapeutic use in the repair of skin [50] and bone [51], and thus it is an attractive option from a regulatory standpoint. Often, collagen I is mixed with Matrigel, a tumor extract containing ECM proteins and growth factors, which promotes cell differentiation, improves graft survival, and helps repair damaged tissues [52]. Matrigel has also been used alone as an injectable scaffold [53]. However, because Matrigel has unspecified composition and is derived from a tumor, it cannot be safely used in clinical settings, thus limiting its relevancy *in vitro*.

Fibrin is an attractive alternative biopolymer for cardiac tissue engineering because it can be easily formed into fibrillar networks, similar to type I collagen [54]. In addition, it has the potential to be autologous because it can be extracted from the patient's blood, alleviating

the risk of a foreign body reaction to the scaffold [55]. Due to its role in the body's natural wound healing mechanism, fibrin gel is very bioactive, stimulating the cells entrapped in the gel to replace the initial scaffolding with their own extracellular matrix. This ultimately allows the construct to serve as a temporary scaffold [56] with the end stage graft being composed only of the patient's own cells and the ECM that they create. In addition, fibrin has been FDA approved as a surgical sealant and thus has a history of use in the clinical setting. Fibrin glue [20], and hydrogel scaffolds [37,57,58] have both been explored as possible cardiac grafts.

Native decellularized extracellular matrix (ECM) from donor organs is gaining momentum as a natural biomaterial choice for cardiac tissue engineering, used both as a preformed scaffold [59] and as an injectable scaffold [60]. Myocardial ECM [60], as well as small intestinal submucosa (SIS) [61], and urinary bladder matrix (UBM) [62] have been assessed as potential decellularized ECM based-biomaterials for cardiac repair. Decellularization removes all cellular content, while preserving the ECM biochemical composition and mechanical integrity [63]. While myocardial tissue has unique cell-to-cell interconnections that are mediated by the ECM [64], the complete composition of the decellularized ECM is still under investigation. Further, concerns still exist as to whether ECM from non-transplantable hearts would still be of beneficial use and whether or not xenogeneic sources of ECM will have issues with immunogenicity when implanted in humans as part of a cardiac graft.

Other biomaterials used in cardiac tissue engineering include hyaluronic acid, a macromolecule made of linear glycosaminoglycans (GAGs) that is critical for embryonic development. Highly controlled HA aids in the neovascularization of tissues [65] and has been shown to increase capillary density and normalize left ventricular function in infarcted rat hearts [66]. Another natural biomaterial that has been used in cardiac tissue engineering is derived from algae. Alginate is a polyanion that readily and reversibly forms cross-linked hydrogels when exposed to calcium [67]. Fetal cardiac cells seeded onto alginate scaffolds stimulated neovascularization and healing of the infarcted myocardium in a rat model of MI [68]. Similarly a collagen – glycosaminoglycan composite has been used to help deliver MSCs to the infarct region resulting in an induction of neovascularization at the site of the infarct and increased MSCs in both the scaffold and the heart wall [69]. Gelatin has also been used to generate tissue engineered myocardium and the use of this natural biomaterial has been shown to allow for cell attachment and integration to the epicardial surface of the infarcted heart [70].

Though natural polymers are biocompatible and aid in the attachment and proliferation of cells, natural polymers have the potential to be degraded enzymatically by the body's natural proteases, which can inhibit the functionality of the constructs created from them, especially over longer periods of time *in vivo*. Furthermore, because the polymers often come from animal sources, isolating large quantities of individual polymers can be difficult and a risk of contamination exists [71].

Synthetic Biomaterials

Synthetic polymers are a beneficial choice for scaffolds for cardiac tissue engineering because it is easier to modify the polymer in order to control the final properties of the scaffold such as the structure, pore size and orientation. The polymer composition can be tailored to create mechanical strength and controllable degradation patterns [72]. Furthermore, the binding sites presented to cells can be specifically controlled to alter how the cells sense the scaffold [73], allowing for optimal stimulation of specific integrin-mediated pathways.

Poly(lactic-co-glycolic acid), PLGA, a polymer created with lactic and glycolic acid, has been used extensively because it can provide a controllable degradation profile, which can be easily modified by changing the concentrations of the constituents in the polymer composition [74]. PLGA scaffolds have been made by lyophilization [75], extrusion [76], and salt leaching methods [77]. Engelmayr created an accordion-like honeycomb scaffold with poly(glycerol sebacate) [78]. Poly(glycerol sebacate) is an elastomeric biodegradable polyester, that degrades very slowly, allowing the polymer to maintain its mechanical strength over an extended period of time [79]. It can also be used to create porous scaffolds using a salt leaching technique [80]. Poly(N-isopropylacrylamide) (PNIPAAm), a temperature-sensitive polymer is used to enable a temperature sensitive cell adhesion binding to tissue-culture plates [81]. At 37°C, the cells adhere to the plates, while at a lower temperature, the cells detach, providing cell sheets that can be used to create scaffoldless constructs, explored later in this review. Other polymers used for engineered myocardial constructs include polycaprolactone [82], poly(trimethylene carbonate-co-lactide) (PTMCLA) [83], and polyurethane [84-86].

While synthetic polymers allow for greater control of internal architecture and degradation profiles, there are still drawbacks of their use in cardiac tissue engineering. For example, they often have poor biocompatibility in comparison to natural polymers. In part this is because the degradation process can release acidic degradation products that at high concentrations can lead to inflammation or fibrous encapsulation [87]. Furthermore, it is often difficult to generate stiffnesses that mimic that of the native myocardium using synthetic biomaterials [88]. This can have significant effects on cell function as well as overall graft function upon implantation on the heart.

Scaffold Structure

In addition to the choice of material when generating engineered myocardial constructs, one must also consider the structure of the scaffold that is being used to create the tissue. Preformed scaffolds are the crux of the classical tissue engineering methodology as envisioned by Langer and Vacanti [89]. These scaffolds that are initially created without cells and the cells are then seeded into the scaffold before culturing *in vitro*. Hydrogels are scaffolds that are cross-linked after the addition of cells, allowing for complete encapsulation of the cells into the construct structure. In addition, methods have been developed to generate engineered cardiac tissue without the use of a scaffold (so-called scaffoldless constructs). The types of each of these scaffold structures used in cardiac tissue engineering and their benefits and drawbacks are discussed in the following sections.

Preformed Scaffolds

Preformed scaffolds have the potential to be biodegradable and non-immunogenic, while containing a large surface area for seeding and high porosity necessary for capillary growth [88]. Furthermore, preformed scaffolds allow for greater control of structure, pore size and orientation as compared to hydrogel scaffolds. Cardiac grafts have been created with preformed PLGA [75], and collagen [90] sponges. Collagen sponges are used clinically as a wound dressing and are FDA approved and commercially available. Preformed sponges can be formed by lyophilizing the biomaterial solution [91] or using salt extraction [77] to create highly porous sponges with interconnected pores. The sponges can also be combined with chemical signals to create more suitable environments for the cells. For example, a collagen matrix containing rat vascular endothelial growth factor (VEGF) produced a cardiac construct that increased the left ventricular ejection fraction of the infarcted hearts compared with the controls [49]. Furthermore, sponges can be created with microfabrication techniques to create scaffolds with complex geometries, such as an accordion-like honeycomb microstructure [78]. The accordion-like scaffolds have mechanical properties

similar to native adult rat right ventricular myocardium, and obtained high cardiac cell alignment and contractility. One of the major limitations of preformed sponges is the difficulty in seeding cells into the scaffold in a uniform and thorough manner. The initial cell densities are highest at the surface of the construct, and seeding efficiency is very low, particularly in the interior [76]. Furthermore, if the production process of the sponges uses harsh chemical conditions, a risk of residual chemicals remaining in the scaffolding exists [92].

Decellularized organs are becoming another method of creating organ level scaffolds that are technically preformed. The decellularized ECM maintains all of the chemical components of the native tissue's ECM, while also preserving the complex geometries and structures that are hard to reproduce with current scaffolding technologies. In particular, the technique of perfusion decellularization [93] has the ability to generate whole organ scaffolds that maintain the complex geometry and special arrangement of ECM molecules. The perfusion decellularized cardiac tissue even maintains the lumens of the vasculature, allowing for thicker constructs to be fabricated (up to 1mm thick[93]). The whole-heart scaffold can then be reseeded with cardiac cells or endothelial cells and after maintaining the constructs with coronary perfusion in a modified Langendorff perfusion bioreactor, the constructs could generate pressures equivalent to approximately 2% of a healthy adult heart [93]. Further research has shown that the ECM contains many growth factors and signals that could affect cell function in an organ specific way [94,95]. Similar to sponges, reseeded cells into the scaffold in a uniform manner mimicking the native myocardium is quite difficult. Functional organs require a heterogeneous population of cells, placed in specific niches to match native distribution [96] and current cell seeding methods obtain cell seeding efficiencies of ~50% [93], with morphology that is not typical of native tissue architecture. Another option that has been investigated that circumvents some of the issues with seeding cells in decellularized whole hearts is the use of portions of the decellularized ventricular myocardium. In this technique, the myocardium is sectioned into 2-mm thick portions and decellularized to create cardiac patches [59].

Electrospinning of polymer solutions is another method of creating preformed scaffolds that mimic the nanofibrous structure of the natural ECM [97]. The fibrous structure provides the physical scaffold for cell adhesion and proliferation, while being porous enough to allow for uniform cell seeding and sufficient oxygen and nutrient delivery [98]. The electrospinning process involves dispensing the biopolymer out of a syringe needle and exposing the solution through a charged voltage gradient between the needle and a collecting wheel [99]. By changing the solution and the voltage gradient, one can control the diameter, density, and alignment of the electrospun fibers. The scaffolds contain a large surface-to-volume ratio, with high permeability and an interconnecting pore structure, allowing the infiltration and proliferation of cells [100]. A wide range of polymers such as collagen [97], PLGA [101], silk fibroin [102] and fibrinogen [103] have been used for electrospinning. Electrospinning is typically done acellularly, because the forces seen in the electrospinning process can be damaging to cells. Thus, the cells must be seeded into the system after creation. Composite materials comprised of layers of different polymers can also be created [85] or functional groups can be added to the polymers to increase bioavailability or increase biological responses [104]. However, due to the thinness of the fibers and the inability to entrap cells during the creation of the scaffold, it is difficult to create truly 3D scaffolds with this method.

Hydrogel Scaffolds

Hydrogels are cross-linked networks of polymers with high water content between the polymer chains [105]. One of their main benefits for use in tissue engineering is the ability to entrap cells throughout the thickness of the generated construct as the scaffold cross-links.

They are also a popular choice because of the ease with which one can control the shape, stiffness, and thickness of the resulting construct [106]. Furthermore, the ability to injection mold the construct into any geometry is of significant benefit. This fabrication method also allows the scaffold to be injected directly to the site of the infarction, thus creating an *in situ* construct (discussed later in this review). Hydrogels provide crosslinked networks with tissue-like elastic properties, resulting in a stable mechanical environment for the cells to attach and grow in a 3D construct [107]. However, the microstructure of the construct is often not as controllable as with preformed constructs. Rather, the cells are allowed to produce their own ECM, and remodel the construct into a more suitable environment.

Hydrogels, like preformed scaffolds, have been created with both native and synthetic polymers. Fibrin [37,108], collagen [35], and chitosan [109] are three major natural biomaterials used in hydrogel systems, while PLGA [110] and PEG [111] are two major synthetic polymers. There have also been composite hydrogels created by combining natural and synthetic polymers such as PEGylated fibrin [112,113]. Hydrogels can be formed through numerous cross-linking methods, including temperature modulation, enzymatic activation, pH change, or ionic cross-linkage [114]. Fibrin gelation, for example, is dependent on the concentration of thrombin, an enzyme that cleaves fibronopeptides from fibrinogen, revealing the binding sites that interact with other fibrin monomers to form a protein mesh. By changing the ratios between fibrinogen and thrombin, one can control the properties of the hydrogel that forms [115]. Similarly, the use of temperature modulated cross-linking hydrogels allow for *in situ* injection of polymers to the infarction site [20]. There are some drawbacks with the use of hydrogels, as they can suffer from significant batch variations and their inherent heterogenous nature can dramatically influence the mechanical properties of the construct and thus cellular function [116]. Furthermore, hydrogels cannot respond effectively to the mechanically dynamic myocardial environment because of their intrinsic mechanical properties [117]. Because mechanical signals are very important for the cell signaling in myocardium, appropriate dynamic mechanical matching is needed for proper construct integration [118].

Scaffoldless

A scaffoldless process is another approach that has been explored in cardiac tissue engineering. Scaffoldless approaches involve seeding cells onto surfaces and allowing them to create their own support structure without additional material to hold the cells together. These techniques are beneficial because they reduce the number of extraneous materials that need to be added into the system and potentially implanted into the body. Biomaterials can cause immunological problems due to rejection or foreign body reaction, which can have a negative impact on the therapeutic effect [119]. The concept is that one can ensure that the engineered tissue is most similar to native tissue by removing the biomaterial and forcing cells to create their own scaffold.

One specific embodiment of the scaffoldless approach is cell sheet engineering. Cell sheets were developed by the Okano group in 1999, using a temperature-responsive culture surface for the cardiac cells [120]. Poly (N-isopropylacrylamide), (PNIPAAm), is grafted onto culture surfaces with electron beam exposure, creating surfaces that are slightly hydrophobic at 37°C, causing the polymers to bind tightly to each other and thus allowing cells to adhere and proliferate. The cells are cultured on the substrate until confluency, at which point, they are connected to each other through cell-to-cell junctions and deposited ECM [121]. When the temperature is decreased below the polymer's critical solution temperature of 32°C, the polymer becomes highly hydrophilic, causing it to swell and creating a hydration layer between the cell sheet and the culture surface. The cells thus detach spontaneously in a sheet, which can be used individually or can be stacked together to form 3D structures. Typical trypsinization cell harvest uses an enzymatic digestion that disrupts the adhesive

proteins and cell-cell interactions, causing the cells to come apart individually [121] and potentially forcing alterations in the gene expression program in the process. The method of detachment used here allows cells to retain the critical connections to the ECM they have produced. Cell sheets can be adhered to the myocardium without additional mediators due to the presence of deposited ECM on the basal surface. One major disadvantage of the original technique to create cell sheet tissue constructs is the lack of control over the cell spatial placement and orientation, which limited the complexity of the constructs that could be created. To combat this issue, Isenberg et al. created cell sheets on top of culture dishes with microtexturing, allowing for high levels of control over the orientation and elongation of the cells in each sheet [122]. The microtextured cell sheets are created by conventional photolithography techniques. By creating a variety of microtexturing patterns for each layer of the cell-sheet stack, researchers could combine the sheets to form complex structures of highly anisotropic tissue that changed throughout the thickness of the construct. The major limitation that still exists for the scaffoldless approach is that although the cell sheets can be stacked together to create thicker constructs, it is difficult to create 3D constructs with this method.

Culture Conditions

Culture conditions are an important aspect of engineering myocardial tissue that need to be considered as a part of the construct creation process. This is especially true in myocardial tissue where there is an abundance of physiological signals that impact the tissue's growth and function (e.g. mechanical stretch, electrical stimulation, and high degree of perfusion).

In vitro

Much of the current research on engineered myocardial constructs has involved assessments of the effects of alterations in the *in vitro* culture environment on engineered myocardial tissue functional performance. The factors that have been considered to date are discussed in further detail below.

Geometries

Cardiac tissue is highly anisotropic in nature and the morphological anisotropy leads to significant electromechanical functional anisotropy that is critical for normal heart function. As such, the geometry of the construct is an important consideration for creating functional engineered myocardial constructs. A tissue construct's geometry can mediate cell growth and viability by modulating the mechanical stress gradient to the cells [123]. Further, the stimulation of mechanotransduction pathways allows the cells to adapt to the changing environment, by increasing the production of growth factors, and the deposition of ECM [124]. The geometry of the construct can be used to create alignment in the engineered tissue which organizes the cells into an interconnected network. For example, anisotropy generated by cell compaction of a fibrin hydrogel with specific physical constraints has been shown to improve the contractile force of engineered myocardial constructs to a greater degree than what was to be expected by merely aligning all of the cells uniaxially [37]. This improvement was attributed to alterations in connexin 43 expression, a gap junction protein used to electrically couple cardiac cells [125], which in turn plays an important role in the spread of electrical current and the establishment of regular synchronous contractions. Because scaffolds can be made into various shapes, including rings [36,37], strands [86], sheets [121], as well as more complex shapes [78], one needs to carefully consider how these geometries affect the cell alignment and the mechanical behavior of the construct. Alignment of cells in the tissue engineered myocardium can also be created using mechanical [35] or electrical stimulation [48] during culture, or through co-culture with cardiac fibroblasts [126].

One aspect of geometry that has not been successfully addressed in to date is the issue of scaling engineered myocardial constructs to human size. As the size of the cardiac patch needed increases, the construction process becomes more complicated due to the issues in maintaining homogenous physical properties and cellularity of the scaffold over larger dimensions. Furthermore, because oxygen and nutrient diffusion into tissues is extremely limited (often less than 100 microns in dense cardiac tissue *in vivo*), vascularization of larger constructs is essential. This is a significant issue that needs to be addressed before cardiac patches are a viable option for clinical applications in humans, and a significant amount of ongoing research is devoted to developing methods for vascularizing engineered myocardial tissue [57,127-129].

Bioreactors

Constructs are generally cultured *in vitro* because of the ability to have a high degree of control over the culture parameters by the use of bioreactors, which allows for more reproducible results and a better understanding of the mechanisms behind functional development of the tissue. Standard bioreactors can control parameters such as temperature, pH, and oxygen concentrations. However, due to the complexity of the *in vivo* biophysical environment of myocardium, increasingly sophisticated designs that account for more involved parameters, such as oxygen and nutrient transport, and mechanical and electrical stimulation, need to be considered when designing a bioreactor for culturing engineered myocardial tissue. An assessment of how these various bioreactor design parameters have been addressed in research to date is discussed below.

Oxygen and Nutrient Transport

In static culture, oxygen dissolved in culture medium is only transported into cells through molecular diffusion, and thus can only support tissue thicknesses of approximately 100 μm [77]. The oxygen limitation is the main reason that engineered constructs without vascularization are limited in size. Bioreactors can be used to increase the oxygen and nutrient transport by culturing constructs in mixing, rotating, or perfusion bioreactors that all aid in increasing transport throughout the thickness of the construct [130]. Each type of bioreactor creates different mass transport conditions. Though mixing helps with oxygen and nutrient transport, spinner flasks should not be used for cultivating cardiac cells because the turbulent flow generated at the surface of the construct can create eddies that are destructive for seeded cells [131]. Rotating-wall vessels provide a dynamic environment with high mass-transfer and low shear stresses [29]. However, rotating-wall vessels do not create the mechanical stresses that are typically present in the cardiac tissue environment *in vivo* [132]. Perfusion bioreactors maintain the best oxygen transport into the construct but can introduce shear stresses on the cells, which could lead to differences in cell function [133]. The focus of much of the current research is on the development of methods for increasing oxygen levels within the tissue construct via perfusion of media through the constructs [134], oxygen carriers in the culture medium [80], or the addition of vascularization into the system [57,77,129].

Controlled Delivery of Bioactive Molecules

Regulatory factors and cytokines are often added to cells in a construct as a method of controlling the chemical environment of the cells in the construct and/ or the infarcted region [19,21,135-137]. The microenvironment in an injured myocardium is a hypoxic environment with high levels of oxidative stress and pro-inflammatory cytokines, which limits cell viability [138]. The addition of bioactive molecules helps to mitigate these effects by altering both the cells in the implanted construct and those resident in the infarcted region.

For example, bioactive materials, created through the addition of peptides, can create biomaterials more similar to natural ECM, allowing cells to respond dynamically to the environment around them. RGD, a common integrin adhesion peptide, has been added to scaffolds, allowing for increased myofiber formation and increased Connexin-43 expression [139]. Likewise, the addition of a tethered RGD peptide and microencapsulated VEGF in a dextran-based hydrogel system increased the fraction of hESCs cells expressing VEGF receptor KDR/Flk-1, a vascular marker, up to 20-fold as compared with spontaneously differentiated embryoid bodies [137].

Molecules can also be used to stimulate local tissue repair of the infarcted region. As bioactive molecules are released from the scaffold in controlled rates through diffusion or bulk degradation of the hydrogel, the cells in contact with the stimuli become activated [140]. The activated cells stimulate many generations of cells to self-assemble into tissues [141]. Thus, the growth factors can prevent heart failure by sustaining organ function, while providing a chemoattractant signal to promote stem cell migration, differentiation, and proliferation [142].

Furthermore, angiogenic factors, such as VEGF or basic fibroblast growth factor (bFGF), can be added to more rapidly create the vascularization needed for the support of a thick tissue construct, especially since the native angiogenic process is inadequate to create the capillary density needed [140]. By delivering the growth factors locally, serious adverse effects can be avoided while simultaneously stimulating localized vessel formation and improving cell survival [143].

Though the heart contains progenitor cells that can be used to regenerate heart tissue [144], they do not induce significant regeneration, likely due to insufficient numbers or due to the harsh microenvironment of the infarcted region [6]. Thus, though bioactive molecules alone in acellular constructs have been tested as a therapeutic for MI [19,67], they are unlikely to be the ultimate solution. More likely, a combination approach of cells and bioactive molecules will be necessary. For example, when rat cardiac progenitor cells (CPCs) were injected together with insulin-like growth factor 1 (IFG-1) modified peptide nanofibers (NP-IFG-1), left ventricular function was preserved and greater myocardial regeneration was detected as compared with CPCs and NP-IFG-1 alone [21].

Mechanical Stretch

The heart is a dynamic mechanical system, where the tissue undergoes mechanical stretch as the chambers of the heart are filled with blood. Mechanical stretch of cardiac constructs *in vitro* has been used for the last decade to create constructs with more similar functional properties to native tissues. Zimmerman et al. created ring like constructs that were mechanically stimulated with 10% stretch at a frequency of 2 Hz [36]. With this method, they could create large (1-4 mm thick, 15 mm diameter) force-generating cardiac tissues. The cyclic mechanical stimulation activates mechanotransduction pathways in the cells that help to improve the development and function of the cardiomyocytes [134]. Cyclical stretch upregulates β 1D-integrin and activates FAK and RhoA, which help to initiate myotube formation [145]. The physical stimulation also aids in the alignment of the cells and collagen [146], and helps stimulate the formation of ECM [147]. Higher cyclic stretch amplitude causes cells to become less stiff at the higher distension strains. Because higher stretch amplitudes lead to this softening of the cells, these strains do not lead to higher cell or tissue forces [148]. The Frank-Starling mechanism has also been observed in mechanically stimulated engineered constructs indicating that the creation of twitch force in mechanically distended constructs is similar to that of native myocardium [149].

Electrical Stimulation

Electrical pacing has also been used to induce synchronous contractions in cardiac constructs and improve their function during *in vitro* culture. Radisic *et al.* showed that 2 ms, 5 V/cm, 1 Hz monophasic square wave electrical pulses created constructs with enhanced contractile properties [48]. The results of this work also demonstrated that the electrical stimulation of the construct needs to be initiated during a specific window of time during the culture period (after 3 days of cultivation). If the stimulation is applied too early, the electrical stimulation inhibits the accumulation of cardiac proteins, yielding poor contractility [150]. However, if applied too late, the electrical stimulation has no effect on the functional assembly of the cells [48]. The electric field is carried by ions in the media, causing a redistribution of charge. After extended periods of electrical stimulation with monophasic pacing, a pH gradient can be observed that has significant effects on cell function [151]. However, biphasic stimulation creates a hyperpolarizing prepulse that reactivates the sodium current, which disrupts the synchronized contractions needed for good construct development [152].

Electric pulses induce transient calcium levels which, over time, improve the volume fraction and organization of sarcomeres in the tissue [153]. Electrical stimulation creates hyperpolarization and depolarization on the ends of the cells, causing the cells to align with the electric field [154] and promoting an increase in gap junction formation and localization [155]. The increases in gap junctions allow the cells in the tissue to communicate the signal initiating contraction more rapidly, and help the tissue produce more synchronized contractions. However, high currents can create harmful byproducts which can affect cell growth [150].

Combined Stimulation

As discussed above, electrical and mechanical stimulation are two important influences on cardiomyocytes *in vivo* and have both shown to improve construct properties *in vitro*. However, the data generated by electrical and mechanical stimulation of constructs are difficult to compare because the outcomes assessed following each type of stimulation are often different. As such, it is currently unclear which method of stimulation has a greater effect on engineered myocardial tissue function. Additionally, the effects of combined electromechanical stimulation on cardiac constructs have not been fully explored. When electrical pacing is combined with mechanical stimulation on electrospun cardiac constructs, the fibers had more pronounced elongation, better alignment, and upregulation of contractile proteins when compared to unstimulated controls [86]. However, the benefits of dual stimulation over electrical or mechanical stimulation individually were not explored in this work and remain unclear. Further, the timing of the combined stimulation in this work was not physiologically relevant as the pacing was conducted after the stretch was released. Future studies should investigate a more appropriate combination of electrical and mechanical stimulation to determine the functional effects on *in vitro* engineered myocardium.

Lastly, it is important to note that regardless of the method of *in vitro* stimulation, the force generation of the engineered cardiac constructs is still an order of magnitude less than native tissue [156] and thus a better understanding of how stimulation leads to enhanced force generation would be of large significance.

In Situ

To counter the issues that arise with constructs that are cultured *in vitro*, constructs can also be formed *in situ*. In this approach, an injectable biomaterial and cells are delivered minimally invasively to the infarcted myocardium in a viscous liquid form, after which the

biomaterial solidifies. The biomaterial provides a method of controlling the environment of the implanted cells, offering a mechanical scaffold for the cells to adhere to and thereby improving retention of cells. In addition, it also allows the body's natural regenerative abilities to remodel the damaged tissue. This technique can also be used to deliver therapeutic molecules to the infarct both with and without the cells to increase angiogenesis, and cellular proliferation [157]. Furthermore, *in situ* tissue formation can be implanted into patients in a quicker time frame than with many *in vitro* solutions. Currently, injectable biomaterials used for *in situ* constructs include fibrin [20], alginate [67], self-assembling peptides [158], and ECM [159]. For example, fibrin glue with skeletal myoblasts helped preserved the fractional shortening and wall thickness of the myocardium after infarction. The fibrin glue supported the weakened heart tissue and increased the blood flow into the infarct [20].

Issues with *in situ* engineering of myocardial tissue still remain. For example, since the construct is created *in situ*, variability of the injury in each patient will change the effectiveness of the construct, limiting control over the culture process, cell placement, orientation, and engraftment [124]. Furthermore, the infarct environment is not ideal from a tissue formation standpoint because the remodeling process often isolates the damaged area, which can limit the communication and integration between the native myocardium and the construct [32]. Furthermore, the biomaterials used in *in situ* engineering need to maintain a similar mechanical stiffness to the myocardium, while also integrating into the host myocardium, in order to transfer mechanical stimuli effectively and in a synchronizing manner [160]. Lastly, the biomaterial can be degraded by the host, leaving the cells vulnerable to be lysed by the immune system [119]. Thus, slowly degrading biomaterials are important in maintaining a favorable environment for cellular growth and proliferation.

In Vivo

Cardiac constructs can also be cultured *in vivo*, where the construct is grown in a protected chamber implanted in another location in the patient, and then transplanted to the myocardium after culture. For example, an arteriovenous blood vessel loop (AVL) can be used to create a cardiac graft *in vivo*. A portion of the left femoral vein is harvested and interposed between the right femoral vein and artery [161], which is then placed inside a semi-sealed chamber that is implanted subcutaneously in a rat along with a tissue graft [128]. The wound healing environment of the region is highly angiogenic [33], creating a thick and highly vascularized cardiac tissue construct, which can then be transplanted to the infarcted myocardium. When cardiomyocytes are seeded into the chamber, cardiac tissue with a mean thickness similar to the adult rat right ventricular wall can be created [128]. Birla *et al.* created cardiac constructs by incorporating a vascular pedicle within the construct to induce angiogenesis [57]. Neonatal rat cardiomyocytes in a fibrin hydrogel were injected inside a silicone tube, surrounding the femoral vein and the constructs were cultured for three weeks. After this three week *in vivo* culture period, the constructs were electrically excitable, generating a force of approximately 843 μN and a maximum specific force of 6.45 kPa, an order of magnitude less than for normal cardiac tissue.

Because the graft is grown outside of the infarcted myocardium, the construct can develop without the harsh microenvironment of the infarct. However, this approach requires multiple procedures and would result in secondary donor site morbidity, due to the removal of the femoral vein. In addition, similar to *in situ* constructs, constructs created *in vivo* do not offer the ability to control alignment and orientation. Studies have shown transplanting *in vivo* cardiac patches on an infarcted heart maintains wall thickness and prevents left ventricular dilatation [162].

Preclinical Animal Studies

Though many methods of construct creation have been explored in the laboratory, the constructs must be able to demonstrate functional improvements in myocardial infarction animal models before they can be applied in a clinical setting. Some measures of functional improvement post-implantation measured in studies conducted to date include improved fractional shortening (FS), increased left ventricular ejection fraction (LVEF), decreased infarct size, and increased infarct neovascularization. A brief summary of some of the *in vivo* animal studies conducted to date and the functional improvements recorded for each is provided in Table 1. Initial animal studies of each cardiac construct scaffolding type have shown improvements in the myocardial infarction, and indicate the potential of using cardiac grafts over current cell therapy methods. However, more studies are needed to fully understand how the constructs affect the infarcted region, in order to develop improve construct design parameters with the end goal of enhancing their functional performance *in vivo*. Furthermore, many of the studies conducted to date have sought to repair early phase remodeling infarctions where the implantation of the construct occurs anywhere from immediately following the induction of MI to 3 weeks post-injury. More studies are needed to assess the functional effects following engineered myocardium implantation in a more chronic model of infarction.

Zimmermann *et al.* 2006's mechanically stimulated ring EHTs were stacked together and implanted on the myocardial infarcted heart of Wistar rats [36]. After four weeks, the grafts showed electrical coupling to the host myocardium, improved fractional area shortening of the infarcted hearts, and better diastolic and systolic function as compared with the sham-operated controls. In addition, blood vessels were observed inside the implanted EHTs, demonstrating the ability of the construct to integrate with the host myocardium.

Other cardiac grafts, such as collagen-GAG grafts [69] and gelatin meshes [70], have shown neovascularization, cell growth and proliferation, and the ability to form junctions with the recipient myocardial cells. The gelatin meshes survived *in vivo* for five weeks. Similarly, many types of *in situ* constructs [20,67,158,159] have shown improvements in the myocardial wall thickness and neovascularization in the construct.

When neonatal rat cardiomyocytes are used to create cell sheets, the layered cell sheets begin to pulse simultaneously, indicating that electrical and morphological communications are established between layers [81]. Once implanted into the subcutaneous tissues of nude rats, the construct showed stratified tissues with little necrotic area, and they were able to survive up to 3 months *in vivo*. However, due to the thinness of the cell sheets, they are extremely fragile, which causes difficulties in handling the cell sheets for stacking purposes or during surgery [163]. Furthermore, it is difficult to generate constructs as thick as native tissues. Multistep transplantation procedures, the process of implanting a few layers of cell sheets every three days, allow researchers to create ~1 mm thick pulsatile myocardial tissue grafts after 10 transplantations [164]. However, increasing the number of transplantations dramatically increases possible complications and costs.

Conclusion

Many processes for creating and culturing cardiac patches have been established in the last decade, using a wide range of biomaterials, scaffold types, and culture methods. Both natural and synthetic polymers have been used to create preformed and hydrogel scaffolds. The scaffolds can be cultured *in vitro*, *in situ*, or *in vivo*. Each combination uses creative ways to deal with the complex issues involved in obtaining constructs that can mechanically and electrically couple to, and remodel, the infarcted myocardium. Vascularization,

alignment, orientation, and electrical/mechanical coupling are all needed to effectively repair the myocardium and prevent congestive heart failure. Construct tissue composition can be improved through chemical and physical stimulation, and improved oxygen and nutrient transport.

Tissue engineering constructs have made huge strides improving the potential therapies available to patients after MI as compared to the current regenerative therapy options. With cell therapies, only a minimum numbers of cells survive [15,16,166], and the transient paracrine effects from cell therapies do not prevent the deterioration of the heart into heart failure. Likewise, the addition of bioactive molecules, such as cytokines, improves the in-situ recruitment of cells to allow the native population to self-regenerate, but the recovery is often slow and not well understood [167]. The addition of a scaffolding biomaterial provides support and control of implanted cells to remodel the injured myocardium and to improve cardiac function following injury or disease.

While many of the methods of culture have resulted in tissue engineered constructs that have demonstrated improved function of infarcted hearts, ultimately, at their current stage of development, they do not completely repair the affected tissue. This is in large part due to the inability to generate constructs that have contractile forces in the range of native tissue, as well as issues with graft thickness due to limitations of oxygen and nutrient diffusion. Furthermore, much of the research is currently conducted on small rodents, and the ability to size up constructs for clinical needs is still in its infancy. The complex architecture of the myocardium is difficult to recapitulate especially on larger scales, and vascularization and tissue organization is needed to support the additional tissue size. An improved understanding of the remodeling and regenerative mechanisms that occur post-implantation will help to direct the creation and culture of cardiac constructs towards fully functional cardiac tissue grafts. To this end, more studies need to be conducted to assess the impact of engineered myocardium implantation at a point that is further along in the remodeling process. Further studies on the impact of electromechanical stimulation on tissue construct function are also warranted.

Though vast challenges in the field of cardiac tissue engineering still exist, engineered cardiac grafts have shown great promise in repairing infarcted myocardial tissue. As methods of creating increasingly complex constructs develop, the use of cardiac tissue engineering grafts for cardiac regeneration and repair becomes more of a clinical reality.

Bibliography

1. Minino AM, Xu J, Kochanek KD. National vital statistics reports deaths : preliminary data for 2008. 2008
2. World Health Organization. The top ten causes of death, 2008. 2008
3. Beltrami AP, Urbanek K, Kajstura J, Yan S-M, Finato N, Bussani R, Nadal-Ginard B, Silvestri F, Leri A, Beltrami CA, Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med*. 2001; 344(23):1750–1757. [PubMed: 11396441]
4. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. experimental observations and clinical implications. *Circulation*. 1990; 81(4):1161–1172. [PubMed: 2138525]
5. Gaudron P, Eilles C, Kugler I, Ertl G. Progressive left ventricular dysfunction and remodeling after myocardial infarction. potential mechanisms and early predictors. *Circulation*. 1993; 87(3):755–63. [PubMed: 8443896]
6. Segers VFM, Lee RT. Protein therapeutics for cardiac regeneration after myocardial infarction. *J Cardiovasc Transl Res*. 2010; 3(5):469–77. [PubMed: 20607468]
7. Boyle A. Current status of cardiac transplantation and mechanical circulatory support. *Curr Heart Fail Rep*. 2009; 6(1):28–33. [PubMed: 19265590]

8. Jeevanandam V, Furukawa S, Prendergast TW, Todd BA, Eisen HJ, McClurken JB. Standard criteria for an acceptable donor heart are restricting heart transplantation. *Ann Thorac Surg.* 1996; 62(5):1268–75. [PubMed: 8893556]
9. Lindenfeld J, Miller GG, Shakar SF, Zolty R, Lowes BD, Wolfel EE, Mestroni L, Page RL, Kobashigawa J. Drug therapy in the heart transplant recipient: part i: cardiac rejection and immunosuppressive drugs. *Circulation.* 2004; 110(24):3734–40. [PubMed: 15596559]
10. Laflamme MA, Zbinden S, Epstein SE, Murry CE. Cell-based therapy for myocardial ischemia and infarction: pathophysiological mechanisms. *Annu Rev Pathol.* 2007; (2):307–39. [PubMed: 18039102]
11. Beitnes J, Hopp E, Lunde K, Solheim S, Arnesen H, Brinchmann J, Forfang K, Aakhus S. Long-term results after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: the astami randomised, controlled study. *Heart.* 2009; 95(24):1983–9. [PubMed: 19833610]
12. Schächinger V, Assmus B, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Yu J, Corti R, Mathey DG, Hamm CW, Tonn T, Dimmeler S, Zeiher AM. Intracoronary infusion of bone marrow-derived mononuclear cells abrogates adverse left ventricular remodelling post-acute myocardial infarction: insights from the reinfusion of enriched progenitor cells and infarct remodelling in acute myocardial infarction (repair-ami) trial. *Eur J Heart Fail.* 2009; 11(10):973–9. [PubMed: 19789401]
13. D'Alessandro DA, Michler RE. Current and future status of stem cell therapy in heart failure. *Curr Treat Options Cardiovasc Med.* 2010; 12(6):614–27. [PubMed: 21063937]
14. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, Hecker H, Schaefer A, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled boost (bone marrow transfer to enhance st-elevation infarct regeneration) trial. *Circulation.* 2006; 113(10):1287–94. [PubMed: 16520413]
15. Terrovitis JV, Smith RR, Marbán E. Assessment and optimization of cell engraftment after transplantation into the heart. *Circ Res.* 2010; 106(3):479–94. [PubMed: 20167944]
16. Hattori F, Fukuda K. Strategies for ensuring that regenerative cardiomyocytes function properly and in cooperation with the host myocardium. *Exp Mol Med.* 2010; 42(3):155. [PubMed: 20164677]
17. Choi YS, Dusting GJ, Stubbs S, Arunothayaraj S, Han XL, Collas P, Morrison WA, Dilley RJ. Differentiation of human adipose-derived stem cells into beating cardiomyocytes. *J Cell Mol Med.* 2010; 14(4):878–889. [PubMed: 20070436]
18. Coppen SR, Fukushima S, Shintani Y, Takahashi K, Varela-Carver A, Salem H, Yashiro K, Yacoub MH, Suzuki K. A factor underlying late-phase arrhythmogenicity after cell therapy to the heart: global downregulation of connexin43 in the host myocardium after skeletal myoblast transplantation. *Circulation.* 2008; 118(14 Suppl):S138–44. [PubMed: 18824745]
19. Zhang J, Ding L, Zhao Y, Sun W, Chen B, Lin H, Wang X, Zhang L, Xu B, Dai J. Collagen-targeting vascular endothelial growth factor improves cardiac performance after myocardial infarction. *Circulation.* 2009; 119(13):1776–84. [PubMed: 19307480]
20. Christman KL, Fok HH, Sievers RE, Fang Q, Lee RJ. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng.* 2004; 10(3-4):403–9. [PubMed: 15165457]
21. Padin-Iruegas ME, Misao Y, Davis ME, Segers VFM, Esposito G, Tokunou T, Urbanek K, Hosoda T, Rota M, Anversa P, Leri A, Lee RT, Kajstura J. Cardiac progenitor cells and biotinylated insulin-like growth factor-1 nanofibers improve endogenous and exogenous myocardial regeneration after infarction. *Circulation.* 2009; 120(10):876–87. [PubMed: 19704095]
22. Kreutziger KL, Murry CE. Engineered human cardiac tissue. *Pediatr Cardiol.* 2011; 32(3):334–341. [PubMed: 21293854]
23. Martinez EC, Kofidis T. Myocardial tissue engineering: the quest for the ideal myocardial substitute. *Expert Rev Cardiovasc Ther.* 2009; 7(8):921–8. [PubMed: 19673670]
24. St. John Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation.* 2000; (101):2981–2988. [PubMed: 10869273]

25. 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(11):1388–96. [PubMed: 18316487]
26. O'Blenes SB, Li AW, Chen R, Arora RC, Horackova M. Engraftment is optimal when myoblasts are transplanted early: the role of hepatocyte growth factor. *Ann Thorac Surg*. 2010; 89(3):829–35. [PubMed: 20172138]
27. Hu X, Wang J, Chen J, Luo R, He A, Xie X, Li J. Optimal temporal delivery of bone marrow mesenchymal stem cells in rats with myocardial infarction. *Eur J Cardiothorac Surg*. 2007; 31(3):438–43. [PubMed: 17239611]
28. Sakamoto T, Kojima S, Ogawa H, Shimomura H, Kimura K, Ogata Y, Sakaino N, Kitagawa A. Effects of early statin treatment on symptomatic heart failure and ischemic events after acute myocardial infarction in Japanese. *Am J Cardiol*. 2006; 97(8):1165–71. [PubMed: 16616020]
29. Martin I, Wendt D, Heberer M. The role of bioreactors in tissue engineering. *Trends Biotechnol*. 2004; 22(2):80–86. [PubMed: 14757042]
30. Vunjak-Novakovic G, Tandon N, Godier A, Maidhof R, Marsano A, Martens TP, Radisic M. Challenges in cardiac tissue engineering. *Tissue Eng Part B Rev*. 2010; 16(2):169–87. [PubMed: 19698068]
31. Jawad H, Ali NN, Lyon AR, Chen QZ, Harding SE, Boccaccini AR. Myocardial tissue engineering : a review. *Tissue Engineering*. 2007:327–342.
32. Matsushita T, Oyamada M, Fujimoto K, Yasuda Y, Masuda S, Wada Y, Oka T, Takamatsu T. Remodeling of cell-cell and cell-extracellular matrix interactions at the border zone of rat myocardial infarcts. *Circ Res*. 1999; 85(11):1046–55. [PubMed: 10571536]
33. Lokmic Z, Stillaert F, Morrison WA, Thompson EW, Mitchell GM. An arteriovenous loop in a protected space generates a permanent, highly vascular, tissue-engineered construct. *Faseb J*. 2007; 21(2):511–22. [PubMed: 17172640]
34. Tee R, Lokmic Z, Morrison W, Dilley RJ. Strategies in cardiac tissue engineering. *Anz J Surg*. 2010; 80(10):683–93. [PubMed: 21040327]
35. Zimmermann W-H. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res*. 2001; 90(2):223–230. [PubMed: 11834716]
36. Zimmermann W-H, Melnychenko I, Wasmeier G, Didié 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. *Nat Med*. 2006; 12(4):452–8. [PubMed: 16582915]
37. Black LD, 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 Eng Part A*. 2009; 15(10):3099–108. [PubMed: 19338433]
38. Huang C-C, Liao C-K, Yang M-J, Chen C-H, Hwang S-M, Hung Y-W, Chang Y, Sung H-W. A strategy for fabrication of a three-dimensional tissue construct containing uniformly distributed embryoid body-derived cells as a cardiac patch. *Biomaterials*. 2010; 31(24):6218–6227. [PubMed: 20537702]
39. Wei H-J, Chen C-H, Lee W-Y, Chiu I, Hwang S-M, Lin W-W, Huang C-C, Yeh Y-C, Chang Y, Sung H-W. Bioengineered cardiac patch constructed from multilayered mesenchymal stem cells for myocardial repair. *Biomaterials*. 2008; 29(26):3547–56. [PubMed: 18538386]
40. Colazzo F, Sarathchandra P, Smolenski RT, Chester AH, Tseng Y-T, Czernuszka JT, Yacoub MH, Taylor PM. Extracellular matrix production by adipose-derived stem cells: implications for heart valve tissue engineering. *Biomaterials*. 2011; 32(1):119–27. [PubMed: 21074262]
41. Garbade J, Schubert A, Rastan AJ, Lenz D, Walther T, Gummert JF, Dhein S, Mohr F-W. Fusion of bone marrow-derived stem cells with cardiomyocytes in a heterologous in vitro model. *Eur J Cardiothorac Surg*. 2005; 28(5):685–91. [PubMed: 16194609]
42. Jacot JG, Martin JC, Hunt DL. Mechanobiology of cardiomyocyte development. *J Biomech*. 2010; 43(1):93–8. [PubMed: 19819458]
43. Cavalcanti-Adam E, Tomakidi P, Bezler M, Spatz J. Geometric organization of the extracellular matrix in the control of integrin-mediated adhesion and cell function in osteoblasts. *Prog Orthod*. 2005; 6(2):232–7. [PubMed: 16276432]

44. Li T-S, Komota T, Ohshima M, Qin S-L, Kubo M, Ueda K, Hamano K. Tgf-beta induces the differentiation of bone marrow stem cells into immature cardiomyocytes. *Biochem Biophys Res Commun.* 2008; 366(4):1074–80. [PubMed: 18158919]
45. Bouten CVC, Dankers PYW, Driessen-Mol A, Pedron S, Brizard AMA, Baaijens FPT. Substrates for cardiovascular tissue engineering. *Adv Drug Deliv Rev.* 2011; 63(4-5):221–41. [PubMed: 21277921]
46. Schwartz MA, DeSimone DW. Cell adhesion receptors in mechanotransduction. *Curr Opin Cell Biol.* 2008; 20(5):551–6. [PubMed: 18583124]
47. Yoneno K, Ohno S, Tanimoto K, Honda K, Tanaka N, Doi T, Kawata T, Tanaka E, Kapila S, Tanne K. Multidifferentiation potential of mesenchymal stem cells in three-dimensional collagen gel cultures. *J Biomed Mater Res A.* 2005; 75(3):733–41. [PubMed: 16110495]
48. 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. *Proc Natl Acad Sci U S A.* 2004; 101(52):18129–34. [PubMed: 15604141]
49. Kutschka I, Chen IY, Kofidis T, Arai T, von Degenfeld G, Sheikh AY, Hendry SL, Pearl J, Hoyt G, Sista R, Yang PC, Blau HM, Gambhir SS, Robbins RC. Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation.* 2006; 114(1 Suppl):I167–73. [PubMed: 16820568]
50. Bauman L. Cosmoderm/cosmoplast (human bioengineered collagen) for the aging face. *Facial Plast Surg.* 2004; 20(2):125–128. [PubMed: 15643578]
51. Geiger M, Li RH, Friess W. Collagen sponges for bone regeneration with rhbmp-2. *Adv Drug Deliv Rev.* 2003; 55(12):1613–1629. [PubMed: 14623404]
52. Kleinman HK, Martin GR. Matrigel: basement membrane matrix with biological activity. *Semin Cancer Biol.* 2005; 15(5):378–86. [PubMed: 15975825]
53. Kofidis T, de Bruin JL, Hoyt G, Lebl DR, Tanaka M, Yamane T, Chang C-P, Robbins RC. Injectable bioartificial myocardial tissue for large-scale intramural cell transfer and functional recovery of injured heart muscle. *J Thorac Cardiovasc Surg.* 2004; 128(4):571–8. [PubMed: 15457158]
54. Williams C, Johnson SL, Robinson PS, Tranquillo RT. Cell sourcing and culture conditions for fibrin-based valve constructs. *Tissue Eng.* 2006; 12(6):1489–502. [PubMed: 16846346]
55. Jochenhoevel S, Zund G, Hoerstrup SP, Chalabi K, Sachweh JS, Demircan L, Messmer BJ, Turina M. Fibrin gel - advantages of a new scaffold in cardiovascular tissue engineering. *Eur J Cardiothorac Surg.* 2001; 19(4):424–30. [PubMed: 11306307]
56. Grassl ED, Oegema TR, Tranquillo RT. A fibrin-based arterial media equivalent. *J Biomed Mater Res A.* 2003; 66(3):550–61. [PubMed: 12918038]
57. Birla RK, Borschel GH, Dennis RG, Brown DL. Myocardial engineering in vivo: formation and characterization of contractile, vascularized three-dimensional cardiac tissue. *Tissue Eng.* 2005; 11(5-6):803–13. [PubMed: 15998220]
58. Linnes MP, Ratner BD, Giachelli CM. A fibrinogen-based precision microporous scaffold for tissue engineering. *Biomaterials.* 2007; 28(35):5298–306. [PubMed: 17765302]
59. Wang B, Borazjani A, Tahai M, Curry ALDJ, Simionescu DT, Guan J, To F, Elder SH, Liao J. Fabrication of cardiac patch with decellularized porcine myocardial scaffold and bone marrow mononuclear cells. *J Biomed Mater Res A.* 2010; 94(4):1100–10. [PubMed: 20694977]
60. Singelyn JM, DeQuach Ja, Seif-Naraghi SB, Littlefield RB, Schup-Magoffin PJ, Christman KL. Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. *Biomaterials.* 2009; 30(29):5409–16. [PubMed: 19608268]
61. Crapo PM, Wang Y. Small intestinal submucosa gel as a potential scaffolding material for cardiac tissue engineering. *Acta Biomater.* 2010; 6(6):2091–6. [PubMed: 19887120]
62. Robinson KA, Li J, Mathison M, Redkar A, Cui J, Chronos NAF, Matheny RG, Badylak SF. Extracellular matrix scaffold for cardiac repair. *Circulation.* 2005; 112(9 Suppl):I135–43. [PubMed: 16159805]
63. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials.* 2006; 27(19):3675–83. [PubMed: 16519932]

64. Simpson DG, Terracio L, Terracio M, Price RL, Turner DC, Borg TK. Modulation of cardiac myocyte phenotype in vitro by the composition and orientation of the extracellular matrix. *J Cell Physiol.* 1994; 161(1):89–105. [PubMed: 7929612]
65. Allison DD, Grande-Allen KJ. Review. hyaluronan: a powerful tissue engineering tool. *Tissue Eng.* 2006; 12(8):2131–40. [PubMed: 16968154]
66. Ventura C, Cantoni S, Bianchi F, Lionetti V, Cavallini C, Scarlata I, Foroni L, Maioli M, Bonsi L, Alviano F, Fossati V, Bagnara GP, Pasquinelli G, Recchia FA, Perbellini A. Hyaluronan mixed esters of butyric and retinoic acid drive cardiac and endothelial fate in term placenta human mesenchymal stem cells and enhance cardiac repair in infarcted rat hearts. *J Biol Chem.* 2007; 282(19):14243–52. [PubMed: 17363374]
67. 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(2):565–78. [PubMed: 20889201]
68. 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(suppl III):III-56–III-61. [PubMed: 11082363]
69. Xiang Z, Liao R, Kelly MS, Spector M. Collagen-gag scaffolds grafted onto myocardial infarcts in a rat model: a delivery vehicle for mesenchymal stem cells. *Tissue Eng.* 2006; 12(9):2467–78. [PubMed: 16995780]
70. Li R-K, Jia Z-Q, Weisel RD, Mickle DAG, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. *Circulation.* 1999; 100(suppl II):II-63–II-69. [PubMed: 10567280]
71. Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliv Rev.* 2007; 59(4-5):207–33. [PubMed: 17482309]
72. Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater.* 2003; (5):1–16. [PubMed: 14562275]
73. Davis ME, Hsieh PCH, Grodzinsky AJ, Lee RT. Custom design of the cardiac microenvironment with biomaterials. *Circ Res.* 2005; 97(1):8–15. [PubMed: 16002755]
74. Kenar H, Kose GT, Hasirci V. Design of a 3d aligned myocardial tissue construct from biodegradable polyesters. *J Mater Sci Mater Med.* 2010; 21(3):989–97. [PubMed: 19862604]
75. Park H, Radisic M, Lim JO, Chang BH, Vunjak-Novakovic G. A novel composite scaffold for cardiac tissue engineering. *In Vitro Cell Dev Biol Anim.* 2005; 41(7):188–96. [PubMed: 16223333]
76. 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. *Biotechnol Bioeng.* 1999; 64(5):580–9. [PubMed: 10404238]
77. Caspi O, Lesman A, Basevitch Y, Gepstein A, Arbel G, Habib IHM, Gepstein L, Levenberg S. Tissue engineering of vascularized cardiac muscle from human embryonic stem cells. *Circ Res.* 2007; 100(2):263–72. [PubMed: 17218605]
78. Engelmayr GC, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nat Mater.* 2008; 7(12):1003–10. [PubMed: 18978786]
79. Wang Y, Kim YM, Langer R. In vivo degradation characteristics of poly (glycerol sebacate). *In Vivo.* 2002; (85)
80. Radisic M, Park H, Chen F, Salazar-Lazzaro JE, Wang Y, Dennis R, Langer R, Freed LE, Vunjak-Novakovic G. Biomimetic approach to cardiac tissue engineering: oxygen carriers and channeled scaffolds. *Tissue Eng.* 2006; 12(8):2077–91. [PubMed: 16968150]
81. Shimizu T. Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. *Circ Res.* 2002; 90(3):40e–48.
82. Shin M, Ishii O, Sueda T, Vacanti JP. Contractile cardiac grafts using a novel nanofibrous mesh. *Biomaterials.* 2004; 25(17):3717–23. [PubMed: 15020147]

83. Pego AP, Siebum B, Van Luyn MJA, Seijen XJGYV, Poot AA, Grijpma DW, Feijen J. Preparation of degradable porous structures based on 1,3-trimethylene carbonate and d,l-lactide (co)polymers for heart tissue engineering. *Tissue Eng.* 2003; 9(5)
84. Blumenthal B, Golsong P, Poppe A, Heilmann C, Schlensak C, Beyersdorf F, Siepe M. Polyurethane scaffolds seeded with genetically engineered skeletal myoblasts: a promising tool to regenerate myocardial function. *Artif Organs.* 2010; 34(2):E46–54. [PubMed: 20420589]
85. Meng J, Kong H, Han Z, Wang C, Zhu G, Xie S, Xu H. Enhancement of nanofibrous scaffold of multiwalled carbon nanotubes/polyurethane composite to the fibroblasts growth and biosynthesis. *J Biomed Mater Res A.* 2009; 88(1):105–16. [PubMed: 18260129]
86. Liao I-C, Liu JB, Bursac N, Leong KW. Effect of electromechanical stimulation on the maturation of myotubes on aligned electrospun fibers. *Exp Cell Res.* 2008; 1:133–145.
87. Thomson R, Wake M, Yaszemski M, Mikos A. Biodegradable polymer scaffolds to regenerate organs. *Adv Polym Sci.* 1995; (122):245–274.
88. Yang S, Leong KF, Du Z, Chua CK. The design of scaffolds for use in tissue engineering. part i. traditional factors. *Tissue Eng.* 2001; 7(6):679–89. [PubMed: 11749726]
89. Langer R, Vacanti JP. Tissue engineering. *Science* (80-). 1993; 260:920–926.
90. Glowacki J, Mizuno S. Collagen scaffolds for tissue engineering. *Biopolymers.* 2008; 89(5):338–44. [PubMed: 17941007]
91. Shapiro L, Cohen S. Novel alginate sponges for cell culture and transplantation. *Biomaterials.* 1997; 18(8):583–590. [PubMed: 9134157]
92. Mikos AG, Temenoff JS. Formation of highly porous biodegradable scaffolds for tissue engineering. *Electron J Biotechnol.* 2000; 3:114–119.
93. Ott HC, Matthiesen TS, Goh S-K, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med.* 2008; 14(2):213–21. [PubMed: 18193059]
94. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater.* 2009; 5(1):1–13. [PubMed: 18938117]
95. Rosso F, Giordano A, Barbarisi M, Barbarisi A. From cell-ecm interactions to tissue engineering. *J Cell Physiol.* 2004; 199(2):174–80. [PubMed: 15039999]
96. Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng.* 2010 March.:27–53.
97. Zhong S, Teo WE, Zhu X, Beuerman RW, Ramakrishna S, Yue L, Yung L. An aligned nanofibrous collagen scaffold by electrospinning and its effects on in vitro fibroblast culture. *J Biomed Mater Res A.* 2006; 79(3):456–463. [PubMed: 16752400]
98. Blakeney BA, Tambralli A, Anderson JM, Andukuri A, Lim D-J, Dean DR, Jun H-W. Cell infiltration and growth in a low density, uncompressed three-dimensional electrospun nanofibrous scaffold. *Biomaterials.* 2011; 32(6):1583–90. [PubMed: 21112625]
99. Sell SA, McClure MJ, Garg K, Wolfe PS, Bowlin GL. Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering. *Adv Drug Deliv Rev.* 2009; 61(12):1007–19. [PubMed: 19651166]
100. Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B. Electrospun cellular microenvironments: understanding controlled release and scaffold structure. *Adv Drug Deliv Rev.* 2011; 63(4-5):209–20. [PubMed: 21145932]
101. Zong X, Bien H, Chung C-Y, Yin L, Fang D, Hsiao BS, Chu B, Entcheva E. Electrospun fine-textured scaffolds for heart tissue constructs. *Biomaterials.* 2005; 26(26):5330–8. [PubMed: 15814131]
102. Liu H, Li X, Zhou G, Fan H, Fan Y. Electrospun sulfated silk fibroin nanofibrous scaffolds for vascular tissue engineering. *Biomaterials.* 2011; 32(15):3784–3793. [PubMed: 21376391]
103. McManus MC, Boland ED, Simpson DG, Barnes CP, Bowlin GL. Electrospun fibrinogen : feasibility as a tissue engineering scaffold in a rat cell culture model. *J Biomed Mater Res A.* 2006; 81(2):299–309. [PubMed: 17120217]
104. Ma Z, Kotaki M, Inai R, Ramakrishna S. Potential of nanofiber matrix as tissue-engineering scaffolds. *Tissue Eng.* 2005; 11(1-2):101–9. [PubMed: 15738665]

105. Drury J. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials*. 2003; 24(24):4337–4351. [PubMed: 12922147]
106. Nicodemus GD, Bryant SJ. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng Part B Rev*. 2008; 14(2):149–65. [PubMed: 18498217]
107. Bian W, Liao B, Badie N, Bursac N. Mesoscopic hydrogel molding to control the 3d geometry of bioartificial muscle tissues. *Nat Protoc*. 2010; 4(10):1522–1534. [PubMed: 19798085]
108. Birla RK, Huang YC, Dennis RG. Development of a novel bioreactor for the mechanical loading of tissue-engineered heart muscle. *Tissue Eng*. 2007; 13(9):2239–48. [PubMed: 17590151]
109. Lu W-N, Lü S-H, Wang H-B, Li D-X, Duan C-M, Liu Z-Q, Hao T, He W-J, Xu B, Fu Q, Song YC, Xie X-H, Wang C-Y. Functional improvement of infarcted heart by co-injection of embryonic stem cells with temperature-responsive chitosan hydrogel. *Tissue Eng Part A*. 2009; 15(6):1437–47. [PubMed: 19061432]
110. Krebs MD, Sutter KA, Lin ASP, Guldberg RE, Alsberg E. Injectable poly(lactic-co-glycolic) acid scaffolds with in situ pore formation for tissue engineering. *Acta Biomater*. 2009; 5(8):2847–59. [PubMed: 19446056]
111. Patterson J, Hubbell JA. Enhanced proteolytic degradation of molecularly engineered peg hydrogels in response to mmp-1 and mmp-2. *Biomaterials*. 2010; 31(30):7836–7845. [PubMed: 20667588]
112. Zhang G, Wang X, Wang Z, Zhang J, Suggs L. A pegylated fibrin patch for mesenchymal stem cell delivery. *Tissue Eng*. 2006; 12(1):9–19. [PubMed: 16499438]
113. 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(6):1025–36. [PubMed: 18476809]
114. Ruel-Gariépy E, Leroux J-C. In situ-forming hydrogels--review of temperature-sensitive systems. *Eur J Pharm Biopharm*. 2004; 58(2):409–26. [PubMed: 15296964]
115. Ryan E. Structural origins of fibrin clot rheology. *Biophys J*. 1999; 77(5):2813–2826. [PubMed: 10545379]
116. Jia X, Kiick KL. Hybrid multicomponent hydrogels for tissue engineering. *Macromol Biosci*. 2009; 9(2):140–56. [PubMed: 19107720]
117. Lee KY, Mooney DJ. Hydrogels for tissue engineering. *Chem Rev*. 2001; 101(7):1869–1880. [PubMed: 11710233]
118. Akhyari P, Kamiya H, Haverich A, Karck M, Lichtenberg A. Myocardial tissue engineering: the extracellular matrix. *Eur J Cardiothorac Surg*. 2008; 34(2):229–41. [PubMed: 18502661]
119. Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Semin Immunol*. 2008; 20(2):86–100. [PubMed: 18162407]
120. 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(4):355–62. [PubMed: 10321708]
121. Masuda S, Shimizu T, Yamato M, Okano T. Cell sheet engineering for heart tissue repair. *Adv Drug Deliv Rev*. 2008; 60(2):277–85. [PubMed: 18006178]
122. Isenberg BC, Tsuda Y, Williams C, Shimizu T, Yamato M, Okano T, Wong JY. A thermoresponsive, microtexture substrate for cell sheet engineering with defined structural organization. *Biomed Eng (ny)*. 2008; 29(17):2565–2572.
123. Dike LE, Chen CS, Mrksich M, Tien J, Whitesides GM, Ingber DE. Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. *In Vitro Cell Dev Biol Anim*. 1999; 35(8):441–8. [PubMed: 10501083]
124. Guo, D.; Kassiri, Z.; Oudit, GY. Role of signaling pathways in the myocardial response to biomechanical stress and in mechanotransduction in the heart. In: Kamkin, A.; Kiseleva, I., editors. *Mechanosensitivity and Mechanotransduction*. Vol. 2011. Dordrecht: Springer Netherlands; 2011. p. 141-166.
125. Oyamada M, Kimura H, Yoamada Y, Miyamoto A, Ohshika H, Mori M. The expression, phosphorylation, and localization of connexin 43 and gap-junctional intercellular communication

- during the establishment of a synchronized contraction of cultured neonatal rat cardiac myocytes. *Exp Cell Res*. 1994; 212:351–358. [PubMed: 8187829]
126. Nichol JW, Engelmayr GC, Cheng M, Freed LE. Co-culture induces alignment in engineered cardiac constructs via mmp-2 expression. *Biochem Biophys Res Commun*. 2008; 373(3):360–5. [PubMed: 18559256]
 127. Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, Marini R, van Blitterswijk CA, Mulligan RC, D'Amore PA, Langer R. Engineering vascularized skeletal muscle tissue. *Nat Biotechnol*. 2005; 23(7):879–84. [PubMed: 15965465]
 128. Morritt AN, Bortolotto SK, Dilley RJ, Han X, Kompa AR, McCombe D, Wright CE, Itescu S, Angus JA, Morrison WA. Cardiac tissue engineering in an in vivo vascularized chamber. *Circulation*. 2007; 115(3):353–60. [PubMed: 17200440]
 129. Sasagawa T, Shimizu T, Sekiya S, Haraguchi Y, Yamato M, Sawa Y, Okano T. Design of prevascularized three-dimensional cell-dense tissues using a cell sheet stacking manipulation technology. *Biomaterials*. 2010; 31(7):1646–54. [PubMed: 19962187]
 130. Chen Q, Harding S, Ali N, Lyon A, Boccaccini A. Biomaterials in cardiac tissue engineering: ten years of research survey. *Mater Sci Eng R Rep*. 2008; 59(1-6):1–37.
 131. Papadaki M, Bursac N, Langer R, Merok J, Vunjak-Novakovic G, Freed LE. Tissue engineering of functional cardiac muscle : molecular , structural , and electrophysiological studies tissue engineering of functional cardiac muscle : molecular , structural , and electrophysiological studies. *Am J Physiol Heart Circ Physiol*. 2001; 280(1):H168–H178. [PubMed: 11123231]
 132. Bilodeau K, Mantovani D. Bioreactors for tissue engineering: focus on mechanical constraints. a comparative review. *Tissue Eng*. 2006; 12(8):2367–83. [PubMed: 16968176]
 133. Radisic M, Yang L, Boublik J, Cohen RJ, Langer R, Freed LE, Vunjak-Novakovic G. Medium perfusion enables engineering of compact and contractile cardiac tissue. *Am J Physiol Heart Circ Physiol*. 2004; 286(2):H507–16. [PubMed: 14551059]
 134. Fink C, Ergün S, Kralisch D, Remmers U, Weil J, Eschenhagen T. Chronic stretch of engineered heart tissue induces hypertrophy and functional improvement. *Faseb J*. 2000; 14(5):669–79. [PubMed: 10744624]
 135. Kraehenbuehl TP, Ferreira LS, Hayward AM, Nahrendorf M, van der Vlies AJ, Vasile E, Weissleder R, Langer R, Hubbell JA. Human embryonic stem cell-derived microvascular grafts for cardiac tissue preservation after myocardial infarction. *Biomaterials*. 2010; 32(4):1102–1109. [PubMed: 21035182]
 136. Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. *J Biomed Mater Res A*. 2003; 65(4):489–97. [PubMed: 12761840]
 137. Ferreira LS, Gerecht S, Fuller J, Shieh HF, Vunjak-Novakovic G, Langer R. Bioactive hydrogel scaffolds for controllable vascular differentiation of human embryonic stem cells. *Biomaterials*. 2007; 28(17):2706–17. [PubMed: 17346788]
 138. Sy JC, Davis ME. Delivering regenerative cues to the heart: cardiac drug delivery by microspheres and peptide nanofibers. *J Cardiovasc Transl Res*. 2010; 3(5):461–8. [PubMed: 20628908]
 139. Sapir Y, Kryukov O, Cohen S. Integration of multiple cell-matrix interactions into alginate scaffolds for promoting cardiac tissue regeneration. *Biomaterials*. 2011; 32(7):1838–47. [PubMed: 21112626]
 140. Spadaccio C, Chello M, Trombetta M, Rainer A, Toyoda Y, Genovese JA. Drug releasing systems in cardiovascular tissue engineering. *J Cell Mol Med*. 2009; 13(3):422–39. [PubMed: 19379142]
 141. Leor J, Amsalem Y, Cohen S. Cells, scaffolds, and molecules for myocardial tissue engineering. *Pharmacol Ther*. 2005; 105(2):151–63. [PubMed: 15670624]
 142. Chen RR, Mooney DJ. Polymeric growth factor delivery strategies for tissue engineering. *Pharm Res*. 2003; 20(8):1103–1112. [PubMed: 12948005]

143. Epstein SE, Kornowski R, Fuchs S, Dvorak HF. Angiogenesis therapy: amidst the hype , the neglected potential for serious side effects. *Circulation*. 2001; (104):115–119. [PubMed: 11435348]
144. Christoforou N, Gearhart JD. Stem cells and their potential in cell-based cardiac therapies. *Prog Cardiovasc Dis*. 2007; 49(6):396–413. [PubMed: 17498520]
145. Zhang SJ, Truskey GA, Kraus WE. Effect of cyclic stretch on beta1d-integrin expression and activation of fak and rhoa. *Am J Physiol Cell Physiol*. 2007; 292(6):C2057–69. [PubMed: 17267546]
146. Rubbens MP, Driessen-Mol A, Boerboom RA, Koppert MMJ, van Assen HC, TerHaar Romeny BM, Baaijens FPT, Bouten CVC. Quantification of the temporal evolution of collagen orientation in mechanically conditioned engineered cardiovascular tissues. *Ann Biomed Eng*. 2009; 37(7):1263–72. [PubMed: 19415496]
147. Akhyari P, Fedak PWM, Weisel RD, Lee T-YJ, Mickle DAG, Li R-K. Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. *Circulation*. 2002; 106(suppl 1):I-137–I-142. [PubMed: 12354723]
148. Wille JJ, Elson EL, Okamoto RJ. Cellular and matrix mechanics of bioartificial tissues during continuous cyclic stretch. *Ann Biomed Eng*. 2006; 34(11):1678–90. [PubMed: 17033741]
149. Asnes CF, Marquez JP, Elson EL, Wakatsuki T. Reconstitution of the frank-starling mechanism in engineered heart tissues. *Biophys J*. 2006; 91(5):1800–10. [PubMed: 16782784]
150. Tandon N, Cannizzaro C, Chao P-HG, Maidhof R, Ting H, Au H, Radisic M, Vunjak-Novakovic G. Electrical stimulation systems for cardiac tissue engineering. *Nat Protoc*. 2009; 4(2):155–173. [PubMed: 19180087]
151. Tandon N, Marsano A, Maidhof R, Wan L, Park H, Vunjak-Novakovic G. Optimization of electrical stimulation parameters for cardiac tissue engineering. *J Tissue Eng Regen Med*. 2011; 5(6):e115–125. [PubMed: 21604379]
152. Kavanagh KM, Duff HJ, Clark R, Robinson KV, Giles WR, Wyse DG. Monophasic versus biphasic cardiac stimulation: mechanism of decreased energy requirements. *Pacing Clin Electrophysiol*. 1990; 13(10):1268–1276. [PubMed: 1701542]
153. Huang G, Pashmforoush M, Chung B, Saxon LA. The role of cardiac electrophysiology in myocardial regenerative stem cell therapy. *J Cardiovasc Transl Res*. 2010:61–65. [PubMed: 21128127]
154. Wikswo JP, Lin SF, Abbas RA. Virtual electrodes in cardiac tissue: a common mechanism for anodal and cathodal stimulation. *Biophys J*. 1995; 69(6):2195–210. [PubMed: 8599628]
155. McDonough PM, Glembotski CC. Induction of atrial natriuretic factor and myosin light chain-2 gene expression in cultured ventricular myocytes by electrical stimulation of contraction. *Biochemistry*. 1992; 267(17):11665–11668.
156. Zimmermann W-H, Didié M, Döker S, Melnychenko I, Naito H, Rogge C, Tiburcy M, Eschenhagen T. Heart muscle engineering: an update on cardiac muscle replacement therapy. *Cardiovasc Res*. 2006; 71(3):419–29. [PubMed: 16697358]
157. Iwakura A, Fujita M, Kataoka K, Tambara K, Sakakibara Y, Komeda M, Tabata Y. Intramyocardial sustained delivery of basic fibroblast growth factor improves angiogenesis and ventricular function in a rat infarct model. *Heart Vessels*. 2003; 18(2):93–9. [PubMed: 12756606]
158. Davis ME, Hsieh PCH, Takahashi T, Song Q, Zhang S, Kamm RD, Grodzinsky AJ, Anversa P, Lee RT. Local myocardial insulin-like growth factor 1 (igf-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc Natl Acad Sci U S A*. 2006; 103(21):8155–60. [PubMed: 16698918]
159. Seif-Naraghi SB, Salvatore MA, Schup-Magoffin PJ, Hu DP, Christman KL. Design and characterization of an injectable pericardial matrix gel: a potentially autologous scaffold for cardiac tissue engineering. *Tissue Eng Part A*. 2010; 16(6):2017–27. [PubMed: 20100033]
160. Gaudette GR, Cohen IS. Cardiac regeneration: materials can improve the passive properties of myocardium, but cell therapy must do more. *Circulation*. 2006; 114(24):2575–7. [PubMed: 17159071]

161. Mian R, Morrison WA, Hurley JV, Penington AJ, Romeo R, Tanaka Y, Knight KR. Formation of new tissue from an arteriovenous loop in the absence of added extracellular matrix. *Tissue Eng.* 2000; 6(6):595–603. [PubMed: 11103081]
162. 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. *Proc Natl Acad Sci U S A.* 2009; 106(35):14990–5. [PubMed: 19706385]
163. Kelm JM, Fussenegger M. Scaffold-free cell delivery for use in regenerative medicine. *Adv Drug Deliv Rev.* 2010; 62(7-8):753–764. [PubMed: 20153387]
164. Shimizu T, Sekine H, Yang J, Isoi Y, Yamato M, Kikuchi A, Kobayashi E, Okano T. Polysurgery of cell sheet grafts overcomes diffusion limits to produce thick, vascularized myocardial tissues. *Faseb J.* 2006; 20(6):708–10. [PubMed: 16439619]
165. Giraud M-N, Ayuni E, Cook S, Siepe M, Carrel TP, Tevæarai HT. Hydrogel-based engineered skeletal muscle grafts normalize heart function early after myocardial infarction. *Artif Organs.* 2008; 32(9):692–700. [PubMed: 18684206]
166. Dimmeler S, Zeiher AM. Cell therapy of acute myocardial infarction: open questions. *Cardiology.* 2009; 113(3):155–60. [PubMed: 19122455]
167. Haider HK, Ashraf M. Preconditioning and stem cell survival. *J Cardiovasc Transl Res.* 2010; 3(2):89–102. [PubMed: 20560023]

Abbreviations

MI	Myocardial infarction
ECM	Extracellular matrix
FDA	US Food and Drug Administration
SIS	small intestinal submucosa
UBM	urinary bladder matrix
GAGs	glycosaminoglycans
PLGA	poly(lactic-co-glycolic acid)
PNIPAAm	Poly(N-isopropylacrylamide)
PTMCLA	poly(trimethylene carbonate-co-lactide)
VEGF	vascular endothelial growth factor
PEG	polyethylene glycol
3D	three-dimensional
FAK	Focal adhesion Kinase
RhoA	Ras homolog gene family, member A
AVL	arteriovenous blood vessel loop
FS	Fractional shortening
LVEF	left ventricular ejection fraction
EHTs	engineered heart tissue
LVEDD	left ventricular end-diastolic dimension
LVEDV	left ventricular end-diastolic volume
bFGF	Basic fibroblast growth factor
CPCs	rat cardiac progenitor cells

IFG-1 insulin-like growth factor 1

Figure 1.

Overview of cardiac tissue engineered construct process. (Left Panel) Natural polymers, such as collagen or fibrin, as well as synthetic polymers, such as PLGA, or PNIPPA, have been used as the biomaterial for the cardiac constructs. (Middle Panel) Preformed scaffolds, such as sponges or decellularized organs, have been used in *in vitro* systems. Hydrogels have been used *in vitro*, *in situ*, where the hydrogel is injected as a liquid directly into the heart wall, or *in vivo*. Cell sheets can be created *in vitro*. (Right Panel) When culturing constructs *in vitro*, oxygen/nutrient delivery of the type of bioreactor used should be taken into consideration. Furthermore, physical stimulation, such as mechanical and/or electrical stimulation can be used to condition the constructs.

Table 1
Summary of functional improvements observed in preclinical animal studies of tissue engineered constructs

Paper	Biomaterial	Scaffold Type	Cell Source	Culture Conditions	Animal Model	Post-MI Implantation Time	Post-Repair Functional Assessment Time	Fractional Shortening	Wall Thickness	Neovascularization	Left Ventricular Function
Leor <i>et al.</i> 2000 [68]	Alginate	Preformed Sponge	Fetal cardiac cells	Static culture	Sprague-Dawley rat	7 days	9 weeks	Improved [*]	---	---	LVID no change
Ruvinov <i>et al.</i> 2011 [67]	Alginate	Hydrogel microbeads	Neonatal rat myocytes	In situ	Sprague-Dawley rat	6 days	4 weeks	---	Increased [*]	increased vessel density [*]	---
Dvir <i>et al.</i> 2009 [162]	Alginate	Preformed sponge	Neonatal rat myocytes	In vivo	Sprague-Dawley rat	7 days	4 weeks	---	Increased [*]	Increased vessel density	---
Landa <i>et al.</i> 2008 [25]	Alginate	Hydrogel	N/A	In situ	Sprague-Dawley rat	7 days; 2 months	8 weeks	Improved [*] ; no change	increased [*] ; increased	---	LVESD, LVEDD improved [*] ; LVESD, LVEDD no change
Kutsebka <i>et al.</i> 2006 [49]	Collagen I, Matrigel	Preformed Sponge	Cardiac myoblasts	Abdomen of syngeneic rats	Lewis rat	60 minutes	4 weeks	improved [*]	Increased [*]	78±23% vascular ingrowth	LVEF improved
Xiang <i>et al.</i> 2006 [69]	Collagen-GAG	Preformed Sponge	MSCs	Static culture	Wistar rat	7 days	3 weeks	---	Increased	Vessel growth	---
Giraud <i>et al.</i> 2008 [165]	Collagen I, Matrigel, Fibrin	Implantable Patch	Skeletal muscle cells	Static culture	Lewis rat	14 days	4 weeks	Improved	Unchanged	No change vessel density	LVESD improved LVEDD no change
Zimmermann <i>et al.</i> 2006 [36]	Collagen I, Matrigel	Injection molded rings	Neonatal rat myocytes	Mechanical strain	Wistar rat	14 days	4 weeks	No change	Decreased	Vessel formation	LVEDV increased LVEDP increased [*]
Singhyn <i>et al.</i> 2009 [60]	Decellularized myocardial ECM	Injectable hydrogel	Neonatal rat myocytes	In situ	Sprague-Dawley rat	No infarct	11 days	---	---	Increased arteriole density [*]	---
SciE-Naraghi <i>et al.</i> 2010 [159]	Pericardial ECM	Injectable hydrogel	N/A	In situ	Sprague-Dawley rat	No infarct	2 weeks	---	---	Increased vessel density [*]	---
Zhang <i>et al.</i> 2008 [113]	Fibrin, Pegylated	Injectable hydrogel	Murine BMNCs	In situ	Balb/C female mice	Immediately	4 weeks	Improved [*]	---	---	LVEF increased [*]
Christman <i>et al.</i> 2004 [20]	Fibrin glue	Injectable hydrogel	Skeletal myoblasts	in situ	Sprague-Dawley rat	9 to 10 days	5 weeks	No change	No change	---	---
Li <i>et al.</i> 1999 [70]	Gelatin mesh	Preformed sponge	Fetal rat myocytes	Static culture	Lewis rat	3 weeks	5 weeks	---	Increased [*]	---	---
Kofidis <i>et al.</i> 2004 [53]	Matrigel	Injectable hydrogel	Mouse ESCs	In situ	Lewis rat	Immediately	2 weeks	Improved [*]	Increased [*]	---	No LV dilation
Shimizu <i>et al.</i> 2002 [81]	PIPAAm	Cell sheets	Neonatal rat myocytes	Culture dish	F344 nude rats	No infarct	3 weeks	Improved [*]	---	Vessel growth	---
Davis <i>et al.</i> 2006 [158]	Self-assembling peptides	Injected peptide fibers	Neonatal rat myocytes	In situ	Sprague-Dawley rat	Immediately	3 weeks	improved [*]	---	---	Ventricular volume improved [*]

* Statistical significance (P<0.05)

LVID – Left ventricular internal dimension, LVEF – left ventricular ejection fraction, LVEDD – left ventricular end-diastolic dimension, LVEDV – left ventricular end-diastolic volume, LVEDP – left ventricular end-diastolic pressure, LVESD – left ventricular end-systolic dimension