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Review

Hydrogels for Cardiac Tissue Engineering

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Abstract: Cardiac tissue regeneration is an integrated process involving both cells and supporting matrix. Cardiomyocytes and stem cells are utilized to regenerate cardiac tissue. Hydrogels, because of their tissue-like properties, have been used as supporting matrices to deliver cells into infarcted cardiac muscle. Bioactive and biocompatible hydrogels mimicking biochemical and biomechanical microenvironments in native tissue are needed for successful cardiac tissue regeneration. These hydrogels not only retain cells in the infarcted area, but also provide support for restoring myocardial wall stress and cell survival and functioning. Many hydrogels, including natural polymer hydrogels, synthetic polymer hydrogels, and natural/synthetic hybrid hydrogels are employed for cardiac tissue engineering. In this review, types of hydrogels used for cardiac tissue engineering are briefly introduced. Their advantages and disadvantages are discussed. Furthermore, strategies for cardiac regeneration using hydrogels are reviewed.

Keywords: hydrogel; cardiac tissue engineering; stem cells; biofunctional polymers

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1. Introduction

The heart is a fascinating engineering marvel of nature. In the heart, cardiac muscle contraction pumps nutrient- and oxygen-rich blood to supply the entire body, including the cardiac muscle itself. Coronary arteries (CA) supply blood for the cardiac muscle [1]. CA narrowing or clogging reduces the blood supply and causes heart cell death within minutes, leading to myocardial infarction (MI). This

initiates a decrease in heart function. After MI, the death of muscle cells triggers a remodeling cascade. The infarcted area is gradually filled with collagen-containing scar tissue to withstand the higher pressure during the contraction cycle (systole). As the scar tissue becomes thinner, the heart function further decreases; this finally becomes congestive heart failure (CHF) [2].

Current clinical intervention for MI is mainly concentrated on coronary reperfusion, which aims to reintroduce oxygen in the infarcted heart to lower cell death. Reperfusion therapy, however, is a conservative method that does not involve new cardiac muscle regeneration because adult cardiomyocytes are non-regenerative. Delivering cells into the heart for cardiac regeneration is accepted to be the ultimate therapeutic approach. These cells rebuild the muscle and integrate with the native heart, leading to an increase in heart function. Many clinical trials are ongoing to deliver stem cells into the heart. A commonly used delivery approach is to suspend cells in a buffer and inject into the heart. However, the injected cells have failed to show a satisfied engraftment rate [3]. One of the possible reasons is the harsh environment at the infarcted area. The blockage of the coronary artery causes very low oxygen and nutrient levels. Meanwhile, phagocytosis releases apoptotic cytokines and cell toxic reactive oxygen species (ROS). All of these factors limit the survival of the delivered cells. The engraftment rate may be improved by an approach called regenerative cardiac tissue engineering, in which cells are delivered within a supporting matrix (scaffold or hydrogel). The supporting matrix prevents cell loss during the injection, and provides an environment protecting cells from attack by the harsh environment in the MI heart. In addition, it acts as a mechanical support to alleviate the elevated wall stress caused by the loss of normal cardiac muscle cells, leading to cardiac function improvement. This approach is also versatile, as biomolecules can be readily incorporated into the matrix to build a cell-friendly environment for better regeneration.

Cardiac tissue engineering mainly uses two classes of materials: cardiac scaffold patches and hydrogels. This review mainly discusses hydrogels, which are water-insoluble, crosslinked polymer matrices with high water content (>30%).

2. Physiology of Cardiac Muscles

The heart wall is composed of the endocardium, myocardium, and epicardium layers [4]. The myocardium is the major layer, providing strength in the systole and diastole. The myocardium has a unique structure (Figure 1) in which multiple layers made of extracellular matrix (ECM) and highly oriented cells are packed together. The ECM is considered to be a major factor directing the alignment of cardiomyocytes [5].

Besides its anisotropic structure, the cardiac muscle exhibits unique biomechanical behavior. It is strong but ductile in the systole phase, while it is more elastomeric in the diastole phase. The Frank-Starling relationship describes the mechanical response of cardiac muscle during a full pump cycle (Figure 2). This unique biomechanical behavior allows cardiomyocytes in the heart to function normally. Many studies show that cardiomyocytes behave poorly on a matrix stiffer than cardiac muscle [6,7]. Therefore, for cardiac tissue regeneration, mimicking cardiac muscle's native mechanical environment is important.

Figure 1. The aligned structure of cardiomyocytes in the myocardium [8]. The alignment is uniform in each layer with a gradual transition of alignment between layers from the endocardium to the epicardium.

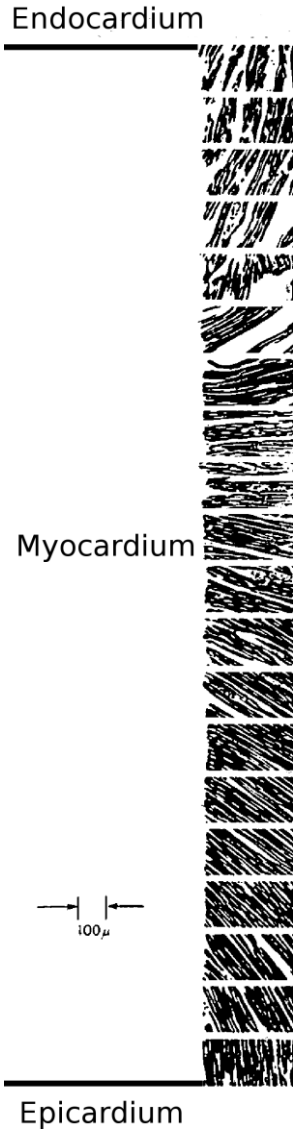
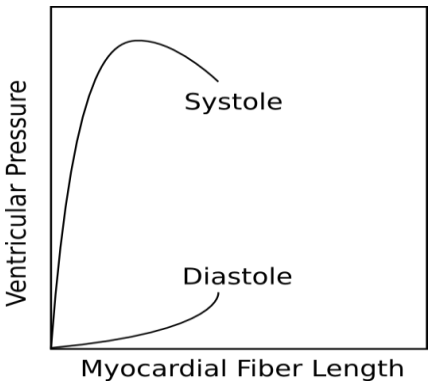


Figure 2. Frank-Starling law for mechanical behaviors of the ventricle muscle [9-11].

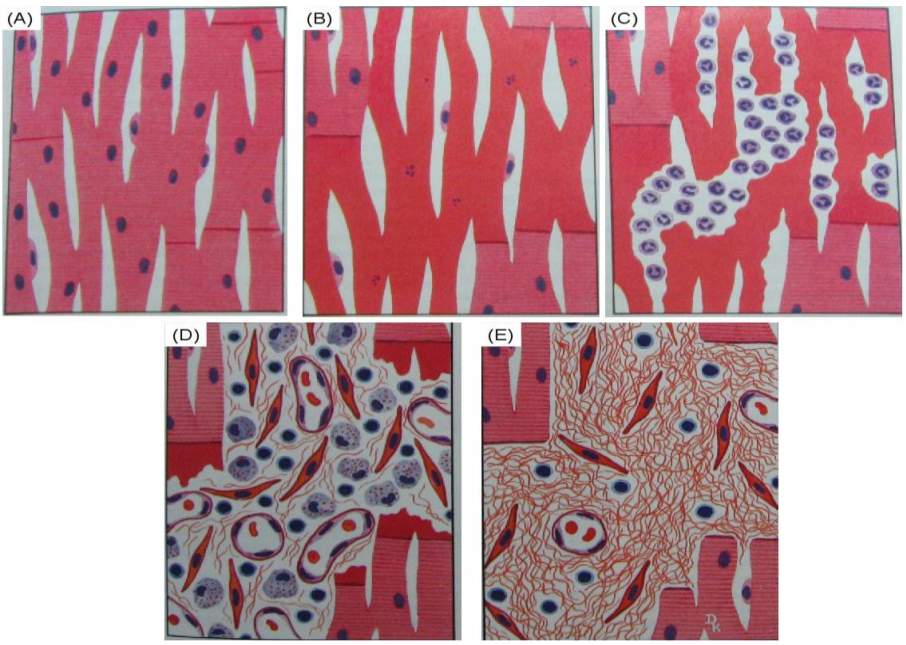


The heart also exhibits a unique electrophysiological property. A continuous integrated electrical conductive network is built inside the heart to direct the spontaneous heart cycle. Lack of connection to the existing cardiac electrical network induces arrhythmia, and reduces heart function.

3. Pathology of MI: The Remodeling Process

For acute MI, if the blood supply resumes within 30 min, the injured myocardium can revert into normal state, although a depression in contractility may persist for hours (Figure 3(A)). After 30 min, the myocardium undergoes an irreversible necrosis process, beginning with swollen mitochondria, broken nuclei, and disruptions in the cell membrane, and disordered tissue structure. Within 12–18 h, proteins in the cytosol start to leak out and the dead myocardial fibers form "wavy fiber" under the stretching of the systole (Figure 3(B)). Within 2 days, polymorphonuclear leukocytes are attracted to the infarcted area and accumulate in the periphery of the infarcted sites. The neutrophils start to infiltrate until they fill the infarcted area. The phagocytosis of dead cardiomyocytes can then be clearly seen in the infarcted area (Figure 3(C)). At day 5, fibroblasts start to proliferate and collagen deposition begins (Figure 3(D)). After 3 weeks, the inflammation process withdraws and collagen deposition continues. In the fourth week, a dense fibrous scar tissue is formed, and the remodeling of the MI is nearly finished [12] (Figure 3(E)). The gradual maturation of the scar tissue will further induce heart wall dilation to compensate for the lost heart function by accommodating a larger volume of blood.

Figure 3. Myocardial infarction and the remodeling process: (**A**) normal myocardium, with clear nucleus and elongated actin fibers and (**B**) 12–18 h post-MI, the nucleus starts to dissociate and a wavy actin fiber emerge at the infracted sites; (**C**) 24 h post-MI, macrophages (cells with multiple nuclei) start to penetrate and the dead cells are removed by phagocytosis; (**D**) 3 weeks post-MI, fibroblasts are attracted to the infracted area and start to deposit collagen fibers in the area; (**E**) 3 months post-MI, the remodeling process is nearly complete. Collagen scar tissue has formed [12].



Timely reperfusion is crucial for acute MI. A bypass surgery can be done to reestablish blood to the blocked arteries by grafting a short vein (normally from the saphenous veins). However, if the myocardium has already undergone permanent damage, reperfusion cannot regain the lost cardiac muscle due to the non-regenerative nature of adult cardiomyocytes. This leads to a permanent decrease of heart function. The ultimate goal for post-MI therapy is to attenuate the remodeling process and regenerate the new cardiomyocyte-based muscle. This can be achieved by a cell delivery system consisting of a supporting matrix and suitable cells. The function of the supporting matrix is to stop or slow down the remodeling and scar formation process by mechanically preventing the cardiac muscle from dilating [13]. Meanwhile, suitable cells are delivered to replace the dead cardiomyocytes and integrate with the neighboring cardiac tissue. The supporting matrix should also have a cell-friendly environment to support the survival of the delivered cells and facilitate the regeneration process. The properties of an ideal cell delivery system are summarized in Table 1. In the following part of this review, how the hydrogel materials addressing these requirements is discussed.

Table 1. Criteria for successful cardiac tissue engineering: the requirements for cells and supporting matrix.

Categories	Assessments
Cells	Survive in the harsh infarcted area
	Protected from neutrophils attacks
	Potential to be cardiomyocytes
	Integrate with neighboring cardiac tissue
	Easy to isolate and expand *in vitro*
	Non-immunogenic
	...
Matrix	Sufficient strength to reinforce weakened heart wall
	Cell-friendly micro-environment for survival and differentiation
	Cardiac mimicking mechanics
	Nontoxic
	Degradable
	...

4. Hydrogels for Cardiac Tissue Engineering

Both natural and synthetic hydrogels are suitable for cardiac tissue engineering because their soft and viscoelastic nature mimic the native tissue. Collagen, gelatin, laminin, matrigel, hyaluronic acid (hyaluronan), alginate, and chitosan are typical natural hydrogels. They have similar or even identical structures to the molecules in biological organisms, thereby reducing the possibility of immune response when implanted *in vivo*.

Synthetic materials used for cardiac tissue engineering include poly(ethylene glycol) (PEG), polylactide (PLA), polylactide-glycolic acid (PLGA), polycaprolactone (PCL), polyacrylamide (PAAm), and polyurethane (PU). It is easy to tailor the physical and chemical properties of these synthetic polymers, such as modulus, water affinity, and degradation rate, to meet the requirements of cardiac muscle tissue engineering. Therefore, they are considered to be good candidates for tissue engineering. However, potential cytotoxicity is a major concern for synthetic polymers. So far, only PEG, PLA, and PLGA have been approved by the FDA for clinical applications. In fact, many other polymers like PAAm and PU have already been confirmed to be non-toxic *in vitro* and *in vivo*. An alternative method is to use natural/synthetic hybrid hydrogels that combine the advantages of both natural and synthetic polymers. This can be achieved by blending or covalently grafting/crosslinking (discussed in Section 4.3).

4.1. Natural Hydrogels

Human tissues and organs are assembled by cells and ECMs [5]. The native ECM components are suitable materials to be used as the delivery vehicles of cells because of their high biocompatibility and biodegradability [14]. In this section, the commonly used natural hydrogels are discussed.

4.1.1. Collagen and Decellularized Matrix

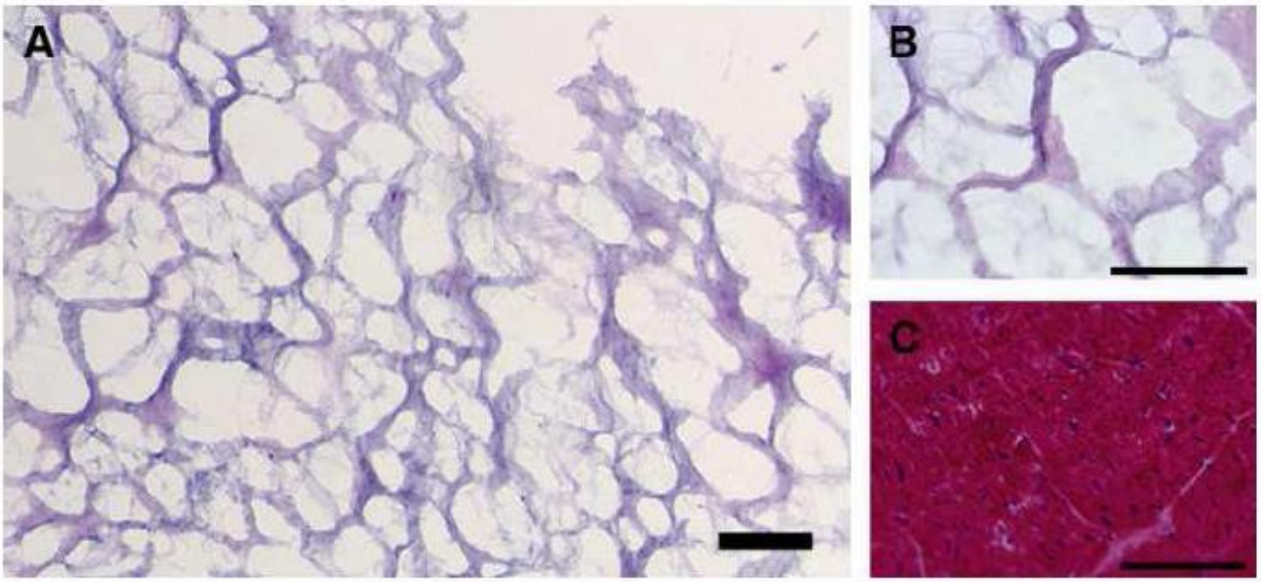
Collagen provides most of the mechanical strength in tissues [1]. There are many types of collagens in the body. Among these, type I collagen is ubiquitously distributed. Because of its excellent biocompatibility, type I collagen has been used in tissue engineering [15-21]. Type I collagen hydrogel can be formed by dissolving the collagen in an acid solution (for example, in 0.3% glacial acetic acid) and then neutralizing the solution to pH = 7.4 [15]. However, the solution-neutralization method is not ideal for cell encapsulation because the initial acid condition may cause cell death. Additionally, injection of acidic solution in an infarcted heart may provoke the local inflammatory reaction. Recently, a method of forming collagen gel via incubating the neutralized solution in physiological conditions has been reported [16]. However, the slow gelation time of this gel may be a problem when used for cardiac tissue engineering, since the encapsulated cells may be flushed away before the gel is formed.

Alternatively, a collagen-containing decellularized matrix can be used. It is prepared by decellularizing native tissues like bladder, pericardium, and heart [17]. Mirsadraee *et al.* decellularized the pericardium and found that the chemical compositions of the native and acellular pericardium are not significantly different [18,19]. In addition, no cytotoxicity was observed for human dermal fibroblasts seeded on the acellular sample. Further study on foreign body reaction showed that the acellular pericardium provoked minor macrophage response [20]. These results demonstrate the feasibility of using acellular ECM as a potential scaffold for cardiac tissue engineering. Recently, a groundbreaking study by Ott *et al.* showed that a decellularized rat heart can be used as a template to make a functional heart by recellularizing it with cardiomyocytes, endothelial cells (ECs), and smooth muscle cells (SMCs) [21].

A soluble ECM hydrogel capable of thermally gelling at body temperature can be obtained by an additional digestion process to the above decellularized matrix. The advantage of using acellular ECM hydrogel is that it may be delivered into the heart by a minimally invasive cardiac surgery. Singelyn *et al.* generated injectable porcine myocardium hydrogel [22] (Figure 4). *In vitro* tests showed that the gel supported the survival of cardiomyocytes, and the migration of ECs and SMCs. Further *in vivo* study demonstrated the infiltration of ECs and SMCs into hydrogel. Additionally, vascularization was enhanced within the hydrogel. However, the *in vivo* study was conducted on a normal rat heart instead of an MI heart. An MI model is needed to verify these findings.

There are disadvantages concerning the decellularized ECM for cardiac tissue engineering. First, any remaining cells or immunogenic proteins will provoke immune rejection. No existing methods can guarantee that the decellularized ECM will be free of immunogenic proteins. Second, the long gelation time may cause the injected gel being flushed away shortly after injection. Rapid gelation (less than one minute) is necessary to retain the delivered material and cells after injection.

Figure 4. Decellularized heart matrix by H&E staining: (**A**) intact acellular matrix before lyophilization, a porous structure can be clearly seen; (**B**) acellular matrix before being milled; (**C**) normal heart tissue before decellularization [22]. Scale bars are 100 μm.

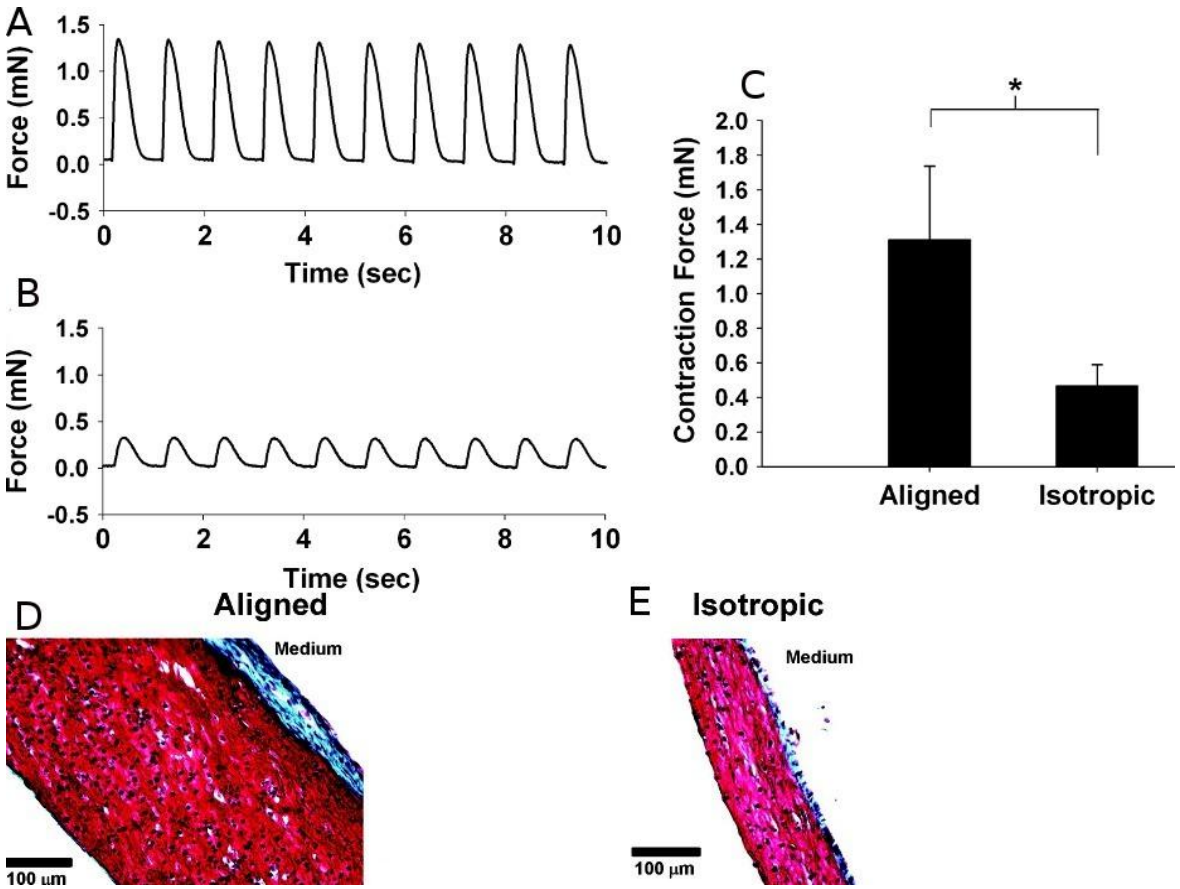


4.1.2. Fibrin Gel

Fibrin is formed during the haemostatic coagulation process by combining fibrinogen and thrombin under the catalysis of calcium ions [23]. Fibrin gel is biodegradable and nontoxic. Therefore, it is suitable for tissue engineering. Birla *et al.* fabricated hollow fibrin gel tubes populated with neonatal cardiomyocytes and implanted into the femoral artery of adult rats [24]. After 3 weeks of implantation, the fibrin gel/cardiomyocytes construct formed a mature cardiac tissue with a dense capillary network. It possessed all normal cardiac functions, including contractility under electric stimulation and synchronous pacing with an outside electric signal. Huang *et al.* embedded rat cardiomyocytes within fibrin gel and found that their contractility can be retained up to two months with normal pacing

ability [25-27]. The embedded cardiomyocytes showed aligned morphology. Black *et al.* cultured the fibrin/cardiomyocytes constructs under circumferential/radial contraction condition ("isotropic" group) and circumferential/radial/axial contraction condition ("aligned" group), respectively [28]. Although the "isotropic" group had a high collagen content and cell density, the mechanical contract response under electrical pacing stimulation was significantly less than that in the "aligned" group (Figure 5(A–C)). This difference can be explained by the different contents of connexin 43 (CX43) protein under different conditions.

Figure 5. The pacing signal of cultured cardiomyocytes in an (**A**) "aligned" and (**B**) "isotropic" construct. The contract force generated by cells on aligned fibers shows a higher force compared to those on isotropic fibers under electric stimulus (**C**) and Lillies' trichrome staining for (**D**) "isotropic" and (**E**) "aligned" samples. The aligned cell construct has a thicker cell layer compared to that of isotropic structure [28].



4.1.3. Matrigel

Matrigel, composed of basement membrane proteins as well as growth factors, is an ECM-mimicking hydrogel produced by mouse Engelbreth-Holm-Swarm tumors [29]. It closely resembles the native ECM with a similar composition and assembling structure. Thus, it has been considered as a cytocompatible gel. In addition, Matrigel shows the ability of faster vascularization compared to other natural hydrogels. These properties make Matrigel a potential candidate for cardiac

tissue engineering. Injection of cells with Matrigel into the heart significantly increased the cell retention [30], indicating that the viscous Matrigel solution can increase the cell retention in the infarcted area. Copland *et al.* used a Matrigel plug as a delivery carrier to deliver genetically modified human mesenchymal stromal cells (MSCs) into an infarcted heart, and showed that MSC survival was significantly enhanced under the ischemic and apoptotic environment [31]. Matrigel can also be combined with other natural materials to improve cell proliferation and angiogenesis *in vivo*. Giraud *et al.* demonstrated that Matrigel/collagen hydrogel significantly improved heart function after implantation into acute MI rat hearts [32]. The implantation of Matrigel/collagen hydrogel and H9C2 cardiomyoblasts in an acute rat MI model was found to significantly increase cell engraftment rate [33]. Matrigel was also combined with fibrin gel to encapsulate cardiomyocytes. The cardiomyocytes maintained normal function throughout the entire experiment time (10 days) [34].

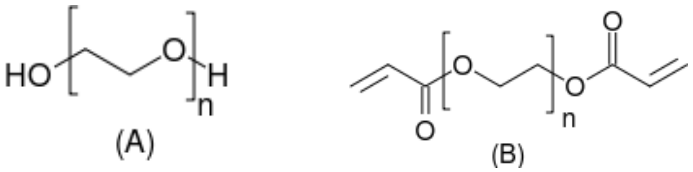
Matrigel and fibrin gel are both naturally derived ECMs. They are free of cytotoxic issues. However, fibrin gel and Matrigel are still far from ideal for cardiac tissue engineering. For fibrin gel, the relatively slow gelation rate and lack of sufficient mechanical strength are major challenges. Slow gelation time, as discussed above, causes a loss of delivered cells and low cell retention during the injection. Low mechanical strength leads to the possible breakdown of gels during heart contraction and relaxation. In addition, fibrin gel has a fast degradation (fibrinolysis) rate. Normally, in the fibrin gel culture medium, a chemical like aprotinin is used to inhibit fibrinolysis. However, it is possibly toxic to the cells [35]. Without aprotinin, a high degradation rate results in a rapid loss of the supporting matrix before the maturation of the delivered cells and the establishment of angiogenesis. This interrupts the regeneration process. For Matrigel, a biosafety concern exists, since it is derived from tumors.

4.2. Synthetic Macromolecules for Cardiac Tissue Engineering

4.2.1. Poly(ethylene glycol)

Poly(ethylene glycol) (PEG, Figure 6(A)) is a water soluble polymer synthesized by the ring-opening polymerization of ethylene oxide [36]. It is biocompatible and has been approved by the FDA. PEG hydrogel is typically made by the polymerization of diacrylate-modified PEG (Figure 6(B)) via photo-polymerization under ultraviolet (UV) irradiation. PEG gel has been widely used as a supporting matrix in almost every field of tissue engineering (nerve, cartilage, liver, pancreas, bladder, skin) because its low protein adsorption and inert surface reduce the inflammation after implantation. However, low protein affinity is not beneficial for cell adhesion. Methods like conjugating cell adhesive peptides or proteins and incorporating growth factors are used to increase cell adhesion [6,37]. PEG hydrogel has been used to study cardiomyocyte-matrix interactions in a three-dimensional (3-D) environment. For example, it was found that Arg-Gly-Asp (RGD) peptide modification largely increased the viability of encapsulated cardiomyocytes [38]. Kraehenbuehl *et al.* constructed a series of PEG hydrogels with different moduli by varying crosslinking density [7]. Embryonic stem cells (ESCs) were encapsulated inside. The results showed that hydrogels with lower moduli (soft) induced the ESCs differentiation into cardiomyocyte lineage. The differentiated cells exhibited a cardiac-like function.

Figure 6. Poly(ethylene glycol) molecular structure: (**A**) PEG; (**B**) PEG-diacrylate.

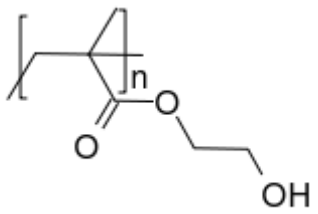


PEG hydrogel can also be formed by PEG-cyclodextrin interaction. This could avoid a potential toxic issue associated with the commonly employed photo-polymerization method. Wang *et al.* used a PEG–PCL–PEG triblock copolymer mixed with α-cyclodextrin to encapsulate bone marrow mesenchymal stem cells and delivered to the rabbit MI site [39]. The retention and survival of the delivered MSCs were significantly increased compared to the control group (cells suspended in saline). Dense vessel networks at the injection sites and the reduction of infarcted areas were observed.

4.2.2. Poly(2-hydroxyethyl methacrylate)

Poly(2-hydroxyethyl methacrylate) (PHEMA) is a hydrophilic polymer with pendant hydroxyl groups (Figure 7). PHEMA hydrogel has been used in cardiac tissue engineering. Walker *et al.* implanted poly(ethylene terephthalate) (PET) mesh reinforced PHEMA gel into the canine epicardium [40,41]. No significant fibrosis or thickening was observed 12 months after implantation. However, there was trace calcification on the gel after 9 and 12 months of implantation, raising the concern of biocompatibility of the PET/PHEMA constructs over a long timeframe.

Figure 7. Chemical structure of PHEMA.

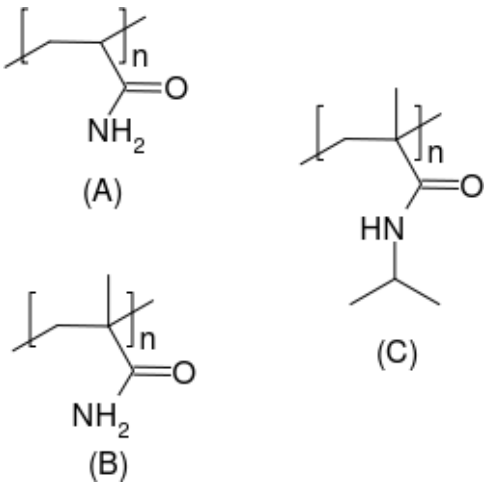


4.2.3. Polyacrylamide and Its Derivatives

The polyacrylamide family is an amide derivative of poly(acrylic acid) (Figure 8). Polyacrylamide gels, because of their peptide/protein mimicking amide structure, are also candidates for cardiac tissue engineering. Similar to PEG hydrogels, crosslinking is needed to fabricate polyacrylamide hydrogels [7]. What makes polyacrylamide gel different from other gels mentioned above is its thermal sol-gel transition. Such a transition allows aqueous polyacrylamide solution to be liquid when the temperature is low; it then solidifies to form a gel at body temperature. The detailed mechanism of its thermosensitivity is still under debate. A widely accepted theory is that hydrogen bonds between amide groups and water molecules are dissociated and the pendant amide groups tend to collapse together as the temperature increases. Thus, the water molecules are repelled and a hydrophobic solid

gel is formed [14]. Regardless of the mechanism, this thermosensitive property has been used to design novel injectable hydrogels that can be delivered *in vivo* in a liquid form through a needle and then turn into solid gels after contact with the warm tissue.

Figure 8. Chemical structure of (**A**) polyacrylamide, (**B**) polymethacrylamide, and (**C**) poly(N-isopropylacrylamide).



Poly(N-isopropylacrylamide) (PNIPAAm) is a typical thermosensitive polymer with a thermal transition temperature (LCST) of 32 ℃. Its aqueous solution is in liquid state at room temperature while forming hydrogel at 37 ℃. Okano *et al.* utilized this property to generate cell sheets for tissue engineering. Tissue culture plates were coated with PNIPAAm and neonatal cardiomyocytes were cultured on top at 37 ℃ [42]. After cells reached confluence, the culture plate was cooled down to room temperature and cells were lifted to form a monolayer cell sheet. The cardiomyocyte monolayers were stacked together and implanted into the infarcted myocardium. The recovery of heart function was observed 4 weeks after implantation. PNIPAAm based hydrogel was also used to encapsulate cells. A gelatin grafted PNIPAAm hydrogel was employed to encapsulate rat cardiac cells [43]. The best contractility was obtained with a seeding density of 50 million/mL. Guan *et al.* developed a family of protein conjugated, biodegradable PNIPAAm hydrogels based on NIPAAm, acrylic acid, acrylic N-succinimide ester and HEMA-poly(trimethylene carbonate) [44]. The hydrogels changed their LCSTs from room temperature before degradation to above 40 ℃ after degradation. The degradation products are therefore soluble in body fluid. The hydrogels and their degradation products were non-cytotoxic. Fujimoto *et al.* synthesized similar hydrogels and injected into infarcted hearts and an improvement of heart function was observed [45]. Guan *et al.* further functionalized the developed PNIPAAm hydrogels with growth factor [46] and antioxidants [47]. It was found that functionalization significantly enhanced MSC growth within the hydrogels. It is expected that the functionalized hydrogels will provide a suitable environment for cells delivered into heart to survive and function.

Many synthetic polymers are used to make hydrogels for cardiac tissue engineering. The advantage of synthetic polymers is their versatility in controlling physical and biochemical properties, including stiffness, water content and cell adhesion. They are easy to make and cost-effective compared to the

natural polymers. However, biocompatibility and possible inflammatory reaction may be an issue. To combine the advantages of both natural and synthetic hydrogels, the approach that blends synthetic and natural hydrogels (gelatin, chitosan, fibrin and hyaluronan) seems to be a good alternative.

Table 2. Summary of commonly used hydrogels in cardiac tissue engineering.

Category	Name	Properties	*Concerns*
Natural materials	Collagen and decellular ECM	Biocompatible, biodegradable,	*weak strength, immune rejection, slow gelation*
	Fibrin gel	Biodegradable, biocompatible, availability	*Slow gelation and fast degradation* in vivo
	Matrigel	Closely resemble native ECM structure	*Potentially carcinogenic concerns*
	…		
Synthetic materials	PEG	Bio-inert, biocompatible, FDA approved	*Low cell adhesion, non-injectable and toxic concerns of small crosslinkers, not degradable*
	PHEMA	Biocompatible and available for functionalization	*Modulus mismatch, non-degradable*
	Polyamides	Fast gelation time, injectibility, versatile for chemical modification	*Pure polyamides are not degradable, non-elastic*
	...		

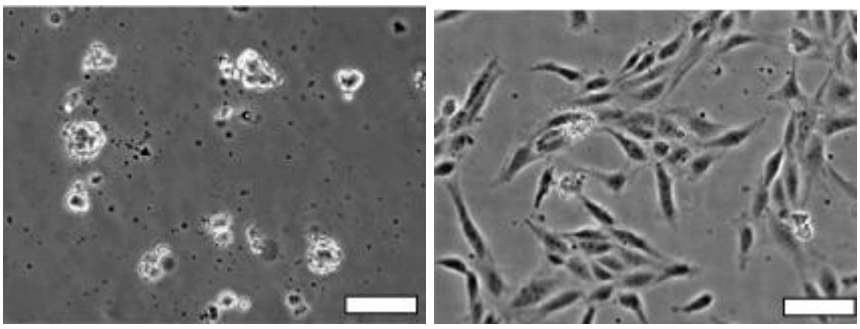
4.3. Natural/Synthetic Hybrid Hydrogels for Myocardial Tissue Engineering

In general, natural materials exhibit better biocompatibility and cell affinity than synthetic polymers, but are less versatile in terms of tailoring properties like mechanical strength, water content, and degradation rate. Combining natural and synthetic hydrogels to make a blend seems to be a good solution when it comes to capitalizing on the advantages of both. For example, the biocompatibility of synthetic polymers can be improved by introducing collagen, fibronectin, laminin, or fibrinogen. Natural/synthetic hydrogels can also be used to modulate cell phenotype. Putnam *et al.* introduced fibrinogen into PEG gel and cultured SMCs in the gel [48]. The cells showed a spindle-like shape as compared to the round shape normally observed in purely synthetic PEG gels. In addition, synthetic hydrogels can greatly enhance the function of natural hydrogel. Zhang *et al.* PEGylated fibrin hydrogel and loaded it with stromal derived factor (SDF) [49]. It was found that incorporation of PEG prolonged the half-life of the encapsulated growth factor.

Introduction of naturally derived peptide sequences into synthetic hydrogels is another approach to fabricate natural/synthetic hydrogels. The peptide sequences can be cell adhesive motifs, enzyme-sensitive segments, and anti-inflammatory segments. RGD is a peptide sequence widely used to improve cell adhesion and biocompatibility [8,37,50-54]. The PEG gel containing RGD sequence has a significantly higher cell adhesion compared to the pure PEG gel [50] (Figure 9). The enzyme-sensitive peptides are used to adjust the degradation of the gel matrix to match the rate of the new ECM deposition during the tissue maturation. For example, a matrix metalloproteinase (MMP)-sensitive peptide was introduced to hyaluronic acid hydrogel and it was found that the gel had

a faster degradation rate than the control (non-MMP-sensitive peptide incorporated gel) [52]. Kraehenbuehl *et al.* used a PEG gel that contains MMP-sensitive peptide to culture P19 embryonic stem cells, and found that MMP-mediated degradation facilitated stem cell differentiation into cardioprogenitor cells [7].

Figure 9. Human umbilical endothelial cells cultured on bare PEG gel (left) and PEG/RGD gel (right) surfaces [50]. Scale bars are 100 μm. Cells on RGD modified PEG surface showed an elongated morphology, while the cells on the bare PEG surface showed a round morphology.



In summary, blending natural and synthetic hydrogels represents a feasible approach to design hydrogels with controllable mechanical and biochemical properties without compromising biocompatibility and biodegradability. Recent progresses in bioconjugation chemistry provide the new possibility for functionalizing a synthetic matrix with naturally derived proteins or peptides [56]. The natural/synthetic hybrid hydrogels can be a future direction in hydrogel design for cardiac tissue engineering.

5. Application of Hydrogels for Cardiac Tissue Engineering and Challenges

Hydrogels for cardiac therapy can be used in two forms, *i.e.*, hydrogel only and hydrogel with cells [3,57-59]. The application of each form for cardiac tissue engineering is discussed in this section.

5.1. Hydrogel-Based Therapy

From a pathology point of view, collagen deposition is the last step of remodeling after MI. If myocardium hypotension cannot be relieved, further ventricle dilation will occur. This will lead to CHF. To break this process, it has been proposed to inject a supporting gel into the infarcted heart to release the elevated wall stress. Injection of PNIPAAm-based copolymers has been shown to attenuate ventricular dilation [45]. This suggests that hydrogel-only therapy can be effective in delaying the cascade that leads to CHF by providing sufficient mechanical support to the infarcted area. However, lack of cells makes this therapy only “passive,” unable to regenerate new myocardium.

5.2. Cell/Hydrogel Delivery Strategy

A promising strategy for cardiac tissue engineering lies in hydrogel-based cell therapy. The viscous hydrogel holds the cells in the target place during injection. The gel itself provides mechanical support to the weakened heart wall [13,22,60,61]. Meanwhile, the gel environment may allow the delivered cells to survive and differentiate into cardiomyocytes to regenerate cardiac muscle. To facilitate cell survival, growth, and differentiation, biochemicals can be co-delivered with hydrogels.

5.2.1. Cells Available for Delivery

The ideal cell type for cardiac tissue engineering is cardiomyocytes. Fetal and neonatal cardiomyocytes have been tested for this purpose [62,63]. However, the implanted cardiomyocytes were unable to integrate with the native myocardium and caused arrhythmias [64]. In addition, the source for human fetal or neonatal cardiomyocytes is a concern. Skeletal myoblasts have also been tested. These cells are fatigue-resistant and have a great tolerance to the ischemic environment [65]. However, the arrhythmia issue persists [66].

Stem cells, including adult stem cells, ESCs, and induced pluripotent stem cells (iPSCs) are possible sources. These stem cells can proliferate and differentiate into cardiomyocytes. For example, bone marrow- and adipose-derived MSCs were reported to differentiate into cardiomyocyte lineage through treatment them with dimethyl sulfoxide and 5-azacytidine (5-aza) for 24 hours *in vitro* [67], or through delivery them into the heart *in vivo* [68]. ESCs cannot be used directly because they cause teratoma formation [69], but they can be differentiated into cardiomyocytes and then used [69-72]. iPSCs have recently been invented with a review to replace ESCs, which are involved in ethical controversy. The iPSCs are generated by transfecting four genes into skin fibroblasts [73,74]. They can be induced to differentiate into cardiomyocytes [75].

Despite the success of using stem cells in cardiac therapy, some challenges still remain. First, strategies that precisely control stem cell differentiation into a specific lineage *in vitro* and *in vivo* need further exploring. Current approaches are either toxic to cells (for example, 5-aza to MSC and cell signaling inhibitor to ESCs) or have potential biosafety concerns (like genetic modification). Recent progresses suggest that cells' surrounding microenvironment directs stem cell differentiation. The microenvironment is a small surrounding space that cells can feel and to which they respond. It includes matrix's global properties like biomechanics, hydrophilicity, and cell adhesion affinity, as well as localized properties like neighbored conjugated bioactive molecules (signal niches) and 3-D patterns [76]. Discussing the detailed relation between microenvironment and stem cell differentiation is beyond the scope of this review, but the design of a microenvironment regulating stem cell differentiation into cardiac lineage is a very interesting topic from a materials perspective.

Second, a better cell source for cardiac tissue engineering is in demand. The ability of MSCs to differentiate into cardiomyocytes is still debated. ESCs involve ethical problems. iPSCs represent a good alternative to ESCs. However, both the virus-involved transfection process and the potential oncological issue have limited their progression to clinical trials at present. Therefore, new cell sources still need to be sought. Recently, a new source of cardiac stem cells has been discovered from heart biopsy [77-79]. These cells originate from the patient's own small heart apex biopsy, and can

proliferate quickly *in vitro*. The *in vivo* studies showed that they are able to differentiate into cardiomyocytes.

5.2.2. Choosing Appropriate Hydrogel Matrix for Cardiac Tissue Engineering

Precisely controlling stem cell differentiation is critical for cardiac tissue engineering. As mentioned above, stem cell differentiation may be mediated by its surrounding microenvironment. It is hypothesized that a native myocardium-mimicking microenvironment will facilitate stem cells differentiation into cardiac lineage. Thus, tuning hydrogel properties to mimic the structure and properties of the native heart appears to be a feasible approach for cardiac tissue engineering.

5.2.3. Aligned *vs.* Isotropic

As shown in Section 2 and Figure 1, cardiomyocytes are highly aligned in the heart. This unique structure affords myocardium with unique physical and electrical properties. Mimicking the alignment structure of myocardium may facilitate cardiac tissue development. A study of cardiomyocytes on the PEG hydrogel surface demonstrated that cells on the patterned surface can align during culture [6]. A larger contractile force (in the aligned direction) and a directional conductive current were observed in the aligned direction. This indicates that cardiomyocytes are more similar to their native phenotype and function if assembled on an aligned structure. These results are based on two-dimensional (2-D) culture. However, achieving alignment of cardiomyocytes in a 3-D hydrogel is challenging [80,81].

To achieve 3-D alignment, a possible approach is to apply mechanical stimulation to induce cell arrangement. An elastic hydrogel is likely needed to withstand the mechanical cycling. The highly extensible PNIPAAm-based hydrogels developed in our laboratory may be good candidates for this approach [44,46,47].

5.2.4. Soft *vs.* Stiff

As discussed above, the fate of stem cells depends on the surrounding microenvironment. Biomechanics represent one of the determining factors of a microenvironment. The relationship between stem cell fate and substrate matrix was first reported by Engler *et al.* [82]. Naive MSCs can differentiate into brain, muscle, and bone lineages simply by varying modulus of the substrate. ESCs show a similar response to the matrix modulus. They can differentiate into cardiac lineage by using the gel with Young's modulus of 300 Pa [9]. Adult cardiomyocytes also respond to the matrix modulus. Cardiomyocytes on the soft polyacrylamide showed an upregulated cardiac specific protein Tropinin I expression and a higher response in electrical stimulation than on stiff hydrogel [7]. Thus, a successful material design should consider matrix modulus. Currently, tuning gel modulus is mostly accomplished by varying the crosslinking density. However, this might also change other physical properties like degradation rate, water content, and cell affinity. A better design is needed to independently tune modulus.

5.2.5. Functionalization of Hydrogels

The cardiac ischemic environment is a harsh environment lacking nutrients/oxygen and rich in apoptotic species (e.g., superoxide) (Section 3). Suitable biomolecules can be co-delivered with the hydrogel to neutralize the harsh environment and protect the delivered cells. Prosurvival growth factors can be used to address the nutrient/oxygen supply issue, while antioxidants can be used to protect cells from cytotoxic superoxide. However, these substances are highly unstable *in vivo*. Therefore, they need to be protected and gradually released from the hydrogels. Chemical conjugation [46] and physical entrapping [47] have been shown to be good approaches.

6. Conclusions

Cardiomyocytes and stem cells are utilized to regenerate cardiac tissue and restore heart functions after MI. However, the injection of cells directly into the infarcted area involves the problems of low cell retention and engraftment rate. A proper supporting matrix can hold cells at the infarcted area initially and further provide support for cell survival and functioning. Hydrogels, because of their native tissue-like properties, are widely used as the cell delivery carriers and supporting matrices for cardiac tissue regeneration. A bioactive, biocompatible hydrogels mimicking native tissue biochemical and biomechanical environment are needed for successful cardiac tissue regeneration.

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