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Injectable Acellular Hydrogels for Cardiac Repair

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Abstract Injectable hydrogels are being developed as potential translatable materials to influence the cascade of events that occur after myocardial infarction. These hydrogels, consisting of both synthetic and natural materials, form through numerous chemical crosslinking and assembly mechanisms and can be used as bulking agents or for the delivery of biological molecules. Specifically, a range of materials are being applied that alter the resulting mechanical and biological signals after infarction and have shown success in reducing stresses in the myocardium and limiting the resulting adverse left ventricular (LV) remodeling. Additionally, the delivery of molecules from injectable hydrogels can influence cellular processes such as apoptosis and angiogenesis in cardiac tissue or can be used to recruit stem cells for repair. There is still considerable work to be performed to elucidate the mechanisms of these injectable hydrogels and to optimize their various properties (e.g., mechanics and degradation profiles). Furthermore, although the experimental findings completed to date in small animals are promising, future work needs to focus on the use of large animal models in clinically relevant scenarios. Interest in this therapeutic approach is high due to the potential for developing percutaneous therapies to limit LV remodeling and to prevent the onset of congestive heart failure that occurs with loss of global LV function. This review focuses on recent efforts to develop these injectable and acellular hydrogels to aid in cardiac repair.

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Introduction

Left ventricular (LV) remodeling caused by a myocardial infarction (MI) is responsible for almost 70% of the 5 million cases of heart failure that have occurred in the USA in recent years [1]. Early infarct expansion or stretching has been associated with poor long-term prognosis [2–4] and has been identified as the mechanical phenomenon that initiates and sustains the process of adverse post-MI LV remodeling that leads to heart failure [5–10]. Infarct expansion causes abnormal stress distributions in myocardial regions outside the infarction, especially in the adjacent border zone (BZ) region, putting this region at a mechanical disadvantage. With time, increased regional stress is the impetus for several maladaptive biologic processes, such as myocyte apoptosis and matrix metalloproteinase activation that inherently alter the contractile property of the normally perfused myocardium [11–13]. Once initiated, these maladaptive processes lead to a heart failure phenotype that is difficult to reverse by medical or surgical means.

Previous work has demonstrated that the use of ventricular restraints, such as polymeric meshes wrapped around the heart or sutured to the surface of the infarcted myocardium, reduces infarct expansion by mechanically stabilizing the heart and forcing it to maintain its original shape, thus limiting long-term global LV remodeling in large animal models [10, 14–17]. However, these approaches are limited by the invasive procedure in which they are applied, and clinical adoption has not occurred. In order to circumvent the invasive surgical placement of

restraining devices early post-MI, our group and others have begun to explore the use of injectable materials, and specifically hydrogels, to limit infarct expansion and normalize the regional stress distribution [18–35].

Hydrogels are water-swollen polymer networks that exhibit many tissue-like properties and have been explored for numerous tissue engineering and drug delivery applications [36–38]. Hydrogels can form through numerous techniques, including via self-assembly, through non-covalent interactions with ionic species, through covalent crosslinking via chemical reaction, and through thermal transitions that lead to gelation [36–38]. These techniques are advantageous as they could potentially translate to catheter delivery for minimally invasive, percutaneous therapies. Hydrogels are finding application in cardiac therapy alone as a means for thickening and stabilizing the myocardium via tissue bulking, as well as for the delivery of a wide variety of therapies, such as cells and growth factors [39, 40]. This review will specifically focus on the application of hydrogels for (1) tissue bulking and (2) molecule delivery approaches that may translate quickly to the clinic since difficulties related to finding an adequate cell source for transplantation are eliminated.

Injectable Hydrogels as Bulking Agents

Over the past decade, it has become clear that the mechanical changes that occur after MI must be considered when developing post-MI therapies [35, 41, 42]. MI leads to extracellular matrix (ECM) breakdown and results in geometric changes in both the infarcted and healthy myocardium. LV dilation results in stretched cardiomyocytes in the BZ and healthy myocardium, which subsequently lose the ability to efficiently contract [43]. Globally, this is manifested as a shift to a more spherical shape and a reduction in the transmural wall thickness. The Law of Laplace (Eq. 1) illustrates how the resulting dilation (increased radius) and thinning of the myocardium post-MI leads to increased stress, where stress (T) is directly proportional to pressure (P) and the radius of curvature (R), and inversely to the thickness of the myocardial wall (h).

$$T = \frac{P \cdot R}{h} \tag{1}$$

Past surgeries to attenuate LV expansion have included surgeries to reconstruct the dilated ventricle and restraints placed around the myocardium or infarct to physically prevent dilation [16, 44–47]. Injectable hydrogels may present a material system that is applied in a non-invasive manner (liquid-to-solid transition) and leads to limited LV expansion. In this section, we will review common natural and synthetic hydrogels that have been investigated to this end, as well as comment on the underlying mechanisms of therapy. The reader should refer to Table 1 throughout this section, which summarizes the various materials that have been investigated in animal models of MI. In most cases, the specific material properties for these studies were not reported.

Natural Hydrogels

Christman et al. pioneered the field of acellular injectable biomaterials by exploring the effects of fibrin glue as a bulking agent [18, 23, 34, 48]. Fibrin has natural binding domains for soluble growth factors and cellular integrin receptors, motivating its use for wound healing applications. Although fibrin is commonly utilized for these biological properties, it can also be used as a mechanical support for the myocardium [18, 29, 34, 48]. Specifically, fibrin forms a crosslinked 3-D hydrogel in the myocardium upon injection with a dual-barreled syringe. One barrel contains fibrinogen and aprotinin, a fibrinolysis inhibitor, and the second barrel contains thrombin, factor XIIIa, and $CaCl_2$ [18, 23, 29, 48]. Following a similar mechanism to that involved in the normal clotting cascade in vivo, when fibrinogen and thrombin are mixed, fibrinogen is converted to fibrin which self-assembles and is crosslinked via the factor XIIIa.

Christman et al. injected these fibrin hydrogels into the ischemic LV 1 week following induced MI in rats (reperfusion-MI model), and animals were sacrificed 5 weeks later. Echocardiograph and explant data showed that fibrin is capable of maintaining fractional shortening (FS) and preserving infarct scar thickness after the material was resorbed [18]. In later studies, using the same model, Christman et al. demonstrated the ability of fibrin to substantially decrease infarct size and increase arteriole density in the infarct area compared to control BSA injections [48]. These results imply that in addition to its bulking effects, fibrin may also elicit a bioactive response that influences LV remodeling. Significant increases in neovasculature formation (capillary density) following fibrin injection in rat models of MI were later confirmed by Huang et al. [23].

Natural materials that are relatively bioinert such as alginate have also been explored as injectable hydrogels to treat MI. Alginate is a linear seaweed-derived copolymer consisting of linked β-D-mannuronate (M) and α-L-guluronate (G) residues and can be crosslinked into hydrogels with the addition of divalent cations [49]. Unlike fibrin, alginate must be modified with adhesive peptides to facilitate cell binding. Both non-modified alginate and alginate modified with adhesive peptides such as Arg-Gly-Asp (RGD) or Tyr-Ile-Gly-Ser-Arg (YIGSR) have been explored as bulking agents [26, 31,

Table 1 Summary of injectable hydrogels and their assessment in animal models of MI

Hydrogel type	Animal/model	Inject time (post-MI)	Gelation mechanism	Injection volume	End point
Fibrin [18, 48]	Rat: LAD ligation/reperfusion	1 week	Peptide-self assembly	50 μL	6 weeks
Fibrin, collagen, matrigel [23]	Rat: LAD ligation/reperfusion	1 week	Peptide-self assembly/ thermal	50 μL	6 weeks
Fibrin and alginate [34]	Rat: LAD ligation/reperfusion	5 weeks	Peptide-self assembly/ionic		10 weeks
Alginate [51]	Rat: LAD ligation/reperfusion	5 weeks	Ionic		10 weeks
Alginate [31]	Rat: ligation of proximal left coronary artery	1 week	Ionic	130 μL	9 weeks
Alginate [26]	Rat: ligation of proximal left coronary artery	1 week and 2 months	Ionic	100 to 150 μL	2 and 4 months
Alginate [50]	Swine: transient balloon occlusion of LAD	4 days	Ionic	1, 2, and 4 mL	2 months
Alginate/fibrin composite [29]	Swine: ligation of OM1 and OM2	1 week	Peptide-self assembly/ ionic	200×25 μL	4 weeks
Chitosan [28]	Rat: LAD ligation	1 week	Thermal	100 μL	5 weeks
Hyaluronic acid [33]	Rat: ligation of left circumflex arteries	2 weeks	Michael-type addition	50 μL	6 weeks
Hyaluronic acid [61]	Sheep: LAD ligation and second diagonal coronary artery	30 min	Redox initiation	300×20 μL	2 months
Collagen [19]	Rat: ligation of proximal left coronary artery	1 week	Thermal	100 μL	6 weeks
Matrigel [25]	Mouse: LAD ligation	Immediately	Thermal	50 μL	2 weeks
Matrigel [62]	Rat: LAD ligation/heterotopic transplant	Immediately	Thermal	125 μL	2 weeks
Dex-PCL-HEMA/PNIPAAm [32]	Rabbit: ligation of the proximal left coronary artery	4 days	Thermal	50×4 μL	34 days
poly(NIPAAm-*co*-AAc-*co*-HEMAPTMC) [22]	Rat: LAD ligation	2 weeks	Thermal	100×5 μL	10 weeks
αCD-MPEG-PCL-MPEG [64]	Rat: LAD ligation	5 min	Self-assembly	100×3 μL	1 month
αCD-MPEG-PCL-MPEG [24]	Rabbit: LAD ligation	1 week	Self-assembly	200 μL	5 weeks
PEG-VS [21]	Rat: LAD ligation	2 min	Redox initiation	100 μL (via 2 or 3)	13 weeks

34, 50, 51], and comparisons have been performed between the two [31, 51]. Yu et al. compared modified alginate to non-modified alginate using a rat reperfusion-MI model with injections 5 weeks post-MI, more indicative of a chronic response [51]. Five weeks after hydrogel injections, both alginate groups improved fractional shortening (FS), reduced LV dimensions, and significantly increased myocardial wall thickness compared to control BSA injections. Although both non-modified and modified groups also increased the number of arterioles in the infarct area, modified alginate resulted in higher densities, indicating the ability of adhesive peptide modifications to promote angiogenesis following MI. Tsur-Gang et al. also observed improved geometry and function following injection of non-modified alginate hydrogels; however, they observed conflicting data with modified alginate hydrogels [31]. Specifically, modified alginate showed a reduced benefit compared to non-modified alginate in terms of LV diastolic and systolic dimension (LVDD and LVSD), LV diastolic and systolic areas (LVDA and LVSA), FS, and fractional area change; although, no significant differences in relative scar thickness or blood vessel densities were observed.

Utilizing a large animal swine model, Mukherjee et al. injected composite hydrogels containing both fibrin and alginate to prevent geometric LV remodeling [29]. One week post-MI, 200 μL injections (25 total) were applied to the infarct area via a double-barreled injection device; one component was comprised of fibrinogen, fibronectin, factor XIII, plasminogen, and gelatin-grafted alginate dissolved in an aprotinin solution, while the second consisted of thrombin and 40 mM $CaCl_2$. Therapeutic outcomes included increased posterior wall thickness 1 week post-injection and a reduction in infarct expansion 21 and 28 days post-MI; however, no functional improvements were observed. Other interesting findings included a significant reduction of soluble collagen in the treatment groups, suggesting that collagen was less vulnerable to protease degradation. This observation was supported by a significant decrease in protease levels (e.g., MMP-2) in the composite hydrogel injection group, which could favor infarct stiffening and, thus, attenuate maladaptive remodeling in the future.

Chitosan is a linear polysaccharide that is biocompatible and biodegradable and therefore has been used in a wide

variety of tissue engineering applications [52]. Chitosan hydrogels can be formed upon mixing commercially produced chitosan with a glycerol phosphate and glyoxal solution. These gels exhibit a thermoresponsive gelation that is tuned to occur at 37°C by changing the glyoxal concentration, while hydrogel degradation is controlled by the degree of deacetylation [53, 54]. In a rat infarct model, a thermally responsive chitosan was injected 1 week post-MI [28]. Four weeks after hydrogel injection, the myocardium thickness was significantly increased compared to PBS controls, even though the amount of chitosan present in the myocardium after 4 weeks had substantially decreased due to hydrogel degradation. There were also significant improvements in infarct size, FS, ejection fraction (EF), end systolic diameter (ESD), end diastolic diameter (EDD), and microvessel density. Although the material was not completely degraded at the end of this study, like fibrin, this is an example of a degradable material that was effective in not only preserving thickness but also function.

Hyaluronic acid (HA) is a polysaccharide that is abundant in the body and plays a role in several biological processes that include angiogenesis, cell migration, and scar reduction depending on its molecular weight, and the addition of functional groups allows for tunability in material properties [55–60]. In one example, acrylated HA was mixed with a thiol-terminated PEG crosslinker (PEG-SH_4) and crosslinked via Michael-type addition; the mixture was injected into a rat MI model 2 weeks post-MI [33]. Four weeks after treatment, heart function was evaluated; HA treatment led to significantly decreased infarct size, increased EF, and increased arteriole and capillary density. Interestingly, results showed significant increases in infarct thickness, while histology showed complete degradation of the HA gels. Improvements with hydrogel injection were attributed to the biological role of HA, which like fibrin has proven to play a large role in wound healing processes. Additional work with engineered HA hydrogels [61] will be discussed in a section below on modulating hydrogel properties.

Collagen is a natural ECM protein that has been applied for LV remodeling therapies due to the ability to inject as a liquid, which subsequently gels at 37°C [19, 23]. Collagen injections in 1-week-old rat infarcts substantially increased infarct thickness, stroke volume (SV), and EF compared to saline injection controls; there was also a trend for improved end systolic volume (ESV) [19]. While this study did not show any evidence of angiogenesis or cell infiltration, Huang et al. using a reperfused model were able to demonstrate both increased angiogenesis and myofibroblast infiltration in the infarct zone compared to controls [23]. Contradicting results may be attributed to variables in methodology (Table 1) or differences in collagen types and concentrations used. Dai et al. used a mixture of collagen I (95%) and collagen III (5%) at 65 mg/mL, while Huang et al. used collagen I at 1 mg/mL. As an alternative to hydrogels composed solely of isolated collagen, Matrigel is a commercially available hydrogel derived from the ECM that is primarily composed of collagen, but also contains numerous other molecules derived from the basement membrane. Studies with Matrigel alone in a mouse model showed trends towards increased scar thickening and improved function compared to infarct controls [25]; thickening and improved EDD were also observed in a rat model [62]. In addition, studies by Huang et al. showed significantly increased capillary density with Matrigel injection [23].

ECM components isolated from healthy myocardium have been recently explored to treat MI. Singelyn et al. decellularized and solubilized the ECM from pig hearts for use as an injectable scaffold [30]. The isolated ECM material maintained a complex composition including collagen and glyocosaminoglycan content and exhibited a natural thermoresponsive behavior as it self-assembled into a nanofibrous gel at 37°C from a liquid precursor at 25°C. Interestingly, the cocktail of isolated ECM components stimulated the migration of human coronary artery endothelial cells (HCAEC) and rat aortic smooth muscle cells (RASMC) in vitro. For in vivo application, 90 μL hydrogels were successfully pushed through a catheter into the non-infarcted myocardium of rats where they induced a significant increase in arteriole formation 11 days post-injection. In later work, Seif-Narahi et al. isolated both porcine and human pericardial ECM (PPM and HPM, respectively) to evaluate their potential as autologous scaffolds for treating MI [63]. Similarly, these gels polymerized under thermal stimulation and maintained native components of the pericardial ECM. While in vitro results demonstrated that PPM was more effective in promoting migration of HCAEC, RASMC, and rat epicardial cells (RECs) compared to HPM, in vivo data 2 weeks post-injection (90 μL) indicated that both PPM and HPM similarly promoted neovascularization (76±13 and 51±42 arterioles/mm^2, respectively). Interestingly, stem cell evaluation revealed that although very slight, c-kit+ cells were present within the injection regions, eluding to a role in endogenous homing of these materials. These studies demonstrate that providing cardiac-specific cues to the injured myocardium via decellularized ECM injectable hydrogels provides a useful strategy to promote cardiac-specific tissue formation.

Synthetic Hydrogels

Natural materials may provide numerous important cellular-interactive cues (e.g., adhesion and cell-mediated degradation), but are generally limited in the extent that their properties can be adjusted (i.e., mechanics, degradation,

and gelation). In contrast, synthetic materials provide additional potential in engineering a variety of gelation mechanisms and physical properties. One synthetic thermosensitive polymer, comprised of dextran (Dex) grafted poly (caprolactone)-2-hydroxyethyl methacrylate (PCL-HEMA) and copolymerized with poly(*N*-isopropylacrylamide) (PNIPAAm) termed Dex-PCL-HEMA/PNIPAAm, was developed to gel in situ. Material injections were performed 4 days post-MI in a rabbit model and resulted in significant reductions in infarct scar and improvement in EF and LV end diastolic and systolic diameter (LVEDD and LVESD) compared to PBS control injections when assessed 30 days after injection [32]. Significant thickening was observed despite no histological evidence of material remaining.

Similarly, Fujimoto et al. synthesized a biodegradable, temperature-responsive hydrogel composed of N-PNIPAAm, acrylic acid, and hydroxyethyl methacrylate-poly(trimethylene carbonate) (poly(NIPAAm-*co*-AAc-*co*-HEMAPTMC)) with slower degradation than the previously discussed polymer (Fig. 1) [22]. Like Dex-PCL-HEMA/PNIPAAm, poly(NIPAAm-*co*-AAc-*co*-HEMAPTMC) was engineered to undergo gelation at body temperature. In this particular study, a hydrogel with a maximum tensile strength of 6.1 kPa and complete hydrogel degradation after 5 months was evaluated for its efficacy in preventing LV remodeling in a rat MI model. The polymer was injected 2 weeks post-MI; after 8 weeks, the myocardium thickness, EDA, and fractional area change were significantly improved compared with PBS injection controls.

Another synthetic material consisting of α-cyclodextrin (α-CD) and poly(ethylene glycol) (MPEG-PCL-MPEG) triblock copolymer that has the ability to gel in situ has also demonstrated therapeutic benefits when injected to target LV remodeling [24, 32]. Degradation can be controlled by the PCL block, and hydrogels were formed upon mixing the linear MPEG-PCL-MPEG polymer with α-CD. When injected 5 min post-MI in a rat model, the hydrogel-treated groups showed a significant reduction in infarct size, LVEDD, LVESD, and an increase in FS compared to PBS injection controls. No increase in neovascularization was observed [32]. In 1-week-old infarcts in a rabbit model, significant improvements in thickness, infarct size, LVEDD, LVESD, and EF were observed with no increase in microvessel density [24].

The aforementioned synthetic materials have all been degradable. In contrast, a non-degradable vinyl sulfone derivatized PEG (PEG-VS) has also been investigated to treat MI [21]. PEG-based hydrogels are bioinert and can be tailored to have high mechanical properties. PEG-VS was polymerized upon combination with dithiothreitol (DTT) and injected into a rat MI model 2 min post-MI. PEG-VS significantly increased the wall thickness at 4 weeks and, although no longer significant, was still thicker than saline

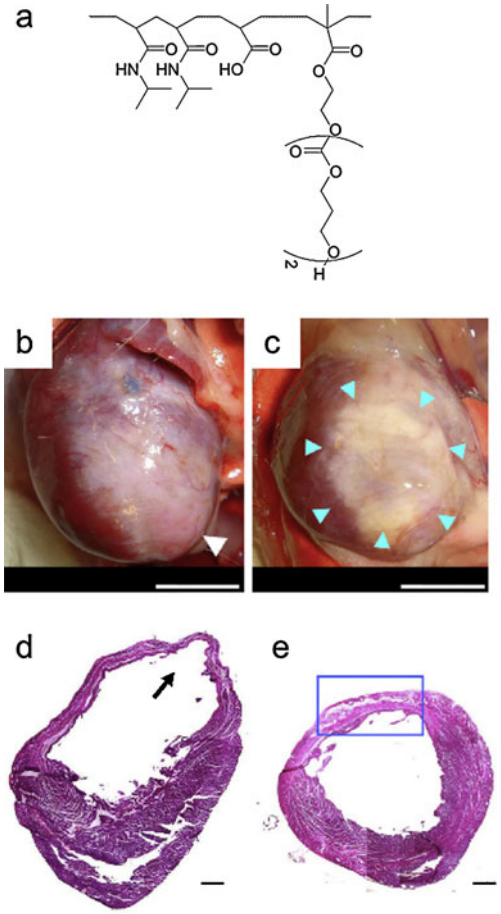

Fig. 1 In vivo application of injectable synthetic polymers. Chemical structure of poly(NIPAAm-*co*-AAc-*co*-HEMAPTMC) (**a**), representative images of PBS (**b**) and gel (**c**) injected hearts at 8 weeks, and hematoxylin and eosin stained section of PBS control (**d**) and gel treatment (**e**) hearts at 8 weeks. *Scale bar* 5 mm in **b**, **c**, 500 μm in **d**, **e**. Figure adapted from [22]

controls at 13 weeks. Despite PEG-VS thickening the myocardial wall, echocardiograph analysis showed significant improvements in EDD only 4 weeks after MI; this was not maintained at 13 weeks. FS was also not improved in treatment groups. Here, in this small animal model, it was observed that prolonged material presence (or stabilization) is not sufficient to attenuate LV remodeling.

Limitations of Experimental Assessment of Bulking Agents

From the reviewed results above, it is clear that bulking agents may attenuate remodeling post-MI; however, the mechanism involved in their success still remains to be elucidated. If anything, these results reveal the complexity in the material interaction with the myocardial tissue, including both the biological (e.g., material remodeling and inflammatory response) and mechanical (e.g., stress reduction) responses. Variable results from these studies indicate that material thickness [21, 26, 29, 34], infarct size

[34], and/or increased angiogenesis [19, 24, 64] do not necessarily correlate directly with improved heart function. However, discrepancies in results could be due to inconsistencies in methodology (Table 1; e.g., animal models, infarct, amount of material injected, and timing of injection), and material properties and the importance of these parameters should be considered when investigating LV remodeling. Specifically, several animal models have been used to study the efficacy of bulking agents. The most popular model is by far the rat model, due to costs and ease of implementation; however, this model has several limitations. The most obvious is the lack of clinical relevance associated with a small animal model, as well as infarct consistency. In the clinical setting, factors such as the LV volume and structure, material injection volume, and method for injection will be very different than in the rat.

Material injection in these studies has been performed as early as immediately post-MI [21, 25] and as late at 8 weeks post-MI [26]. In separate studies with fibrin, injection at 1 week was more effective in improving myocardium function [18] versus injection 5 weeks post-MI [34]. Utilizing a permanent ligation rat model, Landa et al. directly compared injections into new (1 week post-MI) and old (2 months post-MI) infarcts and demonstrated the efficacy of alginate in both (although to different extents) [26]. Recent infarcts resulted in improvements in wall thickening and LV dilation; while older infarcts also showed improvements, results were more pronounced in new infarcts, implying that post-MI therapies are more effective when applied early in the LV remodeling process, before irreversible processes have occurred. Similar to injection time, data collection time points are also important to consider. The longest study evaluated in this review was 3 months post-treatment [21] and 4 months post-MI [26].

Material Optimization: Theoretical Evaluation

Theoretical models have implied that material properties, specifically mechanics and volume, are important to consider when selecting the type of bulking agent to ameliorate dilation and increased stress in the myocardial wall [35, 65–67]. Using a finite element (FE) model to simulate the effects of injecting a non-contractile material into the myocardium, Wall et al. showed that bulking the myocardium was sufficient to attenuate post-MI geometric changes and, thus, decrease stress in the myocardial wall [35]. More specifically, they demonstrated that injections of 4.5% of the LV wall volume and 20% of the stiffness of the natural myocardium into the BZ were able to decrease the fiber stress by 20% compared to control simulations with no injections. Other approaches have validated the importance of infarct compliance using FE, as well as lump-parameter models; results reveal similar overall beneficial outcomes [35, 65, 66]. FE models have also been employed to evaluate the effects of material volume and distribution in the myocardium and showed that they influence the extent of remodeling, depending on their pattern of injection (Fig. 2) [35, 67]. After testing several injection patterns modeled with an objective function that was weighted to minimize mean end-diastolic and end -systolic myofiber stress and LV stroke volumes, it was determined that the maximum number of injections leads to the highest reduction in fiber stress [67]. These simulations provide insight to the relevance of bulking material properties, specifically mechanics and volume distribution, and present more evidence to pursue injectable material therapies to control LV remodeling.

Material Optimization: Comparing Different Materials

Only two studies have directly compared different materials and their efficacy in preventing LV remodeling [23, 34]. Utilizing an infarct-reperfusion rat model, Yu et al. compared non-modified alginate to fibrin with injections applied 5 weeks post-MI [34]. Two days after injection, both materials were similarly effective in improving FS, LV dimensions, and wall thickness. However, 5 weeks post-injection, alginate demonstrated greater improvements than fibrin in FS, LV dimensions, and wall thickness. This could be attributed to the extended presence of alginate in the myocardium while fibrin was no longer detectable; however, fibrin treatment did result in thicker myocardial walls when compared to BSA control injections. Interestingly,

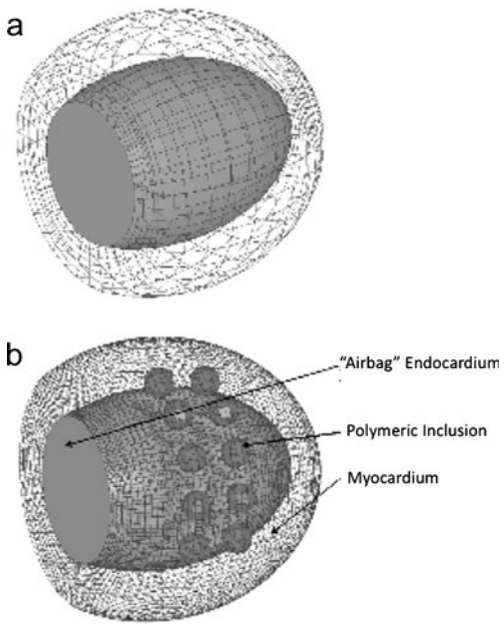

Fig. 2 Finite element (FE) model of injected polymeric materials. Reference FE model for infarcted canine heart (**a**) and modified FE model with polymeric inclusion injection pattern into dilated LV (**b**) can be used to assess the effects of material volume and distribution on myofiber stress. Figure adapted from [67]

while unable to promote cell adhesion, alginate, like fibrin, resulted in significantly higher arteriole density. Despite alginate's superiority in long-term functional outcomes, fibrin treatment groups had significantly smaller infarcts compared to controls. This comparison highlights potential differences in the efficacy of material stabilization in preventing LV remodeling, due to differences in material degradation and biological activity. In another study, Huang et al. compared the extent of angiogenesis between fibrin, collagen, and Matrigel post-MI and determined that while all three polymers significantly increased capillary density, only collagen significantly increased the degree of myofibroblast infiltration [23].

Material Optimization: Comparing Properties and Introducing Biological Cues

Few studies have experimentally evaluated the role of material properties (e.g., volume and mechanics) on LV remodeling, and in most cases, these properties are not even reported. A review of injectable materials illustrates that a wide range of volumes have been injected as bulking agents (Table 1). As a single example of differences in injected volumes, Leor et al. injected an alginate-calcium solution into the LAD 4 days post-MI via a coronary catheter in swine [50]. During this time post-MI, the vasculature of the infarcted myocardium is leaky, allowing the alginate mixture to be delivered to the infarct site. Due to inadequate levels of calcium in the vasculature, the alginate solution does not crosslink until released into the myocardium where levels of calcium suffice to stimulate gelation. Various volumes of 1, 2, and 4 mL were injected; 2- and 4-mL injections led to superior LVDA and LVSA with 2-mL injections resulting in significant thickening (despite material degradation) and more pronounced trends for functional improvements. These findings illustrate the importance of injection volume in stabilizing the myocardial wall [67]. Other parameters, such as number and pattern of injections, although relevant through theoretical analysis [67], have yet to be investigated in a clinically relevant model.

The influence of material properties may also be an important parameter to control, yet few studies have investigated this, particularly in a controlled manner. This may be due to limitations in material systems where various important properties cannot be decoupled. For example, fibrin properties can be varied by adjusting the concentration of fibrinogen and thrombin [68], and Martens et al. adjusted these parameters to optimize fibrin viscosity and gelation for catheter delivery [69]. Similarly, alginate properties can be adjusted by varying the weight percent and the ratio of M and G units [70]. However, in both of these systems, viscosity may be changed during injection and lead to differences in not only final mechanics but also material dispersion and biomaterial concentration. Thus, it is imperative to design systems that allow exploration of how specific properties influence outcomes while maintaining all other properties the same.

A recent study by Ifkovits et al. was the first to explore how the mechanical properties of injectable materials influence LV remodeling (Fig. 3) [61]. A highly modified HA polymer (methacrylated HA (MeHA)) with a high compressive modulus (43 kPa) was directly compared to lower modified MeHA with a low compressive modulus (7.7 kPa). This study demonstrated that although both materials similarly thickened, or bulked, the infarcted myocardial wall, high MeHA was able to also decrease infarct size and dilation as well as improve function under stress compared to the infarct control. This provides evidence that the mechanical properties of the injectable

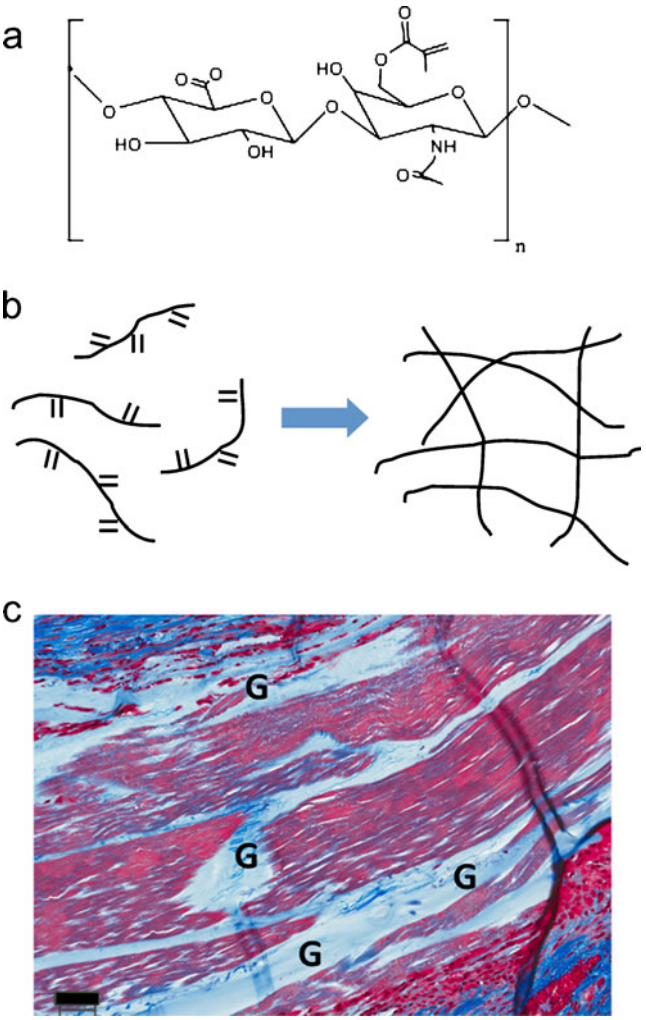

Fig. 3 Injectable hyaluronic acid hydrogel. Chemical structure of methacrylated hyaluronic acid (**a**), schematic of gel formation process where functional macromers react via a radical polymerization to form a gel (**b**), and a representative Masson's trichrome stain of the gel in myocardium 8 weeks post-MI (**c**). *G* gel; *scale bar* 50 μm. Figure adapted from [61]

material are important to consider for attenuating LV remodeling. Studies with tunable injectable materials will broaden the understanding of factors, such as mechanics and degradation that should be regarded to target LV remodeling via bulking agents.

Likewise, the biological activity of materials cannot be overlooked. A study by Ryan et al. injected a biocompatible dermal and soft tissue filler (Radiesse) composed of calcium hydroxyapatite microspheres suspended in an aqueous gel carrier of water, glycerin, and carboxymethylcellulose [71]. The uncrosslinked gel carrier allows endogenous cells to access the encapsulated microspheres to promote collagen synthesis. Radiesse was injected into the myocardium 45 min after ligation in an ovine MI model and analyzed at 4 weeks; injection resulted in a thickened myocardial wall, increased global EF, and reduced LV end systolic volumes compared to controls. Unlike the previously mentioned hydrogel systems that directly bulk the myocardium through hydrogel crosslinking, this approach provides an effective means to induce tissue bulking by promoting the biological response to materials for attenuated LV remodeling and preserved cardiac function [72–77].

Injectable Hydrogels for Molecule Delivery

In addition to mechanically supporting the injured myocardium as described above, injectable hydrogels provide a water-swollen matrix to encapsulate therapeutic molecules for targeted molecule delivery to the myocardium following MI. Exogenous delivery of therapeutic molecules including growth factors, cytokines, and DNA plasmids can be used to manipulate endogenous post-MI remodeling processes; however, the high rates of diffusion and short half-life during which these molecules retain their biological activity in vivo make successful application of these molecules to treat MI difficult [78]. To this end, injectable hydrogels provide a useful platform to sustain the release of therapeutic molecules in the setting of MI. Molecules are encapsulated in the hydrogel matrix and released locally over time to sustain target levels of the molecule in the myocardium while preventing detrimental systemic effects. The pioneering work of Dr. Robert Langer and Dr. Judah Folkman showed that polymer matrices can be used to sustain the release of encapsulated molecules for up to 100 days [79]. Molecule release from these hydrogels exhibited an initial “burst release” in which molecule release rates are rapid, followed by sustained released profiles with slower release rates (Fig. 4). This initial burst release was significantly decreased with higher polymer concentrations (i.e., higher polymer-to-water ratios), and the overall release kinetics were unique for each polymer–molecule combination. Polymer–molecule interactions, polymer hydrophobicity, and hydrogel degradation all influence the diffusion of encapsulated molecules in the hydrogel matrix and therefore determine encapsulated molecule release kinetics [80]. The following sections will review the range of combinations of therapeutic molecules and injectable hydrogel formulations that have been applied to treat MI.

Anti-apoptotic Molecules

In order to attenuate the loss of viable myocardium following MI, protective growth factors have been delivered locally from injectable hydrogels to the at-risk myocardium. Ruvinov et al. developed alginate hydrogel microparticles with sulfate group modifications to affinity-bind encapsulated insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF) for sustained molecule release in the setting of MI [81]. IGF-1 has been shown to be cytoprotective, and HGF has been shown to be pro-angiogenic and anti-fibrotic; therefore, this group hypothesized that delivery of both of these molecules following MI would have an additive effect on preserving the viability and structure of the myocardium. Modifying hydrogels with negatively charged groups is a common way to mimic the natural affinity of glycosaminoglycans (GAGs) for proteins in order to localize and sustain the release of exogenously delivered proteins. In this particular system, the modified alginate microparticles exhibited a dual release behavior with distinct release kinetics for IGF-1 and HGF (IGF-1 release was faster than HGF release), indicating a difference in the affinity of each molecule for the sulfate group modifications (Fig. 5). Importantly, the molecules released from the microparticles remained active (confirmed in vitro with cell culture assays) and were protected by protease degradation when bound to the sulfate modified alginate (as evidenced by mass spectroscopy). When injected 1 week after induced MI in rats, the alginate microparticles facilitated significant improvements in IGF-1/HGF-mediated repair compared to IGF-1/HGF injected in saline and alginate microparticles injected without growth factors. These improvements include reductions in fibrotic area (collagen staining), apoptotic cells (caspase-3 expression), and increases in vessel densities and areas (α-SMA expression), proliferating cardiomyocytes (Ki67 expression), and evidence of cardiogenesis (GATA4 expression) 4 weeks following injection.

In addition to GAG affinities, the natural affinity of streptavidin for biotin has been exploited to sustain the release of IGF-1 from injectable hydrogels [20]. Davis et al. biotinylated self-assembling (SA) oligopeptides and IGF-1 to form a streptavidin–biotin complex upon mixing the biotinylated oligopeptides and IGF-1 with tetravalent streptavidin. The oligopeptides were designed with alter-

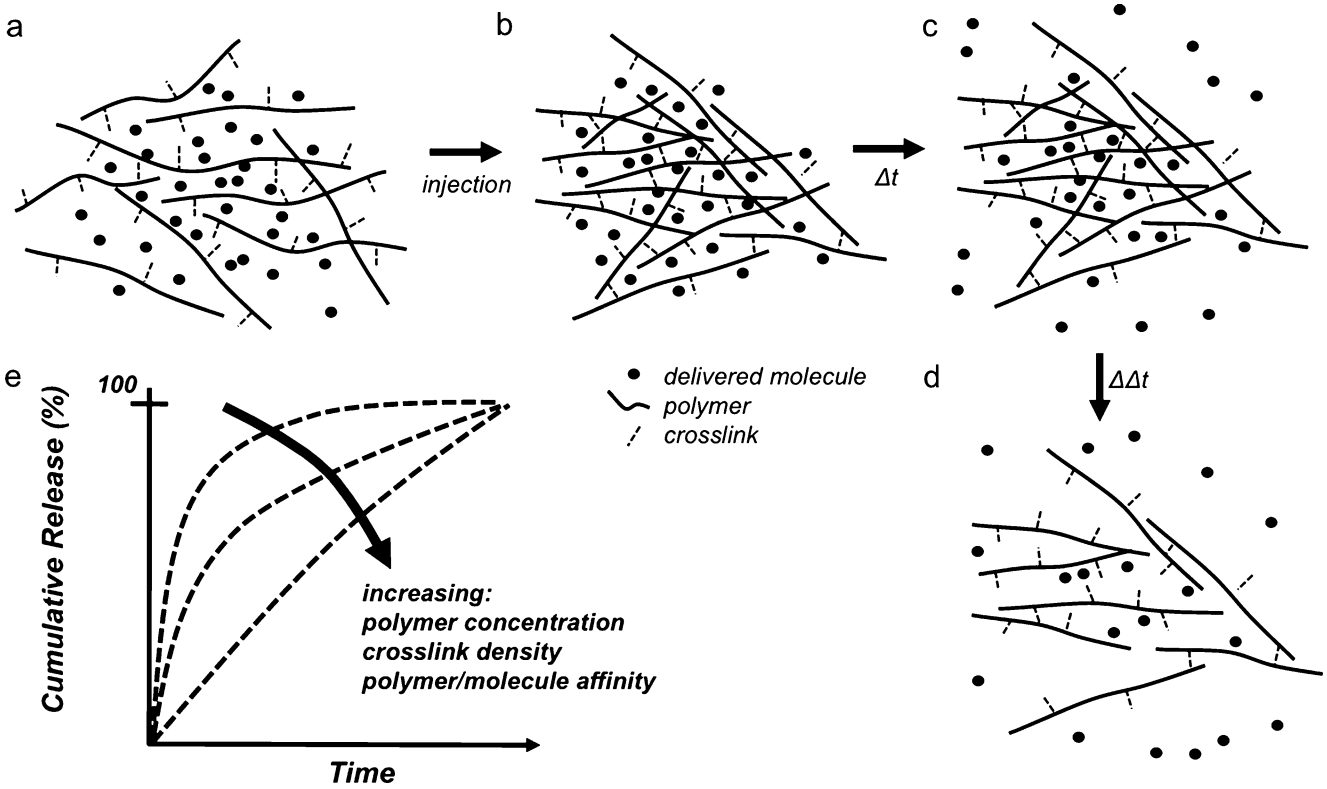

Fig. 4 Representative release behavior from injectable hydrogels. Molecules are mixed into a hydrogel precursor solution (**a**), encapsulated after hydrogel formation upon injection (**b**), and are released from the network through both diffusion (**c**) and degradation (**d**) mechanisms. Molecule release from these systems can be controlled through polymer concentration, crosslink density, and by incorporating affinity between the encapsulated molecule and hydrogel (**e**). Release profiles are unique for each polymer–molecule combination and are therefore often determined experimentally

nating hydrophilic and hydrophobic amino acids in order to "self-assemble" into nanofibrous hydrogels upon exposure to physiologic pH and osmolarity. IGF-1 release from the hydrogels was sustained for up to 84 days in vivo after injection into the myocardium of rats. Tethering IGF-1 to the peptide scaffold using the streptavidin–biotin strategy had significant effects on improving cardiomyocyte survival in vitro (cardiomyocyte [^{3}H]phenylalanine incorporation) and in vivo (caspase-3 cleavage of implanted neonatal cardiac myocytes) confirming the activity of released IGF-1. Combining the IGF-1/SA peptide hydrogels with neonatal cardiac myocytes significantly improved functional outcomes post-MI; however, the contribution of the IGF-1/SA peptide hydrogels alone was not investigated.

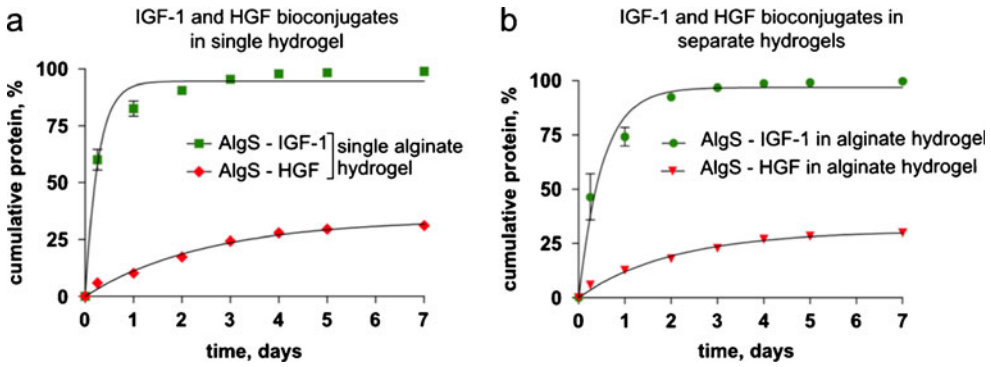

Fig. 5 Molecule release from affinity-binding alginate hydrogels. Release kinetics of IGF-1 and HGF from the same hydrogel (**a**) and from separate hydrogels (**b**). Cumulative protein released (percent)= (cumulative released protein up to each time point/total protein recovered from the hydrogel)×100. Residual protein entrapped in the hydrogels at the end of the study was recovered with sodium citrate treatment. Figure adapted from [81]

These self-assembling peptide hydrogels have also been explored to deliver platelet-derived growth factor (PDGF) to prevent cardiomyocyte apoptosis post-MI [82]. The amphiphilic nature of the oligopeptides provided sustained release of PDGF through weak molecular interactions between the oligopeptides and PDGF. PDGF release was sustained from hydrogels for 14 days following injection into the myocardium of rats. The released PDGF activated its receptor (PDGFR-β) in cardiomyocytes adjacent to the injection areas and attenuated cardiomyocyte death (caspase-3 cleavage) 14 days after induced MI and PDGF/SA peptide hydrogel injection. In addition, global LV geometry and LV function (FS) was preserved 14 days following MI with the PDGF/SA peptide hydrogels. A subsequent study confirmed the therapeutic benefit of these PDGF-releasing hydrogels [83]. LV geometry and function (hemodynamic parameters) and myocardial tissue structure (vascular density, regional blood flow, and infarct size) were significantly improved 4 months following induced MI in rats with injection of the PDGF/SA peptide hydrogels. Importantly, the locally delivered PDGF did not have adverse systemic effects (pulmonary hypertension) as confirmed by no observed change in pulmonary artery thickness.

Another group of proteins that can recover cell viability during periods of environmental stress, the heat shock family of proteins, has also been delivered in the setting of MI using injectable hydrogels. Lee et al. used calcium crosslinked alginate hydrogels as a delivery matrix for poly (lactic-*co*-glycolic acid) (PLGA) microspheres loaded with heat shock protein 27 (HSP27) fused to a transcriptional activator (TAT; to facilitate cell uptake of HSP27) [84]. HSP27-TAT was encapsulated in the PLGA microspheres using water/oil/water emulsions, a frequently used method for preparing microspheres containing proteins, and these loaded microspheres were embedded in the alginate hydrogel during calcium crosslinking upon injection. Loading HSP27-TAT in microspheres prevented the burst release that is typical of proteins encapsulated in hydrogels. Released HSP27-TAT remained active as evidenced by reduced cardiomyocyte apoptosis and restored cardiomyocyte proliferation under ischemic conditions in vitro, although this system was not tested in the setting of MI.

Angiogenic Factors

Another common strategy to preserve viable myocardium following MI is to restore blood flow to the ischemic myocardium by stimulating neovasculature formation in the infarct area with localized release of pro-angiogenic growth factors. Sustained release of fibroblast growth factor (FGF) from injectable hydrogels has been extensively explored to stimulate angiogenesis following MI [85–90]. FGF is a powerful angiogenic molecule, but has a very short half-life in vivo. Sakakibara et al. showed that radiolabeled FGF delivered to the myocardium via bolus venous injections, intracoronary injections, or intramyocardial injections is nearly undetectable 24 h after administration [88]. However, by using gelatin hydrogel microspheres as a carrier matrix, effective concentrations of radiolabeled FGF were sustained in the peri-infarct area of the myocardium 24 and 72 h after injection (all groups injected 4 weeks after induced MI in rats). To assess the therapeutic effects of sustaining FGF levels in the myocardium with this hydrogel system, FGF-loaded microspheres were injected 4 weeks after induced MI in swine. Upon evaluation at 4 weeks after injection, LV remodeling was significantly attenuated (echocardiography), and neovasculature was significantly increased (capillary density) compared to a control MI group. Other groups have also reported the efficacy of using gelatin microspheres to deliver FGF in the setting of MI [88, 89]. In addition to gelatin microspheres, injectable chitosan hydrogels have been used to deliver FGF [85]. Wang et al. encapsulated FGF in thermoresponsive chitosan hydrogels upon injection into the myocardium by mixing FGF with the chitosan solution. This system significantly improved heart function compared to injecting FGF alone in a rat model of chronic MI (hydrogels injected 1 week post-MI).

While the sustained delivery of pro-angiogenic factors to ischemic tissue has stimulated neovasculature formation, as evidenced by increases in capillary densities, the stability and connectivity of these newly formed vessels has been a concern [91]. In order to form more stable vessels with supporting smooth muscles cells, dual delivery of angiogenic growth factors using injectable hydrogels has been explored to treat MI. Hao et al. encapsulated vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) in alginate hydrogels by mixing a solution of purified alginate and growth factors with a calcium sulfate solution upon injection into the myocardium [92]. The rationale for choosing these two molecules was to first stimulate endothelial vessel formation with VEGF delivery, followed by smooth muscle cell recruitment with PDGF delivery to support the immature vessels. To this end, VEGF release preceded PDGF release from the alginate hydrogels, and release of both molecules was sustained for over 30 days in vitro as the hydrogel degraded. When applied 1 week following induced MI in rats, the dual growth factor releasing hydrogels significantly increased the number of α-SMA containing blood vessels around the injection site 4 weeks after injection compared to hydrogels loaded with each growth factor alone.

To further control VEGF delivery in the setting of MI, Wu et al. conjugated VEGF to *N*-hydroxysuccinimide

(NHS) terminated PVL-*b*-PEG-*b*-PVL block copolymers (NHS reacts with primary amines on the protein) [93]. The block copolymers were designed to form a hydrogel at 37° C for injectability and completely degraded after 42 days when implanted subcutaneously in rats. When injected into the myocardium 1 week following induced MI in rats, the VEGF-conjugated hydrogels showed significant improvements in heart function (FS, EF, LV EDV and ESV, dP/dt max, and dP/dt min) and structure (scar area and capillary density) 4 weeks after injection compared to VEGF alone and VEGF injected with the hydrogel but not conjugated (Fig. 6).

Finally, plasmid DNA encoding angiogenic molecules has been delivered with injectable hydrogels. Kwon et al. synthesized multiblock copolymers with acid-labile acetal linkages (for controlled degradation) that form hydrogels at 37°C for VEGF plasmid delivery following MI [94]. The injectable hydrogels significantly enhanced the efficacy of the VEGF plasmid over injecting the naked plasmid, as confirmed by VEGF expression in the infarct area 2 weeks after injection. This enhanced expression was correlated with increases in capillary density and a more preserved myocardium in the infarct area. To further enhance angiogenesis, injectable hydrogels with adhesive sites can be used as a carrier of angiogenic plasmids to provide a matrix that facilitates cell migration. The combination of plasmid and adhesive matrix (termed gene-activated matrix) enhances gene transfection by localizing the plasmid until endogenous cells migrate into the implanted scaffold and become transfected to express the encoded protein [95–97]. For example, Christman et al. used an injectable fibrin glue that forms upon mixing fibrinogen and thrombin to localize a plasmid encoding the angiogenic molecule pleiotrophin (PTN) in the infarct myocardium [95]. Injecting the PTN plasmid in fibrin glue 1 week following induced MI in rats increased the formation of arterioles (α-SMA staining) that were functionally connected to existent coronary vasculature (confirmed with fluorescent microbead perfusion) 5 weeks after injection.

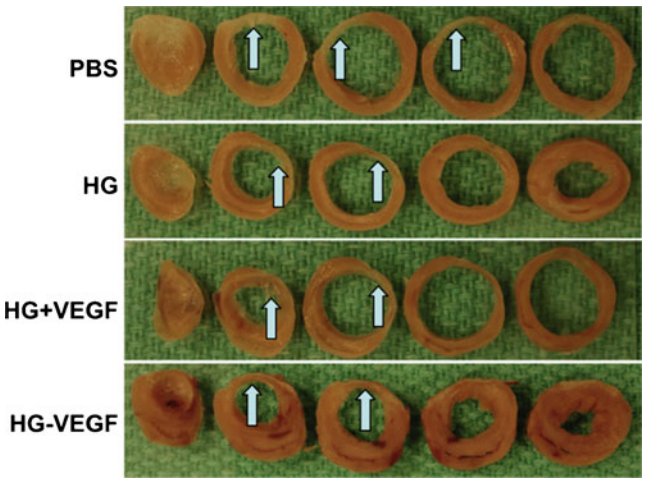

Fig. 6 Tissue response to delivered growth factors. Representative heart slices obtained at 35 days after post-MI injection of PBS, hydrogel (*HG*), hydrogel with soluble VEGF (*HG+VEGF*), or hydrogel with VEGF tethered through covalent linkage (*HG-VEGF*). *Arrows* indicate the location of the infarct in individual slices. Figure adapted from [93]

Chemoattractants

For control of endogenous cell recruitment, chemokines that selectively recruit stem/progenitor cells have been delivered from injectable hydrogels. For example, stromal derived factor-1 alpha (SDF-1α) has been locally delivered in the setting of MI using the self-assembling peptide hydrogels described in the previous section [98]. Release of SDF-1α was controlled by tethering the protein to the oligopeptide scaffold through a peptide sequence that is cleaved by endogenous proteases. The amino acid sequence of SDF-1α was engineered to resist protease degradation to ensure that active SDF-1α was released from the scaffold in the presence of proteases. Released SDF-1α was detectable for up to 7 days around the injection site in vivo and led to significant increases in cells expressing the receptor for SDF-1α in the MI region, which co-expressed markers of stem/progenitor cells and endothelial cells. In addition, capillary (isolectin) and arteriole (α-SMA) densities increased significantly 4 weeks after injecting the SDF-1α-containing hydrogels compared to MI controls in rats. These tissue-level observations were correlated with significant improvements in LV function.

Summary

Although only in its infancy, it is clear that the field of injectable hydrogels to treat cardiac tissue post-MI has high potential as a translatable therapy. The lack of a cell source needed for transplantation will only accelerate development of percutaneous delivered hydrogels, whether they act as tissue bulking agents or for the controlled delivery of therapeutic molecules. A wide range of both natural and synthetic materials have been investigated, and each has unique properties, including mechanics, degradation, and cellular interactions. Future work should further investigate the various mechanisms in which these materials act, both biologically and mechanically, and focus on clinically relevant parameters, such as the animal model and mode of delivery. This information will lead to a clear understanding and illustration of the efficacy of injectable

hydrogels to treat patients that have experienced an MI and to prevent the LV remodeling events that can lead to heart failure.

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