Antifibrotic therapies to control cardiac fibrosis

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Abstract
Cardiac fibrosis occurs naturally after myocardial infarction. While the initially formed fibrotic tissue prevents the infarcted heart tissue from rupture, the progression of cardiac fibrosis continuously expands the size of fibrotic tissue and causes cardiac function decrease. Cardiac fibrosis eventually evolves the infarcted hearts into heart failure. Inhibiting cardiac fibrosis from progressing is critical to prevent heart failure. However, there is no efficient therapeutic approach currently available. Myofibroblasts are primarily responsible for cardiac fibrosis. They are formed by cardiac fibroblast differentiation, fibrocyte differentiation, epithelial to mesenchymal transdifferentiation, and endothelial to mesenchymal transition, driven by cytokines such as transforming growth factor beta (TGF-β), angiotensin II and platelet-derived growth factor (PDGF). The approaches that inhibit myofibroblast formation have been demonstrated to prevent cardiac fibrosis, including systemic delivery of antifibrotic drugs, localized delivery of biomaterials, localized delivery of biomaterials and antifibrotic drugs, and localized delivery of cells using biomaterials. This review addresses current progresses in cardiac fibrosis therapies.

Keywords: Myocardial infarction, Cardiac fibrosis, Myofibroblasts, Cardiac fibroblasts, Antifibrotic therapy

Background
Myocardial infarction (MI) is the leading cause of death in the western countries. Cardiac fibrosis naturally occurs following MI. It is characterized by the excessive deposition of extracellular matrix (ECM) typically collagen in the infarcted area. Cardiac fibrosis increases stiffness and decreases compliance of the infarcted heart tissue. This negatively affects both contraction and relaxation behavior of the heart, resulting in a decrease in cardiac function. While the fibrotic tissue that forms initially may protect the heart from rupture, it gradually expands to the non-infarcted area when the MI evolves from early to late stages. The continuous increase of cardiac fibrosis leads to a progressive decrease in heart tissue contractility [1–5], and finally causes heart failure [6–8]. Cardiac fibrosis occurs not only after MI, but also from congenital defects, dilated cardiomyopathy and hypertension [9].

A therapy that can inhibit cardiac fibrosis from progressing in the infarcted hearts will preserve cardiac function and prevent heart failure. However, there is currently no efficient therapies available. Myofibroblasts are widely accepted to be responsible for cardiac fibrosis. They secrete excessive ECM directly leads to the formation of scar tissue. They also express highly contractile protein α-smooth muscle actin (αSMA) that remodels the surrounding ECM [10]. Understanding the origin of myofibroblasts may help to develop approaches to control tissue fibrosis.

Sources of myofibroblasts in infarcted hearts
Cardiac fibroblasts differentiation into myofibroblasts
Cardiac fibroblasts have greater quantities than cardiomyocytes in the heart tissue [11]. They are quiescent in healthy heart tissue, and are responsible for ECM secretion to keep the integrity of the interstitial matrix. They can also transduce survival signals and therefore control the conduction of electrical and mechanical stimuli to help maintaining the systolic and diastolic function in the heart tissue. Cardiac fibroblasts quickly respond to the changes to the surrounding microenvironment. After MI, the death of cardiomyocytes activates immune response that induces cytokine and chemokine expression. This initiates the infiltration of neutrophils and mononuclear cells to the infarcted area. The neutrophils are

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then phagocytosed by macrophages after apoptosis. The macrophages are able to secrete profibrotic cytokines like transforming growth factor beta (TGF-β), Angiotensin II, and platelet-derived growth factor (PDGF) [12]. TGF-β binds to TGF-β receptors type I and II, and activates the TGF-β/Smad pathway to differentiate the cardiac fibroblasts into myofibroblasts (Fig. 1) [10, 12]. TGF-β has been demonstrated as a major mediator of myofibroblast formation after MI. The formed myofibroblasts then produce excessive ECM to initiate the cardiac fibrosis. The myofibroblasts also secrete cytokines such as TGF-β, Angiotensin II, PDGF, tumor necrosis factor alpha (TNFα), and interleukin 1 beta (IL-1β) to further enhance the differentiation of cardiac fibroblasts into myofibroblasts. Angiotensin-II and PDGF indirectly promote myofibroblast differentiation by increasing TGF-β secretion [10].

Following myofibroblast differentiation, its number increases over a period of a few months in the infarcted area. More ECM is thus generated and deposited, leading to the increase of scar size. In the scar, the content of collagen type III-rich fibers increases in a few weeks. The fibers are then gradually replaced by stiffer type I collagen. The scar tissue matures when the collagen fibers are crosslinked. Unlike other scar tissues, myofibroblasts exist in the cardiac scar for many years, and continue generating ECM [13].

Fibrocytes differentiation into myofibroblasts

Fibrocytes are a type of fibroblast-like peripheral cells [14]. These cells express fibroblast specific proteins, cluster of differentiation 31 and 45 (CD34 and CD45). In response to chemokines such as chemokine (C-C motif) ligand 21 (CCL21) and chemokine (C-X-C motif) ligand 12 (CXCL12), fibrocytes migrate towards the injured area [15]. Under the stimulation of TGF-β or endothelin-1, fibrocytes differentiate into myofibroblast-type cells with expression of α-SMA, production of fibronectin and collagen, and loss of expression of CD34 and CD45 [16]. Besides TGF-β and endothelin-1, cytokines including IL-13, IL-14 and PDGF also promote the fibrocytes to differentiate into myofibroblasts [15, 17].

Epithelial to mesenchymal transdifferentiation

Epithelial to mesenchymal transdifferentiation (EMT) is another origin of myofibroblasts, which is a process of transdifferentiation from epithelial cells into myofibroblast-like cells. In the EMT process, the expression of mesenchymal marker is up-regulated while the expression of epithelial marker is down-regulated [18, 19]. TGF-β1 plays a key role in this process. While the roles of other cytokines in EMT are still in debate, strong evidences suggest that TNFα and IL-1β are capable of accentuating the effect of TGF-β1 in driving EMT [20, 21].

Endothelial to mesenchymal transition

Endothelial to mesenchymal transition (EnMT) is currently thought to be a potential origin of myofibroblasts. EnMT was first proposed to be a phenomenon related to embryonic development until evidence indicated that up to 35% of fibroblasts in fibrotic heart muscle are converted from endothelial cells [22]. Similar to EMT, EnMT can be driven by TGF-β (Types 1 and 2) and be augmented by TNFα and IL-1β [22–24].

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**Fig. 1** TGF-β signaling in fibroblasts. Latent TGF-β binds to its type I and II receptors, and activates the canonical Smad3/4 pathway and the noncanonical TGF-β–activated kinase-1 (TAK1)/p38/c-Jun N-terminal kinase (JNK) and NADPH oxidase 4 (NOX4)/reactive oxygen species (ROS) pathway resulting in induction of fibrogenic genes, such as α-smooth muscle actin (α-SMA) and collagen. (Reprinted from Leask A. [10])
Therapies for cardiac fibrosis

After MI, myofibroblasts differentiated from cardiac fibroblasts are mainly responsible for cardiac fibrosis. Therefore, control of cardiac fibroblast differentiation into myofibroblasts is critical to attenuate cardiac fibrosis. As discussed above, growth factors and cytokines like TGF-β, Angiotensin-II, and PDGF directly and indirectly involve in myofibroblast differentiation. Current therapies are thus focused on reducing the secretion of these growth factors and cytokines, and decreasing the amount of active growth factors and cytokines. These therapies include systemic delivery of antifibrotic agents, localized delivery of biomaterials, localized delivery of biomaterials and antifibrotic agents, and localized delivery of biomaterials and stem cells.

Systemic delivery of antifibrotic agents

Antifibrotic agents that decrease the activity or level of growth factors and cytokines such as TGF-β, Angiotensin-II, PDGF, TNF-α and IL-1β can decrease myofibroblast activation, thus decreasing cardiac fibrosis. Since TGF-β is considered as a major mediator for cardiac fibroblasts to differentiate into myofibroblasts, inhibiting TGF-β from attacking cardiac fibroblasts and decreasing the amount of active TGF-β in the infarcted hearts may decrease the number of myofibroblasts. TGF-β receptor type I (ALK5) inhibitors like GW 788388 have been developed to decrease TGF-β activity [25]. Anti-TGF-β antibodies decrease the amount of active TGF-β [10]. In addition, angiotensin-converting enzyme (ACE) inhibitors are associated with reducing TGF-β level [26, 27].

In cardiac fibroblasts, Angiotensin-II induces expression of TGF-β1 through angiotensin type-I receptor [28]. It also induces expression of collagen through TGF-β/Smad pathway and extracellular signal-regulated kinase by IL-6 dependent mechanism [29, 30]. Angiotensin receptor inhibitors like losartan have been shown to reduce cardiac fibrosis in animal and human trials [31, 32]. Inflammation plays a role in the formation and progression of cardiac fibrosis. The cytokines released from macrophages and T cells, such as IL-1β and TNF-α, can promote the proliferation of cardiac fibroblasts and upregulate the expression of tissue inhibitor of matrix metalloproteinase (TIMP)-1, leading to cardiac fibrosis [33]. Use of drugs to suppress inflammation in the heart has shown benefits in reducing cardiac fibrosis. For example the administration of the selective p38 MAPK inhibitor blocked the secretion of TNFα and decreased cardiac fibrosis [34]. The drugs inhibiting cardiac fibroblast growth can also be used to inhibit cardiac fibrosis, such as β-blockers, relaxin, and statins [35–37].

The above drugs for control of cardiac fibrosis are generally administered by a systemic delivery approach through either oral intake or injection. The major advantage of this approach is that it is convenient. Yet the drug dosage allocated to the infarcted heart may be low, which decreases the therapeutic efficacy. Increase of the initial drug dosage may cause toxic effect. In addition, the drugs allocated to other tissues may have side effects on these tissues and cells inside. Localized delivery of antifibrotic drugs has the potential to address those disadvantages.

Localized delivery of biomaterials

Biomaterials can be used to control cardiac fibrosis. These biomaterials include naturally-derived matrices such as collagen [38] and alginate [39], and synthetic biomaterials such as poly(N-isopropyl acrylamide)-based hydrogels [40]. Besides antifibrotic properties, these biomaterials provide mechanical support to the infarcted tissue and decrease elevated wall stress, resulting in improved cardiac function [41].

Decellularized cardiac ECM is a naturally-derived matrix that has shown promise for treating infarcted hearts after MI [42]. It provides cells with tissue specific biochemical cues important for cell migration and differentiation, and tissue regeneration [42]. The major composition of decellularized cardiac ECM includes collagen, elastin, fibronectin, and GAGs. While fibronectin has been shown to promote cardiac fibroblasts to differentiate into myofibroblasts to facilitate cardiac fibrosis [43], the growth factors retained in the matrix such as hepatocyte growth factor (HGF) may inhibit this differentiation [44]. It is also possible that decellularized ECM increases MMP-1 secretion in the infarcted hearts, thus decreasing collagen deposition [45]. Injection of the hydrogel based on decellularized porcine cardiac ECM into rat MI model significantly decreased fibrosis in infarcted area [46].

In the infarcted hearts, elevated wall stress resulting from left ventricle dilation represents a powerful stimulus for intracellular signaling transduced by mechanoreceptors [47, 48]. The increased wall stress leads to the activation of local tissue renin-angiotensin system, causing up-regulation of angiotensin II. The upregulated angiotensin II increases tissue inflammation, and TGF-β, IL-1β and TNF-α secretion [49–51]. These events lead to the enhanced formation of myofibroblasts. Therefore, use of biomaterials that can effectively decrease wall stress will decrease tissue inflammation, resulting in decreased cardiac fibrosis. These biomaterials are soft with modulus typically similar to or lower than that of the heart tissue. On the other hand, biomaterials implantation is always associated with foreign-body response and inflammation, which may compromise the effect from reduced wall stress. The choose of biomaterials that cause less inflammation is thus critical.
Hydrogel based on alginate and chitosan has been shown to decrease cardiac fibrosis [39]. The hydrogel was highly soft with mean storage modulus of $20 \pm 15$ Pa. Injection of this hydrogel into infarcted rat hearts significantly increased wall thickness, leading to the decrease of wall stress. As a result, the number of CD68+ macrophages was significantly decreased compared to phosphate-buffered saline (PBS) injection [39]. The reduced tissue inflammation largely decreased tissue fibrosis after 8 weeks. In addition, injection of alginate and chitosan enhanced tissue vascularization. The reduced cardiac fibrosis and enhanced vascularization significantly increased cardiac function. Similar results were found when injecting soft collagen and poly(N-isopropylacrylamide-co-2-hydroxyl methacrylate-co-methacrylate-poly lactide) hydrogels into infarcted hearts [38, 47].

When using hydrogels to decrease cardiac fibrosis, time of the injection affects therapeutic efficacy [38, 47]. This is because wall stress of the infarcted tissue varies when the MI evolves from early to late stages. In general, the wall stress gradually increases from the necrotic phase to the fibrotic phase [52]. Therefore, the same hydrogel may have different efficacy in reducing wall stress and inflammation. In addition, the inflammation at different stage of MI is different with the most severe inflammation observed at the beginning of the MI [47]. Yoshizumi et al. found that the degree of inflammation was significantly higher in hearts injected with the hydrogel immediately after MI than in those injected with hydrogel 3 days after MI [47]. As a result, the hearts injected with the hydrogel 3 days after MI showed the lowest cardiac fibrosis. When injecting the hydrogels 2 weeks after MI where the cardiac fibrosis was already formed, the hydrogels did not decrease cardiac fibrosis to the extent when they were injected 3 days after MI (Figs. 2 and 3). The above results suggest that timing of hydrogel injection should be considered in order to achieve optimal therapeutic effect.

Localized delivery of biomaterials and antifibrotic agents

Localized delivery of antifibrotic agents has the potential to address the low efficacy issue associated with the systemic delivery as the dosage in the infarcted area is higher. Yet the inherent disadvantage is that repeated

![Fig. 2](image-url)

**Fig. 2** Effect of time and hydrogel injection on the expression of TNF-α (a), IL-1β (b) and IL-6 (c) in infarcted left ventricle. * indicates significant differences between groups. Rats were divided into 3 injection treatment groups (immediately after MI (IM), 3 d after MI (3D) and 2 w after MI (2 W)) and 2 control groups (healthy and MI without treatments). (Reprinted from Yoshizumi et al. [47])
Fig. 3 Ventricular wall histology for rat hearts 10 w after MI. Rats were divided into 3 injection treatment groups (immediately after MI (IM), 3 d after MI (3D) and 2 w after MI (2 W)) and 2 control groups (healthy and MI without treatments). Representative Masson’s trichrome stained cross-sections: a Healthy control, b MI control, c, f, i IM group, d, g, j 3D group, e, h, k 2 W group. A-H scale bars = 1 mm. Orange arrows point to the hydrogel residues, black arrows point to foreign body giant cells. Wall thickness (l) and infarction size (m) were measured from the complete set of these images. * indicates significant differences between groups. (Reprinted from Yoshizumi et al. [47])
delivery by open surgery is impractical. Delivery of drugs using minimally invasive surgery can avoid this issue. However, repeated delivery increases cost for the therapy and thereby is not ideal. These disadvantages may be overcome by using drug delivery systems that continuously release drugs. In these systems, the drugs are encapsulated in injectable biomaterials such as hydrogels and microspheres. The drugs then gradually release from the biomaterials by diffusion and biomaterial degradation. In addition, the biomaterials can increase drug retention in the heart tissue.

Sustained delivery of drugs that decrease inflammation in the infarcted hearts after MI may decrease the inflammation cytokines-associated myofibroblast formation, thereby reducing cardiac fibrosis. Ibuprofen is a cyclooxygenase inhibitor with anti-inflammatory property. It directly inhibits leukocyte activation and accumulations, and decreases the production of leukocyte attractant and activator leukotriene B4 [53]. Vu et al. injected ibuprofen-containing hyaluronic acid hydrogel into infarcted pig hearts, and found that cardiac fibrosis was significantly decreased compared to the hydrogel only group [53]. Erythropoietin (EPO) shows a cardioprotective effect after acute MI [54]. It augments cell survival in the infarcted hearts by activation of prosurvival signals Stat3, Akt, and ERK. Stat3 has been found to be closely related to tissue inflammation. A deletion of Stat3 is susceptible to dramatic increase of inflammation-induced cardiac fibrosis [54]. The activation of stat3 is thus expected to decrease cardiac fibrosis. Kobayashi et al. encapsulated EPO in the gelatin hydrogel and implanted into infarcted hearts after acute MI [54]. The EPO was able to gradually release from the hydrogel for 14 days. The released EPO significantly decreased cardiac fibrosis and infarct size 14 days and 2 months after MI, leading to the increase in cardiac function.

Delivery of antifibrotic growth factors represents an effective approach to attenuate cardiac fibrosis. One of the strong candidates is HGF. It is a potent agonist for the tyrosine kinase surface receptor c-MET. HGF exerts its antifibrotic property in two ways: inhibition of collagen synthesis by suppression of TGFβ expression, and degradation of collagen by activation of matrix metalloproteinase-1 (MMP-1) [55]. In addition, HGF may attenuate inflammation in the tissue, thereby decreasing inflammation-associated myofibroblast formation [56]. Besides antifibrotic property, HGF has proangiogenic, anti-apoptotic and cardioprotective activity [44, 55, 57–60]. Therefore, HGF is an attractive growth factor for cardiac therapy. Taniyama et al. transfected HGF gene in the cardiomyopathic hamsters and found that cardiac fibrosis was significantly reduced and angiogenesis was enhanced [55]. The disadvantage of gene transfection approach lies in safety concern and inflammation associated with viral vectors. Direct delivery of HGF may be an approach but has low efficacy due to its short half-life in solution form. Sustained delivery of HGF using biomaterials addresses this issue. Nakano et al. developed a HGF delivery system using porous cross-linked gelatin patch [61]. HGF was able to release from the scaffold over 2 weeks. After 2 and 4 weeks of implantation on the epicardium surface of the infarcted rat hearts, the fibrotic area was significantly decreased from 17.5 % (control, without implantation) to 8.8 %. The decrease of cardiac fibrosis increased cardiac function as fractional shortening and end-systolic elastance were significantly greater in the HGF treatment group.

Clinical application of HGF especially recombinant human HGF for cardiac therapy is obstructed by the high cost, challenging to manufacture and short half-life [62]. To increase the translational potential of HGF, Sonnenberg et al. engineered a HGF biomimetic - HGF fragment. The HGF fragment can be produced at a high yield and show similar potency as HGF [63, 64]. In addition, it is more stable than HGF. To deliver HGF fragment into infarcted heart, it was encapsulated into a hydrogel based on decellularized porcine epicardium. The HGF fragment exhibited slow release kinetics, resulting from the binding of HGF fragment with GAGs in the hydrogel [46]. After delivery into infarcted hearts, the released HGF fragment significantly downregulated TGFβ expression and upregulated MMP-1 expression in cardiac cells. Four weeks after injection, collagen content in the infarcted area was significantly decreased compared to control, indicating that the cardiac fibrosis was attenuated.

Co-delivery of HGF and other growth factors can also inhibit cardiac fibrosis. For example, HGF and insulin-like growth factor-1 (IGF-1) were co-delivered into infarcted hearts using alginate hydrogel as a carrier [65, 66]. IGF-1 has cytoprotective effect. It increases cell survival in the infarcted hearts. The decreased tissue apoptosis reduces fibrotic tissue formation. The advantage of dual release is that IGF-1 and HGF can be sequentially released from the hydrogel. HGF released slower than IGF-1 due to a much higher molecular weight. The first released IGF-1 promoted cell survival while the latter released HGF decreased fibrotic tissue formation and stimulated angiogenesis. Both growth factors were able to release from the alginate for 7 days. The amount of released IGF-1 was much higher than that of HGF [66]. The released growth factors remained bioactive. After being delivered into infarcted rat hearts for 4 weeks, the dual release group significantly decreased fibrotic area compared to the alginate only group (Fig. 4). The released growth factors also augmented cardiac cell survival.
Delivery of HGF together with vascular endothelial growth factor (VEGF) can also decrease cardiac fibrosis [67]. Salimath et al. encapsulated HGF and VEGF into collagenase degradable PEG hydrogel [67]. When incubated in PBS, both growth factors exhibited nearly linear release from the hydrogel. In collagenase solution, complete release was achieved in 4 days. After delivering the release system into infarcted hearts for 3 weeks, the non-treatment animals had fibrotic area of 41.5% while those treated with VEGF and HGF had only 13.9%. The combined VEGF and HGF treatment showed significantly higher efficacy than individual growth factor treatment. These results suggested that VEGF played a role in reducing cardiac fibrosis. This may be the result of VEGF promoting angiogenesis in the infarcted area [68, 69]. The vascularized tissue has reduced fibrosis. It is also possible that VEGF and HGF synchronously recruited progenitor cells for myocardial regeneration [67].

Basic fibroblast growth factor (bFGF) is another growth factor that has been demonstrated to inhibit cardiac fibrosis. The mechanism is that bFGF attenuates cardiac fibroblasts to differentiate into myofibroblasts in the presence of TGFβ [70]. While it is not clear whether this effect is resulted only from blocking TGFβ/Smad signaling pathway, studies based on valvular interstitial cells suggested that TGFβ/Erk1/2 pathway may be also involved [71–75]. One of the approaches to deliver bFGF into infarcted hearts is to encapsulate it into crosslinked albumin-alginate microcapsules followed by injection [76]. The released bFGF substantially decreased collagen content in the infarcted area. Co-delivery of bFGF with HGF further reduced collagen content. This study demonstrated that delivery of two antifibrotic growth factors may more efficiently attenuate cardiac fibrosis.

Studies have shown that Notch1 signaling plays a critical role in the cardiac fibroblast-myofibroblast transformation, thereby affecting cardiac fibrosis [77]. Notch1 activation is expected to inhibit the transformation and prevent cardiac fibrosis. This can be achieved by using Notch ligand Jagged-1 (peptide CDDYYYYGFCNKFCRPR) [78]. To deliver the ligand into heart, it was encapsulated into a self-assembling peptide-based hydrogel (peptide RARADADARADADA). Three weeks after delivery, picrosirius red staining results demonstrated that the fibrotic area was significantly decreased compared to self-assembling peptide control. Besides preventing cardiac fibrosis, the Notch ligand also improved angiogenesis, induced cardiac cell proliferation and stem cell recruitment. Compared with growth factors like HGF, VEGF, IGF-1 and bFGF, the peptide based Notch ligand is less expensive yet possesses the function of these growth factors in terms of prosurvival, proangiogenic, and antifibrotic properties. Therefore, the peptide based cardiac therapy may have greater translational potential.

**Localized delivery of biomaterials and cells**

Cell therapy represents a promising approach to treat cardiac fibrosis. It may also lead to heart tissue vascularization and regeneration. In fact, tissue vascularization and regeneration can decrease fibrotic tissue area. Various cell types have been explored in clinical and preclinical models for cardiac therapy.
Some stem cell types do not differentiate into cardiomyocytes to directly regenerate cardiac tissue, but can indirectly promote the regeneration. These cell types typically provide paracrine effects to inhibit cardiac fibrosis, augment the survival of resident cardiac cells, recruit endogenous stem cells, and vascularize the damaged heart tissue [41, 79–84]. Some cell types also directly participate in tissue vascularization. Examples include bone marrow mesenchymal stem cells (BMMSCs) [85–99] and adipose-derived stem cells (ADSCs) [100–103]. Cardiomyocytes and stem cells capable of differentiating into cardiomyocytes can be used to regenerate cardiac tissue. These stem cells include cardiac stem/progenitor cells [104–108], pluripotent stem cell [embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)]-derived cardiovascular progenitor cells [109–111], and cardiosphere-derived cells [112–122]. To deliver cells into infarcted hearts, direct injection experiences low efficacy because of inferior cell retention in the tissue. To increase cell retention, cells can be encapsulated into injectable materials such as hydrogels or microspheres. The cells can also be loaded into porous scaffolds or hydrogels and then patch on the tissue surface.

For those stem cells that indirectly promote heart tissue regeneration, the cell paracrine effects may contribute to decreased fibrosis by decreasing inflammation and profibrotic factor expression. Sun et al. encapsulated ADSCs into platelet-rich fibrin and then patched on the surface of infarcted hearts. Use of fibrin significantly increased cell survival and paracrine effects, which led to the significant decrease of the expression of fibrotic mediators TGF-β and Smad3 (Fig. 5). In addition, the expression of antifibrotic markers Smad1/5 and BMP-2 was significantly increased (Fig. 5) [123]. The enhanced cell survival and paracrine effects also decreased the expression of inflammation markers CD3, CD40, CD68 and CD19. As a result, the fibrotic area was significantly decreased as determined by Masson’s Trichrome staining (Fig. 5). When transplanting ADSCs in collagen patches, the level of procollagen C-proteinase that is responsible for procollagen processing into mature collagen was significantly reduced. In addition, the level of lysyl oxidase, the enzyme involved in collagen crosslinking in the peri-infarct region, was decreased [124]. These events led to the decrease of cardiac fibrosis.

BMMSCs also contribute to decreased cardiac fibrosis. BMMSCs have been found to attenuate cardiac fibroblast proliferation and collagen synthesis through paracrine effects [125]. While it remains unclear how paracrine effects decrease cardiac fibroblast proliferation, the paracrine effects do downregulate the expression of Col1a1 and Col3a1 [125]. BMMSCs also promote cardiac fibroblasts to secrete matrix metalloproteinases that degrade collagen [98]. Transplantation of BMMSCs into infarcted hearts can attenuate cardiac fibrosis at different stages of MI [126–128]. At the acute stage, the cardiac
fibrosis is not formed while at later stages it is formed and progresses with time. Therefore, control of cardiac fibrosis at acute MI stage may more efficiently attenuate cardiac fibrosis. Ceccaldi et al. seeded BMMSCs into microporous alginate-chitosan scaffolds and implanted the constructs on infarcted area using acute MI model [128]. After 33 days, the fibrosis percentage was decreased more in the BMMSCs seeded scaffolds than in the pure scaffolds. Similar results were found when injecting BMMSCs-encapsulated hydrogel into infarcted mice hearts after acute MI. Xia et al. encapsulated BMMSCs into a thermosensitive hydrogel based on N-isopropylacrylamide/acrylic acid and 2-hydroxyethyl methacrylate-polycaprolactone [126]. After 28 days of implantation, the collagen content in the scar tissue was significantly decreased compared to transplantation of BMMSC only. The decease of fibrosis may be resulted from increased cell survival in the hydrogel, which provided greater paracrine effects to inhibit cardiac fibrosis. BMMSC transplantation can also decrease cardiac fibrosis when the MI is in the subacute stage. Fiumana et al. seeded BMMSCs into porous hyaluronan-based scaffolds and placed on the top of the infarcted hearts two weeks following MI [128]. After two weeks of implantation, cardiac fibrosis was largely attenuated.

Implantation of cardiomyocytes that promote cardiac tissue regeneration also has the potential to decrease cardiac fibrosis. Joanne et al. seeded induced pluripotent stem cells-derived cardiomyocytes (iPSC-CMs) into collagen scaffolds with modulus similar to that of the native heart tissue (10–15 kPa) [129]. After 3 days of in vitro culture, the constructs were patched on the dilated mouse hearts. Following 2 weeks of implantation, the transplanted iPSC-CMs integrated with the native myocardium and contributed to the cardiac fibrosis decrease. The hearts implanted with constructs showed significantly higher expression of osteopontin that regulates matrix metalloproteinases than those implanted with pure collagen scaffolds.

Conclusions
Control of cardiac fibrosis is essential to prevent the infarcted hearts from progressing into heart failure. The cardiac fibrosis should ideally be controlled immediately after MI so that the processes that initiate the cardiac fibrosis can be inactivated. Yet the heart tissue may rupture without the protection of the initial fibrotic layer. Cardiac fibrosis naturally expands upon the initial fibrotic tissue is formed. Inhibiting cardiac fibrosis from progressing may prevent progressive deterioration of cardiac function. Different approaches have been explored to treat cardiac fibrosis, such as systemic delivery of antifibrotic drugs, localized transplantation of biomaterials, localized delivery of antifibrotic drugs using biomaterials, and localized delivery of cells and biomaterials. Compared to localized delivery approaches, the systemic delivery approach is more convenient. However, the drug dosage allocated to the heart is limited, resulting in lower therapeutic efficacy.

Localized transplantation of biomaterials controls cardiac fibrosis by decreasing left ventricular wall stress to decrease the elevated wall stress-induced inflammation. When using decellularized ECM, the growth factor released from the matrix may also decrease cardiac fibrosis. Selection of biomaterials with suitable mechanical properties is critical to decrease wall stress. The ideal biomaterials should have elasticity and stiffness matching those of the heart tissue. During the biomaterial degradation, these mechanical properties may decrease. Yet the cells from surrounding tissue may penetrate into the biomaterials to vascularize the tissue, and promote regeneration. Besides mechanical properties, the biomaterials should have excellent biocompatibility without provoking significant inflammation.

Localized delivery of drugs and biomaterials exhibited higher efficacy in controlling cardiac fibrosis than delivery of biomaterials only. The drugs and biomaterials may be delivered into infarcted hearts shortly after MI and before the initial fibrotic tissue is formed since the biomaterials may provide adequate mechanical support to prevent tissue rupture. The encapsulated drugs gradually release from the biomaterials allowing for long-term attenuation of cardiac fibrosis. The efficacy of cardiac fibrosis inhibition is determined by drug release kinetics. When the released drug is sufficient to prevent new myofibroblast formation and ECM synthesis especially collagen, high efficacy can be achieved. Duration of drug release also determine the therapeutic efficacy. Longer time delivery better controls cardiac fibrosis. Thus tailoring properties of the biomaterials to enable the drugs to release for prolonged time period is essential.

Localized delivery of cells using biomaterials has been shown to be an effective approach to control cardiac fibrosis. The cells either provide paracrine effects or directly regenerate the infarcted heart tissue. Those cells that provide paracrine effects may release antifibrotic factors to directly control cardiac fibrosis. They may release anti-inflammatory factors to control inflammation thus indirectly controlling cardiac fibrosis. In addition, the released angiogenic factors promote tissue vascularization and regeneration. To long-term control cardiac fibrosis, high rate of cell survival in the infarcted hearts are necessary. However, the infarcted heart tissue is characterized by a low nutrient and oxygen environment. The delivered cells thus experience low survival rate. The inflammation condition in the infarcted hearts also causes cell death. Use of biomaterials as cell carriers
may increase the cell survival by protecting the cells from attack by inflammatory cytokines, but do not necessarily improve cell survival under the low nutrient and oxygen conditions. Approaches that can be used to increase cell survival under these conditions include encapsulation of cells in biomaterials that release prosurvival growth factors such as bFGF, IGF-1 and HGF [130–135], and release oxygen [136].

In summary, different approaches have been used to inhibit cardiac fibrosis. Localized drug delivery represents a major approach. Yet the widespread clinical application of current approaches is obstructed by the low therapeutic efficacy. Development of new and translational delivery approaches to improve therapeutic efficacy is essential to push the anti-cardiac fibrosis therapy towards clinical application. In addition, development of new drugs that not only prevent myofibroblast formation but also revert existing myofibroblasts back into the cardiac fibroblasts may fundamentally prevent cardiac fibrosis.

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Authors’ contributions
ZF and JG drafted and reviewed the manuscript. Both authors approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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