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Can Heart Function Lost To Disease Be Regenerated By Therapeutic Targeting Of Cardiac Scar Tissue?

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Abstract

Myocardial infarction results in scar tissue that cannot actively contribute to heart mechanical function and frequently causes lethal arrhythmias. The healing response after infarction involves inflammation, biochemical signaling, changes in cellular phenotype, activity, and organization, and alterations in electrical conduction due to variations in cell and tissue geometry and alterations in protein expression, organization, and function – particularly in membrane channels. The intensive research focus on regeneration of myocardial tissues has, as of yet, only met with modest success, with no near-term prospect of improving standard-of-care for patients with heart disease. An alternative concept for novel therapeutic approach is the rejuvenation of cardiac electrical and mechanical properties through the modification of scar tissue. Several peptide therapeutics, locally applied genetic therapies, or delivery of genetically modified cells have shown promise in improving the characteristics of the fibrous scar and post-myocardial infarction prognosis in experimental models. This review highlights several factors that contribute to arrhythmogenesis in scar formation and how these might be targeted to regenerate some of the electrical and mechanical function of the post-MI scar.

Keywords

myocardial infarction; regeneration; fibrosis; structure; tissue properties

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1. Introduction

Myocardial infarction (MI) results in scar formation to repair and replace myocardium lost to ischemic insult. While the scar has mechanical assignments in preventing the heart wall from rupturing, scar tissue does not have the same structure or function as native myocardium. The fibrous scar that forms is unable to contract rhythmically and is not an efficient conductor of electrical signals - two key functions of myocardial tissue. The presence of scar places elevated stress on the heart to fulfill its contractile role, further affecting cardiac performance.

Taking a cue from the neonatal heart and hearts of non-mammals [1–3], many recent studies have been aimed at regenerating myocytes lost to MI [4,5], or reprogramming mature adult non-muscle cells to become myocytes in an attempt to restore cardiac function [6–8]. Recent advances in this area include strategies that enhance fibroblast reprogramming rates, at least in cultured cells [9], and the identification of small molecules that prompt conversion of fibroblasts into myocytes [10]. Though conceptually exciting, these novel findings have, as yet, not demonstrated levels of efficacy in preclinical models or early-stage clinical trials that would justify translation to the next level. Thus far *in vivo*, such approaches have yielded low numbers of regenerated muscle cells of immature phenotype, and have proven more difficult to elicit from human cells than in model species. Emerging, and perhaps more immediately tractable, approaches to mitigating the loss of myocardial tissue to disease in humans are therapeutic strategies aimed at re-engineering scar properties.

While many factors affect the characteristics of the healed MI, targeted alterations in electrical or mechanical function of the scar and its adjacent infarct border zone (IBZ), may be achievable and could lead to improvements in cardiac performance in hearts damaged by disease [11,12]. However, the ability to therapeutically modify cardiac scar tissue is still limited by a lack of knowledge on what constitutes benefit. Achieving healed infarct that performs similarly to intact cardiac muscle is not possible, though attaining a useful intermediate tissue, in which some biomechanical and/or electrical conduction properties are restored may be on the horizon. This review focuses on regenerating cardiac function lost to disease through manipulation of scar and IBZ tissues. Perfect healing may not be possible, but clinicians could eventually be able to give “*Mother Nature an assist*”, inhibiting or slowing the presently unstoppable progression toward heart failure faced by many patients.

2. Changes in the structure and function of the infarcted heart

2.1. Formation of a (mostly) non-electrically conducting, non-contractile scar

Prolonged cardiac ischemia is the cause of tissue death in MI [13], with cells dying within minutes to hours of the onset of ischemia [13]. The ischemic tissue then undergoes a healing and remodeling response in the following days and months. Since adult human myocardium has limited to no ability to regenerate, a fibrous scar that cannot perform as normal myocardium replaces lost cardiac muscle (Fig. 1A). The scar does serve mechanical roles - showing some level of compliance during active pumping, as well as preventing the heart wall from rupturing [14]. Interestingly, increased numbers of myofibroblasts in the scar have been associated with less dilation in mouse models of MI [14]. However, fibrotic tissue

cannot rhythmically contract, nor is it an efficient conductor of electrical signals, both critical functions of myocardium. Since the majority of patients now survive MI, these alterations result in arrhythmia generation, left ventricular (LV) dysfunction, and often, eventual decline into heart failure and death.

In the ischemic portion of the heart, myocytes die, and cardiac repair begins with inflammatory cells infiltrating the injury within the first day after MI [15,16]. This inflammatory response clears the infarct region of dead cells and debris and is thought to be a necessary step in healing of the infarct. Reperfusion and other clinical interventions can significantly alter the course of healing and the characteristics of the healed infarct [17–20]. Inflammation is significantly increased and accelerated in reperfused tissue (which results from the common clinical intervention coronary angioplasty), though reperfusion improves tissue repair because of this increased inflammatory response [21–25]. On the other hand, delayed resolution of inflammation leads to LV enlargement and adverse remodeling in mouse MI models [26–28]. Resident macrophages appear to have important roles in the chronic phase after myocardial infarction [29], while knockout or depletion of macrophages results in impaired healing [30,31]. Additionally, several inflammatory molecules contribute to the healing process after MI and are implicated in post-MI arrhythmogenesis, including interleukin-1 (IL-1) and transforming growth factor- β (TGF- β) [32]. This being said, factors such as TGF- β play a number of contrasting roles in myocardial healing [33,34]. Thus, although modulation of inflammation represents an attractive strategy for development of new treatments for ischemic heart disease, cell and molecular targets and timing may prove critical to optimizing outcomes from novel approaches to therapy.

Together with macrophages and endothelial cells, fibroblasts are major cellular contributors to cardiac granulation tissue formation and infarct scar remodeling [35–39]. Mesenchymal populations responding to the MI come mainly from resident fibroblasts [40], which in turn largely have epicardial/mesothelial origins [41,42]. Fibroblasts recruited into the wound assume an activated status, differentiating into myofibroblasts - exhibiting among other characteristics elevated levels of collagen secretion and cytoskeletal differentiation, as well as an increased ability to generate mechanical force [43–46].

Remodeling of the scar is a balance between extracellular matrix (ECM) degradation and generation. The rapid degradation of ECM occurs via the action of matrix metalloproteinases (MMPs) and collagenases, released either by damaged cells within the myocardium, or by immune cells that have infiltrated the infarct [47–49]. The fibroblasts infiltrating the ischemic area construct a new network of collagen-rich granulation tissue, changing the normal fibrous ECM skeleton of myocardial tissue within the first week. Remodeling of the infarct is time-dependent and a healed infarction may not differentiate substantively until several weeks after the MI, though ongoing scar maturation may continue for months and years, and in some cases never reaches an equilibrium state [13]. As the heart is less able to efficiently pump due to the loss of myocytes during the scar maturation process, the heart begins to dilate and remaining myocytes hypertrophy [13].

Cardiac injury leads to an initial abrupt decline in cardiac performance, with changes occurring within minutes of the onset of ischemia. This is well demonstrated in isolated

heart models, where acute MI can be shown to rapidly impair both force development and contraction [50]. As the infarct heals, the newly generated fibrotic tissues remodel – a process involving extension, expansion, and re-organization of its cellular and acellular components. This not only results in changes to the size and geometry of the infarcted region, but also modifies the myocardium's ability to contract and transfer electrical signals. Ultimately, functional myocardium is replaced by non-contractile and non-conductive scar. The next section details why this is a problem.

2.2. Electrophysiological remodeling of the scar and border zone after MI

Transmission of electrical signals through the myocardium is held to be mediated by GJs, which are preferentially localized at intercalated discs (IDs) between adjacent myocytes (Fig. 1b). There is also growing evidence for an ephaptic contribution to cardiac conduction [51,52]. Ephaptic conduction of electrical impulses has been proposed to occur via transients in extracellular ions in the ID that prompt activation of adjacent myocytes – albeit that the mechanism appears to be localized in the perinexal region of cell membrane surrounding GJs. GJs are comprised of connexin proteins, the most common of which in the mammalian ventricle is connexin 43 (Cx43) [53]. Post-MI, there is a loss in both the density of GJs and in gap junctional intercellular communication (GJIC) in the ischemic myocardium compared to the remote regions [54,55]. Further, a hallmark of the IBZ is the reorganization of Cx43 from IDs at zones of end-to-end contact between myocytes to non-ID lateral membranes (Fig. 1c) [56–59]. Decreased electrical coupling during acute MI has been correlated with dephosphorylation and reorganization of Cx43, as well as translocation from the cell surface to intracellular pools [60,61]. While the reduction in Cx43 is likely a protective mechanism (GJs are implicated in the spread of injury signals in skin [62], neural tissues [63], and heart [64]), Cx43 lateralization interrupts normal electrical conduction patterns in the heart, and may contribute to arrhythmias [10–13].

Bordering the infarct area is the IBZ, which is a few cell layer-thick region of transitional tissue between the scar and adjacent viable myocardium that has survived infarction. The changes in tissue electrophysiology, cell organization, collagen deposition, and inefficient removal of toxic biochemical products, among other factors, can contribute to the generation of lethal arrhythmias in the IBZ [65–67]. Previous studies demonstrate action potential (AP) slowing through the IBZ [68,69], though propagation of activation increases in the scar, possibly due to the presence of surviving islands of myocytes [70]. Passive conduction via fibroblasts may also contribute to within-scar increases in AP conduction speed [70,71].

In the population of Cx43 that remains at the ID in an ischemic setting, there is an increase in phosphorylation at serine (S) 368 [72] and S373 [73]. Additionally, ischemic preconditioning has been shown to augment protein kinase C- ϵ -mediated Cx43 phosphorylation of S262 and S368, and prevent Cx43 lateralization following an ischemic insult [74]. Further, Akt-mediated phosphorylation of S373 is elevated in hypoxia. Though increasing GJ size and GJIC, S373 phosphorylation is thought to precede internalization of Cx43 [73]. Phosphorylation events in Cx43 are known to regulate channel effects like unitary conductance, so connexin phosphorylation, in addition to connexin downregulation,

may be protective mechanisms against the spread of injury effects into otherwise undamaged tissues surrounding the initial ischemic field.

Surviving myocytes in the IBZ have reduced Na⁺ [75,76] and repolarizing K⁺ currents that can lead to prolonged action potential duration (APD) [77,78], and altered intracellular calcium handling, which may result in decreases in cellular excitability and triggered activity [79]. The change in repolarization results in altered and variable resting membrane potential [77,80–82]. Expression of sarcoplasmic reticulum (SR) calcium ATPase (SERCA) is also reduced, as is the activity of the sodium/calcium exchanger [83] - changes that are associated with LV dysfunction. Lower levels of SERCA2a may contribute to alterations in intracellular calcium that are linked to variability in APD and the onset of arrhythmias [84,85]. While reentrant arrhythmias are the most common after MI, triggered and automatic arrhythmias may also occur.

Cx43 is *increased* in activated fibroblasts in the scar and IBZ [86–88], enhancing the possibility for myocytes and fibroblasts to be electrically coupled. Additionally, infarcted fibroblasts show more hyperpolarized membrane potentials, increased outward currents [89], and higher membrane resistance compared to fibroblasts isolated from normal hearts [90].

Non-muscle cells migrating to the MI from the blood circulation may also affect cardiac electrophysiological properties [40,91,92]. Bone marrow derived cells (BMCs) are mobilized by injury signals and recruited to the infarct from the peripheral circulation. Since the first report in 2001 [93], BMCs have been extensively studied for their utility in cardiac repair, though there have been varying accounts of the numbers of these cells recruited following MI [40,91,94,95]. Some clinical trials of exogenously applied BMCs post-MI indicate modest benefit from paracrine factors [96] and exosome release [97,98]. However, studies of large patient cohorts in meta-analyses have raised questions as to whether experimental cell therapies of this type will provide significant benefit beyond existing standard-of-care, in terms of favorable clinical events or improvement in metrics of LV function [99,100].

2.3. Changes in cellular organization and interactions

The changes in electrophysiological characteristics of scar tissue may be related to sub-acute and longer-term remodeling that occurs following MI. While nearly all myocytes in the ischemic area die, numerous myocytes at the infarct periphery in the IBZ survive. Structural remodeling in the IBZ involves an interspersed of myocytes with fibroblasts that have migrated into, and/or proliferated in the wound, and the ECM that these mesenchymal cells produce - leading to separations between strands of surviving myocardial cells and enhanced opportunity for electrical connection between myocytes and fibroblasts (Fig. 1c) [65,87,101,102]. Fibroblasts have higher membrane resistance and capacitance than myocytes, potentially making them better sustainers of electrical signals when paired to each other, or to myocytes [103]. However, fibroblasts also have slightly depolarized resting membrane potentials compared to myocytes [104–106]. Electrical coupling between fibroblasts and myocytes in diseased tissue thus may result in myocardial cell depolarization, increasing the risk of conduction disturbance and arrhythmia - especially

with the potential for numerous heterocellular contacts of this type to occur over the extent of the IBZ [107].

In addition to reorganization of cells and increased deposition of ECM proteins post-MI, one of the alterations in fibroblasts in the scar is an increase in connexin expression (Fig. 1d) [86–88], which enhances the capability of fibroblasts to be paired with myocytes via GJs [87]. Myocyte-fibroblast coupling is relatively rare in the uninjured myocardium in homeostasis and has only been definitively demonstrated in sinoatrial tissue [108]. However, immunolabeling studies suggest that GJ-based heterocellular contacts may accrete in the IBZ (Fig. 1c) [87,88,109]. While intercellular communication of myocardial cells is well-established, as electrical impulse spread through the myocardium relies primarily on the passage of ions through GJs [110,111], the presence and activity of heterocellular GJ coupling is not well understood [103].

In an elegant optical mapping study of infarction in rabbit, AP propagated into post-MI scar tissue, indicating a possible role for myocyte-fibroblast interconnection in sustaining electrical signals [70]. The AP measured within the scar demonstrated profiles similar to those of cardiac fibroblasts connected to myocytes [104,106]. Additionally, fibroblasts in the infarct scar were shown to lack the calcium transients occurring in myocytes [68], suggesting that these cells passively conduct electrical signals through the scar. However, alterations in cell organization and communication may also contribute to the arrhythmogenic propensity of the IBZ [112].

Myocyte-fibroblast coupling is well established *in vitro*, and is reinforced by the fact that fibroblasts removed from native myocardium upregulate their expression of connexin proteins [113–116]. Heterocellular interactions mediated by GJs are also increased in disease [88], as fibroblast phenotype changes in response to injury, making interactions more likely [117–119]. The overall effect of increased intercellular contact in the context of other changes to the post-MI environment is currently unknown. Changes in myocyte-fibroblast coupling may nonetheless be responsible for key aspects of long-term and pathologic changes to conduction at the IBZ.

Tunneling nanotubes – thin, extended membrane-bound projections – are a further type of myocyte-fibroblast interaction known to occur within cardiac tissues [120]. The function of these ephemeral structures is presently unknown. Interestingly, there are reports of Cx43 enrichment in tunneling nanotubes in a number of cell types [121,122], though the existence of punctate Cx43 labeling of nanotubes, suggestive of GJ formation, was not found between myocardial cells and fibroblasts isolated from uninjured neonatal ventricles [120]. The presence of tunneling nanotubes between myocytes and fibroblasts within the IBZ, and the extent to which such structures in the injured heart express connexins, seem pertinent questions for ongoing study.

2.4. Mechanical properties and infarct size

Infarct size is a major predictor of outcome in MI patients [123]. Diabetic and dysglycemic individuals tend to have larger infarcts and higher post-MI mortality [124]. This may be related to GJ coupling in the cardiac injury, as insulin administration was found to decrease

GJIC and increase phosphorylation of Cx43 at S368 [64], which is associated with lower channel conductance. Large infarcts result in extensive cardiac remodeling, including changes in gross morphology, histology, and molecular function and interactions [125]. Within a few days of infarction, fibroblasts begin to deposit ECM, and collagen content increases significantly in the first month [67,126]. Scar stiffness is widely held to increase with escalating collagen levels [126,127], though some studies suggest that even whilst the amount of collagenous tissue deposition continues to increase over time, stiffness peaks a few weeks after infarction and then declines [128,129]. This may be accounted for by the differences in collagen content and MMP profiles in rodents versus large animals.

MI often results in systolic and diastolic dysfunction and can predispose patients to arrhythmias because of the extensive remodeling that occurs [130]. The remodeling process is typically ongoing; with continued dilation, cardiac performance deteriorates over time [131], leading to heart failure and the eventual death of the patient.

3. Experimental approaches to improving myocardial function

The intention of a number of emerging therapies is to modify the properties of the infarct scar, preventing adverse remodeling or functional events, and to mitigate the progression to heart failure. These therapies are directed at restoring several important heart characteristics that are not usually attributes of cardiac scar tissue.

3.1. Amelioration of electrical properties

The scar and IBZ have been the object of many therapies aimed at ameliorating the electrophysiological medium of these tissues. While traditional pharmacological solutions are limited by their global action, resulting in many off-target effects, several directed therapeutic approaches can be taken to alter electrical transfer in the scar and reduce the propensity for arrhythmias. If the border zone and scar tissue could efficiently propagate electrical signals instead of acting as a structure promoting reentrant arrhythmia generation via disruption of uniform conduction, a path to abating the effects and incidence of lethal arrhythmias could be provided.

3.1.1. Connexins—Several experimental treatments that attempt to modify electrical communication post-MI have focused on the connexins. Timing is critical in therapies aimed at increasing connexin expression, as these proteins are known to be involved in the spread of injury signals [62,132]. Cx40 and Cx43 have been used to speed conduction in the pig atria [133,134]. Upregulation of these two connexins enhanced propagation in the atria, as well as prevented atrial fibrillation and lessened arrhythmia susceptibility. Cx43 was found to improve the electrical substrate of the ventricular scar, as Cx43-expressing cells engrafted into murine infarct were shown to prevent arrhythmogenesis post-MI [11]. Following Cx43 gene transfer via intracoronary adenoviral delivery to infarcts in pig, hearts experienced fewer inducible arrhythmias and faster CV through the border zone, results which were correlated with a doubling of Cx43 expression in the IBZ [135]. Interestingly, the ratios of ID-localized Cx43 and phosphorylated Cx43 were similar in transduced infarcts versus controls. This suggests that heightened Cx43 levels in the IBZ may be sufficiently therapeutic to reduce arrhythmias post-MI.

Low pH in infarcted tissue during ischemia can cause Cx43 GJ channels to close [136]. In contrast, Cx32 remains open during low pH [137]. However, when Cx32 was ectopically expressed in an attempt to increase CV through the border zone, though GJIC was increased, no effect on arrhythmia incidence was found [138]. Importantly, Cx32 expression was found to significantly increase infarct size [138], highlighting a long-established role of connexins in injury spread [139]. Cx43 can increase CV in the IBZ of a *healed* infarct [135], but Cx32 added at the time of injury leads to an increase in the extent of injured myocardial tissue [138]. This provides a cautionary example that timing and channel properties of connexin isoforms are essential considerations in any therapeutic strategy involving manipulation of these proteins.

Connexins have also been modulated by microRNAs to improve scar characteristics. Wang et al. noticed that overexpression of microRNA-1 (miR-1) increased arrhythmogenesis in the infarcted heart [140]. Amplified miR-1 expression was associated with slowed conduction spread and depolarized membrane potential, likely caused by reduced expression of Cx43 and inward rectifying potassium channel 2.1 (Kir2.1) [140]. Using antagomirs to block miR-1 activity in a rat model of infarction relieved arrhythmogenesis.

Several Cx43 mimetic peptides have shown potential for novel therapeutic approach to improve prognosis after experimental MI. α CT1, a mimic of the Cx43 carboxyl-terminus, was shown to reduce arrhythmia incidence and severity in a murine cryo-injury model of infarction [141]. This likely occurred because of an enhanced CV through the IBZ, and a tendency for Cx43 to be maintained at IDs. The opening of hemichannels, which are localized to the perinexus at the edges of GJ plaques [51,142,143], is thought to contribute to the phenomenon of connexin-mediated injury spread following MI, with an accompanying efflux of ATP and other pro-inflammatory molecules and influx of toxic levels of ions [143–145]. Gap 26/27, mimetics of extracellular loop domains of Cx43, inhibited electrical connection via GJs and hemichannel activity [146], and diminished infarct size in a rat MI model [144]. Gap19, an intracellular loop mimetic that reportedly blocks Cx43 hemichannels, but not GJs formed by this isoform [143], modestly decreased infarct size in a murine model of MI [143]. Gap19 and Gap 26/27 thus appear to have cardioprotective effects, though no study to date has examined the effects of these peptides on CV post-injury or probed their ability to inhibit arrhythmias.

3.1.2. Myocyte-fibroblast coupling—While myocytes are widely thought to be the prime cellular medium for improving the propagation of cardiac APs, the question of whether non-myocytes could be targets for therapeutically improving conduction through the IBZ and scar remains open. Fibroblasts in the normal heart do express connexins [147,148], albeit at low levels, and dye transfer studies in rabbit sinoatrial node have shown that fibroblasts can functionally couple to myocytes [108]. After MI, activated fibroblasts upregulate expression of Cx43 [86–88], potentially further enhancing myocyte-fibroblast interconnections. Optical mapping studies of experimental MI in rabbit show that, even after chemical ablation of surviving myocytes in the infarct, the remaining cells within the scar can propagate electrical signals [70]. Additionally, a recent report of conduction between myocytes and non-myocytes in the IBZ was demonstrated by a fluorescent voltage-reporting protein under the Wilm's Tumor 1 (WT1) promoter [149]. In light of these studies, instead

of ensuring the scar is non-conducting, it may be beneficial to patients in some instances to augment propagation of electrical signals through the scar. This would not regenerate the contractile attributes of fibrotic tissues in the heart, but it could recapitulate aspects of electrical transfer and reduce propensity for conduction disturbance and arrhythmia.

3.1.3. Ion channels—The skeletal muscle voltage-gated sodium channel isoform 4a (SCN4a) has been used in attempts to speed up CV through the border zone into the infarct scar [150]. Lau et al. observed that the skeletal muscle sodium channel isoform was more active at the depolarized membrane potentials found in the damaged myocytes of the IBZ than was the cardiac sodium channel, SCN5a. Adenoviral transduction of SCN4a into the epicardial border zone reduced arrhythmia inducibility and increased CV, with no change in infarct size in a canine infarct model [150]. In a simulation model of Nav1.5 redistribution in IBZ myocytes, Na⁺ channel blockade with class I antiarrhythmic drugs tended to result in conduction block and reentrant arrhythmia generation at the border zone [151]. Additionally, it was found that Nav1.5 expression is reduced in the IBZ of mouse hearts, and that the transcription factor FoxO1 negatively regulates Nav1.5 α - and β 3-subunits and sodium current density [152], indicating that FoxO1 could be an interesting target for modulating sodium channel expression in the IBZ.

Variability in APD is linked to the onset of arrhythmias [84], and one of the mechanisms that may be responsible for this is variation in intracellular calcium handling that can result, in part, by a reduction in SERCA2a [85]. When SERCA2a was transfected into rat hearts 16 weeks post-MI, the hearts had fewer spontaneous premature ventricular contractions and spontaneous or induced non-sustained VT episodes compared to controls. There were fewer spontaneous SR calcium release events, reduced total SR calcium leak, and fewer triggered arrhythmias [153]. Similar observations were made in a sheep model, with enhanced contractility and stroke volume, and smaller and fewer apoptotic myocytes in the IBZ [154]. As SERCA2a gene therapy can improve the mechanical pump capacity of the heart, Phase I/Phase II clinical trials have been completed [155,156]. However, heart failure patients in the Phase IIb CUPID 2 trial received no benefit from treatment [157].

Since potassium channel alterations can lead to prolonged APD, they have been the subject of several studies. A dominant negative potassium channel mutation in which a glycine (G) was mutated to an S at position 628 (KCNH2-G628S) increased APD by eliminating a key myocyte repolarization current. Localized *in vivo* gene transfer of this mutant channel to the VT site prolonged refractory period, extending the reentrant wavelength and preventing arrhythmias [158]. Similarly, voltage-gated potassium channel 1.3 (Kv1.3)-expressing fibroblasts engrafted into rat heart led to a prolonged local refractory period, but had no effect on the regions of the heart not receiving cells [159]. This effect was maintained at slower pacing cycles in pig hearts with fibroblasts expressing Kv1.3 and Kir2.1 [159]. These genetically engineered non-myocytes were reported to be able to couple with myocytes, as well as being associated with reduced levels of cardiac automaticity and prolonged refractoriness. Additionally, semaphorin 3a is known to decrease sympathetic neural remodeling after MI, and may be involved in electrical remodeling at the IBZ, as levels of potassium channels were restored by adenoviral delivery of the signaling molecule in a rat model of MI [160].

3.2. Targeting scar structure

Resolution of the inflammatory phase of healing after infarction and the proper timing of reparative fibrosis are critical for healing of the scar. An overactive reparative phase of healing can lead to collagen accumulation outside the ischemic area, contributing to interstitial fibrosis in the IBZ. Modifying the inflammatory response could have an impact on both electrophysiological and mechanical properties by altering the proteins directly involved in electrogenesis [161–163], the tissue architecture that supports efficient activation spread within the scar and IBZ [161], and the extent of fibrosis contributing to tissue stiffness.

3.2.1. Altering inflammation and the fibrotic response—One aspect of the healing process that can affect the amount of fibrous ECM deposition is the inflammatory response. Delayed resolution of inflammation leads to LV dilation and adverse remodeling in mouse MI models [26–28]. Consistent with this, knockout or depletion of macrophages results in impaired healing [30,31] and inflammation may be required for regeneration in the zebrafish heart [164]. This suggests the necessity of balancing the inflammatory response for normal healing of the infarct scar.

Non-steroidal anti-inflammatories should NOT be used in MI patients, as they have been shown to worsen clinical outcomes [165]. On the other hand, the literature on the ability of steroidal anti-inflammatories to reduce fibrosis is controversial, with some studies showing decreased mortality and no effect on risk of rupture [166], while others demonstrated detrimental effects, including an increased risk of ventricular rupture [167].

A number of established clinical interventions appear to modulate fibrosis in an “off-target” manner, including angiotensin converting enzyme inhibitors [168], angiotensin receptor blockers [169], and statins [170–174], which have been shown to reduce fibrosis and inhibit detrimental myocardial remodeling. One study suggests that the anti-fibrotic effect of statins is mediated by inhibiting TGF- β -induced differentiation of fibroblasts to myofibroblasts [175].

Modulation of neurohormonal factors can also affect cardiac fibrosis. Endothelin is known to increase collagen deposition by heart fibroblasts [176–179], and endothelin antagonism has been shown to have cardioprotective effects [180,181]. However, clinical trials to reduce LV remodeling have demonstrated that endothelin antagonism is mostly ineffective, though it does not adversely affect remodeling [182–184]. Inhibiting the angiotensin II pathway by administration of losartan was reported to decrease interstitial fibrosis, increase CV, and raise GJ conductance in infarcted hearts [185,186]. Losartan mode-of-action is thought to include the deactivation of MMPs [187–189].

Several studies directed at inhibiting specific inflammatory molecules or the conversion of fibroblasts to myofibroblasts have been completed. Studies in mouse and human using IL-1 inhibition attenuated LV remodeling and preserved cardiac function, while allowing normal infarct healing [190–193]. Inhibition of the TGF β pathway can prevent the conversion of fibroblasts to myofibroblasts [194], and has been shown to reduce fibrosis and progression to heart failure in a mouse model of pressure overload [195].

Remodeling of the infarct area after injury is a balance between ECM degradation by enzymes and ECM synthesis by fibroblasts. Even though a number of reports have demonstrated that MMPs play a role in LV myocardial remodeling after MI [49,196], preventing excess degradation of collagen by MMPs has produced controversial results in preclinical studies. The overall collagen area of the MI scar was found to change very little with broad spectrum MMP inhibition [197–199] and changes in collagen content were modest and mostly not significant in targeted reduction or transgenic knockouts of MMP-1, -3, -9, -12, and MT1-MMP [200–203]. However, broad spectrum MMP inhibition did limit LV dilation [197,199], reduce myocyte hypertrophy in the non-infarcted regions of myocardium [198], and decrease tissue and chamber compliance [198,199], suggesting a beneficial effect on cardiac structure.

Although much progress has been made in understanding the contribution of cardiac fibroblasts to fibrosis, cell lineage analyses suggest differences in fibroblast response to ischemic versus hypertrophic diseases [40,92,204], as well as differences in fibroblast response in atrium versus ventricle [34,205–208]. These studies emphasize the importance of examining potential remedies in appropriate disease models.

3.2.2. Altering scar structure and ultrastructure—The ultimate long-term goal of regenerative therapy is to replace MI scar with new myocytes. Unfortunately, experiments performed thus far *in vivo* suggest low efficiency of myocyte regeneration and immature myocyte phenotypes, which frequently display lateralized GJs, immature calcium handling, slower conduction velocity, and residual pacemaking currents, all of which can contribute to aberrant AP propagation and arrhythmia [209–211]. In line with this, there has been a shift in interest among researchers from a sole emphasis on the prospect of myocardial regeneration to the paracrine effects of experimental cellular therapies – raising interesting unanswered questions as to whether the target for such paracrine effect is cardiac muscle or scar. To date, studies of regenerative and direct reprogramming strategies have focused on myocardial properties, paying less attention to effects on scar electrical and mechanical properties, CV, arrhythmogenesis, and/or cardioprotection.

At the border zone, interstitial fibrosis leads to separation of myocytes, interfering with normal electrical connections. Because of the variation in angle of myocardial sheetlets from the surface of the epicardium through to the endocardium [212,213], fibrosis at the border of the scar may be nearly parallel, nearly perpendicular (or some intermediate orientation) to surviving myocytes [214]. This results in a heterogeneous tissue, which is a well-known predictor of arrhythmia risk [215,216]. Few experimental studies have examined how electrical cues propagate in a 3D heterogeneous tissue. This being noted, an image-based modeling study of the IBZ demonstrated that heterogeneous IBZ composition is correlated with tortuous communication pathways and conduction block [214]. Understanding the geometry of cells and tissue that contribute to conduction in this context may be important for modifying scar configuration and ultrastructure post-MI.

Infarct size is a critical predictor of outcome in MI patients [123,125]. Weakened ventricular function [217], adverse LV remodeling [218], and heart failure [219] are more likely to result from larger infarcts. Revascularization of infarct tissue has been posited as the first

step in reducing infarct size and has indeed found to be associated with extended survival of MI patients [220]. There is some evidence that infarcts may compact spontaneously [221]. Other groups have also addressed the potential of the Wnt pathway in minimizing cardiac scars [222–224]. Mechanisms of cardioprotection may also lead to smaller infarcts. Gap 19/26/27, which are known to inhibit hemichannel activity, reduced infarct size in rat and murine models of MI [143,144]. Additionally, though an unlikely remedy to precede an MI, ischemic preconditioning confers some level of cardioprotection, even when performed on extremities [225].

Another aspect of scar ultrastructure that can shed some light on arrhythmia mechanism is the cellular composition of the IBZ. Mathematical models have demonstrated that when myocyte-fibroblast coupling via GJs and ionic current remodeling is considered in the IBZ, activated fibroblasts at intermediate densities prompt AP shortening and elevated arrhythmia propensity. By contrast, at high density, fibroblasts cause resting depolarization and block propagation, protecting against arrhythmias [226]. While this study did not take into account the *organization* of cells in the IBZ, it does suggest that varying the cellular profile could be critical for arrhythmia prevention.

In simulation models of AF, fibroblast proliferation, but not collagen accumulation alone, was suggested to be responsible for disturbances to electrical activity [227]. Additionally, spatial variations in accumulation of fibrosis may affect electrical propagation in the heart, and patient-specific modeling of this fibrosis may allow identification of areas-at-risk for developing arrhythmias [228]. Rotors in AF were trapped by fiber discontinuities, and fibrosis or scarring of sufficient size could terminate this [229]. Patient-specific distribution of fibrosis, thus, may be critical in causing and maintaining electrical abnormalities [230].

Few studies have focused on changing the patterns of cellular contact in the IBZ, though 2D studies have shown that the geometry of cell interactions may play a role in arrhythmia generation [231,232].

3.2.3. Altering the mechanical properties of the infarct—Mechanical properties of healed infarct are critical, as the scar must be stiff enough to prevent rupture or stretching that leads to poor force generation, but not so stiff that filling during diastole is limited. The scar must also maintain its mural thickness over time to avoid LV dilation.

Early computer modeling work, that treated infarcts as isotropic tissue, suggested that increasing stiffness of the infarct resulted in confounding changes to cardiac LV metrics [233,234]. In a finite-element model in dog heart, Holmes and co-workers determined that isotropic stiffening of the scar did not alter stroke volume, but longitudinal stiffening, though maintaining some level of compliance in the circumferential direction, led to increases in stroke volume [233]. A follow-up study in dogs confirmed this finding by mechanically reinforcing the infarct in the longitudinal direction [12]. While these studies demonstrated a key proof-of-concept for the field, a therapeutic strategy aimed at generating a longitudinally stiff and (somewhat) circumferentially compliant scar requires further development. In an attempt to better understand scar anisotropy, this group generated a mathematical model that

shed light on the mechanical cues that influence collagen alignment [235], though this model does not as yet take into account biochemical effects on this process.

Several studies have examined the effect of polymer injection to control mechanical characteristics of the scar [236–238]. Polymer injection into the scar seems to only provide benefit to cardiac mechanics if a stiff polymer is used [237]. These experimental results were reflected by a finite element modeling study that suggested injections into the IBZ at multiple locations might be the most beneficial, while injections into the scar produced mixed results on cardiac pressure-volume relationships [234].

Another approach to increasing stiffness of infarct scar is to amplify collagen content, either by inhibiting MMPs (as reviewed in section 3.2.1) or boosting tissue inhibitors of metalloproteinases (TIMPs) [239–241]. Hydrogel or adenoviral delivery of TIMPs to infarcts led to reduced MMP activity and LV remodeling in a porcine model [239] and greater fibrillar collagen deposition and LV ejection fraction in mice [241].

Though we still don't fully understand the ideal mechanical properties of a healing or healed infarct scar, many experimental and modeling studies have provided a wealth of information. Hearts with small scars that are longitudinally stiff seem to have the best prognosis.

4. Conclusion

The MI scar and IBZ present significant clinical challenges, as these tissues cannot contribute actively to the contractile ability of the heart, do not efficiently pass electrical signals, and are associated with life-threatening arrhythmias. Advances have been made, including, in a few instances, the progression of promising early-stage therapies to clinical testing. However, the attributes of the healing infarct that are the most amenable to decreasing the likelihood of adverse outcomes are not well understood. Further research is required to gain insight into the basis of variation in scar mechanical and electrical properties between individuals, and how patient-to-patient differences in fibrotic tissue organization and function ultimately contribute to lethal arrhythmias and heart failure. It is also notable that extant preclinical and early clinical-stage studies have mostly examined the effects of therapies based on manipulation of a single factor. In the long term, it is envisaged that improvements beyond the current standard-of-care, as well as mitigation of off-target effects, may require the orchestration of effects on several facets of scar and IBZ microenvironment during the healing process. This being said, that the electrical and mechanical properties of diseased hearts may be partially restored by targeting cardiac scar tissue is a proposition that requires serious consideration.

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Abbreviations

αCT1	alpha-carboxyl-terminal peptide 1
AP	action potential
APD	action potential duration
BMCs	bone marrow derived cells
CV	conduction velocity
Cx43	connexin 43
ECM	extracellular matrix
GJ	gap junction
GJIC	gap junction intercellular communication
IBZ	injury border zone
ID	intercalated disc
Kir2.1	inward rectifying potassium current 2.1
KCNH2	voltage gated potassium channel
Kv1.3	voltage gated potassium channel 1.3
LV	left ventricular
MI	Myocardial infarction
miR-1	microRNA-1
MMP	matrix metalloproteinase
SERCA	sarcoplasmic reticulum calcium ATPase
SCN4a	voltage gated sodium channel isoform 4a
SCN5a	voltage gated sodium channel isoform 5a
SR	sarcoplasmic reticulum
TIMP	tissue inhibitor of metalloproteinase
TGF-β	transforming growth factor-β

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Highlights

- The adult heart cannot regenerate, so myocardial infarction (MI) results in a scar.
- Significant electrical and mechanical remodeling occurs after MI, resulting in arrhythmias.
- Modifying scar properties is a viable strategy for improving post-MI prognosis.
- Proteins involved in conduction and fibrous tissue structure are primary targets for altering scar properties.
- Further study is required to understand how modifying scar tissue affects heart function.

Figure 1.

Structural changes in healed infarct scar. A) A scar differentiates in the ischemic portion of the heart. b) Cx43 (green) is localized to the IDs in remote myocardium. c) Fibroblasts (blue) interrupt normal myocyte (red) connections in the IBZ adjacent to the scar and Cx43 reorganizes away from the IDs. Cx43 is often found between myocytes and fibroblasts. d) Punctate Cx43 is found within the scar.

Table 1

Therapeutic targets for regeneration of electrical and mechanical properties of MI scar tissue.

Target	Targeting strategy	Stage	Findings I	Mode	References
Connexin 40, 43	Adenovirus; engraftment of cells expressing Cx43	Preclinical	Enhanced atrial conduction, prevented atrial fibrillation, reduced arrhythmia susceptibility, faster CV through ventricular border zone	Pig, mouse	[11,133–135]
Connexin 43	Mimetic peptides: α CT1, Gap19/26/27	Preclinical	Reduce arrhythmia incidence and severity, increased CV through IBZ, Cx43 maintained at ID; Decreased infarct size; inhibit electrical coupling and/or hemichannel activity	Mouse, rat	[141,143,144,146]
Connexin 32	Adenovirus	Preclinical	Increased GJIC, no change in arrhythmia incidence, increased infarct size	Dog	[138]
miR-1	Antagomir	Preclinical	Decreased arrhythmogenesis	Rat	[140]
Cardiac sodium channel 4a (SCN4a)	Adenovirus	Preclinical	Reduced arrhythmia inducibility, increased CV through border zone	Dog	[150]
Sarcoplasmic reticulum calcium-ATPase 2a (SERCA2a)	Adeno-associated virus	Preclinical	Fewer spontaneous premature ventricular contractions and spontaneous or induced non-sustained VT episodes, fewer spontaneous SR calcium release events, reduced total SR calcium leak, fewer triggered arrhythmias	Rat, Sheep	[153]
Sarcoplasmic reticulum calcium-ATPase 2a (SERCA2a)	Adeno-associated virus	Clinical, Phase II	Decreased incidence of ventricular arrhythmias and triggers, improved arrhythmogenic substrate	Human	[155,156]
KCNH2-G628S	Adenovirus	Preclinical	Increased APD; prolonged refractory period, extending the reentrant wavelength and preventing arrhythmias	Pig	[158]
Potassium channel (Kv1.3, Kir2.1)	Engraftment of Kv1.3- and Kir2.1-expressing fibroblasts	Preclinical	Prolonged local refractory period, engrafted cells coupled with myocytes leading to reduced automaticity and prolonged refractoriness	Rat, Pig	[159]
Semaphorin 3a	Adenovirus	Preclinical	Increased levels of potassium channels at the border zone	Rat	[160]
Endothelin	ET-1 antagonists	Preclinical, Clinical	Cardioprotective effects; no improvements in cardiac remodeling in clinical trials, but also no adverse effects	Rat, Human	[180–184]
Angiotensin II pathway	Ang-II inhibition	Preclinical, Clinical	Decreased interstitial fibrosis, increased CV, and increased GJ conductance	Hamster, Human	[185,186]
Interleukin-1	IL-1 inhibition	Preclinical, Clinical	Attenuated LV remodeling, preserved cardiac function, allowed normal infarct healing	Mouse, Human	[190–193]
TGF- β pathway	TGF- β inhibition	Preclinical	Reduced fibrosis and progression to heart failure in a model of pressure overload	Mouse	[195]
MMP	Broad spectrum MMP inhibition; targeted MMP inhibition	Preclinical	Overall collagen area of MI scar changes very little with broad spectrum inhibition, but did limit LV dilation, reduced myocyte hypertrophy in non-	Mouse, Rat, Pig	[197–203]

Target	Targeting strategy	Stage	Findings I	Mode	References
			infarcted regions, decreased tissue and chamber compliance; No significant changes in collagen content in targeted reduction or transgenic knockouts of MMP-1, -3, -9, -12, and MT1-MMP		
Wnt pathway	Wnt pathway transgenics or interfering peptides	Preclinical	Reduced MMP level and leukocyte infiltration, decreased scar size, improved cardiac function, reduced fibrosis, reduced infarct expansion and development of heart failure	Mouse	[222–224]
Fibroblast density	NA	Modeling	At high density, fibroblasts cause resting depolarization and block propagation, protecting against arrhythmias	<i>In silico</i>	[226]
Scar stiffness	Scar anisotropy	Modeling, Preclinical	Increasing stiffness of isotropic infarct tissue does not alter LV pump function of the heart, stiffening scar in longitudinal direction (or longitudinal mechanical restraint) increases pump function	<i>In silico</i> , Dog	[12,233,234]
Scar stiffness	Intra-scar polymer injection	Preclinical, Modeling	Maintained fractional shortening and wall thickness; Stiff polymers improve cardiac mechanics; Injections into IBZ at multiple locations is most beneficial, while injections into scar produce mixed results on cardiac pressure-volume relationships	Rat, Sheep, <i>In silico</i>	[234,236–238]
Increase scar stiffness by increasing collagen content	Inhibiting MMPs or increasing TIMPs by hydrogel or adenoviral delivery	Preclinical	Reduced MMP activity and LV remodeling, increased fibrillary collagen content and LV ejection fraction	Mouse, Pig	[239–241]