Mesenchymal stem cell delivery strategies to promote cardiac regeneration following ischemic injury

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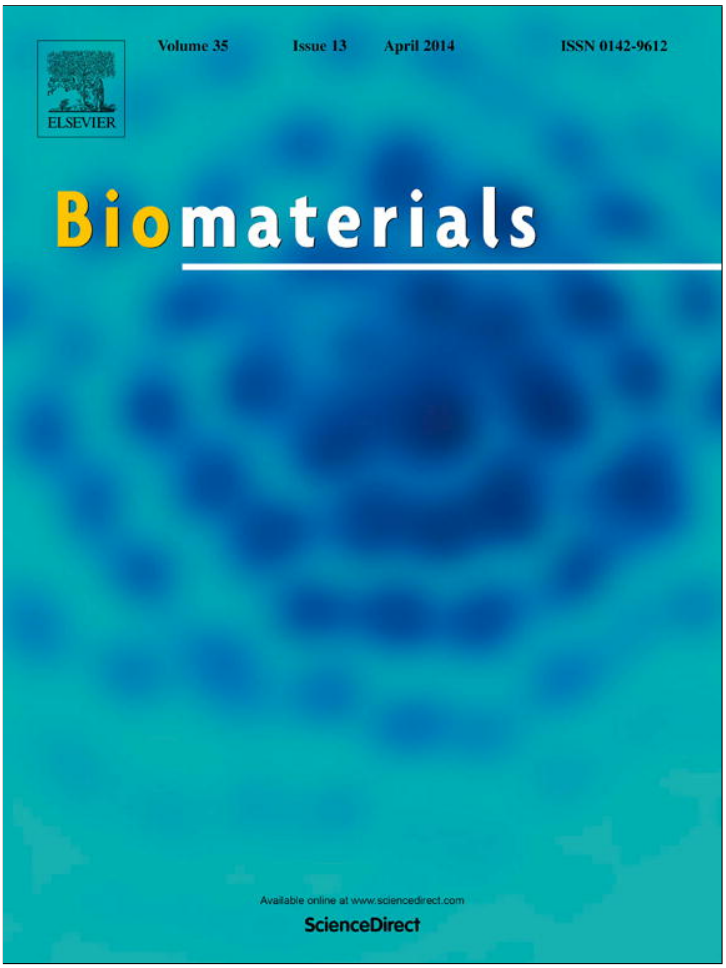

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Review

Mesenchymal stem cell delivery strategies to promote cardiac regeneration following ischemic injury

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ABSTRACT

Myocardial infarction (MI) is one of the leading causes of mortality worldwide and is associated with irreversible cardiomyocyte death and pathological remodeling of cardiac tissue. In the past 15 years, several animal models have been developed for pre-clinical testing to assess the potential of stem cells for functional tissue regeneration and the attenuation of left ventricular remodeling. The promising results obtained in terms of improved cardiac function, neo-angiogenesis and reduction in infarct size have motivated the initiation of clinical trials in humans. Despite the potential, the results of these studies have highlighted that the effective delivery and retention of viable cells within the heart remain significant challenges that have limited the therapeutic efficacy of cell-based therapies for treating the ischemic myocardium. In this review, we discuss key elements for designing clinically translatable cell-delivery approaches to promote myocardial regeneration. Key topics addressed include cell selection, with a focus on mesenchymal stem cells derived from the bone marrow (bMSCs) and adipose tissue (ASCs), including a discussion of their potential mechanisms of action. Natural and synthetic biomaterials that have been investigated as injectable cell delivery vehicles for cardiac applications are critically reviewed, including an analysis of the role of the biomaterials themselves in the therapeutic scheme.

1. Introduction and clinical scope

Cardiovascular disease (CVD) is the leading cause of mortality in Canada, the United States, and many European countries. In fact, CVD is the most lethal human medical condition in the developed world, accounting for 17.4 million deaths in 2008, or 30% of all deaths worldwide [1]. In the USA alone, more than 2150 people die each day from conditions associated with CVD, which is more than the combined total for cancer, chronic lower respiratory disease and accidents [2]. In addition to these dramatic mortality rates, the treatment of CVD places a significant economic burden on the healthcare system. In the USA, the direct and indirect costs of treating CVD were estimated to be more than $312.6 billion in 2009 [2].

CVD encompasses a broad range of medical conditions that affect the heart and vasculature. Focusing on coronary heart disease (CHD), the most common adverse event is myocardial infarction (MI). When the vascular flow is interrupted in MI, a portion of the heart experiences ischemic necrosis and is surrounded by a "marginal zone", which can recover function, but is susceptible to irreversible damage [3]. As a result of subsequent inflammation during the early phase of post-infarction remodeling, the marginal zone is expanded into the surrounding tissue. In the late remodeling stage, collagen scar maturation causes tissue fibrosis as well as ventricular dilation and wall-thinning. The process continues until the tensile strength of the scar balances the distending forces of the heart [3]. The infarcted tissue does not contribute to pressure generation during systole, leading to impaired cardiac function and increasing the work and energy consumption of the remaining viable segments of the ventricular myocardium. The rate of electrical signal

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propagation in the marginal zone may be heterogeneous and may lead to development of arrhythmias, such as ventricular tachycardia, with resultant inefficient cardiac contraction. In more serious cases, multiple chaotic electrical wavefronts can cause ventricular fibrillation where effective cardiac output is impossible [4].

Although current therapies (Fig. 1) have decreased mortality rates from acute MI, many survivors are left with chronic ischemia or complications related to myocardial necrosis such as congestive heart failure. It has been estimated that at least 12% of patients undergoing cardiac catheterization at tertiary medical centers for angina or cardiac ischemia have obstructive coronary disease not amenable to standard revascularization therapies [5]. Attempting further traditional revascularization in this group has not resulted in decreases in morbidity or mortality [6]. The present treatment of congestive heart failure mainly targets its symptoms, failing to address the underlying weakness of the ventricular muscle. When this approach becomes inadequate, ventricular assist devices and cardiac transplantation are considered, although potential demand for these expensive and resource-intensive therapies far exceeds availability [7].

As such, there is an evolving need for new strategies to promote non-traditional coronary revascularization and restoration of functional cardiac tissue following ischemic necrosis. Cell-based strategies involving the delivery of cells with reparative or regenerative potential into the afflicted regions of the heart have shown promise for the treatment of cardiac ischemia. Primarily, these approaches seek to replace damaged cardiomyocytes, promote revascularization, reduce fibrosis and improve ventricular compliance, pressure, and hemodynamics.

Numerous studies in animal models of MI have demonstrated functional benefits following the injection of bone marrow-derived mesenchymal stem cells (bMSCs) and adipose-derived stem cells (ASCs). The lack of significant *in vivo* cardiomyogenic differentiation and cell engraftment observed in the majority of these studies suggests that paracrine action may play a pivotal role in the improved cardiac function. Cell delivery scaffolds may have the potential to enhance the outcomes of MSC therapy by overcoming the limitations of poor localization, retention and viability associated with injecting cell suspensions [8]. With this view, recent research has focused on injectable hydrogels as delivery systems, which can be designed to provide temporary passive reinforcement to the myocardium and improve MSC retention within the site of delivery.

In this review, we will discuss the key elements that are critical for the development of an effective cell-based therapeutic strategy with MSCs for myocardial regeneration, including cell selection and elucidation of their biological mechanisms of action. Additionally, we will address a major challenge in the clinical translation of this strategy: effectively delivering and retaining viable cells in the heart. Biomaterials that could serve as injectable delivery vehicles for cardiac applications are critically reviewed, including an analysis of the role of biomaterials themselves in the therapeutic scheme.

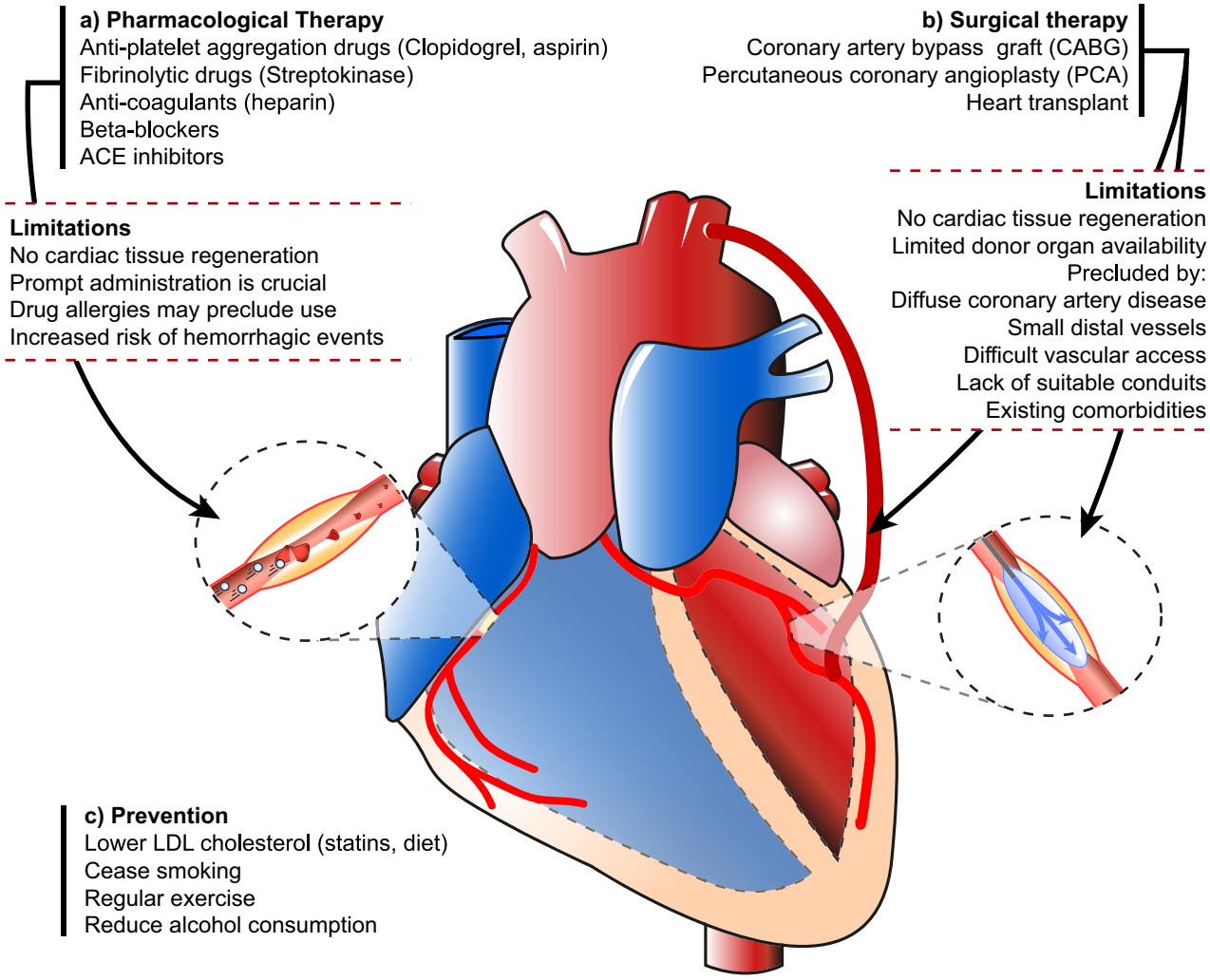

Fig. 1. Overview of the current clinical approaches for the prevention and treatment of myocardial ischemic injury.

2. MSCs for cardio-regenerative applications

For clinical applications, a regenerative cell source should be (i) abundant, (ii) accessible, (iii) easy to deliver within the scope of a single operation, (iv) tested and optimized in appropriate animal models, (v) non-tumorigenic and non-immunogenic, and (vi) should stimulate regeneration in a predictable manner with a well-characterized dose response. With this view, autologous cell therapies have advantages, particularly if the cells can be isolated and available for immediate use in the procedure suite during the time frame of a planned cardiac intervention. As the number of cells required for cardiac cell therapy is predicted to be high ($\sim 10^8$–10^9 per application [9,10]), an ideal source would yield a large number of the desired cell type with a harvesting process that is neither complex nor painful.

To date, many different cell types have been investigated both *in vitro* and *in vivo* for cardiac regeneration, including skeletal myoblasts, embryonic stem cells (ESCs), cardiac stem cells (CSCs), and MSCs [11]. Clinical trials with skeletal myoblasts have demonstrated only modest improvements in cardiac function and remodeling [12,13]. In rat models, transplanted ESCs have improved myocardial function, but the effects were temporary. Further, ESCs demonstrate immunological incompatibility, may form teratomas, and are associated with ethical issues that have limited their broad-scale application [14]. The clinical use of CSCs is impeded by the limited availability and accessibility of cardiac tissue, which contains only a small population of regenerative cells, necessitating large-scale cell expansion. The SCIPIO clinical trial is investigating autologous CSCs extracted from a portion of the atrial appendage resected during cardiac surgery. However, this can be risky in the elderly or in patients who have fragile atria or high right-side venous pressure [15–17]. While studies to date with MSCs have not convincingly demonstrated extensive differentiation into functional cardiomyocytes, their injection into the damaged myocardium can yield improved functional outcomes in the treatment of cardiac ischemia.

MSCs are multipotent adult stem cell populations that have differentiation potential typically associated with their mesodermal lineage (adipogenic, chondrogenic, osteogenic, myogenic). MSCs have been successfully harvested from a variety of adult tissues including bone marrow [18], adipose tissue [19], periostium [20], synovium [21], skeletal muscle [22], and deciduous teeth [23]. In this review, we will focus on two promising MSC sources for cardiac tissue engineering: bMSCs and ASCs (Table 1).

bMSCs have been one of the most extensively studied stem cell populations for regenerative approaches since they were first described by Friedenstein et al. in 1976 [48]. The development of protocols for bMSC isolation, purification and culturing have been reviewed in detail previously [18,49,50]. Typically, bMSCs are extracted from the bone marrow of the iliac crest. This procedure can be challenging for patients with end-stage ischemic heart disease. However, the promise of regeneration with bMSCs shown in animal models of MI (Table 2) has motivated further research and several clinical trials.

ASCs represent an attractive alternative due to their relative abundance, accessibility, multipotent differentiation capacity and demonstrated lack of immunogenicity [62]. Most patients would be able to donate a sample of fat for stem cell isolation with minimal adverse effects. In adult bone marrow, the fraction of MSCs to total nucleated cells is estimated to range between 1:50,000 and 1:1 million, and the limited volume of bone marrow that can be safely harvested per donor may necessitate significant *in vitro* expansion [24–26,63,64]. In contrast, ASC frequency in adult human adipose tissue is estimated to range between 1:30–1:100 per total nucleated cells [27,28]. As a potential allogenic cell source, large volumes of adipose tissue are routinely discarded during lipo-reduction surgeries. Due to the promising availability of adipose tissue as a regenerative cell source, numerous groups have been investigating the therapeutic potential of ASCs for the treatment of MI with encouraging results (Table 3).

Recently, our group has isolated and characterized ASCs derived from pericardial adipose tissue, which is a depot that would be readily accessible and expendable for intraoperative ASC isolation during open heart surgery [47]. As outlined in Table 1, while MSCs isolated from adipose tissue and bone marrow express similar cell surface markers, there is variability in the specific levels of expression between the sources that may have implications in terms of their regenerative capacity [30–36,75].

Importantly in the context of cardiac regeneration, injected bMSCs and ASCs migrate to sites of injury or ischemic tissues and contribute to regeneration [66,72,76]. While the mechanisms involved are not yet fully resolved, gradients of chemotactic factors

Table 1
Key characteristics of bMSCs and ASCs in the context of tissue regeneration.

	bMSCs	Both	ASCs
Cell yield (MSCs:total nucleated cells)	1:50,000 − 1 × 10^6 [24–26]		1:100 [27,28]
Immunophenotype	• $CD34^-$ and $CD73^-$ in freshly isolated cells [29] • $CD106^+$, $CD49f^+$, $PODXL^+$, $CD49d^-$ and $CD54^-$ upon culturing [30–34]	• $CD29^+$, $CD44^+$, $CD73^+$, $CD90^+$, $CD105^+$ and $CD166^+$ upon culturing [35,36]	• Expression of CD34 in freshly isolated cells [29,30] • $CD49d^+$, $CD54^+$, $CD106^-$, $CD49f^-$ and $PODXL^-$ upon culturing [30–34]
Strengths	• Demonstrated short-term safety in allogenic cardiovascular applications [37] • Extensively characterized	• Multilineage differentiation potential and CFU-F formation [35] • Immuno-modulatory activity	• Abundant, easily harvestable and uniquely expendable cell source associated with low donor site morbidity • High cell yield • Higher proliferation [38,39], angiogenic capacity [40]and immunomodulation [41–43] and lower senescence ratio [35] compared to bMSCs
Limitations	• Procedure sometimes complex and not suitable for patients with end-stage ischemic disease • Conflicting results in clinical trials • Risk of ectopic bone formation [44] • Limited quantity of aspirates collectable without adverse effects • Cell expansion challenging	• Low engraftment when delivered as a cell suspension • Limited *in vivo* differentiation • Influence of donor age and health status on cell response currently not well characterized	• Variability in the ASC population with gender, body mass index, and source depot [28,45–47] • Population not as extensively characterized to date; relative potency compared to bMSCs not fully elucidated • Safety for allogenic applications not yet demonstrated

Table 2
Summary of key studies investigating cell therapy with bMSCs in animal models of MI.

Animal model/ delivery method	Cell population	Time-points	Quantification of engraftment/retention	Differentiation into cardio-vascular components	Effects on cardiac function and/or ventricular remodeling	Ref.
Rat/i.m.	Autologous, (i) freshly isolated bMSCs, (ii) 5-aza treated bMSCs and (iii) untreated bMSCs	5 wks	• Engrafted cells at 5 wks (not quantified)	• Donor cells TnI$^+$ • ↑ Capillary density in all MSC-treated groups	• ↑ LVSP and LVDP in 5-aza treated group • ↓ Scar area and LV chamber size	[51]
Rat/i.m.	Autologous bMSCs + CMEC	3 - 12 wks	n.d.	• Donor cells factor VIII$^+$ • ↑ Capillary density and# of collateral vessels	• ↑ Myocardial perfusion and LVEF to 45 ± 2.2%	[52]
Rat/i.m.	Autologous bMSCs	8 wks	n.d.	• Donor cells vWF$^+$ • ↑ Capillary density	• ↑ LVEF and perfusion in scar	[53]
Rat/i.m.	Autologous bMSCs or peripheral blood mononuclear cells (PB-MNC)	1 – 60 days	n.d.	• Donor cells factor VIII$^+$ • ↑ Capillary density	• bMSCs restored LVD and LVEDV to baseline • ↑ FS • ↕ Collagen deposition for bMSC-treated group only	[54]
Rat/i.m.	Allogenic bMSCs	1, 2 and 4 wks	• Engrafted cells at 1–2 and 4 wks (not quantified)	• Donor cells CD31$^+$, α-SMA$^+$ and TnT$^+$ (low number) at 2 wks • ↑ Capillary density	• ↕ LVSP and wall thickness • ↓ LVEDP and infarct size	[55]
Mouse/i.m.	Allogenic c-kit$^+$ bMSCs	9 ± 2 days	n.d.	• Donor cells cardiac myosin$^+$, factor VIII$^+$ and α-SMA$^+$ • Genetic expression of cx-43, MEF2 and GATA-4	• ↕ LVDP • ↓ LVEDP and infarct area	[56]
Mouse/i.m.	Allogenic lin$^-$ bMSCs	2 wks	• Engrafted cells at 2 wks (not quantified)	• Donor cells α-sarcomeric actin$^+$ • New cardiomyocytes in infarct region • bMSCs incorporated into blood vessels (low number)	• ↓ Infarct size and fibrosis	[57]
Mouse/i.m.	Allogenic c-kit$^+$ bMSCs	5 – 10 days	• Engrafted cells at 10 days (not quantified)	• Donor cells positive for GATA-4, Nkx2.5, MEF2C, α-sarcomeric actin, cardiac MHC, TnI, desmin, cx-43, GATA-6, ETS-1, vWF and α-SMA • ↑ Capillary density and# of arterioles	• ↓ LVEDP and infarct size	[58]
Mouse/i.m.	Allogenic c-kit$^+$ bMSCs	30 days	• Engrafted cells at 2 days (~25% of donor cells detected in border zone)	• Donor cells vWF$^+$ and α-SMA$^+$ • Cx-43 and Nkx2.5 expressed at 48 h • Gap junctions between donor and host cells	• Restored contraction • ↕ LVDP and wall thickness • ↓ LVEDP, scar formation and chamber volume	[59]
Pig/i.m.	Autologous bMSCs	4 wks	• "Significant" engraftment at 6 months (not quantified)	• Donor cells TnT$^+$, MHC$^+$ and tropomyosin$^+$	• Preservation of wall thickness	[60]
Dog/i.m.	Allogenic bMSCs	30 days	n.d.	• Donor cells factor VIII$^+$ and α-SMA$^+$ • ↑ Vascular density	• ↕ LVEF • ↓ Collagen fibrosis	[61]

5-aza, 5-azacytidine; α-SMA, alpha smooth muscle actin; CMEC, coronary microvascular endothelial cells; FS, fractional shortening; i.m., intramyocardial; i.c., intracoronary; n.d., no data; LV, left ventricle; LVEDP, left ventricular end diastolic pressure; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVDP, left ventricular developed pressure; LVSP, left ventricular systolic pressure; LVD, left ventricular dysfunction; MHC, myosin heavy chain; TnI, troponin I; TnT, troponin T; vWF, von Willebrand factor; cx-43, connexin-43.

that are up-regulated in the ischemic myocardium can promote the homing of exogenous MSCs in cell-based therapies and stimulate the migration of endogenous stem cells [77]. MSCs express a variety of membrane receptors involved in the recognition of chemo-attractant cytokines, including stromal derived factor-1 (SDF-1), transforming growth factor-β1 (TGF-β1) and tumor necrosis factor-α (TNF-α), which are expressed in inflamed and injured tissues [78,79]. In particular, signaling through SDF-1/CXCR4 and HGF/c-MET has been identified as being of importance in stimulating the migration of reparative cells to the ischemic heart [79–83]. MSCs also secrete a range of growth factors and cytokines that play pivotal roles in mediating inflammation, immune responses, cell

Table 3
Summary of key studies investigating cell therapy with ASCs in animal models of MI.

Animal model/ delivery method	Cell population	Time points	Quantification of engraftment/retention	Differentiation into cardiovascular components	Effects on cardiac function and/or ventricular remodeling	Ref.
Rat/implanted cell sheet	Autologous ASCs	4 – 8 wks	• Thickening of cell sheet at 4 wks • <1% TUNEL positive cells at 48 h	• Donor cells cardiac troponin$^+$, vWF$^+$ and α-SMA$^+$	• ↑↓ LV maximum dP/dt • ↓ LVEDP • Inhibition of LV enlargement	[65]
Rat/i.m.	Allogenic ASCs, bMSCs, or AD-CMG	1 – 4 wks	• Low ASC and bMSC engraftment at 1 wk • No engraftment for any treatment group at 4 wks	• Donor cells MCL2V$^-$, α-SMA$^-$ and BSL-I lectin$^-$ at 1 and 4 wks • ↑ Capillary density	• ↑ LVEF at 4 wks in ASC group only • ↓ Infarct size in all treatment groups	[66]
Rat/i.m.	Allogenic ASCs	12 wks	• Engrafted cells in 2 of 11 rats at 12 wks (not quantified)	• Donor cells cardiac troponin$^+$ (very rare) • ↑ Capillaries density and# of arterioles	• ↑ LVEF, SV, CO and LV end-diastolic dimension	[67]
Rat/i.m.	Allogenic ASCs	1 – 4 wks	• n.d.	• Donor cells sarcomeric α-actinin$^+$ and α-MHC$^+$ (low levels) • ↑ Capillary density	• ↑ LVEF and % viable myocardium in infarct region • Preservation of LV wall thickness	[68]
Athymic nude rat/i.m.	Human ASCs	4 wks	• Engrafted cells in 3 of 10 rats at 4 wks (not quantified)	• Donor cells α-sarcomeric actin$^-$ and α-SMA$^-$ at 4 wks • ↑# of arterioles	• ↑ LVEF and FS • ↓ LVESV and LVEDV and infarct size	[69]
Nude rat and SCID mouse/i.m.	Human ASCs from epicardial fat	15 – 30 days	n.d.	• Donor cells TnI$^+$ and CD31$^+$	• ↑↓ EF, FS and wall thickness • ↓ Infarct size	[70]
SCID mouse/i.m.	Freshly-isolated or cultured human ASCs	1 – 4 wks	• Engraftment of both types of cells at 28 days (not quantified)	• Donor cells TnI$^+$, vWF$^+$ and α-SMA$^+$ • Cx-43 between donor and host cells • ↑ Capillary density in both treatment groups	• ↑↓ LVEF • ↓ LVESV	[71]
Pig/i.c.	Autologous ASCs or bMSCs	30 days	• Engrafted cells at 30 days (not quantified)	• Donor cells cardiac TnI$^-$, vWF$^+$, α-SMA$^+$ and desmin$^+$ • ↑ Capillary density	• ↑ LVEF and wall thickness with both populations	[72]
Pig/i.c.	Autologous freshly-isolated ASCs	4 – 8 wks	n.d.	• ↑ Capillary density in infarct border	• ↑ LVEF, myocardial salvage and wall thickness • ↓ Myocardial perfusion defects	[73]
Rabbit/i.m.	Autologous ASCs or 5-aza treated ASCs	5 wks	• Engrafted cells at 5 wks (not quantified)	• Donor cells α-sarcomeric actin$^+$, cardiac-specific TnT$^+$ and vWF$^+$ • ↓ Capillary density	• ↑↓ LVEF • ↓ Infarct size (more significant with 5-aza treatment)	[74]

5-aza, 5-azacytidine; α-SMA, alpha smooth muscle actin; CO, cardiac output; dP/dt, rate of rise of left ventricular pressure; FS, fractional shortening; EF, ejection fraction; i.c., intracoronary; i.m., intramyocardial; n.d., no data; LV, left ventricle; LVEDP, left ventricular end diastolic pressure; LVEDV, left ventricular end diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end systolic volume; LVDP, left ventricular developed pressure; LVSP, left ventricular systolic pressure; MHC, myosin heavy chain; SV, stroke volume; TnI, troponin I; TnT, troponin T; vWF, von Willebrand factor; cx-43, connexin-43; MLC2V, myosin light chain 2v; AD-CMG, adipose-derived cardiomyoblasts; BSL-I lectin, *Bandeiraea simplicifolia* lectin I.

migration and importantly, in the context of cardiovascular regeneration, angiogenic and vasculogenic factors. This natural secretome has been exploited by many groups assessing cell-based therapies for the treatment of ischemia using induced MI and ischemic hind limb animal models [52,84–86].

Another important feature of MSCs in the context of cell-based therapies is their immunoprivileged status, which may enable the application of allogenic cells with fewer concerns about the initiation of undesired immune responses. This characteristic, which is believed to be associated with very low expression of the major histocompatibility complex II (MHC II) antigen, has been examined both *in vitro* and *in vivo*. Furthermore, results with bMSCs and ASCs indicate that both cell populations modulate T-cell activation, proliferation, migration and inflammatory cytokine secretion [43,87–90]. Recently, Melief et al. showed that ASCs elicit a more potent suppression of peripheral blood mononuclear cell (PBMC) proliferation and a more effective induction of a shift from Th1 response to Th2 response *in vitro* when compared to bMSCs [43]. The assessment of the specific mechanisms by which MSCs mediate the immune response is an area that warrants further investigation, to develop a deeper understanding of how MSCs may promote a more regulatory versus inflammatory condition within the transplant environment.

2.1. Mechanisms of MSC-based cardiac regeneration

Several possible mechanisms by which exogenous MSCs can enhance cardiac regeneration have been proposed: (i) the mobilization of endogenous stem cells capable of cardiomyogenesis to the

site of injury through chemotactic signaling, (ii) the reduction of fibrosis and improvement in cardiomyocyte survival mediated in part through neo-angiogenesis and vasculogenesis, (iii) the mechanical reinforcement of the infarct scar, allowing endogenous repair mechanisms to improve left ventricular function, and (iv) the reduction in the initial tissue damage through the modulation of the inflammatory response.

It is important that *in vivo* studies are designed to assess the mechanisms of MSC-mediated regeneration. Key endpoints that should be considered in assessing the efficacy of a cell-based therapy for MI include:

i. The engraftment, retention, and survival of the transplanted cells
ii. The re-population of lost cardiomyocytes through *de novo* functional cardiomyogenesis and/or the promotion of endogenous cardiomyocyte proliferation
iii. The integration of *de novo* cardiomyocytes with the host electromechanical network
iv. The induction of angiogenesis and vasculogenesis through paracrine-mediated effects or direct differentiation of the transplanted cells into vascular structures
v. Improvements in cardiac function, as measured by left ventricle stroke volume (LVSV), ejection fraction (LVEF), and fractional shortening (LVFS), both at rest and under stress
vi. The prevention or attenuation of left ventricular remodeling in terms of tissue fibrosis, infarct size and ventricular chamber dilation, measured by end diastolic and end systolic diameters (EDD, ESD)

For bMSCs (Table 2), in 2001, Orlic et al. first demonstrated that intramyocardial injection of undifferentiated lineage-negative (Lin^-) bMSCs in a mouse model of MI resulted in the formation of new cardiac tissue and also improved cardiac contractility [56]. Two years later, Kudo et al. demonstrated that bMSC transplantation reduced fibrosis and infarct size, with evidence of bMSC differentiation into cardiomyocytes and endothelial cells within the ischemic myocardium [57]. Similar results were reported by Zhang et al. in a study comparing the effects of intramyocardial injection of bMSCs versus PBMCs that demonstrated functional improvements with only bMSCs [54]. In a more recent study, a highly proliferative bMSC subpopulation was isolated from human bone marrow by Yoon et al. Intramyocardial injection of this subpopulation in a nude rat model resulted in a higher degree of engraftment and differentiation as compared to unselected bMSCs, and also induced endogenous cardiomyogenesis [91].

Similarly for ASCs (Table 3), Valina et al. first compared the effect of intracoronary injection of ASCs and bMSCs in a porcine model. Although cardiac differentiation was not detected, some exogenous cells expressed endothelial lineage markers. Furthermore, both cell types induced comparable improvements in cardiac function and increased capillary density within the infarct zone [72]. Zhang et al. observed similar outcomes in a rat model of MI, comparing ASCs induced towards a cardiomyocyte phenotype using 5-azacytidine (5-aza) with untreated ASCs. In their model, LVEF and capillary density were dramatically increased in both treatment groups as compared to the controls. Additionally, cardiomyogenic differentiation of the ASCs was observed, along with a reduction in the infarct size, with indications that 5-aza pre-treatment was beneficial in reducing the infarcted region [74].

2.1.1. *In vitro differentiation potential*

In vitro studies have demonstrated the potential for MSCs to differentiate into the major cellular components of cardiac tissue when the culture medium is supplemented with specific factors, as outlined in Table 4. It is important to note that this induced *in vitro* differentiation may not be reflective of the typical cell response following *in vivo* transplantation. Further, it has been postulated that the use of some differentiation factors may result in unpredictable reprogramming patterns that could increase the potential for the malignant transformation of the cells [106].

Co-culture systems with MSCs and cardiomyocytes have allowed scientists to develop models to better understand the cell–cell interactions that can occur at the site of transplantation. Both bMSCs [107] and ASCs [108] have been induced to undergo cardiomyogenic differentiation using this approach. This microenvironment-induced differentiation has been hypothesized to be mediated by cell–cell contact [109,110], although similar results have been obtained through indirect co-culturing [108] as well as exposure to cardiomyocyte lysates [111] or ischemic cardiac tissue-conditioned medium [112]. These results suggest that *in vivo*, where a broad array of microenvironmental cues are present, MSC cardiomyogenic differentiation could result from multiple pathways.

2.1.2. *In vivo regeneration through MSC engraftment and differentiation*

The results of several *in vivo* studies support the possibility that transplanted MSCs could contribute to regeneration through direct engraftment and differentiation. Cell fusion, rather than transdifferentiation, has been proposed as a driving mechanism for these events [58,113]. However, in strategies to date, cardiomyogenic differentiation is often not observed [61,66,69,72] or at best occurs at extremely low levels. Even when there is evidence of differentiation, most studies include only limited qualitative assessments. In seeking to quantify the findings, based on two studies, only 0.5–5% of the engrafted cells have been reported to differentiate [65,68]. Further, histological evidence of MSC differentiation has not always been paralleled by a functional benefit [51]. In particular, although the coupling between *de novo* and host cardiomyocytes through gap junction formation has been documented with both bMSCs [59] and ASCs [71], transplanted bMSCs failed to generate spontaneous and electrically-evoked intracellular calcium transients 9–10 days after delivery to the peri-infarct region in a mouse MI model [114].

Neovascularization must proceed in parallel with cardiomyogenesis to compensate for the ischemic conditions. Although at low levels, integration of transplanted MSCs into vascular structures has been reported in many animal models of MI (Tables 2 and 3). The critical importance of vasculogenic differentiation in improving cardiac function has been recently demonstrated by Yoon et al. Through an elegant suicide gene-based system, it was shown that depletion of cells differentiating toward the vascular lineages drastically reduced functional benefits, whereas elimination of cardiomyogenic cells had no significant effect [115]. In a few studies that have compared ASCs and bMSCs, capillarization and ventricular remodeling have been reported to be more prominent following ASC injection [66,72].

2.1.3. *Regeneration through paracrine-mediated effects*

Despite the modest levels of engraftment and differentiation, the injection of bMSCs or ASCs increases capillarization and neoangiogenesis, in some cases almost doubling vascular density as compared to controls [51,53,61]. Similarly, echocardiographic analysis has shown that MSC-based therapies can significantly improve left ventricular diastolic and systolic pressure, LVEF, ESD and EDD, as well as vascular perfusion in the scar. These functional improvements are likely associated with a reduction in infarct size, preservation of wall thickness, and reduction in tissue fibrosis, which all contribute to enhanced ventricular compliance. The

Table 4
Factors investigated for the *in vitro* differentiation of MSCs towards cardiovascular lineages.

Lineage	Cell population	Supplements	Differentiation response	Ref.
Cardiomyogenic	bMSCs/mouse	3 μM/L 5-aza + 20% FBS	• Spontaneously beating cardiomyocytes	[92]
Cardiomyogenic	bMSCs/human	10 μM/L 5-aza + 10 μg/L bFGF + 0.25 mg/L amphotericin + 10% FBS	• MEF-2A and MEF-2D gene expression	[93]
Cardiomyogenic	bMSCs/human	1.0 mg/mL insulin + 10^{-9} M dex + 10^{-4} M ascorbate phosphate + 0.47 μg/mL linoleic acid + 0.55 mg/mL transferrin + 0.5 μg/mL sodium selenite + 10% FBS	• Expression of GATA-4 and MEF2, cardiac TnI, cx-43, cardiac-specific α/β-MHC, and L-type cardiac a_{1c} Ca^{2+} channel	[94]
Cardiomyogenic	ASCs/rabbit	1, 3, 6, and 9 μM/L 5-aza + 15% FCS	• Spontaneous beating • Expression of MHC, actinin and Troponin-I	[95]
Cardiomyogenic	ASCs/human	9 μM 5-aza + 0.12 μg/cm^2 laminin coating or 2.5 μg/cm^2 fibronectin coating + 15% FBS	• Expression of SERCA2α and MLC2α	[96]
Cardiomyogenic	ASCs/mouse and human	1% methylcellulose medium + 10 ng/mL IL-3 + 10 ng/mL IL-6 + 50 ng/mL SCF + 100 μM 2-mercaptoethanol + 2 mM L-glutamine + 10 μg/mL recombinant human insulin + 200 μg/mL transferrin + 15/30% FBS	• Contractile activity • Expression of GATA-4 and Nkx2.5, MLC-2v and MLC-2a	[97,98]
Endothelial	bMSCs/human	50 ng/mL VEGF + 12% FCS	• Expression of vWF • Ability to form capillaries in semi- solid medium	[99]
Endothelial	bMSCs/human	100 ng/mL VEGF + 50 ng/mL EGF + 1 μg/mL hydrocortisone + 5% FBS	• Expression of vWF, flK-1 and VE-cadherin • Ability to uptake acLDL	[100]
Endothelial	ASCs/human and rat	20 − 50 ng/mL VEGF + 10 − 2% FCS	• Expression of vWF and ability to uptake acLDL • Expression of CD31	[55,101]
Endothelial	ASCs/rat	EGM2 media + 1 μg/mL ascorbic acid + 22.5 μg/mL heparin + 0.2 μg/mL hydrocortisone + 5 ng/mL EGF + 10 ng/mL FGF2 + 20 ng/mL IGF-1 + 0.5 ng/mL VEGF + 2% FBS	• Expression of endothelial markers CD31, vWF, and eNOS • Formation of endothelial-like tube structures	[102]
Smooth muscle	bMSCs/human	1 ng/mL TGF-β1 + 2 ng/mL ITS + 10% FBS	• Expression of α-SMA	[103]
Smooth muscle	ASCs/human	100 units/mL heparin + 1% FBS	• Expression of α-SMA, smoothelin, calponin, caldesmon • Dose dependent contraction in response to muscarinic agonists	[104]
Sinus-node like cells	bMSCs and ASCs/human	10 μM 5-aza + 10% FBS	• Expression of HCN2 and HCN4	[105]

5-aza, 5-azacytidine; dex, dexamethasone; ITS, insulin, transferrin, selenium; α-SMA, alpha smooth muscle actin; MHC, myosin heavy chain; MLC, myosin light chain; vWF, von Willebrand factor; acLDL, acetylated low density lipoprotein.

positive effects observed in the absence of long-term engraftment and differentiation suggest that the MSCs primarily function through paracrine-mediated effects to establish a more regenerative milieu within the host tissues. This theory is supported by the work of Kinnaird et al. who showed that injecting bMSC-conditioned medium increased the collateral perfusion in a murine model of peripheral ischemia [84].

Table 5 highlights some key factors secreted by MSCs and their potential biological effects within the ischemic heart. Among the many proteins secreted by MSCs, VEGF is potentially one of the most important mediators of angiogenesis in the ischemic heart. Tang et al. assessed the presence of VEGF in the infarct region through immunohistochemistry and detected its up-regulation following bMSC injection [53]. The critical role of VEGF in MSC-induced myocardial revascularization was further demonstrated by Markel et al., who showed that small interference RNA (siRNA) directed against VEGF negatively affected the recovery of myocardial function in a rat model of MI [126].

While genetic engineering approaches with MSCs are beyond the scope of the current review, the importance of chemotactic signaling pathways has been demonstrated by studies where the forced over expression of chemotactic receptors or ligands, including CXCR4, insulin-like growth factor-1 (IGF-1) and chemokine receptor-1 (CCR1), have enhanced the homing, viability and/or engraftment of injected MSCs [127–131].

Finally, MSCs respond to variations in oxygen tension through hypoxia-inducible factors (HIFs), which can up-regulate the expression of a broad array of genes associated with metabolism [132,133], proliferation and viability [84,118,134–136], angiogenesis and vascular remodeling [137–145], and cellular motility [146,147]. As such, a number of groups are trying to better understand the biological mechanisms of this hypoxia-induced gene up-regulation, in order to harness it as a means to improve the regenerative capacity of the transplanted cells in the context of the ischemic heart [142,144–146,148].

2.2. Clinical trials with MSCs for myocardial regeneration

Based on the positive results observed in animal models, several clinical trials investigating bMSCs in the treatment of MI have been

Table 5
Paracrine factors secreted by MSCs in the context of tissue regeneration in ischemic tissues.

Biological effect	Factors	References
Angiogenesis	VEGF	[52,53,65,70,84,86,116–120]
	bFGF	[52,70,86,118]
	Ang-1	[52,119,120]
	PDGF	[84,119,121]
Inflammation mediation/ angiogenesis	IL-1, 6, 7, 8, 11, 12, 14	[70,84,86,121,122]
	CINC	[86]
	MCP-1	[86,121]
	TGF-β	[84,117,119]
	TNF-α	[70,84]
Cytoprotection and chemotaxis	HGF	[65,117,123,124]
	IGF	[116,118,125]
	SDF	[118,124]

Ang-1, angiopoietin 1; CINC, cytokine-induced neutrophil chemoattractant; MCP-1, monocyte chemotactic protein 1.

initiated. An initial short-term trial demonstrated that intracoronary injection of autologous bMSCs was safe and beneficial in terms of improved cardiac function at 3 months [149]. However, longer-term trials with autologous bMSCs have yielded conflicting data. The TOPCARE-AMI (8.5% treatment *vs*. 2.5% control), REPAIR-AMI (5.5% *vs*. 3.0%), and BOOST (5.9% *vs*. 3.1%) trials have all shown increased LVEF compared to control groups at 4, 12 and 18 months post-treatment, respectively [150–152]. The TOPCARE-AMI trial also compared the effects of bMSC treatment to cardiac progenitor cell (CPC) infusion, with the bMSCs showing a greater improvement in terms of left ventricular contractility [153]. However, a study conducted in Belgium, as well as the ASTAMI and BONAMI trials, demonstrated no significant improvement in cardiac function between control and bMSC-treated patients [154–156]. Further, a 5-year follow up of the patients enrolled in the BOOST trial showed that the beneficial effects observed at 18 months post-treatment in terms of cardiac contractility were not sustained. In contrast, the 5-year follow up of the TOPCARE-AMI study showed a significant improvement in LVEF in the bMSC-treated group, as well as reductions in end-systolic volume and end-diastolic volume. The observed differences between the trials may result from donor variability, the diverse methods of cell isolation and delivery employed, as well as different baselines for assessing left ventricular function [157,158].

Numerous ongoing clinical trials, including the REVITALIZE (NCT00874354), BAMI (NCT01569178), REGEN-AMI (NCT00765453) and REPAIR-ACS (NCT00711542) trials, are seeking to further elucidate the effects of autologous bMSCs in the treatment of MI through multinational, multicenter studies. Given the potential benefits of allogenic transplantation, the results from the first phase I clinical trial to test the safety and efficacy of intravenously-injected allogenic bMSCs were reported in 2009. Promisingly, in 53 patients who suffered MI, there were no rejection issues and heart function was improved, as measured by LVEF that showed a 6.7 ± 2.2% increase at 6 months. Nevertheless, when patients' physical performances were assessed using the 6 min walk test, no differences between the treated and control groups were observed [37].

Clinical trials for the treatment of MI with autologous human ASCs have also been initiated. More specifically, the APOLLO [159] and the PRECISE [160] trials have investigated the transplantation of freshly-isolated ASCs in 14 patients with MI that have demonstrated acute ST-elevation by electrocardiography, as well as 27 patients with non-revascularizable cardiac ischemia. Although statistically limited by the low number of subjects, these two trials have demonstrated the feasibility, safety and efficacy of autologous ASC administration and motivated the initiation of ATHENA (NCT01556022), a PHASE II clinical trial enrolling 45 patients, in June 2012. Additionally, MyStromalCell is a PHASE II clinical trial initiated in April 2010 that was designed to assess the efficacy of expanded autologous ASCs stimulated with VEGF-A prior to injection [161].

3. Cell delivery to the ischemic heart

The efficacy of cell therapy depends on the ability of the delivery mechanism to provide sufficient localization, survival, and retention of the donor cells in the affected tissues [162]. These challenges are particularly apparent in the field of cardiac tissue regeneration following MI, where the host environment presents numerous unique obstacles to cellular transplantation. Nevertheless, in the vast majority of clinical trials, an isotonic saline solution was routinely employed as the cell delivery vehicle [37,163]. These solutions possess physiological osmolarity but do not have the capacity to localize or retain the administered cells within the target site while maintaining long-term cell viability.

3.1. Challenges of cell delivery: localization, survival, and retention

Compressive forces produced by continuous, periodic systolic contraction, as well as shear forces caused by hemodynamic flow, can cause the dissemination of cells injected in saline. One hour following delivery of PBMCs via intramyocardial, intracoronary, and interstitial retrograde coronary venous injection, retention rates were 11 ± 3%, 2.6 ± 0.3%, and 3.2 ± 1%, respectively, with the greatest fraction lost to the pulmonary circulation [164]. Further, confocal microscopy of green fluorescent protein (GFP)-labeled MSCs injected intramyocardially has shown delivery in saline results in variable cellular distribution throughout the depth of the ventricle, with a decrease in retention from 31% to 1% over five days post-injection [8]. An uneven distribution could further exacerbate the already disrupted alignment of the surviving cardiomyocytes, interrupting the conduction of electrical and mechanical signals through the parallel bundles of cardiomyocytes, potentially producing a pro-arrhythmic heterogeneous milieu [165].

The short-term survival of the retained cells represents a further challenge [166]. In the post-MI heart, ischemia results in localized hypoxia and nutrient depletion [167], while the acute inflammatory reaction is associated with leukocyte and macrophage infiltration, fibrotic tissue deposition, oxidative load, and the generation of apoptotic cytokines [168]. Postponing delivery may avoid some inflammatory effects of acute-MI, but later stages of MI present additional challenges: advancing tissue fibrosis, ventricular dilation and wall thinning, and reduced vascularization all result in an apoptotic environment deficient in oxygen and nutrients [169]. Continuing cardiomyocyte death contributes to the release of apoptotic factors that further threaten the survival and function of the transplanted cell population [170].

Considering these events, timing of cell delivery emerges as an important factor in regenerative efficacy. A study by Hu et al. concluded that delivery of MSCs one week post-MI provided greater functional benefit as compared to delivery after 1 h or 2 weeks [171]. However, there is motivation to initiate stem cell therapy before the localized inflammatory response has subsided, as inflammatory cytokines are known to be involved in the homing, differentiation, and activation of MSCs [172].

3.2. Designing a cell delivery vehicle

Recent efforts have focused on biomaterial scaffolds as a means of delivering cells to the heart. An ideal scaffold would (i) introduce a critical cell population at the site of infarction, (ii) maintain their localization and viability over the course of the wound healing response, and (iii) promote the sustained production and release of beneficial paracrine factors into the ischemic region. Two general methods have been explored: surgical implantation of cells in pre-formed scaffolds, and the injection of cells in a polymer precursor solution that forms a cell-encapsulating hydrogel scaffold *in situ*. While implantation allows greater control over physical structure, it also necessitates open surgery. Injectable scaffolds represent a less invasive approach that could minimize damage to the already injured tissue, require shorter procedure and recovery times, and utilize existing catheter delivery technology to expedite translation to clinical practice. Emphasizing the role of cells as trophic mediators, this review focuses on injectable biomaterials that have demonstrated potential for delivering cells to the heart, their required material properties in terms of chemical, physical, and biological action, and their lifecycle in the body, from delivery to degradation.

Table 6
Representative natural biomaterials investigated *in vivo* as potential injectable cell-delivery scaffolds for cardiac cell therapy.

Material class	Cell type/animal model	Experimental design	Gelation mechanism	Ref.
Alginate	Cell-free/rat	i.m. (100–150 μL) 7 or 60 days post-MI	Ca^{2+} dependent physical gelation	[173]
Alginate	Cell-free/swine	i.c. (1,2, or 4 mL) 4 days post-MI	Ca^{2+} dependent physical gelation	[174]
Alginate (±RGD, ±YIGSR)	Cell-free/rat	i.m. (130 μL) 7 days post-MI	Ca^{2+} dependent physical gelation	[175]
Alginate microspheres (+RGD)	Human bMSCs/nude rat	Closed chest, echocardiography guided injections (100 μL) 7 days post-MI	Ca^{2+} dependent physical gelation	[176]
Chitosan	Mouse ESCs/rat	i.m. at infarct border (1×10^7 ESCs in 100 μL) 1 wk post-MI	Temperature responsive, glycerol phosphate-dependent physical gelation	[177]
Chitosan	Allogenic ASCs/rat	i.m. at infarct border (4×10^6 ASCs in 100 μL)	Temperature responsive, glycerol phosphate-dependent physical gelation	[178]
Collagen	Cell-free/rat	i.m. at infarct centre (100 μL) 1 wk post-MI	Physical fibrillogenesis of Zyderm® type II	[179]
Collagen	Allogenic bMSCs/rat	i.m. injection at infarct centre (2×10^6 bMSCs in 70 μL) 1 wk post-MI	Physical fibrillogenesis of Zyderm® type I	[180]
Fibrin	Allogenic skeletal myoblasts/rat	i.m. (5×10^6 myoblasts in 50 μL) 1 wk post-MI	Physical assembly of fibrin after co-injection of Tisseel VH sealant with thrombin and $CaCl_2$	[181]
Fibrin (PEGylated)	Allogenic BMNCs/mouse	i.m. in infarct and border zone (5×10^5 BMNCs in 50 μL) immediately post-MI	Physical assembly of fibrin after injection of PEGylated fibrinogen pre-mixed with thrombin	[182]
Fibrin	Allogenic ASCs/rat	i.m. to infarct border (5×10^5 ASCs in 100 μL) 1 wk post-MI	Physical assembly of fibrin after co-injection of Tisseel VH sealant with thrombin and $CaCl_2$	[183]
Hyaluronic acid (methacrylated)	Cell-free/sheep	i.m. to infarct area (20 × 0.3 mL) 30 min post-MI	Covalent radical polymerization with APS and TEMED initiators	[184]
Hyaluronic acid (HEMA)	Cell-free/sheep	i.m. to infarct area (20 × 0.3 mL) 30 min post-MI	Covalent radical polymerization with APS and TEMED initiators	[185]
Matrigel	Allogenic ESCs/mouse	i.m. to infarct (1×10^6 in 50 μL) immediately post-MI	Temperature responsive physical gelation	[186]
Matrigel (+collagen)	Allogenic neonatal cardiomyocytes/rat	i.m. to infarct ($2–3 \times 10^6$ in 150 μL) 3 wks post-MI	Temperature responsive physical gelation	[187]
Matrigel (+pro-survival factors)	hESC-derived cardiomyocytes/ athymic nude rat	i.m. to infarct (10×10^6 hESCs in 70 μL) 4 days post-MI	Temperature responsive physical gelation	[188]

i.m., intramyocardial; i.c intracoronary; PEG, Poly(ethylene glycol); HEMA, hydroxyl ethyl methacrylate.

Tables 6 and 7 highlight the major classes of injectable biomaterials that have been identified as potential delivery scaffolds.

3.3. Material requirements

3.3.1. Injectability

The injectable scaffold approach introduces several design requirements:

i. The material and cell suspension must be a catheter-transferable liquid for either intracoronary, epicardial, or transendocardial injection;
ii. Once injected, the solution must quickly undergo a gelling process to prevent dissemination of the material and cells without damaging surrounding tissues or reducing stem cell viability and function;
iii. The gelled scaffold must maintain structural integrity over the course of the cell therapy to negate the risk of peripheral or cerebral embolism;
iv. To obviate the requirement of surgical removal, the biomaterial should ultimately degrade in a manner that does not harm the local tissue or adversely affect its function, while yielding non-toxic and readily metabolized or eliminated degradation products.

The three routes of injection feature unique risks and benefits. Intracoronary delivery is similar to balloon angioplasty, and therefore utilizes well-established and common tools and techniques. While it is the least invasive approach and does not directly puncture the myocardium, it provides the lowest control over the amount and location of the delivered material and raises the risk of microembolization [198]. Epicardial injection allows for precise control over delivery and good visualization; however, the necessary open-chest or endoscopic surgery requires the puncture or removal of the pericardium, significantly increasing the risk to the patient. The endocardial route provides similar access directly to the ischemic tissue, while the use of a catheter bypasses the need for surgery. However, visualization requires indirect imaging, and injecting material into the beating myocardial wall is technically challenging, increasing the risk of material dissemination into the ventricle [199]. Particularly in the cardiac setting, material dissemination must be tightly controlled to mitigate the potentially critical risk of systemic embolization.

Various non-cytotoxic mechanisms of gel-formation have been investigated, including two-component chemical cross-linking, UV photo-cross-linking, and thermal- or pH-induced physical gelling. The timescale and conditions of gelation are critical factors. If gelation occurs prematurely or too rapidly, the catheter could become clogged; if gelation is delayed, the biomaterial would provide no benefit over liquid-phase delivery. Ideally, the gelation mechanism would allow for consecutive injections at multiple sites.

3.3.2. Mechanical properties

Appropriate mechanical properties of the gelled scaffold are critical for supporting the damaged ventricular wall. A scaffold providing strength and thickness to the myocardium could temporarily compensate for the loss of contractile tissue, thereby reducing the extent of remodeling and scar formation required to balance hemodynamic forces [200,201]. Many groups have focused on designing gels with stiffness matching healthy myocardium, based on an end-diastolic elastic modulus of approximately 10 kPa

Table 7
Representative synthetic biomaterials investigated *in vivo* as potential injectable cell-delivery scaffolds for cardiac cell therapy.

Material class	Cell type/animal model	Experimental design	Gelation mechanism	Ref.
Self-assembling peptides (with IGF-1)	Allogenic CPCs/rat	i.m. to infarct border (1×10^5 CPCs in 5 μL + 5 μL peptides) immediately post-MI	Physical self-assembly	[189]
Self-assembling peptides	Autologous BMNCs/swine	i.m. to infarct (1×10^8 BMNCs in 2 mL) immediately post-MI	Physical self-assembly	[190]
mPEG-PCL-mPEG with α-cyclodextrin	Allogenic bMSCs/rabbit	i.m. to infarct (2×10^7 bMSCs in 4 x 50 μL) 7 days post MI	Physical assembly of copolymer within α-cyclodextrin cavities	[191]
8a-PEG-VS + cysteine-flanked peptide	hESCs-derived ELCs and SMLCs/nude rat	i.m. to infarct (0.66×10^6 ELC and 0.33×10^6 SMLC in 60 μL) 1 h post-MI	Covalent Michael type addition between peptide thiol groups and vinyl sulfone groups	[192]
Oligo (PEG fumarate) + PEGDA	Mouse ESCs/rat	i.m. to infarct border (1×10^6 ESCs in 3 x 33 μL) 1 wk post-MI	Covalent radical polymerization with APS and TEMED initiators	[193]
PVL-PEG-PVL	Cell-free/rat	i.m. to infarct border (4 x 25 μL) 7 days post-MI	Physical gelation via temperature-dependent hydrophobic aggregation	[194]
PNIPAAm-co-(HEMA-PCL -grafted dextran)	Cell-free/rabbit	i.m. (4 × 50 μL) 4 days post-MI	Physical gelation via temperature-dependent hydrophobic aggregation	[195]
PNIPAAm-co-AAc-co-HEMApTMC	Cell-free/rat	i.m. into infarct and border (5 x 100 μL) 2 wks post-MI	Physical gelation via temperature-dependent hydrophobic aggregation	[196]
PNIPAAm-co-AAc + peptide cross-linker + RGD-grafted pAAc	Allogenic bMSCs/mouse	i.m. injection into infarct border (1×10^5 bMSCs in 10 μL) immediately post-MI	Physical gelation via temperature-dependent hydrophobic aggregation	[197]

i.m., intramyocardial; i.c, intracoronary; CPCs, cardiac progenitor cells; PEG, Poly(ethylene glycol); PCL, poly(ε-caprolactone); 8a-PEG-VS, 8-arm PEG vinyl sulfone; ELC, endothelial-like cells; SMLC, smooth muscle-like cells; PEGDA, PEG diacrylate; PVL, poly (δ-valerolactone); pNIPAAm, poly(*N*-isopropyl acrylamide); AAc, acrylic acid; HEMA, hydroxyl ethyl methacrylate; pTMC, poly(trimethylene carbonate).

[200]. There is evidence that stiffer gels with elastic moduli over 40 kPa are more effective at preventing maladaptive remodeling [184]. This approach somewhat counter-intuitively mimics the properties of fibrotic scar, as a stiffer material may better stabilize the myocardium and bear more wall stress, leading to reduced infarct expansion and LV dilation [184]. An optimal biomaterial should provide the mechanical stiffness to passively balance peak distending forces during end-systole, since these forces are a cause of the maladaptive remodeling process [184]. Subsequent material degradation should occur in response to cellular infiltration, vascularization, and formation of contractile tissue, such that the load originally borne by the scaffold is transferred to the regenerated myocardium. The scaffold must also be pliable and robust, withstanding fatigue failure at least until the tissue has recovered. Aligning mechanical properties with the surrounding tissues may additionally minimize physical irritation, promote integration at the site of delivery, and recreate a myocardial-like microenvironment for the delivered MSCs.

3.3.3. Cell–material interactions

Complex signaling interactions between the scaffold, the surrounding extracellular matrix (ECM), and the cells (both transplanted and host) must be considered across a range of length scales:

i. Direct contact interactions: including mechanical properties such as stiffness of the material and bioactive surface properties, whether inherent or engineered.
ii. Microenvironmental interactions: including the microstructure and porosity of the scaffold; paracrine signaling molecules released by transplanted cells or the scaffold itself; and the formation of a regenerative milieu by locally concentrating cytokines.
iii. Systemic interactions: the recruitment of host stem cells from the systemic circulation and the prevention of donor cell migration; promotion of vascularization within and surrounding the scaffold; mediation of the host immune response.

Scaffold properties are influential in directing the cellular response, including migration [202], phenotype [203], and differentiation [204]. The microstructural properties of the scaffold, including cross-link density and porosity, also play critical roles in cell therapy by influencing the diffusion of nutrients, oxygen, and cytokines [205,206].

4. Injectable biomaterials as scaffolds for MI cell therapy

4.1. Alginate

Alginate, a natural polysaccharide derived from algae, is composed of (1-4)-linked β-D-mannuronic acid (M unit) and α-L-guluronic acid (G unit) arranged in block and atactic regions (Fig. 2a). The addition of divalent cations such as Ca^{2+} causes the binding of G unit blocks on adjacent chains, rapidly forming an ionic cross-linked network. The elevated concentration of calcium ions in the post-MI environment has been exploited as a location-dependent *in situ* cross-linking mechanism [173]. Dissociation of G unit blocks occurs due to the outward flux of ions to the surrounding medium in a process that can potentially result in uncontrolled and typically slow hydrogel degradation kinetics. This mechanism, however, is well suited to myocardial applications: as the post-infarction environment recovers, the local calcium ion concentration decreases, increasing the rate of alginate dissociation and clearance from the region. Dissociated alginate that enters the circulation may be cleared by the kidneys if below the renal excretion limit of 48 $kg \cdot mol^{-1}$ weight average molecular weight [207]. Degradation rates may be increased by combining alginates with cross-linking G blocks of varying length [208] and by partial oxidation to facilitate hydrolysis [209]. As the hydrophilic nature of alginate limits protein adsorption, scaffolds are commonly functionalized with cell-adhesive peptides [175,176,210]. The functionalizable and non-thrombogenic [173,175] nature of alginate has motivated its use as an injectable cardiac biomaterial, both cell-free and as a delivery vehicle for cell therapy.

Yu et al. compared the injection of alginate and fibrin glue in a chronic (5 week old) LV aneurysm model [211]. Both materials increased arteriogenesis compared to saline controls, thickened the infarct wall, and improved LV function 2 days after administration. After 5 weeks, alginate, which was still present, improved LVFS, while fibrin glue, which had resorbed, resulted in LVFS similar to controls. Morphometric improvements remained however, indicating a beneficial effect of fibrin even after resorption.

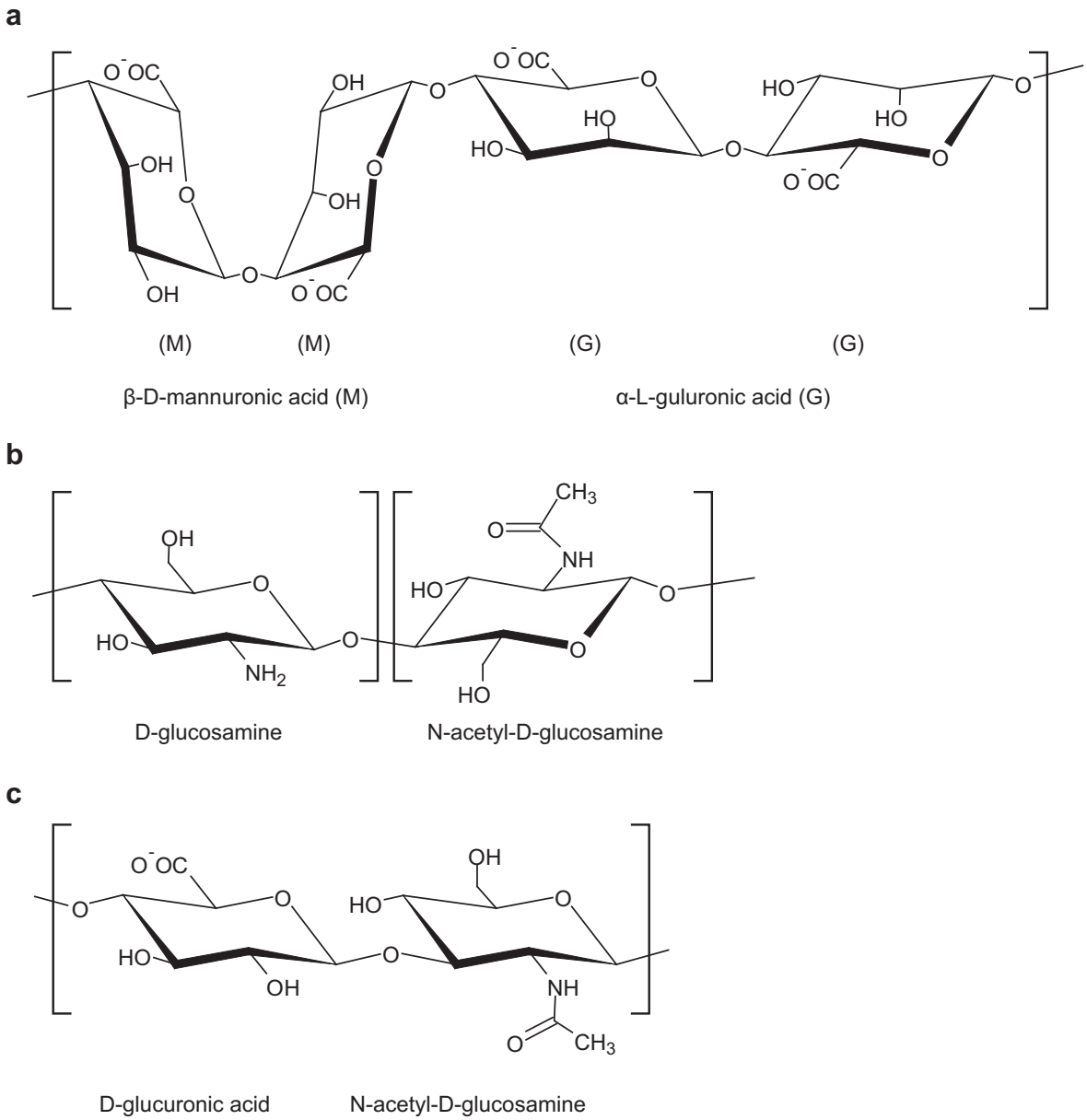

Fig. 2. Chemical structures of common naturally-derived hydrogels explored as cell delivery vehicles for cardiac regeneration. (a) Alginate, composed of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G). (b) Chitosan, composed of β-(1,4)-linked D-glucosamine and *N*-acetyl-D-glucosamine. (c) Hyaluronic acid, composed of β-(1,4)-D-glucuronic acid and β-(1,3)-*N*-acetyl-D-glucosamine.

Landa et al. injected an aqueous solution of 30–50 $kg \cdot mol^{-1}$ sodium alginate combined with calcium gluconate via catheter into recent (7 day) and aged (2 month) infarcts in a rat MI model [173]. Histological results indicated that the material had been replaced by connective tissue within 6 weeks. In both groups, after 8 weeks alginate resulted in a thicker scar and attenuation of systolic and diastolic dysfunction compared to saline controls. When delivered in recent infarcts, alginate also reduced ventricular dilation. This material was also used to evaluate the efficacy of intracoronary injection in a pig model, 3–4 days after MI. The alginate diffused from the leaky coronary vasculature into the infarcted myocardium, without generating thrombi or further infarction. By 60 days, the material was replaced by myofibroblasts and collagen, reversed LV enlargement, and increased scar thickness.

Alginate has been modified with synthetic peptide sequences in attempts to induce cellular infiltration, adhesion, matrix deposition, and vascularization at the infarct site. These attempts have met with mixed results. Tsur-Gang et al. functionalized 30–50 $kg \cdot mol^{-1}$ alginate with RGD and YIGSR motifs at a degree of substitution of 0.2% of uronic acid monomer, which improved cardiofibroblast adhesion *in vitro*, but increased solution viscosity when cross-linked with calcium gluconate [175]. Injected in a rat MI model, the modified alginate gel reduced the therapeutic efficacy in terms of scar thickness, ventricle diameter, and function, and did not induce any cellular infiltration or angiogenesis. The reduced physical benefit could potentially result from the poor material distribution upon injection due to increased viscosity, while the lack of cellular response to the peptide modification was likely due to the low level of functionalization. Yu et al. mixed alginate with a commercially available high-density RGD-alginate complex at a ratio of 1:4 [210]. Injected with $CaCl_2$ 5 weeks post MI in a rat model, alginate with and without RGD-modification

improved LV function, while RGD-modification induced a greater angiogenic response. While the level of RGD functionalization was not reported, this study demonstrates the importance of cell–material interactions for angiogenesis. The same material was used to deliver human MSCs via microsphere encapsulation in a rat MI model [176]. Both encapsulated MSCs and microspheres alone maintained LV shape, limited remodeling, reduced the infarct area, and increased arteriole density. Compared to MSCs delivered in saline, which were only retained for one day, MSCs delivered in microspheres were detected after up to two weeks, although the overall retention rate was not reported.

4.2. Chitosan

Chitosan is the deacetylated form of the linear polysaccharide chitin, a component of crustacean exoskeletons. Its cationic molecular structure of random β-(1,4)-linked D-glucosamine and *N*-acetyl-D-glucosamine facilitates electrostatic interactions with anionic glycosaminoglycans (GAGs) and proteoglycans of the ECM (Fig. 2b) [212]. *In vivo*, chitosan undergoes biodegradation primarily by lysozyme at a rate inversely related to the degree of deacetylation. Degradation produces oligosaccharides [213], which have been suggested to promote vascular endothelial cell migration and angiogenesis [214]. Combined with innate biocompatibility, antibacterial, and wound healing properties, the amenability of chitosan to chemical modification has drawn interest to it as an injectable biomaterial.

Chenite et al. developed a temperature-responsive hydrogel system by combining chitosan with a polyol-phosphate salt at physiologic pH [215]. The resulting liquid solution gels when heated to body temperature through hydrogen bonding and electrostatic and hydrophobic interactions. Lu et al. used this mechanism to encapsulate ESCs and deliver them 1 week post-infarction in a rat MI model [177]. Compared to PBS, chitosan reportedly improved ESC retention in terms of cellular graft area from 9.91 ± 1.80% to 17.48 ± 3.30% (24 h) and from 6.41 ± 1.15% to 12.93 ± 1.90% (4 weeks). At 4 weeks, the scaffold, both with and without cells, significantly improved heart function including ESD, EDD, LVEF, wall thickness, and microvessel density within the infarct. Liu et al. utilized the same material to deliver ASCs in a rat MI model, and investigated the ability of chitosan to protect the ASCs from reactive oxygen species (ROS) generated in the ischemic zone [178]. They found that ROS, specifically H_2O_2, inhibited the expression of matrix adhesion molecules β1 and αV, as well as other cell-adhesion pathways, and contributed to apoptosis. Chitosan attenuated these effects, improving adhesion and significantly increasing ASC viability. *In vivo*, ASCs delivered in chitosan resulted in increased stem cell homing and revascularization. The authors suggest that GAGs electrostatically associated with the chitosan may have enhanced the paracrine effects of ASCs by improving retention and concentration of cytokines and growth factors.

4.3. Collagen

Collagen has been used in numerous tissue engineering applications and is available in several commercial forms. Injectable collagen hydrogels are prepared by acid-solubilization of collagen, primarily type I [216]. Solution stability is determined by the electrostatic and hydrophobic interactions between amino acid groups, which are respectively affected by solution pH/ionic strength and temperature. Upon *in vivo* injection at neutral pH and 37 °C, collagen undergoes entropy-driven self-assembly through fibrillogenesis to form a physically cross-linked hydrogel capable of encapsulating cells [217]. While supporting cell attachment, viability, and proliferation, a drawback of injectable collagen is that the process of solubilization results in a loss of structural properties, including stiffness and elasticity. Collagen hydrogels formed under physiological conditions typically have compressive moduli between 7 and 14 kPa, depending on salt concentration [218].

Using radio-labeled MSCs injected in a rat MI model, Dai demonstrated that the commercially available collagen scaffold Zyderm at 35 mg/mL reduced MSC dissemination compared to delivery in saline [180]. Specifically, when collagen scaffold delivery was compared to saline, fewer of the 13 rats had MSCs present in the lungs (2 *vs.* 3), liver (0 *vs.* 4), spleen (4 *vs.* 11), and non-infarcted myocardium (5 *vs.* 9) at 4 weeks following injection. However, compared to the LVEF resulting from saline treatment (54.9 ± 3.4%), MSCs in collagen failed to improve LVEF (53.2 ± 1.4%), while MSCs in saline, as well as collagen alone increased the LVEF (58.6 ± 2.4%, 58.1 ± 2.5% respectively) [180]. The authors postulated that the collagen may have reduced long-term MSC survival by impairing the diffusion of oxygen and nutrients to the encapsulated cells, and by limiting microvascular ingrowth into the interstitial space.

4.4. Fibrin

Fibrin is a natural polymer involved in the coagulation cascade that contains intrinsic sites for cell binding. A fibrin hydrogel is formed when fibrinogen is cleaved by the enzyme thrombin, yielding insoluble fibrin peptides, which rapidly aggregate to form a fibril network mesh capable of encapsulating cells [219]. The physical properties of fibrin depend on the fibrinogen concentration and self-assembly kinetics. Higher thrombin concentrations result in faster gelation times and produce a tighter network of finer, thinner fibers [220]. By varying thrombin concentrations, cell-seeded fibrin scaffolds have been produced with elastic moduli of approximately 10–30 kPa, a range suitable for myocardial tissue applications [221]. Depending on fibrin concentration, fibrinolysis occurs naturally over days to weeks, and can be slowed by inclusion of aprotinin, a protease inhibitor [219]. Degradation produces non-immunogenic amino acid products with known angiogenic properties [222]. Under anoxic and glucose-deprived conditions *in vitro,* fibrin encapsulation of marrow-derived CSCs resulted in significantly improved survival rates over 7 days [223].

Christman et al. compared injecting bovine serum albumin (BSA) as a control to fibrin, with and without skeletal myoblasts, in a rat MI model [224]. With and without cells, fibrin reduced the percentage of infarcted ventricle wall (17.5 ± 3.4% and 19.7 ± 3.8%, respectively, compared to 26.5 ± 2.2% for cell-free BSA), and also increased arteriole density in the infarct and border zone (13 ± 3 mm^{-2} and 16 ± 1 mm^{-2}, compared to 6 ± 2 mm^{-2} for cells in BSA).

Zhang et al. utilized a two-component fibrin/thrombin material with aprotinin to deliver ASCs in a rat MI model. ASCs delivered in fibrin covered significantly more of the infarct compared to ASCs delivered in PBS after 24 h (19.10 ± 3.13% *vs.* 14.16 ± 2.73%), and this significant difference persisted after four weeks (11.52 ± 2.34% *vs.* 5.85 ± 1.35%). After four weeks, ASCs in fibrin compared to ASCs in PBS and fibrin alone resulted in significant reductions in EDD (5.91 ± 0.24 mm *vs.* 6.60 ± 0.38 mm and 6.70 ± 0.41 mm) and ESD (4.24 ± 0.26 mm *vs.* 5.04 ± 0.22 mm and 5.24 ± 0.35 mm), as well as modest improvements in LVEF (61.77 ± 4.14% *vs.* 55.75 ± 3.41% and 53.14 ± 3.59%) and LVFS (28.16 ± 2.23% *vs.* 24.11 ± 1.97% and 23.24 ± 2.44%) [183].

4.5. Hyaluronic acid

Hyaluronic acid (HA) is an anionic linear glycosaminoglycan disaccharide polymer of β-(1,4)-D-glucuronic acid and β-(1,3)-*N*-

acetyl-D-glucosamine, ranging in size from 5 to 20,000 $kg \cdot mol^{-1}$ *in vivo* (Fig. 2c). It is a widely distributed component of the ECM in mammalian tissues, and has been chemically modified to produce numerous biomaterials [225]. HA is involved in many cellular processes including proliferation and differentiation, and its abilities to promote angiogenesis and suppress fibrous tissue formation have drawn attention to its potential as a cardiac biomaterial [226].

In 2007, Kim et al. developed an acrylated 170 $kg \cdot mol^{-1}$ HA derivative, which when combined with 10 $kg \cdot mol^{-1}$ tetrathiolated four-armed poly(ethylene glycol), underwent a Michael-type addition reaction to form a hydrogel in 10 min under physiological conditions possessing an elastic modulus of 1.8 kPa [227]. Originally intended for bone regeneration, *in vivo* tests indicated the gel was a suitable scaffold for MSC and growth factor delivery. Despite its low modulus, this material was tested *in vivo* as a cell-free scaffold in a rat MI model, injected 2 weeks post-infarction [228]. Compared to untreated rats at 4 weeks post-implantation, the gel significantly increased wall thickness by 200%, decreased infarct area by 53%, and enhanced generation of arterioles and capillaries in the border region by 152% and 148%, respectively.

In 2010, Ifkovits et al. adapted a process used to methacrylate dextran [229] to produce a methacrylated 74 $kg \cdot mol^{-1}$ HA (MeHA) which underwent radical polymerization when initiated with ammonium persulfate (APS) and tetramethylethylenediamine (TEMED) [184]. At low and high levels of methacrylation (30% and 60% substitution), the elastic modulus increased from 8 kPa to 43 kPa. By increasing the initiator concentration from 5.0 to 12.5 mM APS and 5.0–6.25 mM TEMED, the gelation time was reduced from 4.5 to 2.5 min. While not used to deliver cells, these investigations illustrate the influence of scaffold mechanical properties. The lower initiator concentrations facilitated deeper tissue penetration during injection, independent of methacrylation levels. This enhanced infiltration allowed for evaluation of the effects of hydrogel mechanical properties on tissue remodeling, independent of degradation rates and other material properties, which were consistent between the materials. MeHA was injected at 4% w/v in an ovine MI model, 30 min post-infarction. While both hydrogels significantly increased apex wall thickness (7.02 and 6.54 mm, respectively, versus 2.13 mm in controls), only the higher-modulus gel significantly increased the elastic modulus of the infarcted myocardium (from 5.8 ± 1.5 kPa to ~40 kPa). The slow gelation of the less-modified MeHA resulted in a wider distribution through the myocardium and no change in myocardial stiffness. Only the higher modulus gel produced significant reductions in infarct area (23.9% versus 28.6% in controls) and improvements in end-diastolic and end-systolic volumes (increasing only 1.7 and 1.9 times over baseline, respectively, versus 2.1 and 2.4 times in controls).

Similarly, Tous et al. produced hydroxyethyl methacrylate HA (HeMA-HA) hydrogels that underwent tuneable hydrolytic degradation [185]. At low and high levels of functionalization, *in vitro* degradation increased from approximately 3–10 weeks, and the elastic modulus increased from 8 to 33 kPa, respectively. Using the same ovine MI model, HeMA-HA gels were compared to hydrolytically stable MeHA gels. 8 weeks after injection, HeMA-HA with low functionalization was no longer present in the tissue, and only small amounts of HeMA-HA with high functionalization remained. While both HeMA-HA increased inflammation, potentially due to degradation product release, all HA gels increased vessel formation. The stable MeHA gels maintained myocardial thickness more effectively. While both high-modulus gels prevented LV enlargement at 2 weeks, only the stable MeHA maintained the effects at 8 weeks, implying that long-term wall stabilization is required for volume maintenance.

4.6. Matrigel™

Matrigel™ is a commercialized material composed of an assortment of ECM proteins, primarily laminin, collagen IV, and entactin, derived from mouse tumor cells. Based on their previous success in directly injecting ESC-derived human cardiomyocytes to uninjured hearts in nude rats [230], Laflamme et al. investigated a similar cell delivery approach in an infarcted rat heart model [188]. Cell retention was much lower in the MI model, where only 18% of hearts contained grafted cells compared to 90% in the uninjured model, and the observed grafts were generally much smaller. This failure was attributed to cell death from mitochondrial damage, ischemic shock, and anoikis. Cardiomyocyte delivery was reattempted with a cocktail of pro-survival factors and a Matrigel™ delivery vehicle to prevent anoikis. Consistent with previous results, no engraftment was observed when the cells were delivered alone. When delivered in Matrigel™, 100% of the infarcts had detectable grafts, which were four-fold larger when combined with the delivery of pro-survival factors. Transplanted cardiomyocytes persisted and were proliferating after 4 weeks, with the vast majority localized in the central regions of the infarct. Despite these promising results, the sarcoma cell line origin of Matrigel™ limits its clinical translatability [231].

4.7. Self-assembling peptides

Self-assembling peptides (SAPs) are oligopeptides composed of alternating hydrophilic and hydrophobic amino acids, 8 to 16 residues in length, capable of forming cell-supportive scaffolds under physiological conditions. At low pH and osmolarity, SAPs remain in solution, but when exposed to physiological conditions upon injection, rapidly self-assemble into fibers forming a stable macroporous membranous matrix [232]. SAPs can be modified to present growth factors and cellular signals to further promote cell attachment, growth, and differentiation, as well as matrix vascularization. Further, SAPs are capable of binding clinically relevant levels of growth factors such as platelet-derived growth factor (PDGF), VEGF, basic fibroblast growth factor (bFGF), and angiopoietin-1, making them potential candidates for growth factor delivery [233].

Davis et al. developed an SAP system that provided sustained, controlled release of IGF-1 tethered to the scaffold via a biotin-tetravalent streptavidin complex [234]. In a rat MI model, CPCs delivered in the IGF-1 tethered peptides significantly attenuated ventricular dilation and LVFS compared to CPCs alone and peptides with tethered IGF-1 alone. Compared to CPCs alone and peptides with IGF-1 alone, the combined CPC and IGF-1 therapy also increased the number of newly formed myocytes by 32% and 230%, respectively, and increased the length and density of newly formed arterioles by 73% and 83%, respectively [189].

4.8. Injectable hydrogel scaffolds based on synthetic polymers

Synthetic polymers offer several advantages over natural materials, including batch-to-batch consistency, precise control over mechanical and degradation properties, and amenability to chemical functionalization [235]. However, synthetic polymers often lack the innate bioactivity of natural materials, affecting cellular interactions, including migration, adhesion, and remodeling.

4.8.1. Poly(ethylene glycol)-based hydrogels

Poly(ethylene glycol) (PEG) is a water soluble polymer exhibiting low protein adsorption and immunogenicity and is widely used as a synthetic matrix in tissue engineering [235]. Wu et al.

developed a supramolecular tri-block methoxy PEG-*b*-poly(ε-caprolactone)-*b*-methoxy PEG (MPEG-PCL-MPEG) hydrogel with corresponding block weights of 5000-6110-5000 Da [236]. Upon mixing with α-cyclodextrin, a matrix was formed by linear MPEG-PCL-MPEG threading into cavities of α-cyclodextrin. Injected in a rabbit MI model, this material prevented scar expansion and wall thinning, and also improved ESD and EDD after 4 weeks. However, unlike most natural biomaterials, it did not induce neovascularization [237]. This hydrogel was used to deliver bMSCs in a rabbit MI model. After four weeks, delivery via scaffold compared to culture media significantly improved cell retention (2150 ± 235 cells/mm^2 *vs*. 845 ± 156 cells/mm^2), LVEF (62% *vs*. 47%), EDD (145 mm *vs*. 175 mm), and infarct size (20% *vs*. 30%), and the hydrogel was completely absent from the infarct, likely by chain dissociation and diffusion [191].

Kraehenbuehl et al. have developed a hydrogel system using a multi-arm PEG vinyl sulfone that undergoes *in situ* cross-linking via Michael addition with a cysteine residue-flanked peptide sequence [238]. The peptide sequences were engineered to include a matrix metalloproteinase (MMP) substrate site, allowing for complete hydrogel degradation within 6 weeks, as well as an RGDSP cell adhesive ligand. This material was used to deliver endothelial- and smooth muscle-like cells derived from human ESCs, along with thymosin β4 (Tβ4), a pro-angiogenic and pro-survival factor, in a rat MI model 1 h post-infarction [192]. *In vivo*, there was approximately 80% release within 3 days of the 2.5 μg of Tβ4, and 95% after 6 weeks. At both time points, combined ESC and growth factor delivery, the hydrogel was found to preserve contractile function performance, attenuate LV dilation, and decrease infarct size. The density of small vessels (2–50 μm in perimeter) in the infarct area was approximately three-fold greater than in PBS-treated controls, potentially due in part to the elevation in the human ESC-derived cytokines VEGF, EGF, and HGF that were detected after 1 day.

4.8.2. Poly(N-isopropylacrylamide)

Poly(N-isopropylacrylamide) (PNIPAAm)-based polymers have been investigated as hydrogel scaffolds due to their thermo-gelling nature. With a lower critical solution temperature slightly below body temperature, PNIPAAm hydrogels undergo a phase change into an insoluble gel when injected into the body. However, as PNIPAAm is non-biodegradable, the eventual solubilization *in vivo* may produce high molecular weight fragments that cannot be cleared by the kidneys. For this reason, PNIPAAm is often combined with degradable copolymers to improve biocompatibility [196,239].

Wang et al. developed a thermosensitive composite hydrogel by combining PNIPAAm with the polysaccharide dextran [195]. First, 60–90 kg·mol^{-1} dextran was grafted with hydrophobic poly(ε-caprolactone)-2-hydroxyl ethyl methacrylate to form an amphiphilic Dex-PCL-HEMA, which was subsequently copolymerized with NIPAAm. In solution at 8 wt%, the composite underwent reversible phase transition to gel in 30 s when raised from 20 to 37 °C. The products of *in vivo* degradation were found to be below 10 kg·mol^{-1}, and thus expected to be cleared from renal filtration. The hydrogel was first tested 4 days post-infarction in a rabbit MI model [195]. After 30 days, the material prevented scar expansion and wall thinning, and improved LV morphology and hemodynamic performance; however, no neovascularization was reported. The same material was used to deliver bone-marrow-derived mononuclear cells (BM-MNCs) in a similar animal model [240]. While the polymer alone produced similar benefits, when combined with BM-MNCs it also enhanced neovascularisation and prevented scar expansion, illustrating the positive influence cells can have when combined with an otherwise non-angiogenic material.

Wall et al. addressed biodegradability by designing a semi-interpenetrating network (sIPN) based on PNIPAAm and acrylic acid (AAc) copolymers [197]. NIPAAm and AAc at 95:5 were combined with 0.3 mol % of a diacrylated, MMP-labile peptide cross-linker, and a range of concentrations of 450 kg·mol^{-1} poly(AAc) functionalized with a cell-binding RGD sequence. These components were initiated with APS to form the thermo-responsive sIPN. The sIPN was used to deliver bMSCs to a rat infarct and compared to saline alone, bMSCs in saline, sIPN alone, and bMSCs in Matrigel™. All injections with bMSCs versus cell-free controls resulted in significant functional improvements after 2 weeks in LVFS and LVEF. However, these benefits decreased after 6 weeks to levels no different than saline-treated rats. Over 6 weeks, only sIPN alone improved LV function to a level not significantly different from sham-operated, non-infarcted rats. This result could be due to increased degradation of the sIPN or Matrigel™ by encapsulated bMSCs, reducing its structural benefit to the myocardium. A higher percentage of hearts (38%) contained bMSCs when delivered in sIPNs as opposed to Matrigel™ (25%) or saline (0%). Separately, these results indicate the sIPN polymer has potential to improve LV function and promote bMSC retention and survival. Together, they reveal the challenge of timing the degradation of a scaffold to match tissue recovery, and indicate that many benefits seen in other studies may be transient during early time points.

5. The future of biomaterials for cell delivery to the ischemic heart

Regardless of the particular application, the first factor to take into consideration when designing a cell-based therapy is the selection of an appropriate regenerative cell population. Studies in animal models have demonstrated significant benefits following the injection of either bMSCs or ASCs, primarily in terms of increased vascularization and modest improvements in cardiac function. When directly comparing the two MSC populations, the abundance, ease of harvest, and high regenerative cell yield of adipose tissue are advantageous, and the presence of fat depots in the intra-thoracic cavity could allow for intraoperative isolation without requiring a secondary harvest site [47,70,241]. If *ex vivo* cell expansion was necessary, the higher proliferation rate of ASCs would minimize the culture times required as compared to bMSCs to obtain a clinically-relevant cell population [39]. Studies in animal models have also challenged the original hypothesis that direct differentiation of the exogenous populations into cardiovascular components represents the primary mechanism in stem cell-mediated tissue regeneration. In fact, while engraftment and differentiation of administered cells have been demonstrated to be possible, these events are rare and occur at best at very low levels. However, studies have consistently demonstrated improvements in cardiac function and ventricular remodeling following MSC therapy, providing evidence that paracrine-mediated effects may be the driving mechanism in cardiac regeneration in this context. Further, there is a growing body of work that suggests that treatment efficacy could be augmented through enhanced cellular retention and/or engraftment.

A fair appraisal of regenerative cell types depends on a delivery strategy that can consistently deliver and retain a clinically relevant number of cells to the heart. In this role, injectable biomaterials have repeatedly demonstrated therapeutic efficacy towards improving cellular localization and retention in the infarcted myocardium in a minimally invasive manner. In small and large animal models, injected hydrogels consistently ameliorated negative effects on cardiac morphology and function caused by MI. However, at present, the field faces several challenges. Most critically, the therapeutic mechanisms of injectable hydrogels are

unclear. Research has variously focused on structurally reinforcing the myocardium, activating the intrinsic healing response through bioactive molecules or degradation products, encouraging host cell infiltration, and delivering therapeutic cells. Until the relative contributions of these factors are explored, it is difficult to optimize any specific material for stem cell delivery. In this context, because of the vast inconsistency in research methods it is difficult to compare studies, and to select the most promising approaches that should be advanced to clinical consideration. Therefore, broader studies should be designed keeping consistency in animal models, methodology and time points of both administration and assessment, and only varying the material and cells employed.

While natural polymers offer innate biological activity, synthetic polymers are emerging as a highly-customizable platform for hydrogel formulation. Though the scaffolds described here have all exhibited some degree of efficacy in delivering cells, appropriate mechanical properties for physically supporting the myocardium during the course of healing has emerged as a critical factor, and should also be a focus of future delivery strategies. Future materials will likely borrow from both fields, combining the pro-angiogenic activity of natural polymers with the consistent and tailorable degradation and mechanical properties of synthetics.

Furthermore, when employing natural polymers, batch-to-batch variability and regulatory approval are among the largest challenges; synthetic polymers, however, face issues in scaling-up intricate reaction processes and sourcing some precursors. Due to specific gelation kinetics and conditions, new injection equipment may be required, as current catheter technology is inappropriate for many of these systems. Special conditions must be met to maintain the liquid polymer before injection, facilitate rapid gelation, and provide repeated deliveries during the period of operation. Entirely new equipment may need to be designed, including catheterized double-barrel injectors for two-component mixing, cooled catheters for thermal gelation, and UV light guides for photo-cross-linking. Ultimately, the cyto-compatibility of these processes will be the determinant factor. Researchers must consider these criteria, and the input from medical professionals, when designing gelation mechanisms.

Finally, clinical translation must be the ultimate consideration throughout development of the biomaterial scaffold, specifically in terms of material selection and delivery method. While functional benefits are observed in terms of LVEF, it is unclear if these metrics will translate into any clinical benefit for a patient, such as overall health, activity, and life expectancy. Also, the clinical significance of improved LVEF has been questioned since it is often not paralleled by variations in other parameters such as left ventricular end systolic volume and ejection fraction [152,242]. The end point of any therapy is to improve the patient's quality of life, ideally restoring the pre-pathology health status. In the particular case of heart conditions, this means allowing a patient to perform routine physical activities with no adverse effects. Therefore, clinical trials should always include physical performance analysis, as the success of a treatment should not only be evaluated by measuring changes in cardiac parameters but also by assessing if there are paralleled improvements in patient exercise tolerability.

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