Review

Regenerative Medicine

# Myocardial infarction: stem cell transplantation for cardiac regeneration

It is estimated that by 2030, almost 23.6 million people will perish from cardiovascular disease, according to the WHO. The review discusses advances in stem cell therapy for myocardial infarction, including cell sources, methods of differentiation, expansion selection and their route of delivery. Skeletal muscle cells, hematopoietic cells and mesenchymal stem cells and embryonic stem cells-derived cardiomyocytes have advanced to the clinical stage, while induced pluripotent cells are yet to be considered clinically. Delivery of cells to the sites of injury and their subsequent retention is a major issue. The development of supportive scaffold matrices to facilitate stem cell retention and differentiation are analyzed. The review outlines clinical translation of conjugate stem cell-based cellular therapeutics post-myocardial infarction.

**Keywords:** cardiac regeneration • bench to clinic • myocardial infarction

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A total of 11.2% of deaths worldwide are caused by ischemic heart disease according to the WHO statistics 2012 [1]. Ischemic injury of the heart causes loss of blood flow along the coronary arteries supplying the heart mainly affecting the flow to the ventricular portion of the heart. The relatively low regenerative potential of the resident cardiac stem cells (CSCs) is insufficient to replace the approximately 50 g of heart muscle, in other words, two billion cells that follows scar formation [2,3]. Infiltration of fibroblasts with the deposition of collagen and fibrin results in scar tissue formation [4]. The resultant damage leads to an increase in tensile strength, elongation and wall thinning of the heart, commonly known as 'infarct expansion' [5]. Details of the mechanisms are beyond the scope of this article and can be found in a thorough review by Pfeffer and Braunwald [4].

Pluripotent stem cells, are proliferative cells that can differentiate into cardiomyocytes fibroblasts, endothelial cells and smooth muscle cells are most sought after to replace post infarct scar tissue. There is also a need to arrest the progression of the infarct with various means immediately after the infarct. In this regard, adult bone marrow stem cells (BMCs) and mesenchymal stem cells (MSCs) have moved into extensive clinical trials, although CSCs and pluripotent stem cell-derived cardiovascular cell types are also showing promise. Methods such as cardiac restraints, hydrogels and patches have been proposed for the infarct condition and, while some of the methods are still in nascent stages of validation, others have reached clinical trials. Furthermore, scaffold materials are used to deliver cells temporarily or permanently to support the infarcted section of the heart. In this regard, scaffold materials are being envisaged as combination therapies along with stem cells and growth factors. The strategy required to mitigate the extent of damage due to infarction involves control and treatment at various levels of infarct progression. This can involve administration of anti-apoptotic agents in order to reduce cellular necrosis and resultant apoptosis that occur due to lack of oxygen [6]. The second goal should be the replacement of the scar tissue with cellular/molecular mediators that promote cardiac tissue regeneration. The

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primary goal should be to target the infarct as early in its progression as possible, ideally before scar tissue is formed. Clinical therapies look at the infusion of cells immediately after, in other words, 2–3 days to a week after the infarct and up to a year and have found that some cell types are more suitable than others. This review focuses on the various aspects of the sources of stem cells, their expansion and finally their clinical application in the stages of deterioration after a myocardial infarction (MI) episode. Various currently researched materials are compared and the criteria of the scaffold materials for cardiac applications are discussed.

## Cell sources

There are different choices available to clinicians as given in Figure 1 for transplantation to the heart. Depending on the cell type in question the protocol will involve various steps of isolation, expansion and finally delivery as given in Figure 2. Endothelial cells and smooth muscle cells, the two most predominant cell types, must be represented in the cellular populations to be delivered. The modes of delivery of cellular payload can be systemic or localized for which various strategies of transplantation of cells have been elucidated. Resistance to ischemia is one of the major hurdles for stem cell populations to differentiate toward a cardiomyocyte population at the infarct site, especially with regard to electromechanical integration, and cellular retention.

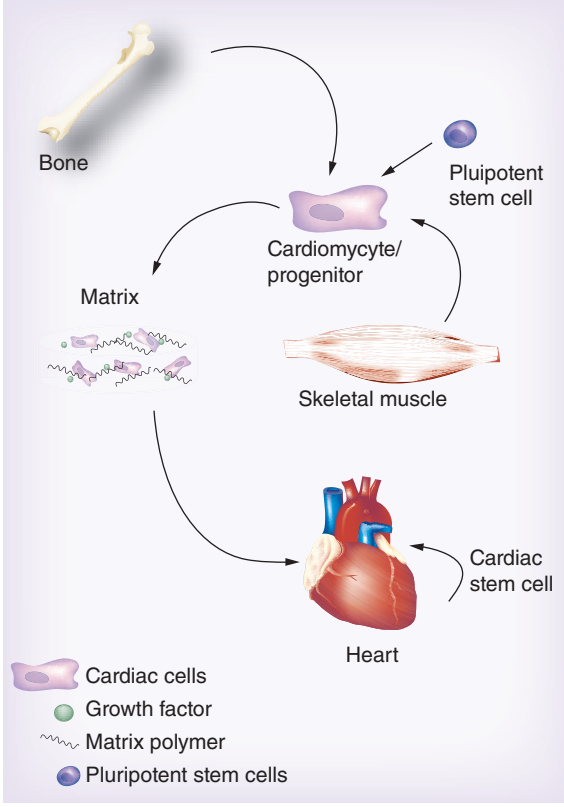


**Figure 1. Cell sources and their methods of application for cardiac regeneration.**

### Adult stem cells

#### Skeletal muscle myoblast

Skeletal myoblasts (satellite cells) have been classically identified as a stem cell population resident within non-cardiac musculature. These can differentiate into various lineages, such as bone, cartilage and fat and are identified by the marker Pax7 [7]. Recently, non-satellite $CD34^-$, $CD45^-$ and $Sca1^-$ stem cell populations isolated from skeletal muscle cells have demonstrated rhythmic beating similar to cardiomyocytes in *in vitro* culture [8]. The autologous nature of these cells ensures their suitability for transplantation. These cells can further be modified to express markers like VEGF before transplantation into the heart.

Non-satellite skeletal myoblast cells, when transplanted into adult mice, have shown transdifferentiation into cardiac tissue [8]. The resistance of satellite cells to ischemia *in vivo* has resulted in better retention times as well as higher survival rates [9].

The suitability of autologous *ex vivo* expanded skeletal myoblasts to form viable muscle in severely scarred myocardium has been established [10]. It was also found that the catheter-mediated delivery of cells resulted in increased wall thickening at the target site and improved ejection fractions [11,12]. A consequent study reported a 3–8% change in the ejection fractions, even to the extent of ventricular remodeling [13]. Ongoing clinical trials are further looking into catheter-mediated delivery of cells [14].

The autologous nature of the satellite cells, along with the structural benefits that these cells endow, does create a case for the suitability of this stem cell population for transplantation. Nevertheless, there is doubt as to whether the cells provide only structural benefits rather than form new cardiac tissue, due to lack of trans-differentiation to cardiac tissue [15]. Furthermore, there are issues with engraftment; studies have reported low engraftment with over 90% injected cells dying within the first few days. A high number of cells, in other words, 600–800 million cells, when transplanted, have caused arrhythmia [16].

#### Adult bone marrow- & blood-derived stem cells

BMCs have been known to supply the entire repertoire of cells in the hematopoietic lineages, cardiomyocytes and various other lineages. Among the populations present, $Lin^-c\text{-}kit^+$, $CD133^+$, $CD133^-CD34^+$, $c\text{-}kit^+$ and $Sca1^+$ cells are found to be suitable for cardiac regeneration [17,18]. *In vitro* encapsulation of cells, within

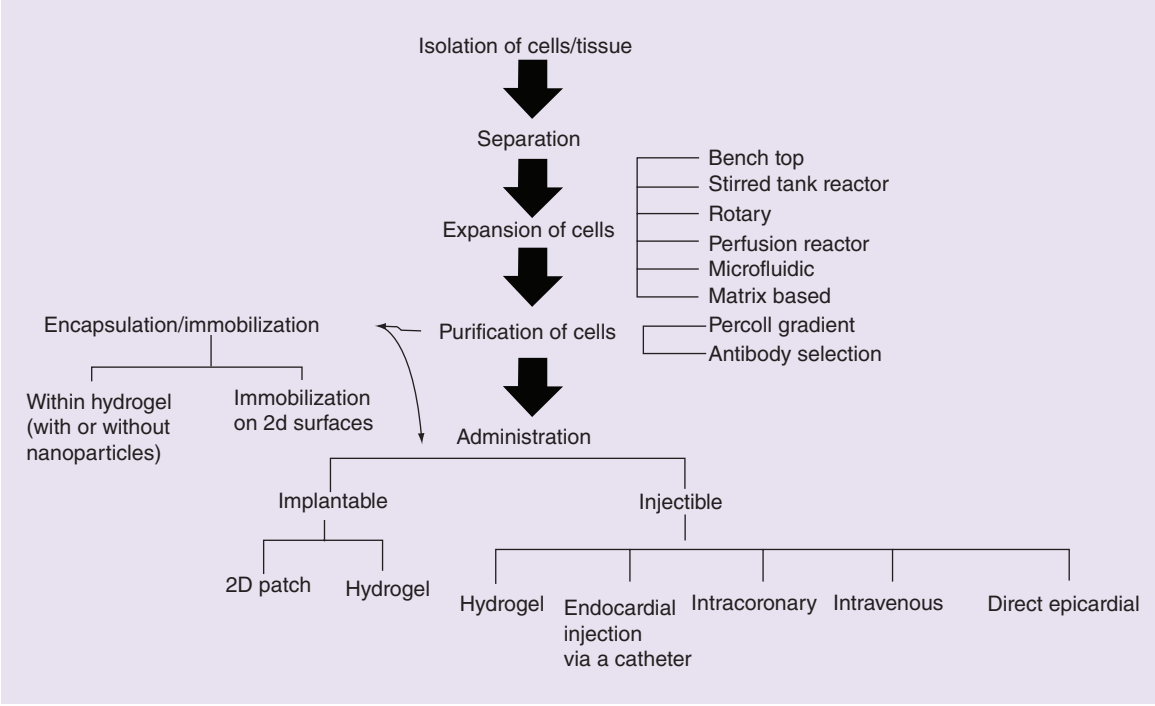


**Figure 2. Strategies for cell therapy of cardiac tissue after a myocardial infarction.**

porous type I collagen 3D conduits scaffolds resulted in expression of cardiac structural genes like α-myosin heavy chain (MHC) and β-MHC to sustained high levels for 28 days in culture [19].

c-kit$^+$ Sca1$^-$ cells improved survival, enhanced cardiac function, reduced regional strain, attenuated remodeling and decreased infarct size in mice [20]. While Lin$^-$c-kit$^+$ cells resulted in significant occupation of the infarct areas, when transplanted [18]. Other authors have suggested that the c-kit$^+$ BMCs do not fuse but differentiate to the endothelial and cardiac lineage after transplantation [21]. Canine models have been studied for a comparison of catheter-based endocardial to direct epicardial injections of blood-derived endothelial progenitors, as an alternative to intravenous administration of cells as shown in Table 1 [22]. Endocardial and epicardial administration routes of administration presented similar kinetics.

The TOPCARE-AMI clinical trial administered blood- and bone marrow-derived mononuclear cells post infarction with a beneficial effect through the prevention of remodeling [44,45]. Reversal of remodeling through paracrine signaling has been suggested as a probable mechanism [46]. Furthermore, bone marrow mononuclear stem cell administration has a negligible effect on left ventricular ejection fractions (LVEF), but a positive effect on remodeling at 6 months [47].

Improvements in ejection fractions varying from a minimum of 2% to a maximum of 7% have been reported with the administration of adult BMCs [48–51], but these improvements do not include left ventricular (LV) remodeling [52], local or global wall thickening [53] changes in LV end-diastolic volume and infarct size [54]. Other studies have demonstrated that these stem cells do not transdifferentiate into cardiomyocytes in an infracted heart [55]. Breitbach *et al.* have reported calcification and ossification at the infarct site, with the use of BMCs [56]. In studies using the CD34$^+$ cell population, retrieval of clinically relevant numbers is possible only through *in vitro* expansion before administration [57]. The CD133$^+$-purified hematopoietic stem cells (HSCs) when tested showed only limited improvement in cardiac function [17,58]. To promote further work in this area, ongoing clinical trials are trying to assess the efficacy further [59,60].

## Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are defined by the expression of antigenic receptors for CD105$^+$/CD90$^+$/CD73$^+$, CD34$^-$/CD45$^-$/CD11b$^-$ or CD14$^-$/CD19$^-$ or CD79alpha$^-$/HLA-DR1$^-$-specific antibodies and their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages [61]. Koninckx *et al.* have shown that TGF-β enhances the myocardial differentiation of bone marrow-derived MSCs by the expression of TnT in monoculture and MHC in coculture with rat neonatal cardiomyocytes [62]. They have also suggested that co-cultured hMSCs expressed the transcription factor GATA-4, but did not express Nkx2.5 [63]. 5-azacytidine (5-aza) or dimethylsulfox-

Table 1. Large animal models for myocardial infarction.

| Animals | Cells | Route | Parameters for study | Outcomes | Ref. |
|---|---|---|---|---|---|
| Canine | Endothelial progenitor cells | Subendocardial and subepicardial injection | Comparison of subendocardial and subepicardial cellular retention and clearance kinetics | Similar subepicardial and a subendocardial technique kinetics | [22] |
| Canine | MSCs | Intramyocardial injection | MSC transplantation | Cells differentiate to endothelial cells and smooth muscle cells and enhance vascularization | [23] |
| Ovine | Autologous endothelial cell within fibrin matrix | Intramyocardial injection | Assessment of improvements through angiogenesis | Neovascularization improves blood flow, and improves left ventricular function | [24] |
| Ovine | Mouse cardiac committed ESCs | Intramyocardial injection | Cross species transplantation of committed ESCs | ESCs are immune privileged, and can improve heart function | [25] |
| Ovine | Allogenic STRO-3-positive mesenchymal precursor cell | Intramyocardial injection | Cell transplantation and dosage assessment | Attenuation of remodeling through vascularization | [26] |
| Ovine | Allogenic mesenchymal precursor cells | Intracoronary infusion | Safety, efficacy of transplant of cells | Infarct size decreased by 40%, blood vessel density increased by >50%, 8 weeks postinfarction | [27] |
| Porcine | BMSCs | Intramyocardial injection | Stem cell transplantation and cellular retention | Cellular retention better at infarct border zone | [28] |
| Porcine | Bone marrow-derived MSCs | Intramyocardial injection | Functional recovery after transplantation | Improve bioenergic and contractile function; improvements via paracrine effects | [29] |
| Porcine | Bone marrow-derived MSCs | Intramyocardial injections | Intracoronary catheter-mediated transplantation | Long-term engraftment, reduction in scar formation | [30] |
| Porcine | ADSCs and BMSCs | Intracoronary injection | Comparison of ADSCs and BMSCs | Improvement in cardiac function via angiogenesis | [31] |
| Porcine | Allogenic bone marrow MSCs | Intramyocardial injection | Assessment of improvement in left ventricular function | Improved vascularization through differentiation into cardiomyocytes and endothelial cells | [32] |
| Porcine | Bone marrow-derived MSCs | Transendocardial injections | Stimulation of CSCs to proliferate and differentiate | 20-fold increase in endogenous c-kit$^+$ CSCs | [33] |
| Porcine | MSCs | Intravenous injection | Infusion of cells immediately after infarct hypothesized to reduce remodeling | Improves left ventricular ejection fractions, prevents wall thickening in noninfarcted myocardium | [34] |
| Porcine | Allogenic MSCs | Endomyocardial injection | Safety of endomyocardial delivery and dosage | Reduction of infarct size attributed to paracrine effects | [35] |
| Porcine | MSCs | Intracoronary infusion | Assessment of localization of MSC | Remodeling prevented up to 2 months after myocardial infarction | [36] |

ADSC: Adipose tissue-derived stem cell; BMSC: Bone marrow-derived stem cell; CDC: Cardiosphere-derived stem cell; CS: Cardiosphere; CSC: Cardiac stem cell; ESC: embryonic stem cell; hCSC: Human cardiac stem cell; hMSC: Human mesenchymal stem cell; MSC: Mesenchymal stem cell.

Table 1. Large animal models for myocardial infarction (cont.).

| Animals | Cells | Route | Parameters for study | Outcomes | Ref. |
|---|---|---|---|---|---|
| Porcine | Peptide-1-eluting MSCs | Intracoronary infusion | Study of engraftment, survival and dosage | Improvement in ejection fraction by 9.3%, alginate facilitates retention of cells | [37] |
| Porcine | Autologous MSCs | Intracoronary perfusion | To assess whether MSCs mobilize bone marrow progenitors to myocyte proliferation | Increase in circulating c-kit$^+$ and CD133$^+$ cells with increase in myocardial c-kit$^+$/CD133$^+$ and c-kit$^+$/CD133$^-$ bone marrow progenitor cells | [38] |
| Porcine | ADSCs | Intracoronary, transendocardial | Comparison of modes of delivery of cells | Both modes are similar in engraftment and differentiation, intracoronary mode induces neovascularization | [39] |
| Porcine | Allogenic ADSCs | Intracoronary | Assessment of immune response | Illicit an immune response | [40] |
| Porcine | Allogenic CDCs | Intracoronary infusion | Safety and efficacy of CDCs as adjunctive therapy to reperfusion | Infarct size reduction, attenuation of remodeling, microvascular occlusion prevention | [41] |
| Porcine | CS | Transendocardial injection | Safety, efficacy and dosage of CSs injection | Regeneration through increase in viable myocardium | [42] |
| Porcine | hCSCs/hMSCs, hCSCs alone, hMSCs alone | Intramyocardial injection | Combination of hMSCs with c-kit$^+$ CSCs hCSCs produces greater infarct size reduction | Twofold reduction infarct size in hCSCs/hMSCs as compared with, hCSCs alone and hMSCs alone | [43] |

ADSC: Adipose tissue-derived stem cell; BMSC: Bone marrow-derived stem cell; CDC: Cardiosphere-derived stem cell; CS: Cardiosphere; CSC: Cardiac stem cell; ESC: embryonic stem cell; hCSC: Human cardiac stem cell; hMSC: Human mesenchymal stem cell; MSC: Mesenchymal stem cell.

ide induce rat MSC differentiation toward cardiomyocytes, but have no such effect on hMSCs [64,65]. BMP-2 or FGF-4 have also been used to enhance the differentiation potential of rat MSCs *in vitro* [66]. Novel ways in which entrapment of cells within hyaluronic acid-based 3D scaffolds have demonstrated that cell spreading occurs when there are matrix degradation moieties present within the scaffold, especially in the presence of the RGD peptides [67]. Furthermore, matrix stiffness has been shown to be a key factor in MSC proliferation within fibrin scaffolds [68].

Some studies suggest that MSCs, when injected intramyocardially, differentiated to vascular smooth muscle cells or endothelial cells *in vivo* and showed improvements via angiogenesis in a porcine model of ischemia [69]. Furthermore, human umbilical cord blood-derived MSCs, when transplanted into mice, resulted in improvements through paracrine effects [70]. Preconditioning of these stem cells with 5-aza resulted in differentiation of MSCs to cardiomyogenic cells, when transplanted into mouse models of MI; this prevented infarct expansion and eventually improved heart function [66,71]. BMP-2 and FGF-4 can alternatively be used for the differentiation of MSCs toward cardiomyocytes. Studies report that BMP-2- and FGF-4-treated MSCs, when transplanted into rat models, demonstrated improvements similar to 5-aza-treated cells [66]. Contrary to claims made about the lack of differentiation potential of MSCs, studies have shown that they could differentiate to cardiomyocytes or fibroblast scar tissue, when transplanted in rats [72]. Furthermore, adipose-derived MSCs (ATMSCs) have been used as cell sheets to repair the infracted myocardial cells in rats, resulting in the reversal of wall thinning of the myocardium [73]. ATMSCs have induced vascularization with VEGF expression, additionally eliciting an immune response [39,40]. Cellular retention studies in porcine animal models transplanted with bone marrow-derived MSCs have indicated that infarct border zone injection retained more cells than direct injection into the heart [29]. Cardiac functional improvements in porcine models after transplantation of bone marrow-derived MSCs have attributed improvements to paracrine effects, while reporting retention of as low as 0.035% cells at the infarct site after peri-infarct injection of cells [28]. A study by Toma *et al.* has shown that hMSCs, when injected intraventricularly into SCID mice, differentiated into cardiomyocytes with the expression of cardiac-specific TnT, α-MHC, α-actinin and phospholamban with visible-striated fibers [74]. Furthermore, ablation of proinflammatory receptors TNF-α on MSCs has been linked to increased survival and reduced infarct size [75].

Clinical studies have shown that bone marrow-derived MSCs are safe for use through the transendocardial route of administration [76]. Intravenous transplantation of allogenic hMSCs at various single dosages of 0.5, 1.6 and 5 million cells/kg resulted in marked improvements with reduction of arrhythmias and improved LV function but no dosage response for most parameters [77]. Clinical studies have shown that the administration of MSCs to the heart leads to a therapeutic result via a paracrine effect rather than differentiation of MSCs to cardiomyocytes, while others have suggested differentiation toward a lineage based on the environment. To realize the full potential of MSCs as therapeutic agents, their differentiation to cardiomyocytes, in order to replace the cellular losses caused due to an infarct, is vital. Cardiomyocytes as well as angiogenic progenitors, if produced by the MSCs, will replenish the cells from the depleted heart and increase circulation to the affected area. Clinical studies are underway to assess the comparison of transplantation of autologous hMSC transplantation versus allogenic hMSCs, transendocardially [78,79].

### Cardiac stem cells

The presence of a self-renewing, clonogenic and multipotent population of cells within the heart that is induced by paracrine signaling in the event of ischemia, has been established [80]. However, these cells cannot overcome the local loss of cells after an infarct [81]. The renewal rate of these cells declines at the rate of 1% per year at age 25 years to 0.45% at age 75 years [82,83]. The different cell populations isolated and characterized are c-kit$^+$ cells, Sca1$^+$ (CD31$^-$) cells, isl-1$^+$ (c-kit1$^-$ and Sca1$^-$) cells and cardiosphere-derived cells [84]. Cardiospheres (CSs) are clusters of self-adherent cells formed when heart biopsy specimens are expanded *in vitro* [85]. The core of the CS is composed of c-kit$^+$ cells, while cells that exhibit endothelial and stem cell markers (Sca-1, CD34 and CD31) are on the periphery [86].

Sca1$^+$, when induced by 5-aza-C [81] or oxytocin [87], result in the expression of cardiac transcription factors cardiac troponin1, sarcomeric α-actin, MHC and Nkx2.5. Oxytocin induces differentiation of the Sca1$^+$/c-kit$^+$ population to cardiomyocytes. Sca-1$^+$/CD31$^-$ cells differentiate to cardiac myocytes and endothelial cells in the presence of FGF, 5-aza-C and Wnt antagonist Dkk-1 [87]. Extracellular matrix (ECM) stiffness can induce differentiation as well; a matrix modulus of 31–35 kPa can support CSs and results in a high expression of cardiac markers cardiac TnT (cTnT) and cardiac MHC (MYH6) [88]. FGF-2 has been shown to play a critical role in the mobilization and differentiation of resident cardiac precursors in the treatment of cardiac diseases *in vivo* [89]. c-kit$^+$ cells have been found

to solely mitigate regeneration of a damaged heart [90]. They also induce neovascularization on transplantation via a paracrine effect [87,91–92]. Oxytocin-activated c-kit$^+$ cardiac progenitor cells, when injected at the site of coronary occlusion, differentiate to smooth muscle cells and endothelial cells [93]. Sca1$^+$ cells, on the other hand, show connexin 43, cTnI and sarcomeric α-actin expression after intravenous infusion into mouse hearts following ischemia/reperfusion [81]. Cardiosphere-derived cardiac progenitor cells contribute to improving ventricular function in mouse and swine models [86,94–95]. Furthermore, these cells do not induce immune reactivity, when transplanted [96]. Coronary infusion of CDCs in porcine models also provides a good model for the safety of the delivery of cells, modes of delivery as well as the benefits of such delivery [41].

Autologous c-kit$^+$ CSCs isolated from the right atrial appendage have been clinically administered in the SCIPIO trial through coronary infusion into patients after expansion [97]. LV ejection fraction increased from 30 to 38% and the infarct volume decreased from on average 32.6 to 7.2 g within 4 months of infusion [97]. However, doubts were cast as to why results were published before the trial was completed, further as to why patient from the non-randomized part of the trials were analyzed and results displayed [98]. Controlled double-blinded and randomized trials overturning positive results of non-randomized or partially randomized trials were cited to be the reason behind the objections. A separate study has harvested CDCs after generating CSs from end myocardial autografts and demonstrated reduction in scar mass and increase in viable tissue in the Phase I CADUCEUS clinical trials [99]. Follow-up studies with the patients revealed increase in viable myocardium, consistent with regeneration; furthermore, patients 1 year after MI are also eligible for the treatment and show improvements similar to those treated 2–3 months post-MI [100,101].

Clinical relevance of CDC transplantation is possible only after autologous cardiac tissue is harvested from patients during procedures like coronary artery bypass grafting (CABG). Although the CADUCEUS Phase I clinical trials points to improvements of LVEF over bone marrow cell transplantation and the SCIPIO trial of the improvements due to c-kit$^+$ cells, there is further need for clinical data to ascertain the efficacy of these cells [99]. Recently, van Berlo *et al.* cast doubts on the actual effective populations of c-kit$^+$ cells to mediate a regenerative response [102]. But the effectiveness of the cre-lox recombination system used to come to their conclusions has been elaborated [103]. Furthermore, the benefits of double-blinded, randomized and placebo-controlled clinical trials have to be understood to design effective clinical trials.

## Pluripotent stem cells

### Embryonic stem cells

Embryonic stem cells (ESCs) are cells isolated from the inner cell mass of blastocysts and which can give rise to the three germ layers, as well as giving rise to all the cardiac subtypes. ESCs have demonstrated differentiation toward a cardiac lineage and expression of cardiac functions [104–110] and further to prove their proliferative capacity, since a large number of cells are required at the site of infarct [111,112]. *In vitro* differentiation of ESCs has been optimized in mouse cell lines as well as human; while some protocols of differentiation work for mouse cell lines, some others work for human cell lines [113]. Furthermore, the use of gelatin, agarose and poly(lactide-*co*-glycolide) (PLGA)-based microparticles within cellular aggregates for differentiation has improve gene expression [114]. Apart from simple spontaneous differentiation protocols, to usage of mediators like BMP-4 and activin A and to coculture pluripotent stem cells with endothelial cell lines (END-2) have been used for the direct differentiation of ESCs to the cardiac lineage and to improve the yield of cardiomyocytes population generated therein [106,115–116]. ECM material stiffness is another aspect that is being studied to direct differentiation. A study showed that a dynamic module of ~8.6 Pa is suitable, and that differentiation was better in the presence of ECM as against collagen hydrogels supplemented with cardiac growth factors alone [117]. Hyaluronic acid/polyethylene glycol (PEG) hydrogel scaffolds with a dynamic modulus ranging from 1 to 8 kPa influenced differentiation of chicken embryonic cells [118]. Differentiation toward a cardiac lineage has led to the production of ECM proteins versican and hyaluronan [119]. While differentiated cells migrate toward fibronectin and noncanonical Wnt gradients [120]. Taken together methods of differentiation, isolation, enrichment and storage have been optimized to facilitate transplantation [121]. Allogenic transplantation of undifferentiated ES cells did not lead to a cardiomyocyte fate in either normal or infarcted hearts, neither was an allogenic immune protection observed. However, xeno transplants of cardiac-committed mouse ESCs into ovine models have proved that ESCs are immune privileged, as shown in Table 1 [25]. Cardiomyocytes derived from human ESCs have been able to repopulate rat hearts, suggesting an encouraging scenario for their use with humans [122]. Guinea pig injury models have shed light on the protective effects of transplanted ES-CMs against arrhythmias while beating in sync with host cardiac tissue [123]. Frozen human ESCs-derived cardiomyocytes could be revived and administered to nonhuman primates, leading to remuscularization and electromechanical integration albeit with the occurrence of nonfatal arrhythmias [124]. Matrix-

impregnated ES-CMs have utilized matrix properties to revascularize host tissue while controlling the immune response [125], Ongoing clinical trials are testing the use of fibrin gel embedding human ESC-derived CD15$^+$ Isl-1$^+$ progenitors [126].

### Induced pluripotent stem cells

With the advent of induced pluripotent stem cells (iPSCs) in 2006 [127], a new opportunity presented itself toward the generation of pluripotent ES-like cells from somatic cells. It was shown that normal somatic cells could be converted to what are known as 'iPSCs' by the forced expression of four crucial factors transcription factors: Oct4, Sox2, c-Myc and Klf4 [127]. This technology has proved itself by its application across various species and tissues [128]. iPSCs too have shown properties of differentiation similar to ESCs [129–133]. Although differentiation protocols have succeeded in increasing the efficiency of differentiation, a cause for concern with respect to the final administration of iPSCs is the undesirable transfer of pathogens and ethical approval for transfer of cells cocultured with other cells lines [134], and third isolation of cardiomyocytes from the undifferentiated population [135]. Immunological safety of iPSCs were raised by Zhao *et al.* [136], but implanted tissue grafts obtained from iPSCs-derived cells implies that these cells are safe to take to the next level in tissue engineering of patient-specific cells [137]. Furthermore, cardiomyocytes, endothelial cells and smooth muscle cells derived from these cells have been tested on porcine infarct models, along with fibrin-encapsulated IGF. The results indicate a substantial reduction in infarct size, ventricular wall stress and apoptosis [138].

Various aspects of cardiac regeneration such as effective differentiation of stem cells, electrical and mechanical integration and especially long-term effects without adverse side effects – are yet to be dealt with in addressing the issue of regeneration of the heart at the site of MI [139]. Although it has been indicated that hESC-CMs and hiPSC-CMs 80–120 days in culture compare well with host cardiac tissue enough to elicit better integrative effects on transplantation [140]. Novel protocols of reprogramming fibroblast with cardiac genes *Gata4*, *Tbx5* and *Mef2c* have resulted in fibroblasts with limited survival and low cardiac molecular or electrophysiological change [141]. To further the iPSCs potential, the Japanese government recently gave permission for the conducting of clinical trials for the treatment of macular degeneration using iPSCs, suggesting a paradigm shift toward the use of iPSCs for therapy [142]. To harness ES cell potential, somatic cell nuclear transfer (SCNT) or somatic cell reprogramming offers a solution for the isolation of patient-specific cells for treatment. Furthermore, pluripotent stem cells generated from parthenogenesis have shown potential toward cardiac differentiation and efficient integration within host tissue [143].

### Combinational therapy

Graft transplantation strategies required to address the reduction of vascular endothelial as well as smooth muscle cells to effectively address the site of infarct. Combined cell approaches like the transplantation of skeletal muscles and BMCs have indicated improvements in LVEF in the combined group against the skeletal muscle only group [144]. Human CSCs (hCSCs) c-kit$^+$ combined with bone marrow MSCs (hMSCs) administered to porcine MI models brought about a twofold greater reduction in the infarct size as compared with the use of the cell populations alone [43]. Further studies report that the administration of just bone marrow MSCs results in a 20-fold increase in c-kit$^+$ CSCs to synergistically mediate improvements [33]. Pluripotent stem cell differentiation to cardiac subtypes and transplantation of cardiomyocytes, endothelial cells and smooth muscle cells population together served to compensate losses to muscle as well as vasculature [138]. The transplantation of multiple cell types opens up an undiscovered area of cell therapy with the potential to study synergistic effects of complimentary cell population in MI therapy. A complementarity between the cells also gives the opportunity to reduce the final number of cells administered along with benefits greater than administration of each of the cell types alone [144].

## Modes of application of stem cells in myocardial infarction

Various modes of delivery of cells to the site of infarct have been discussed extensively by Jezierska-Woźniak *et al.* and the resulting inefficiencies of the methods involved [139]. There have been issues with retention of cells as well as homing of cells, with methods like intravenous infusion [145], intracoronary injection [146] and direct epicardial [147] or endocardial injection via a catheter [148,149]. Although catheter-based clinical trials for transplantation of skeletal myoblast show improvements in the infarcted heart [11,150], there are other studies that suggest a completely contrary scenario to the transplantation of these cells [15]. A method that will allow a small population of progenitor cells either unipotent, multipotent or pluripotent to be encapsulated and delivered to the site of infarct is desirable. This will facilitate retention until differentiation, create a barrier between the undifferentiated population and the adult cells, preventing any adverse effects due to the undifferentiated population and

reduce the final cell number required for transplantation. This should facilitate paracrine effects, if any, without the harmful effects of the delivered cells, such as ossification and calcification. Furthermore, there is a need for direct contact of the tissue with the delivered material and cells.

## Implantable systems

### Cardiac patches

2D approaches have been pioneered in order to have strict control on the constructive elements that go into the scaffold, namely growth factors, cells and small molecules. Cardiac patches were developed to place elastic support with/without cells along the external ventricular wall of the myocardium for regeneration. ECM collagen has been used to prepare patches for treatment of MI by the transplantation of $CD133^+$ cells. Although there was visible angiogenesis at the site, the cells failed to differentiate to cardiomyocytes [151]. Polyurethane (PU) and poly(ester urethane) (PEU) rubbers are suitable candidates for the heart [152,153]. When cardiomyocytes were grown on biodegradable polyester urethane urea (PEUU), the membrane could contract the patch [154]. Other studies have shown that phytic acid cross-linked peptides, prepared by electrospinning, mimic the ECM in the heart [155]. Mouse iPSCs-derived cardiomyocyte cells have been used to prepare tissue sheets on thermoresponsive polymers [156]. Poly(glycerol sebacate) (PGS), another material whose mechanical characteristics can be tailored to match the heart, promoted the growth and beating of ES cell-derived cardiomyocytes *in vitro* [157]. Constructs with a combination of polytetrafluoroethylene, polylactide mesh, and type I and IV collagen hydrogel have been used to encapsulate MSCs [158].

PU is elastic and degradable *in vivo*. Animal trials of biodegradable PU-conducted patches promoted contractile phenotype smooth muscle tissue formation and improved cardiac remodeling and contractile function at the chronic stage [154]. iPSc-derived tissue sheets, when implanted in mice, reduced LV remodeling [156].

Poly(tetrafluoroethylene) reinforced porous poly(L-lactic acid) mesh seeded with bone marrow-derived mesenchymal cells and soaked in type I and IV collagen were sutured onto the rat infarct wall after a ventriculotomy. This resulted in a reduction in aneurysm elongation [158].

### *Ex situ* gelled: hydrogel scaffolds

Hydrogels have been widely used as their mechanical properties can be fine-tuned to match those of cardiac tissue. Table 2 compares the stiffness of various gels and the cardiac matrix.

Hydrogels with stiffness lower than heart tissue can be used as temporary space-filling moieties, and further can be used to deliver stem cells and/or molecules for growth. In this regard, collagen injections into the ventricular wall have been shown to prevent progressive wall thinning, a sequel to permanent heart dysfunction, in rats [171]. Furthermore, hydrogels made up of ECM and collagen were able to differentiate human ESCs *in vitro* to cardiomyocytes [117]. Growth factor bFGF, along with MSC delivery, was demonstrated by encapsulating within thermoresponsive N-isopropylacrylamide (NIPAAm), N-acryloxysuccinimide, acrylic acid and hydroxyethyl methacrylate-poly(trimethylene carbonate). These hydrogels were able to sustain the growth of the cells through bFGF release [172]. bFGF has also been used for improvement in vasculature by Iwakura *et al.* [173].

ESCs encapsulated in collagen type I transplanted into intramural pouches at the infarct wall, resulted in reduction of fractional shortening. Carbohydrate polymers, like alginate, have been used for seeding cells and further implantation into mice to prove their efficacy as carriers for cells. These implants reduced LV remodeling, and it is further proposed as a carrier scaffold for iPSCs [174]. Zimmerman *et al.* have developed tissue by casting a mixture of collagen type I along with neonatal rat cardiomyocytes into moulds to form engineered heart tissue (EHT). These constructs were developed into ring-shaped flexible structures and sutured onto pericardiectomized rat hearts [175]. The EHT transplant became vascularized and electrically integrated *in vivo* and since these were prepared in serum-free media conditions, immunosupression was not required during transplantation [175,176]. Engineered heart muscle was developed with a similar approach by assembling cardiomyocytes derived from the differentiation ESCs onto EHT [177].

Of all the constructs developed, the ones that were successful were those derived from native heart tissue. Furthermore, collagen types I and IV have also been successful in being able to support cellular growth, cellular vascularization and to allow electrical integration within the heart. In case of transplantation of pluripotent stem cells, it will be essential to differentiate these on site with molecular mediators entrapped within the hydrogel; alternatively, one could use the stiffness characteristics of the hydrogel to differentiate the cells. Although robust, the hydrogel approaches can be employed only by surgical intervention.

## Injectable systems

### *In situ* gelling systems

Implantable systems can only be administered through invasive surgical intervention. Thus, implantation of these constructs will have to accompany procedures like CABG. *In situ* gelling systems, on the other hand,

Table 2. Matrix molecules and the relevant stiffness they can provide.

| Congestive heart failure | Material stiffness | Ref. |
|---|---|---|
| Fibrin | 50 Pa | [159] |
| Matrigel1 | 30–120 Pa | [160] |
| Type I collagen gels | 20–80 Pa for 1–3 mg/ml | [161] |
| N-isopropyl acryl amide | 100–400 Pa | [162,163] |
| Alginate | 100 Pa to 6 kPa | [164] |
| Polyethylene glycol | 1–3 kPa | [165] |
| Heart | 50 kPa in normal hearts or 200–300 kPa in congestive heart failure hearts | [166–170] |

are defined by a sol-to-gel transition from *in vitro* to *in vivo* setups, respectively. This method of gelation can assist the administration of the gelling polymer through a catheter, facilitating a minimally invasive method to cardiac treatment. In regard to this, the materials that have been studied extensively are fibrin glue [178,179], collagen [171], matrigel [180], hyaluronic acid [181], keratin [182], ECM [183,184], alginate [174,185]. There are many potentially useful materials that can fulfill this role and are yet to be tested in this application. Endothelial cells home to a self-assembling injectable RAD16-II peptide scaffold and cause more angiogenesis as compared with matrigel. Potential myocyte progenitors also populate the peptide microenvironment created *in vivo*, and the retention of myocytes is higher as compared with matrigel [186]. Furthermore, this study demonstrated that ESCs spontaneously differentiated to αMHC-positive cells *in vivo* within the peptide scaffold. Cell survival was better within fibrin glue when delivered through injectable fibrin glue scaffolds compared with the cellular cardiomyoplasty technique, additionally inducing neovascularization and reducing infarct expansion [179]. This was followed up with a study that suggested short-term improvements of the alginate fibrin blends at the site of infarct [187]. *In vivo* studies via injection through a catheter to a rat heart demonstrated the injectability of a porcine heart-derived matrix as well as endothelial cell infiltration within the matrix [183,184]. The method of delivery has been known to induce improvements within the cardiac environment with and without bone marrow mononuclear cells when injected with fibrin, collagen and matrigel, albeit separately [188,189]. Other methods have been studied, such as collagen through catheter encapsulated with bone marrow cells [190] and without cells [171]. Both these studies showed improvement in LV function without vascularization, but in the study by Huang *et al.*, there was also an improvement in vascular density [189]. Another widely available tissue culture matrix called Matrigel™, has been used as an *in situ* gel. Studies have demonstrated improvement in LV function with the gel, and ESC delivered along with it caused increased vascularization at the site of infarct [191,192]. Simulation of injection of material to the heart injected at various sites postinfarct suggests that administration of a noncontractile material at the site of infarct helps reduce stresses on the myocardium [193]. Self-assembling peptides have been useful in the delivery of IGF to the heart and permit the sustained release of the growth factor along with aiding the positive effects accrued to the cells delivered along with the peptide matrix [194]. Ungerleider and Christman have dealt with injectables and large animal models in detail, and according to their opinion, shorter gelling times are the not suitable for the delivery of injectable gels through catheters [195]. Furthermore, expansion and encapsulation through current good manufacturing practices, if not performed with adequate robustness, result in inefficient scaffolds. Despite positive results on a range of materials as injectables, alginate without cells is being currently clinically tested for its efficacy to prevent ventricular remodeling [196–198]. Radhakrishnan *et al.* have emphasized the importance of appropriate mechanical properties and electrical conductivity of the polymers used as injectables to be important in their overall regenerative potential [199].

## Translational & future perspective

With the innovations in cardiac support devices to provide care immediately after an infarct and to prevent cardiac remodeling, it was envisaged that the devices and innovations market in the cardiac space would get a boost [200,201]. But after a 10-year battle with the US FDA, the cardiac mesh support device has not seen the light of day, even after positive clinical results. Regulations are established for implantable devices, like cardiac stents, valves, pace makers and LV assist devices (LAVD) such as HeartMate® I and II, CentriMag, SynCardia Total Artificial Heart. However, there is no regulation for implant materials, with or without cells, to mitigate therapy. On the

other hand, heart injectable regulations are structured toward delivery of small molecules via intracoronary, intracardiac injections or with transcatheters [195].

Most present clinical trials are performed with established autologous stem cell populations. Although these have accrued benefits, the loss of cells, paracrine effects and differentiation away from the cardiac lineage are an associated issue with transplantation ESCs have proved their immune privilege, their propensity for teratoma inhibits their usage, but a differentiated population of committed ESC-derived cardiomyocytes are a useful proposition as a cell source. Although a significant hurdle therein is the efficient differentiation toward a cardiac lineage, obtaining functional and viable cells after differentiation and most importantly electromechanical integration with host tissue after implantation is desired. iPSC technologies, on the other hand, are yet to be realized in their complete potential and require more work for application. Real-time monitoring of cells within embedded matrices has been made possible, through nanoparticulate approaches giving us robust tools to monitor and assess tissue regeneration *in vivo* [202]. This will surely bring down investment of time into the selection of cells.

Bioreactor systems are being optimized with suitable conditions for the expansion and differentiation of stem cells [203]. Devices, such as those prepared by Kofidis *et al.* [204] and Ting *et al.* [205], can be used for simultaneous expansion and differentiation of cells *in vitro* to prepare grafts for transplantation *in vivo*. Antibody purification of cardiomyocytes is available through antibody to SIRPA, resulting in a high efficiency for selecting cardiomyocytes [206]. These cells can further be encapsulated to prepare a 3D architecture and then delivered, or grown, in a 2D matrix and layered to have a 3D structure for implantation. Good manufacturing practice requires that the growth, propagation and differentiation of cells for commercial use have to be done with animal product-free material [207].

Implant material properties of toxicity, biodegradability and physical characteristics, like stiffness, are established in the literature. Implantation of patches, cardiac assist devices and injectable noncontractile supports have been studied. Cardiac support patches can be administered in the event of superficial scarring of the heart, leading to loss of contractile tissue. Injectable hydrogels accompanied with cells can also be administered at the border zone to prevent remodeling due to scarring. Furthermore, reperfusion procedures, such as CABG, can be accompanied with such implantation of hydrogel grafts at multiple sites along the epicardium. This, along with reperfusion, will facilitate the ingrowth of stem cells and their final differentiation to cardiomyocytes. Additionally, with degradable materials, it is possible after a period, the cells will be the only remnant of the procedure. Injectable materials like self-assembling peptide matrices, for example, RAD-16, fibrin glue, alginate, agarose can be administered via a transcatheter system, or normal cardiomyoplasty, epicardially or endocardially. Pluripotent stem cells accompanying the implant could address the problem of remodeling. The administration of hydrogel material serves as a two pronged strategy; first, to act as a support matrix to the heart and prevent any remodeling due to the infarct; and second, to allow retention of cells administered within it, further improving LVEF. Furthermore, the hydrogel must be able to degrade over time and allow cells to take over the supporting role after tissue regrowth [208]. Although the regulatory hurdles and the translational challenges in just administration of hydrogels materials are immense, making the journey of hydrogel material scaffold along with cells and growth factors a strategy with long-term fruition [195].

## Executive summary

**Stem cells for cell therapy**

- Stem cells are available with various levels of benefits for an infarct condition.
- Pluripotent stem cells offer the better solution in terms of the final cell population that can be derived and applied.

**Clinical studies**

- Clinical studies have reported safety, efficacy and dosage response to stem cell populations. Cardiac stem cells and mesenchymal stem cells offer by far the best alternatives for auto and allogenic transfer. Pluripotent stem cells are yet to be evaluated clinically.

**Conclusion**

- Stem cell and biomaterial approaches are being investigated separately under clinical conditions. Furthermore, small molecule delivery for differentiation is still not under consideration.

**Future perspective**

- The dosage, delivery and integration will have to be an approach where, biomaterials act as carriers, support for the heart and matrix for differentiation, small molecules aid differentiation and cells compensate for the loss.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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• of interest; •• of considerable interest

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