Interaction between myofibroblasts and stem cells in the fibrotic heart: balancing between deterioration and regeneration

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Signalling between the various cell types in the heart has been investigated for decades. However, relatively little is known about the interplay between the cardiac fibroblasts and myofibroblasts, which help to maintain myocardial tissue structure and function, and resident cardiac or extracardiac stem cells involved in tissue homeostasis and repair. Much of our knowledge about these interactions is derived from experimental animal models, especially those of myocardial infarction and stem cell transplantation. However, it still remains incompletely understood how stem cell therapy could modulate cardiac fibrosis in a beneficial manner and, how on the other hand, fibrotic processes in the heart may affect the therapeutic potential of stem cell therapy. A detailed and mechanistic insight into these matters would expedite the therapeutic optimization of cardiac cell therapy for the fibrotic heart and may even provide a basis for future biological therapies aiming for a reversal of cardiac fibrosis. Therefore, the main focus of this review is to discuss interactions between myofibroblasts and stem cells, especially in the adult and diseased, fibrotic myocardium, and emphasize those aspects that require more investigation using dedicated models and tools.

Keywords: Myofibroblasts • Stem cells • Cardiac fibrosis • Cell therapy • Heterocellular communication

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1. Introduction

The adult heart is rich in fibroblasts, which, by producing extracellular matrix (ECM), help to establish and maintain well-organized, three-dimensional tissue structures with defined spatial characteristics. Besides their role in modulating cardiac muscle structure and function under physiological conditions, these cells play a crucial role in the adaptation and/or repair processes triggered by cardiac aging, stress, and injury. One aspect of these processes involves the transdifferentiation of fibroblasts into myofibroblasts, cells that express contractile proteins, like α-smooth muscle actin, and secrete pro-fibrotic and anti-inflammatory factors to preserve structural integrity of the affected myocardium. Although ensuring the organism’s short-term survival, such myocardial scarring after stress or injury may eventually contribute to heart failure and cardiac arrhythmias. Hence, the ischaemic and fibrotic myocardium has been subject of intense research into underlying pathophysiological mechanisms and therapeutic targets to counteract these detrimental effects. Through these investigations, it has become clear that myocardial scars are biologically active, instead of largely a-cellular accumulations of invariable ECM. As such, infarcted myocardial tissue has become an interesting target for modification. Along with the search for novel drug- and surgery-based interventions to reduce, slow, or even stop the progression of cardiac fibrosis (i.e. reactive fibrosis), in recent years, (stem) cell therapy has been introduced as a possible option to minimize myocardial scar formation following myocardial infarction. Although there is currently no consensus on the actual beneficial effects nor the underlying mechanisms of cardiac cell therapy, numerous studies did show an improvement in cardiac function/perfusion upon transplantation of different stem cell types in ischaemic and/or fibrotic hearts, both in experimental and clinical settings. In most of these studies, cells were transplanted into myofibroblast-rich regions, e.g. the infarct border zone or stressed myocardium. While cardiac cell therapy aims to have beneficial effects on surrounding, damaged tissue, not much is known about the effects of recipient tissue on the implanted cells. Cardiac tissue targeted by cell therapy often comprises a relatively hostile environment and therefore provides suboptimal conditions for cell retention and survival. However, there is increasing evidence that not only these environmental factors affect the biological and therapeutic activity of transplanted stem cells, but that also cells residing in the recipient tissue, mostly myofibroblasts,
may directly or indirectly influence the potential of transplanted stem cells to improve cardiac function. Detailed and more mechanistic insights into these matters of cell transplant–recipient tissue interactions would aid to further optimization of cardiac cell therapy for the fibrotic heart. Such insights may even provide a basis for future, biological therapies aiming for a reversal of cardiac fibrosis. The main focus of this review is therefore to discuss the interactions between myofibroblasts and stem cells, especially in the diseased, fibrotic myocardium, and emphasize those aspects of donor cell–recipient tissue interactions requiring more investigation.

First, the effects of endogenous and various types of transplanted stem cells on different aspects of cardiac fibrosis will be discussed. Secondly, the impact of fibrotic myocardium on the function of stem cells, either endogenously present or transplanted, will be discussed. Thirdly, a number of future perspectives will be outlined, including those related to genetic engineering of myofibroblasts for mechanistic studies on their role in the pathogenesis of cardiac diseases and to endow them with properties favouring myocardial regeneration.

2. Effects of stem cells on cardiac fibrosis

Stem cells are undifferentiated cells that can be found throughout the human body, including the heart. These cells can proliferate and, upon proper stimulation, differentiate into more specialized cells. Several stem cell types have been studied for their effects on cardiac fibrosis, including those derived from the heart itself, but also others like mesenchymal stem cells (MSCs) isolate from bone marrow (BM) and adipose tissue. While different cardiac pathologies have been targeted with cell therapy, most data come from experimental and clinical studies on stem cell transplantation into the failing, infarcted heart. Although the outcome of these studies differs, both in terms of molecular, histological, and clinical parameters, most studies seem to report at least some beneficial effects on cardiac fibrosis.

2.1 Effects of cardiac progenitor cells on cardiac fibrosis

Little is known about the natural role of endogenous cardiac stem cells [also referred to as cardiac progenitor cells (CPCs)] in modulating the fibrotic responses of the heart to aging and injury. Although transgenic animal models allow tracing of defined stem cell populations in the healthy and damaged heart, these models have not yet been combined with lineage tracing of cardiac fibroblasts and myofibroblasts. Such investigations would provide important insights into the natural effects these endogenous stem cells may have on cardiac fibrosis. However, local stimulation of CPCs by cytokines has shed some light on their potential role in counteracting myocardial scarring. Rota et al. used local injections of hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) to boost the accumulation, survival, and proliferation of endogenous CPCs at the site of myocardial infarction. This resulted in degradation of collagen fibres, the main constituents of myocardial scars, by matrix metalloproteinases (MMP-2, -9, and -14) secreted by these progenitor cells, while the amount of tissue inhibitor of MMP-4 (TIMP-4) was decreased. Hence, these CPCs could now invade the scarred area and contribute to the formation of new cardiomyocytes. These investigations suggest that the beneficial effects of local CPC activation on cardiac fibrosis are mainly mediated by degradation of the ECM components produced by the myofibroblasts in the scar, and not by direct inhibition of ECM production.

On the other hand, the effects of CPCs on cardiac fibrosis, once isolated, expanded ex vivo and subsequently transplanted at sites of myocardial injury, have been investigated more intensively. These studies also point towards a more indirect effect of CPCs on fibrosis, by (i) facilitating the degradation of the ECM in the myocardial scar and (ii) replacing it with new cardiomycocytes and vasculature, ultimately leading to a reduction in scar size and an improvement of cardiac function. In addition, also CPCs derived from the epicardium, instead of the myocardium, appear to have beneficial effects on cardiac fibrosis after myocardial infarction. Interestingly, besides its role as a cell source for transplantation after cardiac injury, the epicardium also acts as an endogenous source of fibroblast progenitor cells in both healthy and disease conditions. Recently, CPCs have been transplanted together with other stem cells into the damaged myocardium in an attempt to further improve the therapeutic effects of such interventions. Also in these studies, degradation of ECM appeared to play an important role in the beneficial effects observed after cell injection near the areas of myocardial fibrosis. Taken together, CPC activation or transplantation seems a promising approach for the treatment of cardiac fibrosis upon ischaemic cardiac injury. However, obtaining these CPCs in sufficient numbers for therapeutic purposes is technically challenging. Although myocardial biopsies have been proposed as a safe and rich source of CPCs, for practical reasons stem cells from more accessible sources are currently being explored for their ability to counteract cardiac fibrosis.

2.2 Effects of non-cardiac stem cell transplantation on cardiac fibrosis

Various non-cardiac stem cell types have been tested for their beneficial effects on cardiac fibrosis, in most cases in the context of myocardial infarction. The vast majority of reports about the effects of exogenous stem cells on cardiac fibrosis deal with cells derived from BM. The BM contains many different nucleated cell types, which can be separated in different fractions on the basis of (i) relative density, (ii) cell surface marker profile, or (iii) vital dye exclusion ability. Different stem cell types can be found in these fractions, including endothelial progenitor cells, haematopoietic stem cells and, although relatively low in abundance, but intensively investigated in especially experimental studies, MSCs. Although no consensus has yet been reached about the limits of their differentiation range, several studies have indicated that these BM cells can differentiate into cells with characteristics of endothelial cells, fibroblasts, pericytes (PC), myofibroblasts, smooth muscle cells (SMCs), and possibly even cardiomycocytes. Besides their ability to differentiate into many different mesodermal cell types, BM-derived MSCs secrete a large number of cytokines and chemokines, and especially this paracrine activity is held responsible for the therapeutic effects following transplantation near myofibroblast-rich areas. Regardless of the exact cellular composition of the transplant used for intervention, most, but not all, studies with cells derived from BM show a modest inhibitory effect on cardiac fibrosis in the infarcted heart as deduced from left ventricular function measurements and immunohistochemical analyses.

The effects of BM cell therapy in the clinical setting have been discussed extensively in recent review articles. However, although paramount for determining the relevance of such interventions in
In similar experiments, Mias type I and III expression in cardiac fibroblasts. However, the supposed medium. Moreover, exposure to such medium also reduced collagen 3, was associated with the anti-proliferative effects of MSC-conditioned MSC cultures (presumed to contain MSC-secreted factors) and vitro models to show that medium obtained from adult rat BM-derived further mechanistic insight was provided by lekushi et al. who showed that BM-derived mononuclear cells secrete IGF-1 leading to a reduction in the expression of the pro-apoptotic miR-34a. In addition, expression of the pro-fibrotic miR-21 was significantly decreased upon transfection of these cells near infarcted murine myocardium. Likewise, paracrine signalling through HGF released from BM cells transplanted into the infarcted myocardium appeared to suppress pro-fibrotic signalling by miR155, thereby inhibiting cardiac fibrosis. As mentioned earlier, BM harbours different cell types, including MSCs. The therapeutic potential of MSCs in the damaged heart has been investigated in numerous studies, often with promising results. These beneficial outcomes may at least in part be explained by the inhibitory effects of MSCs on cardiac fibrosis. It seems that transplantation of these cells into infarcted myocardial areas leads to a decrease in ECM production due to secretion of paracrine factors (e.g. HGF) by the MSCs. ECM production is also attenuated by MSC-dependent suppression of TGF-β signalling, which, among others, reduces the rate of fibroblast to myofibroblast differentiation. Apart from their ability to indirectly affect cardiac fibrosis by inducing ECM degradation, a number of studies have indicated that MSCs could also exert direct effects on cardiac fibroblasts. Ohnishi et al. used in vitro models to show that medium obtained from adult rat BM-derived MSC cultures (presumed to contain MSC-secreted factors) and applied to cardiac fibroblasts strongly reduced their proliferation rate. Further studies indicated that up-regulation of various genes known to inhibit proliferation, like elastin and DNA damage-inducible transcript 3, was associated with the anti-proliferative effects of MSC-conditioned medium. Moreover, exposure to such medium also reduced collagen type I and III expression in cardiac fibroblasts. However, the supposed paracrine factors responsible for these effects remain to be identified. In similar experiments, Mias et al. were able to prove that conditioned medium of rat MSCs also decreased cardiac fibroblast viability based on ATP quantification assays. The decrease in cell viability was accompanied by lower α-smooth muscle actin expression, suggesting myofibroblast loss or change of phenotype. In addition, less ECM was produced by these fibroblasts after exposure to MSC-conditioned medium, and transplantation of rat MSCs into infarcted rat myocardium attenuated the fibrotic response as assessed by Sirius red staining. Several factors could be responsible for these anti-fibrotic effects, like HGF, basic fibroblast growth factor (bFGF), IGF-1, and adrenomedullin (ADM). ADM was studied in more detail by Li et al. who showed that ADM secretion by MSCs was associated with inhibition of cardiac fibroblast proliferation and collagen I synthesis, which is in line with the results of earlier studies. In addition, after MSC transplantation into the fibrotic myocardium of rats suffering from dilated cardiomyopathy, cardiac fibrosis was less pronounced than in the vehicle-treated group. Besides the effects, MSCs may have on ECM production, these cells are also known for their immunomodulatory effects. These effects result from interactions between MSCs and cells involved in both innate and adaptive immune responses (e.g. dendritic cells, neutrophils, natural killer cells, mast cells, monocytes/macrophages, B cells, and T cells), which involve, for example, secretion of various factors like interleukin-6 (IL-6), HGF, and TGF-β. More details about the immunomodulatory effects of MSCs in the damaged heart can be found in recent review articles. As chronic inflammation is an important driving force in the development of cardiac fibrosis, transplantation of MSCs near the site of inflammation may have beneficial effects after the initial phases of inflammation through anti-inflammatory actions. Indeed, studies using rat models of myocardial infarction suggested that MSC transplantation inhibited nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), attenuated tumour necrosis factor alpha (TNF-α), and IL-6, and increased IL-10 expression in remote myocardium. For such in vivo studies, it is difficult to investigate whether stem cell injection merely inhibited cardiac fibrosis or actually reversed the processes of fibrosis. Nevertheless, the vast amount of studies on MSC therapy in fibrotic hearts points towards beneficial effects. However, a number of recent studies on MSCs resulted in some cautionary notes. First of all, MSCs derived from the heart appeared to perform less than those derived from more conventional MSC sources in terms of therapeutic potential, thought to be mainly due to pro-inflammatory features of cardiac MSCs. In line with this study, it was shown that, after infarction, cardiac MSCs can give rise to fibroblasts, which in turn contribute to scar formation by ECM production. The same research group showed that, upon myocardial infarction in the aged murine heart, MSCs could give rise to dysfunctional and poorly maturing fibroblasts, thereby contributing to diminished scar formation (i.e. reparative fibrosis). These detrimental effects could be counteracted by treatment with activators of AMP-activated protein kinase, leading to MSC mobilization, their differentiation towards myofibroblasts, increased collagen deposition, and fibre maturation in the scar, accompanied by reduced adverse remodelling when compared with that in control animals. On a different note, studies by Arslan et al. indicated that exosomes (i.e. small extracellular vesicles) are, at least partly, responsible for the MSC-mediated paracrine effects and upon injection prior to reperfusion of murine myocardium resulted in a 45% reduction in infarct size when compared with that in control animals receiving saline. With such impressive effects, exosome-based therapy might be favoured over cell-based therapy for particular cases considering the potential adverse effects of cell injection. In addition, other cell types have been investigated for their anti-fibrotic potential, including embryonic stem cells (ESCs), skeletal myoblasts (SMBs), SMCs, and PCs. A study by Burt et al. revealed that transplantation of irradiated ESCs, and therefore non-proliferative and prone to apoptosis, in infarcted murine or monkey hearts resulted in enhanced survival and proliferation of cardiomyocytes, but also less cardiac fibrosis. These data were supported by microarray analysis showing a decrease in pro-fibrotic gene expression. From a more mechanistic point of view, direct cell–cell interactions, secretion of paracrine factors, and a so-called scaffolding effect between transplanted and host cells seemed to play an important role in the observed beneficial effects. However, more research is needed to identify the exact ESC-mediated modes of action. Transplantation of SMBs into the infarcted myocar-
medium has been reported to also have a beneficial effect on fibrosis. This may result, on one hand, from expansion of the cellular graft, thereby compensating for the local increase in fat and fibrous tissue, and on the other hand from factors secreted by these transplanted cells.63,64 Quantitative RT-PCR analyses showed that, 3 days after transplantation of SMBs in infarcted adult rat myocardium, MMP-2 and TIMP-4 were up- and down-regulated, respectively, when compared with control.65 Interestingly, Farahmand et al.66 showed that SMB transplantation after myocardial infarction in rats also preserved ECM architecture outside the area of cell injection as well as global cardiac function, suggesting a more widespread effect of these implanted cells that was associated with a decrease in both MMP-2 and -9 activities. In addition to the more commonly found effects of cell therapy on myocardial fibrosis (e.g. effects on ECM-modulating factors, like TIMP),67,68 intramyocardial transplantation of SMCs was shown to also have a direct effect on myofibroblasts.69 In the cell group, hearts expressed significantly less of the myofibroblast marker α-smooth muscle actin, while expression of bFGF was increased. This latter factor is known to suppress transdifferentiation of fibroblasts into myofibroblasts.70 PCs is another group of cell that has been studies for its anti-fibrotic effects. These contractile cells line small blood vessels and represent multipotent precursors.71 Transplantation of saphenous vein-derived PC progenitor cells into the borderzone of infarcted murine myocardium resulted in, among others, a reduction in scar size and interstitial fibrosis, which was associated with long-term improvement of cardiac function.72 Mechanistically, it was shown that conditioned medium of these cells, containing secreted miR-132, limits myofibroblast formation in vitro by inhibition of at least two targets of this miR; Ras-GTPase activation protein and methyl-CpG-binding protein 2. Similar results were found by Chen et al.73 using human PCs.

As with the cell types discussed in previous sections, secretion of paracrine factors by abovementioned cells acting on ECM-modulating factors like MMPs and TIMPs seems a plausible underlying mechanism of the anti-fibrotic effects (see Figure 1), but further investigation is certainly warranted, especially with regard to identification and long-term therapeutic potential of these factors.

Besides different types of native stem cells, also stem cells that were genetically modified prior to transplantation have been injected in fibrotic myocardium to further improve their therapeutic potential and to increase our knowledge of their possible mode(s) of action. The effects of stem cells, either genetically modified or not, on cardiac fibrosis by other means than intramyocardial injection, e.g. tissue engineering, are discussed in more detail elsewhere.13,45,74 – 76 As stem cell transplantation could modulate the fibrotic processes in a beneficial way, the short- and long-term consequences of cardiac fibrosis, like the induction of cardiac arrhythmias and development of heart failure, may also be counteracted. However, further investigation is needed to better comprehend the complex interplay between molecular and biochemical processes underlying the different aspects of cardiac fibrosis at different time-points and how the unique features of stem cell therapy, or derivatives like cytokine therapy, could be exploited for maximal therapeutic benefit in defined patient populations.

**Figure 1** Overview of effects and proposed mechanisms of cell transplantation on various aspects of cardiac fibrosis. In addition, MSCs may exert immunomodulatory effects. These effects could have an impact on inflammation and therefore also on different features of cardiac fibrosis, as inflammation is one of its key driving forces. Also, MSCs are known to secrete exosomes, containing paracrine factors listed above, and thereby affect cardiac fibrosis. FB, fibroblast; MFB, myofibroblast; CMCs, cardiomyocytes; ECM, extracellular matrix; ADM, adrenomedullin; TGF-β, transforming growth factor-β; bFGF, basic fibroblast growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP; IGF-1, insulin growth factor-1; MSCs, mesenchymal stem cells; BMCs, bone marrow cells; SMCs, smooth muscle cells; PCs, pericytes; CPCs, cardiac progenitor cells; ESCs, embryonic stem cells.
3. Effects of cardiac fibrosis on stem cells

Most studies on cardiac cell therapy have investigated whether cardiac function improves after stem cell transplantation and thereby primarily focused on the effects of the transplanted cells on cells residing in the damaged myocardium. However, relatively little information is available about the effects of cardiac cells at the site of injury (e.g. myofibroblasts) on the transplanted stem cells.

Originally, the main goal of cardiac stem cell therapy was to regenerate the damaged heart by replenishing lost cardiomyocytes, SMCs, and endothelial cells either through differentiation of the transplanted cells or by inducing differentiation of resident CPCs, angiogenesis, or myocardial cell survival via paracrine factors released by the transplanted cells. However, a number of studies have shown important insights indicating that the recipient tissue may affect the properties of transplanted cells and thereby affect their therapeutic potential.

3.1 Effects of ECM elasticity on stem cells

After myocardial infarction, the damaged myocardium loses compliance due to cardiac fibrosis, including the production of large amounts of ECM by myofibroblasts. A study by Engler et al. investigated the influence of matrix elasticity on stem cell differentiation. It was shown that the lineage specification of MSCs is strongly influenced by matrix rigidity. From these studies it became clear that naive stem cells are able to sense their microenvironment and adapt their differentiation pathway accordingly. The non-muscle myosin II isoforms, present in MSCs, were identified to be involved in sensing matrix elasticity. Matrix elasticity was mimicked in vitro by seeding MSCs on collagen I-coated inert polycrylamide gels with various degrees of cross-linking allowing control over the coating's elasticity. Cell morphology, RNA profiles, and cytoskeletal protein and transcription factor content indicated that MSCs grown on a matrix with a particular stiffness tend to become cells that experience a similar matrix rigidity in their natural environment. For example, soft matrices were neurogenic, stiffer matrices myogenic, and rigid matrices osteogenic. The influence of matrix elasticity on the differentiation of MSCs was confirmed in this study by blocking their mechanosensing mechanism with blebbistatin, a myosin II inhibitor, which blocked all elasticity-induced lineage specification. The same research group also investigated the importance of the rigidity of the microenvironment in striated muscle differentiation. In this study, they showed that myosin/actin striation only appeared in myotubes that were seeded on gels with an elasticity specific for skeletal muscle tissue. In another study, they confirmed that matrix elasticity is important for differentiation of CPCs into more specialized cells. Of note, the stiffness of the microenvironment changes during the different phases of cardiac development. Mimicking these dynamics of the ECM in vitro enhanced the differentiation of CPCs. CPCs that were allowed to differentiate on a substrate with increasing rigidity much better recapitulated the developmental changes in expression of the immature cardiac marker NKX2.5 and the late cardiac marker troponin T than those grown on a matrix of constant stiffness. Similar results were found for BM-derived MSCs. These findings suggest that loss of compliance during the process of cardiac fibrosis should be taken into account in the further development of cardiac cell therapy, especially if cardiomyogenic differentiation is considered to be part of the underlying mechanistic mechanisms. In this respect, Zhang et al. have performed an interesting study in which they hypothesized that timing of stem cell transplantation in the damaged myocardium is relevant to achieve an optimal therapeutic effect of cell therapy due to changes in myocardial stiffness. This hypothesis was based on the in vitro studies described above, but also on several clinical studies showing that the therapeutic effect of transplantation of BM-derived cells after myocardial infarction differs with the time point at which the cells are injected. One study showed that stem cell transplantation 1 week after myocardial infarction was superior to transplantation within 1 h or after 2 weeks. The REPAIR-AMI study yielded similar results, namely that transplantation of stem cells 5 days or more after myocardial infarction was more beneficial than injection within the first 4 days after infarction. Zhang et al. also conducted in vitro experiments in which they cultured BM-derived cells in an environment with similar stiffness as the infarcted myocardium at Day 7. The ability of these BM cells to differentiate into endothelial-like cells was greater than that of cells cultured in an environment that mimicked infarcted myocardium after 1–24 h. However, coronary occlusion does not only result in cardiac fibrosis and scar formation, but also induce time-dependent changes in many other biological and biochemical processes. It is therefore difficult to determine the direct impact of myocardial stiffness on the transplanted stem cells and their therapeutic potential at the site of injury. A recent study by Huang et al. suggested that the potential detrimental effects of myocardial scarring on cardiac cell therapy could also result from the physical barrier produced by the ECM accumulated at the site of injury. In control hearts, such an ECM barrier was associated with hampered penetration of epicardially applied cells into underlying infarcted areas and lower therapeutic efficacy, when compared with miR-29b-treated and therefore less fibrotic rat hearts.

In conclusion, the studies discussed in this section point towards important roles for ECM composition and elasticity as factors that may influence the therapeutic effect of stem cell therapy in the fibrotic myocardium.

3.2 Effects of heterocellular coupling on stem cells

Besides cell–ECM interactions, cell–cell contact is another factor that appears to play a major role in stem cell differentiation in the damaged myocardium. After myocardial infarction, a fraction of the lost cardiomyocytes is replaced by myofibroblasts. Different studies in which several types of stem cells were co-cultured with cardiomyocytes have shown that stem cell fate is influenced by contact with neighbouring cells. For example, skeletal muscle-derived cells were shown to differentiate towards cardiomyocyte-like cells if these cells were cultured adjacent to native cardiomyocytes. In more detail, contraction of the cardiomyocytes was essential in inducing cardiomyogenic differentiation as inhibition of contraction abolished expression of the cardiac marker proteins NKX2.5, atrial natriuretic peptide, troponin T, and GATA4, and cardiomyocyte-like action potentials could no longer be recorded in the skeletal muscle-derived cells. Our research group has shown that co-culture of foetal human MSCs with neonatal rat cardiomyocytes is necessary to induce cardiomyogenic differentiation, whereas co-culture with neonatal rat cardiac fibroblasts did not lead to expression of cardiac-specific proteins and the presence of cardiomyocyte-like action potentials. Next, we also investigated the role of gap junctional coupling in cardiomyogenic differentiation of these MSCs. In this study, we showed that gap junctional coupling with neighbouring cardiomyocytes was necessary to induce cardiomyogenic differentiation in foetal human MSCs. Knockdown of the gene encoding the gap junction protein connexin43 inhibited cardiomyogenic differentiation of the
MSCs, while rescue of gap junctional coupling by up-regulation of connexin45 expression in these cells restored their cardiomyogenic differentiation potential. These studies may indicate that stem cell transplantation in an area where no or very few viable cardiomyocytes are present, but myofibroblasts represent the majority of cells could impair the regenerative potential of these stem cells, at least in terms of their cardiomyogenic differentiation ability.

As discussed in detail in the earlier section, the paracrine activity of transplanted stem cells appears to be an important determinant of their therapeutic potential in the damaged heart. However, to our knowledge, no studies have been reported that have explicitly investigated the influence of fibrotic myocardium on the paracrine activity or actions of stem cells transplanted into myofibroblast-rich areas. Since the therapeutic effect of adult stem cells used in cell therapy are nowadays suggested to be mainly based on paracrine effects signaling, it is important to unravel the influence of cardiac fibrosis on these paracrine effects.

In conclusion, cardiac fibrosis could have an influence on the therapeutic potential of stem cell-based therapy in the damaged heart by changing ECM composition and elasticity. Also, contact with adjacent cells appears to be an important determinant of the regenerative potential of stem cells (see Figure 2). Finally, although not well investigated as of yet, paracrine effects of stem cells transplanted into the damaged myocardium may also be affected by cardiac fibrosis, but of all points discussed above this issue requires the most investigation before any evidence-based statements can be put forward.

4. Conclusions and future perspectives

The biological role of myofibroblast–endogenous stem cell interactions, in terms of cardiac fibrosis, remains unclear, although a number of studies suggest that endogenous CPCs may affect myocardial scar integrity by secretion of ECM-modulating factors. More is known about the interaction between myofibroblasts and stem cells injected at the site of injury. Paracrine signaling of these stem cells, influencing ECM composition and myofibroblast proliferation and viability, appears the dominant mode of action of these stem cells. From a different point of view, not much is known about the effects of myofibroblasts residing in the infarcted myocardium on the stem cells being transplanted into these regions. Based on the current literature, ECM elasticity and myofibroblast–stem cell coupling seem to be two important factors. Various studies have indicated that lineage specification of stem cells is strongly influenced by matrix rigidity, a factor that is directly related to myofibroblast activity. In addition, co-culture studies have shown that cardiac differentiation of stem cells depends on the adjacent cell type and gap junctional coupling, e.g. cardiac (myo)fibroblasts fail to induce cardiomyogenic differentiation of adjacent MSCs. One aspect of myofibroblast–stem cell interaction that is very poorly understood, but certainly warrants dedicated research, concerns to effects of a fibrotic environment on the paracrine activity and variety of different factors secreted by transplanted stem cells. More detailed and mechanistic insights into all of these matters are likely to facilitate therapeutic optimization of cell therapy for the fibrotic heart and may also lead to the development of biological therapies aiming for the reversal of cardiac fibrosis. With regard to such therapies, the recent discovery of direct cardiac reprogramming is of special interest. Inspired by the work of Yamanaka and co-workers, who were the first to convert adult fibroblasts into pluripotent stem cells by genetic engineering, other laboratories succeeded to reprogramme cardiac fibroblasts directly into functional cardiomyocyte-like cells by forced expression of selected transcription factors in these fibroblasts. The true promise of such reprogramming strategies became evident when follow-up studies indicated that also myofibroblasts present in the infarcted myocardium could be reprogrammed into cardiomyocyte-like cells. Intriguingly, since myofibroblasts replace cardiomyocytes after infarction and transplanted stem cells may contribute to formation of new cardiomyocytes,
the direct cardiac reprogramming of myofibroblasts seems to combine these different aspects of cardiac deterioration and regeneration in a truly beautiful manner.

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