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Recovery of cardiac function mediated by MSC and interleukin-10 plasmid functionalised scaffold

Carolyn A. Holladay, Aoife M. Duffy, Xizhe Chen, Michael V. Sefton, Timothy D. O'Brien, Abhay S. Pandit

Network of Excellence for Functional Biomaterials, National University of Ireland, Galway, Ireland
Regenerative Medicine Institute, National University of Ireland, Galway, Ireland
Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada

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A B S T R A C T
Stem cell transplantation has been suggested as a treatment for myocardial infarction, but clinical studies have yet to demonstrate conclusive, positive effects. This may be related to poor survival of the transplanted stem cells due to the inflammatory response following myocardial infarction. To address this, a scaffold-based stem cell delivery system was functionalised with anti-inflammatory plasmids (interleukin-10) to improve stem cell retention and recovery of cardiac function. Myocardial infarction was induced and these functionalised scaffolds were applied over the infarcted myocardium. Four weeks later, stem cell retention, cardiac function, remodelling and inflammation were quantified. Interleukin-10 gene transfer improved stem cell retention by more than five-fold and the hearts treated with scaffold, stem cells and interleukin-10 had significant functional recovery compared to the scaffold control (scaffold: −10 ± 7%, scaffold, interleukin-10 and stem cells: +7 ± 6%). This improved function was associated with increased infarcted wall thickness and increased ratios of collagen type III/type I, decreased cell death, and a change in macrophage markers from mainly cytotoxic in the scaffold group to mainly regulatory in scaffold, stem cells and interleukin-10 group. Thus, treatment of myocardial infarction with stem cells and interleukin-10 gene transfer significantly improved stem cell retention and ultimately improved overall cardiac function.

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1. Introduction

Cardiovascular disease, a major component of which is myocardial infarction (MI), is the leading cause of death in the developed world and is responsible for approximately 33% of deaths worldwide. Mesenchymal stem cell (MSC) transplantation has been proposed as a treatment for a number of cardiovascular diseases including lower-limb ischemia [1], stroke [2], and myocardial infarction (MI) [3–10]. In MI, some early preclinical studies have reported significant therapeutic improvements that were associated with stem cell transplantation [3–5] but clinical trials have failed to translate these results into humans [7,9,10].

A potential reason for this failure is the very poor retention rate of stem cells transplanted into the ischemic myocardium; in a recent study, only 2% of injected stem cells remained within the injection zone after 7 days [11]. It is possible that this poor retention of transplanted cells may be related to the inflammatory response associated with ischemia/reperfusion (IR) injury [12–15]. In fact, a recent study found a significant decrease in MSC retention (95% loss after 7 days) in a syngeneic model where the cells were delivered in a collagen scaffold [16]. As the cells were syngeneic, the adaptive immune-mediated response was relatively minor and the inflammatory reaction appeared to be the primary cause of transplanted MSC death.

An emerging method for modulating host response to transplants is localized anti-inflammatory gene transfer [17,18]. This effectively decreased rejection of whole organ transplants and was recently shown to improve the survival of MSC transplants, where transfection with the anti-inflammatory gene interleukin-10 (IL-10)
was used to modulate the inflammatory response after implantation of a collagen scaffold seeded with rat mesenchymal stem cells (rMSCs) [16]. IL-10 is considered the most potent anti-inflammatory cytokine produced naturally and has been used in a number of studies to decrease or control inflammation [19–22].

It was hypothesized that in vivo transfection with IL-10 could be used to increase the retention rate of stem cells in a collagen scaffold when delivered to the ischemic myocardium. The primary objectives were to quantify the effects of scaffold-mediated IL-10 gene transfection on stem cell retention, overall cardiac function and the overall inflammatory response.

2. Materials and methods

2.1. Animals

A total of 45 female Lewis rats weighing between 180 and 250g were obtained from Charles River and allowed to acclimatise for at least 7 days. All animal procedures were approved by the institutional animal ethics committee and the federal board under the Cruelty to Animals Act. All animals received humane care in compliance with federal and institutional guidelines.

2.2. Materials

Rat mesenchymal stem cells (rMSCs) were generously donated by Dr. Mary Murphy (Regenerative Medicine Institute, Galway, Ireland). These cells were isolated and characterized as previously described [16,23]. CMV-promoter driven mouse interleukin-10 plasmids were generously donated by Dr. Jeffrey Medin (University of Toronto). These plasmids were propagated and isolated according to standard protocols [16]. Partially degraded polyamidoamine (PAMAM) dendrimers, commercially available as SuperFect™ (Qiagen, IE) were used to complex and deliver the plasmids. All other standard chemicals and reagents were obtained from Sigma Aldrich (IE).

2.3. Preparation and loading of scaffolds

A 0.3w/v% type I atelocollagen solution was freeze-dried and crosslinked with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide and N-hydroxysuccinimide (EDC/NHS) to make the collagen scaffolds, as described elsewhere [24]. Interleukin-10 plasmid-dendrimer polyplexes (pIL-10) were prepared by incubating IL-10 plasmids with SuperFect™. 2 µg of plasmid complexed to 30 µg of dendrimer was added to each scaffold and the IL-10 polyplexes (pIL-10) were allowed to adsorb for 3 h.

2.4. Seeding of cells onto scaffolds

Flasks containing male rMSCs between passage 4 and 6 were washed with DPBS and stained with a 4 µM solution of Celltracker™ CM-DiI (Invitrogen, IE) for 30 min at 37 °C. These labelled rMSCs were pipetted onto the polyplex-loaded scaffolds and the entire system was incubated overnight to allow the cells to attach.

2.5. Induction of myocardial infarction

Each rat was anaesthetized with isoflurane (5% induction, 2% maintenance), intubated and ventilated using a volume-controlled ventilator with a mixture of oxygen (±isoflurane) and room air. The tidal volume (1.2 ml/100 g) and respiration rate (65–70/min) were automatically calculated using the animal’s weight. A Small Animal Monitoring and Gating System (Harvard Apparatus, UK) was used to monitor the animal’s vital statistics throughout the procedure. The left thoracic region was

Fig. 1. Stem cell retention. The retention of rMSCs (red) in collagen scaffolds after 28 days attached to an infarcted rat heart is shown qualitatively (a) and quantitatively (b, c). The representative sections in (a) are shown at lower (top) and higher (bottom) magnifications to illustrate the distribution of cells. The quantifications of volume fraction and rMSC numbers per mm² in the scaffold alone ( ), scaffold + rMSCs ( ), and scaffold + rMSCs + pIL-10 ( ) groups illustrate the increase in cell retention associated with the inclusion of IL-10 encoding polyplexes. (Data expressed as mean ± 95% CI, * represents statistical significance, p < 0.05, n = 8, scale bar represents 20 µm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
shaved and swabbed with 10% povidone iodine to disinfect the region. Thoracotomy was performed by opening the chest at the fourth or fifth intercostals space to expose the heart. Permanent coronary artery ligation of the left anterior descending artery (LAD) was used to induce MI and was confirmed by discolored of the affected myocardium. The suture location was set at approximately 1 cm from the apex of the heart.

2.6. Implantation of scaffolds

The rMSC and pIL-10 loaded scaffolds were applied directly to the surface of the heart where discoloration marked the infarct and a single suture was used to secure the scaffold in place. The animal was then closed and placed in a Small Animal Recovery Chamber (Harvard Apparatus, UK) overnight. All mortalities that occurred within 24 h of the procedure were assumed to be due to post-operative complications unrelated to the therapy and were thus excluded from the remainder of the study.

2.7. Echocardiography

Transthoracic echocardiography was used to assess overall cardiac function. Baseline echocardiograms were obtained prior to any procedures. Post-operative echocardiograms were obtained 24 h after LAD ligation and the final echocardiograms were obtained immediately prior to euthanasia, 28 days after infarction. M-mode analysis allowed estimation of the left ventricular (LV) ejection fraction (LVEF %). Any animal found to have less than a 15% decrease in overall LVEF% or an LVEF% over 80% after MI was induced was excluded from the remainder of the study. The diameters of the hearts in systole and diastole were also examined as a measure of ventricular dilation.

2.8. Tissue preservation and sectioning

Each rat was euthanized and its heart excised immediately after the sacrifice echocardiogram was obtained. The hearts were flushed with saline before preservation in 10% buffered formalin. After a minimum of 48 h in fixative, the hearts were sectioned at 2 mm thickness and perfused using an ASP300 Tissue Processor (Leica Microsystems, Meyer Instruments, USA). The heart slices were then embedded in paraffin and sectioned at 5 μm thickness using a microtome. The sections analyzed immunohistochemically were embedded in OCT and flash-frozen for cryosectioning.

2.9. Staining

Standard Masson’s Trichrome and Picrosirius red staining protocols were used with rehydrated sections. After staining, the sections were dehydrated in an ascending series of ethanol baths, cleared in xylene, and mounted using DPX mounting media. In all analysis, the areas discussed are as described in Supplementary Fig. 1.

2.10. Stem cell volume fraction quantification

Fluorescent micrographs of rehydrated sections, counter-stained with 4',6-diamidino-2-phenylindole (DAPI) (Invitrogen, Ireland), were analyzed stereologically to quantify the volume fraction of rMSCs in each tissue sample. These sections

Fig. 2. Cardiac function. Mean ejection fraction data from all animals from the scaffold alone (a), scaffold + rMSCs (b) and scaffold + rMSCs + pIL-10 group (c). The infarcted hearts treated with collagen scaffolds tended to have progressively decreasing LVEF%. The scaffold + rMSCs group was more variable, with some hearts recovering significantly while others continued to lose function. The scaffold + rMSCs + pIL-10 group had mostly constant or improved LVEF% and the mean recovery in LVEF% (d) was statistically higher in the scaffold + rMSCs + pIL-10 group (□) than the scaffold alone control (■). The scaffold + rMSCs group yielded highly variable data which was not significantly different from either group (▲). There was no change observed in left ventricular diameter in systole (LVDs) but there was a trend towards less dilation in the scaffold + rMSCs + IL-10 group (e). (Data expressed as mean ± 95% CI, * represents statistical significance, p < 0.05, n = 8. The intra-observer correlation coefficients were: 0.947, 0.980 and 0.982, while the inter-observer correlation coefficient was 0.976).
were mounted using aqueous mounting media (Vectorshiedt, Vectorlabs, USA). At least 3 sections, each separated by 400 μm, and a minimum of 3 images per section were analyzed.

2.11 Immunohistochemistry

Immunohistochemistry (IHC) was used to visualize IL-10 production using goat anti-IL-10 primary antibody (1:100, R&D Systems, MN) and FITC-anti-goat IgG secondary (1:100, VectorLabs, USA). Macrophage phenotype was investigated as described elsewhere [25,26] using IHC for M1 (anti-CD80, 1:10, AdiB Serotec, UK) and M2 (anti-CD163, 1:50, AdiB Serotec, UK). Collagen type III was stained for using mouse anti-rat collagen type III (1:100, Abcam, UK). FITC-anti-mouse IgG (1:500, VectorLabs, USA) was used as the secondary antibody for all of these primary antibodies. To control for non-specific binding of all secondary antibodies, negative control sections were incubated with PBS instead of primary antibody and used to determine maximum integration time and gain.

2.12 Image analysis

All image analysis was conducted using ImageJ (NIH). rMSC volume fraction was estimated stereologically as a function of the area fraction of red fluorescence within the scaffold. rMSC number/mm² was calculated by counting the number of nuclei that co-localized with the CM-DiI fluorescence. The number of apoptotic particles/mm² and the macrophage densities (CD68, CD80, and CD163) were determined similarly.

Fibrotic fraction measurements were made using low magnification Masson’s Trichrome images of the entire heart section as described elsewhere [27]. The infarct length was traced and divided by the LV perimeter to obtain a second estimate of the infarct area. Collagen area fraction was calculated similarly using Picrosiris red stained sections. It was assumed that the majority of collagen within the ischemic area would be type I or III and thus the fraction of collagen type III/I was calculated as the fraction of collagen type III/LV, then dividing that fraction by the (total collagen - area would be type I or III)/LV fraction.

2.13 Statistics

In all figures, data is expressed as mean ± 95% confidence interval. Cumulative averages from the stereological measurements were calculated for each heart and used in all further analysis. All statistical comparisons were carried out using SPSS Statistics 18.0. ANOVA was used to determine statistical significance, followed by Bonferroni’s multiple test correction to determine statistically different groups. Statistical significance was set at p < 0.05 and post-hoc corrections were used where applicable.

3. Results

3.1. Stem cell retention

Retention of rMSCs was significantly higher (>5x) in the scaffolds loaded with pIL-10 than the control scaffolds, as shown in Fig. 1. This effect was statistically significant both in terms of volume fraction (Fig. 1b) and density of rMSCs/mm² (Fig. 1c). The fraction of the implanted cells retained in the group without pIL-10 was statistically negligible.

3.2. Cardiac function

The most important finding of this study was that there was a significant and consistent improvement in overall function in hearts treated with a collagen scaffold loaded with IL-10 polypelexes and rMSCs (scaffold + rMSCs + pIL-10). Fig. 2a–c) show the left ventricular (LV) ejection fraction (LVEF%) of all 8 animals in each group at baseline, 24 h after MI was induced and scaffold applied, then 28 days after MI. In Fig. 2a), where the hearts were treated with scaffold alone, the LVEF% in all cases either decreased or remained constant when comparing 28 days post-MI with immediately post-MI. Recovery of LVEF% (28 days post-MI LVEF% - 24 h post-MI LVEF%) was the main evaluation parameter. In the group treated with scaffolds containing only rMSCs (scaffold + rMSCs), as shown in Fig. 2b), some animals had dramatic improvements while others had significant losses in LVEF% by 28 days post-MI. In the group treated with scaffold + rMSCs + pIL-10, however, all animals had either constant or improved LVEF%, as illustrated in Fig. 2c).

When the average recovery of LVEF% was compared between groups, (Fig. 2d) a statistically significant increase in the recovery of LVEF% was observed as compared to the scaffold alone control. On average, there was a 7% recovery in LVEF% in the scaffold + rMSCs + pIL-10 group while there was a 10% loss in the scaffold alone control. The variability in the response in the scaffold + rMSCs group resulted in no statistically significant differences between that group and either of the other two. This variability also may have masked any differences in left ventricular diameter changes in systole (LVDs) as there was a trend towards reduced dilation of the LV in the scaffold + rMSCs + pIL-10 group but it was not statistically significant (Fig. 2e). Finally, increased dilation of the LV was correlated to decreased recovery in LVEF% (R = 0.81, p < 0.0001).

3.3. Inflammatory response

As the inflammatory response had been previously implicated as a potential cause of cell death [28], the numbers of macrophages and their phenotypes were determined. By the 28 day timepoint, there was no statistically significant difference in the overall macrophage numbers (CD68+) within the implant area (Fig. 3b). However, there were significantly more CD80+ macrophages and significantly fewer CD163+ macrophages within the implant area in the scaffold alone control than in the scaffold + rMSCs + pIL-10 group, as illustrated in Fig. 3a), c), and d). It should also be noted that many of the cells which were positive for CD68 did not appear to carry either the cytotoxic (CD80) or regulatory (CD163) markers, as shown in Fig. 3e). The overall macrophage numbers within the ischemic zone were not statistically different between groups, nor were there any statistically significant decreases in CD80+ macrophage numbers in the scaffold + rMSCs + IL-10 group as compared to either control, but there was a statistically significant increase in the number of CD163+ macrophages (Fig. 4).

3.4. Cell death

While the primary effect of transfection with pIL-10 was hypothesized to be modulation of the inflammatory response, endogenous IL-10 is also known to have anti-apoptotic properties. To investigate whether this played any role in the modulation of left ventricular function, the numbers of dead cells in each region were quantified using TUNEL analysis, as shown in Fig. 5. In all cases, the trend was towards lower levels of apoptosis in the scaffold + rMSCs + pIL-10 treatment group than either of the other groups. In the border zone, there was a statistically significant decrease in the number of dead cells in the scaffold + rMSCs + pIL-10 group and in the implant area both the scaffold + rMSCs and the...
scaffold + rMSCs + pIL-10 groups had significantly reduced cell death compared to the control.

3.5. Infarct size and remodelling

Histological analysis of the infarct, as shown in Fig. 6, indicated that there was no statistically significant change in the volume fraction or perimeter fraction of damaged tissue within the left ventricle (Fig. 6b,c) but there was a slight increase in the average thickness of the infarcted wall (Fig. 6d). There was no statistically significant change in the volume fraction of collagen in the left ventricle, as shown qualitatively and quantitatively in Fig. 7a and c) respectively. While immunostaining for collagen type III (Fig. 7b) showed no difference in overall collagen type III volume fraction (Fig. 7d), comparing the two measurements revealed that there was increased ratio of collagen type III/collagen type I within the infarcted areas in the group treated with scaffold + rMSCs + pIL-10, as shown graphically in Fig. 7e.

4. Discussion

Collagen scaffolds loaded with polyplexes encoding IL-10 were tested as a stem cell delivery system and regenerative cardiac therapeutic in a rat model of myocardial infarction. It was found that rMSCs delivered in a scaffold loaded with pIL-10 had significantly better survival in the ischemic myocardium. Furthermore, hearts treated with scaffold + rMSCs + pIL-10 had significantly improved recovery of left ventricular function. This improvement was associated with a change in the inflammatory response characterized by decreased CD80+ and increased CD163+ macrophages numbers and decreased numbers of dead cells. The ratio of collagen type III to collagen type I was also increased in the scaffold + rMSCs + pIL-10 group as compared to the scaffold alone control group, implying a reduction in adverse remodelling was associated with the scaffold + rMSCs + pIL-10 treatment.

The stem cell retention observed with the scaffold + rMSCs + pIL-10 system was relatively high (1–2% of original implanted number) 28 days after implantation, as previous studies have found similar retention rates after only 7 days [11]. Furthermore, the increased numbers of cells observed after 28 days implies significantly improved retention over the entire course of the 4 weeks, as observed in previous studies [16]. Without inclusion of pIL-10, the retention of the rMSCs was negligible (Fig. 1) as has been observed previously in similar studies [11,29]. Thus, pIL-10 incorporated into a collagen scaffold-based stem cell delivery device improved the retention rate of these cells.

Increased stem cell retention was associated with a statistically significant improvement in LVEF%, the most commonly used measure of overall cardiac function. Detailed analysis of the individual animals LVEF% at each timepoint provided an insight into the value of the full combination therapy. The infarcted hearts treated with scaffold alone had an average of a 10% loss in overall LVEF%. All of the hearts in this group, as highlighted in Fig. 2a), continued to lose LVEF% in the 28 days following coronary artery ligation. Similar

Fig. 4. Macrophage phenotype within the ischemic zone. Qualitative (a) and quantitative (b–d) assessments of macrophage numbers within the ischemic zone in groups treated with scaffold alone [ ], scaffold + rMSCs [ ], and scaffold + rMSCs + pIL-10 [ ]. There was no statistically significant change in overall CD68+ or CD80+ macrophage numbers but there was a statistically significant increase in CD163+ macrophages in the scaffold + rMSCs + pIL-10 group as compared to the control. (Data expressed as mean ± 95% CI, * represents statistical significance, p < 0.05, n ≥ 3, scale bar represents 20 μm).

Fig. 5. Apoptosis analysis. Qualitative (a) and quantitative (b) analysis of cell death in different areas of damaged myocardium in the scaffold alone [ ], scaffold + rMSCs [ ], and scaffold + rMSCs + pIL-10 [ ]. A trend towards fewer apoptotic cells in certain areas was observed, but the only statistically significant differences were observed in the border zone where the scaffold + rMSCs + pIL-10 group had significantly fewer apoptotic cells, in the healthy tissue where the scaffold + rMSCs group had significantly more apoptotic cells, and in the implant area, where the scaffold + rMSCs had significantly fewer apoptotic cells. (Data expressed as mean ± 95% CI, * represents statistical significance, p < 0.05, n ≥ 3, scale bar represents 20 μm).
loss of LVEF% has been observed previously in the weeks following MI [29–31]. In the group treated with rMSCs, four of the animals had improvements in LVEF%—some quite dramatic—while the other four continued to lose LVEF%, as shown in Fig. 2b). This suggests that some hearts respond better to the scaffold + rMSCs treatment than others. Further investigation into the differences between the responsive and unresponsive hearts might yield insight into both the optimal candidates for stem cell therapy and the actual mechanism of stem cell mediated cardiac protection. Of the hearts treated with scaffold + rMSCs + pIL-10, five had significant improvements and the remaining three were not significantly altered in the 27 days between the measurements.

While there was no statistically significant difference between the LVDs in the treatment groups compared to the control, there...
Fig. 7. Analysis of extracellular matrix remodelling. Picrosirius red staining indicated no statistically significant effects on total collagen volume fraction within the left ventricle (a, c) and immunohistochemistry for collagen type III volume fraction within the infarct zone indicated no statistically significant differences (b, d), although there was a trend towards higher collagen type III content in the scaffold + rMSCs + pIL-10 group. However, there was a statistically significant increase in the ratio of collagen type III/collagen type I in the scaffold + rMSCs + pIL-10 group as compared to the scaffold alone group (e). (Data expressed as mean ± 95% CI, * represents statistical significance, $p < 0.05$, $n = 8$, scale bar represents 1 mm).
was a positive correlation between the LVDs and the recovery in LVEF%. This suggests that the systolic stretch of the LV, or an increase in LVDs, was a contributing factor in loss of LVEF%. This may seem self-evident in view of the accepted pathophysiology of MI, but has two important consequences. Firstly, the recovery in LVEF% observed in the scaffold + rMSCs + IL-10 group was predominantly due to decreased dilation of the LV which implies that the infarcted tissue in this group had better mechanical properties than the tissue in the scaffold alone control. Secondly, the recovery in LVEF% was not primarily due to hypertrophy of the unaffacted LV and therefore the beneficial actions of MSC therapy are not restricted to the surviving cardiomyocytes.

Although the combined scaffold + rMSCs + pIL-10 therapy appeared to significantly improve LVEF%, no significant changes were observed in infarct size or infarct perimeter fraction. This lack of correlation between functional improvement and infarct size echoed the findings of many other studies, where no statistically significant relationships between function and histological infarct size were observed [32–35]. This disparity may be due to a number of factors. Firstly, histological infarct measurement techniques are subject to several limitations. Thicker infarcted tissue decreases correlations between infarct volume and overall cardiac function, because the increased thickness of the infarcted LV wall may improve LVEF% by decreasing systolic stretch and thereby increasing LV efficacy, but may also result in overestimation of the infarcted volume [27]. Furthermore, induction of MI by coronary artery ligation yields highly variable infarct sizes [36] and because these parameters can only be measured once the heart has been explanted for analysis the initial infarct size is unknown. Thus it is impossible to assess changes in infarct size. LVEF%, conversely, can be assessed as frequently during the course of the experiment and therefore allows quantification of the changes over time.

Another limitation of conventional infarct size estimates is that these estimates do not account for the composition of the infarcted area. However, this can be a critical parameter in predicting cardiac function. Low ratios of collagen type III to collagen type I, for example, are associated with reduced compliance of the tissue, as observed in the later stages of cardiomyopathy [37–39]. When the ratio of collagen type III/I was investigated in this study it was found that while there were no significant changes in the volume fractions of either type, there was a statistically significant increase in the ratio of collagen type III/I in the group treated with scaffold + rMSCs + pIL-10. This implies that the infarcted tissue in these hearts was more compliant than in the hearts treated with scaffold alone. Poor compliance is often associated with stretch over time which may have contributed to the dilation of the hearts in the scaffold alone control group.

The changes in the inflammatory response appear to have improved the overall cardiac function both directly and indirectly. It is hypothesized that over-active inflammatory cells may be responsible for unnecessary cell death during the post-ischemic response, as decreased serum levels of proinflammatory cytokines and decreased infiltration of inflammatory cells are associated with improved prognosis post-MI [40,41]. The reduction in CD80 + macrophages and increase in CD163 + macrophages observed in the rMSCs and IL-10 treatment group implies a diminished macrophage-mediated inflammatory response. This may have directly improved overall cardiac function, but it is also possible that the change in macrophage phenotype may have been indirectly beneficial via the remodelling response [42,43].

It should be noted that CD80 and CD163 are cell surface markers that have been previously associated with the cytotoxic (M1) and regulatory (M2) macrophage phenotypes respectively [25,26,42]. While these definitions of macrophage phenotypes may be sufficient for the current study, it may be over-simplistic in other contexts. Furthermore, it would be ideal to check a panel of markers to confirm the macrophage phenotypes. Thus, to ensure clarity, we have referred to the cells in question by their marker and not by the associated phenotype (i.e. CD80 + instead of cytotoxic macrophage).

When this system was tested in a skeletal muscle, both host tissue and implanted rMSCs were transfected [16]. Furthermore, increased IL-10 levels were associated with an increase in the CD163 + macrophage phenotype and a decrease in the CD80 + macrophage phenotype. The association between these macrophage markers and the overall inflammatory response was demonstrated by comparing immunostained sections and tissue cytokine levels from the same samples [16]. As this modulation of the inflammatory response appeared to be directly correlated to the macrophage phenotypes, the increase in CD163 + macrophage density observed in this study is likely to be associated with decreased proinflammatory and increased anti-inflammatory cytokine concentrations.

The decrease in CD80 + macrophage density was associated with improved rMSC retention (R = 0.874), implying a connection between the change in the inflammatory response and the improvement in rMSC retention. It is possible that the improvement in rMSC retention directly improved LVEF% but it seems more likely that the rMSCs mediated an improved remodelling response, thereby resulting in improved overall cardiac function. This is supported by bivariate correlation analysis, which showed a positive correlation between rMSC retention and wall thickness and collagen type III/I ratio (R = 0.530 and R = 0.812, respectively, p < 0.05). Fig. 8 illustrates the postulated inter-relationships between the treatments and therapeutic effects. pIL-10 transfection appeared to directly affect the rMSC retention and the inflammatory response. These two factors were likely linked, as increased levels of inflammation would have decreased the survival of the stem cells and vice versa. Furthermore, both factors could have improved the remodelling response as well as directly improving cardiac function.

As IL-10 is known to have anti-apoptotic functions in addition to its anti-inflammatory effects, TUNEL analysis was conducted. As hypothesized, treatment with scaffold + rMSCs + pIL-10 was associated with decreased cell death in both the implant area and the border zone compared to the scaffold alone control. This is
significant as cell survival versus cell death in the border zone may have significant effects on cardiac function. Furthermore, the IL-10 treatment may have minimized apoptosis of the implanted rMSCs, as has been observed previously [16].

The main limitation of this study is in the clinical translation of the preclinical model. In this study, the scaffolds were implanted immediately after MI was induced. However, it is unlikely that an invasive procedure would be clinically feasible in a human immediately after MI. However, a very similar collagen scaffold can be prepared as an injectable system which would allow minimally invasive cell delivery. Alternatively, a pre-formed collagen scaffold seeded with cells and anti-inflammatory polyplexes could be implanted into a chronically ischemic heart as an adjunct as part of an invasive surgical intervention like coronary artery bypass grafting or ventricular reconstruction. As this was the first study to describe a combined gene and stem cell therapy approach, a commonly accepted model of MI was used. However, future studies may examine the efficacy of the treatment with an injectable scaffold or in a chronic MI model.

5. Conclusions

Scaffold-mediated transfection with pIL-10 improved stem cell retention by more than five-fold, improved cardiac function by approximately 17% compared to the scaffold alone control, and modulated the remodeling response by decreasing the numbers of CD80 + macrophages, increasing the numbers of CD163 + macrophages, decreasing apoptosis and altering the ratio of collagen type III/I. Thus, the combination of interleukin-10 polyplexes and stem cell therapy in a biomaterial carrier improves outcome after stem cell transplantation following myocardial infarction. This system represents an important innovation in stem cell therapy as it could be used to protect and deliver a variety of cell types, thereby minimizing the limitation of low stem cell survival after implantation.

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Disclosures

The authors have no conflicts of interest or other disclosures to declare.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.biomaterials.2011.10.019.

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