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

Carolyn A. Holladay [a], Aoife M. Duffy [b], Xizhe Chen [b], Michael V. Sefton [c], Timothy D. O'Brien [b], Abhay S. Pandit [a,*]

[a] Network of Excellence for Functional Biomaterials, National University of Ireland, Galway, Ireland
[b] Regenerative Medicine Institute, National University of Ireland, Galway, Ireland
[c] Institute of Biomaterials and Biomedical Engineering, University of Toronto, Canada

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ABSTRACT

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: $-10 \pm 7\%$, scaffold, interleukin-10 and stem cells: $+7 \pm 6\%$). 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.

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)

Abbreviations: MSC, mesenchymal stem cells; MI, myocardial infarction; IR, ischemia/reperfusion injury; LV, left ventricle; LVEF%, left ventricular ejection fraction; pIL-10, IL-10 plasmid polyplexes; LVDs, left ventricular diameter changes in systole; CD(#), cluster of differentiation (#); PAMAM, polyamidoamine; EDC/NHS, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide and N-hydroxysuccinimide; LAD, left anterior descending artery; OCT, optimal cutting temperature media; DAPI, 4′,6-diamidino-2-phenylindole; IHC, immunohistochemistry.

* Corresponding author. NFB Building, IDA Business Park, Dangan, Newcastle, Galway, Ireland. Tel.: +353 91 49 2758; fax: +353 91 495 585.
E-mail address: abhay.pandit@nuigalway.ie (A.S. Pandit).

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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 isofluorane (5% induction, 2% maintenance), intubated and ventilated using a volume-controlled ventilator with a mixture of oxygen (±isoflorane) 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

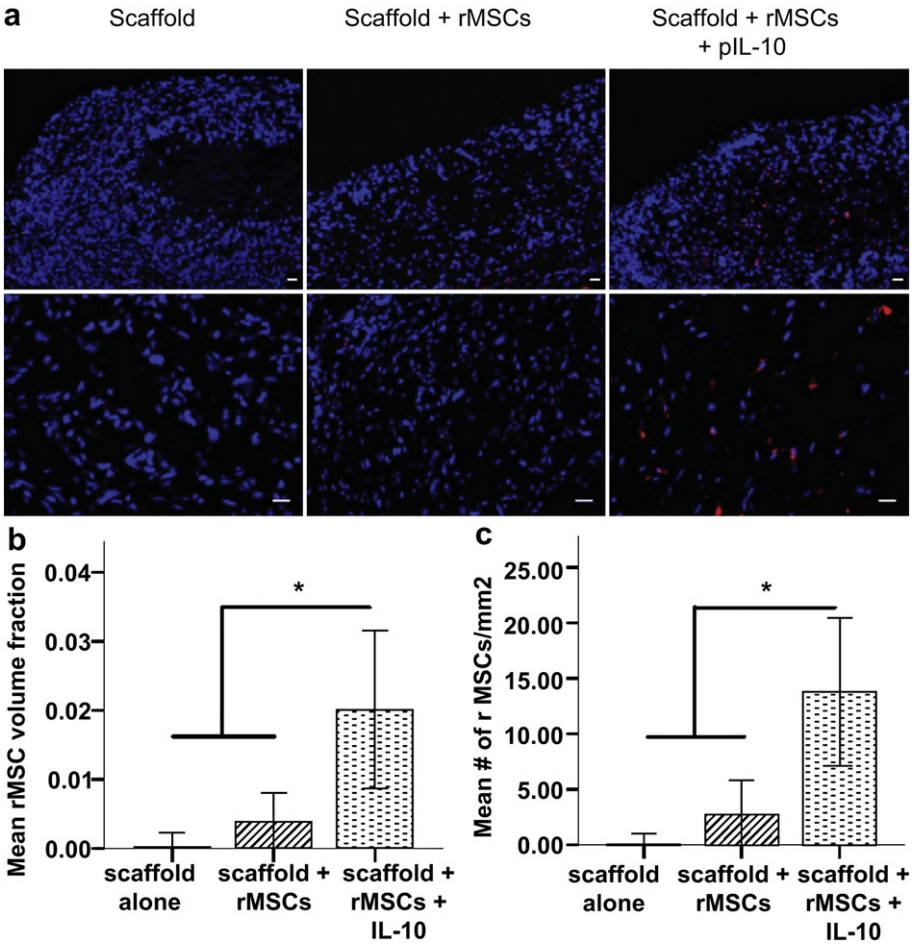

Fig. 1. Stem cell retention. The retention of rMSCs (red) in collagen scaffolds after 28 days attached to an infarcted rat heart is shown qualitatively (a) and quantitatively (b, c). The representative sections in (a) are shown at lower (top) and higher (bottom) magnifications to illustrate the distribution of cells. The quantifications of volume fraction and rMSC numbers per mm^2 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.)