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Macrophages Modulate the Viability and Growth of Human Mesenchymal Stem Cells

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ABSTRACT

Following myocardial infarction, tissue repair is mediated by the recruitment of monocytes and their subsequent differentiation into macrophages. Recent findings have revealed the dynamic changes in the presence of polarized macrophages with pro-inflammatory (M1) and anti-inflammatory (M2) properties during the early (acute) and late (chronic) stages of cardiac ischemia. Mesenchymal stem cells (MSCs) delivered into the injured myocardium as reparative cells are subjected to the effects of polarized macrophages and the inflammatory milieu. The present study investigated how cytokines and polarized macrophages associated with pro-inflammatory (M1) and anti-inflammatory (M2) responses affect the survival of MSCs. Human MSCs were studied using an in vitro platform with individual and combined M1 and M2 cytokines: IL-1β, IL-6, TNF-α, and IFN-γ (for M1), and IL-10, TGF-β1, TGF-β3, and VEGF (for M2). In addition, polarization molecules (M1: LPS and IFN-γ; M2: IL-4 and IL-13) and common chemokines (SDF-1 and MCP-1) found during inflammation were also studied. Indirect and direct co-cultures were conducted using M1 and M2 polarized human THP-1 monocytes. M2 macrophages and their associated cytokines supported the growth of hMSCs, while M1 macrophages and their associated cytokines inhibited the growth of hMSCs in vitro under certain conditions. These data imply that an anti-inflammatory (M2) environment is more accommodating to the therapeutic hMSCs than a pro-inflammatory (M1) environment at specific concentrations. J. Cell. Biochem. 114: 220–229, 2013.

KEY WORDS: MESENCHYMAL STEM CELLS; MACROPHAGES; INFLAMMATION

Human adult mesenchymal stem cells (hMSCs) are considered a potential cell source for tissue repair due to their ability to grow and differentiate in culture and immediate availability from the patient's bone marrow or adipose tissue. Not only are hMSCs able to differentiate into a variety of cell types, but they have also been shown to increase angiogenesis and improve local cell function by mechanisms involving the release of growth factors and signaling molecules [Bartunek et al., 2008; Mathieu et al., 2009; Godier-Furnemont et al., 2011]. In addition, there is growing evidence that hMSCs are immuno-regulatory and immuno-privileged, and able to overcome the limitations of using non-autologous cells in cell-based and tissue engineering therapies. Instead, hMSCs home into sites of active inflammation and are recruited as active participants in the healing response and the stabilization of new vasculature. Therefore, hMSCs are a promising cell source for tissue repair [Baksh et al., 2004; Bartunek et al., 2008; Lasala and Minguell, 2009; Mathieu et al., 2009; Godier-Furnemont et al., 2011].

One frequent use of hMSCs is the repair of cardiac tissue following myocardial infarction [Bartunek et al., 2008; Mathieu et al., 2009; Godier-Furnemont et al., 2011]. Damaged heart muscle has no significant ability to regenerate itself following injury, often leading to the development of congestive heart failure [Bergmann et al., 2009]. Currently, the most promising cell-based therapeutic approach is the introduction of the repair cells into the ischemic site to induce angiogenesis and ultimately achieve tissue regeneration and improve heart function.

hMSCs have not been proven effective at creating functional cardiomyocytes [Murry et al., 2004]. Instead, their injection into the infarct bed results in increased angiogenesis, reduced myocardial wall remodeling, and improvements in global cardiac function [Bartunek et al., 2008; Lasala and Minguell, 2009; Mathieu et al., 2009]. However, the effectiveness of cell injections has been hindered by poor engraftment and retention of cells once delivered to the infarct site (as low as 11% in swine model [Hou et al., 2005]). Cardiac patches can be designed to encapsulate and deliver cells within the region of

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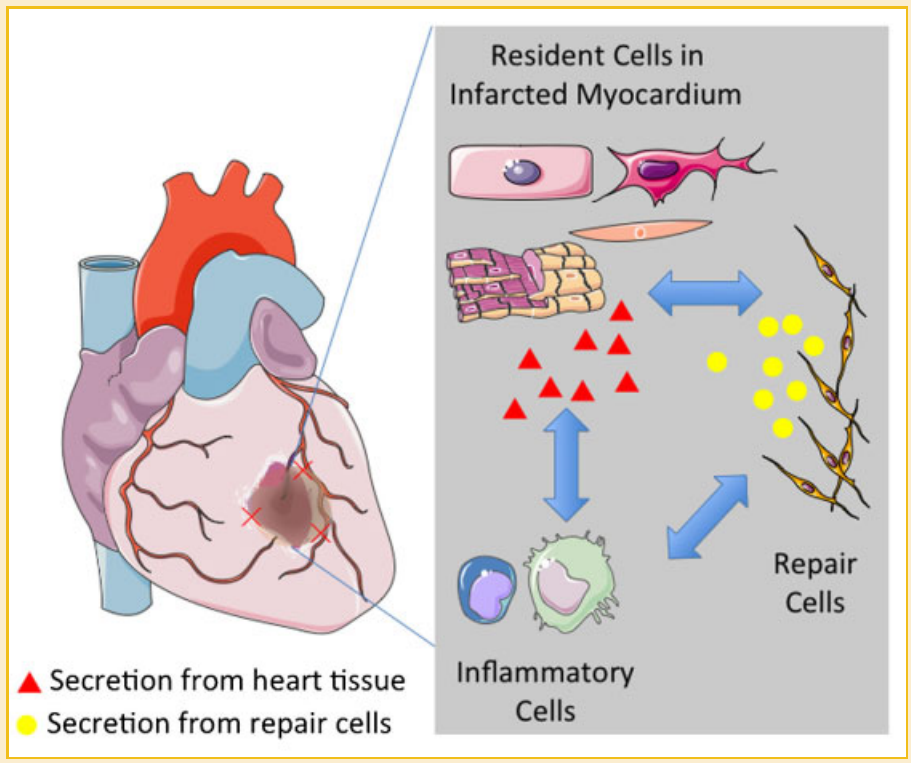

Fig. 1. Summary of the interactions between the host tissue and the repair cells after myocardial infarction (MI). Following MI, resident cells will interact with the infiltrating inflammatory cells such as neutrophils and monocytes [Lambert et al., 2008]. Monocytes will differentiate into macrophages that can be polarized into pro-inflammatory (M1) and anti-inflammatory phonotypes (M2). Once the repair cells are introduced, the macrophages, the repair cells (cardiac or vascular), and the native host cells (cardiomyocytes, smooth muscle cells, endothelial cells) will interact with each other via direct contact and/or secretion of molecules. There is a dynamic reciprocity between the repair cells, the inflammatory cells, and the cardiac cells that will ultimately dictate the outcome of the repair process. Figures were produced using Servier Medical Art (www.servier.com).

interest, where they will be subjected to the milieu present as a result of the host tissue response [Godier-Furnemont et al., 2011].

Following myocardial infarction, the heart undergoes a three-step healing process characterized by the inflammatory, proliferative and maturation phases. Within 24 h, the myocardium is invaded by monocytes, which phagocytose the apoptosed neutrophils, leading to the release of cytokines that initiate fibrotic tissue remodeling [Frangogiannis, 2008]. Initially, classical (M1) activation of macrophages takes place, leading to the production of nitric oxide (NO) and pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. M1 activation is typically associated with inflammation, tumor resistance, and graft rejection. The initial pro-inflammatory response is followed by phase II with the activation of anti-inflammatory macrophages (alternative macrophage activation, M2). M2 macrophages exhibit a non-inflammatory profile with the expression of IL-10, TGF-β, and VEGF. M2 activation is associated with immunoregulation, matrix deposition, remodeling, and graft acceptance [Martinez et al., 2006; Nahrendorf et al., 2007; Brunelli and Rovere-Querini, 2008; Lambert et al., 2008].

M1 and M2 macrophages in the ischemic heart follow a bimodal response, in which early events, dominated by M1 macrophages, gradually shift towards an M2 dominated response over time [Troidl et al., 2009]. M2 macrophages turn over the extracellular matrix during scar formation, and promote angiogenesis by activation of both resident and infiltrating endothelial cells and fibroblasts. There is also a dynamic change of cytokines and chemokines within the tissue (Figs. 1 and 2). Both the repair cells and engineered tissue constructs will have to function within this environment with a milieu of signals affecting cellular engraftment and survival (Fig. 1). Such consideration should be included during the design process of cardiac constructs. It is thus critical to understand how inflammatory cells affect the survival of repair cells and to determine if such environment can be exploited to improve current therapies.

The goal of our study was to determine the effects of macrophages and their associated cytokines at the early and late stages of infarction (M1 vs. M2) on the survival of hMSCs. To this end, we established an in vitro platform for studying the effects of individual cytokines, using indirect (transwell) and direct co-cultures of polarized macrophages and hMSCs. We show that the type of macrophages present at the time of implantation affects the survival of the repair hMSCs. We propose that the inflammatory response could be harnessed to improve the effectiveness of engineered cardiac constructs in the setting of cardiac ischemia.

METHODS

EXPERIMENTAL DESIGN

The overall approach of the present study (Fig. 2) was to quantify the viability and growth of hMSCs in the presence of pro- and anti-inflammatory cells and cytokines. The hMSCs were cultured in the

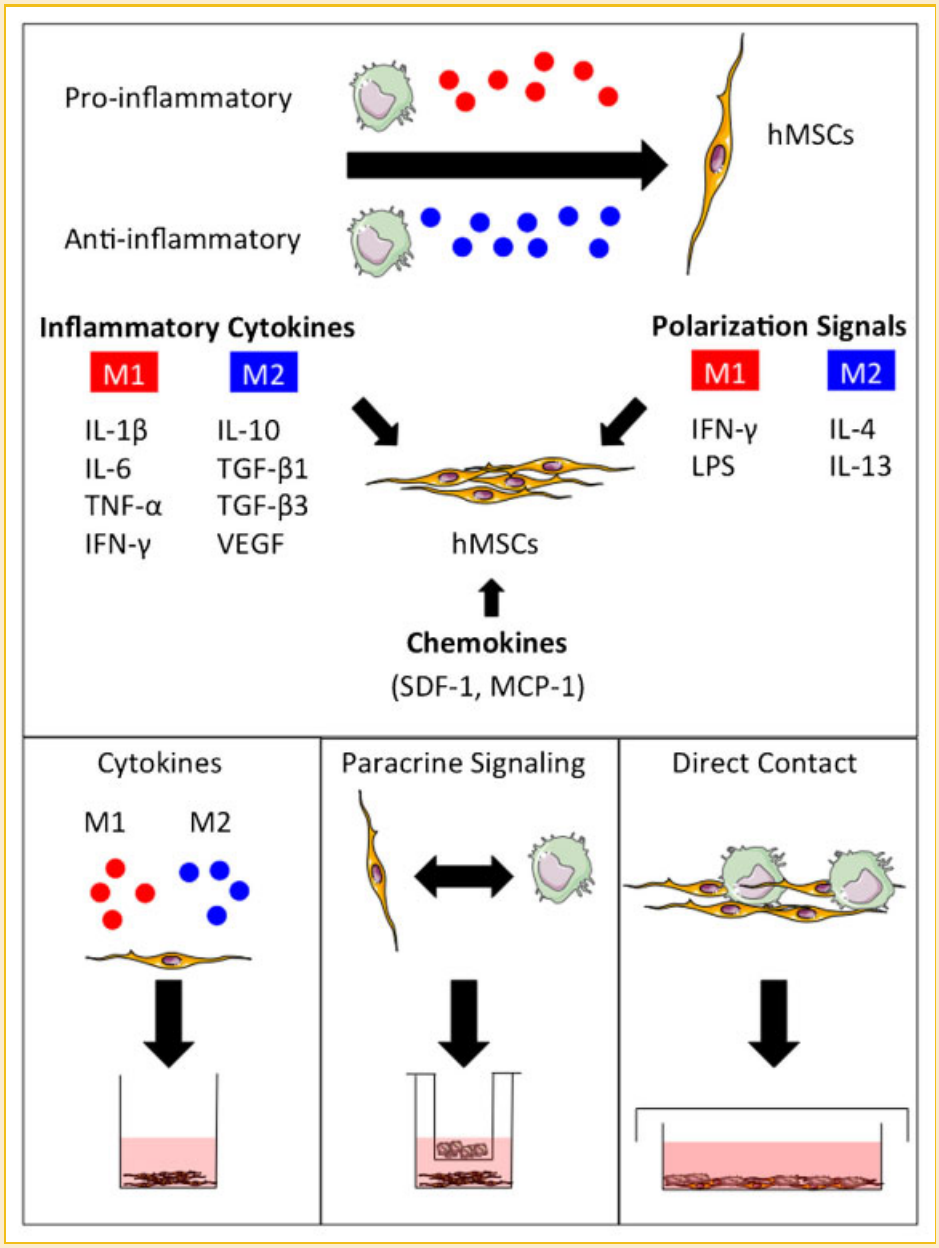

Fig. 2. Overall experimental design. The goal of the study was to determine how pro- and anti-inflammatory signals affect hMSC growth in vitro. The different signals that were tested include inflammatory cytokines, polarization signals, and chemoattractants. Three different conditions were used: (1) Culturing hMSCs with cytokines, polarization signals, and chemokines found during inflammation. (2) Co-culturing hMSCs with polarized macrophages that are separated by a permeable membrane. (3) Directly co-culturing hMSCs with polarized macrophages. Figures were produced using Servier Medical Art (www.servier.com).

presence of individual cytokines and chemokines that are normally found within the inflammatory environment of an infarcted myocardium. The results were corroborated by culturing hMSCs with polarized macrophages (M1 and M2) using a transwell system. Finally, direct co-cultures of hMSCs with M1 and M2 macrophages were used to determine if the observed results were due to paracrine signaling.

HMSC CULTURE

hMSCs were obtained from Cambrex Life Sciences (East Rutherford, NJ), expanded as previously described [Grayson et al., 2010], and cultured in 10% fetal bovine serum (FBS), 1% penicillin–streptomycin (PS), and 1 ng/ml of basic fibroblast growth factor (bFGF) in Dulbecco's Modified Eagle Medium (DMEM) with high glucose. hMSCs were used between passages 3 and 4.

CYTOKINE AND CHEMOKINE CULTURE

In order to determine the effects of cytokines and chemokines commonly associated with pro- and anti-inflammatory environments, hMSCs were cultured in the presence of cytokines, chemokines, and the signals used to differentiate macrophages towards M1 and M2 phenotypes. The cytokines tested include: (1)

M1 cytokines: TNF-α, IL-1β, IL-6, and IFN-γ; and (2) *M2 cytokines*: IL-10, TGF-β1, TGF-β3, and VEGF. The chemokines tested were MCP-1 and SDF-1. The polarization signals were: (1) *M1*: LPS and IFN-γ; (2) *M2*: IL-4 and IL-13. Cytokines were obtained from Peprotech (Rocky Hills, NJ).

hMSC were cultured overnight on a 96-well plate at 5×10^3 cells/well in 0.1 ml of DMEM supplemented with 10% FBS, 1% PS, 1 ng/ml bFGF and allowed to attach. The cells were washed with PBS once and cultured with 0.1 ml/well of complete RPMI-1640 medium [10% heat-inactivated FBS (HI-FBS) and 1% PS] supplemented with individual M1 and M2 cytokines or a mixture of all the M1 and M2 cytokines. hMSCs were also cultured with medium supplemented with chemokines and polarization signals as stated above. Each was tested at three different concentrations: 50, 25, and 12.5 ng/ml. After 48 h of incubation, the medium was aspirated and the samples were frozen at −80°C, and DNA quantified using the CyQuant Cell Proliferation Assay (Life Technologies, Grand Island, NY).

MACROPHAGE DIFFERENTIATION

THP-1 cells (a human monocytic cell line) were purchased from the American Type Culture Collection (Manassas, VA) and cultured in RPMI-1640 medium supplemented with 10% FBS and 1% PS. THP-1 cells were differentiated into macrophages using a standard protocol [Tjiu et al., 2009], by culturing the cells with 50 ng/ml of 12-myristate 13-acetate (PMA) for 48 h. After differentiation, the cells were washed and cultured with polarization medium for 48 h. M1 polarization medium consisted of 240 ng/ml of LPS and 20 ng/ml of IFN-γ in RPMI medium supplemented with antibiotics and heat-inactivated fetal bovine serum (HI-FBMS). M2 polarization medium consisted of 20 ng/ml of IL-4 and IL-13 in RPMI-1640 medium supplemented with PS and HI-FBS. Following polarization, the supernatant was collected and the cells were washed twice with 1× PBS. The cells were then used for characterization or for co-culture with hMSCs.

TRANSWELL CO-CULTURE

Macrophages were differentiated and polarized on 24-well plate inserts (3-μm size pore; Millipore) or 96-well plate inserts (1-μm size pore, Corning). Co-cultures in a 24-well plates were performed at different initial THP-1 cell concentrations (15 k, 30 k, 60 k, and 90 k) with 15 k hMSCs pre-plated on the bottom wells. The medium was collected from each well after the polarization step. Co-cultures in 96-well plates were performed at an initial seeding density of 5 and 10 k THP-1 cells per insert and pre-plated with hMSCs at 5 k hMSC/well.

Differentiation and polarization of the THP-1 cells was performed as described in the previous section. The volumes used for the 24-transwell system were 0.25 ml for the insert and 0.935 ml for the well. The volumes used for the 96-transwell system were 0.1 ml for the insert and 0.3 ml for the well. The inserts were washed twice with PBS and placed on a new 24- or 96-well plate that contained hMSCs (bottom well) that had been cultured for 2 days in DMEM supplemented with 10% FBS, 1% PS, and 1 ng/ml bFGF. For the 24-well plate configuration, the supernatants were collected after 2 days of co-culture and the plates frozen at −80°C. The frozen cell samples were used to quantify DNA using the CyQuant Cell Proliferation Assay (Life Technologies), according to the manufacturer's protocol. On selected 24-well plates with an initial seeding density of 30,000 THP-1 cells per well, Cell Viability/Cytotoxicity kit (Life Technologies) was used according to the manufacturer's protocol to stain for live cells (shown in green) and dead cells (shown in red).

ELISA

Supernatant samples collected from the transwell assays were used to perform ELISA measuring the amounts of tumor necrosis factor-alpha (TNF-α) and transforming growth factor-beta (TGF-β). ELISA kit was purchased from BD Bioscience (San Jose, CA) and used by following the manufacturer's instructions. Colorimetric changes were measured using a SpectraMax Plus spectrophotometer (Molecular Devices).

DIRECT CO-CULTURE AND FLOW CYTOMETRY ANALYSIS

Macrophages were differentiated and polarized in 100 mm dishes with an initial seeding density of 0.7×10^6 THP-1 cells per dish as described above. The cells were washed twice with PBS and co-cultured with the same number of hMSCs (1:1 ratio) in fresh RPMI-1640 medium (10% HI-FBS, 1% PS) for 3 days. hMSCs and macrophages were detached, stained for CD14 and CD73 (BD Bioscience), fixed and examined using a FACSCalibur flow cytometer. Flow cytometry data were analyzed using FlowJo version 7.6. Cell Viability/Cytotoxicity kit was used to stain for live and dead cells after co-culture according to the manufacturer's protocol.

STATISTICAL ANALYSIS

Two-way ANOVA was performed using Prism 5.0 ($\alpha = 0.05$) with Bonferroni's multiple comparisons test. $P < 0.05$ was considered statistically significant. Minimum of $n = 4$.

RESULTS

EFFECT OF M1- AND M2-ASSOCIATED CYTOKINES ON PROLIFERATION OF HMSCS

The M1-associated cytokines (TNF-α, IL-1β, IL-6, and IFN-γ) suppressed the proliferation of hMSCs, in a manner dependent on the types of cytokines and their concentration levels. IL-1β and IFN-γ decreased the numbers of hMSCs in culture at high concentrations, as compared to the non-supplemented (control) group. The combination of all four M1 cytokines at low concentrations (12.5 ng/ml each) resulted in a significant decrease in numbers of hMSCs. However, TNF-α alone increased the numbers of hMSCs (Fig. 3A).

In contrast, the M2-associated cytokines (IL-10, TGF-β1, TGF-β3, and VEGF) enhanced or at least maintained the proliferation of hMSCs when compared to controls. Both VEGF alone and the combination of all four M2 cytokines showed an increase in numbers of hMSCs as compared to controls. IL-10 alone was the only M2 associated cytokine without significant positive effect on hMSC proliferation (Fig. 3B).

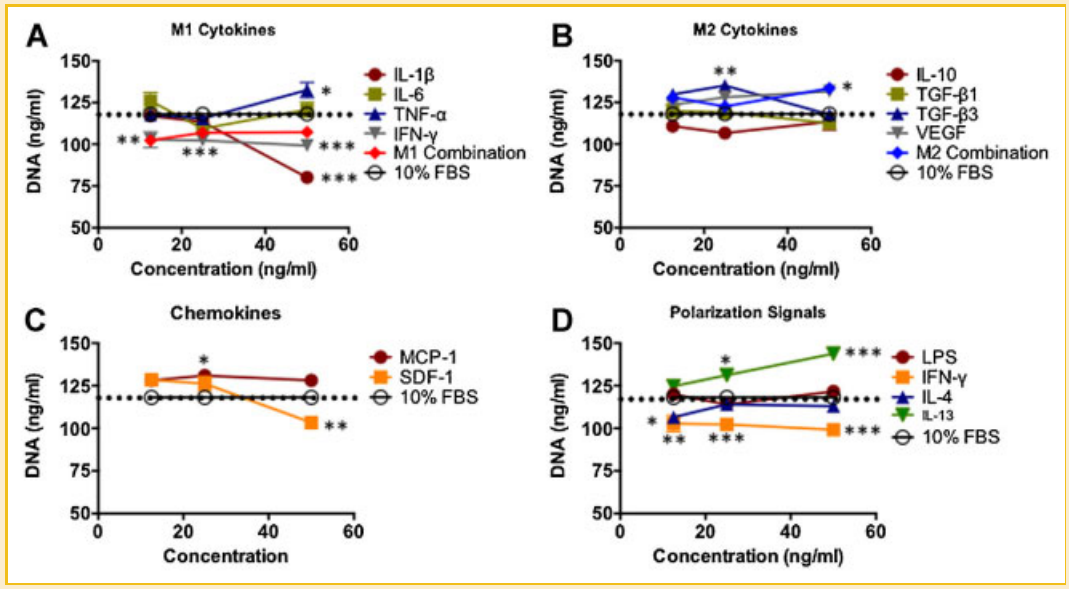

Fig. 3. Growth of hMSCs in medium supplemented with M1 and M2 macrophage cytokines. A: DNA per well after culture for 48 h with medium supplemented with the individual M1 associated cytokines or the combination of all M1 associated cytokines. B: DNA per well after culture for 48 h with medium supplemented with the individual M2 associated cytokines or the combination of all M2 associated cytokines. C: DNA per well after culture for 48 h with medium supplemented with chemokines. D: DNA per well after culture for 48 h with medium supplemented with the polarization cytokines. Dotted line shows values for 10% FBS. All values were compared to 10% FBS. Data represent mean ± SE, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

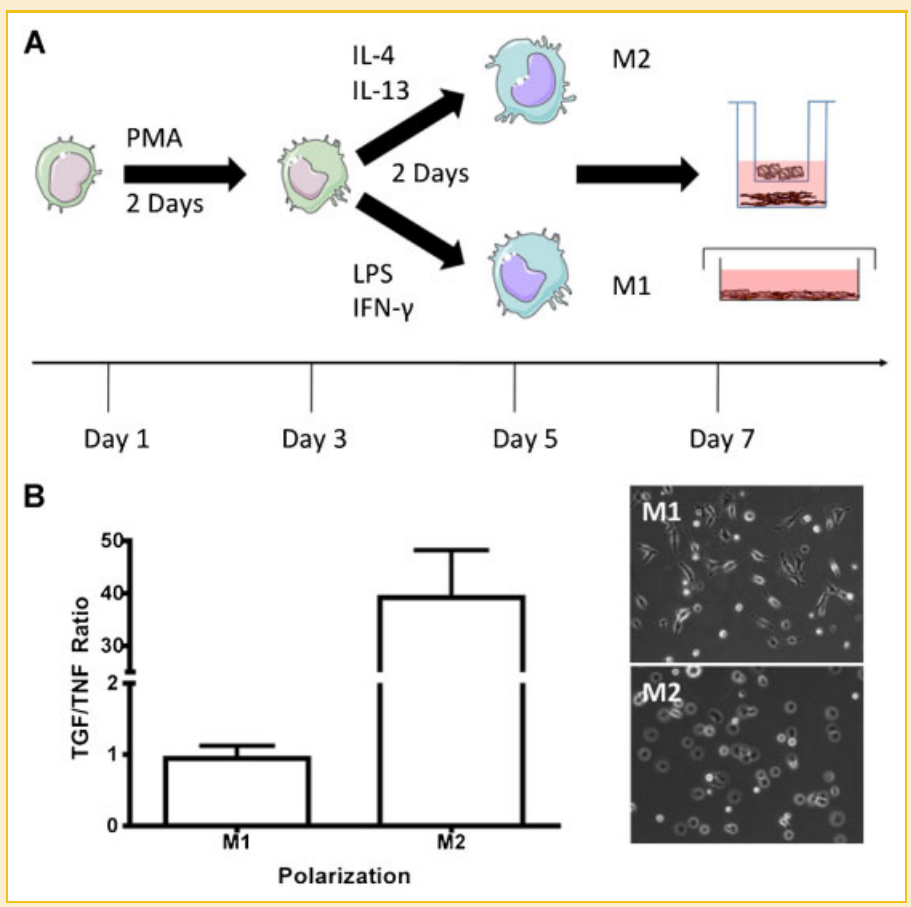

Fig. 4. Polarization of THP-1 cells into M1 and M2 macrophages. A: Schematic showing the differentiation of THP-1 monocytes into polarized macrophages. THP-1 cells were cultured in PMA for 2 days followed by polarization with M1 and M2 inducing cytokines. Polarized macrophages are then used for co-culture using a transwell system or for direct co-culture. B: Ratio of TNF-α and TGF-β showing the polarization of M1 and M2 macrophages (left) and their morphological appearance (right).

The supplementation of MCP-1 and SDF-1 increased the number of hMSCs (Fig. 3C) at low concentrations. At a concentration of 50 ng/ml, SDF-1 showed a significant decrease in hMSC number when compared to MCP-1. IFN-γ was the only polarization cytokine that decreased the number of hMSCs. IL-13 = 3 enhanced hMSCs growth in a concentration-dependent manner (Fig. 3D).

CHARACTERISTICS OF POLARIZED M1 AND M2 MACROPHAGES

THP-1 cells were differentiated into macrophages after activation with PMA, and further polarized to M1 and M2 macrophages following 2 days of culture in the presence of the appropriate cytokines (Fig. 4A). M1 macrophages expressed more TNF-α than M2 macrophages (Fig. 4B), confirming their polarization [Tjiu et al., 2009]. Morphologically, M1 macrophages showed a more spindle like shape when compared to M2 macrophages.

EFFECTS OF FACTORS SECRETED BY M1 AND M2 MACROPHAGES

Polarized macrophages were co-cultured with hMSCs using a transwell system to limit macrophage–hMSC interactions to occur only by diffusing factors. The effects of co-culture on the survival of hMSCs were assessed by DNA assay and Live/Dead assay after 48 h of co-culture. The testing scheme is summarized in Figure 5A. In parallel, hMSCs were cultured in conditioned medium obtained during the polarization step between Day 2 and Day 4 (Fig. 5A).

As shown in Figure 5B, viability of hMSCs in the M1-hMSC co-culture was markedly lower than in the M2-hMSCs co-culture or culture of hMSCs alone. The same effect was observed, in a dose-dependent manner, when hMSCs were cultured in medium conditioned with different concentrations of polarized macrophages (Fig. 6A). However, when the same polarized macrophages that produced the conditioned medium were cultured with hMSCs in a transwell, there were no differences between M1 and M2 cultures. There was a decrease for both types of macrophages at the lowest concentration (Fig. 6B).

EFFECT OF DIRECT CONTACT OF M1 AND M2 MACROPHAGES

Polarized macrophages were cultured in direct contact with hMSCs in order to compare the effects of direct cellular contact with paracrine effects of the same cells. hMSCs quickly attached and took most of the space that was previously populated by the macrophages. After 3 days of co-culture, there were fewer dead cells in the M2-hMSCs culture than in the M1-hMSCs co-culture. Interestingly, there were a slightly larger percentage of CD14+ cells in the M2-hMSCs group (Fig. 7B).

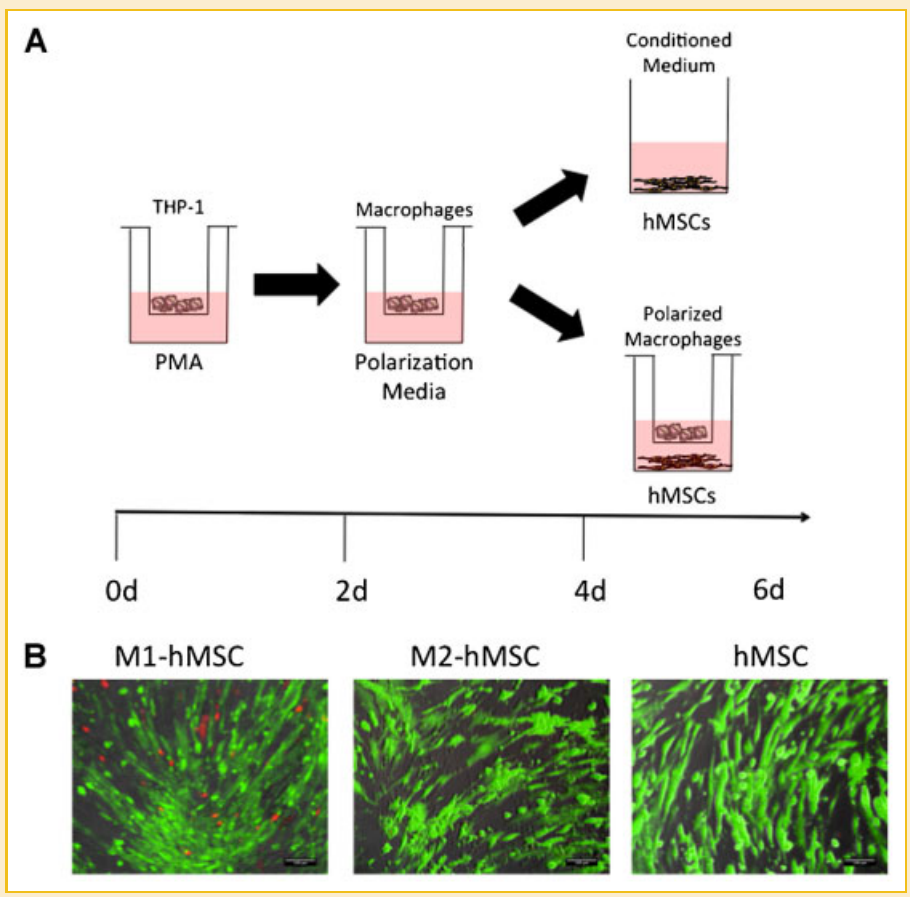

Fig. 5. Transwell co-culture of hMSCs and polarized macrophages (24-well plate). A: Schematic showing the transwell system used to co-culture polarized macrophages and hMSCs. B: Live/dead assay of hMSCs when co-cultured with polarized macrophages. Green staining signifies healthy cells and red staining signifies dead cells.

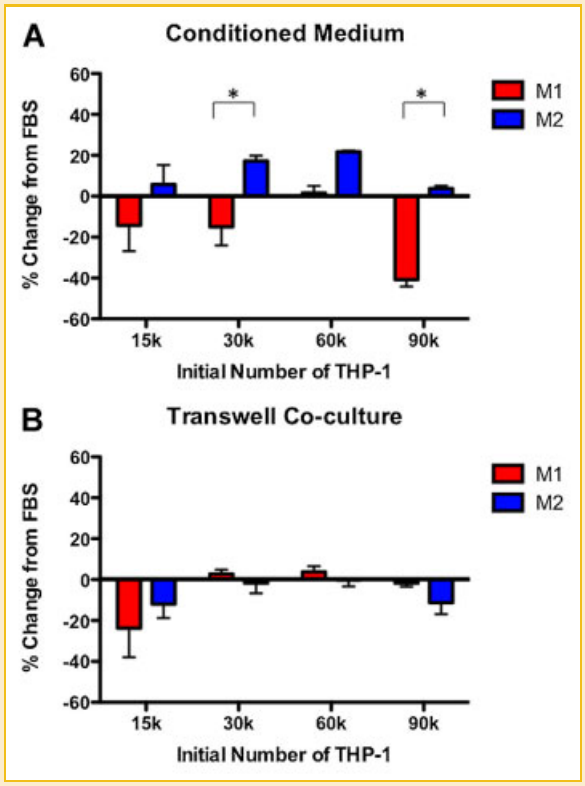

Fig. 6. Effect of initial THP-1 concentration on hMSC growth. A: Culture of hMSCs with conditioned medium from polarized macrophages at different starting concentrations. B: Culture of hMSCs and polarized macrophages in a transwell system at different starting concentrations. Data represent mean ± SE, $^{*}P < 0.05$.

DIFFERENTIAL EFFECTS OF CYTOKINES AND FACTORS SECRETED BY POLARIZED MACROPHAGES

The effects of M1- and M2-associated cytokines on hMSCs were compared between the supplementation of pure cytokines and the culture medium and transwell co-cultures of hMSCs with M1 and M2 macrophages. Supplementation of M1-associated cytokines IL-1β and IFN-γ resulted in a decrease in the number of hMSC as compared to M2-associated cytokines at a concentration of 50 ng/ml (Fig. 8A).

Polarized macrophages were co-cultured with hMSCs at two different initial concentrations (0.5×10^4 and 1×10^4 cells/well) for 48 h to confirm the effects observed using the purified cytokines. Transwell co-culture (96-transwell system) with M1 macrophages resulted in a larger decrease in cell number than the co-culture with M2 macrophages (Fig. 8B). It was observed that the higher the starting number of THP-1 cells the less detrimental effect on cell number. The inverse relationship between starting number of THP-1 cells and hMSCs was consistent with the effects observed using the 24-transwell system, and opposite from the effect of conditioned medium (Fig. 6A,B).

DISCUSSION

The present study investigated the survival and function of hMSCs in the presence of pro-inflammatory and anti-inflammatory macrophages (M1 vs. M2) and their associated cytokines, using an in vitro platform. We found that pro-inflammatory (M1 polarized) macrophages and the cytokines they secrete were detrimental to the growth and survival of hMSCs in vitro. In contrast, M2 associated cytokines and M2 polarized macrophages did not affect, and in some cases improved, the growth of hMSCs in vitro. Profound understanding of the interactions between the inflammatory cells and repair cells can lead to increased efficacy of cell-based therapy targeting the heart.

Myocardial infarction initiates a set of dynamic cellular events that are characterized by distinct waves of pro- and anti-inflammatory cells recruited to remove necrotic tissue and begin the healing process. Since any therapeutic cells used to treat myocardial infarction will be subjected to such environment and will inevitably interact with the cells present at the site of implantation, it is important to understand how repair cells survive and react in such an environment. A more complete understanding of these interactions and how they affect the survival and function of repair cells can lead to more efficient cell-based therapies by aiding the selection of biomaterial used for delivery. In addition, the timing selected for the application of the therapy can also be maximized to increases the survival and attachment of the repair cells once implanted.

Polarized macrophages are normally found at the site of myocardial infarction during the inflammatory response with early stages dominated by M1 macrophages followed by a gradual shift towards more M2 macrophages [Trial et al., 2004; Lambert et al., 2008]. Interestingly, anti-inflammatory cytokines and anti-inflammatory macrophages (M2) in some cases improved the viability of hMSCs in vitro. The effects seem to be paracrine in nature and dependent on cytokine concentration (Fig. 3) and the number of macrophages present (Figs. 6 and 8). Inhibition of hMSC growth was found for most concentrations of inflammatory chemokines and polarization signals (Fig. 3). These effects suggest that wound environments dominated by M1 macrophages may result in lower survival and retention of hMSCs. Furthermore, polarized macrophages may secrete factors such as TGF-β and VEGF that could potentially trigger differentiation and linage commitment that could affect their role as repair cells. The present study show evidence that macrophages may play an important role in hMSC survival and engraftment following implantation and possibly also affect the hMSC differentiation potential. In addition, these findings need to be validated in vivo in order to establish the relative contribution of macrophages to the overall survival and function of hMSCs in a myocardial infarct environment.

The role of macrophages during the healing of myocardial tissue has been well documented in studies that show improper healing, decreased wound debridement, lower activity of resident fibroblasts, and overall decrease in wound healing when macrophages are depleted [Takayama et al., 2000; Danenberg et al., 2002; Espinosa-Heidmann et al., 2003; Lambert et al., 2008]. Some researchers focused on delivering activated macrophages into ischemic myocardium following coronary artery ligation and showed improvements in the healing response, vascularization, tissue repair, and heart function [Leor et al., 2006]. The interactions between some inflammatory cells such as T cells, B cells and natural

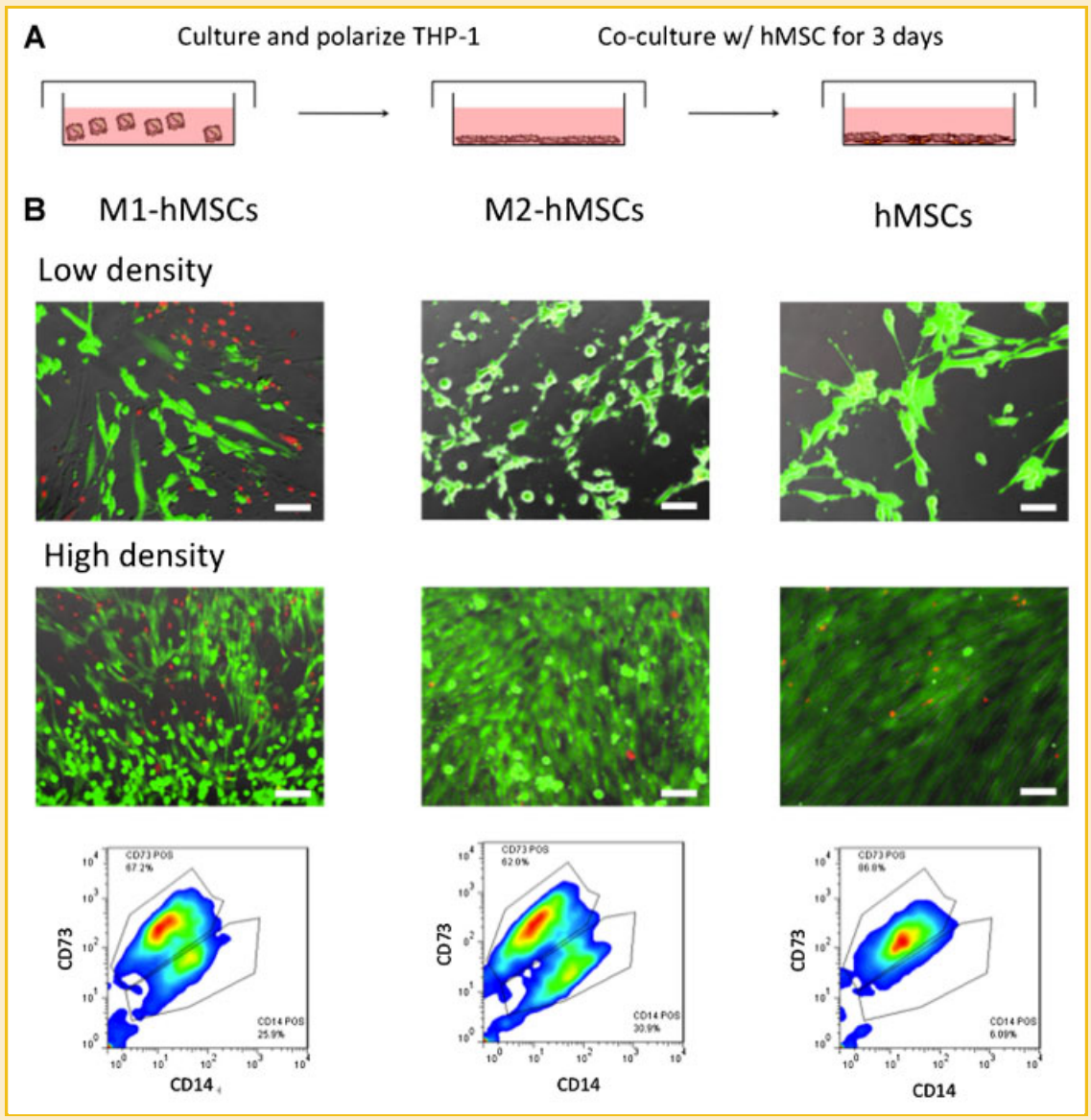

Fig. 7. Direct co-culture of hMSCs and polarized macrophages. A: Diagrammatic representation of the direct co-culture of macrophages and hMSCs. B: Results of the direct co-culture of polarized macrophages with human mesenchymal stem cells for 3 days. Composite of live/dead assay and bright field images of the direct co-cultures of polarized macrophages and hMSCs (green staining signifies healthy cells and red staining signifies dead cells). Top row images are representative of areas of the plate with a low density of cells. Bottom row images are representative of areas of the plate with high density of cells. Flow cytometry data showing CD73 and CD14 positive cells are shown below.

killer cells and MSCs have been the focus of recent studies with emphasis on the immune-regulation of T cells by hMSCs. For example, activated MSCs can suppress activation of T cells via secretion of indoleamine-2,3-dioxygenase (IDO) [Singer and Caplan, 2011]. However, the interactions of macrophages and repair cells such as MSCs remain largely unknown, although being important for the survival of cardiac constructs and their engraftment with the host tissue.

We found that hMSCs survive better when exposed to M2 macrophages or anti-inflammatory cytokines, as compared to M1 macrophages and pro-inflammatory cytokines (Fig. 3). Taken together, these data suggest that an anti-inflammatory (M2 macrophage-related) environment within the infarct bed could improve the engraftment and retention of hMSCs delivered into an infarct site either in suspension or as part of a cardiac construct. Alternatively, a cardiac patch containing hMSCs could be engineered for controlled release of cytokines or small molecules to promote or maintain a predominately anti-inflammatory environment, thus maximizing the survival rate of the cells and ultimately improving the overall healing response.

During healing following myocardial infarction, cytokines are released from cells residing within the injured tissue that are undergoing apoptosis or necrosis and interact with neighboring host cells, cells infiltrating from circulation, and cells within the repair construct [Lambert et al., 2008; Copland and Galipeau, 2011]. This creates rich and complex wound environment full of chemical cross

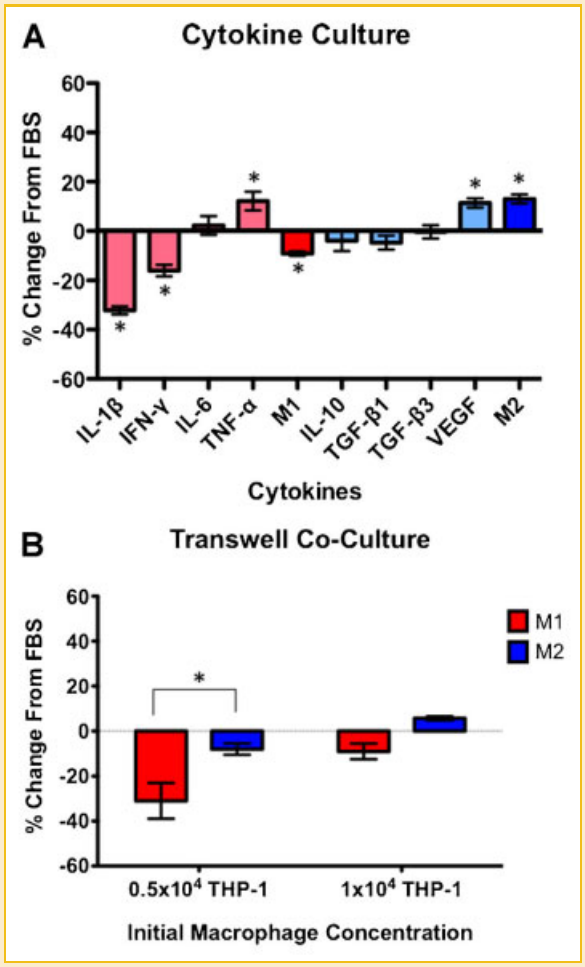

Fig. 8. Comparison between the effects of cytokines and the M1 and M2 paracrine signaling on hMSC growth (96-well plates). A: Effect of M1 and M2 associated cytokines on the growth of hMSCs. Cytokines were tested individually or combined (M1 and M2). Data represent mean ± SE for eight replicates in a 96-well plate. B: Effect of a transwell co-culture of polarized THP-1 cells and hMSCs at two different starting concentrations in a 96-well plate format. Data represent mean ± SE for four replicates in a 96-well transwell plate.

talk between cells that can affect the repair process. The cytokines associated with inflammation include: TNF-α, IL-1β, IL-6, and IFN-γ (For M1) and IL-10, TGF-β1, TGF-β3, and VEGF (for M2) [Frangogiannis et al., 2002; Lambert et al., 2008; Frangogiannis, 2008]. TNF-α, a cytokine associated with an M1 response, has been shown to increase MSC proliferation, basement membrane invasion, cytokine expression, and inhibit chondrocyte and myocyte differentiation [Guttridge et al., 2000; Ladner et al., 2003; Markel et al., 2007; Bocker et al., 2008]. In addition to secretion of cytokines as a result of inflammatory signals, external mechanical and chemical stimuli have been shown to affect cytokine production of hMSCs [Sumanasinghe et al., 2009]. Taken together it seems that paracrine cross talk between multiple types of cells present at the site of injury is unavoidable and can determine the success or failure of an engineered cardiac construct. Therefore, in vitro screening platforms may be used to maximize the success rate by selecting the optimum configuration prior to implantation.

M1 associated cytokines, with the exception of TNF-α, and conditioned medium were shown to inhibit the growth of hMSCs. The supportive effect of TNF-α found in the present study is consistent with the results of several other studies [Sharma and Das, 1997; Ladner et al., 2003; Abarbanell et al., 2009]. However, the protective effect of TNF-α is not sufficient to counteract the negative effects of other cytokines such as IL-1β and IFN-γ (shown in Fig. 3). Co-cultures of hMSCs with M1 or M2 macrophages in a transwell system showed similar trends, but only at the lowest initial macrophage concentration, suggesting that there is a difference between a one-way versus a two-way cross-talk between macrophages and MSCs (Figs. 6 and 8). Under co-culture conditions, macrophages are also exposed to cytokines secreted by the hMSC and may change polarization. The effects seem to be dose-dependent and the exact mechanisms and the influence of hMSC on macrophage polarization remain to be investigated. Future studies will focus on the effect of hMSC on macrophages that have not received polarization signals prior to exposure and how macrophage polarization changes over time. Further insights into the cross talk between both cell types are needed to evaluate any potential impact of the inflammatory response on engineered cardiac constructs.

Both types of culture systems—direct and transwell, consistently showed lower viability in the M1 co-culture group at low initial macrophage density (shown in Figs. 5 and 7). Direct co-culture of hMSCs and THP-1, although both human cells, represents an autologous system and therefore hMSCs are subject to recognition by the macrophages. However, the same effect is observed when a membrane physically separated the cells. The observed effects are presumably due to paracrine signaling and most likely affect hMSCs delivered to an infarct site even when they are not in direct contact with infiltrating and resident macrophages. This has direct impact on engineered cardiac constructs containing hMSCs since diffusion of molecules from the infarct site could affect the repair cells. How these finding translate to the in vivo environment still needs to be investigated. In addition, the present study ignores the contribution from other cell types present at the site of infarction such as T cells. Other cell types may be added to the mixture in the future to increase the complexity of the in vitro model and help identify the key players that dictate the potential success of a cardiac patch.

CONCLUSIONS

The type inflammatory environment at the site of injury can potentially impact the survival and engraftment of hMSCs in cell-based therapies used to treat myocardial infarction. Anti-inflammatory (M2 macrophages)-associated cytokines support the growth of hMSCs while pro-inflammatory (M1 macrophages) associated cytokines inhibit the growth of hMSCs in vitro. Direct cultures of hMSCs with polarized M1 and M2 macrophages showed the same effects, suggesting that a M2 environment is more accommodating to the therapeutic hMSCs than an M1 environment. The most interesting implication of this study is that it may be possible to harness the inflammatory host tissue response towards enhanced survival and function of the repair cells. The observed differential effects of M1 and M2 macrophages suggest that the timing of

implantation of cardiac constructs may be important for the ultimate success of tissue-engineered heart repair.

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