Mesothelial cell transplantation in the infarct scar induces neovascularization and improves heart function

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Abstract

Objectives: Cell transplantation and associated neovascularization in vivo may be beneficial in ischemic disease. We hypothesized that transplanted mesothelial cells (MCs) could improve neovascularization in the post-myocardial infarct scar in rats.

Methods: Myocardial infarction was created by left coronary artery ligation in Lewis rats. After 3 weeks, surviving rats with left ventricular (LV) ejection fraction (EF) <50% were randomized into 2 groups which received, via injection into the infarct scar, either syngeneic rat peritoneal MCs (transplanted group) or vehicle alone (control group). Rats were followed-up echocardiographically for 4 weeks. Before transplantation, cells were transfected in vitro or labeled by a fluorescent dye for subsequent tracking in vivo. Transplanted cells and neovascularization were assessed histologically in the infarct scar by immunostaining or intravenous FITC-dextran injection prior to sacrifice, from 1 to 30 days post-transplantation.

Results: Among other pro-angiogenic chemokines, cultured MCs released stromal cell-derived factor (SDF-1α) (15.9 ± 1.8 μg/mg protein) in vitro. At 1 month, some transplanted MCs were visualized (surviving or proliferating) in the LV scar and were incorporated in new vessels. The transplanted rats presented an increased vascular density in the scar, improved LV-EF (44.0 ± 8.6% vs. 24.0 ± 4.5%, p < 0.01) with decreased LV end-diastolic diameter (9.6 ± 0.6 vs. 11.1 ± 0.6 mm, p < 0.01) and volume (0.47 ± 0.1 vs. 0.63 ± 0.1 ml, p < 0.01) vs. controls. One week post-transplantation, higher levels of SDF-1α were extracted from LV peri-infarct tissue (32.3 ± 5.8 vs. 22.6 ± 3.1 pg/mg protein in controls, p < 0.01).

Conclusions: Since autologous MCs can be obtained easily and cultured in large quantities, MC transplantation may represent a new angiogenic strategy in the prevention of ischemic remodeling.

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Keywords: Remodeling; Gene transfer; Cell therapy; Angiogenesis; Contractile function

1. Introduction

In situ cell therapy for preventing heart failure after myocardial infarction (MI) has been recently proposed in view of successful experimental approaches in animals and clinical feasibility in human patients. Several cell strategies have been applied, including seeding of muscle cells such as cardiomyocytes [1], or skeletal myoblasts [2] in order to recuperate contractile function, and seeding of non-cardiac cells such as smooth muscle cells (SMCs) [3,4], endothelial cells [5] or bone marrow (BM) stem cells [6–8] in order to promote therapeutic angiogenesis and to induce differentiation of endogenous progenitors into myocytes in the infarct scar. Nevertheless, whatever the cell type, the angiogenic effect appears to be predominant.

We selected mesothelial cells (MCs) for cell therapy in MI because they are accessible in human patients by excision and digestion of epiploon or from peritoneal fluid or lavage [9,10], they are easy to culture to obtain large quantities in vitro and they can be genetically modified.

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These cells also display angiogenic properties that could be of interest in infarct scar remodeling. The important potential of MCs in tissue engineering has recently been underlined [12] and this cell type is probably the precursor of coronary arteries during embryogenesis [13]. They have already been proposed for use in patients to seed vascular prostheses [10,14] and also to prevent adhesions in the peritoneal cavity [15]. MCs secrete a broad spectrum of angiogenic cytokines including SDF-1α, growth factors and extracellular matrix [16,17]. Indeed, MCs are transitional mesodermal-derived cells, but share similar morphological and functional properties with endothelial cells [10] and conserve properties of transdifferentiation [12]. Therefore, it is of interest to explore the potential of MC seeding on neoangiogenesis in an experimentally induced infarct scar.

The aim of the present study was to investigate the therapeutic effect of MC seeding in the infarct scar in a rat model. For this purpose, MCs were isolated from the peritoneal cavity of Lewis rats, cultured in vitro and seeded in vivo in the infarct scar of syngeneic rats, three weeks after the experimental induction of MI. Neo-vascularization in the scar and functional improvement of the left ventricle (LV) were evaluated one month later.

2. Methods

2.1. Animals and infarction

All experimental procedures were performed in accordance with European Community Standards on the care and use of laboratory animals (Ministère de l’Agriculture, France, authorization 75-214) conform to NIH Guidelines. Male syngeneic Lewis rats (250–300 g, Janvier, France), were used throughout in order to avoid any problem of histocompatibility.

A left thoracotomy was performed under general anesthesia and the left coronary artery was ligated in order to obtain an LV infarction as described previously [2,4].

Table 1

<table>
<thead>
<tr>
<th>Detection by</th>
<th>Source</th>
<th>Dilution</th>
<th>Method</th>
<th>Treatment</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Anti-cytokeratin (AE1/AE3)</td>
<td>Dako</td>
<td>1:10</td>
<td>IHC, IF</td>
<td>Heating+pepsin</td>
<td>[21]</td>
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<tr>
<td>Anti-α-actin smooth muscle (SM)</td>
<td>Dako</td>
<td>1:50</td>
<td>ICC, IF</td>
<td>–</td>
<td>[3,22]</td>
</tr>
<tr>
<td>Anti-rat endothelial cell antigen-1 (RECA-1)</td>
<td>Serotec</td>
<td>1:10</td>
<td>ICC, IF</td>
<td>–</td>
<td>[22]</td>
</tr>
<tr>
<td>Anti-proliferating cell nuclear antigen (PCNA)</td>
<td>Zymed</td>
<td>1:100</td>
<td>IHC</td>
<td>heating+Pepsin</td>
<td>[1,8]</td>
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<tr>
<td>Anti-stromal cell-derived factor (SDF-1α)</td>
<td>R&amp;D</td>
<td>10 μg/ml</td>
<td>IHC</td>
<td>heating</td>
<td>[17,23]</td>
</tr>
<tr>
<td>Lectin Bandeiraea simplicifolia (BS-1)</td>
<td>Sigma</td>
<td>50 μg/ml</td>
<td>ABC–DAB</td>
<td>–</td>
<td>[23]</td>
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All primary antibodies are mouse monoclonal IgGs. Immunocytochemistry (ICC), immunohistochemistry (IHC) and lectin BS-1 were revealed by Avidin–Biotin Complex (ABC) conjugated to peroxidase using diaminobenzidine (DAB) as substrate. Immunofluorescence (IF) detection in cells or tissues was performed using either green fluorescent secondary antibody Alexa Fluor 488 or red 594 nm goat anti-mouse IgG. Antigen retrieval was required for some detections: heating at 95 °C for 5 min in 0.01 mol/L citrate buffer, pH 6.0, followed or not by 0.005% pepsin in 0.01 N HCl at 37 °C for 15 min.

2.2. MC and BM stromal cells culture in vitro

MCs were obtained from anesthetized Lewis rats by intraperitoneal injection of 0.05% Trypsin–0.53 mM-EDTA and harvesting by aspiration after 20 min [10,11]. They were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 15% fetal calf serum. Cultured MCs were passaged twice before transplantation. Lewis rat BM stromal cells were obtained from femoral bones as previously described [7,18] and cultured in vitro in similar condition to MCs.

2.3. Transplantation of MCs into the infarct scar

Three weeks after ligation of the coronary artery, surviving rats with an LV ejection fraction (EF) below 50% in ultrasonic assessment were randomized into control and transplanted groups. Through an inferior midline mini-sternotomy, the infarct scar was exposed [2,4]. Since fibronectin promotes survival and integration of transplanted cells [19], syngeneic MCs (4 x 10⁶ cells) in a volume of 250 μl of serum-free DMEM containing 100 μg/ml fibronectin (purified from rat plasma by gelatin-Sepharose affinity chromatography [20]) were injected subepicardially at 2 opposite sites in the border zone of the scar with the tip of a needle (30-gauge needle, Microlance ™3, BD) (transplanted group, n=56). The same volume of serum-free DMEM containing fibronectin at a similar concentration was injected in an identical manner into the ischemic area of control rats (control group, n=29). Bulging over the myocardial infarction area was confirmed in every case after injection. The sternotomy was then sutured, the skin closed and the rat ventilated until complete recovery.

2.4. Tracking of transplanted MCs

In order to assess the transplantation yield and the fate of the injected MCs in vivo, MCs were labeled ex vivo before in vivo seeding. Some batches of MCs were transfected one day before transplantation with human secreted alkaline phosphatase (SEAP) reporter gene using a non-liposomal cationic lipid (FuGene ™6 (Roche)/pcDNA3-SEAP; v/w ratio 3:1, Transfection Reagent FuGene ™6 (Roche), v/w ratio 3:1, Invitrogen) and harvested by aspiration after 24 h. The transfection efficiency was checked with the fluorometric SEAP detection kit (Promega).

Table 2

<table>
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<th>Detection by</th>
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<th>Dilution</th>
<th>Method</th>
<th>Treatment</th>
<th>Ref</th>
</tr>
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<tbody>
<tr>
<td>MC transfected</td>
<td>Transfection Reagent FuGene ™6 (Roche)/pcDNA3-SEAP; v/w ratio 3:1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>[–]</td>
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Later on, the injected MCs in vivo, MCs were labeled ex vivo before injection. Some batches of MCs were transfected one day before transplantation with human secreted alkaline phosphatase (SEAP) reporter gene using a non-liposomal cationic lipid (FuGene ™6 (Roche)/pcDNA3-SEAP; v/w ratio 3:1, Transfection Reagent FuGene ™6 (Roche), v/w ratio 3:1, Invitrogen) and harvested by aspiration after 24 h. The transfection efficiency was checked with the fluorometric SEAP detection kit (Promega).
ratio 3:1) as previously described [4]. SEAP activity was monitored in plasma every 2 days following transplantation. In addition, nuclei of MCs were labeled by overnight incubation with 50 μg/ml DAPI fluorescent dye (4′,6-diamidino-2′-phenylindole, Sigma) [6] which permits subsequent in situ detection.

2.5. Histology and immunostaining

Each heart was cut into transverse slices which were fixed in buffered formalin and embedded in paraffin. Sections from each heart were stained with hematoxylin and eosin (H and E). Immunostaining of cultured MCs in vitro, or of myocardial sections, was performed using the primary antibodies listed in Table 1 and a detection system based on the Avidin–Biotin Complex peroxidase-diaminobenzidine method (Vector Kit) or double stain systems (Dako), followed by nuclear counterstaining by hematoxylin, or using fluorescence-labeled secondary antibodies.

2.6. Evaluation of neovascularization in infarct scar

One month post-transplantation, rats were injected intravenously with 150 mg/kg FITC-dextran (MW 150 000, Sigma) [24] in order to visualize blood vessels. The heart was rapidly excised, fixed and embedded in paraffin. Sections were observed by fluorescence microscopy to visualize the perfused vessels in the infarct scar. Alternatively, the vessels in the infarct scar were immunostained (α-actin SM, lectin BS-1 and PCNA) and counted by an observer blinded to the treatment group using light microscopy at 200× magnification. Five fields (0.07 mm²) in the scar in 3 sections from each rat were randomly selected and the vascular density was averaged and expressed as the number of blood vessels/mm².

2.7. Quantification of SDF-1α levels

Levels of SDF-1α, one of the angiogenic mediators released by MCs and BM stromal cells in vitro and by infarct scar and surrounding “peri-infarct” tissue ex vivo, were assessed using a commercial ELISA (R&D systems). Cells were cultured in serum-free DMEM for 48 h in presence or absence of 10 or 100 μg/ml fucoidan (Sigma), a sulfated polysaccharide, known to displace SDF-1α sequestered in heparan sulfate proteoglycans on the cell surface [23]. The conditioned medium and cell extracts were then collected for quantification of SDF-1α protein. The infarct scar and the peri-infarct areas of LV from control and transplanted MC groups were excised and likewise incubated in 24-well plates containing 500 μl serum-free DMEM supplemented with 100 μg/ml fucoidan for 24 h at 37 °C and the conditioned media assayed for SDF-1α. In addition, the incubated tissues were then homogenized in 0.1 M Tris,
4 mM EDTA, 0.1% Triton X-100, pH 7.6, sonicated and centrifuged (11 000 g, 15 min at 4 °C) to extract the SDF-1α remaining within the tissue. SDF-1α was quantified in the supernatant and the results expressed as SDF-1α picograms per milligram of protein.

2.8. Evaluation of LV function

LV function after transplantation was measured by echocardiography as previously described [2,25]. In brief, rats were mildly anesthetized with Isoflurane. LV dimension and function were assessed just before cell seeding, and followed-up 2–4 weeks later by an observer blinded to the treatment groups. Images were recorded using an 8 to 14 MHz phased-array transducer (Toshiba Powervision 6000) and subsequently analysed (Toshiba Workstation-300 A Image). The rats were placed in a supine position. Echocardiograms were recorded through parasternal long-axis views followed by short-axis view. LV dimensions were measured with M-mode tracings from short-axis view of the LV at the papillary muscle level. LV-EF percentage was calculated by a modified version of Simpson’s biplane analysis. All measurements were performed in systolic and diastolic phases from at least three consecutive cardiac cycles.

2.9. Statistical analysis

Statistical analysis was performed with Statview 5.0 software. All values were expressed as means ± standard deviation. Statistical comparison of the data regarding levels of SDF-1α, SEAP activity, number of vessels and LV parameters was performed using either the Student t-test, a one-way ANOVA or a mixed model of ANOVA, followed by Bonferroni’s test for intergroup comparisons. p < 0.05 was considered significant.

3. Results

3.1. Characterization of MCs and production of SDF-1α in vitro

Cultured peritoneal MCs appeared as a cobblestone monolayer at confluence (Fig. 1A). These cells stained positively for cytokeratin (Fig. 1B) and lectin BS-1 (data not shown), but were negative for RECA-1 (Fig. 1C) confirming pure cultures of MCs, distinct from endothelial cells (RECA-1 positive [22]) (n = 3). We tested the production of SDF-1α in primary cultures of MCs and compared it with the SDF-1α produced by BM stromal cells. Large amounts

Fig. 2. Identification of mesothelial cells (MCs) in the infarct scar in vivo. One day after in vitro transfection by SEAP or empty plasmid, 4 × 10⁶ MCs were injected into the MI scar. SEAP was assessed in plasma before and at two-day intervals after seeding. Results are expressed in RLU/µl (A). Immunostaining showed that some grafted cells still expressed cytokeratin at 15 (B, green fluorescence, ×400) and 30 days (C, arrows, brown cytoplasmic staining, ×600). Some MCs were proliferating within the scar as they were PCNA(brown)-cytokeratin(red) positive double-stained and in vessel luminal position (D, inset arrow, ×400).
of SDF-1α were detected in conditioned medium (Fig. 1D) from MCs (15.9 ± 1.8 vs. 0.25 ± 0.4 µg/mg protein in BM stromal cells, \( p < 0.0001 \)) and cell extracts (data not shown) in 6-well plates (\( n = 8 \) wells for each condition), the release of which was increased 10-fold by addition of 100 µg/ml fucoidan.

### 3.2. Fate of transplanted MCs in infarct scar in vivo

Preliminary experiments showed that MCs could be successfully transfected in vitro with plasmid SEAP, with a peak of secretion at 48 h followed by a gradual decline up to 2 weeks (data not shown). One day after successful in vitro transfection of MCs, these cells were injected in vivo into the infarct scar (\( n = 5 \) per group, Fig. 2A). The plasma level of SEAP activity peaked at 48 h (15-fold) (\( p < 0.0001 \)) and then gradually decreased thereafter but still remained significantly elevated for at least 10 days, providing evidence of the persistence of transfected MCs in the scar and their capacity to express the transgene in situ. A potential trapping of MCs by other organs such as lung, liver or spleen was assessed by injection of DAPI-labeled MCs in the scar, followed by paraffin sectioning after 48 h (data not shown). A very limited number of DAPI-labeled cells were found in organs other than the heart, suggesting that SEAP expression is mainly attributable to cells residing in the scar. Rats were studied histologically for identification of MCs in the infarct scar at 15 (\( n = 20 \)) and 30 days (\( n = 22 \)). Cytokeratin positive cells were observed in the infarct scar after 2 weeks (Fig. 2B) and one month post-implantation (Fig. 2C). Many proliferating cells were observed at one month (nuclear positive PCNA staining, brown) and incorporated in neo-vessel formation (Fig. 2D and inset). Some PCNA-positive cells were also positive for cytokeratin (double staining, Fig. 2D).

### 3.3. MCs as a component of vessel formation

DAPI-labeled cells were still observed in the infarct scar at 30 days after implantation (\( n = 5 \) per group, Fig. 3, all blue nuclei). We looked at this time for evidence of colocalization within cells of DAPI (blue) and cytokeratin (in red, Fig. 3).

![Fig. 3. MCs as a component of vessel formation. One month post-implantation, DAPI-positive cells were observed in the infarct scar (all blue nuclei). Fluorescent immunostaining followed by image merging showed that some blue DAPI-labeled cells express red cytokeratin (A, arrow, other colors than blue and red resulting from image merging should not be taken into account). Some DAPI-positive cells (blue) were localized in a luminal position of large and small vessels 1 month after injection of DAPI-labeled MCs (B, Bx and C, arrow heads). Perivascular smooth muscle cells are positive for α-actin SM (B, Bx, arrows) but DAPI-positive cells express neither α-actin SM (B, Bx, arrows) nor RECA-1 (C). In C, the arrow shows DAPI-negative RECA-positive cells (large red areas correspond to staining artifacts of intravascular blood clots). Positive control of RECA-1 staining in normal myocardium is included (D). Merged images of green autofluorescence of the internal elastic lamina (obtained with FITC filter) and DAPI shows that some DAPI-labeled cells are in endothelial position (arrow heads) and others are in the media (arrows).](https://academic.oup.com/cardiovascres/article-abstract/68/2/307/299423? acqust on 14 May 2018)
3A), α-actin SM (in red, Fig. 3B and Bx) or RECA-1 (in red, Fig. 3C) using immunofluorescence. Some of DAPI-positive cells were observed in a luminal position of large and small vessels in some MC-implanted hearts (Fig. 3B, Bx and E). These cells expressed cytokeratin but not α-actin SM or RECA-1. In spite of the possible diffusion of DAPI from transplanted to host cells, the colocalization of cytokeratin and DAPI suggests that some MCs were directly incorporated or indirectly participated in neo-vessel formation. In Fig. 3E, autofluorescence of elastin allows visualization of internal elastic lamina (green) showing that some DAPI-labeled cells are in an endothelial position (arrow heads) and others are localized in the media (arrows).

3.4. Histological evaluation of transplanted infarct scar

The presence of transplanted MCs within the infarct scar was evaluated by H and E staining with corresponding DAPI-labeled cells in serial sections at 3, 15, 30 days post-transplantation (n=14, 28 and 31 respectively). There was no histological evidence of inflammation in the infarct scar in control or transplanted groups in this syngeneic strain. Three days after transplantation, large numbers of exogenous cells were observed within the infarct scar in transplanted rats, but not in control rats (Fig. 4B, n=8 and C, n=4 vs. A, n=6). At this time there was no increase in vascular density in the infarct scar around the transplanted MCs compared with controls. Two weeks post-transplantation, the numbers of transplanted MCs had diminished but some clusters were still visible and the number of blood vessels surrounding them had increased compared to controls (Fig. 4E, n=20 and F, n=5 vs. D, n=8). One month post-transplantation, the clusters of transplanted cells had completely disappeared (Fig. 4H). Numerous developing vessels were observed in the scar, some containing red blood cells in their lumina. This suggested that neovascularization had occurred in the vicinity of the transplanted MC and there appeared to be a greater density of vessels in the infarct scar of the MC transplanted groups compared to controls (Fig. 4H, n=22 and I, n=5 vs. G, n=9).

3.5. Evaluation of neovascularization

In the same rats, vascular density at one month appeared to be markedly increased in the infarct scar of the transplanted rats compared to controls (Fig. 5B vs. A), as

![Fig. 4. H and E staining and corresponding DAPI mesothelial cells in myocardial scar tissue. Rat myocardial infarction scar in hearts excised three days (A–C), two weeks (D–F) and one month (G–I) after injection of vehicle alone (control, A, D, G) or mesothelial cells (transplanted) with (C, F, I) or without (B, E, H) DAPI-labeling. Red blood cells are present in the lumen of vessels (>200).](https://academic.oup.com/cardiovascres/article-abstract/68/2/307/299423)
visualized by FITC-dextran present in the lumina of blood vessels. Vascular structures in infarct scar tissue were counted after identification by (immuno)staining for \( \alpha \)-actin SM, lectin BS-1 and PCNA to assess SMCs, endothelial cells and proliferating new vessels respectively (Fig. 5C–H). Vascular density in the transplanted scar was 2- to 3-fold greater \((p < 0.0001)\) than that in control infarct scar (Fig. 5I).

### 3.6. Transplanted LV tissues express SDF-1\( \alpha \)

Based on our in vitro results showing that MCs secrete large amounts of SDF-1\( \alpha \) (Fig. 1D), we assessed the level of this angiogenic cytokine in the infarct scar. Immuno-staining of myocardial sections for SDF-1\( \alpha \) at 30 days post-transplantation appeared to be markedly increased in the infarct scar and peri-infarct of the transplanted group (Fig. 6C–D \( \eta = 22 \) vs. A–B \( \eta = 9 \) in controls), associated with neovascularization. Globally we show a significant effect of MC transplantation on SDF-1\( \alpha \) secretion measured by ELISA, whatever the site (two-way ANOVA \( F=4.4, p<0.05 \) for EDD). End-diastolic diameter (EDD) was assessed as a marker of LV remodeling. LV-EDD was significantly decreased in transplanted \((9.6\pm0.6\ \text{mm})\) vs. control groups \((11.1\pm0.6\ \text{mm})\) at day 30 \((p<0.01)\). LV-EDD was also lower at 30 days in the transplanted group compared to baseline \((p<0.004)\) and 2 weeks \((p<0.02)\) indicating a progressive decrease of this parameter after transplantation. LV-EDD did not significantly change with time in controls (Fig. 7A). Similarly, the LV-end-diastolic volume (EDV) was significantly decreased in the transplanted group at day

3.7. Effect of transplanted MCs on LV function

At baseline (i.e. after infarction and before transplantation) echocardiographic parameters were not significantly different between transplanted \((\eta = 17)\) and control \((\eta = 9)\) groups. Analysis of echocardiographic parameters revealed strong group–time interactions (two-way ANOVA \( F=4.4, p<0.05 \) for EDD). End-diastolic diameter (EDD) was assessed as a marker of LV remodeling. LV-EDD was significantly decreased in transplanted \((9.6\pm0.6\ \text{mm})\) vs. control groups \((11.1\pm0.6\ \text{mm})\) at day 30 \((p<0.01)\). LV-EDD was also lower at 30 days in the transplanted group compared to baseline \((p<0.004)\) and 2 weeks \((p<0.02)\) indicating a progressive decrease of this parameter after transplantation. LV-EDD did not significantly change with time in controls (Fig. 7A). Similarly, the LV-end-diastolic volume (EDV) was significantly decreased in the transplanted group at day
LV-EDV was also lower in the transplanted group at day 30 vs. base line (p < 0.04) (Fig. 7B).

4. Discussion

Among emerging new therapeutic strategies for preventing heart failure in patients with MI who are not candidates for conventional methods of revascularization, autologous cell therapy has been proposed in many experimental and clinical studies [26]. Most human potential donor cells are difficult to isolate, purify and expand ex vivo and do not yield adequate numbers of cells for transplantation [3,26]. Some donor cells even have deleterious effects and lead to complications such as arrhythmia, calcification, teratoma or tumor formation [26].

For these reasons, we explored the potential of MCs for transplantation into the infarct scar, speculating that this cell type might participate in inducing safe and long-lasting angiogenesis and vascularization of the myocardial scar. MCs are abundant in the peritoneal cavity [9,27] and can be obtained safely in humans [9]. Moreover, MCs may be a suitable clinical tool for ex vivo gene therapy to express therapeutic proteins at useful levels [9,11].

Our in vitro results showing cytokeratin and lectin BS-1 expression in MCs agree with published data demonstrating that the expression of cytokeratin in cultured MCs was positively affected by cell–cell contact or cell spreading [21]. Lectin binding to normal mesothelium is not well-defined. Lectin-binding sites on the cell surface are likely to be involved in cell maturation and transformation [21]. Moreover MCs release large quantities of SDF-1α, much more than other cells such as BM stromal cells, that secrete minimal amounts. This agrees with reports on the low production of SDF-1α by human BM stromal cells [28].

The fate of transplanted MCs was assessed with respect to survival, proliferation and their involvement in neo-vessel formation. When transfected before transplantation, MCs expressed the SEAP reporter gene as assessed by alkaline phosphatase activity in plasma for at least 10 days. Our results show that MCs can survive after transplantation and express a potential therapeutic angiogenic gene for a period of time sufficient to initiate neovascularization. This suggests that MCs can be used for ex vivo gene transfer, confirming previous studies demonstrating that MCs have excellent secretory function following transfection and can devote up to 3% of their total protein synthesis to a single secreted protein [9,11]. Transitory transfection did not allow tracking of transplanted cells for long periods [4]. Therefore, other markers were used, such as cytokeratin immunostaining or DAPI-labeling.

MCs labeled ex vivo were visualized in the infarct scar of myocardial sections and immunostained for cytokeratin after 30 days, indicating that transplanted MCs were still present in the scar and continued to express cytokeratin, reflecting their reactive state [21]. The use of DAPI for...
tracking implanted cells has recently been criticized [29] since it can be taken up by surrounding cells. The DAPI positivity observed in our sections thus cannot be attributed solely to transplanted MCs, but the colocalization of focal DAPI staining and cytokeratin [21] suggests that some DAPI-labeled cells were indeed transplanted MCs. Our results agree with other studies of transplantation of DAPI-labeled cells [6,7,30] in rat MI models. Moreover, some transplanted cells were double-stained for PCNA and cytokeratin suggesting that some MCs were proliferating [1,8] within the infarct scar.

Some engrafted MCs were localized in an endothelial position but their staining remained more mesothelial (cytokeratin) than endothelial (RECA-1). It has been shown that mesothelium-derived cells can transdifferentiate into endothelial cells in the liver with remaining transient cytokeratin expression [12] and that the precursors of the coronary endothelium are probably derived from epicardial mesothelial cells, which are able to differentiate into fibroblasts, endothelial, smooth muscle and valvuloseptal mesenchymal cells [13,31]. Other studies showed that transplanted endothelial cells stimulated angiogenesis and were incorporated into the new vessels [5], whereas, transplanted stromal cells differentiated in situ and contributed to about 10–22% of the endothelial cells of newly formed blood vessels [6,7,18].

Neovascularization was significantly increased in the infarct scar of the transplanted group compared to controls at day 30. Since MCs can release numerous angiogenic growth factors in vitro including SDF-1α, we assessed the expression of this cytokine in the infarct scar compared to control LV. SDF-1α levels were higher in transplanted groups, indicating that MCs promote a pro-angiogenic environment. This increase in SDF-1α cannot be attributed to an inflammatory reaction resulting from the mechanical trauma of injection into the myocardial scar since the control group was treated similarly with DMEM and fibronectin, and no inflammatory cells were observed by histology. These results agree with reports of stem cell therapy, where para-secretion of vascular endothelial growth factor (VEGF) [7,32], basic fibroblast growth factor (bFGF) [32] or cytokines such as interleukin-1β [32] is achieved in MI.

The possible role of SDF-1α in endogenous angiogenesis (direct or via certain secondary cytokines), as well as in vasculogenesis, is now well documented [33–36]. However, it is not the aim of our work to assess the role of SDF-1α in infarct scar remodeling after transplantation. The role
of MCs is not limited to the release of a single protein such as SDF-1α as they also secrete a variety of other growth factors in vitro such as VEGF, bFGF, TGF-β, PDGF, IGF, HGF, EGF and KGF [12,27] that may be involved in neovascularization. MCs have also anti-inflammatory and immunomodulatory properties mediated by secreting proangiogenic factors in vitro such as VEGF, bFGF, TGF-β, PDGF, IGF, HGF, EGF and KGF [12,27] and pro-angiogenic plasminogen activator inhibitor-1 [37], all of which could be useful for decreasing infarct scar remodeling [34,38].

Finally, the end point of our study showed a significant prevention of cardiac function impairment after MC therapy, with a reversal of LV remodeling reflected by smaller LV-EDD and EDV values in the transplanted group. Other studies showed that cell types associated with neo-vessel formation after transplantation may improve cardiac function [8,32,39] by limiting the remodeling process in the scar and/or decreasing apoptosis of hypertrophied myocytes in the peri-infarct region [7,8,40,41]. As previously reported, diastolic diameter and volume appear sensitive and reliable echographic parameters in the rat model of infarction providing evidence of LV remodeling [25]. Also, implanted cells can alter matrix metabolism and thereby prevent ventricular dilatation and improve heart function [41]. Regardless of the mechanism involved, MC transplantation and associated neovascularization lead to prevention of LV dysfunction.

In conclusion, we show for the first time using an experimental model that mesothelial cells, easily obtainable in large amounts, could be proposed for cell therapy in the reversal of infarct scar remodeling. Our data highlight the suitability of these cells for clinical applications and open up new perspectives in the search for therapeutic strategies.

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