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Bone marrow stromal cells improve cardiac performance in healed infarcted rat hearts


Bone marrow stromal cells improve cardiac performance in healed infarcted rat hearts. Am J Physiol Heart Circ Physiol 287: H464–H470, 2004. First published March 25, 2004; 10.1152/ajpheart.01141.2003.—Postinfarct congestive heart failure is one of the leading causes of morbidity and mortality in developing countries. The main purpose of this study was to investigate whether transplantation of bone marrow stromal cells (BMSC) directly into the myocardium could improve the performance of healed infarcted rat hearts. Cell culture medium with or without BMSC was injected into borders of cardiac scar tissue 4 wk after healed infarcted rat hearts. BMSC-treated rat hearts showed electrical and mechanical parameters more similar to those in control than in medium-treated animals: a normal frontal QRS axis in 6 of 10 BMSC-treated animals and in 8 of 10 medium-treated animals. BMSC treatment, assessed by echocardiography, improved fractional shortening (39.00 ± 0.74%) and QRS axis (18.20 ± 4.03%) compared with medium-treated hearts (18.20 ± 4.03%) and prevented additional changes in cardiac geometry. Immunofluorescence microscopy revealed colocalization of 4’,6-diamidino-2-phenylindole-labeled nuclei of transplanted cells with cytосkeletal markers for cardiomyocytes and smooth muscle cells, indicating regeneration of damaged myocardium and angiogenesis. These data provide strong evidence that BMSC implantation can improve cardiac performance in healed infarctions and open new promising therapeutic opportunities for patients with postinfarction heart failure.

myocardial infarction; cell therapy

IN HUMANS, the healed myocardial infarction is characterized by the presence of scar tissue that develops after the initial insult. Structurally, the damaged myocardium undergoes a series of changes that ultimately lead to formation of a transmural or subendocardial fibrous scar, composed of a dense layer of collagen fibers and fibroblasts that form the so-called “healed myocardial infarction.” The extensive loss of myocardial tissue and the progressive ventricular remodeling that follow the ischemic insult contribute to abnormal cardiac function and heart failure.

Despite its limitations, such as shortage of donors, problems with immunosuppression, and graft failure, cardiac transplantation was, until recently, the only available solution for treatment of severe terminal heart failure. New therapeutic approaches such as cellular cardiomyoplasty (CCM), in which appropriate donor cells are delivered to the injured myocardium, target the pathophysiological basis of congestive heart failure by attempting to regenerate the damaged myocardium through the transplantation of healthy cells. Many cell types have been transplanted into injured myocardium: embryonic (15), fetal (16, 21), and adult cardiomyocytes (9), skeletal myoblasts (24), immortalized myoblasts (14), smooth muscle cells (8), fibroblasts (5), bone marrow-derived stromal (27) and hematopoietic (12) stem cells, and human bone marrow-derived endothelial precursors (7).

The efficiency of CCM therapy to improve cardiac function in models of ischemia-reperfusion (18), cryoinjury (9, 24), and dilated cardiomyopathy (29) has been reported. CCM has also been extensively performed in models of permanent ligation of the left anterior descending coronary artery (LAD), but mainly within a short period of time after the ischemic injury: 7 days (2), 2 days (7), 3–5 h (12), 1 h (23), 30 min (11), and immediately after surgery (19).

Thus, the main purpose of this study was to investigate whether CCM can improve the performance of healed infarcted rat hearts when a fibrous scar is present in the wall of the left ventricle and heart failure has developed.

METHODS

This investigation conforms with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85-23, Revised 1996, Office of Science and Health Reports, Bethesda, MD 20892] and was approved by the institutional animal welfare committee.

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Animals

Male inbred Wistar rats (200–250 g) were obtained from the Centro de Pesquisa Gonçalo Muniz (Fiocruz-Bahia/Brazil). Animals were housed at controlled temperature (23 °C) with daily exposure to a 12:12-h light-dark cycle and free access to water and standard rat chow. These isogenic rats were used as donors and recipients of bone marrow-derived stromal cells (BMSC) to simulate clinical autologous implantation.

Cell Isolation and Culture Procedures

The isolation and primary culture of BMSC from femoral and tibial bones of donor rats were performed as described by Caplan (1). BMSC were cultured in DMEM supplemented with 20% fetal bovine serum (GIBCO-BRL), 2 mM l-glutamine (Sigma), and antibiotics (100 U/ml penicillin G and 100 µg/ml streptomycin; GIBCO-BRL) and maintained at 37°C in a 5% CO2 incubator. The cultures were maintained for 1 wk, during which medium was changed at least twice, which washed away all floating hematopoietic cells. At ~80–90% confluence, the cells were detached from the culture flasks with 0.25% trypsin-EDTA (Sigma), resuspended in DMEM, and labeled with 4’,6-diamidino-2-phenylindole (DAPI; Sigma) for 30 min. The morphology of the cells just before labeling with DAPI is shown in Fig. 1A. The cells were rinsed six times in balanced salt solution to remove unbound DAPI and kept in warm DMEM (100% labeling of cell nuclei). At ~100% labeling of cell nuclei.

Flow Cytometry Analysis

On the day of injection, BMSC were detached from culture flasks with ice. Briefly, 10^6 cells were stained with anti-CD34-phycocerythrin (Becton Dickinson, San Jose, CA) and anti-CD45-FITC (Caltag Laboratories, Burlingame, CA) for 30 min at 4 °C and washed twice before flow cytometry (FACSCalibur, Becton-Dickinson). Figure 1B shows that >99% of the cells were negative for both hematopoietic markers. Mouse isotype controls IgG1-phycocerythrin and IgG1-FITC were also used. All antibody dilutions were 1:100.

Myocardial Infarction and Cell Transplantation

Left ventricular myocardial infarction was induced following the procedure described by Johns and Olson (6). Briefly, male Wistar rats (200–250 g) were anesthetized with halothane (Merck), and a 2-cm incision was made on the left side of the thorax, parallel to the sternum. The fifth and sixth ribs were separated, exposing the heart, and the LAD was permanently occluded as it passes under the left atrial appendage by ligation with a 6-0 silk thread. The chest was then closed with continuous silk stitch, and the rats were allowed to recover.

The infarcted rats were randomized for injection with BMSC or culture medium alone. For the injection procedure, the animals were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg ip) and maintained under positive-pressure ventilation with room air supplemented with oxygen (2 l/min; model 680 rodent respirator, Harvard Apparatus) at 60–80 cycles/min with a tidal volume of 1 ml/100 g body wt. The heart was exposed, the macroscopic scar area was visualized, and 200 µl of a BMSC suspension containing a total of 1–3 × 10^6 cells were injected with a tuberculin syringe into two to three sites along the borders of the left ventricular wall scar tissue. The interanimal variability and because of mortality during the delivery procedure, we used a smaller number of infarcted rats (n = 20), in which we determined functional parameters in a serial manner. Electro- and echocardiograms were recorded before and 14 days after treatment with BMSC (n = 10) or culture medium alone (medium, n = 10).

Functional Assessment

Electrocardiographic study. Rats anesthetized with ketamine and xylazine were placed in the supine position for electrocardiographic recording (Cardimax FX-2111, Fukuda Denshi) in classic configuration: six limb leads (L1, L2, L3, aVR, aVL, and aVF) and two chest leads (V1, V2, recorded at the 1/2 distance from the sternal manubrium and the xyphoid process, and V3, V4, recorded at the midaxillary line, at the same height of the 1st chest lead). To obtain reproducible electrocardiographic recordings in the rat, the anesthetized animals were fixed on a board with their anterior paws in the orthogonal direction with respect to the body and their posterior limbs kept free. Additionally, all animals were carefully examined at the time of medium or cell treatment.
injection (in the infarcted group) and postmortem (in all groups) to
detect alterations of the anatomic relations of the heart in the thoracic
cage that might lead to misleading results in the evaluation of the
ventricular depolarization vector. Electrocardiograms were recorded
for 3 min, and the anesthetized animals were then subjected to
echocardiographic examination. During the 5- to 15-min echocar-
diographic examination, we continuously recorded an L2 lead
electrocardiogram.

The electrocardiogram parameters were as previously described
(17): heart rate, presence of a P wave >0.1 mV, P-R interval, QRS
duration, Q-T interval, frontal QRS axis, QRS amplitude index (i.e.,
the sum of QRS complex voltage in L1, L2, and L3), and the presence
of a Q wave in L1, L2, L3, aVF, V_anterior, and V_left. In all infarcted animals
(n = 30), the presence of a frontal QRS axis >90° (rightward)
deviation was used as evidence of an extensive infarction as demon-
strated by postmortem pathological analysis that confirmed the
presence of transmural scar tissue in an area corresponding to ~30% of
the left ventricle (data not shown). Thus the data for frontal QRS axis
are represented as frequency of occurrence: animals with frontal QRS
axis >90° (rightward) or between 0 and 90° (leftward).

Echocardiographic study. We used an echocardiographic color
system (Megas/Esaote) equipped with a 10-MHz electronic phased-
array transducer. Under ketamine and xylazine anesthesia, the chests
were opened through the 4th intercostal space in the left flank, the
pericardium was opened, the heart was placed on a wooden
support, and the thoracic cage that might lead to misleading results in the evaluation of the
ventricular depolarization vector.

Arrhythmia was expressed by the fractional shortening obtained from the M-mode
trace. The pulsed-wave Doppler spectra of mitral inflow were re-
corded from the apical four-chamber view with the guidance of the
color Doppler. All Doppler spectra [mitral flow velocity pattern: peak
ear diastolic filling velocity (E velocity), peak filling velocity at atrial contraction (A velocity), and their ratio (E/A)] were recorded,
and morphological parameter values were measured during the echo-
cardiographic exam.

Immunohistochemistry. At 3 wk after CCM, BMSC-treated (n = 3)
and medium-treated (n = 3) animals were anesthetized with ether and
killed by cervical dislocation, and their hearts were perfused with 4%
paraformaldehyde in phosphate buffer. The tissues were cryoprotected
and cooled in liquid nitrogen, and 10-μm consecutive cryostat sec-
tions were obtained. Thereafter, the sections were processed for
immunofluorescence using a monoclonal anti-α-smooth muscle actin
antibody (clone 1A4, Dako) and a monoclonal anti-α-sarcomeric actin
(clone 5C5, Sigma). Both antibodies were used at 1:100 dilutions.
Goat secondary antibodies were used as follows: anti-mouse IgG
FITC conjugate (catalog no. F-2012, Sigma) and anti-mouse IgM
FITC conjugate (catalog no. F-9259, Sigma), both at 1:50 dilutions.
The grafted BMSC were identified by DAPI-labeled nuclei. An
Axiovert 135 microscope coupled to a high-resolution AxioCam HR
charge-coupled device videocamera (Zeiss) was used to obtain differ-
ential interference contrast and fluorescence images from regions of the
infarcted left ventricle displaying nuclear and cytoskeletal mark-
ers. Images were overlaid and processed using Photoshop 7 software
(Adobe).

Statistical Analysis

Values are means ± SE. Differences between electro- and echo-
cardiographic measurements before and after medium or BMSC
treatment and between groups were evaluated by paired and unpaired
Student’s t-test, respectively. Values were considered different when
P < 0.05 after Bonferroni’s correction. When frequency data were
compared, the nonparametric Fisher’s exact test with the 0.05 level of
probability was used.

RESULTS

Functional Assessment

Electrocardiographic study. Electrocardiographic parameters
from nonoperated (normal) and sham-operated groups
were not statistically different. However, interesting differ-
ences were detected between electrocardiographic parameters
from infarcted and normal or sham animals (Table 1). The
most outstanding difference is the significant rightward shift of
the frontal QRS axis in all 30 animals from the infarcted
group. This group also differed from the sham-operated and normal
groups in relation to the presence of the Q wave in L1 and V_left
and the significant decrease in the QRS amplitude index,
confirming previous reports (17).

At 2 wk after BMSC treatment, the electrocardiographic parameters
of the healed infarcted rats were significantly al-
tered (Table 2): the QRS amplitude index increased, the frontal
QRS axis returned to normal values in 6 of 10 animals, and the
Q waves in L1 and V_left disappeared in 5 of 9 animals. The
effectiveness of the BMSC treatment in restoring the electro-
cardiographic parameters of the healed infarcted rat hearts to
values resembling those of the sham-operated animals was
different from the results obtained in the infarcted group
injected solely with medium. Compared with the electrocar-
diogram of BMSC-treated rats, the electrocardiographic traces
of medium-treated animals did not show improvement in any
of the electrocardiographic parameters: QRS amplitude index
showed no alteration, Q waves in L1 and V_left were present in
9 of 9 animals, and the rightward deviation of the frontal QRS
axis persisted in all 10 animals.

Regarding the development of arrhythmias, one BMSC-
treated rat displayed four isolated episodes of ventricular pre-
mature beats during the first electrocardiogram recorded 2 wk
after cell injection. This arrhythmia was not present in subse-
quent recordings performed 1 and 2 days later.

All other electrocardiographic parameters that are not listed
in Tables 1 and 2 were similar in all groups.

Echocardiographic study. Heart rate was constantly moni-
tored during echocardiography and was not statistically differ-

Table 1. Electrocardiographic data of control, sham, and
infarcted rat hearts

<table>
<thead>
<tr>
<th>Presence of Q wave in</th>
<th>Normal (n = 34)</th>
<th>Sham Operated (n = 33)</th>
<th>Infarcted (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IQRS, mV</td>
<td>1.27±0.05</td>
<td>1.30±0.16</td>
<td>0.98±0.06*</td>
</tr>
<tr>
<td>Presence of rat</td>
<td>34 left/0 right</td>
<td>33 left/0 right</td>
<td>0 left/30 right*</td>
</tr>
<tr>
<td>L1</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>L2</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>L3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>aVF</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>V_anterior</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>V_left</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of rats. IQRS, QRS amplitude index; 
A_QRS, number of rats with left or right orientation of frontal QRS axis. *P <
0.01 vs. normal and sham-operated rat hearts.
ent between all groups studied, ranging from 250 to 300 beats/min under anesthesia (data not shown). As shown in Table 3, 4 wk after infarction, a significant increase in left atrium-to-aorta ratio, left ventricular end-diastolic dimension, and E/A velocity ratio (restrictive mitral flow) and a significant decrease in relative wall thickness and fractional shortening were observed in the infarcted group compared with the other two groups. These differences point to the fact that 4 wk after infarction the animals have already developed heart failure. No major changes were detected in other echocardiographic parameters.

BMSC treatment did not reverse the enlargement of the left atrial and ventricular chambers induced by the infarction (Fig. 2, A and B). In contrast, the left atrium-to-aorta ratio was significantly increased in the medium-injected group. The decrease in relative wall thickness observed after infarction was also not altered by BMSC treatment (Fig. 2C).

Systolic function of the BMSC-treated rats was significantly improved. Fractional shortening increased from 23.90 ± 3.16 before treatment to 39.0 ± 4.03 in the same animals 2 wk after BMSC (P < 0.01; Fig. 2D). In the medium-injected group, fractional shortening was 21.88 ± 1.08 before medium injection and, 2 wk later, decreased to 18.20 ± 0.74 (P < 0.05; Fig. 2D). The difference in fractional shortening attained after medium or BMSC injection was highly significant (P < 0.01).

Doppler analysis of the BMSC-treated infarcted hearts shows that, 2 wk after treatment, the mitral flow pattern measured by the E/A velocity ratio had decreased from 7.49 ± 2.37 to 2.66 ± 0.91 (P < 0.05). In contrast, in the medium-injected animals, the E/A velocity ratio increased from 6.43 ± 2.64 to 10.52 ± 2.61 (P < 0.05). The values attained 2 wk after medium or BMSC injection are statistically significant (P < 0.01).

Representative M-mode traces of one infarcted rat before (Fig. 3A) and 2 wk after (Fig. 3B) CCM show clear improvement in left ventricular contractility, which in this animal, as in most animals studied, was particularly striking in the anterior wall.

Postmortem study. The hearts from medium- and BMSC-treated groups were carefully examined postmortem, and none was found to present tissue adherence or abnormal heart position in the thoracic cage, which might otherwise lead to misinterpretation of electrocardiographic parameters, mainly in the frontal QRS axis.

DAPI-labeled transplanted cells could be easily identified in consecutive sections obtained from the middle portion of the left ventricle of the BMSC-treated rat hearts. Figure 4 shows representative images of positive immunostaining for smooth muscle and sarcomeric actin colocalized with DAPI-labeled nuclei in individual smooth muscle cells from the tunica media of coronary vessels and in cardiomyocytes (Fig. 4, A–E). These double-labeled vessels and myocardium seemed to be structurally integrated with the healthy ventricular wall. However, not all the cells presenting nuclear and cytoskeletal fluorescent labels seemed to have achieved a fully differentiated state (Fig. 4F). When this was the case, cell shape was round, and the cells were frequently found in groups or distributed along the cardiomyocytes. By counting the numbers of DAPI-labeled nuclei in an 6-mm² area around the scar (which should account for the localization of most of the injected cells), we determined an average of 3 × 10⁴ nuclei, suggesting that ≥6–10% of the injected cells were still present in the heart 3 wk after cell injection.

**DISCUSSION**

CCM with different cell types in animal models of acute myocardial infarction has been shown to be effective in improving cardiac performance (7, 10–12, 23). It has also been reported that CCM performed within a short period of time after permanent ischemic injury can prevent ventricular remodeling (2, 7, 23). In the present report, we evaluated whether the use of BMSC in CCM improves the cardiac performance of rats with healed infarction when heart failure has already taken place.

BMSC are a good choice of cell type to be used in the regeneration of the damaged myocardium. These cells have the potential to differentiate into cardiomyocytes “in vitro” (10) and express functional adrenergic (β₁ and β₂) and muscarinic (M₁ and M₂) receptors (4) after treatment with 5-azacytidine. In addition, BMSC can also exhibit a cardiomyogenic phenotype when implanted into myocardium (27). Moreover, from a clinical point of view, the use of BMSC in CCM would be extremely convenient, inasmuch as these cells can easily be harvested from patients during bone marrow aspiration and then expanded in culture. Although the therapeutic effects of BMSC have been recognized (3, 20, 25, 26, 28), it is still not known whether a specific subpopulation in the BMSC is actually responsible for these effects. Phenotypic analysis by flow cytometry showed that the BMSC used in our transplants are mainly CD34⁻ and CD45⁻ and extremely homogeneous from a morphological standpoint. Many groups isolate the so-called “mesenchymal stem cells” from the bone marrow by

Table 2. Electrocardiographic data of infarcted rat hearts before and after treatment with medium or BMSC

<table>
<thead>
<tr>
<th></th>
<th>Medium (n = 10)</th>
<th>BMSC (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>IQRST, mV</td>
<td>1.01 ± 0.12</td>
<td>1.08 ± 0.15</td>
</tr>
<tr>
<td>L1</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>VLS†</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. BMSC, bone marrow-derived stromal cells. *P < 0.05 vs. before treatment with BMSC; †P < 0.05 vs. after treatment with culture medium.

Table 3. Echocardiographic data of control, sham, and infarced rat hearts

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 34)</th>
<th>Sham Operated (n = 33)</th>
<th>Infarced (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA to Ao dimension</td>
<td>0.99 ± 0.02</td>
<td>0.95 ± 0.04</td>
<td>1.37 ± 0.07*</td>
</tr>
<tr>
<td>LVEDd, mm</td>
<td>0.54 ± 0.01</td>
<td>0.56 ± 0.03</td>
<td>0.92 ± 0.06*</td>
</tr>
<tr>
<td>RWT</td>
<td>0.40 ± 0.01</td>
<td>0.41 ± 0.09</td>
<td>0.24 ± 0.01*</td>
</tr>
<tr>
<td>FS%</td>
<td>65.40 ± 2.67</td>
<td>58.04 ± 2.95</td>
<td>21.29 ± 1.92*</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.63 ± 0.09</td>
<td>1.56 ± 0.08</td>
<td>6.32 ± 0.12*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. LA, left atrium; Ao, aorta; LVEDd, left ventricular end-diastolic diameter; RWT, relative wall thickness; FS%, fractional shortening; E/A ratio, ratio of peak early diastolic filling velocity to peak filling velocity at atrial contraction. *P < 0.01 vs. normal and sham-operated rat hearts.
the adherence protocol we used. The mesenchymal stromal cells were originally isolated by Caplan (1) using such a method. Because classical markers for bone marrow stem cells are scant for rats, we opted to call our cells stromal cells and used the absence of CD34 and CD45 markers to prove their nonhematopoietic lineage. Further phenotypic analysis is rendered difficult by the lack of availability of specific markers for rat bone marrow cells.

The functional data presented here provide strong evidence that use of BMSC in CCM can improve cardiac performance even after the healing process has occurred. Electro- and echocardiographic parameters of BMSC-treated animals improved significantly compared with medium-treated animals, suggesting the participation of BMSC in the chain of events that ultimately led to the improvement of cardiac function. Additionally, the histological examination suggests that implanted BMSC engrafted in the myocardium give rise to new myocardium and blood vessels. However, one limitation of our study is that we cannot rule out that the colocalization in the fluorescent images results from structures that are not in the same focal plane, because we did not use confocal microscopy. Furthermore, because our histological studies were conducted 3 wk after cell injection, we cannot speculate about the mechanisms involved in new myocardial and blood vessel formation. Investigating the responsible mechanisms for new muscle and vessel formation would require sequential histological evaluation at much earlier time points. Our study was clearly not designed to address this point but, rather, to investigate the therapeutic value of bone marrow cell transplantation in postischemic heart failure.

Sakakibara et al. (16), using the same infarction model and fetal cardiomyocyte transplantation, did not report improvement in cardiac function 4 wk after treatment. It is possible that the different cell types used for transplantation may account for the diverse results. This hypothesis is supported by work developed in pig models where Fuchs and colleagues (3).
showed that bone marrow cells secrete angiogenic factors that induce endothelial cell proliferation and, when injected transendocardially, augment collateral perfusion and myocardial function of ischemic myocardium. More recently, three other groups (20, 25, 28) reported that bone marrow mesenchymal stem cells do engraft, generate cardiomyocytes, and restore cardiac function in porcine hearts subjected to ischemic injury 2–4 wk before cell therapy.

If it is considered that BMSC treatment prevented additional left atrial enlargement, restored normal mitral flow pattern, and substantially increased fractional shortening, it is tempting to hypothesize that cellular therapy with BMSC can reverse or at least delay the evolution of the processes that, after myocardial infarction, lead to heart failure. Whether a reversion of the heart failure associated with myocardial infarction can be sustained by CCM deserves further investigation with long-term follow-up. The use of mononuclear bone marrow cells (MBMC) has proven efficient in patients with acute myocardial infarction (22), and our group has reported improvement in heart function after MBMC transplantation in patients with healed infarcts (13). MBMC contain the stromal cells. The percentage of BMSC in a mononuclear fraction of bone marrow cells varies from ~0.5 to 1%.

Data presented here suggest that improvement of cardiac function may result from new heart vessel and muscle formation even after scar tissue has formed. These findings are extremely relevant clinically, because postischemic congestive heart failure is the major cause of death in patients who survive myocardial infarction, and there are no effective therapies to stop the progression of this disease.

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GRANTS

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Fig. 4. Immunofluorescence overlays and differential interference contrast images from left ventricular wall 3 wk after injection with 4',6-diamidino-2-phenylindole (DAPI)-labeled BMSC (blue) using antibodies against smooth muscle actin (A–C; red label) and sarcomeric muscle actin (D–F; red label). A: panoramic view of myocardium showing part of transplanted BMSC colocalizing with a blood vessel, indicated by the presence of smooth muscle actin. B: magnified portion of vessel wall revealing BMSC origin (blue-labeled nuclei) of tunica media cells. C: detail of another vessel exhibiting cells with nuclear and cytoskeletal fluorescent markers. D and E: sarcomeric actin-labeled myocardium in a region presenting DAPI-marked nuclei in low magnification and in an enlarged field, respectively. Note exact position of each DAPI-stained nucleus in the overlay of the two color fluorescence and transmitted light images. Colocalization of DAPI-labeled nuclei with typical striated labeling pattern of sarcomeric actin could be recognized in some cells. F: round cells with large nucleus and scant cytosol also harbored both fluorescent markers. Scale bar, 20 μm.
REFERENCES