Intracardiac Transplantation of a Mixed Population of Bone Marrow Cells Improves Both Regional Systolic Contractility and Diastolic Relaxation

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Background: Pre-clinical and clinical studies suggest that transplantation of bone marrow–derived stem cells can improve global cardiac function. However, no quantitative assessment of regional systolic contraction and correlation with phenotype has been made. Therefore, we used our model of cryoinfarcted rabbit myocardium for intracardiac transplantation of a mixed population of bone marrow–derived cells and assessed both regional function and myogenic conversion of the cells.

Methods: Nineteen New Zealand white rabbits underwent cryoinjury of the left ventricle. Autologous bone marrow (BM) cells were expanded in vitro. After 2 weeks, either 1×10^8 mixed BM-derived progenitor cells (BM group, $n = 11$) or vehicle (control group, $n = 8$) were injected into the cryoinjured region. Regional systolic function was measured using micromanometry and sonomicrometry before and 4 weeks after cell injection; cell phenotype was evaluated histologically.

Results: All animals in the BM group significantly improved both systolic shortening (0.11 ± 0.7 vs -0.05 ± 0.05 mm in the control group, $p < 0.05$) and regional stroke work when compared with control (9.6 ± 2.4 vs -1.2 ± 1.2 mm · mm Hg, $p < 0.003$). In addition, the BM group had improved global diastolic function, as measured by minimum dP/dt and end-diastolic pressure. On histologic assessment, BM cells differentiated toward a myogenic phenotype.

Conclusions: Transplanting a mixed population of marrow-derived cells that can adopt a myogenic phenotype improves regional contractility and diastolic relaxation after myocardial infarction.

J Heart Lung Transplant 2005;24:205–14.

Cell therapy for heart failure has arrived at the forefront of cardiac research. Several pre-clinical[1–5] and clinical studies[6–9] have shown that bone marrow–derived stem cells can successfully engraft in injured myocardium and improve cardiac function, but the mechanism by which they do so remains obscure.

One such population of cells is comprised of bone marrow stroma—selected in vitro based on adherence to culture plastic—which contains mesenchymal stem cells (MSC).[10,11] Use of these progenitor cells for cardiac repair has increased recently since it was reported that they can: (1) differentiate into cardiomyocytes both in vitro[12,13] and in vivo[3,4]; (2) improve neoangiogenesis[14]; and, presumably as a result; (3) improve cardiac function in animal models. These cells are also popular because they are readily available, autologous, and can easily be grown at quantities sufficient for cardiac repair.

Although several groups have reported improved global cardiac function after bone marrow stem cell transplantation, it is not clear that this improvement can be attributed to a change in contractility within the infarct scar, especially given reports that these cells differentiate into fibroblasts or fat in the infarct.[15,16] To directly address this, we transplanted a heterogeneous population of bone marrow–derived cells into cryoinfarcted rabbit myocardium and directly measured regional contractility within the scar using sonomicrometry and micromanometry—a model previously validated in our lab.[17,18] Cryoinfarction results in a reproducible, total loss of cardiomyocytes in a well-delineated area and subsequent impaired regional function. Any improvement in regional stroke work and systolic shortening, as assessed by sonomicrometry and micromanometry, can then be attributed to the presence of the transplanted cells. By placing sonomicrometry crystals well within the defined borders of the

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Presented in part at the annual meeting of the International Society for Heart and Lung Transplantation, April 2003, Vienna, Austria.

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 1053-2498/05/$–see front matter. doi:10.1016/j.healun.2003.10.021

homogeneous scar, we can exclude functional improvement secondary to the induction of neoangiogenesis that leads to subsequent salvation of hibernating myocardium in the border zone.

We chose to characterize a mixed population of bone marrow stem cells for the presence of mesenchymal ($CD45^-$), myogenic ($desmin^+$) and endothelial ($CD31^+$/$Tie\text{-}2^+$) progenitors to determine whether this cell population has the potential to regenerate contractile tissue or vasculature. We then transplanted these cells into rabbits 2 weeks after cryoinfarction and measured regional contraction before and 4 weeks after cell injection. After euthanasia, hearts were histologically examined for the presence of transplanted contractile cells in the engrafted areas.

MATERIALS AND METHODS

All experiments were conducted in accordance with guidelines published in the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23, revised 1985) and under protocols approved by the institutional animal care and use committee at Duke University.

Surgery Preparation

New Zealand white rabbits ($n = 26$) were pre-medicated with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg). Endotracheal intubation was performed and the animals were mechanically ventilated. Anesthesia was maintained with 2% isoflurane.

A femoral bone marrow aspirate was performed aseptically from the left hindlimb. A left thoracotomy through the fourth intercostal space was performed, as previously described,[17,18] and the heart was exposed. A cryoprobe (diameter 1.2 cm) cooled to −70°C by continuously circulating nitric oxide (Frigitronics) was applied to the epicardial surface of the anterolateral left ventricular (LV) free wall for 3 minutes to create a transmural cryoinjury of approximately 1.5 cm in diameter. The chest was closed in layers. Fourteen days after cryoinjury, the animals underwent a second thoracotomy for initial data acquisition using sonomicrometry and micromanometry and for injection of 1×10^8 bone marrow cells (BM group, $n = 11$) or medium (control group, $n = 8$) in the infarct area. Injections were performed using a 1-ml syringe and 25-gauge needle. The bone marrow cells were injected in 3 evenly spaced, parallel columns within the short-axis plane of the easily visible infarct.

Four weeks after cell injection, a third thoracotomy was performed and the animals were re-examined using sonomicrometry and micromanometry. After recording the final data, the animals were euthanized and hearts were excised for histologic analysis.

Expansion of Bone Marrow–Derived Stem Cells

The bone marrow aspirates were plated in bone marrow growth medium, consisting of Dulbecco's Modified Eagle Medium (DMEM)/F12 (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), which was selected for rapid growth of MSCs, and 0.5% gentamicin (10 mg/ml, Gibco). Cultures were fed every third day with bone marrow growth medium and maintained at <50% confluence for 14 days, resulting in 3 or 4 passages before time of injection. Cells were labeled with 4′,6-diamidino-2-phenylindole (DAPI; Sigma) for 8 hours before injection. For injection, bone marrow cells were trypsinized (0.25%), washed twice in phosphate-buffered saline (PBS), counted, and re-suspended at 1×10^8 cells/ml of DMEM.

Characterization of Injected Cell Population

To determine the percentage of hematopoietic vs mesenchymal cells in our injectate, cells were analyzed for the presence of the hematopoietic marker CD45 by fluorescence-activated cell sorting (FACS) analysis. The number of endothelial progenitors was determined by analysis of CD31 (PECAM)[19] and the angiopoietin receptor, Tie-2.[20] FACS was performed as follows: cells were trypsinized; washed twice in PBS; blocked for 15 minutes with blocking buffer; stained with polyclonal antibodies to CD45 (Serotec); CD31 (Dako) or Tie-2 (kind gift from Dr. Chris Kontos, DUMC) for 30 minutes on ice; and then washed twice before secondary labeling with phycoerythrin (PE) anti-mouse secondary antibody (BD Pharmingen). After 2 final washes, cells were re-suspended in blocking buffer for analysis using a FACS Vantage SE flow cytometer (BD Biosciences). Blocking buffer consisted of Hank's balanced salt solution (HBSS) supplemented with 3% FBS, 2 mmol/liter ethylene-diamine tetraacetic acid (EDTA) and 10 mmol/liter HEPES buffer.

In vitro desmin staining of the bone marrow–derived population was performed as follows. A portion of the injected cell population was plated on glass coverslips, grown overnight and fixed in 5% paraformaldehyde for 10 minutes. Cells were permeabilized in 0.2% NP-40 and 0.2% Tween in PBS for 10 minutes. Blocking was performed for 1 hour in PBS with 5% HS and 5% FBS. Cells were then incubated in primary monoclonal anti-desmin antibody (1:200; Sigma) for 90 minutes, followed by a PE-conjugated anti-mouse secondary antibody (1:200; Sigma) in blocking buffer. All incubations were done at 37°C. Extensive washing in blocking buffer was performed between steps. Staining was analyzed on a Nikon TE 200 Eclipse inverted microscope and the number of desmin-positive cells was counted and expressed as a percentage of the total cell number.

Physiologic Data Acquisition

Micromanometry and sonomicrometry were performed 14 days after cryoinfarction (baseline) and 4 weeks after cell injection as follows. A pair of ultrasonic dimension transducers (Sonometrics Corp.) were sutured with 6-0 polypropylene to the epicardial surface, within 2 mm of the border of the cryoinjured region in the plane of the left ventricular (LV) short axis. A 3.0F micromanometer (Millar Instruments) was inserted into the LV cavity via the left atrium. Data were then collected using a sonomicrometry acquisition system (Sonometrics) over 10 seconds.

Hemodynamic data, collected prior to cell injection and after 4 weeks, included heart rate, LV end-diastolic pressure, LV developed pressure and dP/dt.

Data Analysis

Physiologic data were analyzed using CARDIOSOFT software (Sonometrics). The cardiac cycle was defined using the first derivative of LV pressure (dP/dt), as detailed previously.[12]

A regional systolic performance index analogous to stroke work (SW_A) was defined as the area within a plot of pressure vs dimension and was calculated as the integral of ventricular pressure (P) with respect to the epicardial segment length (L):

$$SW_A = \int P\,dL$$

LV end-diastolic and peak pressures were obtained from the LV pressure waveform. Maximal and minimal dP/dt were obtained from the first derivative of the LV pressure waveform. Systolic shortening (SS) was defined as the absolute difference in epicardial segment length between end-diastole (L_{ED}) and end-systole (L_{ES}):

$$SS = L_{ED} - L_{ES}$$

All cardiac cycles in a 10-second data acquisition period were analyzed and the beat-to-beat indices were averaged over that period. Ectopic beats and those cycles exhibiting significant catheter artifact or poor ultrasonic dimension signals were not analyzed.

Histologic Analysis

After excision, all hearts were placed immediately in 30% sucrose in PBS. Hearts were cut into 5 transverse slices, embedded in OCT freezing medium, and frozen in isopentane cooled by liquid nitrogen. Twelve serial 6-μm-thick sections were cut from each slice on a cryostat. Sections were air dried overnight, fixed in 5% paraformaldehyde, and permeabilized in 1% Tween. Sections from each treatment group were stained with primary rabbit specific monoclonal antibodies to desmin, slow myosin heavy chain (MHC), connexin-43 and sarcomeric actin (all from Sigma) and a monoclonal antibody against troponin I (Chemicon), all diluted 1:200 in blocking buffer, for 90 minutes. Secondary antibody staining was performed with a PE-conjugated anti-mouse antibody (Molecular Probes) for 1 hour. Sections 11 and 12 from the series were stained with hematoxylin–eosin and Goldner's trichrome, respectively. Sections were examined under standard light and fluorescence microscopy.

Statistical Analysis

All data are reported as a mean ± SEM. For normally distributed data, statistical analysis was performed using repeated-measures analysis of variance (ANOVA) for between-group comparisons. For non-parametric data, change in end-diastolic pressure (EDP), the Mann–Whitney U-test was performed. Results were considered statistically significant at $p < 0.05$.

RESULTS

During the study, 2 animals died at the time of cryoinjury and 4 animals died before cell or sham injection. One animal died within 24 hours of receiving BM cells, leaving a total of 11 BM-treated animals and 8 control animals to complete the study.

Cell Culture

Cultures yielded $>1 \times 10^8$ adherent bone marrow–derived cells for each animal after 2 weeks. By FACS analysis the injected cell population contained >80% CD45-negative mesenchymal cells. As indicated in Figure 1, the morphologic appearance of the injected cell population was fairly homogeneous; however, as indicated in Figure 2, there was a small population of desmin-positive cells that were more elongated than the majority of cells. This population comprised 1.1% (±0.4%) of the injected cell population. FACS analysis also showed that a small fraction of the cells expressed CD31 (1.05%) or Tie-2 (0.98%), suggesting differentiation toward an endothelial phenotype. Together, the data show that these injected cells comprised a heterogeneous population of progenitors with characteristics of myogenic and vascular precursors.

Regional Systolic Performance and Diastolic Relaxation

A stroke work analog using pressure/segment length measurements was obtained for each animal before injection and 4 weeks after injection. There were no statistical differences in stroke work between groups at baseline ($p = 0.08$). Regional stroke work increased after treatment in 11 of 11 (100%) BM-cell-treated animals, but in only 1 of 8 (12.5%) control animals ($p < 0.01$, BM vs control).

Quantitative analysis of both stroke work and systolic shortening showed a significant treatment effect in the cell-treated group compared with control. At baseline,

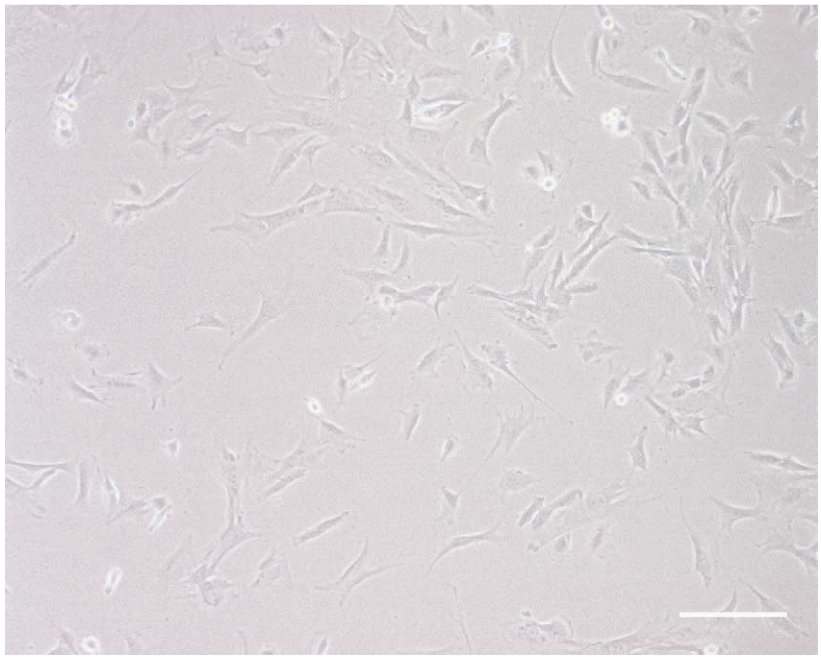

Figure 1. Light micrograph of rabbit bone marrow–derived cells after 1 week in culture (passage 3). Original magnification: 200×; bar = 50 μm.

mean systolic shortening (SS) was 0.15 ± 0.02 and 0.15 ± 0.05 mm in the BM and control groups, respectively. However, cell injection resulted in a significantly improved SS of 0.26 ± 0.06 mm after 4 weeks, whereas in the control group SS decreased to 0.10 ± 0.02 mm ($p < 0.05$ for the BM group vs control; Figure 3A). Analysis of change in stroke work (ΔSW) over time showed the same treatment effect (Figure 3B). ΔSW was 9.6 ± 2.4 mm · mm Hg in the BM group and −1.2 ± 1.2 mm·mm Hg in the control group ($p < 0.003$ for BM vs control).

Furthermore, bone marrow cell transplantation improved diastolic relaxation, as indicated by minimum dP/dt at 4 weeks after cell injection (dP/dt_{min} = −1,976 mm Hg/s vs −1,545 mm Hg/s in the control group, $p < 0.02$). Finally, LV end-diastolic pressure tended to decrease more in the BM group, −6.0 ± 1.6 mm Hg (8 of 11 animals showed decreased EDP), than in the control group, −2.9 ± 2.5 mm Hg (4 of 8 animals showed decreased EDP), although the difference was not statistically significant ($p = 0.17$).

Hemodynamics

There were no statistical differences in heart rate, developed pressures or maximum dP/dt between groups before or 4 weeks after injection.

Histologic Findings

Sections from the left ventricle of each study animal were examined to ensure that a transmural cryoinjury had occurred. Sections through the center of the scar from each cell-treated animal were examined using standard light microscopy and fluorescence microscopy to identify engrafted cells (DAPI-positive). The engrafted cells were widely dispersed throughout the scar, comprising up to 70% of the infarct area in most animals. Despite this dispersal, the engrafted areas were mostly surrounded by dense, collagen-rich scar tissue. However, some groups of cells at the periphery of the infarct appeared to migrate into healthy myocardium (Figure 4C,D).

At random locations throughout the scar, small pockets of bone marrow–derived cells formed muscle-like fibrils, although most cells remained mononuclear. As depicted in Figure 5—small clusters of bone marrow–derived cells throughout the infarct—both fibrils and mononuclear cells, also stained positively for desmin (a myogenic cell marker), the slow isoform of MHC and α-sarcomeric actin, indicating conversion to a contractile phenotype.

None of the animals examined showed positive staining for connexin-43 or cardiac troponin I in the grafted areas. Furthermore, the control animals showed no desmin expression in the scar area, indicating no cardiomyocyte survival 6 weeks after the cryoinjury, which could have confounded the functional results.

Throughout the scar, there was evidence of incorporation of injected cells in the existing vasculature, varying in size from small capillaries to larger arterioles (Figure 4E,F). There was no particular trend in the location of these vessels, as they occurred in both the center and periphery of the infarct.

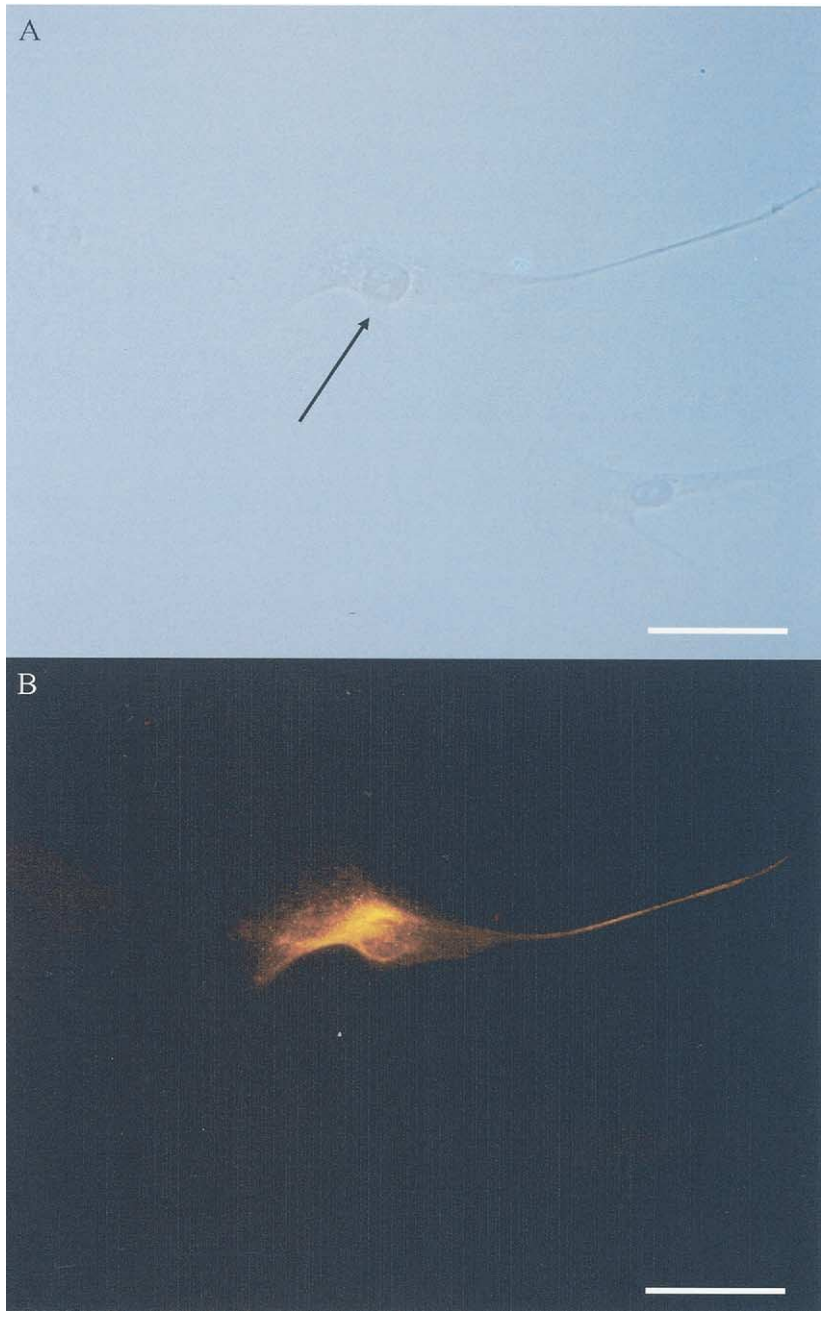

Figure 2. Light micrograph and fluorescent micrograph of desmin-stained bone marrow–derived cell (arrow). The other 2 cells in this field are not stained. Original magnification: 400×; bar = 20 μm.

DISCUSSION

This study demonstrates that a population of mesenchymal-enriched, bone marrow–derived progenitor cells can generate myogenic and vascular cells in the infarct and improve regional systolic function and global diastolic relaxation. The results are supported by histologic data showing engraftment of cells throughout the infarct in all cell-treated animals and differentiation of DAPI-positive cells into cells expressing contractile proteins. This is the first study using parameters of regional function to show that bone marrow cells can actually convert a non-contractile scar into a region capable of systolic contraction and diastolic relaxation secondary to differentiation of BM cells toward a myogenic, contractile phenotype.

Injected Cell Population

We used FACS analysis and in vitro immunocytochemistry to determine the number of mesenchymal

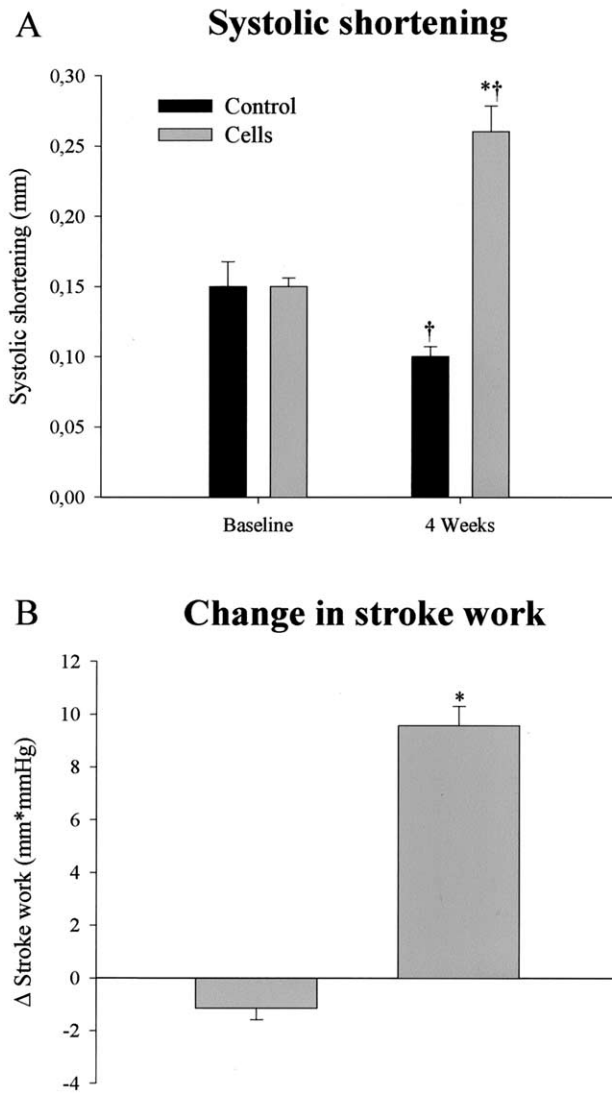

Figure 3. (A) Systolic shortening at baseline and after 4 weeks for control and cell-treated animals. $*p < 0.05$ vs control after 4 weeks; $^{\dagger}p < 0.05$ vs baseline. In (B), the change in stroke work from baseline to 4 weeks is shown, demonstrating significant improvement after cell treatment. $*p < 0.05$ vs control.

and hematopoietic cells, endothelial precursors and myogenic cells in our culture before injection. The injected cells contained a high percentage (>80%) of CD45-negative cells, indicating that the cultured cells were mainly of mesenchymal origin. FACS analysis of CD31 and Tie-2 showed a low percentage of both markers (around 1%), indicating a low but distinct population of endothelial progenitors in the injectate. Furthermore, desmin immunocytochemistry indicated a few myogenic cells in the bone marrow cell population before injection. Desmin is a cytoskeletal protein found in all contractile cells, including skeletal muscle cells, cardiomyocytes and smooth muscle cells.

Mesenchymal stem cells or marrow stromal cells have the ability to differentiate down multiple lineages, into adipocytes, chondrocytes, osteoblasts[21] and cardiomyocytes.[3] Also, it has been shown that a distinct subpopulation of progenitor cells with multilineage capacity and unlimited self-renewal capacity, called multipotent adult progenitor cells (MAPCs), co-purify with the mesenchymal cell fraction.[22]

After injection and engraftment, the transplanted cells clearly underwent what has been described previously as a milieu-dependent differentiation toward a myogenic phenotype, as shown by the expression of contractile proteins. In addition, engrafted cells were incorporated into new vessels. It is very likely that the differentiated myogenic cells arose from the mesenchymal progenitor cell fraction of our population in that the ability of bone marrow mesenchymal cells to differentiate into muscle and to take part in neovascularization has been shown previously.[23] It is also possible, however, that in vivo proliferation and further differentiation of the endothelial and myogenic progenitors, present at low percentages in vitro, account for the histologic observations.

The contribution of the CD45-positive fraction, which contains hematopoietic cells, to engraftment and myogenic conversion is not clear. It is possible that most of these cells are macrophages because they also strongly adhere to culture plastic. However, it cannot be ruled out that hematopoietic progenitor cells were present as well and contributed significantly to the vasculogenesis and myogenesis. However, unlike data reported previously,[3] we did not observe cardiocyte differentiation or regeneration.

Our histologic findings were partly based on identification of the engrafted cells by nuclear DAPI staining. The feasibility of this method for identification of bone marrow stromal cells after transplantation has been shown by other investigators.[24] Although leakage of the dye to neighboring cardiomyocytes or vascular smooth muscle cells could have skewed our interpretation of the histologic findings, the defined nuclear staining and co-localization of desmin, α-sarcomeric actin, and the slow isoform of MHC, but not connexin-43 or cardiac troponin I, makes this unlikely.

Comparison With Previous Studies

Several investigators have isolated purified sub-populations of bone marrow–derived progenitor cells or mononuclear cells and showed functional improvement and regeneration of vasculature or viable myogenic cells.[5,7,8] These studies, however, each have distinct limitations. For example, Toma et al injected human MSCs intravenously into mice and showed intracardiac differentiation into cells expressing contractile proteins, but they did not examine cardiac function.[4] In other studies, investigators

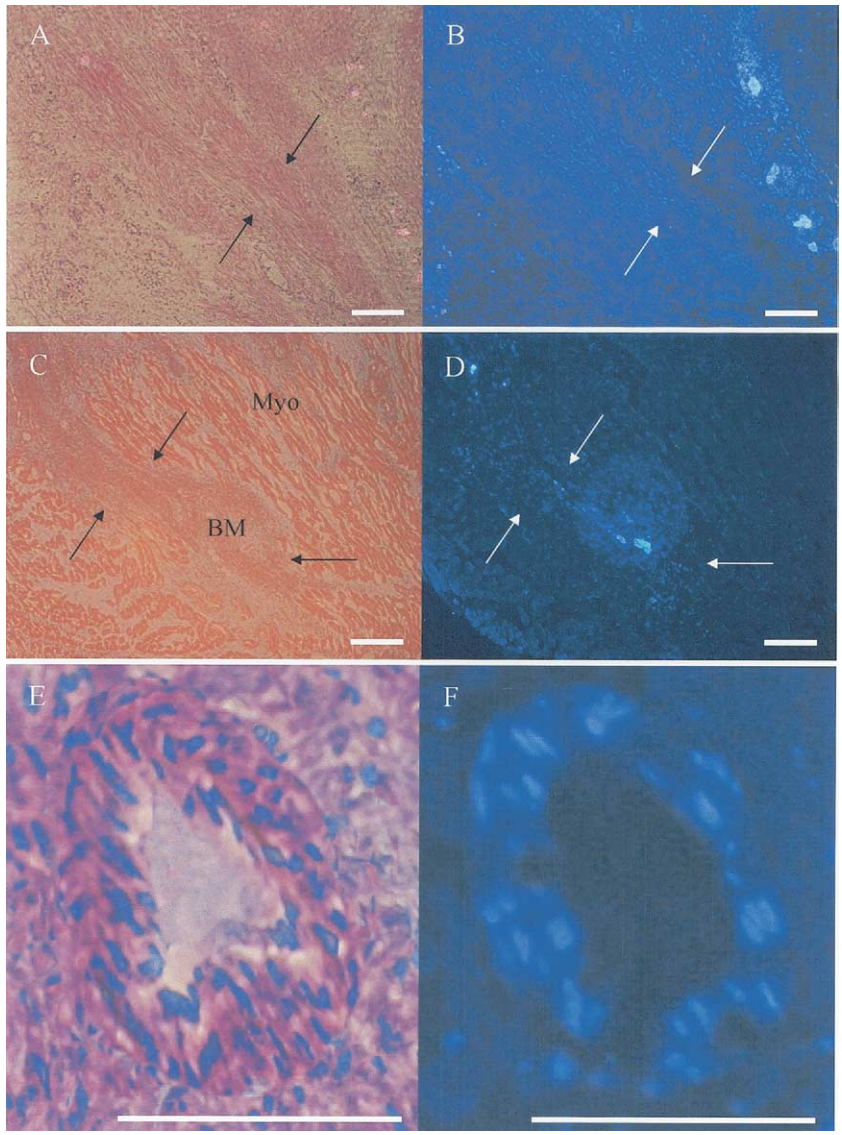

Figure 4. Panels (A) and (C) show a normal light micrograph with hematoxylin–eosin staining of the border zone of the cryoinfarct. Corresponding fluorescence micrographs show nuclear DAPI stain in (B) and (D). Bone marrow cells (BM) migrated into the healthy myocardium (Myo). The engrafted cells are shown by the black and white arrows. In addition to forming contractile tissue, bone marrow–derived cells may contribute to new vessel formation, as shown in (E) and (F). Original magnification: 100×; bar = 100 μm.

showed improved cardiac function after intracoronary injection of monocytes in humans, but were unable to histologically evaluate the myocardia to ascertain cell engraftment, phenotype, or differentiation.[7,8] In studies in which investigators injected marrow-derived cells and examined both phenotype and function, a single population of progenitors was delivered and limited phenotype differentiation was observed. For example, when human $CD34^{+}/117^{+}$ angioblasts (with sub-sets expressing AC133 and Tie-2) were delivered intravenously into mouse myocardium, function improved, but only increased vasculogenesis was observed.[2] Although these studies have shown that several sub-populations of bone marrow–derived progenitors with a specific phenotype may have the potential to repair damaged vasculature or myocardium and improve cardiac function, they give little insight into the regional impact of multipotent progenitors that have the potential to differentiate down multiple lineages in vivo. This is particularly important in light of the findings of Wang et al,[15,16] who showed that mesenchymal stem cells can differentiate not only into muscle, but also into fibroblasts and fat in the infarcted myocardium. Unfortunately, in their study, regional contractile function was not examined.[15,16]

Previously, bone marrow–derived cells have also shown an ability to differentiate into cardiomyocytes both in vivo and in vitro when specific inducers, such as 5-azacytidine,[24] were used or when specific sub-popula-

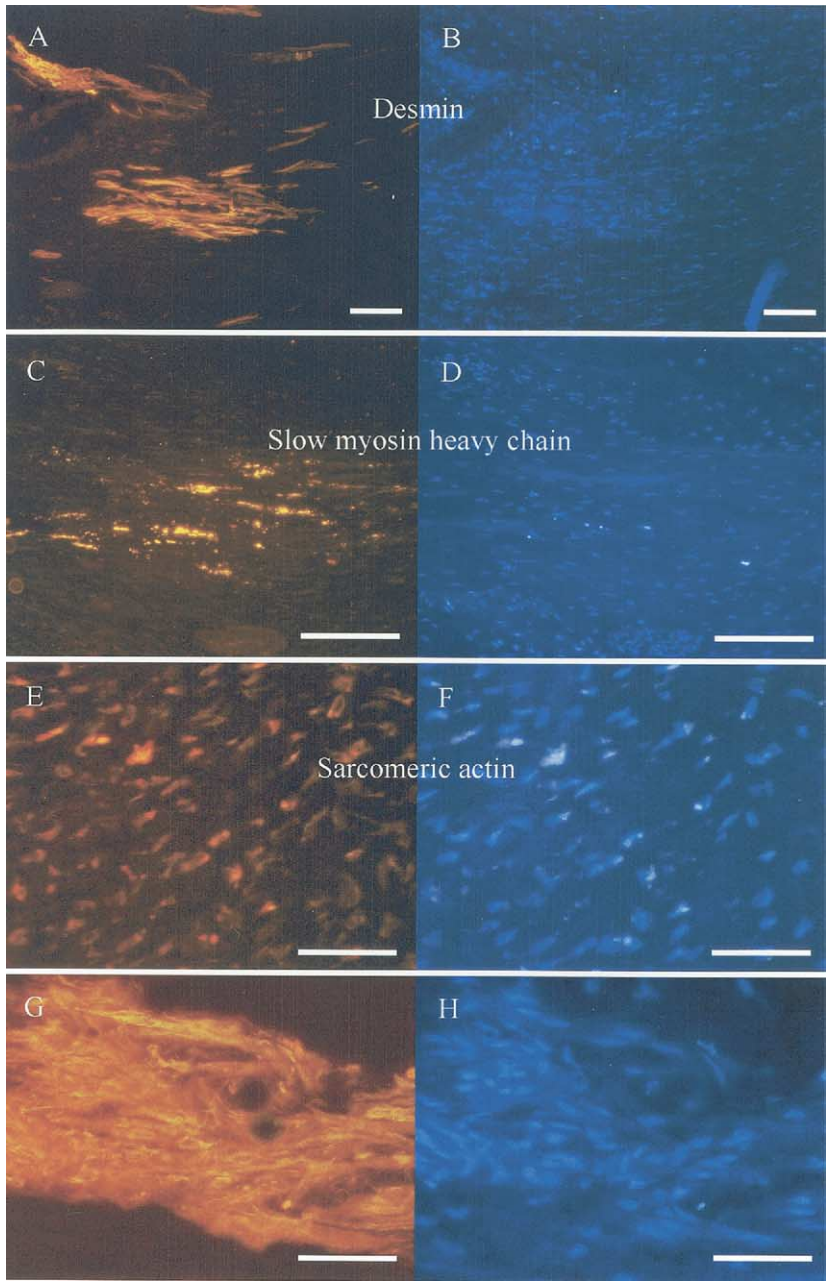

Figure 5. Sections of scar engrafted with bone marrow–derived cells showing patches of positive staining for desmin [(A) and (B) show corresponding fields], slow myosin heavy chain [(G) and (H) show corresponding fields] and α-sarcomeric actin [(E) and (F) and (G) and (H) show corresponding fields] throughout the scar area. Panels (B), (D), (F) and (H) show positive cells. Original magnification: 100$\times$ for (A)–(D) and 200$\times$ for (E)–(H); bar = 100 μm.

tions were selected.[3] Although the engrafted cells in the current study expressed muscle-specific contractile proteins, no differentiation toward a cardiomyocyte phenotype was observed. Pre-differentiation with 5-azacytidine before injection may be required to observe this effect.

Regional and Global Heart Function

In this study we found an improvement in regional contractile function after treatment of injured rabbit myocardium with a heterogeneous population of BM-derived progenitor cells. However, no significant improvement in global systolic function was observed, as indicated by maximum dP/dt and LV-developed pressure. One possible explanation for this lack of global effect is that the infarct area was too small to directly translate regional improvement of contractility into global functional improvement. A second possibility is that our initial decrement in ejection fraction (EF) was

too small to be improved. This is supported by previous findings showing EF improves only after transplantation of contractile cells, such as myoblasts, when baseline values are markedly decreased.[25] Although cryoinfarction caused only a small decrease in baseline heart function after 2 weeks, functional measures do appear to worsen over time, as evidenced by a decrease in stroke work at 4 weeks in the control group. Thus, it is possible that injection at later times could improve global function. It is also possible that no increase in global function was seen because the dose of cells was too low. Although 1×10^8 myogenic cells are sufficient to improve function in our model,[26] a higher dose of non-myogenic cells, such as BM-derived progenitors, may be required. This could reflect the fact that not all cells injected differentiate into a contractile phenotype—in fact, some may become fibroblasts and work toward reversing any contractile function seen.

Electromechanical Integration

Synchronous contraction of bone marrow cells and cardiomyocytes has been observed in vitro during co-culture experiments.[27] However, this phenomenon was only observed when both cell types were in close contact with each other, suggesting the involvement of gap junctions for electrical activation. In this in vivo study, no expression of the gap junction marker, connexin-43, was found in the engrafted areas, which indicates that the engrafted cells lack electromechanical integration with the host myocardium. This was further confirmed by our observation that most cells are surrounded by scar tissue. This does not preclude contractility, however, because other mechanisms of cell activation are possible. For example, although intracardiac-transplanted skeletal myoblasts do not express connexin-43 in vivo, they clearly can contract.[18,28] The proposed mechanism of electromechanical activation of these cells is via their stretch receptors, which record stretch of the surrounding myocardium.[28] Such a mechanism may also be responsible for the activation of differentiated bone marrow cells in this study, especially as they adopt a myogenic phenotype.

Future Studies

It is not clear to what extent the data in this study relate to the latest clinical trials of human intracardiac cell transplantation, where differences in timing of injection and cell injection procedure are evident. Clinically, bone marrow mononuclear cells have been delivered within hours to a few days post-infarction, but stromal cells (as used here) are being proposed for use at several weeks to months post-injury, as is the case in this study. Furthermore, although we chose the center of the infarct as the site of injection, in the clinic, cells are generally injected either at the border zone of the infarct,[6,7] where the scar is less thin, or into the coronary circulation.[8,9] Future studies critically evaluating the timing, dose and location of cell delivery are needed to support and to begin to explain clinical outcomes.

Another matter not addressed in this study is the extent to which bone marrow–derived cells can affect cardiac remodeling and global contractile function. In future studies, echocardiography or MRI should be employed for assessment of remodeling and global contractility.

When clinicians approach the topic of cell choice, it is important that all characteristics of the selected population are considered. Although BM-derived cells represent an exciting new addition to the field of cell therapy, their heterogeneity and capability to become multiple cell types must be carefully considered. Although it may be advantageous to have cells that can differentiate down myogenic and vascular lineages, having cells that can become fibroblasts or adipocytes in the infarct could be deleterious. Learning how to control the outcome of these cells represents the next challenge in this field of study.

We must also consider the role of aging on the treatment of heart disease with bone marrow–derived stem cells. It has been shown that the number and plasticity of bone marrow–derived stem cells decreases with age.[29] In older patients it is possible that there will be insufficient numbers of bone marrow stem cells with the potential to adopt a contractile or vascular state and repair an infarcted myocardium.

In addition, when faced with the task of trying to fully re-populate an infarct, one must consider using multiple cell types together to regenerate a myocardium with vasculature and electrical conduction, as well as functioning muscle. This will likely require either a population of multipotent cells, a combined myogenic and angiogenic approach,[30] or the use of combined cell therapy.[31]

As we continue our efforts to successfully engineer functional myocardium from scar, we will conquer new challenges that will make cell transplantation an even more effective therapy. Bone marrow–derived cells represent a very exciting addition to the pool of potential cell types for use in cell transplantation. As we gain more knowledge of their differentiation in vivo and the varying phenotypes they may be able to generate, new avenues for cardiac and vascular repair may arise. However, until we are able to predict or control their differentiation in vivo, a complete understanding of BM-derived progenitors remains a challenge. It will be important for investigators to compare multiple cell types, alone or in combination, for their safe and effective use in cardiac repair.

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