

Mesenchymal Stem Cell Implantation in a Swine Myocardial Infarct Model: Engraftment and Functional Effects

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Background. A novel therapeutic option for the treatment of acute myocardial infarction involves the use of mesenchymal stem cells (MSCs). The purpose of this study was to investigate whether implantation of autologous MSCs results in sustained engraftment, myogenic differentiation, and improved cardiac function in a swine myocardial infarct model.

Methods. MSCs were isolated and expanded from bone marrow aspirates of 14 domestic swine. A 60-minute left anterior descending artery occlusion was used to produce anterior wall infarction. Piezoelectric crystals were placed within the ischemic region for measurement of regional wall thickness and contractile function. Two weeks later animals autologous, Di-I-labeled MSCs (6×10^7) were implanted into the infarct by direct injection. Hemodynamic and functional measurements were obtained weekly until the time of sacrifice. Immunohistochemistry was used to assess MSC engraftment and myogenic differentiation.

Results. Microscopic analysis showed robust engraftment of MSCs in all treated animals. Expression of muscle-specific proteins was seen as early as 2 weeks and could be identified in all animals at sacrifice. The degree of contractile dysfunction was significantly attenuated at 4 weeks in animals implanted with MSCs ($5.4\% \pm 2.2\%$ versus $-3.37\% \pm 2.7\%$ in control). In addition, the extent of wall thinning after myocardial infarction was markedly reduced in treated animals.

Conclusions. Mesenchymal stem cells are capable of engraftment in host myocardium, demonstrate expression of muscle specific proteins, and may attenuate contractile dysfunction and pathologic thinning in this model of left ventricular wall infarction. MSC cardiomyoplasty may have significant clinical potential in attenuating the pathology associated with myocardial infarction.

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Unfortunately for persons who suffer large myocardial infarctions the adult heart lacks regenerative capabilities. In these patients congestive heart failure develops when a critical threshold of myocardium dies and compensatory mechanisms become overwhelmed. A novel therapeutic option to prevent the progression toward heart failure involves the introduction of healthy cells into the infarct in an effort to repopulate the region, commonly referred to as cellular cardiomyoplasty. This new population of cells may potentially prevent the typical wall thinning, scar formation, and decline in systolic function seen in these patients.

One particularly promising cell transplantation alternative involves the use of mesenchymal stem cells (MSC). These cells possess pluripotential capabilities [1] and have been shown to undergo myogenic differentia-

tion in small animal preparations [2–4]. The purpose of this study was to investigate whether autologous bone marrow derived stem cells are capable of engraftment into host myocardium and differentiation into myogenic lineages, and could contribute to improved regional wall function in a large animal myocardial infarct model.

Material and Methods

Marrow Harvest

Fourteen female swine (25 to 35 kg) were sedated with ketamine (1,000 mg intramuscular [IM]) and brought into the laboratory. Intravenous access was established through an ear vein and the animals were anesthetized with thio-pental (10 mL of 5% intravenous [IV]). They were then placed in a ventral recumbency position with the hind legs tucked under the body. The iliac crest area was prepared and draped using sterile technique and approximately

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14 mL of bone marrow was aspirated into a syringe containing 6,000 units of heparin. All animals received buprenorphine (0.3 mg IM) as an analgesic before returning to the animal facility. The marrow aspirates were then sent to the Osiris Pre-Clinical/Animal Tissue Core Facility for MSC isolation and expansion.

MSC Isolation, Expansion, and Labeling

Mesenchymal stem cell isolation, and confirmation of a uniform population following colony expansion, was performed at Osiris as previously described [1]. Briefly, the bone marrow aspirates were passed through a density gradient to eliminate unwanted cell types. When plated, a small number of cells developed in visible symmetric colonies by days 5 to 7. Hematopoietic cells, fibroblasts, and other nonadherent cells were washed away during medium changes. The remaining purified mesenchymal stem cell population was further expanded in culture until approximately 60 million MSCs were present. On the day of implantation, the MSCs were labeled with a cross-linkable membrane dye, CM-DiI (Molecular Probes, Inc, Eugene, OR) following the manufacturer's protocol. Prior to injection the cells were thoroughly washed and held on ice at an approximate concentration of 20 million cells/mL.

Myocardial Infarction and Instrumentation

Swine were sedated with ketamine (1,000 mg IM), induced with sodium pentobarbital (30 mg/kg IV), endotracheally intubated, and maintained on isoflurane (0.8% to 1.5%) through a Narkomed ventilator. Electrocardiographic monitoring and rectal temperatures were continuously displayed. Animals were then prepared and draped in routine sterile fashion. Benzathine penicillin (1.2 μ IM) and gentamycin (80 mg IV) were given as preoperative antimicrobial therapy. A midline sternotomy was performed and the heart suspended in a pericardial cradle. A tygon catheter (0.06 in inner diameter) was placed in the apex of the left ventricle, sutured in place, and used for left ventricular (LV) pressure and blood gas monitoring. The left anterior descending (LAD) coronary artery was dissected free just distal to the first diagonal branch and isolated with a vessel loop. A brief occlusion of the coronary artery was performed to identify the region to be rendered ischemic. Four piezoelectric crystals were then placed in the area destined for infarction to measure regional wall motion (segment shortening and wall thickening). At completion of the surgical instrumentation, a 15-minute stabilization period was allowed prior to baseline recordings of hemodynamics and regional cardiac function. Lidocaine (2 mg/kg IV bolus, then 0.5 mg/min IV) was started and continued until the time of reperfusion. A 60-minute occlusion of the LAD was used to produce myocardial infarction. At the end of 60 minutes and after baseline recordings, the snare was released and reperfusion was visually confirmed. At this time the piezoelectric crystal leads and the LV catheter were brought together below the xyphoid process, tunneled posteriorly, and externalized near the scapula. The pericardium was approximated as well as possible and any remaining epicardial surface was cov-

ered with a polytetrafluoroethylene (PTFE) sheet (Preclude Pericardial Membrane; Goretex, Flagstaff, AZ). The chest was closed and a single mediastinal tube (18F) was placed to reestablish a negative intrapleural pressure and evacuate any remaining blood or irrigation solution. The inhaled anesthetic was then turned off, the animal extubated when appropriate, and allowed to recover. The chest tube was removed when there was no visible air leak or blood accumulation. Animals received postoperative antimicrobial therapy (amoxicillin 500 mg orally twice daily for 3 days) and buprenorphine (0.3 mg IM twice daily for 3 days) for postoperative pain.

Sonomicrometry

Four piezoelectric crystals (Sonometrics Corp, London, Ontario, Canada) were placed in the infarct region: epicardium, endocardium, and two midmyocardium. The crystals were placed in a spatial orientation in order to measure myocardial wall thickness and segment length throughout the cardiac cycle (see Fig 1). Barbed piezoelectric crystals were used to prevent migration during the remodeling phase. Also all crystals were further fixed in place with sutures as the leads exited the epicardium. Confirmation of proper crystal arrangement was possible through online analysis during placement. Two dimensional analysis of epicardial and endocardial crystals (LV thickness) expressed on an X-Y axis allowed immediate calculation of LV wall thickness and crystal excursion. Transducer wires were externalized as previously described and connected to a Series 5001 Digital Sonomicrometer (Sonometrics Corp, London, Ontario, Canada) for data acquisition. Ventilation was suspended during sonomicrometry measurements if cyclical variations were noted. Off-line analysis was performed on a desktop computer using software from Sonometrics. End-systole was defined as 20 milliseconds prior to the peak dp/dt, while end-diastole was taken at the upward deflection of the dp/dt trace.

MSC Implantation

Two weeks after the production of a myocardial infarction the animals were returned to the operating room for a second procedure. The animals were anesthetized and monitored as usual and the chest reentered with great caution to prevent disruption of the instrumentation. Prior to implantation, the MSCs were Di-I labeled, washed two times, and resuspended in 3 mL of vehicle with 2000 units of heparin. Once the area of infarction was clearly visualized six injections containing either autologous MSCs in 0.5 mL (total 6.0×10^7 cells in 3 mL; $n = 7$), or a comparable volume of vehicle ($n = 5$), was given using a 30-gauge needle. The injections were given in a manner such that the regions between the piezoelectric crystals received all of the cells. This technique was used to optimize the likelihood of demonstrating regional functional changes after MSC cardiomyoplasty. After the MSC/vehicle injection, the chest was closed and the animals recovered as previously described. Every week for the first month and biweekly thereafter the ani-

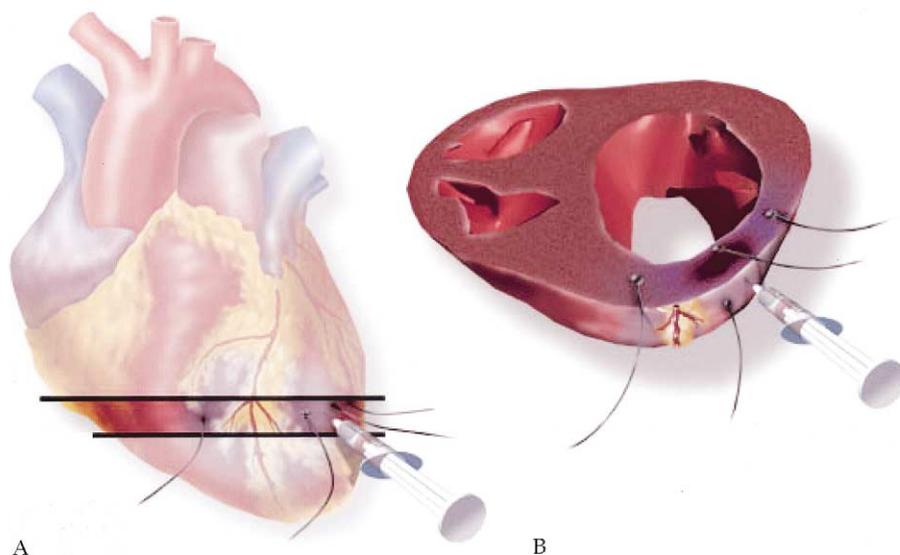


Fig 1. Location of the left ventricle infarct (A) and spatial orientation of the piezoelectric crystals (B). An endocardial and epicardial pair of crystals measured the wall thickness and active wall thickening during the cardiac cycle. Two mid-myocardial crystals measured the segment shortening. Mesenchymal stem cells were directly injected in the localized region between the piezoelectric crystals.

imals were sedated and brought to the laboratory for serial hemodynamic measurements and sonomicrometry.

Histology and Microscopy

At the end of the experimental protocol the animals were anesthetized with ketamine and euthanized with saturated solution of potassium chloride and the heart excised with the instrumentation intact. The appropriate location and alignment of the piezoelectric crystals was verified in the explanted heart. If the endocardial and epicardial crystals were found to be misaligned (not perpendicular to the LV circumference) dimensional data from that animal were not included in the final data analysis. Subsequently, any adhesions fixed to the heart's epicardial surface were sharply dissected free and the atria and great vessels were removed at their origin. The left ventricle was then sliced into 6 or 7 rings parallel to the minor axis of the left ventricle. All rings were then weighed for subsequent determination of infarct size. Both surfaces of the ventricular slices were photographed while immersed in water to eliminate highlights and digital images were obtained. The percentage of each slice that was infarcted was determined using the Scion Image Analysis program (Beta version 4.0.2). First the infarcted area of the ventricular ring was calculated and reported in pixels. Then the regional area of the entire left ventricular ring was determined. Based on these measurements (in pixels), the percent infarction for the ring could be calculated. These percentages were multiplied by the weight of the rings and summed, converting the percentage values to grams, and enabled a reconstruction of infarct size of the left ventricle. The tissue was then prepared for immunohistochemistry and confocal microscopy by freezing in O.C.T. and serial sectioning at 5 μm . Antibodies examined included α -actinin (Sigma, St. Louis, MO), troponin-T (Sigma), tropomyosin (Sigma), myosin heavy chain-MHC (DSHB), and phospholamban (Affinity Bioreagents, Golden, CO). The confocal microscope (PCM 2000; Nikon, Melville, NY) was equipped with two excitation lasers of different wavelengths to simultaneously identify two separate molecular markers.

Statistical Analysis

All data in the text are presented as mean \pm SEM. Between-group comparison of piezoelectric crystal (end diastolic wall thickness and systolic wall thickening) and hemodynamic measurements (LV pressures, heart rate, dp/dt) were made using a repeated measures analysis of variance (ANOVA). Infarct size was analyzed using an unpaired Student's *t* test. Differences were deemed significant when *p* values < 0.05 were obtained.

Animal Care

All work was preapproved by the Johns Hopkins School of Medicine Animal Care and Use Committee and performed according to the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985).

Results

Of the 14 animals entered into the protocol, 12 were used in the final data analysis. The initial two animals were excluded from analysis after discovery of life-threatening infections involving the externalized instrumentation. The infections were discovered prior to randomization into either the MSC or control groups. After the addition of short-term postoperative antibiotics no further animals suffered serious infection. Two additional animals (both MSC) while included in the histology and microscopy evaluation were omitted from regional functional analysis owing to malfunctioning piezoelectric crystals or waveforms incompatible with proper placement.

Hemodynamics

There was no significant difference in any of the monitored hemodynamic variables (heart rate, left ventricular pressure, and dp/dt) noted between groups. It should be noted that this model resulted in severe cardiac injury with reductions in contractile function noted in both groups after infarction. Of these 12 animals presented,

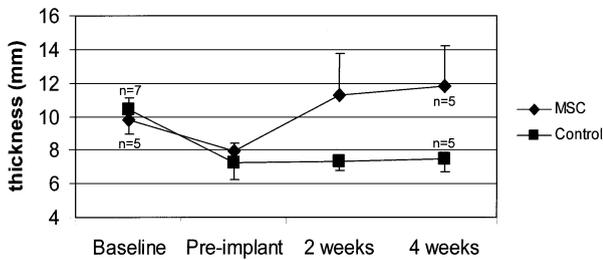


Fig 2. End diastolic wall thickness as measured by sonomicrometry. Note the extent of left ventricular wall thinning in the control group after production of the left ventricular infarct. Mesenchymal stem cell (MSC) implantation resulted in an attenuation of wall thinning when compared with control animals at all time points. Data are group averages and presented as mean \pm SEM.

clinical signs of congestive heart failure (exercise intolerance and pulmonary edema) developed in 8 by the time they were euthanized. This symptomatic reduction in cardiac function was characteristically observed between 6 and 8 weeks and the incidence was not significantly different between groups.

Sonomicrometry

In the 2-week period after myocardial infarction, wall thickness and systolic function were markedly reduced in all animals. Prior to implantation of either MSCs or saline vehicle wall thickness had been reduced approximately 25% (from 9.9 ± 0.8 mm to 7.9 ± 1.6 mm) and systolic wall thickening had been replaced by paradoxical wall thinning. The MSC and vehicle injections (3 mL) were within the region bordered by the piezoelectric crystals. This fact is demonstrated by the acute change in end diastolic wall thickness (EDWT) measured immediately after injection (7.9 ± 1.6 mm preinjection versus 9.4 ± 2.1 mm postinjection, $p < 0.05$). This increase in EDWT persisted in the MSC group and by 2 weeks measured 11.3 ± 2.4 mm. In contrast, the wall thickness in control animals returned to preinjection values by 2 weeks and the region of infarction continued to thin throughout the duration of the study. There was a sustained improvement in EDWT in the MSC-treated animals when compared with control at all time points postinjection (see Fig 2). This augmentation of wall thickness neared statistical significance at 4 weeks ($F = 4.36$, $p = 0.07$).

In addition to preventing pathological wall thinning in the region of treatment, MSC implantation also appeared to locally augment systolic function. More precisely MSC implantation attenuated the degree of contractile dysfunction observed after infarction. These data are summarized in Figure 3. Percent systolic wall thickening was defined as $EDWT - ESWT / EDWT \times 100$, where EDWT = end diastolic wall thickness and ESWT = end systolic wall thickness. Both control and treated animals displayed profound reductions in regional function immediately after infarction ($15.7\% \pm 6.7\%$ at baseline versus $-0.65\% \pm 3.1\%$ preimplant). The decline in function progressed over time in the vehicle-treated animals ($-7.4\% \pm 3.8\%$ at 6 weeks). Systolic wall thickening was often replaced by paradoxical wall thinning such that diastolic wall thickness values were larger than systolic values. In contrast, MSC-treated animals exhibited a

significant augmentation in systolic wall thickening at 4 weeks ($5.4\% \pm 2.2\%$) when compared with control animals (F value = 7.72, p value = 0.04). These data do not demonstrate a global restoration of contractile function to preinfarct levels but rather a significant attenuation in the degree of dysfunction after infarction in the localized region of MSC implantation.

Histology and Microscopy

The hearts of all animals had large anterior/septal infarctions and showed signs of enlargement at sacrifice. The size of the infarctions was not significantly different between groups ($12.45\% \pm 2.07\%$ of LV in control versus $14.69\% \pm 1.65\%$ in MSC). Cross-sections revealed transmural infarctions in all animals and compensatory hypertrophy events in several. Upon gross examination control hearts had marked LV wall thinning in the region of vehicle injection consistent with the sonomicrometry results. In contrast the animals receiving MSC treatment had apparently normal gross LV geometry and maintenance of wall thickness at the site of MSC implantation. Figure 4 illustrates these findings in photographs of LV sections from the site of sonomicrometry crystal placement and MSC injection. Histologic examination of MSC-treated myocardium revealed collagen-rich scar tissue with an open, lattice work appearance as opposed to the dense, tightly pack extracellular matrix found in the thinned walls of control animals.

There was successful engraftment of MSCs in all treated animals as shown by the large number of Di-I-labeled cells seen on microscopy. Labeled MSCs could be identified at the site of implantation for as long as 6 months. Implanted MSCs were not found in regions of myocardium remote from the injection site or systemically. Cells appear to preferentially engraft in regions of necrotic tissue and adhere to the collagen rich matrix. Clusters of cells forming a continuum could occasionally be found in proximity to viable myocardium (Fig 5A and B). In addition to the impressive number of cells found in the infarct region colocalization of immunofluorescence indicates that a significant portion of the surviving cells had begun to express several muscle specific proteins (α -actinin, tropomyosin, toponin-T, myosin heavy chain,

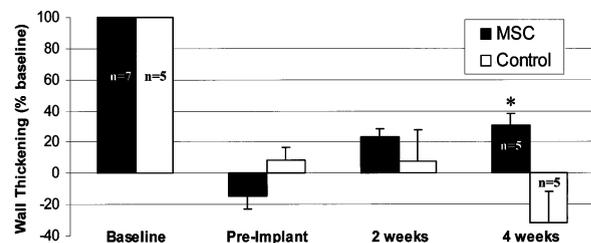


Fig 3. Systolic wall thickening as measured via sonomicrometry and expressed as a percent of baseline values. Myocardial infarction resulted in a marked reduction in contractile function in both control and treated animals. While paradoxical wall thinning was observed in control animals (negative thickening values), mesenchymal stem cell (MSC) implantation significantly reduced the degree of systolic dysfunction. Differences between the two groups were evident by 2 weeks and became statistically significant by 4 weeks. Data are group averages and presented as mean \pm SEM. * $p < 0.05$ versus control.

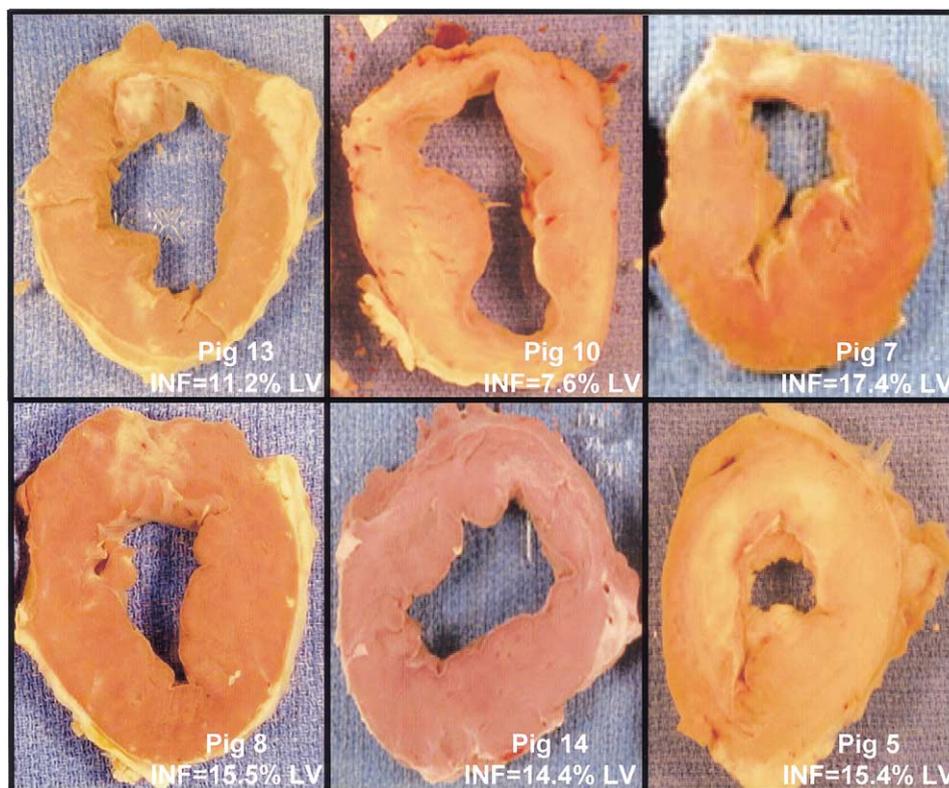


Fig 4. Gross left ventricular (LV) cross-sections at the level of mesenchymal stem cell (MSC) injection and piezoelectric crystal placement. Large transmural infarctions were observed in all animals. Control animals (top row) had wall thinning and loss of normal ventricle chamber geometry associated with remodeled infarctions. MSC-treated animals (bottom row) had preserved wall thickness and LV geometry. (INF = infarction.)

and phospholamban) not present in MSCs prior to implantation (Fig 5C-F). Muscle-specific protein expression was observed as early as 2 weeks postimplantation and persisted as all subsequent time points examined (up to 6 months). No ectopic tissues were observed and no evidence for MSC differentiation to adipose, bone, cartilage, tendon, or any tissue other than muscle was seen in any animal. Similarly, no significant inflammatory infiltrates were identified at the site of MSC implantation.

Comment

The primary findings of the present study were (1) mesenchymal stem cells engraft into host myocardium when implanted by direct injection; (2) the MSCs expressed muscle-specific proteins after implantation into areas of injured myocardium; (3) local milieu dependent factors as opposed to exogenous substances influence differentiation toward a myogenic lineage; and (4) implanted cells had a beneficial impact on cardiac remodeling when implanted 2 weeks postinfarction.

Mesenchymal stem cells are pluripotent cells capable of differentiation into many cell types [1]. Furthermore, muscle-specific differentiation has been seen [3]. What has made MSCs particularly appealing to investigators in cellular cardiomyoplasty is that these cells are easily obtained from bone marrow, can be expanded in culture, and can be cryopreserved for future use.

In this study we observed significant engraftment of

MSCs in host myocardium yet quantification of cell number remained technically difficult. In an effort to offer the MSCs an optimal survival advantage they were introduced at 2 weeks postinfarction to avoid the peak of the postinfarct inflammatory phase.

By 2 weeks postimplantation there was expression of muscle-specific proteins identifiable by immunofluorescence. The lack of true "cardiac-specific" markers for immunohistochemistry has limited our ability to conclusively state that MSCs implanted in the heart differentiate to a cardiac phenotype (as opposed to skeletal muscle). The only muscle marker that gives some degree of insight into this question is phospholamban. The expression of phospholamban indicates that if these MSCs are differentiating to either a cardiac or a slow-twitch skeletal phenotype. Theoretically either may be beneficial in the heart due to their nonfatiguable nature. It should also be noted that no nonmuscle tissue types (ie, bone, cartilage, adipose, parenchyma) were observed in any animal, consistent with the notion of milieu-specific differentiation.

Perhaps the most significant observation in this study is the maintenance of wall thickness seen in the MSC-treated group. This conclusion is supported by both the piezoelectric crystal data and the gross morphology of the ventricle at the time of sacrifice. This MSC-induced maintenance of wall thickness could be due either to the addition of tissue or to an inhibition of remodeling process observed after infarction. Potentially MSCs could alter collagenase activity or other enzymatic pathways

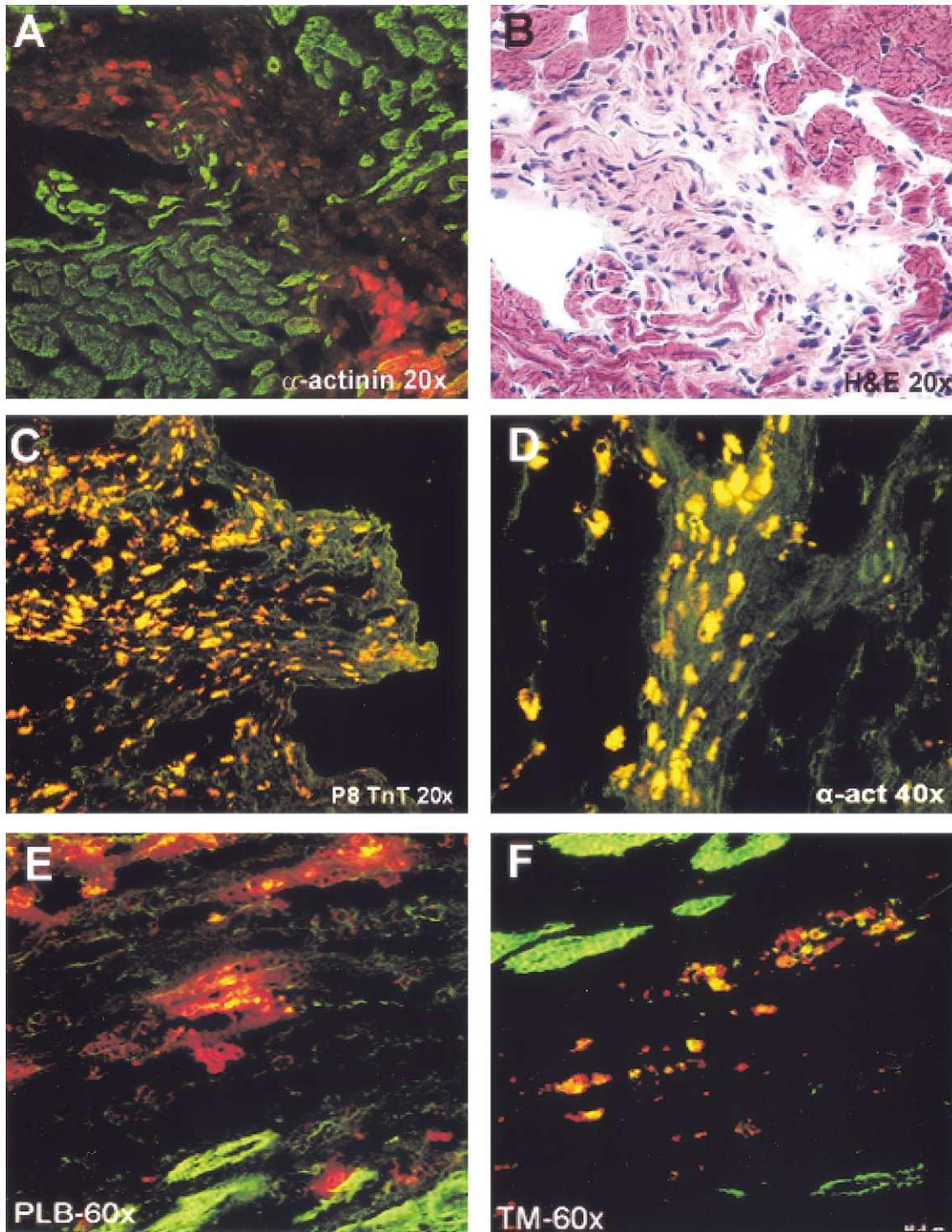


Fig 5. Mesenchymal stem cell (MSC) engraftment and muscle-specific protein-expression using immunohistochemistry. The Di-I labeled mesenchymal stem cells (red) and muscle protein-specific antibodies (green) fluoresce under confocal microscopy. Colocalization (yellow) confirms cells having both probes present. Specifically this represents specific muscle protein expression within the MSCs being imaged. (B) This hematoxylin and eosin (H&E)-stained serial section corresponds to (A) illustrating a cluster of MSC in proximity to host myocardium. Several muscle-specific proteins were identified in treated myocardium. Examples include α -actinin (α -act) (A and D), troponin T (TnT) (C), phospholamban (PLB) (E), and tropomyosin (TM) (F) expression at 4 weeks postimplantation.

responsible for the pathologic wall thinning observed in remodeled myocardium. Although the precise mechanism by which MSC implantation limits the extent of myocardial thinning after infarction has yet to be deter-

mined, it appears that extracellular matrix alterations are likely involved.

The implantation of MSCs had a beneficial effect on systolic wall thickening despite the absence organized

contractile elements ($5.4\% \pm 2.2\%$ in treated animals at 4 weeks versus $-3.4\% \pm 2.7\%$ in control). It is apparent that the mechanism of improvement does not lie in systolic contraction of implanted MSCs but rather in the attenuation of paradoxical systolic wall thinning. Regional contractile function is severely impaired in both groups after infarction. In other words the degree of dysfunction appears exaggerated in the control group when compared with MSC treatment. Taken together the data suggest that the implantation of MSCs may result in a more compliant, less stiff region of ventricle with improve diastolic filling properties. Improvements in diastolic dysfunction have also been reported in other models of cellular cardiomyoplasty [5].

Currently one of the more popular cell types used by cardiomyoplasty investigators has been immature skeletal myoblasts, commonly referred to as "satellite cells." Murry and coworkers [6] have shown conversion of satellite grafts to fatigue-resistant, slow-twitch fibers better suited for cardiac workloads. Taylor and associates [5] have shown improved myocardial performance in a rabbit model where myoblasts were incorporated into cryoinfarcts. Furthermore, Chiu and associates [7] have demonstrated "milieu-influenced" differentiation of satellite cells into cardiac-like muscle cells. Lastly, xenogeneic and allogeneic myoblast cell transplantation has been successful in a large animal model. Additionally these transplanted cells were thought to initiate proliferation of surrounding microvasculature by a presumed angiogenic factor [8].

Cellular cardiomyoplasty has also been done using cells other than skeletal myoblasts. Li and colleagues [9] have shown improved cardiac function in a model of smooth muscle cell transplantation. Despite the socially and politically controversial use of fetal tissue for research several groups have investigated the use of fetal cardiomyocytes as a method to alleviate heart failure [10-12]. Others have used bone marrow derived cells to repair or replace damaged muscle fibers [2, 4]. These cells were exposed to an appropriate microenvironment in order to direct the cells toward a myogenic lineage. Preliminary evidence suggests that exogenous agents such as 5-azacytidine can induce cultured bone marrow cells to differentiate into cardiac-like muscle cells prior to injection into myocardial infarcts [3, 13].

A major limitation of the current model was the fact that all animals progressed to failure by 6 to 8 weeks. As MSCs were only injected cells in a relatively small area within infarct, the overall morbidity associated with such an injury was not altered. This pilot study was done to demonstrate MSC engraftment and differentiation and to examine what effect MSC implantation may have on regional function following infarction. Because of the premature progression toward failure, adequate time may not have been allowed for the cells to synthesize and assemble a functional contractile apparatuses. Future work will determine if injection of a larger number of cells throughout the entire region infarct improves global function and prolongs survival. Such a model may allow time for more complete myogenic differentiation of implanted MSCs.

In conclusion, mesenchymal stem cell implantation may attenuate contractile dysfunction and pathologic remodeling of the ventricular wall after infarction. MSCs show sustained engraftment within infarcted myocardium and exhibit myogenic differentiation as evidenced by expression of muscle-specific proteins. Further study is needed to fully characterize the engrafted MSCs, their integration with host myocardium, optimal cell number and delivery technique, and the degree to which they contribute to global changes in cardiac function.

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DISCUSSION

DR CONSTANTINE MAVROUDIS (Chicago, IL): That was a very nice study. I have a couple of questions. Did you note or measure the orientation of the transplanted cells and whether they aligned with the intercalated disks and whether in fact they took their place alongside the other myocytes? And was the thickening that you demonstrated due to the fact that you injected something into the area, or in fact do you think it was due to the potential cells or the differentiating cells that you placed there? I didn't catch it well—Your groups were one group that you injected yourselves and another group that you injected something other than cells, or you did not?

DR SHAKE: The vehicle was injected.

DR MAVROUDIS: The vehicle. So then in many respects you have answered one part of my question. But I wonder if you had injected some other cells that might have done something to cause the thickness and maybe not have caused the potential for contractility that you're seeking. This is a very nice study. This has a potential for revolutionizing this field and I hope you continue and find all the other answers. Congratulations.

DR SHAKE: Thank you. As far as your first question about orientation, we did not see the MSCs orienting themselves in the center of the infarct. Because, remember, we inject them in the middle of the infarct where the crystals are placed. We did see some stray cells that made their way to the edge of the infarct, very close to the host myocardium. Those cells were orienting themselves more appropriately and appeared to be larger and elongated. Obviously there is some communication between the host myocardium and these cells. In our next study, hopefully we'll be able to answer the question as far as what would be the orientation of the cells adjacent to host myocardium.

And second of all, we were pleased when we saw increased wall thickness and thickening at the site of the piezoelectric crystals. We do not know why it is thickened there. It could be twofold. We do see a large number of cells present. We also see a lot of colocalization demonstrating MSCs with contractile proteins. But we also believe there is, perhaps, a paracrine-like effect ongoing, where there is less remodeling that is occurring in the heart. Perhaps there is actually prevention of the collagenases or other similar kind of enzymes that are breaking down that area. These MSCs may be communicating with the cells secreting these enzymes.

DR GREGOR ZUND (Zurich, Switzerland): Congratulations on your paper. What do you think about the pretreatment of such cells before implantation? And a second question: do you think you would get such cells as well from the blood?

DR SHAKE: Well, we have seen that we do not need to pretreat. As I mentioned, other investigators are certainly trying to send skeletal myoblasts or MSCs down lineages. We have seen that if just injected into the right environment, the MSCs differentiate into what they should be in that environment. Our collaborators at Osiris are using human MSCs for replacement of collagen in osteoarthritic knees, and we have also seen appropriate differentiation when injected into hearts. So I am not sure if we need to send them down the lineage.

DR ZUND: The second question is you got the cells from the bone marrow. Do you think you can get these cells as well from the blood?

DR SHAKE: Potentially. I know Dr. Meyer's group has been doing some work with that. We have very good success with bone marrow aspiration. It works very well. Perhaps the most interesting thing that we have found is that we can use allogeneic bone marrow derived stem cells. They seem to be totally immunoprivileged and we are currently doing a study where we are injecting cells from one animal into another with no immunosuppression and we are seeing no rejection.

DR CHARLES R. BRIDGES (Philadelphia, PA): I was wondering if you looked at remodeling in terms of measuring other parameters, in terms of, for example, ventricular dilatation. Your results with respect to systolic thickening are fascinating and outstanding. But in terms of overall ventricular remodeling, I know that with just the crystals you put there, you really could only assess local changes. But did you look globally? Did you echo any of these animals, for example, to see if you saw changes there?

DR SHAKE: Basically all of those questions are hopefully going to be able to be answered in our next group of animals which are ongoing. We have nearly finished the series. We have injected the entire infarct with allogeneic stem cells, and we are echoing all animals.

And as far as remodeling, this experiment was really a pilot study to determine the mode of delivery as well as the volume and the number of cells to deliver. So we were not trying to look at any of the specific questions that you are asking. But certainly those are great questions and great things to pursue in our follow-up studies.

DR JOHN E. MAYER, JR (Boston, MA): If I might ask a question. I am interested in your speculations on what you think are the local factors, be they either physical or biochemical, cytokine, whatever, signals that you think are inducing these relatively uncommitted cells down a myogenic lineage.

DR SHAKE: That is a great question. We would like to know what the physical or chemical signal is that tells the MSCs to pass down a given lineage.

DR MAYER: I was hoping you could tell me.

DR SHAKE: We do not know what that is, but we think that there is a soluble factor. At Osiris they have seen that there is some evidence for a soluble factor that actually sends MSCs down a lineage. What it is, we do not know, and it is not purified at this point.

DR MAYER: The second question would be whether or not you have tried to deliver any physical signals to these cells in vitro, in culture, and observed what effects that might have?

DR SHAKE: We have not but it would be interesting.