Published in final edited form as:

Biochem Biophys Res Commun. 2009 December 18; 390(3): 902–907. doi:10.1016/j.bbrc.2009.10.074.

Direct Injection of Autologous Mesenchymal Stromal Cells Improves Myocardial Function

Yifu Zhou, MD, Suna Wang, MD, Zuxi Yu, MD, PhD, Robert F. Hoyt Jr., DVM, Vandana Sachdev, MD, Pamela Vincent, BS, Andrew E. Arai, MD, Minjung Kwak, PhD, Sandra Sczerba Burkett, BS, and Keith A. Horvath, MD

Cardiothoracic Surgery Research Program, NHLBI, National Institutes of Health

Abstract

Cell-based therapies have been employed with conflicting results. Whether direct injection of ex-vivo expanded autologous marrow stromal cells (MSCs) would improve the function of ischemic myocardium and enhance angiogenesis is not well defined. In a porcine model of chronic ischemia, MSCs were isolated and cultured for 4 weeks. Sixteen animals were random divided into two groups to receive either direct intramyocardial injection of autologous MSCs, or equal volumes and injections sites of saline. Cine MRI and epicardial echocardiography were performed just prior to the injections and again 6 weeks later at the time of sacrifice at which point tissue was also analyzed. Myocardial function as assessed by regional wall thickening (as measured by dobutamine stress echocardiograms) demonstrated a 40.9% improvement after cell treatment of the ischemic zone ($p = 0.016$) whereas the saline treated animals only had a 3.7% change ($p = 0.82$) compared to baseline. The left ventricular ejection fractions of MSC group showed 19.5% improvement from baseline $35.9 \pm 3.8\%$ to $42.9 \pm 5.8\%$ ($p = 0.049$). Increased vascularity was found in the MSC group compared to controls (0.80 ± 0.30 vs 0.50 ± 0.19 capillary/myocyte ratio, $p = 0.018$). Direct injection of autologous MSCs promotes angiogenesis and enhances the functional improvements following chronic myocardial ischemia. This suggests that the angiogenesis engendered by cell treatment may be physiologically meaningful by improving the contractility of ischemic myocardium.

Keywords

Mesenchymal stromal Cells; Myocardial Ischemia; Myocardial Function

Cell-based therapy with either embryonic or adult stem cells has been broadly used in the animal models of myocardial ischemia or infarction to regenerate damaged myocytes. This practice is challenging the classical concept that adult myocytes do not regenerate after birth. Many investigators now believe that in adults, bone marrow-derived stem cells or endothelial progenitor cells can be recruited to and incorporated into tissues undergoing neovascularization (1–4), and a limited portion of cardiomyocytes may be regenerated by locally sited or recruited circulating stem cells (5–9). Therefore, stem cells which include hematopoietic stem cells, endothelial progenitor cells, mesenchymal stem cells/stromal stem cells, myoblasts, and undifferentiated side population cells have been used as an alternative therapeutic strategy for

Address correspondence to: Yifu Zhou, MD, Cellular Biology Section, CSRP/NHLBI, National Institutes of Health, 10 Center Drive, MSC 1550, Building 10, Room 2N246, Bethesda, MD 20892-1550, zhouyifu@mail.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ischemic cardiovascular diseases that cannot be treated by routine interventional approaches (10–12). Theoretically, embryonic stem cells have more potential to differentiate into cardiomyocytes. However clinical trials are now only limited to adult stem cells due to ethical issues with embryonic stem cells and the fact that adult stem cells are easier to handle and autologous transplantation can be performed in clinical patients. Several centers in the world, have reported improved functional status in experimental animals and clinical patients after such therapy (8–17). However it is still unclear how bone marrow-derived stem/progenitor cells are mobilized and recruited into ischemic tissues. In addition, it is unknown if the injected cells in clinical trial patients actually differentiate into functional cardiomyocytes and what particular cell type is better to use. In this animal study, we chose to use adult bone marrow derived mesenchymal stromal cells (MSC) due to the following considerations: a) Limitations in embryonic stem cells study; b) relative ease of handling; c) the translational ability that autologous transplantation can be performed in clinical patients; d) potential differentiation of these MSCs into myocytes. Our intent was to determine whether these considerations were true and would lead to improved myocardial function after MSC treatment.

Material and Methods

Animals

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, and the investigation conformed to the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 1996, Washington, D.C.). Twenty-two Yorkshire domestic pigs, initially weighing 15–20 kg, were selected for this study. All animals were housed one per cage and allowed free access to water and commercial pig food.

Study design

Sixteen animals underwent a small left thoracotomy under general anesthesia and placement of an ameroid constrictor around the proximal circumflex artery to create a model of chronic myocardial ischemia (18). At this first operation bone marrow (15 ml) was harvested for ex-vivo stem cell expansion. Four weeks later, animals were randomly divided into two groups: Treatment Group (to receive cell injections) or Control Group (saline injections). A second left thoracotomy was performed on each animal, the circumflex territory (ischemic zone) exposed and injected with either ex-vivo expanded MSC or saline. Prior to treatment, all animals underwent pretreatment analysis which included viability assessment by contrast enhanced MRI and functional assessments with rest/dobutamine stress echocardiography and cine MRI analysis to determine baseline myocardial function. Six weeks following cell or saline injection, each animal had follow up MRI and echo evaluation prior to euthanasia. The hearts were then harvested and sectioned. The capillary density and cell differentiation were tested by immunohistochemistry staining with anti-vW factor antibody, smooth muscle actin and desmin. Six additional ischemic animals were created as described and treated with cell injections. Two of these animals were sacrificed at one, two or four weeks after treatment to study the fate of the injected cells.

Bone marrow-derived Cells preparation and culture

Using aseptic technique, the bone marrow was aspirated from either the iliac crest or tibia into a syringe containing preservative free heparin. Peripheral RBCs were separated by gradient centrifugation using lymphocyte separation liquid. The middle layered cells were collected and divided into two populations: one population was cultured in DMEM with 10% FBS and 1X pen/strep in a density of $10^6/\text{cm}^2$ at 37°C with 5% CO_2 in T-75 culture flasks without coating. Three days later, the non-attached floating portion of the mononuclear cells were collected and centrifuged and re-suspended in EGM2 medium. The attached colonies were continued in

culture with DMED with 10% FBS. When the attached cells reached confluence, they were split and expanded for 2–4 passages. The second population of cells were cultured in growth factor enriched culture medium (EGM2) with 5% FBS. At three days, the non-attached floating portion of these cells was treated the same way as described above.

Flow cytometry analysis of cell surface markers

Cells were analyzed for surface markers of CD34, CD31, CD90, CD117(C-kit), CD54, CD45, CD144, CD44 and CXCR4 by BD FACScalibur.

Multi-potency of differentiation assessment

To test the multi-potency differentiation abilities, passage 4 cells were cultured with special adipogenic and osteogenic medium kits by Stem Cell Technology, Inc. (Vancouver, Canada); according to manufacturer's methods.

Karyotype analysis

To ensure that the ex-vivo expanded MSCs do not spontaneously transform over the 4 passages of cell culture, karyotype analysis was performed on randomly selected samples.

Treatment of ischemic zone with bone marrow-derived stem cells

The autologous MSCs (1.2×10^8) in 2.5 ml of saline were then injected intramyocardially with a 25 gauge needle (with an injection depth of 5 mm) around the ischemic zone (25 injection sites, 100 μ l in each site). The same volume of saline was injected for the control group in the same number of sites.

Histological and immunohistochemistry analysis

After euthanization, ischemic (LCX territory) or non-ischemic (LAD territory) myocardium was cut into 5×5 mm-thick pieces, either collected in cassettes and fixed with 10% buffered formalin for paraffin embedding, or in O.C.T. for frozen sections with no fixation. Paraffin embedded sections were stained with H&E and Masson trichrome for morphological analysis. The immunohistochemical or immunofluorescent staining was performed using anti-vWF antibody to detect vascular endothelial cells and anti-smooth muscle actin antibody to detect vascular smooth muscle cells, anti-collagen IV antibody to detect connective tissues surrounding the myocytes and capillaries to facilitate capillary density reading, and anti-desmin antibody to detect myocytes. All above mentioned antibodies were purchased from Dako North America (Carpinteria, CA). The incubations of primary antibodies were followed by detections of FITC conjugated anti-mouse IgG or Rhodamine conjugated anti-rabbit IgG and the nuclei were labeled with DAPI. Anti-CD90 antibody was used to detect injected MSCs, anti-CD68 and CD163 antibodies were used to detect inflammatory cells.

Statistical analysis

For exploratory analysis, data is presented as mean $\pm$ standard deviation (SD) along with box plots. To analyze the changes of stress echo and MRI from baseline to 6 week follow-up in each group, paired *t*-test and Wilcoxon signed rank test were used. To compare the difference between saline and cell treated group, two sample *t*-test and Wilcoxon rank sum tests were performed. Independent two-sided *t*-test and Wilcoxon rank sum tests were used for capillary density analysis. Multivariate analysis of variance was also conducted to study the effect of time (baseline and 6 week) and treatment (saline and cell). All analysis was conducted using R (ver.2.6.0).

Results

Cells

Bone marrow-derived cells showed heterogeneous morphologies at passage 0 (round shaped, irregular shaped and spindle shaped cells) forming different cell colonies after culture with DMEM with 10% FBS. The growth speed of the cultured bone marrow cells was much slower at passage 0 to passage 2, but dramatically increased starting from passage 3, and demonstrated uniformed fibroblast-like morphology (Supplemental figure 1 a, b). The cells cultured with growth factor enriched EGM2 medium grew faster and were smaller in size than the cells in DMEM, but demonstrated similar spindle shape morphology. After four weeks ex-vivo expansion, the total number of cells in both culture conditions reached 1.2×10^8 . Adipogenesis testing revealed that three days after culturing in the adipogenic stimulating supplements, small colonies form bubble cells and gradually increased (about 35% of the total cells) over a two week period. These cells then stained strongly positive with oil-red-o (Supplemental figure 1 c). Osteogenesis testing demonstrated that two weeks after culturing in osteogenic stimulating supplements, some irregular lumps appeared sparsely on top with confluent cells and slightly increased over a 4 week period. The irregular lumps stained strongly positive for alizarin red (Supplemental figure 1 d). These results indicate that cells in our cultural conditions maintained the multi-potency and differentiation ability of mesoderm-derived cells.

Karyotype analysis showed normal karyotyping of 38, XY and 38, XX, and with normal heteromorphisms in chromosome 8. No Robertsonian translocation or other transformation was found (Supplemental figure 2).

FACS analysis at passage 4 revealed cells strongly positive for cell surface markers CD44 and CD 90, but CXCR4, CD34, C-kit, CD144, CD54, CD45, CD31, were all negative (Supplemental figure 3). These results indicate that bone marrow-derived stem cells cultured in the aforementioned two conditions are mesenchymal stem cells (MSC), instead of endothelial progenitor cells.

Functional improvements of left ventricular myocardium

Animals that survived until the scheduled euthanasia were 7 from the control group and 8 from the cell treated group. Ventricular contractility was measured by dobutamine stress echocardiography. A wall thickness increase at the end of systole was noted in both groups. The ischemic zone of saline-injected animals (N=7) showed an insignificant 3.7% improvement from baseline to the six week follow-up (from 40.6 ± 14.7 to 42.1 ± 9.3 , $p = 0.82$ (t -test/0.94 (Wilcoxon)), while the MSC-injected animals (N=8) showed a 40.9% increase from baseline to the six week follow-up (from 26.9 ± 9.8 to 37.9 ± 17.2 , $p = 0.016/0.035$) (Figure 1A). The wall thicknesses measured by resting echo at end diastole (ED) or end systole (ES) showed no significant change from baseline to follow up in saline injected animals. However, they showed significant increases in MSC-injected animals (ED: from 0.80 ± 0.11 to 0.89 ± 0.10 mm, $p = 0.04/0.06$; ES: from 0.91 ± 0.20 to 1.05 ± 0.21 mm, $p = 0.045/0.034$). No statistically significant differences were found between the saline and cell-injected groups for both resting and dobutamine stress echo tests. Additionally, no significant differences were found in non-ischemic areas between two groups.

Cine MRI analysis measured left ventricular ejection fraction. For the saline-injected group (N=6), baseline left ventricular ejection fraction improved by 12% at follow up (from $38.8 \pm 5.7\%$ to $43.5 \pm 5.5\%$). However this improvement was not significant ($p = 0.24/0.28$). In contrast, the MSC-injected group (N=7) showed a 19.5% improvement from baseline to follow-up ($35.9 \pm 3.8\%$ to $42.9 \pm 5.8\%$, $p = 0.049/0.051$) (Figure 1B). The left ventricle end systolic (LVESV) and end diastolic volumes (LVEDV) at the six-week follow-up in both groups

showed increases compared with their baselines (Saline group: from 101.0 ± 16.1 to 140.3 ± 7.7 for LVEDV, $p=0.007/0.031$; from 61.5 ± 9.3 to 80.9 ± 11.2 for LVESV, $p=0.006/0.059$. MSC group: from 92.1 ± 13.0 to 141.7 ± 5.3 for LVEDV, $p=0.000/0.016$; from 58.8 ± 8.8 to 80.4 ± 10.0 for LVESV, $p=0.001/0.016$). However, no significant differences were found between the two groups.

The stress echo and MRI data suggests that MSC treatment improved the left ventricular myocardial function both globally as measured by ejection fraction, and regionally, as noted by wall motion in the treated area. Multivariate F-test yielded that we have significant time effect for MRI data ($p < 0.001$).

Histologic Analysis

Analysis of angiogenesis—The myocardial capillary density, expressed as the capillary/myocyte ratio, was determined by positive staining of vWF of frozen sections from the ischemic zone of left ventricle in both groups harvested at the six week follow up. The vWF positive cells were counted and averaged from 5 randomized high power fields ($\times 380$) of 3 sections from each sampled areas. It was significantly greater in the cell treated group (0.50 ± 0.19 vs. 0.80 ± 0.30 , $p = 0.018/0.022$) (Figure 2).

The fate and differentiation of the injected cells: at one week after MSC injection, clusters of injected cells, small and round shaped, stained strongly with hematoxylin close to the epicardium were seen. Higher magnification revealed many immature capillaries inside the cell cluster (Figure 3, left panel a, b). Desmin and smooth muscle actin stained negative in this area, suggesting that they are neither myocytes nor smooth muscle cells (Figure 3, left panel c, d). The similar cell clusters were also found in animals sacrificed at two weeks. Within the cluster area, positive vWF staining was also found (Figure 3, right panel a, b). To confirm that these cells are the MSC injected, we performed immunofluorescent staining in the correspondent area from a frozen section slide and found that these cells were strongly positive for CD90 and the cells outside the channel were stained negative for CD90 (Figure 3 right panel c). The negative staining for CD163 and CD68 excluded the possibility that cells inside the channel to be the inflammatory cells. Moreover, the similar cell clusters were neither found in the non-ischemic zone of cell-treated animals, nor in saline-injected animals. The unique phenotype associated with the clusters of smooth muscle actin positive cells, strongly suggests they were newly formed vessels derived from the injected MSCs. At six week, clusters of injected cells were also observed. Additional newly formed small vessels were found in the cell injected animals, which were stained positive for smooth muscle actin and vWF. However, they again stained negatively for desmin suggesting that the ex-vivo expanded bone marrow-derived MSC survived and differentiated into vascular smooth muscle cells or endothelial cells, but not myocytes (Figure 4).

Discussion

Most clinical trials of autologous bone marrow-derived cell therapy in cardiovascular disease have employed the route of intracoronary injection using a balloon/catheter device and/or using G-CSF to stimulate the mobilization of bone marrow cells into the circulation (2–6,13–17). These methods have several limitations. Large portions of the intracoronary injected cells flow into the blood stream, and only a small number of the cells actually migrate into the ischemic myocardium. The majority of non-expanded mononuclear cells from the bone marrow are hematopoietic lineage cells. The intracoronary injection is only applicable for non-ex vivo expanded cells (or after short expansion times of 4 days or less), since the cell size of expanded cells are much bigger than non-expanded cells ($10\text{--}15$ vs. $5 \mu\text{m}$ in diameter. This will dramatically limit their benefits. The direct injection of expanded MSC used in this study facilitates the ability to determine both the beneficial effects and the fate of the injected cells.

The optimal way to perform cell therapy is to allogeneically transplant cells to recipients, or use a developed cell line for all recipients, since theoretically such stem cell lines would lack MHC1 and would not incite rejection. However, there is no solid evidence to prove that is always the case. The clinical trials of cell-based therapy in cardiovascular diseases are still limited to autologous use of the patients' own bone marrow cells (2–17). Similarly in order to avoid possible rejection related issues, we chose to use autologous cells in this study. In addition, we carefully tested the karyotype of the cells before injection to ensure the ex-vivo expansion did not result in any transformations or translocations of the chromosomes, which has been reported to happen in mouse and human bone marrow-derived cells following ex-vivo expansion (19–21).

In preparing the cells used in this study, we chose to use two methods used by other investigators for MSC and endothelial progenitor cells (22,23). We found that cells cultured by either method share the same characteristics of morphology and cell surface markers. We also found that they are able to differentiate into adipocytes or osteocytes, except that the growth rate is faster in EGM2 than in DMEM. The cells cultured in both conditions are classified as MSC according to the published guidelines for the nomenclature of the stem cells (24). Furthermore in the time course study, we injected cells from both culture conditions separately and saw no difference in their differentiation.

After cell injection, we found that the cells survived in the myocardial environment, and differentiated into vascular cells, smooth muscle or endothelial cells. We certainly cannot exclude the possibility that those new regenerated cells may be the result of the paracrine effect of the injected cells causing recruitment from other sources. However, the histological findings in our study strongly suggest that these grouped vessels arose directly from the injected cells, because a paracrine effect would likely lead to a more diffuse distribution of cells. In our study, we did not see any evidence that the injected MSC differentiated into functional or morphologically analogous cardiac myocytes.

The functional tests we used for this study are echocardiography for wall motion and MRI for ejection fraction. Both analysis showed statistically significant improvements in cell treated animals at six weeks follow up compared with baseline. No such functional improvement was seen in the saline injected control animals. While the average baseline wall thickness in control animals was higher than in the cell injected animals this difference was not significant ($p = 0.06$). Additionally the baseline difference cannot explain the difference noted at follow up due to the fact that all animals were randomly divided before treatment and data was recorded and analyzed by operators blinded to the treatment that the animals received. Finally, the statistical analysis also showed no significant difference in baseline wall thickness between the two groups both in untreated normal areas as well.

Shake et al (25) reported functional improvement results in a porcine model of acute myocardial infarction using mesenchymal stem cells. Their functional analysis showed preservation of function, albeit without significant improvement, after an infarct was treated with MSC. Our work attempted to mimic the clinical scenario and evaluated the effect MSC has on chronically ischemic myocardium using clinically employed methods of assessment; dobutamine stress echocardiography and cine MRI. Similar to the previous report (27) we also found that mesenchymal stem cells engraft into host myocardium when implanted by direct injection and MSC expressed muscle-specific proteins. Our data further confirmed that in various time points after injection, the cells survived and produced evidence of angiogenesis. The most interesting findings of our study are that we tracked several injection channels which contained CD90 positive and CD68 and 163 negative cells and the clusters of newly formed immature vessels lined with endothelial cells and irregular smooth muscle cells. We also demonstrated that there is no myocardial regeneration incited by MSC transplantation.

Conclusion

In a large animal model of chronic ischemia, autologous injection of MSCs is safe and efficacious. No observable side effects or abnormal cell proliferation were found in our study. Echo and MRI analysis documented functional improvement and capillary density test suggesting more angiogenesis following cell treatment. The formation of new vessels were also detected in ischemic myocardium after cell injection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to express our appreciation to our colleagues in the *Laboratory of Animal Medicine and Surgery* for assisting in animal surgery and Tannia Clark for her help and expertise in the statistical analysis of our experimental data.

References

1. Suri C, Jones PF, Patan S, et al. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell* 1996;87:1171–80. [PubMed: 8980224]
2. Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002;106:3009–17. [PubMed: 12473544]
3. Britten MB, Abolmaali ND, Assmus B, et al. Infarct remodeling after intracoronary progenitor cell treatment in patients with acute myocardial infarction (TOPCARE-AMI)-mechanistic insights from serial contrast-enhanced magnetic resonance imaging. *Circulation* 2003;108:2212–8. [PubMed: 14557356]
4. Schächinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction-final one-year results of the TOPCARE-AMI trial. *J Am Coll Cardiol* 2004;44:1690–9.
5. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106:1913–8. [PubMed: 12370212]
6. Strauer BE, Brehm M, Zeus T, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic coronary artery disease the IACT study. *J Am Coll Cardiol* 2005;46:1651–8.
7. Perin EC, Dohmann HFR, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107:2294–302. [PubMed: 12707230]
8. Fuchs S, Baffour R, Zhou YF, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol* 2001;37:1726–32. [PubMed: 11345391]
9. Fuchs S, Satler LF, Kornowski R, et al. Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease. *J Am Coll Cardiol* 2003;41:1721–4.
10. Chen SL, Fang WW, Ye F, et al. Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am J Cardiol* 2004;94:92–5.
11. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem cells mobilized with granulocyte colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomized clinical trial. *Lancet* 2004;363:751–6. [PubMed: 15016484]

12. Patel AN, Geffner L, Vina RF, et al. Congestive heart failure with autologous adult stem cell transplantation: A prospective randomized study. *J Thorac Cardiovasc Surg* 2005;130:1631–8. [PubMed: 16308009]
13. Ince H, Petzsch M, Kleine HD, et al. Preservation from left ventricular remodeling by front-integrated revascularization and stem cell liberation in evolving acute myocardial infarction by use of granulocyte-colony-stimulating factor (FIRSTLINE-AMI). *Circulation* 2005;112:3097–106. [PubMed: 16275869]
14. Zöhlhörer D, Kastrati A, Schömig A. Stem cell mobilization by granulocyte-colony-stimulating factor in acute myocardial infarction: lessons from the REVIVAL-2 trial. *Nature Clinical Practice* 2007;4(suppl 1):s106–s109.
15. Menasché P, Alfieri O, Janssens S, et al. The myoblast autologous grafting in ischemic cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 2008;117:1189–1200. [PubMed: 18285565]
16. Kang S, Yang Y, Li CJ, et al. Effectiveness and tolerability of administration of granulocyte colony-stimulating factor on left ventricular function in patients with myocardial infarction: A meta-analysis of randomized controlled trials. *Clin Ther* 2007;29:2406–18. [PubMed: 18158081]
17. Ripa RS, Haack-Sørensen M, Wang Y, et al. Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: Results from the stem cells in myocardial infarction (STEMMI) Trial. *Circulation* 2007;116(suppl 1):I-24–I-30. [PubMed: 17846310]
18. Unger EF, Banai S, Shou M, et al. A model to assess interventions to improve collateral blood flow: continuous administration of agents into the left coronary artery in dogs. *Cardiovascular Research* 1993;27:785–91. [PubMed: 7688663]
19. Zhou YF, Bosch-Marce M, Okuyama H, et al. Spontaneous transformation of cultured mouse bone marrow-derived stromal cells. *Cancer Research* 2006;66:10849–54. [PubMed: 17108121]
20. Curtis RE, Rowlings PA, Deeg HJ, et al. Solid cancers after bone marrow transplantation. *N Engl J Med* 1997;336:897–904. [PubMed: 9070469]
21. Burns JS, Abdallah BM, Guldberg P, et al. Tumorigenic heterogeneity in cancer stem cells evolved from long-term cultures of telomerase-immortalized human mesenchymal stem cells. *Cancer Res* 2005;65:3126–35. [PubMed: 15833842]
22. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:984–7.
23. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7. [PubMed: 10102814]
24. Horvitz EM, Le Blanc K, Dominici M, et al. Clarification of the nomenclature for MSC: The international society for cellular therapy position statement. *Cytotherapy* 2005;7:393–5. [PubMed: 16236628]
25. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: Engraftment and functional effects. *Ann Thorac Surg* 2002;73:1919–26. [PubMed: 12078791]

Figure 1.

Figure 1A. By echocardiography, the change in wall thickening in saline group showed no significant difference ($p = 0.82$), while the cell treated groups demonstrating significant improvement in regional wall motion as assessed by stress echocardiography from baseline to 6 weeks after treatment ($p = 0.018$).

Figure 1B. By cine MRI, the average left ventricle ejection fraction in cells group was significantly higher after 6 wks of cell treatment compared with baseline ($p < 0.049$) while the control group showed no statistical difference between baseline and follow up.

Figure 2.

Bar graph shows the average capillary density of saline and cell injected groups expressed by ratio to myocytes at 6 weeks (top); immunofluorescent staining with vWF (red), collagen IV (green) and dapi (blue) represent the difference following treatment with saline or cell injection (bottom left and right, $\times 380$).

Figure 3.

Left panel: Animal sacrificed at 1 wk after MSCs injection. A. Arrow shows injected cells ($\times 100$); B. high magnification ($\times 400$); C and D shows desmin or smooth muscle actin staining alone with vW factor ($\times 380$). Right panel: Animal sacrificed at 2 wks after MSCs injection: a) Arrow shows injected cells $\times 100$; b) high magnification ($\times 400$); c) Arrow shows positive CD90 staining of injected cells ($\times 400$); d) positive control for CD90 staining of MSCs pellets in frozen section ($\times 600$); e) and f) double staining of desmin or smooth muscle actin along with vW factor ($\times 380$). Figure labels: A=artery, Myo=myocardial area, Epi=epicardial area.

Figure 4.

a) At 6 wks, injected cells form groups of small vessels (H&E, x200); b) Smooth muscle actin (green) and vWF staining (red) (x200); c) Higher magnification of b (x400); d) Injected cells showing no sign of cardiomyocytes morphologically and negatively stained for desmin, a marker for myocytes (x400).