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Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology

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Abstract

Aim—A limitation of cell therapy for heart disease is the fact that stem cells injected directly into the myocardium are capable of entering the vasculature and migrating to remote organs. We determined whether retention of mesenchymal stem cells (MSCs) in the infarcted myocardium could be improved by implanting the cells in a collagen matrix.

Methods—A myocardial infarction was induced by ligation of the left anterior descending coronary artery in Fischer rats. A total of 7 days after myocardial infarction, saline (n = 12), saline plus 2 million bone marrow-derived rat MSCs labeled with isotopic colloidal nanoparticles containing europium (n = 13), collagen (n = 13) or collagen plus 2 million labeled MSCs (n = 13) were directly injected into the infarcted myocardium. Tissues from the infarcted myocardium, noninfarcted myocardium, lung, liver, spleen and kidney were sampled 4 weeks later. Distribution of grafted MSCs was quantitatively analyzed by measuring the nanoparticle radioactivity in these tissues. Cardiac function was assessed by left ventriculography.

Results—There were zero nanoparticles detected in the tissues that received saline or collagen alone into the heart. Nanoparticles were detected in the heart and remote organs in the saline plus MSC group. Labeled cells (expressed as cell number/g tissue weight) were present in three out of 13 lungs (mean of $12,724 \pm 7060$ cells/g), four out of 13 livers ($12,301 \pm 5924$ cells/g), 11 out of 13 spleens ($57,228 \pm 11,483$ cells/g), zero out of 13 kidneys, 13 out of 13 infarcted myocardium ($8,006,835 \pm 1,846,462$ cells/g) and nine out of 13 noninfarcted myocardium ($167,331 \pm 47,007$ cells/g). However, compared with the saline plus MSC group, nanoparticles were detected to a lesser extent in remote organs in collagen plus MSC group. Nanoparticles were detected in two out of 13 lungs (4631 ± 3176 cells/g; p = NS), zero out of 13 livers (0 cells/g; p < 0.05 vs saline plus MSC), four out of 13 spleens ($24,060 \pm 17,373$ cells/g; p < 0.05), zero out of 13 kidneys (p = NS) and five out of 13 noninfarcted myocardium ($51,522 \pm 21,548$ cells/g; p < 0.05). In the collagen plus MSC group, nanoparticles were detected in 12 out of 13 infarcted myocardium ($4,830,050 \pm 592,215$ cells/g), which did not

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significantly differ from that in the saline plus MSC group ($p = \text{NS}$). Both saline plus MSCs and collagen alone improved left ventricular ejection fraction compared with saline treatment. However, collagen plus MSCs failed to improve cardiac function.

Conclusions—Collagen matrix as a delivery vehicle significantly reduced the relocation of transplanted MSCs to remote organs and noninfarcted myocardium.

Keywords

cellular cardiomyoplasty; collagen; mesenchymal stem cells; myocardial infarction

Cardiac cell loss after myocardial infarction is replaced by scar tissue, which results in adverse cardiac remodeling and progression of heart failure. Cell transplantation therapy aims to deliver viable cells to the site of damaged myocardium to improve cardiac function in the infarcted heart. Bone marrow derived-mesenchymal stem cells (MSCs) are an attractive cell source for myocardial repair, since they are easily obtained from bone marrow aspirate drawn through the skin and can be expanded in large numbers *in vitro*, and can differentiate into various kinds of cells including myogenic cells [1]. However, poor retention of the transplanted MSCs in the infarcted area remains as a limitation. Müller-Ehmsen and coworkers injected MSCs from male Fischer 344 rats into the border zone of myocardial infarction of syngeneic female rats immediately or 7 days after left coronary artery ligation [2]. Between 34 and 80% of injected cells were detected in the heart right after injection, assessed by quantitative real-time PCR with Y-chromosome specific primers. The retention of donor cells in the heart decreased rapidly to 0.3–3.5% at 6 weeks. Donor MSCs were also found in the lung, liver and kidney immediately after injection. Recently, Zhang and coworkers [3] and our research group [4] demonstrated that intramyocardially injected MSCs migrated from infarcted myocardium to extracardiac organs, such as the spleen, lung and liver. Therefore, a strategy for preventing the migration of transplanted MSCs to extracardiac organs is needed for cell-therapy applications.

In recent years, various biomaterials, such as fibrin glue [5], liquid compound consisting of growth factor-free Matrigel™ [6], collagen matrices [7] and self-assembling peptide (RAD16-I) nanofibers [8], have been used as delivery vehicles to transplant cells into the damaged myocardium and to improve their survival. Wang and coworkers tore sheets of cultured MSCs into pieces, preserving the extracellular matrix around the MSCs [9]. These MSC sheet fragments were injected into the peri-infarct areas of the myocardial infarction of syngeneic Lewis rats. Extracellular matrix as a cell-delivery vehicle provided a favorable environment to retain the transplanted MSCs in the heart and to improve the efficacy of therapeutic cell transplantation.

In the present study, we investigated whether using a collagen matrix as a delivery vehicle could prevent the washout of the transplanted cells from the injection site and reduce the relocation to the adjacent healthy myocardium and other remote organs, including the lungs, liver, spleen and kidneys, in a rat myocardial infarction model.

Materials & methods

This investigation was approved by the Institutional Animal Care and Use Committee, and performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' (NIH publication No. 85–23, National Academy press, Washington DC, revised 1996). The Heart Institute at Good Samaritan Hospital is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

MSC preparation

Lewis rat bone marrow-derived MSCs were purchased from the Tulane University Health Sciences Center (LA, USA). The MSCs used in this study were nonclonal and at passage six, MSCs were expanded and labeled with nanoparticles in our laboratory. The nanoparticles are approximately 20 nm in diameter and are composed of a colloidal material containing europium (BioPAL, Biophysics Assay Laboratory, Worcester, MA, USA). There are 1×10^{16} nanoparticles per ml of purchased labeling solution. It was diluted at a 1:100 concentration in medium for cell labeling. Cultured MSCs were incubated with the medium containing nanoparticles for 24 h. After labeling, the cells were harvested after three washings with phosphate-buffered saline to remove suspended nanoparticles, and were prepared for frozen storage in freezing medium containing 65% complete culture medium, 30% fetal bovine serum (Atlanta Biologicals, GA, USA) and 5% dimethyl sulfoxide. Only one population of cultured MSCs was used for transplantation. All cells were kept frozen in liquid nitrogen until use. MSC viability upon thawing was routinely assessed by trypan blue exclusion and the average viability was 86%.

Collagen matrix for cell transplantation

Zyderm[®] collagen implant (Zyderm type 1) was purchased from INAMED Corporation (5540 Ikwil Street, Santa Barbara, CA, USA). Zyderm collagen implant is a ready-to-use sterile compound stored in a syringe and is used clinically for correction of contour deficiencies of soft tissue. It is composed of highly purified bovine dermal collagen (35 mg/ml) consisting of 95% collagen I and 5% collagen III.

Model of myocardial infarction & MSC injection

Female Fischer rats were weighed and anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). After endotracheal intubation, the rats were ventilated with room air at a rate of 60 cycles/min and a tidal volume of 1 ml/100 g body weight (Harvard Apparatus Rodent Ventilator, Model 683, South Natick Mass). Using sterile procedure, the heart was exposed by a left thoracotomy through the fourth intercostal space, and after removing the pericardium, the proximal left anterior descending coronary artery was permanently ligated. The rats were allowed to recover with postoperative care. Buprenex (0.001 mg/100g body weight, twice daily) was given for 2 days as an analgesic.

A week after myocardial infarction, rats were reanesthetized and hearts re-exposed (as described above) for direct intramyocardial injection. For cell injection, the tip of the needle was placed in the center of the infarcted myocardium. When cells were injected, a raised pale bleb that extended from the center to the border zone of the infarcted area could be seen. Thus, the injected cells were distributed into not only the central area of the infarct, but also into the outer margins of the infarct and border zone of the infarct where neovascularization occurs. Saline (~70 µl), saline plus 2 million MSCs labeled with nanoparticles (MSC in ~70 µl saline), collagen matrix (~70 µl) or collagen matrix plus 2 million MSC labeled with nanoparticles (MSC in ~70 µl collagen) were injected directly into the infarcted myocardium with a 28-gauge needle attached to an insulin syringe.

Hemodynamic measurements

At 4 weeks after treatment, the rats were anesthetized. The right carotid artery was exposed, and a 2F high-fidelity, catheter-tipped micromanometer (model SPR-869, Millar, Inc, TX, USA) was inserted into the carotid artery through a small incision. The catheter was advanced into the ascending aorta to record heart rate and blood pressure, then was further advanced into the left ventricle to record left ventricular (LV) hemodynamic parameters.

Left ventriculography

After recording hemodynamic parameters, a left ventriculography was performed to assess LV function with a XiScan 1000 C-arm X-ray system (XiTec, Inc; 3-inch field of view). Following the injection of 1 ml nonionic contrast into the left jugular vein, video images of anterior–posterior and lateral projections were recorded on half-inch super-VHS videotape at 30 frames/s under constant fluoroscopy. Systolic and diastolic LV volumes were calculated from the video images by an investigator blinded to the treatment. All parameters were averaged over three consecutive cycles in both projections. Ejection fraction (%) was calculated as:

$$\left[100 \times \frac{(\text{volume in diastole} - \text{volume in systole})}{\text{volume in diastole}} \right]$$

and averaged over both projections.

Evaluation of the location of transplanted MSCs

The rats were euthanized by an injection of KCl given through the left jugular vein. Heart, lung, liver, spleen and kidney were harvested from each rat. Hearts were pressure-fixed with formalin (pressure equal to 13 cm water column). After measuring the postmortem LV volumes by filling the cavity with water and weighing (repeated three times and averaged), the hearts were cut into two pieces along the border line between the infarcted and noninfarcted myocardium. Infarcted myocardium at 4 weeks was clearly visible as a thin gray zone compared with the surrounding normal thick, pink muscle.

Tissues from the infarcted myocardium, noninfarcted myocardium, lung, liver, spleen and kidney were sampled, weighed and dried. The samples, as well as a standard curve of labeled cells, were sent to Biophysics Assay Laboratory to quantify the contained nanoparticles. The samples were exposed to a neutron beam. The europium contained within the nanoparticles was activated and became radioactive, which was quantified in an automatic spectroscopic instrument. The disintegration per min (dpm) of each sample was obtained, and the dpm was converted into cell number using a standard curve.

Statistics

All data are presented as mean $\pm$ SEM. Comparisons of postmortem LV volume, infarcted myocardium weight, heart weight, infarcted myocardium/heart weight ratio, heart rate, blood pressure, $\pm$ dP/dt (change in ventricular pressure over the change in time) and LV end diastolic pressure among groups were made by one-way analysis of variance with subsequent Tukey's *post-hoc* test if appropriate. LV ejection fraction (LVEF) versus infarcted myocardium weight among groups were compared by analysis of covariance. Cell numbers tracked by nanoparticles among groups were compared by Wilcoxon scores. Results were considered statistically significant if $p < 0.05$.

Results

A total of 72 rats were included in this study. Seven rats died after the first surgery to induce myocardial infarction (9.7% postoperative mortality in the first surgery). During the second surgery at 1 week after myocardial infarction, 11 rats died (two in the saline group, three in the saline plus MSCs group, three in the collagen group, two in the collagen plus MSCs group and one died after anesthesia before surgery; 16.9% postoperative mortality in the second surgery). At 4 weeks after injection, three rats died after anesthesia (two in the saline group and one in the collagen group). Overall, 51 rats were successfully processed for cardiac function

assessment and postmortem analysis (12 in the saline group, 13 in the saline plus MSCs group, 13 in the collagen group and 13 in the collagen plus MSCs group).

Postmortem LV volumes & infarcted myocardium weight

Mean postmortem LV volumes and heart weights were similar among the four groups at 4 weeks after treatment (Table 1). In the collagen plus MSC group, infarcted myocardium weight was significantly higher than in the other three groups ($p = 0.0017$). Infarcted myocardium/heart weight ratio (mg/g) in the collagen plus MSC group was significantly higher compared with saline group, with a nonsignificant trend toward being higher than the saline plus MSC group and collagen groups (Table 1). There were no significant differences in infarcted myocardium/heart weight ratio (mg/g) among the groups receiving saline, saline plus MSC or collagen (Table 1).

Hemodynamics

At 4 weeks after treatment, heart rate, systolic blood pressure, diastolic blood pressure, mean blood pressure, maximum $+dP/dt$ (LV positive change in pressure over time), minimum $-dP/dt$ (LV negative change in pressure over time), and LV end diastolic pressure were comparable in the four groups (Table 1).

LVEF by LV ventriculography

LVEF was improved in the saline plus MSC group ($58.6 \pm 2.4\%$, $n = 13$; $p = 0.017$) and collagen group ($58.1 \pm 2.5\%$, $n = 13$; $p = 0.055$) compared with the LVEF in the saline group ($54.9 \pm 3.4\%$, $n = 12$), but LVEF was similar in the saline group and collagen plus MSC group ($53.2 \pm 1.4\%$, $n = 13$). As shown in Figure 1, in the saline, saline plus MSCs and collagen groups, higher infarcted myocardium weight correlated with lower LVEF in rats, but this correlation was lost in the collagen plus MSC group. Note that for any infarcted weight, the saline plus MSCs or collagen alone group was associated with an improved LVEF compared with the saline-alone group (Figure 1).

Distribution of transplanted cells tracked by nanoparticles in tissues

Figure 2 demonstrates that the measured dpm of the europium particle content and cell number were linearly related ($r^2 = 0.99$). This standard curve of known quantities of labeled cells was used for computing the numbers of labeled cells in the sampled tissues.

No europium particles were detected in the tissues of rats that received saline or collagen alone 4 weeks after injection, n . In the saline plus MSC group, europium particles were detected in three out of 13 lungs, four out of 13 livers, 11 out of 13 spleens, zero out of 13 kidneys, 13 out of 13 infarcted myocardium and nine out of 13 noninfarcted myocardium. In the collagen plus MSC group, europium particles were detected in two out of 13 lungs, zero out of 13 livers, four out of 13 spleens, zero out of 13 kidneys, 12 out of 13 infarcted myocardium and five out of 13 noninfarcted myocardium. Results of cell numbers that were normalized to sample mass are shown in Table 2. Compared with the saline plus MSC group, transplanted cells were detected to a lesser extent in noninfarcted myocardium and remote organs, including the liver and spleen, in the collagen plus MSCs group, and the relocated cell numbers were significantly lower in these organs ($p < 0.05$). However, engrafted cells within infarcted myocardium were similar in the two groups that received MSCs. There were 23% of labeled cells detected in the whole heart in the saline plus MSC group and 16% of labeled cells were detected in the heart in the collagen plus MSCs group.

Discussion

In this study, we demonstrated that collagen matrix as a delivery vehicle significantly reduced the relocation of transplanted cells to noninfarcted myocardium and remote organs, including the liver and spleen. Compared with saline treatment, saline plus MSCs or collagen injection alone improved LVEF. However, collagen plus MSCs failed to improve cardiac function.

Cell loss after direct injection into the infarcted myocardium remains a primary challenge for stem cell-based therapy in the damaged heart. Zhang and coworkers injected male swine MSCs labeled with iron oxide into the border zone of a 1-week-old myocardial infarction of a female swine [3]. Transplanted cells were identified in the heart, spleen, lung and liver 3 days later, as assessed by MRI and PCR for Y-chromosome (SRY). Recently, our research group labeled rat MSCs with europium *in vitro* and injected these cells into the infarcted myocardium of rats [4]. One week later, 15% of labeled cells were retained in the heart and a number of labeled MSCs were detected in the liver, spleen and lung. In our present study, compared with saline, using collagen as the delivery vehicle significantly prevented the wash-out of transplanted MSCs from infarcted myocardium and reduced the relocation of transplanted cells to remote organs and noninfarcted myocardium. Thus, biomaterial as a delivery vehicle is a promising approach to retain the cells in the injection site and reduce the acute cell loss after cell transplantation due to wash-out through the vascular system of the heart.

A new approach to repairing damaged myocardium is injecting an *ex vivo* mixture of cells and biomaterial (known as *in situ* cardiac tissue engineering) [10,11]. In recent years, some biomaterials, such as fibrin glue [5], growth factor-free Matrigel [6] and collagen matrices [7], have been used as vehicles for cell-transplantation therapy in the heart. Results demonstrated that these biomaterials increased transplanted cell survival and improved heart function. However, other biomaterial, such as RAD16-I nanofibers [8], did not improve transplanted cell survival or cardiac function. In our present study, both saline plus MSCs and collagen alone improved LVEF compared with saline treatment. This finding is consistent with our previous reports [12,13]. In our previous studies, MSCs plus saline improved LV function at 4 weeks after transplantation, perhaps through a transient paracrine mechanism [12], while collagen implantation alone strengthened the infarcted myocardium and limited paradoxical systolic bulging of the infarcted LV wall, preserving cardiac function [13]. However, in the present study, collagen plus MSCs failed to improve cardiac function or remodeling, and the number of detected cells was lower in the collagen plus MSCs than in the saline plus MSCs group, although the difference was not statistically significant. The potential mechanisms of the negative long-term interaction between the transplanted cells and collagen vehicle are unknown. However, one possibility is that collagen may interfere with the diffusion of oxygen and nutrients through the interstitial space. While collagen may hold the cells in place, it may interfere with cell-to-cell communication. Collagen might also inhibit connections between the microvessels within the interstitial space or have some other unknown toxic effect on the transplanted cells.

Limitations of the nanoparticle labeling technique

Nanoparticles have been safely and effectively used for many years in labeling cells for tracking. Nontoxic and biodegradable nanoparticles containing tracking materials are absorbed by spontaneous cell internalization and incorporate into cytoplasmic vacuoles, and have been used to track transplanted cells *in vivo* [14–17]. There is a good correlation between the number of labeled cells and the signal intensity of the absorbed nanoparticles (Figure 1). Riviere and coworkers labeled rat smooth muscle cells with iron oxide nanoparticle, and measured *in vitro* cell proliferation, iron content per cell and MR signal intensity of cells [14]. This *in vitro* labeling was stable, long-lasting, quantitative and non-toxic. In their *in vivo* experiments, transplanted labeled cells were efficiently detected in both healthy and ischemic rat hearts.

Amsalem and coworkers labeled rat MSCs with iron oxide nanoparticles and observed that almost 100% of the cells remained viable after labeling measured by trypan blue exclusion assay [15]. Hill and coworkers demonstrated that MSCs labeled with iron fluorophore particle remained viable for multiple passages and retained *in vitro* proliferation and differentiation capacity [18].

Whether detected labeled nanoparticle represents the viable grafted cells remains unclear. Amsalem and coworkers injected iron oxide nanoparticle-labeled male MSCs into 1-week-old myocardial infarction in female rats [15]. Although labeling signals were detected in the infarcted myocardium by noninvasive MRI 4 weeks later, histological examination demonstrated that the detected signals arose from cardiac macrophages that had engulfed the nanoparticles released by the transplanted MSCs. No transplanted MSCs were detected by real-time PCR for Y chromosome-specific SRY DNA in infarcted hearts. Recently, Terrovitis and coworkers double-labeled MSCs with iron oxide particles and β -galactosidase and injected the cells into rat hearts [16]. At 3 weeks after injection, the signal of iron oxide particles was detected in the hearts by MRI. Histology demonstrated the presence of iron-containing macrophages at the injection site, but very few or no β -galactosidase-positive stem cells were observed. Thus, nanoparticle labeling cannot confirm that the detected labeling signal is associated with viable engrafted cells. The retained cells may die and release the internalized nanoparticles. Although the released nanoparticles may not be counted as viable engrafted cells, they still result from acutely retained cells. Thus, this technique was capable of detecting the number of located transplanted cells, but did not confirm that the detected cells were viable [17].

A limitation of our present study is that we do not know whether the detected nanoparticles signals represent viable or dead transplanted MSCs, or macrophages that intake the released particles by dead transplanted MSCs. In order to answer this question, we performed a study in which we injected 2 million male rat MSCs into ischemic myocardium in female rats ($n = 3$). At 1 week after cell transplantation, tissue from the infarcted myocardium, noninfarcted myocardium, liver, kidney, spleen and lung were sampled to detect transplanted male cells by PCR with Y chromosome-specific primers. The PCR signal was observed in all of the above sampled tissues. The PCR findings of this MSC study are similar to that of our previous study with neonatal cardiac cell transplantation. Previously our research group injected male immature cardiac cells (saline as delivery vehicle) directly into the ischemic myocardium of female Fischer rats [19]. Ischemia was induced by 1 h of coronary artery occlusion followed by 3 h of reperfusion, or 4 h of coronary artery occlusion with no reperfusion. Samples, including heart, lung, liver, spleen and kidney, were taken for histology and PCR examination. In this acute study, injected cells escaped from the ischemic region through the vascular system (visualized by histology) of the heart and relocated in remote organs including lung, liver, spleen and kidney; reperfusion of coronary artery increased the loss of cells. The transplanted cell number was sharply decreased shortly after cell injection into the heart. These findings agree with the current study, suggesting that while some cells can be retained in the desired infarction area, other cells escape to remote organs.

Conclusion

Collagen matrix as a delivery vehicle reduced the relocation of transplanted cells to remote organs and noninfarcted myocardium. However, collagen may have impaired long-term cell survival within the infarcted myocardium, possibly because it produced an environment with a poor supply of oxygen and nutrients. Although injecting a mixture of cells and biomaterial is a promising approach to improve efficiency of cell transplantation therapy for cardiac repair, collagen may not be the optimal biomaterial to use for a cell-delivery vehicle.

Executive summary

- We determined whether retention of mesenchymal stem cells (MSCs) in the infarcted myocardium could be improved by implanting the cells in a collagen matrix.
- Saline (n = 12), saline plus MSCs labeled with isotopic colloidal nanoparticles containing europium (n = 13), collagen (n = 13) or collagen plus labeled MSCs (n = 13) were directly injected into the infarcted myocardium of rats.
- There were zero nanoparticles detected in the tissues that received saline or collagen alone into the heart 4 weeks later.
- Nanoparticles were detected in the infarcted myocardium, noninfarcted myocardium and remote organs, including the lungs, livers and spleens, in the saline plus MSC group.
- Nanoparticles were detected to a lesser extent in noninfarcted myocardium and remote organs in the collagen plus MSC group, and the relocated cell numbers were significantly lower in these organs.
- Detected nanoparticles were similar in the infarcted myocardium in the two MSC groups.
- Both saline plus MSCs and collagen alone improved left ventricular ejection fraction compared with saline treatment. However, collagen plus MSCs failed to improve cardiac function.
- Therefore, collagen matrix as a delivery vehicle significantly reduced the relocation of transplanted MSCs to remote organs and noninfarcted myocardium, but did not enhance cell survival within the infarcted myocardium.

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Figure 1. The association between infarcted myocardium weight and left ventricular ejection fraction

Note that in the saline, saline plus MSC and collagen groups, higher infarcted myocardium weight related to lower LVEF in rats, but this correlation was lost in the collagen plus MSC group. LVEF was improved in saline plus MSC group ($p = 0.017$) and collagen group ($p = 0.055$) compared with the LVEF in saline group. That is, for any given infarcted myocardium weight, LVEF was better in the saline plus MSC group or collagen alone group. LVEF was similar in saline and collagen plus MSC groups.

LVEF: Left ventricular ejection fraction; MSC: Mesenchymal stem cell.

Figure 2. The relationship between the disintegrations per min and the cell number for each sample in the standard curve form the same expansion of cells used for cell transplantation in this study. The variables were linearly related. The correlation between cell number and disintegrations per minute is good ($r^2 = 0.99$).

Table 1
Postmortem parameters and hemodynamics

	Saline (n = 12)	Saline + MsCs (n = 13)	Collagen (n = 13)	Collagen + MsCs (n = 13)
Post-mortem LV volume (μl)	330 ± 20	317 ± 15	325 ± 11	347 ± 13
Infarcted myocardium weight (mg)	43.4 ± 3.7	50 ± 2.6	50.3 ± 2.7	62.8 ± 4.1 *
Heart weight (g)	0.47 ± 0.01	0.46 ± 0.01	0.47 ± 0.01	0.50 ± 0.01
Infarcted myocardium /heart weight ratio (mg/g)	92 ± 6	109 ± 6	107 ± 5	125 ± 6 **
Heart rate (beats/min)	236 ± 9	236 ± 9	219 ± 9	242 ± 9
Systolic blood pressure (mmHg)	133 ± 6	121 ± 3	121 ± 5	133 ± 7
Diastolic blood pressure (mmHg)	96 ± 3	89 ± 2	89 ± 2	93 ± 5
Mean blood pressure (mmHg)	108 ± 4	100 ± 2	100 ± 3	107 ± 6
+dp/dt (mmHg/s)	5875 ± 272	5368 ± 399	5625 ± 185	5829 ± 372
-dp/dt (mmHg/s)	3943 ± 214	3453 ± 327	3525 ± 238	3919 ± 317
LV end diastolic pressure (mmHg)	11 ± 2	8 ± 2	11 ± 2	13 ± 2

* p = 0.0017 (vs other three groups);

** p = 0.0033 (vs saline group).

dP/dt: Change in ventricular pressure over the change in time; LV: Left ventricular; MSC: Mesenchymal stem cell.

Table 2

Cell numbers (per gram of tissue)

Rat #	Group	Lung	Liver	Kidney	Spleen	Infarcted	Noninfarcted
1761	Saline + MSC	0	0	0	82,510	6,098,339	288,344
1762	Saline + MSC	0	0	0	81,100	6,349,317	1438
1763	Saline + MSC	0	0	0	0	7,272,642	396,747
1828	Saline + MSC	0	0	0	0	16,400,476	0
1829	Saline + MSC	76,464	39,940	0	4350	2,655,929	0
1830	Saline + MSC	0	0	0	48,371	3,181,834	130,797
1891	Saline + MSC	0	0	0	55,978	23,027,843	468,118
1892	Saline + MSC	0	59,854	0	120,683	3,367,431	81,042
1893	Saline + MSC	0	0	0	11,091	17,361,962	298,677
1894	Saline + MSC	0	0	0	88,712	5,769,351	0
1896	Saline + MSC	50,760	0	0	85,831	5,042,648	199,843
1897	Saline + MSC	38,185	14,935	0	64,219	6,823,418	310,296
1898	Saline + MSC	0	45,185	0	101,119	737,670	0
<i>Average</i>		12,724	12,301	0	57,228	8,006,835	167,331
<i>SEM</i>		7060	5924	0	11,483	1,846,462	47,007
1747	Collagen + MSC	0	0	0	0	7,912,927	149,143
1749	Collagen + MSC	0	0	0	41,031	3,973,147	0
1770	Collagen + MSC	0	0	0	0	3,146,156	0
1771	Collagen + MSC	0	0	0	0	3,228,201	0
1772	Collagen + MSC	0	0	0	0	5,769,739	237,233
1820	Collagen + MSC	34,620	0	0	4178	5,081,350	0
1821	Collagen + MSC	0	0	0	0	4,494,527	0
1822	Collagen + MSC	0	0	0	226,308	0	0
1823	Collagen + MSC	0	0	0	0	4,711,100	0
1877	Collagen + MSC	0	0	0	0	8,103,938	97,138
1878	Collagen + MSC	0	0	0	0	6,239,805	64,362
1879	Collagen + MSC	0	0	0	0	4,098,552	121,904
1880	Collagen + MSC	25,580	0	0	41,269	6,031,207	0
<i>Average</i>		4631	0	0	24,060	4,830,050	51,522
<i>SEM</i>		3176	0	0	17,373	592,215	21,548
p-value		0.479	0.034	1	0.005	0.317	0.049

Infarcted: Infarcted myocardium; MSC: Mesenchymal stem cell; Noninfarcted: Noninfarcted myocardium.