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The Structural Basis of Functional Improvement in Response to Human Umbilical Cord Blood Stem Cell Transplantation in Hearts with Post-Infarct LV Remodeling

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Abstract

Cellular therapy for myocardial repair has been one of the most intensely investigated interventional strategies for acute myocardium infarction. Although the therapeutic potential of stem cells has been demonstrated in various studies, the underlying mechanisms for such improvement are poorly understood. In the present study, we investigated the long-term effects of stem cell therapy on both myocardial fiber organization and regional contractile function using a rat model of post-infarct remodeling. Human non-hematopoietic umbilical cord blood stem cells (nh-UCBSCs) were administered via tail vein to rats 2 days after infarct surgery. Animals were maintained without immunosuppressive therapy. In vivo and ex vivo MR imaging was performed on infarct hearts ten months after cell transplantation. Compared to the age-matched rats exposed to the identical surgery, both global and regional cardiac function of the nh-UCBSC-treated hearts, such as ejection fraction, ventricular strain and torsion, were significantly improved. More

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importantly, the treated hearts exhibited preserved fiber orientation and water diffusivities that were similar to those in sham-operated control hearts. These data provide the first evidence that nh-UCBSC treatment may prevent/delay untoward structural remodeling in post-infarct hearts, which supports the improved LV function observed in vivo in the absence of immunosuppression, suggesting a beneficial paracrine effect that occurred with the cellular therapy.

Keywords

umbilical cord blood stem cells; ischemic heart injury; diffusion tensor MR imaging; displacement encoding; myocardial wall strain; fiber architecture

INTRODUCTION

Myocardial infarction (MI), induced by coronary artery disease, is one of the major causes of heart failure in the world. Due to the loss of viable myocardium after MI, the heart works continuously under elevated wall stress, which results in progressive myocardial hypertrophy and left ventricular (LV) dilatation that eventually leads to heart failure. In the past ten years, implantation of progenitor cells for myocardial repair in post-infarct hearts has been extensively investigated both experimentally and clinically (16,17,27). Although different levels of functional benefit have been reported from studies using different animal models, delivery modes, and cell types and doses (1,12), the underlying mechanisms of the functional improvement remain poorly understood. Overall, the level of muscle cell regeneration is low (21,41). If we assume that a large infarct irreversibly causes a 30% loss of LV mass and stem cell induced muscle regeneration is 10% of the damaged tissue, this would lead to only 3% of the total LV myocytes from stem cell differentiation. On the other hand, it has been reported that a paracrine effect plays an important role in observed beneficial effects of cellular therapy for myocardial repair (11). Further, a recent mouse study that compared marrow-derived progenitor cells for myocardial repair in immuno-deficient and immuno-competent mice suggests that inflammatory response may also play an important role in the paracrine effect benefiting the recipient myocardium (25). Regardless of whether the beneficial effects are due to the paracrine effect or from engrafted stem cells, the observed functional improvement must be a result of the functional changes of the native ventricular muscle structure, coronary circulation, and pacemaker and conduction system, or the integration of exogenous stem cells into the existing ventricular structure.

In the normal heart, the orientation of left ventricular fibers changes smoothly from left-handed fibers in the subepicardium to circumferential fibers in mid wall, and to right-handed fibers in the subendocardium (28,29). This unique three-dimensional structure of myocardial fibers plays an important role in determining ventricular function and the electromechanical properties of the heart (2,32). Early descriptions of myocardial fiber structure are based on histological measurements, which are destructive and time-consuming (22,29). Recently, diffusion tensor MRI (DTMRI) has emerged as a promising method for non-destructive reconstruction of the three-dimensional fiber structure throughout the whole ventricle at high spatial resolution (4,5). By comparing DTMRI and conventional histological analysis,

several studies have demonstrated a direct correlation between the fiber angle measurements, validating the utility of DTMRI as a fast and nondestructive technique to characterize fiber architecture in myocardium (13,14,26).

Alterations of cardiac fiber architecture after MI have been previously investigated using DTMRI (7,30,33,35). Chen et al. observed a significant increase in water diffusivity and a significant decrease in relative diffusion anisotropy in remodeled rat myocardium one month after infarction (7). The changes in diffusion anisotropy were found to correlate with the degree of microscopic fiber disarray. Recent studies in both humans and porcine models further confirmed these findings. In addition, a redistribution of fiber structure was observed with more left-handed fibers in infarct myocardium (33,35). Furthermore, a recent study on cardiomyopathic Syrian hamsters showed that changes in transverse diffusivity may reflect calcium deposition in the lesion areas (19). These studies demonstrate the capability of multi-parametric DTMRI in delineating various aspects of structural remodeling in diseased hearts.

In the present study, we investigated the long-term effects of stem cell therapy on myofiber orientation and cardiac function in post-infarct rat hearts. Infarct rats were treated with human non-hematopoietic umbilical cord blood stem cells (nh-UCBSCs) in the absence of immunosuppressive therapy. These nh-UCBSCs were previously shown to exhibit neuro-restorative effects in a rodent model of ischemic brain injury (37). Global and regional LV function and myocardial fiber structure were characterized using cardiac functional MRI and DTMRI, respectively. Compared to the age-matched untreated rat hearts with MI, the regional myocardial function of nh-UCBSC-treated hearts was significantly improved, in conjunction with preserved myocardial fiber structure that may serve as an underlying mechanism for the observed functional improvement.

MATERIALS AND METHODS

All animals used in this study were treated in accordance with the guidelines of the Institutional Animal Care and Use Committee of both University of Minnesota and Case Western Reserve University.

nh-UCBSCs were collected from a male donor. Informed consent for donation was obtained before collection. The use of human-derived cells was approved by University of Minnesota.

MI surgery and treatment with nh-UCBSCs

Twelve male Lewis rats (250–275 g, 2–3 months old) were used in the study. MI was induced in seven rats through the occlusion of the left anterior descending artery (LAD). Briefly, the animal was anesthetized in an isoflurane (Butler Schein Animal Health, Dublin, OH) induction chamber (3%), intubated and then placed on a ventilator (Harvard Apparatus, Southnatick, MA). A thoracotomy was performed to expose the heart. The LAD was ligated using polypropylene suture (6-0 Prolene, Ethicon). The chest was closed and animals were allowed to recover with post-surgical monitoring for two hours. The remaining five sham-operated animals were used as the controls (CONT, n = 5).

The seven rats that underwent MI surgery were randomly allocated into two groups: 1) LAD ligation without any further therapy (MI, n=3); 2) LAD ligation with nh-UCBSC injection (MI+Cell, n=4). The procedure to isolate nh-UCBSCs was described in detail previously (37). The isolated cells were cultured in T25 flask (Fisher Scientific, Hampton, NH) in 7 ml Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Ham) 1:1 (D-MEM/F12; Invitrogen) supplemented with 1xN2 supplement (Invitrogen), 10% fetal bovine serum (FBS; Invitrogen), 10 ng/ml recombinant human basic fibroblast growth factor (b-FGF; R&D Systems), 10 ng/ml recombinant human epidermal growth factor (EGF; R&D Systems), 100 U penicillin (Invitrogen), and 1000 U streptomycin (Invitrogen). When cells were 70% – 80% confluent, they were detached with 0.25% trypsin-EDTA (Invitrogen) and split at 1:4 for future usage. Two days after surgery, nh-UCBSCs were administered via tail vein injection ($10^6/500 \mu\text{l}$ PBS). For the rats in MI group, the same volume of PBS (Sigma-Aldrich, St. Louis, MO) without any cells was injected. None of the animals were given immunosuppressive therapy.

In vivo MR imaging

Ten months after the surgery, in vivo functional MRI studies were performed on a 9.4T Bruker Biospec (Billerica, MA) horizontal bore scanner with a 10 cm volume coil (Bruker Biospin, Billerica, MA). Anesthesia was induced by 2% isoflurane in O_2 and was maintained at 1.5% isoflurane in O_2 by a nose cone built in-house during imaging. An MR-compatible small animal gating and monitoring system (SA Instruments, Stony Brook, NY) was used for electrocardiography (ECG) gating, respiration gating and temperature control. The ECG signal was obtained from two ECG electrodes that were attached to the left paw and the right leg of the rat. The respiration patterns were acquired from a respiratory pad placed under the chest wall of the animal in the prone position. A rectal temperature probe was inserted to measure the body temperature. The body temperature was maintained at $34.2 \pm 1.2^\circ\text{C}$ by blowing hot air into the magnet through a feedback control system. A series of scout images were acquired to obtain the LV long-axis plane. After acquiring a horizontal long-axis image (four-chamber view), the LV short-axis planes at the base, mid-ventricle and apex were prescribed as perpendicular to the LV long-axis with an inter-slice distance of 3 to 3.5 mm.

Displacement-encoding with stimulated-echo (DENSE) MRI was used to quantify the myocardial wall motion at high spatial resolution as previously described (43). Multi-phase DENSE images were acquired at the base, mid-ventricle and apex for each rat. The DENSE imaging sequence used a SPAMM11 tagging preparation module with fast low-angle single-shot (FLASH) readout acquisition. To measure displacement in one direction, displacement encoding/unencoding gradients with the same magnitude but opposite polarities were used in two DENSE acquisitions to correct for baseline phase errors and to increase the dynamic range of the displacement encoding. A total of four cine data sets were acquired to quantify myocardial motion in two orthogonal directions. The displacement encoding gradient was chosen to yield an encoding frequency (k_x) of 0.77 cycles/mm. Additional imaging parameters included the following: flip angle, 20° ; TE, 3.0 ms; field of view, $5 \times 5 \text{ cm}^2$; matrix size, 128×128 ; slice thickness, 1.5 mm; number of averages, 6. TR was adjusted according to the heart rate (HR). A total of 17 frames were acquired in one cardiac cycle to

yield a temporal resolution of 10~13 ms. Cine FLASH images with the same geometric parameters were also acquired at each imaging level to calculate the LV volumes and the ejection fraction (EF). The imaging parameters were: flip angle, 20°; TE, 2.1 ms; number of averages, 4.

Hemodynamic measurements with the pressure-volume conductance catheter

Hemodynamic studies were performed one week after the in vivo MRI scan. During the terminal surgery, a 2.0-Fr pressure-volume (P-V) catheter (SPR-838, Millar Instruments, Houston, TX) was inserted into the right carotid artery and advanced into the LV (23,24), and the position was optimized by aiming for maximal stroke volume (SV). A 14-gauge angiocath (BD Biosciences, Franklin Lakes, NJ) was inserted into the right jugular vein for the infusion of saline boluses for calibration. After 15 min of stabilization, steady-state parameters, including heart rate, maximal LV systolic pressure, and LV end-diastolic pressure, were recorded using MPVS-400 signal conditioning hardware with integrated AD Instruments PowerLab DAQ technology (AD Instruments, Mountain View, CA). Maximal slopes of systolic and diastolic pressure (+dP/dt and -dP/dt) were calculated using PVAN 3.2 software (Millar Instruments) (23,24).

Ex vivo diffusion tensor imaging

After hemodynamic measurements, the heart was excised and fixed for DTMRI study as previously described (7,19). Briefly, the heart was first perfused with Krebs buffer (Sigma-Aldrich, St. Louis, MO) and then arrested in diastole by perfusion with 50 ml solution of KCl (20 mM, Sigma-Aldrich, St. Louis, MO) and NaCl (120 mM, Sigma-Aldrich, St. Louis, MO). The perfusate was then switched to 10% formalin (Fisher Scientific, Hampton, NH) for rapid fixation. The procedures were all performed at room temperature (20 °C). The fixed heart was then taken off the perfusion column, immersed in formalin and stored in the refrigerator at 4°C for further fixation. One day before imaging, the heart was taken out from formalin solution, rinsed and suspended in 1x PBS.

Diffusion-weighted, short-axis images of the fixed hearts were acquired in a 9.4T vertical bore magnet (Bruker Biospin, Billerica, MA) with a 2 cm birdcage coil (M2M Imaging, Cleveland, OH). The acquisition utilized a standard Stejskal-Tanner spin-echo sequence. Seven to nine short-axis images were acquired to cover the whole LV. Imaging parameters included the following: TR, 2.5 sec; TE, 18 msec; diffusion time (Δ), 20 msec; diffusion gradient on time (δ), 10 msec; number of diffusion-encoding directions, 6; b, 800 s/mm²; field of view, 2×2 cm²; matrix size, 128×128; slice thickness, 1 mm; number of averages, 8. The total acquisition time for each heart was about 5 h.

Image reconstruction and data analysis

In vivo MR images were reconstructed and analyzed offline using in-house developed, MATLAB-based image analysis tool package with a custom-designed graphic user interface. LV contours were traced using cine FLASH images to calculate LV diameter, wall thickness and LV volume. The LV ejection fraction was then calculated from LV end-diastolic volume and end-systolic volume as previously described (42).

The image processing and displacement calculation from DENSE images were described in detail previously (43). Briefly, a k-space filter with a cut-off frequency of 0.48 cycles/mm was used to eliminate the T_1 -relaxation echo and the residual complex conjugate echo. The raw data was zero-filled and a 2D inverse Fourier transform was applied to generate the complex images. The two phase images obtained from the same direction but opposite encoding gradient polarities were subtracted to eliminate baseline phase errors. Subsequently, displacement-encoded phase images were unwrapped using an algorithm similar to the 2D quality-guided path following method. The 2D displacement map was calculated and the Lagrangian strain tensor, E , was computed within each four-adjacent-pixel element from the 2D displacement as previously described (43). LV twist angles and torsion were then quantified from the displacement. The twist angle was defined as the rotation of each voxel compared to its original position at end-diastole. Torsion was calculated as the net twist between apical and basal slices normalized by the inter-slice distance (20).

The ex vivo diffusion-weighted images were transferred to a PC for analysis with an in-house developed MATLAB software (7). The three eigenvalues of the diffusion tensor, λ_1 , λ_2 , λ_3 , and their corresponding eigenvectors were calculated on a pixel-by-pixel basis for the entire LV. All the eigenvalues were then normalized to the mean diffusivity of the surrounding PBS solution. The mean diffusivity (λ) was computed as the average of the three normalized eigenvalues. The fractional anisotropy (FA) was used to quantify the diffusion anisotropy. The myofiber helix angle was further determined from the primary diffusion eigenvector to delineate myocardial fiber orientation as previously described (7). Angular dispersion, which is defined as the standard deviation of the helix angles, was used to evaluate the coherence of fiber orientation at each transmural depth. For the MI group, 4 or 5 slices that cover the infarct region in the apex and mid-ventricle were selected. The results were compared to the data obtained from similar regions of the LV in both CONT and MI+Cell groups.

The myocardium in diffusion-weighted images was divided into three segments, i.e., infarct, border, and remote zones (Fig. 1) (33). The infarcted myocardium was identified as the region with reduced wall thickness in the MI group (Fig. 1a). In the MI+Cell rats, no obvious wall-thinning was observed and a segment of anterior myocardium with the same size as the average infarct size in MI rats was selected as the infarct segment. The rest of the myocardium was divided into six equiangular segments. The two segments that were adjacent to the infarct region were classified as the border zones, and the four remaining sections were defined as the remote zones. In the CONT rats, sham infarct, border, and remote zones were defined similarly as the MI+Cell rats (Fig. 1c). The ventricular geometry in DENSE images was correlated to DTMRI images based on the position of the right ventricle and papillary muscles (Figs. 1b&d).

Immunohistochemistry analysis

The hearts were fixed in 10% formalin, processed and paraffin embedded. Paraffin sections of 5 μ m thickness were used for immunohistochemistry studies. To visualize the scar tissue, tissue sections underwent trichrome staining (Sigma-Aldrich, St. Louis, USA) as per

instructions. To quantify the infarct zone, trichrome-stained heart sections were scanned. The percentage of infarction was calculated as the ratio of the infarct area to LV free wall area (not including LV septal wall). The percentage of wall thickness in infarct zone was calculated as the ratio of the wall thickness in infarct region to the average LV free wall thickness of normal control rats (n=3 or 4 animals in each group). The percentage of fibrosis in infarct zone was calculated as the ratio of the numbers of pixels representing fibrotic and total myocardial tissue, respectively.

c-Kit⁺ cardiac progenitor cell (CPC) density was calculated as the number of total c-kit⁺ CPC divided by left ventricle free wall area (cm²). Cardiac progenitor cells were quantified at 400x magnification in 8 microscopic fields/heart (n=3 or 4 animals in each group) after double fluorescent immunostaining for c-kit (R&D Systems, USA) and α -sarcomere actin (α -SA, Sigma Aldrich, USA) expressions.

The blood vessel density at the infarct and border zones was measured at 400x magnification in 8 microscopic fields/heart (n=3 or 4 animals in each group) after double fluorescent immunostaining for vWF-VIII (Dako, Denmark) and smooth muscle actin (Sigma-Aldrich, St. Louis, USA) expressions.

Statistical analysis

All the results are presented as mean $\pm$ SD. Comparisons for multiple groups were performed with one-way ANOVA followed by Tukey's test. Significance of differences was assigned at P<0.05.

RESULTS

Animal characteristics and global cardiac function

The morphological and physiological data of the animals are presented in Table 1. The body weight and the LV length were similar among all three groups. Significant increases in LV diameter and end-diastolic volume (EDV) were observed in both MI and MI+Cell groups, which suggests LV dilation. Compared to the MI group, the end-systolic volume (ESV) in the MI+Cell group decreased by 21%. As a result, LV EF in nh-UCBSC-treated rats was significantly improved (P<0.05) and was similar to that of the CONT rats (P=N.S.).

End-diastolic wall thickness at the apex, mid-ventricle and base was measured from *in vivo* CINE images (Table 2). In the MI group, wall thickness in the infarct zone was reduced by 50% compared to the CONT rats. In addition, wall thickness at the border zone was also significantly decreased as compared to the CONT rats. In the MI+Cell group, no statistical difference in wall thickness was observed as compared to the controls.

Hemodynamics under steady-state conditions

Steady-state *in vivo* hemodynamic properties assessed using a PV conductance catheter are reported in Table 3. Peak LV +dP/dt and -dP/dt decreased significantly in the MI group as compared to the CONT group. However, ES pressure and ED pressure were not different. Ejection fraction was also significantly reduced in the MI group. In contrast, no hemodynamic changes were observed in the MI+Cell group. Ejection fraction in MI+Cell

rats was also similar to that of the CONT rats, which is consistent with the in-vivo MRI measurements (Table 1).

Myocardial strain and torsion

Figures 2a&b show the radial (E_r) and circumferential (E_c) strains in the infarct, border and remote zones at apex. Due to the significant wall thinning in MI rats, myocardial strains in the infarct zones cannot be reliably quantified. Compared to the CONT group, a significant decrease in both radial and circumferential strains was observed in the border zone of the MI group. However, in nh-UCBSC-treated hearts, wall strains were similar to the controls in the infarct, border, and remote zones. At the mid-ventricular and basal levels, no significant differences in myocardial wall strains were observed among all three groups (data not shown).

Improved ventricular function in nh-UCBSC-treated rats was further demonstrated by the measurement of ventricular torsion (Fig. 2c). At peak systole, LV torsion was $23.6 \pm 2.6^\circ/\text{cm}$ for the CONT group. It decreased significantly to $14.3 \pm 1.8^\circ/\text{cm}$ in the MI group ($P < 0.05$). However, torsion in the MI+Cell hearts ($21.2 \pm 4.9^\circ/\text{cm}$) was similar to that of the controls, a 48% increase compared to the MI group.

Myocardial fiber structure

The histograms of the helix angle distributions are presented in Fig. 3. Compared to the control rats, there was a significant reduction in the percentage of right-handed fibers (30° to 90°) in all three zones of the untreated hearts, suggesting a loss of endocardial fibers. As a result, the percentage of circumferential fibers (-30° to 30°) increased by 18.0%, 22.8% and 26.9% in the infarct, border and remote zones, respectively. The percentage of left-handed fibers (-90° to -30°) in the remote zone also decreased significantly in MI rats. The nh-UCBSC treatment prevented such shift towards more circumferential orientation in all three zones. No difference was observed in the percentage of the three types of fibers between the control and the therapy group.

Increased angular dispersion of the helix angle was observed in the infarct zone for both MI and MI+Cell rats. Angular dispersion ranged from 11.2° to 17.0° in the sham “infarct” zone for the CONT group. In the infarct zone, the range of angular dispersion increased to $22.8^\circ \sim 28.7^\circ$ for the MI group and $16.1^\circ \sim 21.7^\circ$ for the MI+Cell group. However, in the remote zone, the range of angular dispersion was similar among all three groups (CONT, $10.9^\circ \sim 22.8^\circ$; MI, $13.0^\circ \sim 17.8^\circ$; MI+Cell, $12.4^\circ \sim 23.3^\circ$).

In addition to the changes in fiber helix angles, water diffusivity was also significantly reduced in the nh-UCBSC-treated hearts as compared to the MI group (Fig. 4a). No significant difference in mean diffusivity was observed between the CONT and the MI+Cell group ($P = \text{N.S.}$). Further analysis showed that all three eigenvalues in the infarct zone of the MI+Cell group were significantly reduced as compared to the MI group, which contributed to the overall decrease of the mean diffusivity (Table 4). In addition, the primary eigenvalue (λ_1) in both the border zone and remote zone were also significantly decreased with nh-UCBSC treatment as compared to untreated MI rats (Table 4).

Figure 4b shows the fractional diffusion anisotropy (FA) in the infarct, border and remote zones in all three groups. Compared to the control rats, a trend towards decreased FA was observed in the MI group in the infarct and border zones by 21.1% and 18.9%, respectively. A similar trend of reduction was also observed in nh-UCBSC-treated hearts. No evident difference in FA was observed in the remote region among all three groups.

Histological analysis

Masson trichrome staining showed reduced fibrosis in rat hearts treated with nh-UCBSCs (Fig. 5a–f). Although the area of scar zone was similar between the two groups (Fig. 5g), significantly increased wall thickness was found in the MI+cell group (Fig. 5h). MI+cell group also showed significantly reduced fibrosis in the infarct zone (Fig. 5i). There is no difference in c-kit⁺ cardiac progenitor cells between the MI and MI+cell groups (Fig. 6). As compared to the MI group, a trend of increase in the number of blood vessels was observed (Fig. 7).

DISCUSSION

Stem cell therapy for myocardial repair has been investigated extensively for the past 10 years. Although different levels of improvement in LV function have been reported in response to different cell types, LV injury model, or cell delivery modes, the underlying mechanisms of the beneficial effects are not fully defined. In the current study, the long-term effects of stem cell therapy on both ventricular function and myocardial fiber structure were investigated in post-infarct hearts. In addition to improved contractile performance, nh-UCBSC-treated hearts demonstrated preserved right-handed fibers that were similar to that in control hearts. While previous study reported preserved global ventricular structure by stem cell treatment (10), our results demonstrate for the first time that improvement in LV contractile function is associated with preserved myofiber orientation. The preserved structure observed with nh-UCBSC treatment may provide the structural basis for improved cardiac function in post-infarct hearts.

Global and regional LV function was significantly improved with nh-UCBSC treatment. This functional improvement has been reported in many studies using various imaging methods, including MRI (1,12). However, most of these studies focused on the short-term effects of stem cell treatment on myocardial function, while the prolonged effect remains to be investigated. In the current study, our results demonstrate the long-term effects of improved cardiac function in post-infarct myocardium with nh-UCBSC treatment. Both MI and MI+Cell groups showed significantly increased EDV (Table 1), which may lead to improved ejection fraction by increasing diastolic filling. However, the nh-UCBSC treated hearts also showed decreased ESV as compared to untreated hearts, suggesting that improved systolic contractility also contributed to the observed increase in ejection fraction. Further, nh-UCBSC treated hearts showed increased peak LV +dP/dt and –dP/dt as compared to the MI group (Table 3), providing further evidence of improved systolic and diastolic function.

During the MRI experiments, we typically observed a 1~2°C fluctuation in body temperature measured by a rectal probe. To avoid overheating the animals, the temperature

control system was set to 35°C during MRI scanning, leading to an average body temperature of $34.2 \pm 1.2^\circ\text{C}$. A previous study in rats reported that body temperature measured by rectal probe was typically lower ($\sim 0.6^\circ\text{C}$) than body temperatures recorded by chronically implanted electrodes, with the difference being smaller at low and greater at high basal temperatures (3). Further, the observed torsion and strain values in the current study were similar to that from a previous study when the temperature was maintained at $35.8 \pm 0.8^\circ\text{C}$ (8).

The unique three-dimensional architecture of myocardial fibers renders the heart an efficient pump. Previous studies employing mathematical modeling demonstrated that a 15% shortening of circumferentially oriented fibers can only induce $\sim 30\%$ reduction in ventricular volume from end-diastole to end-systole; however, obliquely oriented fibers can lead to more than 60% volume reduction (15). In addition, the twisting motion and torsion of the LV is a consequence of the obliquely oriented fiber structure. Therefore, the efficacy of cellular therapy relies on the preservation of the structure of ventricular myocardium. In the current study, examination of myofiber structure with DTMRI revealed that nh-UCBSC treatment can preserve fiber orientation within the myocardium, as demonstrated by the similar percentage of left-handed, circumferential, and right-handed fibers as compared to the control hearts. This preserved structure contributed to decreased ESV (Table 1) and elevated ventricular torsion (Fig. 2c) in nh-UCBSC-treated hearts, leading to normalized ejection fraction.

Post-infarct myocardial remodeling is a prolonged process that involves changes in both cell types and structure. In the infarct zone, different cell types exist at different stages of the remodeling process. In the early stage, macrophages differentiated from monocytes in the blood are first recruited to the injury site to remove necrotic cardiac myocytes and apoptotic neutrophils from the myocardium. Later, more fibroblasts and endothelial cells will emerge during the wound healing process. Once the scar is formed, it will gradually expand to the border zone and induce global remodeling of the whole heart (18,39). These cellular remodeling processes can impact DTMRI measurements of myocardial structure.

Previously, DTMRI has been applied to study myofiber structure in both humans and animals (6,7,9,13,14,19,30,33,35). Several studies also examined the fiber structure in post-infarct myocardium (7,30,33,35). Consistent with previous findings in both human and rodent studies, we observed reduced right-handed fibers and increased angular dispersion in untreated infarct hearts (7,34,35). These changes are likely due to the loss of viable myocytes in the subendocardial area after the infarction. As a result, the percentage of circumferential fibers was increased. Interestingly, this redistribution of myocardial fibers occurred not only in the infarct zone, but also in the border and remote zones, suggesting that the whole myocardium underwent structural remodeling as a result of long-term post-infarct remodeling. While the fiber redistribution in all three zones was consistent with a previous ex vivo study in a porcine model (33,36), our current finding of decreased left-handed fibers in the remote zone has not yet been reported. Further investigations are needed to elucidate whether this reduction is due to the long-term remodeling after infarction.

In addition to fiber orientation, we also found preserved water diffusivity in nh-UCBSC-treated rats as compared to the controls, while untreated hearts showed significantly increased water diffusivity in the infarct and border zones. The exact mechanisms for the preserved diffusivity in nh-UCBSC-treated hearts are not completely understood. We have previously shown that the increased water diffusivity was associated with enlarged extracellular space following cell death (7). It is possible that nh-UCBSC treatment and the subsequent paracrine effect caused less cell death and limited the expansion of extracellular space. However, this hypothesis needs to be examined at an earlier time point as myocardial apoptosis is expected to be very low 10 months after acute infarction. Another possibility is that the integration of nh-UCBSCs in the infarct myocardium replenished the extracellular space after initial cell death. However, the sparseness of nh-UCBSCs surrounding the regions of ischemic injury makes this an unlikely possibility (Fig. 6b).

In spite of the preserved fiber orientation and water diffusivity, similar decrease in diffusion anisotropy were observed in the infarct zone in both untreated and treated hearts. In addition, nh-UCBSC-treated hearts also showed increased angular dispersion as compared to the controls. Changes in cell morphology, extracellular space and fiber orientation can all lead to alterations in the diffusion anisotropy. Our previous study demonstrated that decreased anisotropy and increased angular dispersion in infarcted myocardium were associated with microscopic fiber disarray and extracellular space expansion (7). Given that the water diffusivity in nh-UCBSC-treated hearts was similar to that of the controls, the reduced diffusion anisotropy was unlikely due to an enlarged extracellular space. Hence, the decreased diffusion anisotropy and increased angular dispersion suggest fiber disarray in nh-UCBSC-treated hearts.

In the current study, human nh-UCBSCs were used in immune-competent rats without immunosuppressive agents. Previously, we have used human bone marrow derived stem cells on both immune-deficient (NOD/SKID) and immune-competent mice with acute myocardial infarct. Our results show that although the engraftment rate is significantly lower in immune-competent mice, the beneficial effects on LV function is significantly better for the immune-competent mice than the NOD/SKID mice, suggesting a cytokine effect (31). In another study using a swine model of post-infarct LV remodeling (40), we compared animals with or without immunosuppression cyclosporine A (CsA 15 mg/kg/day). The results of that study demonstrate that mesenchymal stem cells have an immune-privilege window of at least 4 weeks, and that cellular therapy induced beneficial effects on LV function was similar between groups with or without immunosuppression.

No significant increase in the number of c-kit positive cells was observed in nh-UCBSC treated hearts (Fig. 6). Although it is possible that the c-kit marker of the engrafted nh-UCBSCs was silenced during differentiation over a period of 10 months, the observed functional benefits are more likely related to the mobilization of the endogenous c-kit positive cells induced by cellular therapy, which was observed in our recent study using a swine model treated with human embryonic stem cell-derived vascular cells (38).

One limitation of the current study is the low heart rate in both MRI and hemodynamic studies (Tables 1 and 3). As myocardial contractility is impacted by heart rate, the

measurements acquired at lower heart rate may deviate from the normal baseline. Another limitation of this study is the small number of animals in each group. Due to the high mortality in older rats and the cost of maintaining the animals for a long period of time, only 3–5 animals were examined in each group. The small sample size may reduce the power of statistical analysis to detect a difference. Such may be the case in anisotropy measurement. While our current study only showed a trend toward decreased diffusion anisotropy in the infarcted hearts, the observed values in both the control and the untreated groups were similar to those reported in the literature where statistical difference was detected (33,36). Furthermore, even with the small sample size, significant changes in both cardiac function and myofiber orientation were still observed with nh-UCBSC treatment.

CONCLUSIONS

In conclusion, we investigated the long-term effect of stem cell therapy on myocardial function and myofiber orientation in the post-infarct rat heart. While the functional data suggested significantly improved global and regional functions in nh-UCBSC-treated hearts, DTMRI studies showed that nh-UCBSC treatment may have the additional benefit of preventing/delaying untoward structural remodeling in post-infarcted hearts. These data demonstrate a novel finding that nh-UCBSC treatment preserves myocardial fiber structure that supports the improved LV regional and chamber function.

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References

1. Amado LC, Saliaris AP, Schuleri KH, StJohn M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G, Lehrke S, Baumgartner WW, Martin BJ, Heldman AW, Hare JM. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci U S A*. 2005; 102:11474–11479. [PubMed: 16061805]
2. Arts T, Reneman RS, Veenstra PC. A model of the mechanics of the left ventricle. *Ann Biomed Eng*. 1979; 7:299–318. [PubMed: 547767]
3. Bae DD, Brown PL, Kiyatkin EA. Procedure of rectal temperature measurement affects brain, muscle, skin, and body temperatures and modulates the effects of intravenous cocaine. *Brain Res*. 2007; 1154:61–70. [PubMed: 17466279]
4. Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. *NMR Biomed*. 1995; 8:333–344. [PubMed: 8739270]
5. Basser PJ, Pierpaoli C. Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. *J Magn Reson B*. 1996; 111:209–219. [PubMed: 8661285]
6. Chen J, Liu W, Zhang H, Lacy L, Yang X, Song SK, Wickline SA, Yu X. Regional ventricular wall thickening reflects changes in cardiac fiber and sheet structure during contraction: quantification with diffusion tensor MRI. *Am J Physiol Heart Circ Physiol*. 2005; 289:H1898–H1907. [PubMed: 16219812]
7. Chen J, Song SK, Liu W, McLean M, Allen JS, Tan J, Wickline SA, Yu X. Remodeling of cardiac fiber structure after infarction in rats quantified with diffusion tensor MRI. *Am J Physiol Heart Circ Physiol*. 2003; 285:H946–H954. [PubMed: 12763752]

8. Chen Y, Somji A, Yu X, Stelzer JE. Altered in vivo left ventricular torsion and principal strains in hypothyroid rats. *Am J Physiol Heart Circ Physiol*. 2010; 299:H1577–H1587. [PubMed: 20729398]
9. Geerts L, Bovendeerd P, Nicolay K, Arts T. Characterization of the normal cardiac myofiber field in goat measured with MR-diffusion tensor imaging. *Am J Physiol Heart Circ Physiol*. 2002; 283:H139–H145. [PubMed: 12063284]
10. Gittenberger-de Groot AC, Winter EM, Poelmann RE. Epicardium-derived cells (EPDCs) in development, cardiac disease and repair of ischemia. *J Cell Mol Med*. 2010; 14:1056–1060. [PubMed: 20646126]
11. Gnechchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J*. 2006; 20:661–669. [PubMed: 16581974]
12. Hendry SL, van der Bogt KE, Sheikh AY, Arai T, Dylla SJ, Drukker M, McConnell MV, Kutschka I, Hoyt G, Cao F, Weissman IL, Connolly AJ, Pelletier MP, Wu JC, Robbins RC, Yang PC. Multimodal evaluation of in vivo magnetic resonance imaging of myocardial restoration by mouse embryonic stem cells. *J Thorac Cardiovasc Surg*. 2008; 136:1028–1037. [PubMed: 18954646]
13. Holmes AA, Scollan DF, Winslow RL. Direct histological validation of diffusion tensor MRI in formaldehyde-fixed myocardium. *Magn Reson Med*. 2000; 44:157–161. [PubMed: 10893534]
14. Hsu EW, Muzikant AL, Matulevicius SA, Penland RC, Henriquez CS. Magnetic resonance myocardial fiber-orientation mapping with direct histological correlation. *Am J Physiol*. 1998; 274:H1627–H1634. [PubMed: 9612373]
15. Ingels NB Jr. Myocardial fiber architecture and left ventricular function. *Technol Health Care*. 1997; 5:45–52. [PubMed: 9134618]
16. Kawamoto A, Tkebuchava T, Yamaguchi J, Nishimura H, Yoon YS, Milliken C, Uchida S, Masuo O, Iwaguro H, Ma H, Hanley A, Silver M, Kearney M, Losordo DW, Isner JM, Asahara T. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*. 2003; 107:461–468. [PubMed: 12551872]
17. Kofidis T, Lebl DR, Martinez EC, Hoyt G, Tanaka M, Robbins RC. Novel injectable bioartificial tissue facilitates targeted, less invasive, large-scale tissue restoration on the beating heart after myocardial injury. *Circulation*. 2005; 112:I173–I177. [PubMed: 16159811]
18. Lambert JM, Lopez EF, Lindsey ML. Macrophage roles following myocardial infarction. *Int J Cardiol*. 2008; 130:147–158. [PubMed: 18656272]
19. Li W, Lu M, Banerjee S, Zhong J, Ye A, Molter J, Yu X. Ex vivo diffusion tensor MRI reflects microscopic structural remodeling associated with aging and disease progression in normal and cardiomyopathic Syrian hamsters. *NMR Biomed*. 2009; 22:819–825. [PubMed: 19434665]
20. Liu W, Ashford MW, Chen J, Watkins MP, Williams TA, Wickline SA, Yu X. MR tagging demonstrates quantitative differences in regional ventricular wall motion in mice, rats, and men. *Am J Physiol Heart Circ Physiol*. 2006; 291:H2515–H2521. [PubMed: 16751290]
21. Menasche P. Current status and future prospects for cell transplantation to prevent congestive heart failure. *Semin Thorac Cardiovasc Surg*. 2008; 20:131–137. [PubMed: 18707646]
22. Nielsen PM, Le Grice IJ, Smaill BH, Hunter PJ. Mathematical model of geometry and fibrous structure of the heart. *Am J Physiol*. 1991; 260:H1365–H1378. [PubMed: 2012234]
23. Pacher P, Liaudet L, Bai P, Mabley JG, Kaminski PM, Virag L, Deb A, Szabo E, Ungvari Z, Wolin MS, Groves JT, Szabo C. Potent metalloporphyrin peroxynitrite decomposition catalyst protects against the development of doxorubicin-induced cardiac dysfunction. *Circulation*. 2003; 107:896–904. [PubMed: 12591762]
24. Pacher P, Nagayama T, Mukhopadhyay P, Batkai S, Kass DA. Measurement of cardiac function using pressure-volume conductance catheter technique in mice and rats. *Nat Protoc*. 2008; 3:1422–1434. [PubMed: 18772869]
25. Pelacho B, Nakamura Y, Zhang J, Ross J, Heremans Y, Nelson-Holte M, Lemke B, Hagenbrock J, Jiang Y, Prosper F, Luttun A, Verfaillie CM. Multipotent adult progenitor cell transplantation increases vascularity and improves left ventricular function after myocardial infarction. *J Tissue Eng Regen Med*. 2007; 1:51–59. [PubMed: 18038392]

26. Scollan DF, Holmes A, Winslow R, Forder J. Histological validation of myocardial microstructure obtained from diffusion tensor magnetic resonance imaging. *Am J Physiol.* 1998; 275:H2308–H2318. [PubMed: 9843833]
27. Strauer BE, Brehm M, Zeus T, Kostering M, Hernandez A, Sorg RV, Kogler G, Wernet P. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002; 106:1913–1918. [PubMed: 12370212]
28. Streeter DD Jr, Hanna WT. Engineering mechanics for successive states in canine left ventricular myocardium. I. Cavity and wall geometry. *Circ Res.* 1973; 33:639–655. [PubMed: 4762006]
29. Streeter DD Jr, Hanna WT. Engineering mechanics for successive states in canine left ventricular myocardium. II. Fiber angle and sarcomere length. *Circ Res.* 1973; 33:656–664. [PubMed: 4762007]
30. Strijkers GJ, Bouts A, Blankesteyn WM, Peeters TH, Vilanova A, van Prooijen MC, Sanders HM, Heijman E, Nicolay K. Diffusion tensor imaging of left ventricular remodeling in response to myocardial infarction in the mouse. *NMR Biomed.* 2009; 22:182–190. [PubMed: 18780284]
31. Tolar J, Wang X, Braunlin E, McElmurry RT, Nakamura Y, Bell S, Xia L, Zhang J, Hu Q, Panoskaltsis-Mortari A, Zhang J, Blazar BR. The host immune response is essential for the beneficial effect of adult stem cells after myocardial ischemia. *Exp Hematol.* 2007; 35:682–690. [PubMed: 17379078]
32. Waldman LK, Nosan D, Villarreal F, Covell JW. Relation between transmural deformation and local myofiber direction in canine left ventricle. *Circ Res.* 1988; 63:550–562. [PubMed: 3409487]
33. Wu EX, Wu Y, Nicholls JM, Wang J, Liao S, Zhu S, Lau CP, Tse HF. MR diffusion tensor imaging study of postinfarct myocardium structural remodeling in a porcine model. *Magn Reson Med.* 2007; 58:687–695. [PubMed: 17899595]
34. Wu MT, Su MY, Huang YL, Chiou KR, Yang P, Pan HB, Reese TG, Wedeen VJ, Tseng WY. Sequential changes of myocardial microstructure in patients postmyocardial infarction by diffusion-tensor cardiac MR: correlation with left ventricular structure and function. *Circ Cardiovasc Imaging.* 2009; 2:32–40. [PubMed: 19808562]
35. Wu MT, Tseng WY, Su MY, Liu CP, Chiou KR, Wedeen VJ, Reese TG, Yang CF. Diffusion tensor magnetic resonance imaging mapping the fiber architecture remodeling in human myocardium after infarction: correlation with viability and wall motion. *Circulation.* 2006; 114:1036–1045. [PubMed: 16940196]
36. Wu Y, Chan CW, Nicholls JM, Liao S, Tse HF, Wu EX. MR study of the effect of infarct size and location on left ventricular functional and microstructural alterations in porcine models. *J Magn Reson Imaging.* 2009; 29:305–312. [PubMed: 19161181]
37. Xiao J, Nan Z, Motooka Y, Low WC. Transplantation of a novel cell line population of umbilical cord blood stem cells ameliorates neurological deficits associated with ischemic brain injury. *Stem Cells Dev.* 2005; 14:722–733. [PubMed: 16433627]
38. Xiong Q, Ye L, Zhang P, Lepley M, Swingen C, Zhang L, Kaufman DS, Zhang J. Bioenergetic and functional consequences of cellular therapy: activation of endogenous cardiovascular progenitor cells. *Circ Res.* 2012; 111:455–468. [PubMed: 22723295]
39. Yang F, Liu YH, Yang XP, Xu J, Kapke A, Carretero OA. Myocardial infarction and cardiac remodelling in mice. *Exp Physiol.* 2002; 87:547–555. [PubMed: 12481929]
40. Zeng L, Hu Q, Wang X, Mansoor A, Lee J, Feygin J, Zhang G, Suntharalingam P, Boozer S, Mhashilkar A, Panetta CJ, Swingen C, Deans R, From AH, Bache RJ, Verfaillie CM, Zhang J. Bioenergetic and functional consequences of bone marrow-derived multipotent progenitor cell transplantation in hearts with postinfarction left ventricular remodeling. *Circulation.* 2007; 115:1866–1875. [PubMed: 17389266]
41. Zhang G, Hu Q, Braunlin EA, Suggs LJ, Zhang J. Enhancing efficacy of stem cell transplantation to the heart with a PEGylated fibrin biomatrix. *Tissue Eng Part A.* 2008; 14:1025–1036. [PubMed: 18476809]
42. Zhong J, Liu W, Yu X. Characterization of three-dimensional myocardial deformation in the mouse heart: an MR tagging study. *J Magn Reson Imaging.* 2008; 27:1263–1270. [PubMed: 18504746]

43. Zhong J, Yu X. Strain and torsion quantification in mouse hearts under dobutamine stimulation using 2D multiphase MR DENSE. *Magn Reson Med*. 2010; 64:1315–1322. [PubMed: 20740659]

Figure 1. Segmentation of LV into three segments. Segmentation of the LV into the infarct, border, and remote zones in a fixed (a) and in vivo (b) heart from the MI group, and a fixed (c) and in vivo (d) heart from the control group.

Figure 2.

Characteristics of *in vivo* cardiac function. Radial (a) and circumferential (b) strains at apex and ventricular torsion (c) in CONT, MI, and MI+Cell groups. †Myocardial strains in the infarct zone of MI rats were not quantified due to significant wall thinning. * $P < 0.05$ CONT versus MI. # $P < 0.05$ MI versus MI+Cell.

Figure 3.

Fiber helix angle distribution in fixed hearts. Histograms of fiber helix angles in the infarct (a), border (b), and remote (c) zones for CONT, MI and MI+Cell groups. * $P < 0.05$ CONT versus MI. # $P < 0.05$ MI versus MI+Cell.

Figure 4. Diffusivity and FA in fixed hearts. Mean water diffusivity (a) and fractional diffusion anisotropy (b) in the infarct, border, and remote zones for the CONT, MI and MI+Cell groups. *P<0.05 CONT versus MI. #P<0.05 MI versus MI+Cell.

Figure 5.

Masson trichrome staining of fixed hearts. (a&c) Magnified pictures of scar zone of rat hearts from the MI group (b). (d&f) Magnified pictures of scar zone of rat hearts from the MI+Cell group (e). Color representation: nuclei, black; cytoplasm, red; muscle fiber, red; collagen, blue. (Bar = 1 mm) (g) Percentage of infarct area. (h) Percentage of wall thickness in infarct zone. (i) Percentage of fibrosis in infarct zone.

Figure 6. Quantification of c-kit⁺ cardiac progenitor cells. (a) Representative picture from a heart in the MI group. (b) Representative picture from a heart in the MI+cell group. (c) Quantification of c-kit⁺ cardiac progenitor cells. (Bar = 100μm; α-SA = α-sarcomere actin).

Figure 7.

Dual fluorescent immunostaining for blood vessel (arterioles) density quantification. (a&b) Representative pictures of blood vessel density in infarct zone (a) and border zone (b) of the MI group. (c&d) Representative pictures of blood vessel density in infarct zone (c) and border zone (d) of the MI+cell group. Quantification of blood vessel density (number/400x magnification field) was based on immune-staining images for von Willebrand Factor-8 (vWF-8; e) and smooth muscle actin (SMA; f). Bar = 100 μ m.

Table 1

Body weights and CINE MRI data

	CONT (n=5)	MI (n=3)	MI+Cell (n=4)
Body Weight (g)	649.6±16.9	670.7±61.3	661.5±67.3
LV Diameter (mm)	9.2±0.5	10.7±0.3 [*]	10.8±1.1 [*]
LV Length (mm)	14.9±1.5	14.4±0.4	14.7±1.1
EDV (ml)	0.53±0.07	0.71±0.03 [*]	0.71±0.13 [*]
ESV (ml)	0.22±0.03	0.43±0.03 [*]	0.34±0.11
EF (%)	59.1±4.9	39.7±4.5 [*]	52.8±7.1 [#]
Heart Rate (bpm)	305±26	274±16	280±13

EDV: end-diastolic volume;

ESV: end-systolic volume;

^{*} P<0.05 compared to CONT group;[#] P<0.05 compared to MI group;

bpm: beats per minute.

Table 2

End-diastolic wall thickness (mm) assessed from *in vivo* CINE MRI images.

		Apex	Midventricle	Base
CONT	Infarct	1.27±0.21	1.34±0.25	1.34±0.29
	Border	1.35±0.20	1.41±0.16	1.39±0.16
	Remote	1.42±0.29	1.53±0.29	1.53±0.24
MI	Infarct	0.63±0.03 [*]	0.92±0.25	1.64±0.05
	Border	0.92±0.08 [*]	1.29±0.51	1.50±0.07
	Remote	1.12±0.09	1.15±0.22	1.36±0.15
MI+Cell	Infarct	1.10±0.21 [#]	1.37±0.06 [#]	1.54±0.40
	Border	1.23±0.10 [#]	1.51±0.16	1.46±0.20
	Remote	1.56±0.33	1.48±0.23	1.57±0.19

^{*} P<0.05 compared to CONT group;

[#] P<0.05 compared to MI group.

Table 3Steady-state hemodynamic properties assessed by *in vivo* PV conductance catheter.

	CONT (n=5)	MI (n=3)	MI+Cell (n=4)
ES Pressure (mmHg)	127.9 ± 10.4	104.3 ± 37.8	116.0 ± 48.9
ED Pressure (mmHg)	5.3 ± 4.9	5.2 ± 2.7	6.9 ± 4.2
+dP/dt _{max} (10 ³ mmHg/s)	8.8 ± 0.9	5.1 ± 2.3*	7.8 ± 2.8
−dP/dt _{max} (10 ³ mmHg/s)	10.8 ± 1.0	5.2 ± 1.5*	8.4 ± 3.0
EF (%)	58.8 ± 5.7	33.3 ± 7.0*	55.9 ± 5.7 [#]
Heart Rate (bpm)	327 ± 33	306 ± 32	286 ± 73

* P<0.05 compared to CONT group;

[#] P<0.05 compared to MI group.

Table 4

Three normalized eigenvalues in the infarct, border, and remote zones for control, untreated and therapy groups.

		λ_1	λ_2	λ_3
CONT	Infarct	0.58±0.04	0.41±0.02	0.31±0.02
	Border	0.59±0.04	0.43±0.02	0.32±0.02
	Remote	0.60±0.04	0.45±0.03	0.34±0.04
MI	Infarct	0.74±0.05 [*]	0.60±0.05 [*]	0.46±0.03 [*]
	Border	0.67±0.01 [*]	0.52±0.02 [*]	0.41±0.05 [*]
	Remote	0.66±0.02	0.48±0.02	0.37±0.04
MI+Cell	Infarct	0.59±0.02 [#]	0.46±0.02 ^{*#}	0.37±0.03 ^{*#}
	Border	0.60±0.04 [#]	0.47±0.04	0.36±0.04
	Remote	0.59±0.02 [#]	0.45±0.01	0.35±0.02

^{*} P<0.05 compared to CONT group;

[#] P<0.05 compared to MI group.