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Transplantation of Cardiogenic Pre-Differentiated Autologous Adipose-Derived Mesenchymal Stem Cells Induced by Mechanical Loading Improves Cardiac Function Following Acute Myocardial Infarction in Rabbit Model

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Abstract

Objective- Investigate myocardial performance after autologous adipose-derived (ASCs) mesenchymal stem cell differentiated under equiaxial cyclic strain, transplantation in rabbits with acute myocardial infarction (AMI).
Design- Prospective, randomized experimental study
Animals- 20 New Zealand White rabbits (2-3 kg)
Procedure- ASCs were studied in four distinct groups of mechanical (ADM), chemical (ADC), undifferentiated (AD) and control (C) groups. According to this categorization, the cells were exposed to cyclic mechanical loading or 5-azacytidine as the chemical factor. 10^6 ASC cells were transplanted intramyocardially in rabbits with AMI (Acute Myocardial Infarction). Echocardiographic study was used to evaluate effects of cells on cardiac function.
Results- Left ventricle ejection fraction (LVEF) was significantly increased in the ADM (mechanically-differentiated adipose-derived mesenchymal stem cell) group at 2 months follow-up. Fractional shortening (FS) also showed a similar pattern as LVEF and increased in ADM group in compare to control and AD (undifferentiated adipose-derived mesenchymal stem cell) group.
Conclusion and clinical prevalence- The results indicate that intramyocardial transplantation of mechanically-differentiated ASCs improves cardiac function of ischemic myocardium. Transplantation of mechanically-differentiated ASCs for myocardial regeneration may become the future therapy for acute myocardial infarction.
Keywords- Adipose-derived mesenchymal stem cell, Equiaxial strain, Acute myocardial infarction, Echocardiography

Introduction

Cardiovascular disease (CAD), particularly myocardial infarction (MI), is counted as one of the significant causes of morbidity and mortality around the world. Although, numerous biomedical researches are allocated to find the best solution to prevent and/or treatment of diseased heart, it has been remained a great challenge.[1-3] Following myocardial infarction or other ischemic associated cardiac diseases, numerous cardiomyocyte are lost. On the other hand, owing to poor regeneration capability of the heart, myocardium is incompetent to replace or regenerate the missed cardiomyocytes. Inflammatory response confines the tissue injury by developing fibrosis. The fibrotic scar tissue within the myocardium impairs the contractile ability of ventricles and in turn deteriorates cardiac function which can be lead to heart failure.[4-6] Nowadays, current treatments try to restore myocardial blood flow after AMI by means of primary percutaneous coronary intervention (PCI), coronary artery bypass graft (CABG) or thrombolytic

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pharmacologic therapy. However, reperfusion of ischemic myocardium is associated with extra deleterious changes. [7] Therefore, to overcome such problems, large volume of studies focused on developing new strategies such as stem-cell based therapies along with tissue engineering and gene therapy for cardiac repair. [8]

Among various types of cell-based products, mesenchymal stem cells (MSCs) showed beneficial effects on improving cardiac function and decreasing infarct size following transplant into infarcted myocardium in several pre-clinical studies. It has been proved that biochemical environment can lead to differentiation of MSCs into different lineage. [12-14] Mechanical stimulation actives some biochemical signals which in return leads to cell change and is considered as an important factor in cell biology. [15, 16] Mechanical stimuli affect proliferation, differentiation and gene expression in stem cells as well as adult cells. [17] Many studies defined the effects of various mechanical stimuli such as shearing force, tension, compression and pressure on cell characteristics *in vitro* and *in vivo*. [19-21] In order to mimic the extracellular matrix (ECM) stretching, some scientists utilized extension forces on silicone or hydrogel membranes. [22, 23] With the knowledge of the stem cells microenvironment and niche impact on cell characteristics and function, researchers will be capable to indicate the role of mechanical environment on stem cells' fate. [24] Although, a considerable amount of literature has been published on role of chemical mediators and macromolecular factors on cell behavior, there are limited investigations on mechanical stimulation. It has been proved that change in stiffness of stem cells microenvironment by means of mechanical forces lead to selectively differentiation into different cell lineages.[25] Admittedly, mechanical forces can induce differentiation into fibroblasts, chondroblasts, myoblasts, osteoblasts and cardiomyocytes. [26-29]

We previously reported the effect of equiaxial cyclic strain on GATA4 (a transcription factor) expression. [30] In the present study, we established a rabbit acute myocardial infarction (AMI) model to evaluate the efficacy of delivering cyclic mechanical stimulated ASCs (adipose-derived stromal cells) on cardiac function and characteristics.

Materials and Methods

Animal care

All animal procedures performed under experimental protocols approved by the University of Tehran Animal Care and Use Committee. The study follows the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Isolation and culture of ASCs

Under general anesthesia, 10 g of adipose tissue was harvested from each animal with sharp and blunt surgical dissection. Samples stored in cold sterile DMEM (Dulbecco's Modified Eagle's Medium) (gibco, Grand Island,NY, USA) and washed with PBS (Phosphate Buffer Serum) (Sigma, St.Louis, MO, USA). Enzymatically digestion was performed by 30 minutes incubation in 0.1% collagenase type I (Invitrogen/Thermo Fisher Scientific, Waltham, MA, USA) at 378C and air plus 5% CO2 along with shaking every 10 min. To stop collagenase activity, DMEM and 20% FBS (Fetal Bovine Serum) (Gibco, Grand Island, NY, USA) were added. For assisting the process of separating ASCs from adipocytes, samples were centrifuged twice - each of 5 min duration. The pellet were re-suspended in DMEM supplemented with 20% FBS, 1% penicillin/streptomycin and 1% L-glutamine (Merck, Germany) and incubated for 24 h. Later, cells were washed with PBS to eliminate residual non-adherent cells. Every 3 days the culture medium was replaced and passaging cells were done.

Flow cytometry

To analyze the expression of cell surface markers of MSCs, cell surface antigens including CD44 and CD90 and absence of CD45 were detected using flow cytometric technique. Passage-3 ASCs were trypsinised, washed and incubated with antibodies including Mouse anti-rabbit CD44 and CD45 (AbD Serotec) for 30 min and CD90 Monoclonal Antibody (Thermo Fisher Scientific) for 1 h in darkness. Thereafter, cells were washed with PBS and 10% FBS to remove the unconjugated antibodies and were incubated with the secondary antibody Rabbit anti-mouse IgG: FITC (fluorescein-4-isothiocyanate) (AbD Serotec) for 30 min. Data were analyzed using FlowJo Cytometry Analysis Software (version 7.6.4). (All cell culture chemicals and supplies were purchased from Sigma (NY, USA) and Gibco‐BRL (Grand Island, NY, USA) otherwise noted)

Multipotency assay

Differentiation capacity of ASCs to mesodermal lineage (i.e. adipocyte, osteoblast, chondrocyte) was assessed.

Adipogenic differentiation was induced by exposing cells to adipogenic culture media containing DMEM with 500 mM IBMX (isobutylmethylxanthine; Sigma, USA), 5 mg/mL insulin (Sigma, USA), 1mM dexamethasone (Sigma, USA) and 60mM indomethacin (Sigma, USA) for 3 weeks. Lipid droplets were stained with oil red O (Sigma, USA). For osteogenic differentiation, the culture media was replaced with osteogenic induction medium contains DMEM supplemented with 10mM b-glycerophosphate (Sigma,

USA), 50 mM ascorbate-2 phosphate (Sigma, USA) and 0.1 mM dexamethasone for 3 weeks. Mineralized colonies detected by Alizarin red s (Sigma, USA) staining.
Chondrogenic media which contained DMEM with 50mM ascorbic acid-2 phosphate (Sigma, USA), 10 ng/mL TGF b1 (transforming growth factorb1; R&D Systems, Invitrogen, USA), 100 nM dexamethasone, 1% ITS-Premix (BD Biosciences) and 1mM sodium pyruvate (Gibco, USA) was utilized for condrogenic differentiation induction. Differentiation were assessed by Alcian blue (Sigma, USA) staining of pellet sections.

Cardiogenic pre-differentiation of ASCs

Cells were cultured in circular silicone membranes which were coated with collagen type I solution (Sigma) (0.8 mg/mL in 0.1% acetic acid). On behalf of cardiogenic induction, cells were exposed to mechanical and chemical stimuli as described below.

Pre-differentiation by chemical stimulus

Cells were incubated in presence of 10 µM 5-azacytidine as a cardiogenic differentiating factor, along with DMEM and 10% FBS for 24 h. Afterwards, culture media was washed with PBS and replaced by DMEM.

Pre-differentiation by mechanical loading

Mechanical pre-differentiation was performed as described before. [30] In brief, cells were cultured in collagen-coated circular silicone membranes and subjected to cyclic equiaxial strain. A custom-made apparatus which was manufactured at Pasteur Institute of Iran (Fig.1) was used to apply mechanical load. [31] 10% cyclic strain at 1Hz was applied on incubated cells for 24 h.[32]

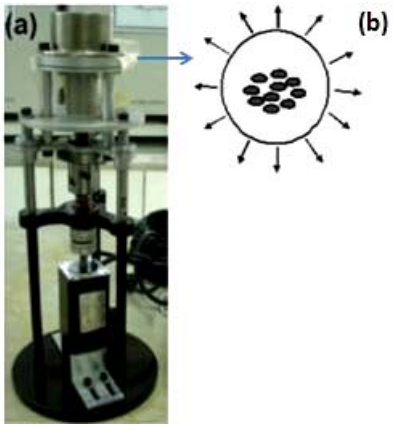

Figure 1. Mechanical loading apparatus. (a) applying Equiaxial strain on ASCs; (b) schematic image of the medium container and the exerted forces direction.

Induction of myocardial infarction

Twenty male New Zealand White rabbits (2-3 kg) were used. Anesthesia was induced by injection of 35mg/kg ketamine and 5 mg/kg xylazine intramuscularly; in addition to 12 mg/kg enrofloxacin and 0.5 mg/kg tramadol subcutaneously and intramuscularly, respectively. After endotracheal intubation, animals were mechanically ventilated and anesthesia was maintained by isoflurane (1% MAC). A limb lead electrocardiogram (ECG) was used to monitor heart rate and ECG changes after MI induction. Left 4th intercostal space was exposed by sharp and blunt dissection and left thoracotomy was performed. Pericardium was incised; the left anterior descending (LAD) coronary artery was ligated using a 5-0 monofilament prolene suture. Myocardial infarction was confirmed by a distinct paleness of the myocardium region. Animals divided in 4 groups (each group n=5), for treatment groups, 5 minute after LAD occlusion mechanically differentiated ASCs cells (ADM group) and chemically differentiated ASCs cells (ADC group) and undifferentiated ASCs (AD group) a total of 1ml agent containing 10^6 cells were delivered intramyocardially into adjacent border zones of infarct region via 5 injections each of 100 µl volume. For control group (n=5) DMEM injected as the same way.
Thoracotomy was closed and animals were placed on a heating pad until full recovery. Routine post-operative care were performed and rabbits were given enrofloxacin (12 mg/kg, bid), tramadol (0.5 mg/kg, bid) for 3 days post-operatively.

Echocardiography

On days -1 (pre-MI), 1 (after MI induction) and 60 (end of study), the transthoracic echocardiographic study was done. Animals were anesthetized, positioned in lateral recumbence and two-dimensional parasternal long and short axis views were obtained (Vivid 7, GE, Norway). Left ventricular (LV) dimensions and wall thickness were measured according to American Society of Echocardiography standards. [33] Two-dimensional LV data sets were acquired to assess left ventricular ejection fraction (EF) and fractional shortening (FS).

Statistical analysis

Numerical values are expressed as mean±SD. For multiple comparisons of more than two groups, one-way analysis of variance (ANOVA) was performed. If the ANOVA was significant, Tukey's test procedure was used as a post hoc test. Comparisons of parameters between two groups were made by unpaired Student's t-test. A value of $P<0.05$ was considered significant.

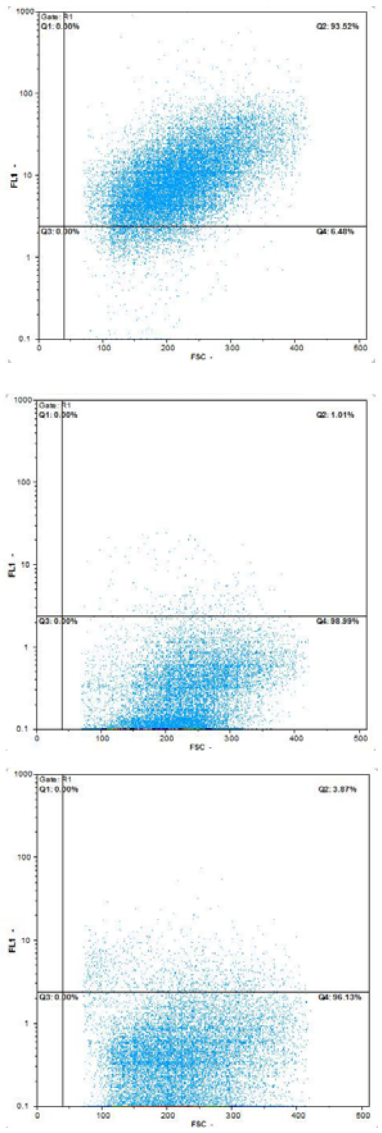

Figure 2. Immunophenotyping of the ASCs. (a) CD90 (expression 98.99%), (b) CD44 (expression 93.52%), (c) CD45 (expression 3.87%)

Results

Characterization and multipotency of ASCs

Flowcytometry was done to characterize of the isolated MSCs and as expected surface markers CD44 and CD90 were expressed whilst CD45 was not. In order to prove multipotency of the isolated ASCs, differentiation into adipogenic, osteogenic and chondrogenic lineage was performed. Oil red stained lipid vacuoles (Fig.3a) after 15 days, alizarin red S stained calcified colonies (Fig. 3b) after 3 weeks and alician blue stained sulfated glycosaminoglycan (Fig. 3c) after 4 weeks confirmed the differentiation capability of studied ASCs into mesodermal lineage.

Cardiac function

In order to assess the effect of transplanted cell on cardiac function echocardiography was performed at baseline (pre-MI), after MI induction and 2 months post-transplantation (table 1). As expected, there was a marked reduction of left ventricular ejection fraction in all four groups from baseline to post-MI which proved a similar ischemic injury among groups. Two months after cell transplantation, Injection of AD (Adipose-derived mesenchymal stem cells) did not induce a statistically significant change in LVEF (Left Ventricular Ejection Fraction) compared to the control. In contrast, transplantation of ADM (Adipose-derived mesenchymal stem cells induced by mechanical factor) significantly improved LVEF compared to the AD and control group as well as post-MI value. ADM and ADC (Adipose-derived mesenchymal stem cells induced by chemical factor) were not statistically different (figure 4a).

Fractional shortening (FS) indicated a similar pattern between groups from post-MI to 2 months after transplantation (figure 4b). There were no differences in other echocardiographic parameters related to heart remodeling and diastolic function (figure 4c and 4d)

Discussion

Mechanical stimuli due to heartbeats and fluid flow forces influence the cardiac function since heart genesis initiates. Heart experiences various forces such as shear stress which creates when blood flow friction acts on endothelium, pressure due to blood flow as a result of beating and cyclic stretch that is produced by pulsatile blood flow. [34,35]

Numerous studies have attempted to demonstrate that mechanical load such as shear stress or cyclic stretch can lead to upregulation of cardiac transcription factor expression such as GATA4 along with mechanical load-responsive transcriptional mediator in cardiogenesis. [14, 36-38] Though, far too little attention has been paid to effect of applications of pre-differentiated cells under cyclic equiaxial stretch in regenerating of myocardial ischemia *in vivo*. Undoubtedly, effects of mechanical loading is vary among different kinds of stem cells, and consequently different results in cardiac repair following implantation such cells into myocardial ischemia have been observed. [39] Eding et all. in 2015 in a systematic review and meta-analysis of animal studies demonstrated that the effect size [difference in mean LVEF (mean±SEM) between treated and placebo per subgroup] was 8.0±0.7 for MSCs (n=536), 7.6±1.3 for BMMNCs (n=286) and 5.2±4.1 for CSCs (n=64).[9]

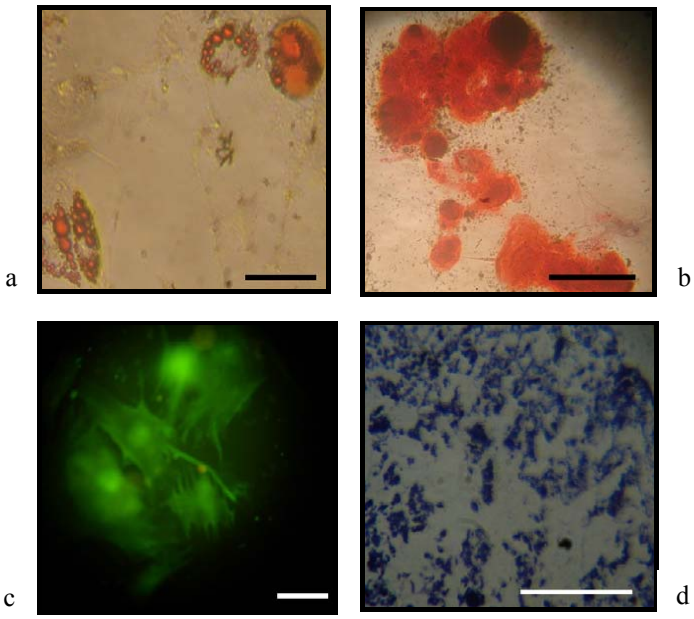

Figure 3. Differentiation capability of ASCs to mesodermal lineage (a) stained lipid vacuoles – photo taken by inverted microscope (400X), (b) stained the calcium deposition - photo taken by inverted microscope (400X), (c) stained glycosaminoglycans in a section of pelleted cells - photo taken by optical microscope (400X), (d) Connexin43 expression after cardiomyogenic differentiation - photo taken by fluorescence microscope (630X).

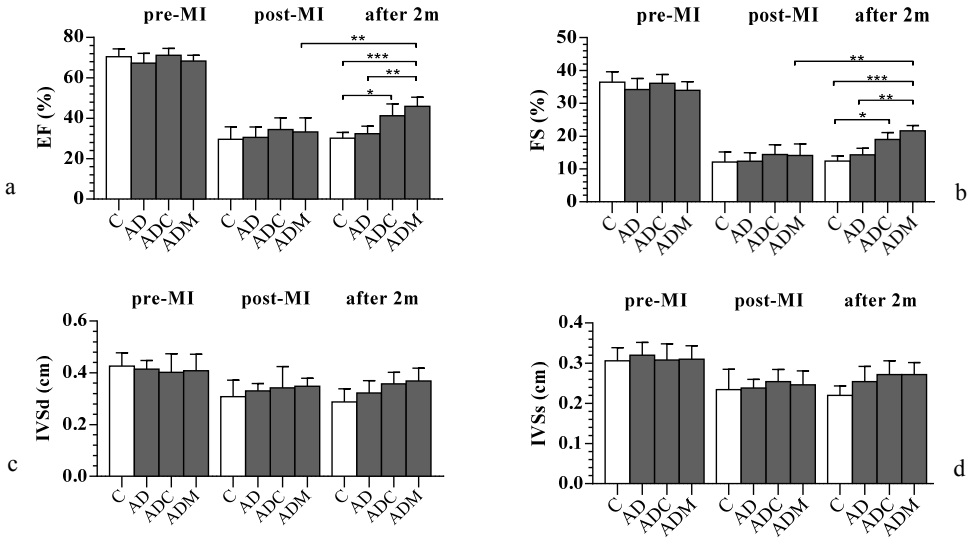

Figure 4. Transplanted ADCs improved cardiac function. (a) LV ejection fraction (LVEF) measured by 2D echocardiography, was significantly increased in the ADM group at 2 months follow-up. (b) Fractional shortening (FS) an overall increase in ADM group in compare with control and AD group. (c and d) interventricular septal diameter end systole and end diastole (IVSs and IVSd) did not showed statistically different between various group.
ADM: mechanical-differentiated ADCs; ADC: chemical-differentiated ADCs; AD: undifferentiated ADCs; C: control group; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$

Among the cell sources, adipose tissue represents an attractive source of stem cells for cardiac cell therapy. This is due to the fact that adipose-derived stem cells (ASCs) (i) can be obtained from patients through an easy procedure with low morbidity, (ii) can be efficiently isolated and cultured, (iii) exert a potent paracrine effect involved in tissue revascularization and reduction of myocardial remodeling, and (iv) show immunomodulatory properties. [40] The efficacy of ASCs has already been demonstrated in animal models of MI

Table 1. Echocardiographic parameters 2 months after cell transplantation

Group	IVSs	IVSd	EF	FS
C	0.22 ± 0.02	0.28 ± 0.05	30.16 ± 2.9 [a, c]	12.42 ± 1.5 [d,f]
AD	0.25 ± 0.03	0.32 ± 0.04	32.37 ± 3.8 [b]	14.29 ± 2.0 [e]
ADC	0.27 ± 0.03	0.35 ± 0.03	41.29 ± 5.7[c]	19.02 ± 2.0 [f]
ADM	0.27 ± 0.2	0.36 ± 0.04	45.96 ± 4.3 [a,b]	21.58 ± 1.6 [d, e]

a,d: significantly different ($p<0.001$)
b, e ,: significantly different ($p<0.05$)
c, f: significantly different ($p<0.01$)
C: control group; AD: Adipose-derived mesenchymal stem cell; ADC: Adipose-derived mesenchymal stem cell induced by chemical factor; ADM: Adipose-derived mesenchymal stem cell induced by mechanical factor; IVSs: Interventricular Septal diameter end systolic; IVSd: Interventricular Septal diameter end diastolic; EF: Ejection Fraction; FS: Fractional Shortening

and clinical trials have recently been completed (APOLLO and PRECISE) exploring the safety and feasibility of ASCs transplantation in MI patients. [41-43]
This study was conducted to evaluate cardiac function of in vivo transplantation of ASCs under chemical and mechanical stimulation. AMI was induced in rabbits and ASCs were transplanted in border zone of infarct site. Echocardiographic study demonstrated a significantly improve in LVEF and FS in mechanical adipose-derived MSCs (ADM) in compare to control group and undifferentiated adipose-derived cells after 2 months by increasing LVEF 13.52% (mean) and 15.66% (mean), respectively. LVEF of ADM group was 4.6% (mean) higher than ADC group, however did not statistically significant difference. EF of ADM group also showed significantly increase in compare with post-MI for 12.68%. Fractional shortening also showed the same pattern and ADM was significantly differ from control and AD group after two months, indeed post-MI value. FS also does not statistically significant change in compare to ADC group. Other echocardiographic parameters related to heart remodeling and diastolic function such as IVSs (interventricular septal diameter end systolic) and IVSd (interventricular septal diameter end diastolic), LVEDs (left ventricular end Systolic diameter) and LVEDd (left ventricular end diastolic diameter) did not statistically change between groups during the study. However, IVSs and IVSd decreased post-MI and slightly increased after 2 month in AD, ADC and ADM groups, although not significantly differ. Improvement of EF in this study was according to others results in which MSCs were used in vivo.
Formerly, we showed that application of cyclic equiaxial mechanical load on bone marrow- and adipose derived mesenchymal stem cells upregulates GATA4 expression. [30] Findings of our previous study are consistent with other research which found the impact of uniaxial strain. Mechanobiology MSC studies of parker et all also have found that shear stress increases the expression of angiogenic factors. Uniaxial strain studies have also showed an upregulation in SM markers with physiological arterial strains, while equiaxial strain decreases SM marker expression. 31 Huang in 2010 demonstrated that utilizing FSS (fluid shear stress) besides 5-azacitidin up-regulates cardiogenic markers expression, probably, due to synergistic interaction. [37] Gue et al carried out an experiment and identified the upregulating of GATA4 expression in MSCs owing to apply uniaxial cyclic strain combined with 5-azacytidine. [44] In contrast, another study claimed that cyclic equibiaxial stimulation is not capable to affect GATA4 expression level. [44] This controversy may be due to usage of a low tensile strain (8%). Preclinical and clinical studies have proved that mesenchymal stem cells regenerate the infarcted myocardial as their paracrine properties. Hence, by bioluminescence imaging, van der bogt found short term survival rate of mesenchymal stem cells after injection into myocardial infarction models. 45 This finding corroborate previous studies which identified less than 2% of originally transplanted mesenchymal stem cells after 6 weeks following intramyocardial transplantation. 46 Indeed, Nakamura and colleagues has consistently shown that only 4.4% of transplanted mesenchymal stem cells were identifiable after 1 week post-transplantation.[47] Scientists proved that genetically modified MSCs or pre-differentiation of MSCs in vitro by the use of mechanical, electrical, chemical and etc. before injection into infarcted myocardium not only can enhance the paracrine effects of MSCs, but also increase the survival time of cells.[48-50] According to these results and consequences of current study, suggest that pre-differentiated mesenchymal stem cells can improve cardiac function more than undifferentiated MSCs. However, more studies are needed to identify the fate of MSCs after in vivo transplantation and survival rate of them.

Conclusion

This study demonstrates that intramyocardial transplantation of mechanically-differentiated ASCs improves cardiac function after acute myocardial infarction through enhancement of regeneration in the ischemic myocardium by the effect of cyclic equiaxial strain on enhancing expression of cardiac proteins and

genes in ASCS. Transplantation of mechanically differentiated ASCs for myocardial regeneration may become the future therapy for acute myocardial infarction.

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References

1. Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, Carnethon MR, Dai S, de Simone G, Ford ES. Heart disease and stroke statistics—2011 update a report from the american heart association. *Circulation*. 2011;123:e18-e209
2. Petersen S, Peto V, Rayner M, Leal J, Luengo-Fernandez R, Gray A. *European cardiovascular disease statistics*. Department of Public Health, University of Oxford; 2005.
3. Thom T, Haase N, Rosamond W, Howard VJ, Rumsfeld J, Manolio T, Zheng Z-J, Flegal K, O'donnell C, Kittner S. Heart disease and stroke statistics—2006 update a report from the american heart association statistics committee and stroke statistics subcommittee. *Circulation*. 2006;113:e85-e151
4. Pasumarthi KB, Field LJ. Cardiomyocyte cell cycle regulation. *Circulation research*. 2002;90:1044-1054.
5. Rubart M, Field LJ. Cardiac regeneration: Repopulating the heart. *Annu. Rev. Physiol.* 2006;68:29-49
6. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nature biotechnology*. 2007;25:1015-1024
7. Windecker S, Bax JJ, Myat A, Stone GW, Marber MS. Future treatment strategies in st-segment elevation myocardial infarction. *The Lancet*. 2013;382:644-657
8. Gálvez-Montón C, Prat-Vidal C, Roura S, Soler-Botija C, Bayes-Genis A. Cardiac tissue engineering and the bioartificial heart. *Revista Española de Cardiología (English Edition)*. 2013;66:391-399
9. Eding JEC, Vesterinen HM, van der Spoel TIG, Sena ES, Duckers HJ, Doevendans PA, Macleod MR, Chamuleau SAJ. Similar effect of autologous and allogeneic cell therapy for ischemic heart disease systematic review and meta-analysis of large animal studies. *Circulation research*. 2015;116:80-86
10. Azene N, Fu Y, Maurer J, Kraitchman DL. Tracking of stem cells in vivo for cardiovascular applications. *Journal of Cardiovascular Magnetic Resonance*. 2014;16:7
11. Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, Granton J, Stewart DJ. Safe ty of cell therapy with mesenchymal stromal cells (safecell): A systematic review and meta-analysis of clinical trials. 2012
12. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002;105:93-98
13. Amado LC, Saliaris AP, Schuleri KH, John MS, Xie J-S, Cattaneo S, Durand DJ, Fitton T, Kuang JQ, Stewart G. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:11474-11479
14. Huang Y, Zheng L, Gong X, Jia X, Song W, Liu M, Fan Y. Effect of cyclic strain on cardiomyogenic differentiation of rat bone marrow derived mesenchymal stem cells. 2012
15. Wang JH, Li B. Mechanics rules cell biology. *BMC Sports Science, Medicine and Rehabilitation*. 2010;2:16
16. Holle AW, Engler AJ. More than a feeling: Discovering, understanding, and influencing mechanosensing pathways. *Current opinion in biotechnology*. 2011;22:648-654
17. Wang JHC, Thampatty BP. Mechanobiology of adult and stem cells. *International Review of Cell and Molecular Biology*. 2008;271:301-346
18. Tschumperlin DJ, Dai G, Maly IV, Kikuchi T, Laiho LH, McVittie AK, Haley KJ, Lilly CM, So PT, Lauffenburger DA. Mechanotransduction through growth-factor shedding into the extracellular space. *Nature*. 2004;429:83-86
19. Chen K-D, Li Y-S, Kim M, Li S, Yuan S, Chien S, Shyy JY. Mechanotransduction in response to shear stress roles of receptor tyrosine kinases, integrins, and shc. *Journal of Biological Chemistry*. 1999;274:18393-18400
20. Ingber DE. Tensegrity: The architectural basis of cellular mechanotransduction. *Annual review of physiology*. 1997;59:575-599
21. Szafranski JD, Grodzinsky AJ, Burger E, Gaschen V, Hung H-H, Hunziker EB. Chondrocyte mechanotransduction: Effects of compression on deformation of intracellular organelles and relevance to cellular biosynthesis. *Osteoarthritis and cartilage*. 2004;12:937-946
22. Katsumi A, Naoe T, Matsushita T, Kaibuchi K, Schwartz MA. Integrin activation and matrix binding mediate cellular responses to mechanical stretch. *Journal of Biological Chemistry*. 2005;280:16546-16549
23. Ahmed WW, Wolfram T, Goldyn AM, Bruellhoff K, Rioja BA, Möller M, Spatz JP, Saif TA, Groll J, Kemkemer R. Myoblast morphology and organization on biochemically micro-patterned hydrogel coatings under cyclic mechanical strain. *Biomaterials*. 2010;31:250-258

24. Ohlstein B, Kai T, Decotto E, Spradling A. The stem cell niche: Theme and variations. *Current opinion in cell biology*. 2004;16:693-699
25. Lee DA, Knight MM, Campbell JJ, Bader DL. Stem cell mechanobiology. *Journal of cellular biochemistry*. 2011;112:1-9
26. Cagri Uysal A, Mizuno H. Tendon regeneration and repair with adipose derived stem cells. *Current stem cell research & therapy*. 2010;5:161-167
27. Sandino C, Checa S, Prendergast PJ, Lacroix D. Simulation of angiogenesis and cell differentiation in a cap scaffold subjected to compressive strains using a lattice modeling approach. *Biomaterials*. 2010;31:2446-2452
28. Nagel T, Kelly DJ. Mechano-regulation of mesenchymal stem cell differentiation and collagen organisation during skeletal tissue repair. *Biomechanics and modeling in mechanobiology*. 2010;9:359-372
29. Bhang SH, Gwak SJ, Lee TJ, Kim SS, Park HH, Park MH, Lee DH, Lee SH, Kim BS. Cyclic mechanical strain promotes transforming-growth-factor-β1-mediated cardiomyogenic marker expression in bone-marrow-derived mesenchymal stem cells in vitro. *Biotechnology and applied biochemistry*. 2010;55:191-197
30. Amin S, Banijamali SE, Tafazoli-Shadpour M, Shokrgozar MA, Dehghan MM, Haghighipour N, Mahdian R, Bayati V, Abbasnia P. Comparing the effect of equiaxial cyclic mechanical stimulation on gata4 expression in adipose-derived and bone marrow-derived mesenchymal stem cells. *Cell biology international*. 2014;38:219-227
31. Park JS, Huang NF, Kurpinski KT, Patel S, Hsu S, Li S. Mechanobiology of mesenchymal stem cells and their use in cardiovascular repair. *Front Biosci*. 2007;12:5098-5116
32. Huang NF, Li S. Mesenchymal stem cells for vascular regeneration. 2008
33. Schiller NB, Shah P, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American society of echocardiography committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. *Journal of the American Society of Echocardiography: official publication of the American Society of Echocardiography*. 1988;2:358-367
34. Patwari P, Lee RT. Mechanical control of tissue morphogenesis. *Circulation research*. 2008;103:234-243
35. Bartman T, Hove J. Mechanics and function in heart morphogenesis. *Developmental dynamics*. 2005;233:373-381
36. Schmelter M, Ateghang B, Helmig S, Wartenberg M, Sauer H. Embryonic stem cells utilize reactive oxygen species as transducers of mechanical strain-induced cardiovascular differentiation. *The FASEB journal*. 2006;20:1182-1184
37. Huang Y, Jia X, Bai K, Gong X, Fan Y. Effect of fluid shear stress on cardiomyogenic differentiation of rat bone marrow mesenchymal stem cells. *Archives of medical research*. 2010;41:497-505
38. Heineke J, Auger-Messier M, Xu J, Oka T, Sargent MA, York A, Klevitsky R, Vaikunth S, Duncan SA, Aronow BJ. Cardiomyocyte gata4 functions as a stress-responsive regulator of angiogenesis in the murine heart. *The Journal of clinical investigation*. 2007;117:3198-3210
39. Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. *Circulation research*. 2002;91:1092-1102
40. Mizuno H, Tobita M, Orbay H, Uysal AC, Lu F. Adipose-derived stem cells as a novel tool for future regenerative medicine. *Stem cells and cancer stem cells, volume 12*. Springer; 2014:165-174.
41. Valina C, Pinkernell K, Song Y-H, Bai X, Sadat S, Campeau RJ, Le Jemtel TH, Alt E. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. *European heart journal*. 2007;28:2667-2677
42. Mazo M, Cemborain A, Gavira JJ, Abizanda G, Araña M, Casado M, Soriano M, Hernández S, Moreno C, Ecay M. Adipose stromal vascular fraction improves cardiac function in chronic myocardial infarction through differentiation and paracrine activity. *Cell transplantation*. 2012;21:1023-1037
43. Araña M, Gavira JJ, Peña E, González A, Abizanda G, Cilla M, Pérez MM, Albiasu E, Aguado N, Casado M. Epicardial delivery of collagen patches with adipose-derived stem cells in rat and minipig models of chronic myocardial infarction. *Biomaterials*. 2014;35:143-151
44. Guo Y. Effects of mechanical stimulus on mesenchymal stem cells differentiation toward cardiomyocytes. *Asian Biomedicine (Research Reviews and News)*. 2011:655
45. van der Bogt KE, Schrepfer S, Yu J, Sheikh AY, Hoyt G, Govaert JA, Velotta JB, Contag CH, Robbins RC, Wu JC. Comparison of transplantation of adipose tissue-and bone marrow-derived mesenchymal stem cells in the infarcted heart. *Transplantation*. 2009;87:642
46. Müller-Ehmsen J, Krausgrill B, Burst V, Schenk K, Neisen UC, Fries JW, Fleischmann BK, Hescheler J, Schwinger RH. Effective engraftment but poor mid-term persistence of mononuclear and mesenchymal bone marrow cells in acute and chronic rat myocardial infarction. *Journal of molecular and cellular cardiology*. 2006;41:876-884
47. Nakamura Y, Wang X, Xu C, Asakura A, Yoshiyama M, From AH, Zhang J. Xenotransplantation of long-term-cultured swine bone marrow-derived mesenchymal stem cells. *Stem Cells*. 2007;25:612-620
48. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, Dzau VJ. Mesenchymal stem cells modified with akt prevent remodeling and restore performance of infarcted hearts. *Nature medicine*. 2003;9:1195-1201
49. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, Noiseux N, Zhang L, Pratt RE, Ingwall JS. Paracrine action accounts for marked protection of

ischemic heart by akt-modified mesenchymal stem cells. *Nature medicine*. 2005;11:367-368

50. Gonzalez-Garza MT, Alcaraz C, Gonzalez-Jara L, Arcos D, Cuevas JEM. Engraftment of pre-differentiated stem cells into cardiomyocytes in an animal model of ischemic cardiopathy. *Stem Cell Discovery*. 2013;2014

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چکیده

بهبود عملکرد قلب پس از پیوند سلول‌های مزانشیمی خودی مشتق از بافت چربی پیش-تمایز یافته قلبی تحت تاثیر بارگذاری مکانیکی در انفارکتوس حاد میوکارد در مدل حیوانی خرگوش

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هدف- بررسی کارایی میوکارد قلب به دنبال پیوند سلول مزانشیمی مشتق از چربی که تحت بار مکانیکی قرار گرفتند، در انفارکتوس حاد قلبی خرگوش

طرح- مطالعه تجربی آینده نگر

حیوانات- ۲۰ سر خرگوش سفید نیوزلندی (وزن ۲ تا ۳ کیلوگرم)

روش کار- سلول‌های مزانشیمی مشتق از چربی در چهار گروه مجزا شامل گروه مکانیکی، شیمیایی، تمایز نیافته و شاهد مطالعه شدند. طبق این دسته بندی، سلول‌ها تحت لود بار مکانیکی و یا ۵-آزاسایتیدین (به عنوان فاکتور شیمیایی) قرار گرفتند. $۱۰^{۶}$ سلول مزانشیمی مشتق از چربی داخل میوکارد قلب خرگوش به دنبال انفارکتوس حاد میوکارد تزریق شد. بررسی اکوکاردیوگرافی جهت ارزیابی عملکرد قلبی مورد استفاده قرار گرفت.

نتایج -کسر جهشی (Ejection fraction) بطن چپ بعد از گذشت ۲ ماه در گروه سلول‌های مزانشیمی مشتق از چربی تمایز یافته به روش مکانیکی به طور معنی‌داری افزایش یافت. کسر کاهشی (Fractional shortening) نیز با الگویی مشابه کسر جهشی افزایش معنی-داری نسبت به گروه کنترل و تمایز نیافته، نشان داد.

نتیجه‌گیری و کاربرد بالینی- نتایج این مطالعه نشان می‌دهد که سلول مزانشیمی مشتق از چربی تمایز یافته به روش مکانیکی به دنبال تزریق داخل میوکارد در انفارکتوس حاد قلبی باعث بهبودکارکرد قلب می‌شود. پیوند سلول‌های مزانشیمی مشتق از چربی تمایز یافته به روش مکانیکی می تواند در آینده برای درمان انفارکتوس میوکارد به کار رود.

کلمات کلیدی- سلول مزانشیمی مشتق از چربی، تحریک مکانیکی، انفارکتوس حاد میوکارد، اکوکاردیوگرافی.