Therapeutic angiogenesis promotes efficacy of human umbilical cord matrix stem cell transplantation in cardiac repair

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A R T I C L E I N F O	A B S T R A C T
	Objective(s): Although previous studies have confirmed the beneficial effects of human umbilical cord matrix stem cell (hUCM) transplantation post myocardial infarction (MI), but this stem cell resource has no potential to induce angiogenesis. In order to achieve the process of angiogenesis and cardiomyocyte regeneration, two required factors for cardiac repair agents were examined namely; hUCM and VEGF on an infarcted heart. The main objective of this research is to investigate the combinatory effect of dhUCM and VEGF transplantation on an infarcted heart. *Materials and Methods:* 45 min of ligating the left anterior descending coronary artery, the MI-induced animals received 50 µl PBS, 5 µg VEGF, 5×10^6 hUCM cells alone, combined with 5 µg VEGF and 5×10^6 differentiated hUCM cells alone or combined with 5 µg VEGF through intramyocardial injection. MI group, without hUCM and VEGF served as the control group. Left ventricular function and angiogenesis were also evaluated. *Results:* After eight weeks post MI, there were significant rise in left ventricular ejection farction in dhUCM+VEGF group compared to the other treated and non-treated groups ($P<0.05$). Fibrosis tissue was markedly lower in the dhUCM+VEGF and hUCM+VEGF groups compared to the other treated and non-treated groups ($P<0.05$). Despite these benefits, vascular density in dhUCM+VEGF group was not markedly different compared to VEGF and hUCM+VEGF groups. The transplanted hUCM and dhUCM cells survived and migrated to the infarcted area. *Conclusion:* Our findings demonstrated that the dhUCM cells transplantation combined with VEGF were more efficient on an infarcted heart.

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Introduction

Despite many advances in medical sciences, coronary disease which may lead to myocardial infarction (MI) is a complex one and still remained the leading cause of mortality in developed countries (1) Previous studies have proven that human umbilical cord matrix stem cell (hUCM) injected intravenously improved the functional recovery of a traumatic brain Injuries in rats (2). Angiogenic and stem cell therapies have generated much excitement as novel therapeutic approaches that might provide additive benefits on conventional treatments to restore left ventricular dysfunction after MI (3-6). Angiogenesis and replacement of lost cardiomyocytes with new, live cardiomyocytes are considered as key factors in cardiac repair (7). Apart from the use of stem cells and angiogenic therapies, the use of antioxidants to protect

he myocardium from isoproterenol-induced MI through reduction of lipid peroxidation would be effective (8). Angiogenesis is defined as formation and development of blood vessels. The use of vascular endothelial growth factor (VEGF) for angiogenic therapies have demonstrated beneficial effects of angiogenesis by decreasing apoptosis and improving heart functions but replacing lost cardiomyocytes with live ones requires stem cell therapy (9-11). Many studies have shown that stem cell therapy is a promising strategy in replacing lost cardiomyocytes and thereby reducing size of infarcted parts of the heart and improvement of cardiac functions (12-14). Currently, scientists have focused on hUCM due to their great expansion capability, non-invasive isolation procedure, low risk of immune system reaction and teratoma formation in the recipient (15-17). Studies

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that have examined the effects of transplantation of hUCM into infarcted heart are rare and therefore its application requires more assessment. Study have demonstrated the beneficial effects of hUCM cells on improvement of cardiac function 4 weeks post MI, however angiogenesis was never observed (18). We assume that of beneficial synergic effects are existed between stem cells combined with VEGF transplantation which may cover the limitations related to stem cells or VEGF alone transplantation. Here we have described the effects of myocardial injection of hUCM and hUCM-derived cardiomyocytes either alone or in combination with VEGF into infarcted heart on cardiac function, 8 weeks post MI.

Materials and Methods
Isolation and culture of hUCM cells
Umbilical cords were obtained after birth and collected in Hank's balanced salt solution (HBSS) (Gibco, USA), with informed consent of the parents. Umbilical cord blood vessels were cleared off and the remaining matrices were divided into fragments and then cultured in culture plates containing a complete medium DMEM-F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) supplemented with 15% FBS (Gibco, USA), 100 U/ml penicillin, 60 µg/ml streptomycin (Gibco, USA) in a humidified 37 °C incubator with 5% CO_2. After expansion of fibroblast shape cells, the fragments were discarded and non-adherent cells were removed by changing the media every three days. Cells at 80-90% confluence were trypsinized and sub-cultured in new medium.

Characterization of hUCM cells
A) *Expression of cell surface molecules*
After trypsinization, the detached cells were washed and suspended in PBS. 1×10^6 cells/ml hUCM cells were incubated with primary antibodies against CD73, CD29, CD34, CD44, CD45, CD90, CD105, HLA-ABC (HLA-1) and HLA-DR (all ebiosciences, USA) as described in previous studies (16, 17) at room temperature followed by fluorescent secondary antibodies. Control hUCM cells were stained with a secondary antibody alone. The cells were assessed in a FAC Scan (Fluorescence Associated Cell Sorter Scan) machine (Becton, Dickinson).

B) *In vitro differential potential*
hUCM cells at passage 4 were plated into adipogenic differentiation media containing complete medium supplemented with 10% FBS and 100 nM dexamethasone [Sigma, St. Louis] and into osteogenic differentiation media containing complete medium, 80 µg/ml ascorbic acid, 10 nM dexamethasone, and 10 nM β-glycerophosphate [Sigma, St. Louis].

Three weeks later, the cells were fixed by 4% paraformaldehyde in PBS and underwent both oil red and Alizarin red staining for detection of adipogenic and osteogenic differentiation, respectively.

Induction of cardiac differentiation
hUCM cells at passage 4 were incubated with cardiogenic differentiation medium containing complete medium and 10 nM Oxytocin (Wako chemical, Germany) for 72 hr. Then the differentiation medium was replaced with complete medium and was regularly refreshed every three days, for three weeks.

Immnuocytochemical analysis
Oxytocin- treated cells (dhUCM) and hUCM cells served as the control and were fixed in 4% formaldehyde (Sigma, USA), permeabilized with 0.1% triton X-100 (Sigma, USA) in PBS for 15 mins and then incubated overnight at 4 °C with primary antibodies against cardiac troponin T (cTnT, abcam, UK), sarcomeric actin (sA, abcam, UK), cardiac actin (cA, sigma, USA) followed by fluorescent secondary antibodies (FITC and Alexa Fluor) for 2 hr at 37 °C (19).

Cell preparation
hUCM and dhUCM cells were labeled by Cell tracker CM-DiI according to manufacturer's instruction (Invitrogen, USA), before cell transplantation.

Induction of MI model and cell transplantation
Eighty white New Zealand male rabbits (weighing 3-3.5 kg) were randomly divided into eight groups of 10 animals each: (a) intact group (non-MI); (b) MI group consisting of MI model; (c) PBS group consisting of MI model and PBS; (d) VEGF group; (e) hUCM group; (f) dhUCM group; (g) hUCM + VEGF group; (h) dhUCM + VEGF group. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally), intubated and ventilated using a ventilator. After the left thoracotomy, a 0.6 mm silk ligature was placed and tied closely around the proximal end of the left anterior descending coronary artery (LAD). 45 min later, 5×10^6 cells with or without 5 µg VEGF in normal saline were injected into the margin of MI region of the VEGF recipient groups (d, e, f, g, h). The injection was administered by 28 gauge needle attached to an insulin syringe. Cyclosporine A (10 mg/kg, subcutaneously) was administered 24 hr before the cell therapy, once daily. This investigation was approved by the Ethical Committee at Tehran University of Medical Science, Iran.

Echocardiography
Left ventricular ejection fraction (LVEF) was measured using a trans-thoracic echocardiographic device (Vivid3; General Electric) equipped with a 5-MHz linear transducer, five days and eight weeks
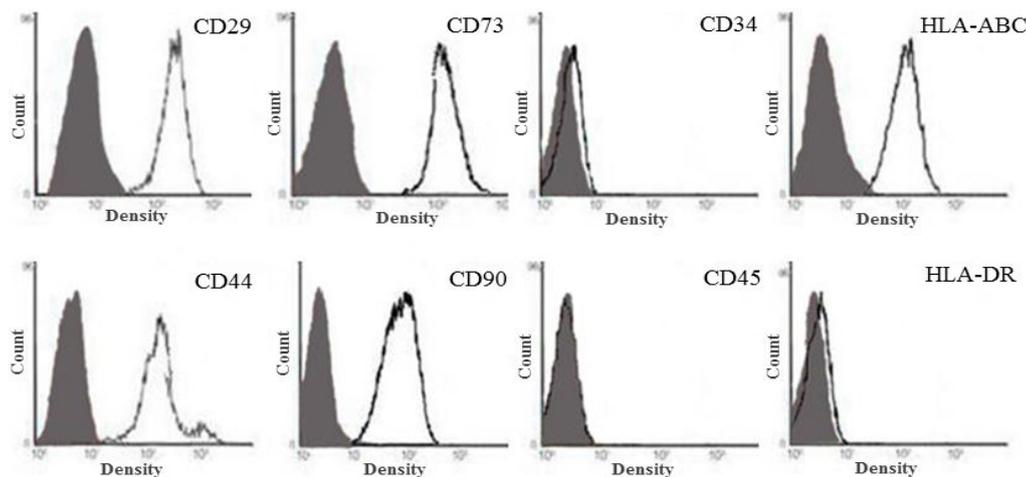
Figure 1. Flowcytometric analysis of hUCM cells. The hUCM cells were positive for mesenchymal specific markers (CD29, CD44, CD73 and CD90) but negative for (CD34, CD45). hUCM cells were positive for HLA-1 but negative for HLA-DR, which indicated the low risk of recipient inflammatory reaction. The represented data obtained after 3 experiments

after MI in all groups. The Cardiologist was blinded to the treatment groups.

Histological and immunohistochemical analysis

Eight weeks post MI, animals were sacrificed and their hearts were then removed, washed in PBS and fixed in 10% formaldehyde. Then, 5 μm thick sections were prepared from infarcted areas and stained with hematoxylin and eosin (H&E), as well as trichrome masson. Some selected sections were incubated with primary antibody against CD31 (Bioscience, USA), followed by peroxidase conjugated secondary antibody, each one for 1 hr at room temperature. CD31-positive cells with a vascular structure were counted as a vessel (capillary or artery). Vascular density and fibrosis tissue of myocardium have been quantified as described in previous studies (17, 18). For immunohistochemical studies, the prepared sections from cell-treated groups were incubated overnight 4 °C with primary antibody against cardiac α-actinin (cAn, Sigma, USA) followed by Fluorescein isothiocyanate(FITC) and Alexa Fluor for one hour.

Statistical analysis

Statistical analyses were performed by ANOVA test using SPSS 19.0 software. A *P*-value of <0.05 was considered significant. Data is expressed as means±SD.

Results

Culture and Characterization of hUCM cells

The hUCM cells have expressed mesenchymal specific markers [CD29 (93%), CD44 (89%), CD73 (97%), CD90 (92%)], but did not expressed CD34 and CD45 hematopoietic specific markers. Moreover, the hUCM cells were positive for HLA-ABC (95%) and negative for HLA-DR (Figure. 1). The primary

isolates appeared in fibroblast shape cells possessing cytoplasmic processes which remained in similar morphology after several passages (Figure 2c). Differential potential was confirmed following their differentiation into adipogenic and osteogenic linages (Figure 2a, 2b).

Cardiac differentiation of hUCM cells

Three weeks after induction of cardiogenic differentiation, the Oxytocin-treated cells appeared as flat shape cells with short cytoplasmic and striped cytoplasm as well as spontaneous beating of these cells was observed under microscope (Figure 2d). Also, dhUCM cells expressed some contractile proteins including sA, cA and cTnT, indicating fibrillar and contractile structure of the dhUCM cells (about 65% of oxytocin-treated cells). The hUCM cells served as the control group and were negative for contractile proteins (Figure 3).
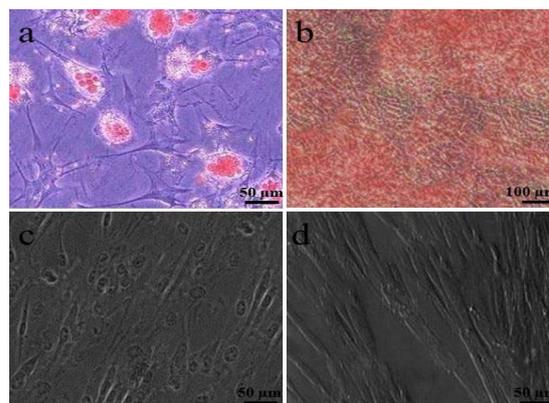
Figure 2. Adipogenic and osteogenic differentiation of hUCM stem cells were verified by (a) Oil-Red and (b) Alizarin Red Staining, respectively. After treating hUCM cells with oxytocin, fibroblast shape hUCM cells (c) were turned into cardiomyocytes with short cytoplasmic extensions and fibrillar structure (d)
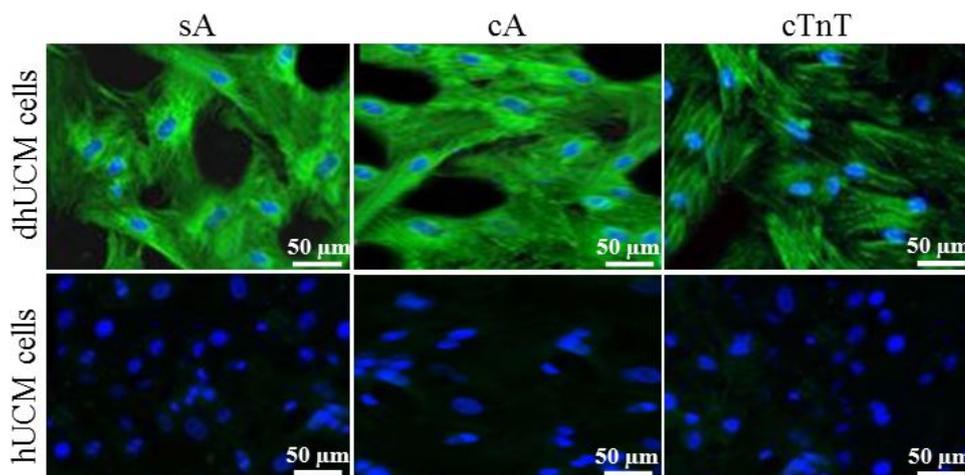
Figure 3. Immunohistological analysis has shown that the dUCM cells expressed sarcomeric actin (sA), cardiac actin (cA) and cardiac troponin T (cTnT). The hUCM cells did not express these contractile proteins. All cells nuclei were stained by DAPI

Evaluation of myocardial function

Five days post MI, LVEF decreased significantly in all MI-induced groups compared to the control group ($P<0.01$) but there was no significant difference among the MI groups. Eight weeks post MI, LVEF improved significantly ($P<0.05$) in all treated groups compared to the MI and PBS ones. The dhUCM+VEGF group exhibited significant increase in LVEF ($P<0.05$) level when compared to other treated groups. Increase in LVEF was significantly higher ($P<0.05$) in dhUCM+VEGF group compared to VEGF, hUCM, hUCM and hUCM+VEGF groups (Figure 4).

Histological and immunohistochemical analysis

Vascular densities were significantly higher in VEGF, hUCM+VEGF and dhUCM+VEGF groups than other treated and non-treated groups ($P<0.05$). There were no significant differences in vascular density among VEGF, hUCM+VEGF and dhUCM+VEGF groups (Table 1). The amount of fibrosis tissue of myocardium was determined by trichrome masson staining. The fibrosis of myocardium in all treated groups were significantly less than to other non-treated groups ($P<0.05$). The hUCM+VEGF and dhUCM+VEGF groups showed significantly the lowest level of fibrosis compared to other treated groups ($P<0.05$) (Table 1). No significant difference was observed between hUCM+VEGF and dhUCM+VEGF groups. The transplanted hUCM and dhUCM cells were observed in infarcted myocardium and in a remote distance from the injection sites (Figure 5a, 5b, 5e, 5f). The α- actinin stained sections confirmed that the transplanted dhUCM cells expressed the α-actinin, but transplanted hUCM cells did not express α-actinin in cardiac microenvironment (Figure 5c, 5d, 5g, 5h).
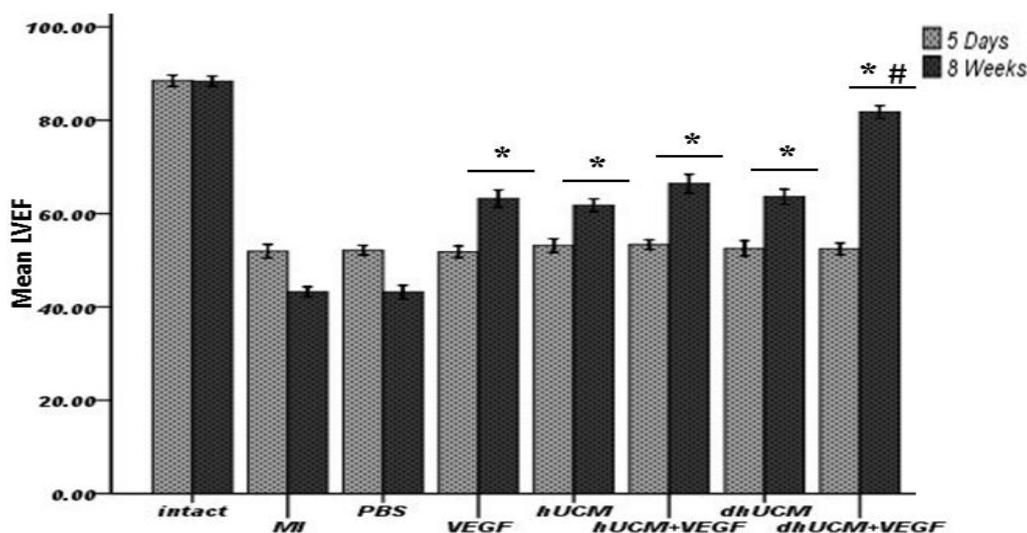

Figure 4. Eight weeks post MI, the animals in all the treated groups showed a significant improvement in LVEF compared to the MI and PBS groups. LVEF in the dhUCM+VEGF group was markedly higher when compared to other treated groups
Keys: * $P<0.05$ compared to MI and PBS. # $P<0.05$ compared to VEGF, hUCM, hUCM+VEGF and dhUCM

Table 1. Vascular density and fibrosis tissue of the myocardium

	Intact	MI	PBS	VEGF	hUCM	hUCM +VEGF	dhUCM	dHUCM+VEGF
CD32+ vessels	108/3±3.6	51.8±4.2	54.8±4.6	* 83.6±2.8	59.3±5.1	* 89.6±3.6	57.3±47	* 86.3±3.7
Fibrosis area	0.4±0.1	34.5±1.8	35.1±2.1	* 17.6±2.1	* 14.5±1.6	*# 9.3±1.7	*# 13.4±16	*# 8.8±1.7

Values are means±SD. *$P<0.05$ compared to myocardial infarction and myocardial infarction Phosphate-buffered saline. # $P<0.05$ compared to vascular endothelial growth factor, human umbilical cord matrix and differentiated human umbilical cord matrix (dhUCM)
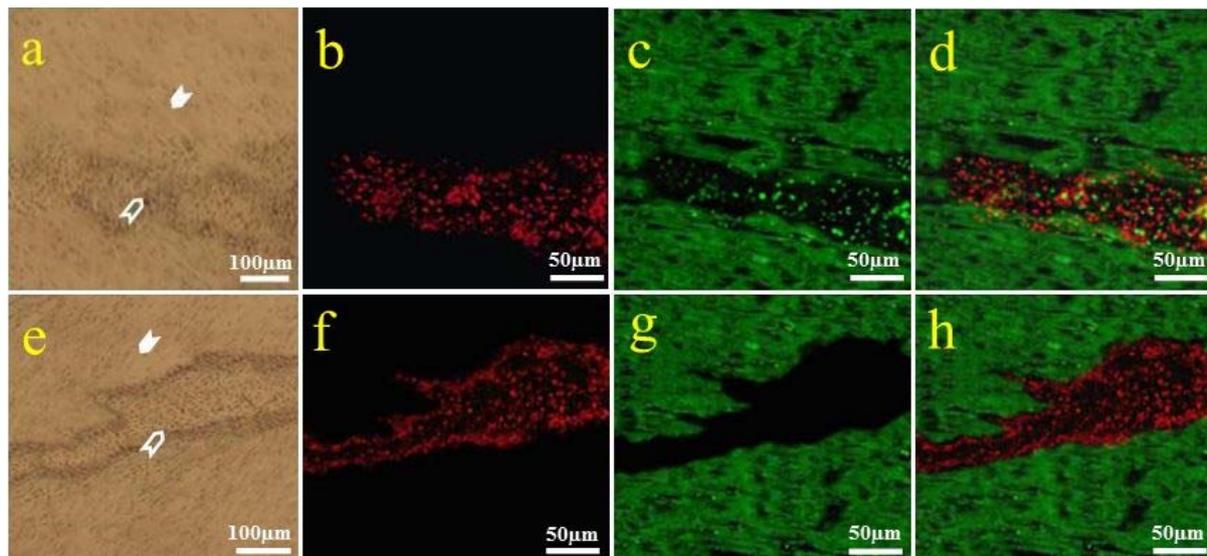
Figure 5. Transplanted differentiated hUCM cells (dhUCM) (a) hUCM cells (e) infarcted regions showed by non-filled chevron and non-infarcted regions by filled chevron, after H&E staining. Transplanted DiI-labeled dhUCM (b) and hUCM cells (f) appeared in spots within dark zone. The α- actinin stained sections confirm that transplanted dhUCM cells expresses α- actinin in cardiac tissue (c), but transplanted hUCM cells did not express α- actinin in cardiac microenvironment (g). Merged images provided Figures (d & h)

Discussion

Our achievement confirmed that transplantation of hUCM and dhUCM cells either alone or in combination with VEGF via direct intramyocardial injection improve cardiac functions and attenuate fibrosis tissue formation in infracted myocardium eight weeks after MI. Although previous studies had confirmed the beneficial effects of various stem cell resources on restoration of ischemic heart function but practical application of these sources is limited because of some associated problems (13, 19-21). The hUCM stem cells were obtained from the umbilical cord that have been routinely discarded at parturition, thus eliminating some of the problems associated with other stem cell resources (15, 18 ,22). Echocardiographic measurements revealed that the LVEF increased markedly in all treated groups compared to MI and PBS groups at eight weeks post MI (*P*<0.05). The dhUCM+VEGF group shows significant increase in level among the treated groups (*P*<0.05) (Figure 4). The dhUCM+VEGF transplantation was more efficient when compared to VEGF, hUCM and dhUCM and hUCM+VEGF

transplantation, one possible reason is because of the synergic effects of dhUCM cells and VEGF.

Scientists have rarely investigated synergic effects of stem cells and pro-angiogenic factors administration on MI. In Yang's study BM-MSCs transplantation combined with HGF (proangiogenic factor) improved cardiac function and angiogenesis, but were not efficient when compared to only BM-MSCs transplantation (23). Our study confirmed that the combination of dhUCM cells and VEGF have more beneficial effects than the administration of dhUCM cells or VEGF on infarcted myocardium. The proposed potential synergistic effect between dhUCM cells and VEGF is explained as thus. Although, cardiac microenvironment potentially induces stem cells to cardiomyocyte differentiation, some studies have shown that less than 5% of cardiomyocytes are derived from stem cells in the recipient cardiac tissue (24, 25). About one billion cardiomyocytes are lost during post MI, therefore the number of transplanted stem cell-derived cardiomyocytes *in vivo*, may not be enough to influence the contractility of the infarcted myocardium (27). We

induced the hUCM cells to undergo cardiogenic differentiation with high quality prior to the *in vivo* administration by oxytocin, which has been confirmed using immunocytochemical analysis (Figure 3). On one hand, the contractile/functional cardiomyocytes have been delivered into the infarcted heart and the loss or mal-differentiation of hUCM cells in cardiac microenvironment has been prevented. So on the other hand, VEGF by itself diminished cardiomyocyte apoptosis, stimulated cell proliferation and mediated the migration of cardiac stem cells to infarcted myocardium post MI (28-29). According to the above statements, we delivered and protected the enough cardiomyocytes into ischemic hearts which hypothetically participated in cardiac regeneration but the exact mechanism by which they act still remain unclear. There was an insignificant increase in LVEF's level in hUCM+VEGF group than in VEGF, hUCM and dhUCM groups. The transplanted hUCM cells did not express α-actinin in recipient cardiac tissue (Figure 5 (g, h)).

Poor synergic effect between hUCM cells and VEGF could be because of the lack of cardiogenic differentiation of the transplanted hUCM cells in recipient cardiac microenvironment. Immunohistochemical study has revealed that vascular density was significantly higher in the VEGF, hUCM+VEGF and dhUCM+VEGF groups than in other treated and non-treated groups (*P*<0.05). Vascular density was insignificantly different among the VEGF, hUCM+VEGF and dhUCM+VEGF groups (Table 1). Our results have suggested that the transplanted hUCM and dhUCM cells cannot stimulate angiogenesis, in accordance with Latifpour's results (18). BM-MSCs potentially induce angiogenesis in infarcted hearts (30) but the hUCM cells do not possess this potential. Lack of the ability to induce angiogenesis is considered as a defect for dhUCM cells but this defect could be eliminated by combination with VEGF (18). VGEF induces angiogenesis through pathways which lead to induction of growth, proliferation and migration of endothelial cells to infarcted myocardium. Many studies have reported the beneficial effects of VEGF administration into infarcted hearts, however, conflicting data about its efficacy has been reported which is primarily associated with an exposed dose of it (31-35). We have considered using 5 µg VEGF in normal saline as a sufficient dose (36). Lost and hibernated cardiomyocytes replaced by fibrosis tissue after MI. Transplanted stem cells have potential to activate signaling pathways that lead to reduction of the fibrosis tissue formation in myocardium. Fibrosis tissue in the treated groups was markedly lower when compared to the MI and PBS groups. Animals in the hUCM+VEGF and dhUCM+VEGF groups markedly have lower myocardium's fibrosis tissue when compared to

animals in the other treated groups (Table 1). Immunohistochemicl analysis confirmed that the hUCM and dhUCM cells survived and migrated to infarcted myocardium, therefore lost cardiomyocytes seems to be replaced by the transplanted cells (Figure 5). Previous studies have documented that VEGF administration into infarcted heart induces some beneficial effects such as: Endothelial, CSC and aortic smooth muscle cells migration to the infarcted myocardium, neovascularization and survival of blood vessels, migration and proliferation of cardiomyocytes as well as prevention of cardiomyoctes apoptosis (28, 29, 36-38). It can be concluded that the chances of fibroblasts to proliferate in the infarcted myocardium were reduced because of the administration of hUCM, dhUCM and VEGF.

Negative influence of the transplanted cells and VEGF fibrosis tissue formation in myocardium has been duplicated in hUCM+VEGF and dhUCM+VEGF groups and as a result, hUCM+VEGF and dhUCM+VEGF groups have significant reduction in fibrosis tissue when compared to the other treated and non-treated groups.

Conclusion

The dhUCM cells combined with VEGF transplantation can improve left ventricular dysfunction, induce angiogenesis and decrease fibrosis tissue formation in infarcted myocardium in eight weeks post MI owing to the synergic effect between dhUCM cells and VEGF. The (dhUCM+VEGF) is more effective when compared to VEGF, hUCM, dhUCM and (hUCM+VEGF) administration on infarcted heart.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Acknowledgment

References

1. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG *et al*. Guidelines for the diagnosis and management of heart failure in adults: a report of the american college of cardiology foundation/american heart association task force on practice guidelines developed in collaboration with the international society for heart and lung transplantation. J Am Coll Cardiol 2009;53:e1–e90.
2. Bakhtiary M, Marzban M, Mehdizadeh M, Joghataei MT, Khoei S, Tondar M, et al. Combination of stem cell mobilized by granulocyte-colony stimulating factor and human umbilical cord matrix stem cell: therapy of traumatic brain injury in rats. Iran J Basic Med Sci 2011; 14:327-339.

3. Seghatoleslam M, Jalali M, Nikravesh MR, Hosseini M, Hamidi D, Fazel A. Therapeutic benefit of intravenous administration of human umbilical cord blood-mononuclear cells following intracerebral hemorrhage in rat. Iran J Basic Med Sci 2012;15:860-872.

4. Wu KH, Mo XM, Han ZC, Zhou B. Stem cell engraftment and survival in the ischemic heart. Ann Thorac Surg 2011; 92:1917–1925.

5. Ahn A, Frishman WH, Gutwein A, Passeri J, Nelson M. Therapeutic angiogenesis: A new treatment approach for ischemic heart disease-Part II. Cardiol Rev 2008; 16:219–229.

6. Pandya NM, Dhalla NS, Santani DD. Angiogenesis-a new target for future therapy. Vascul Pharmacol 2006; 44:265–74.

7. Beohar N, Rapp J, Pandya S, Losordo DW. Rebuilding the damaged heart the potential of cytokines and growth factors in the treatment of ischemic heart disease. Am Coll Cardiol 2010; 56:1287–1297.

8. Mehdizadeh R, Parizadeh MR, Khooei AR, Mehri S, Hosseinzadeh H. Cardioprotective effect of s   Saffron extract and safranal in isoproterenol-induced myocardial infarction in wistar rats. Iran J Basic Med Sci 2013; 16:56-63.

9. Tao Z, Chen B, Tan X, Zhao Y, Wang L, Zhu T, *et al*. Coexpression of VEGF and angiopoietin-1 promotes angiogenesis and cardiomyocyte proliferation reduces apoptosis in porcine myocardial infarction (MI) heart. Proc Natl Acad Sci U S A 2011; 108:2064–2069.

10. Higuchi T, Anton M, Saraste A, Dumler K, Pelisek J, Nekolla SG, *et al*. Reporter gene PET for monitoring survival of transplanted endothelial progenitor cells in the rat heart after pretreatment with VEGF and atorvastatin. J Nucl Med 2009; 50:1881–1886.

11. Xie X, Cao F, Sheikh AY, Li Z, Connolly AJ, Pei X, *et al*. Genetic modification of embryonic stem cells with VEGF enhances cell survival and improves cardiac function. Cloning Stem Cells 2007;9:549–563.

12. Peng C, Yang K, Xiang P, Zhang C, Zou L, Wu X, *et al*. Effect of transplantation with autologous bone marrow stem cells on acute myocardial infarction. Int J Cardiol 2013; 162:158–165.

13. Bayes-Genis A, Soler-Botija C, Farré J, Sepúlveda P, Raya A, Roura S, *et al*. Human progenitor cells derived from cardiac adipose tissue ameliorate myocardial infarction in rodents. J Mol Cell Cardiol 2010; 49:771–780.

14. Moretti A, Bellin M, Jung CB, Thies T-M, Takashima Y, Bernshausen A, *et al*. Mouse and human induced pluripotent stem cells as a source for multipotent Isl1+ cardiovascular progenitors. FASEB Journal 2010; 24:700–711.

15. Fan C-G, Zhang Q, Zhou J. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. Stem Cell Rev 2011;7:195–207.

16. Taghizadeh RR, Cetrulo KJ, Cetrulo CL. Wharton's jelly stem cells: future clinical applications. Placenta 2011; 32:S311–S315.

17. Troyer DL, Weiss ML. Concise Review: Wharton's jelly-derived cells are a primitive stromal cell population. Stem Cells 2008; 26:591–599.

18. Latifpour M, Nematollahi-Mahani SN, Deilamy M, Azimzadeh BS, Eftekhar-Vaghefi SH, Nabipour F, *et al*. Improvement in cardiac function following transplantation of human umbilical cord matrix-derived mesenchymal cells. Cardiology 2011; 120:9-18.

19. Hirata Y, Sata M, Motomura N, Takanashi M, Suematsu Y, Ono M, *et al*. Human umbilical cord blood cells improve cardiac function after myocardial infarction. Biochem Biophys Res Commun 2005; 327:609–614.

20. Chen Y, Liu W, Li W, Gao C. Autologous bone marrow mesenchymal cell transplantation improves left ventricular function in a rabbit model of dilated cardiomyopathy. Exp Mol Pathol 2010; 88:311–315.

21. Bollini S, Smart N, Riley PR. Resident cardiac progenitor cells: at the heart of regeneration. J Mol Cell Cardiol 2011; 50:296–303.

22. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, *et al*. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 2004; 22:1330–1337.

23. Yang ZJ, Ma DC, Wang W, Xu SL, Zhang YQ, Chen B, *et al*. Experimental study of bone marrow-derived mesenchymal stem cells combined with hepatocyte growth factor transplantation via noninfarct-relative artery in acute myocardial infarction. Gene Ther 2006; 13:1564–1568.

24. Müller-Ehmsen J, Krausgrill B, Burst V, Schenk K, Neisen UC, Fries JWU, *et al*. Effective engraftment but poor mid-term persistence of mononuclear and mesenchymal bone marrow cells in acute and chronic rat myocardial infarction. J Mol Cell Cardiol 2006; 41:876–884.

25. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, *et al*. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. Am Soc Clin Invest 2001; 07:1395–1402.

26. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, *et al*. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nat Med 2003; 9:1195–1201.

27. Collins SD, Baffour R, Waksman R. Cell therapy in myocardial infarction. Cardiovasc Revasc Med 2007; 8:43–51.

28. McGinn AN, Nam HY, Ou M, Hu N, Straub CM, Yockman JW, *et al*. Bioreducible polymer-transfected skeletal myoblasts for VEGF delivery to acutely ischemic myocardium. Biomaterials 2011; 32:942–949.

29. Byrne AM, Bouchier Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med 2005; 9:777–794.

30. Li Q, Turdi S, Thomas DP, Zhou T, Ren J. Intra-myocardial delivery of mesenchymal stem cells ameliorates left ventricular and cardiomyocyte contractile dysfunction following myocardial infarction. Toxicology lett 2010; 195:119.

31. Baumgartner I, Rauh G, Pieczek A, Wuensch D, Magner M, Kearney M, *et al*. Lower-extremity edema associated with gene transfer of naked DNA encoding vascular endothelial growth factor. Ann Int Med 2000; 132:880–884.

32. Hariawala MD, Horowitz JR, Esakof D, Sheriff DD, Walter DH, Keyt B, *et al*. VEGF improves myocardial

blood flow but produces EDRF-mediated hypotension in porcine hearts. J Surg Res 1996; 63:77–82.

33. Stewart DJ, Kutryk MJB, Fitchett D, Freeman M, Camack N, Su Y, *et al*. VEGF gene therapy fails to improve perfusion of ischemic myocardium in patients with advanced coronary disease: results of the NORTHERN trial. Mol Ther 2009; 17:1109–1115.

34. Lee RJ, Springer ML, Blanco-Bose WE, Shaw R, Ursell PC, Blau HM. VEGF gene delivery to myocardium: deleterious effects of unregulated expression. Circulation. Am Heart Assoc 2000; 102:898–901.

35. Tsoporis JN, Izhar S, Proteau G, Slaughter G, Parker TG. S100B-RAGE dependent VEGF secretion by cardiac myocytes induces myofibroblast proliferation. J Mol Cell Cardiol 2012; 52:464–473.

36. Bougioukas I, Didilis V, Ypsilantis P, Giatromano-

laki A, Sivridis E, Lialiaris T, *et al*. Intramyocardial injection of low-dose basic fibroblast growth factor or vascular endothelial growth factor induces angiogenesis in the infarcted rabbit myocardium. Cardiovasc Pathol 2007; 16:63-8.

37. Schwarz ER, Speakman MT, Patterson M, Hale SS, Isner JM, Kedes LH, *et al*. Evaluation of the effects of intramyocardial injection of DNA expressing vascular endothelial growth factor (VEGF) in a myocardial infarction model in the rat-angiogenesis and angioma formation. J Am Coll Cardiol 2000; 35:1323–1330.

38. Payne TR, Oshima H, Okada M, Momoi N, Tobita K, Keller BB, *et al*. A relationship between vascular endothelial growth factor, angiogenesis, and cardiac repair after muscle stem cell transplantation into ischemic hearts. J Am Coll Cardiol 2007; 50:1677–1684.