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Improvement in Cardiac Function following Transplantation of Human Umbilical Cord Matrix-Derived Mesenchymal Cells

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Key Words
Human umbilical cord matrix-derived mesenchymal cells · Myocardial infarction · Cell therapy · Cardiac repair

Abstract
Objectives: Human umbilical cord mesenchymal cells (hUCM) can be easily obtained and processed in a laboratory. These cells may be considered as a suitable source in the repair of heart failure diseases. We, therefore, examined whether these cells may contribute to heart regeneration following an acute experimental myocardial infarction (MI). ***Methods:*** MI-induced animals received 5×10^6 hUCM cells, 5×10^6 5-azacytidine-treated cells (dhUCM), or PBS alone, subepicardially. A group of animals with MI and no other former intervention served as controls. dhUCM cells were assessed for F-actin, myogenin and troponin-I expression. ***Results:*** dhUCM cells appeared as binucleated cells with extensive cytoplasmic processes. These differentiated cells were F-actin and myogenin positive. Thirty days after LAD ligation, left ventricular ejection fraction and the percentage of fractional shortening improved significantly in cell-receiving animals. In addition, the amount of scar tissue was significantly reduced in hUCM and dhUCM groups compared to MI group ($p < 0.05$). These parameters were comparable between hUCM and dhUCM groups. Histopathological evaluations revealed that some engrafted cells adjacent to and remote from the MI area expressed troponin-I, F-actin and connexin43. ***Conclusion:*** These findings demonstrated the potential therapeutic use of either differentiated or undifferentiated hUCM cells in treatment of heart failure conditions.

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Introduction

Myocardial infarction (MI), a common disease in the industrialized world, results in loss of cardiomyocytes leading to heart failure. To replace lost cardiomyocytes after infarction, cell transplantation has been suggested as a potential therapy [1]. Autologous adult stem cells are immunologically compatible and can be collected from a variety of sources including bone marrow [2–4], and there is no ethical issue relevant to their use. Meta-analyses have shown the safety of this treatment and a slight but significant improvement in left ventricular (LV) ejection fraction (EF) and a decrease in infarct size [5]. However, not all clinical trials and experimental studies could demonstrate a significant improvement in heart function following mesenchymal stromal cell (MSC) transplantation [6, 7].

In addition to bone marrow as a common source of mesenchymal cells [8], multipotent MSC can be collected from extraembryonic tissues including placental and umbilical cord blood (UCB) [9] as well as umbilical cord matrix (UCM) [10], which needs to be given more attention. Studies have indicated the feasibility of MSCs derived from extraembryonic tissues [11], including UCB cells for the repair of ischemic heart diseases [12–14]. However, there are conflicting data in the literature regarding the use of UCB cells for the repair of ischemic myocardium in different animal models [15, 16]. Several studies have evaluated the effects of UCBs on MI animal models. However, the feasibility of human UCM (hUCM) cells for the repair of acute MI needs to be explored. The hUCM cells have the advantages of being obtained by noninvasive procedures, harvesting done easily in laboratory and, most often, not triggering immune response in host animals [17]. Weiss et al. [18] demonstrated that porcine UCM cells survive, migrate and express markers for mature neurons when transplanted into a rat brain. Importantly, the implanted cells showed no evidence of forming teratomas. In vitro evidences support the idea that UCM cells could differentiate into cardiomyocytes by treating them with 5-azacytidine or by culturing them in cardiomyocyte-conditioned medium [19]. Also, transplantation of hUCM cells into the injured anal sphincter improved the sphincter function after two weeks [20], indicating the potential of hUCM cells to transdifferentiate into muscle cells in vivo.

Here, we describe the feasibility of hUCM cell survival, possible in vitro differentiation into adipogenic, osteogenic and cardiogenic cell lineage, contribution to heart function and possible trans-differentiation into cardiomyocytes in an MI-induced rabbit model.

Materials and Methods

Chemicals

Chemicals were purchased from Sigma-Aldrich Chemical Company, St. Louis, Mo., USA unless stated otherwise.

Isolation and Culture of hUCM Cells

Human umbilical cords were obtained after delivery and collected in HBSS, with written consent provided by the mothers. The umbilical cords were diced into fragments and then cultured in tissue culture plates containing DMEM-F12 supplemented with 15% FBS (Gibco), 100 U/ml penicillin, 60 μg/ml streptomycin (complete medium) in a humidified 37°C incubator with 5% CO_2 in the air. Cells at confluence of >90% were either subcultured or cryopreserved for further use.

Phenotype Analysis of hUCM Cells

hUCM cells were cultured to confluence until some colonies appeared among the propagated cells. The colonies were stained by an alkaline phosphatase kit (R-87; Sigma). Also, 1×10^6 hUCM cells/ml were cultured in hanging drops (50 μl each), and three days later, colony formation was assessed. These colonies were also examined for alkaline phosphatase activity.

Flow Cytometric Analysis of hUCM Cells

A set of antibodies against hUCM cells was selected as reported in the previous studies [17]. 1×10^5 cells/ml hUCM cells were fixed with 4% paraformaldehyde and incubated with 5% normal goat serum in PBS. Cells were labeled by the following antibodies: FITC-conjugated anti-CD44 (Chemicon), FITC-conjugated anti-CD34 (Chemicon), FITC-conjugated anti-CD45 (Ediscience), PE-conjugated anti-CD90 (Dako), and PE-conjugated anti-CD105 (R&D Systems) for 1 h. Cells were also incubated with anti-CD117 and anti-Oct4 for 1 h, followed by FITC-conjugated goat anti-mouse IgG (R&D Systems) for 45 min. The cells were analyzed using FACSCalibur (Becton Dickinson) machine and WinMDI Cellquest software.

Adipogenic and Osteogenic Differentiation

To evaluate the differentiation potential of hUCM cells as a mesenchymal stem cell, these cells were induced to differentiate into adipocyte and osteocyte cells. Adipogenic differentiation was induced by the culture of hUCM cells in complete medium supplemented with 10% FBS and 100 nM dexamethasone. For osteogenic differentiation, the hUCM cells were cultured in osteogenic differentiation medium containing complete medium, 10 nM β-glycerophosphate, 80 μg/ml ascorbic acid and 10 nM dexamethasone. Twenty-one days later, the cultures were fixed by 4% paraformaldehyde in PBS and stained with Oil red and Alizarin Red S (40 nm) for the detection of adipogenic and osteogenic differentiation, respectively. The dish area was observed with a light microscope equipped with a phase-contrast apparatus and the cells cultivated in basal medium served as control.

Induction of Cardiogenic Differentiation

The hUCM cells were incubated for 24 h in serum-free DMEM-F12 containing 3 μM 5-azacytidine [21]. The medium was then changed to complete medium. Cells were maintained in the same culture medium for two to three weeks. The medium was refreshed every 2–4 days.

Immunocytochemistry

5-Azacytidine-treated cells (dhUCM) and hUCM cells as controls were fixed in 4% paraformaldehyde in PBS, incubated with 5% normal goat serum in PBS, treated with 3% H_2O_2 in PBS, and were incubated with F-actin (Dako; 1:400), cardiac troponin-I (Chemicon; 1:2,000) and myogenin (Dako; 1:50) for 45 min, followed by incubation in EnVision™ (Dako). Cells were evaluated under a light microscope.

Cell Preparation

Seventy-two hours before cell transplantation, the hUCM and dhUCM cells were incorporated with 10 μmol 5-bromo-2′-deoxyuridine (BrdU; Chemicon) for 48 h [22]; 5×10^6 viable cells were resuspended in 50 μl PBS, and were transplanted subepicardially at the periphery of the infarcted area.

MI and Cell Transplantation

Thirty-five adult female New Zealand white rabbits (weight range 2.25–3.75 kg; Razi institute, Karaj, Iran) that withstood surgical procedures were enrolled in this study. After echocardiography, the animals were divided into five groups: (1) intact group (n = 7); (2) control group (n = 7) consisting of MI model; (3) PBS group (n = 7); (4) hUCM group (n = 7); (5) dhUCM group (n = 7) consisting of MI model with PBS, hUCM and dhUCM cells injected into the periphery of MI region, respectively.

Animals were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally), were mechanically ventilated, and their hearts were exposed by means of a left thoracotomy through the fifth intercostal space. The proximal end of the LAD artery was ligated with a 6/0 silk suture. After 1 h of ligation, animals in PBS, hUCM and dhUCM groups received 50 μl PBS, 5×10^6 hUCM cells and 5×10^6 dhUCM cells, respectively. Cells were injected subepicardially by a 28-gauge needle attached to an insulin syringe to the border of cyanotic area. Cyclosporine A (10 mg/kg, subcutaneously) was given 24 h before the cell therapy and once daily, thereafter. The investigation was approved by the local ethics committee at Kerman University of Medical Sciences, Kerman, Iran.

Transthoracic Echocardiography

Transthoracic echocardiography was performed using an echocardiographic instrument (Vivid3; General Electric) provided with a 5-MHz linear transducer, 5 and 30 days after MI to measure cardiac function in each group. A blinded cardiologist performed transthoracic echocardiographic evaluations on the rabbits. Left-ventricular end-diastolic dimension (LVEDD) and left-ventricular end-systolic dimension (LVESD) were derived from two-dimensionally targeted M-mode tracings obtained along the parasternal long-axis view of the left ventricle at the papillary muscle level. Fractional shortening (FS) of the LV (LVFS (%) = [(LVEDD – LVESD)/LVEDD] ×100) and LVEF were calculated automatically by the echo equipment.

General Pathology and Immunohistochemistry Studies

Rabbits were killed by an overdose of pentobarbital, the hearts were then removed, rinsed in PBS and fixed in 10% buffered formaldehyde solution. 5-μm-thick sections were prepared from infarcted areas for hematoxylin and eosin as well as immunohistochemistry (IHC) studies. Three different sections were randomly chosen from the infarcted area and the following parameters were assessed: number of small vessels, number of lymphocytes, number of fibroblasts, amount of scar tissue (0–3 scale) and severity of chronic inflammation (0–3 scale). For IHC studies, the slides were blocked in 5% normal goat serum and incubated overnight at 4°C with primary antibodies against BrdU (Abcam; 1:40), troponin-I (Chemicon; 1:2,000) and connexin43 (Abcam; 1:250). The cells were then incubated with fluorescent Alexa-fluor® 594 goat anti-mouse secondary antibody (Molecular Probes; 1:400) for 1 h for detection of troponin-I- and connexin43-positive cells, and subsequently incubated with Alexa-fluor® 647 secondary antibody (Molecular Probes; 1:400) to detect BrdU-positive cells. The slides were also incubated with the primary antibody against connexin43 (Abcam; 1:250) for 24 h at 4°C followed by Alexa-fluor® 594 goat anti-mouse secondary antibody (Molecular Probes; 1:400) and hematoxylin for the background staining. To label the cells with human origin, mouse primary antibody to human specific nuclear antigen (abDSerotec; 1:100) was followed by fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Sigma-Aldrich; 1:100) and DAPI (Sigma-Aldrich). To detect F-actin-positive cells in the transplanted samples, the same procedure for IHC was applied to the slides after preparing the slides in Tris buffer (pH 7) for 10 min. The images were taken using an Olympus IX71 inverted fluorescent microscope equipped with a digital camera (DP72; Olympus).

Statistical Analysis

Data are presented as means ± SEM. The echocardiographic data were compared by oneway ANOVA, followed by post hoc Tukey-Kramer's test, after normality assumption. Statistical comparison of histopathologic postsacrifice data was performed using Kruskal-Wallis test followed by the Mann-Whitney test after Bonferroni adjustment.

Results

hUCM Culture and Cell Marker Expression

The primary isolates were heterogeneous, with fibroblast-like cells possessing short and long processes and small round cells with a relatively high nuclear-to-cytoplasm ratio (fig. 1a). Individual cells propagated easily and formed colonies after being confluent >90% (fig. 1b). The hUCM cells expressed alkaline phosphatase after colony formation in culture plates (fig. 1c) and hanging drops (fig. 1d). Adipogenic (fig. 1e) and osteogenic (fig. 1f) differentiation was confirmed 21 days later. The hUCM cells were positive for mesenchymal stem cell markers, CD44, CD105, CD90, negative for CD34, CD45 and CD117, and slightly (4%) positive for Oct-4 (fig. 2).

Cardiogenic Differentiation of hUCM Cells

We treated the hUCM cells with 5-azacytidine to induce the differentiation of hUCM cells into cardiomyocyte-like cells [23]. Treated cells retained a mesenchymal phenotype with some binucleated cells among them

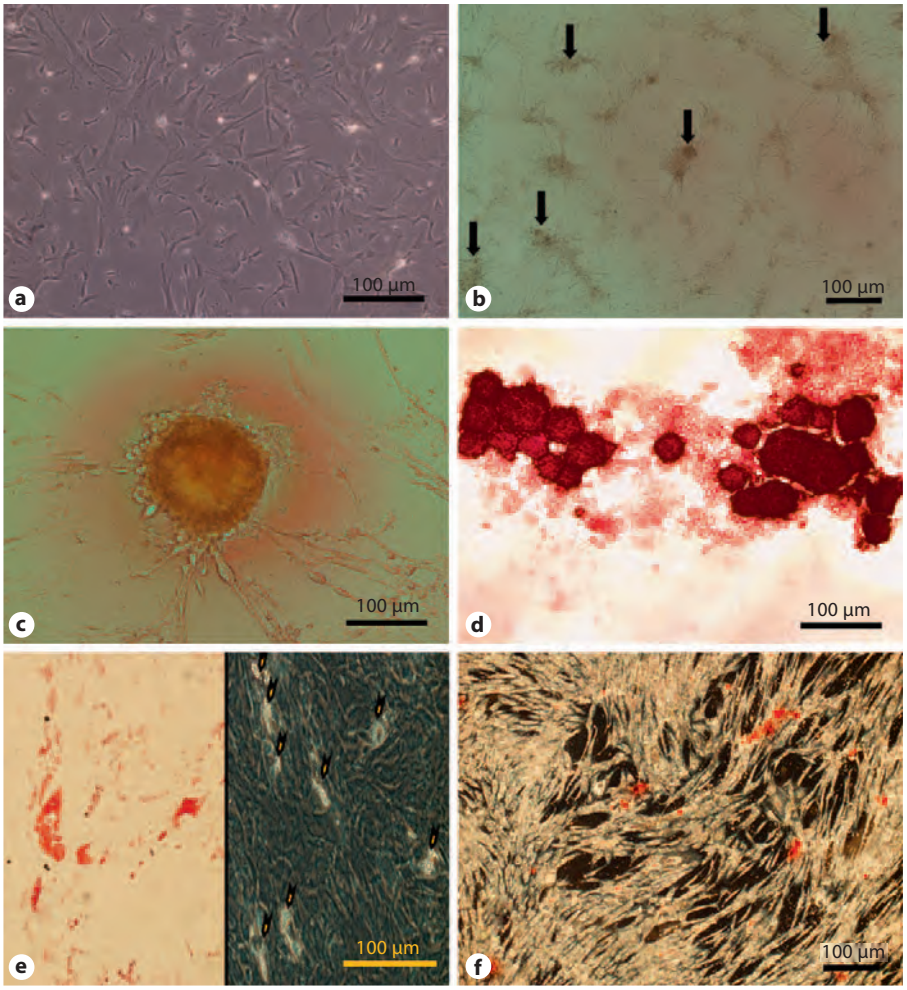

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Fig. 1. The hUCM cells retained a fibroblastic cell shape with numerous cytoplasmic processes (**a**). These cells formed numerous colonies (**b**) that stained positively by alkaline phosphatase in culture plates (**c**) and hanging drops (**d**). These cells underwent adipogenic (**e**; right-side image shows oil red staining and left-side image is a phase-contrast image) and osteogenic (**f**; phase-contrast image showing deposition of minerals in red) differentiation following appropriate induction. Color refers to the online version.

(fig. 3a). Colonies of 5-azacytidine-treated cells expressed F-actin (fig. 3b). After an extended culture period, hUCM cells transformed into flat, large cells strongly positive for F-actin (fig. 3c). Moreover, some cells had a positive reaction with myogenin (fig. 3d) and a negative reaction with troponin-I. Some cells in the control group were F-actin positive, but none were troponin-I and myogenin positive.

Evaluation of Myocardial Function

Five days after LAD ligation, LVEF decreased efficiently in all LAD-ligated animals with no significant difference among the MI groups, and a significant decrease ($p < 0.01$) compared with the intact group (fig. 4). Thirty days after LAD ligation, LVEF increased significantly ($p < 0.05$) in hUCM and dhUCM groups compared with PBS and MI groups. The rise in LVEF was also significantly higher ($p < 0.05$) in the hUCM and dhUCM groups compared with the intact, MI and PBS groups (fig. 4). Although the rise in LVEF was higher in dhUCM group, it was not significantly different from hUCM group. LVFS also significantly improved ($p < 0.05$) in the hUCM and dhUCM groups compared with PBS and MI groups (table 1).

Immunohistochemical Evaluations

Thirty days after transplantation, BrdU-incorporated cells were observed in the transplanted area (fig. 5a). Transplanted cells were HNA positive, indicating the human origin of transplanted cells in the rabbit myocardium (fig. 5b). Some transplanted cells were troponin-I and F-actin positive. Besides, clusters of engrafted cells were observed in a remote distance from the transplantation site (fig. 5c, d; 6c, e). Double staining revealed some BrdU+/troponin-I+ cells in the MI area adjacent to the normal myocardium (fig. 5e, f).

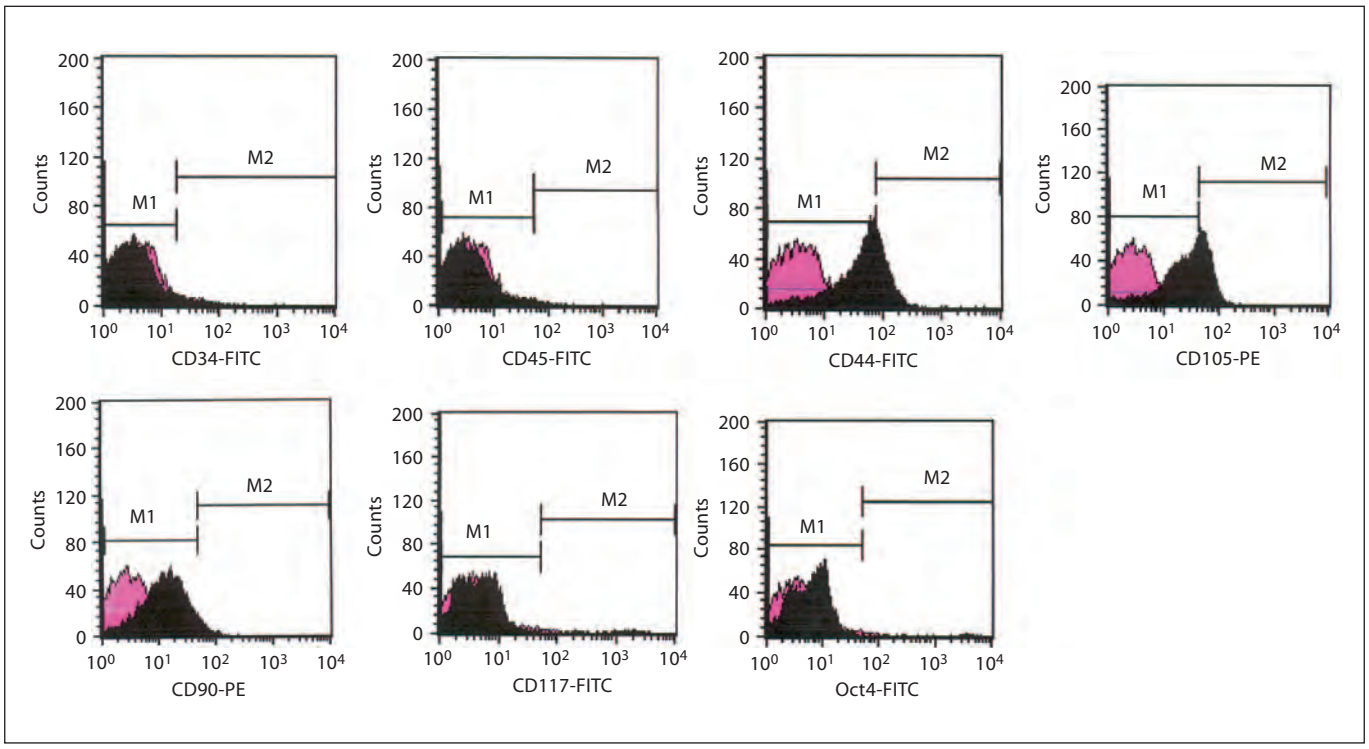

Fig. 2. The hUCM cells were positive for mesenchymal stem cell markers CD44, CD105 and CD90, negative for hematopoietic markers CD34 and CD45, negative for CD117 and slightly positive for Oct-4 as an embryonic stem cell marker.

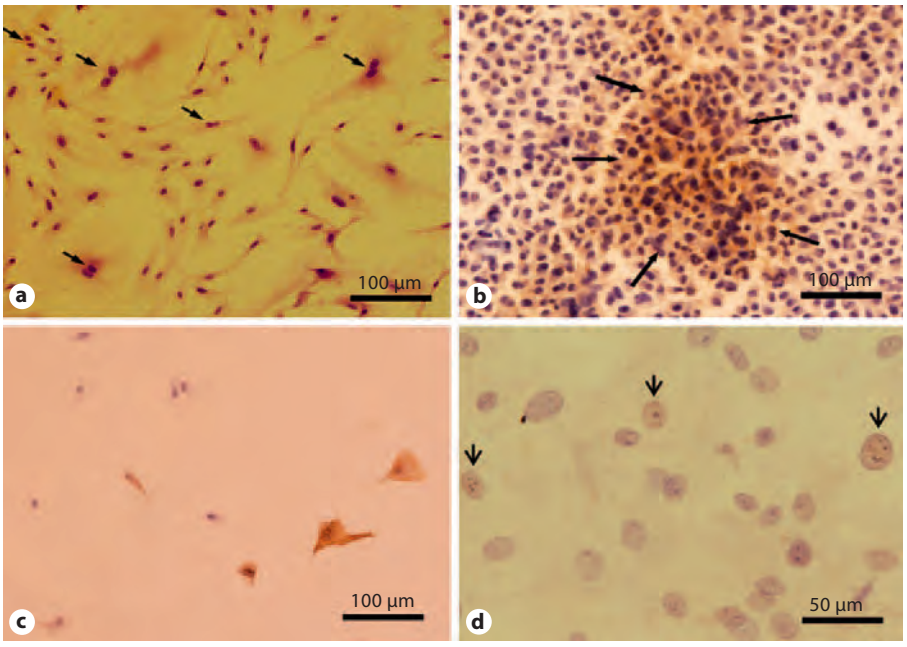

Fig. 3. Treatment of hUCM cells with 5-azacytidine resulted in some binucleate cells (arrows) with extended cytoplasmic processes (**a**), F-actin-positive clusters (**b**) and also individual cells (**c**). The treated cells showed positive reaction with myogenin (short arrows, **d**).

Table 1. Echocardiography results in different groups (n = 7)

Time point and parameter	Intact	Sham	PBS	hUCM	dhUCM
5 days after LAD ligation and cell transplantation					
LVFS%	48.1 ± 3.9	28.3 ± 3.6	32.6 ± 3.2	27.1 ± 3.7	26.8 ± 4.4
LVEDD, cm	1.42 ± 0.21	1.29 ± 0.15	1.22 ± 0.10	1.22 ± 0.12	1.12 ± 0.08
LVESD, cm	0.74 ± 0.16	0.92 ± 0.10	0.81 ± 0.06	0.87 ± 0.07	0.82 ± 0.08
30 days after LAD ligation and cell transplantation					
LVFS%	42.3 ± 0.22	29.6 ± 2.7	28.4 ± 2.1	39.4 ± 2.2*	38.8 ± 3.1*
LVEDD, cm	1.32 ± 0.12	1.58 ± 0.22	1.28 ± 0.15	1.26 ± 0.08	1.27 ± 0.07
LVESD, cm	0.76 ± 0.07	1.11 ± 0.15	0.92 ± 0.11	0.76 ± 0.04	0.79 ± 0.07

Values are means ± SEM. LAD = Left descending artery; LVFS% = left-ventricular fractional shortening; LVEDD = left-ventricular end-diastolic dimension; LVESD = left-ventricular end-systolic dimensions. * $p < 0.05$ compared to PBS.

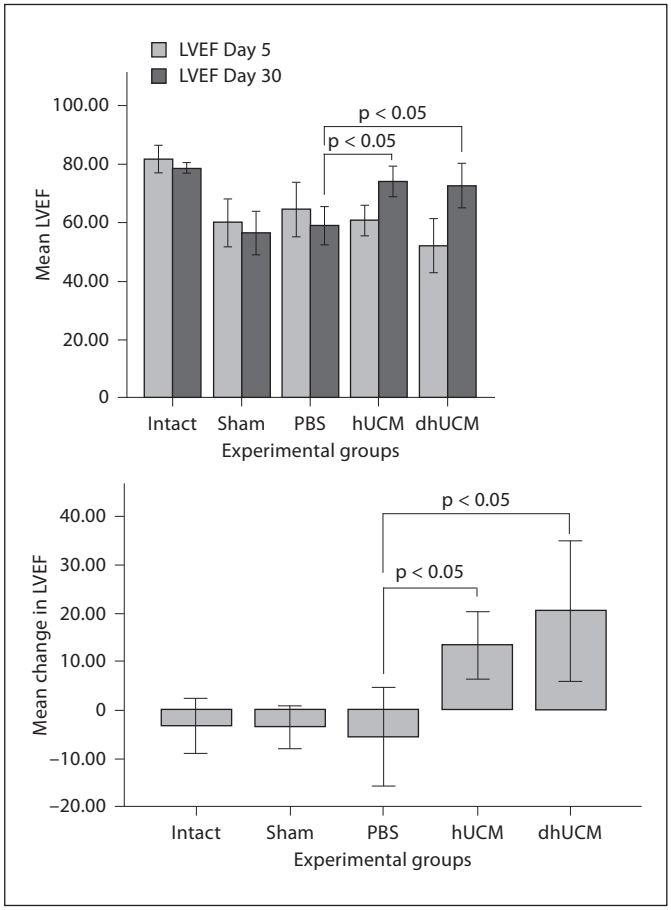

Fig. 4. The animals in the hUCM and dhUCM groups demonstrated a significant improvement in LVEF compared to the MI and PBS groups. Results obtained 5 and 30 days after LAD ligation. A significant change in LVEF was detected when LVEF change in hUCM and dhUCM groups was compared to other groups. Bars are representative of means ± SEM.

The grafted cells also demonstrated to express gap-junction protein connexin43 next to the host myocardium and among the engrafted cells (fig. 6f).

Histopathologic Evaluations

The scar tissue was more abundant in MI (2.29 ± 0.42) and PBS (1.46 ± 0.49) than hUCM (0.71 ± 0.35) and dhUCM (0.57 ± 0.3) groups (fig. 6a, b; table 2). The number of fibroblasts (89 ± 44.7 and 77 ± 33.2 in hUCM and dhUCM groups, respectively) and the severity of chronic inflammation (0.86 ± 0.14 and 1 in hUCM and dhUCM groups, respectively) decreased in the hUCM and dhUCM groups, but no significant difference was observed among the cell-treated animals compared with PBS and MI groups (table 2). Some viable engrafted cells were detected in the MI area interposed between the normal myocardial cells (fig. 6c), and remote from it (fig. 6e).

Discussion

This study demonstrated that subepicardial engrafted hUCM and dhUCM cells could improve experimentally infarcted myocardium function after 30 days. While there was only weak evidence of transdifferentiation of engrafted cells into cardiomyocyte cells, and the presence of engrafted cells in the vicinity of cell-transplanted zone, hUCM and dhUCM cells have significantly improved left ventricle contractile function. Transplantation of hUCM cells also advantageously influenced scar tissue formation and the number of fibroblasts, while inflammatory reactions were minimized in infarcted hearts following cell engraftments. An ideal source of donor cells for cardiovascular diseases therapy should be easily collected

Table 2. Histopathologic findings of the treatment groups (n = 7)

Parameter	Sham	PBS	hUCM	dhUCM
Chronic inflammation (0–3)	1.28 ± 0.18	0.6 ± 0.3	0.86 ± 0.14	1
Small vessels	9.14 ± 3.15	9.91 ± 6.81	8.57 ± 3.70	6.43 ± 2.37
Lymphocytes	18.90 ± 8.2	14.57 ± 2.8	17.14 ± 7.5	22.14 ± 17.3
Scar tissue (0–3)	2.29 ± 0.42	1.46 ± 0.49	0.71 ± 0.35*	0.57 ± 0.3**
Fibroblasts	307 ± 60.2	198 ± 62.3	89 ± 44.7	77 ± 32.2

Values are representative of means ± SEM. * p = 0.056; ** p = 0.042 versus the sham group.

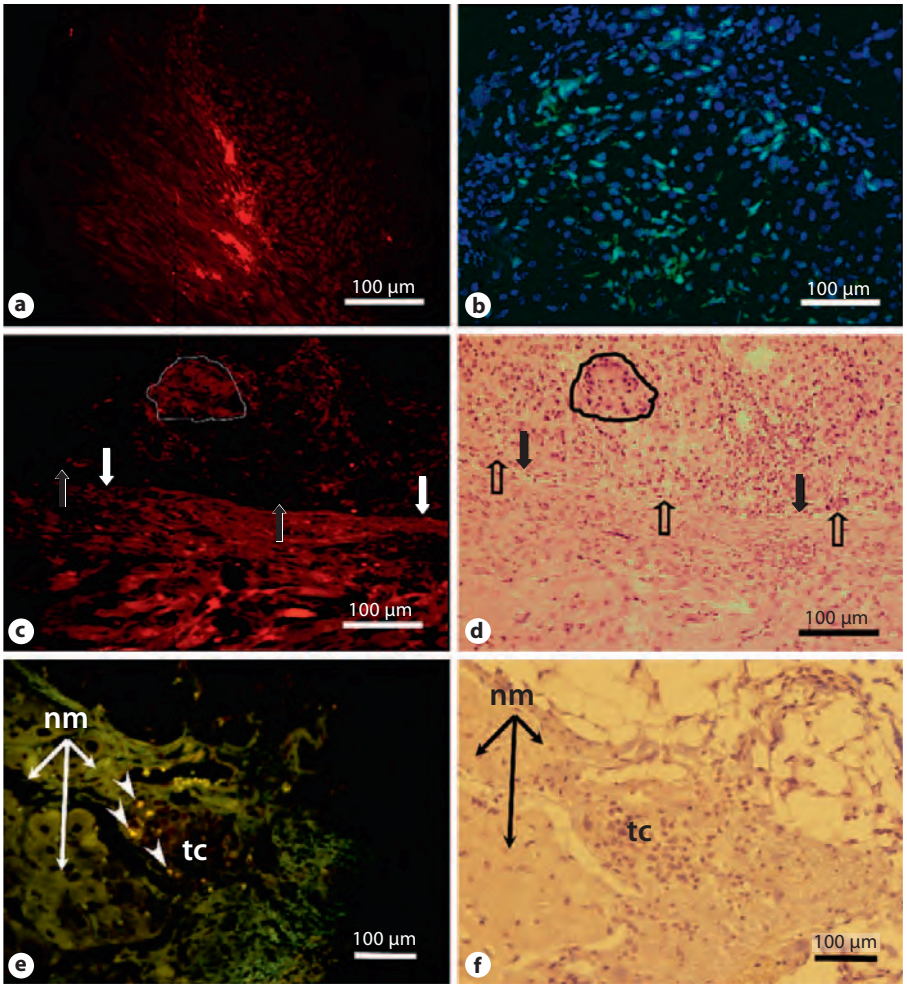

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Fig. 5. BrdU-positive cells were found in the transplanted area (**a**). HNA-positive cells (green), DAPI-positive cells (blue) and a merged image of them (light blue) are shown in the infarcted area (**b**). Anti-troponin-I staining revealed troponin-I+ cells both in normal myocardium (filled arrows) and in engrafted area (encircled zone; hollow arrows) (**c**, **d**). Double staining showed BrdU+/troponin-I+ cells (arrowheads) between transplanted cells (tc); normal myocardium (nm, arrows) was positive for troponin-I (**e**, **f**). Colors refer to the online version.

and rapidly expanded in laboratory, immunologically compatible and capable of long-term survival and integration in the host tissue. hUCM cells can be easily obtained and harvested, compared to bone marrow mesenchymal stromal cells. These cells have greater colony-forming unit fibroblast frequency and shorter doubling time compared with bone marrow mesenchymal stromal cells [24]. In the present study, we used a nonenzymatic method which yielded a sufficient number of cells after two weeks of culture. Isolated cells formed alkaline phosphatase-positive colonies in plastic dishes and hanging drops, a feature common to embryonic stem cells [25].

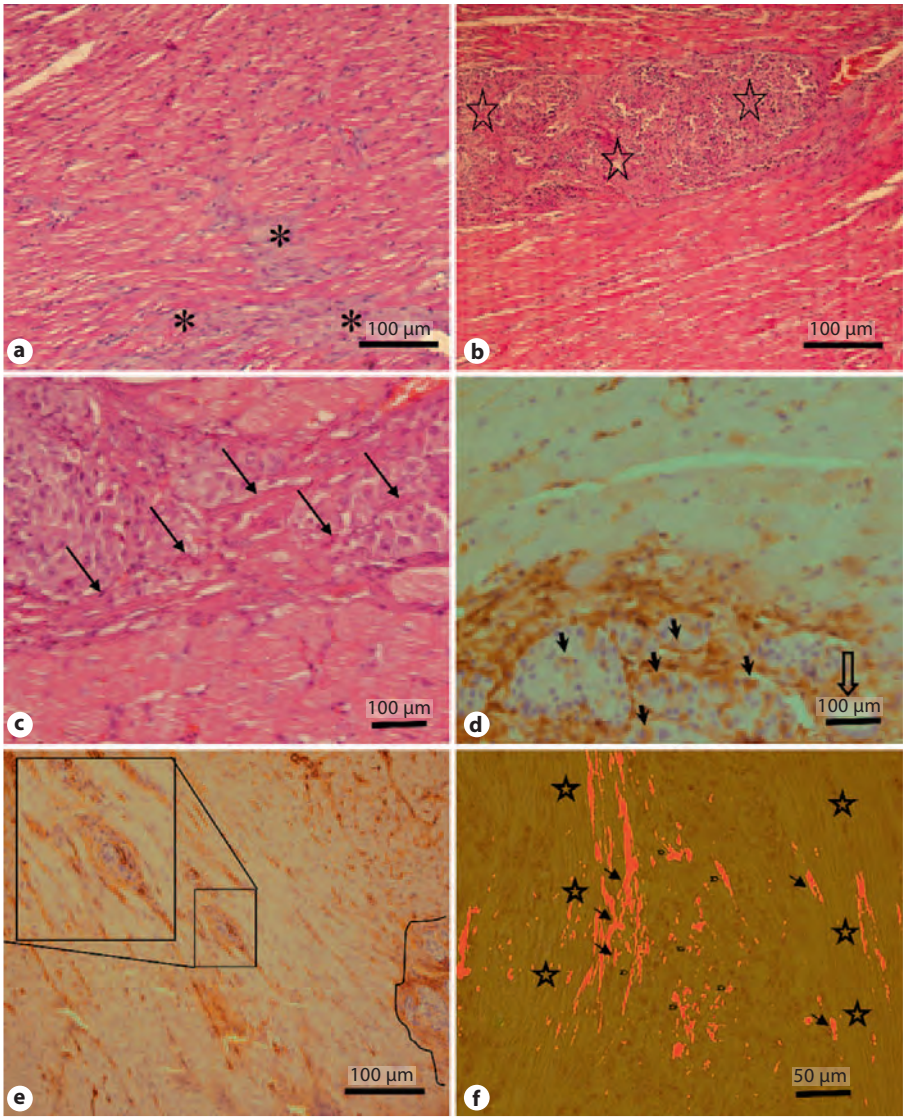

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Fig. 6. Hematoxylin-eosin staining demonstrated more scar tissues in the MI and PBS groups (**a**; asterisks); while in hUCM- and dhUCM-treated myocardium (**b**; hollow stars) fibrous tissue was less abundant. Bundles of myocardium were interposed between transplanted cells (**c**; arrows). Some transplanted cells were F-actin positive (**d**; small arrows) and few vessel-like structures (**d**; hollow arrow) were also present adjacent to the transplanted area. The transplanted cells were found at the transplantation site and remote from it (**e**). A merged image of immunostaining against connexin43 showed connexin43-positive cells in the transplanted area (**f**; arrowhead), at the interphase (arrows) and host myocardium (stars).

These cells also successfully differentiated into adipocytes and osteocytes in vitro.

After cardiogenic differentiation, some dhUCM cells transformed into binucleate cells with extensive cytoplasmic processes, a common feature of cardiac cells. These cells expressed F-actin and myogenin. However, troponin-I expression, as a cardiac marker, was not detected in this study, nor was it confirmed after myogenic differentiation in vitro in human bone marrow stem cells [5, 26]. Presence of troponin-I- and connexin43-positive cells between the transplanted cells in the MI area and in the remote distance from it suggests that cardiac milieu has induced cardiogenic differentiation in the transplanted cells. Exposure of hUCM cells to a cardiomyocyte-conditioned medium has also resulted in differentiation of these cells into troponin-I- and N-cadherin-positive cells [19].

In this study, transplantation of hUCM and dhUCM cells improved LV function with no significant difference. However, a slight nonsignificant increase in LVEF was detected in dhUCM group compared with hUCM group (fig. 2b). Although the hUCM cells differentiated into cardiomyocyte-like cells by 5-azacitidine, dhUCM cells have improved LV function at a comparable rate with the hUCM cells. Gap junctions as the functional unit of grafted cells were also expressed in the both groups. Such results have been reported after treatment of MI-induced animals with differentiated and nondif-

ferentiated UCB cells [12]. The precise mechanism by which the transplanted cells may contribute to the regeneration of MI heart is unknown yet. There are several potential explanations. One possible reason is that stem cells differentiate into myogenic-like cells; if the transplanted cells are in contact with the contracting host myocardium, myocardial factors from the host myocardium might induce the engrafted cells to differentiate into myogenic-like cells, that is, the hypothesis of 'milieu-influenced' differentiation [22]. Another reason is that engrafted cells may fuse to cardiomyocytes in the normal myocardium to trigger the reentry of fused cardiomyocytes into a functional state [27]. We could demonstrate that some engrafted cells next to the host myocardium and remote from the injection site had stained positively with troponin-I and F-actin and connexin43 (fig. 5, 6) as cardiac, mesenchymal and gap junction markers, respectively. Lack of specific detection systems to demonstrate contractile function in transplanted cells at present has limited our ability to finally state that hUCMs implanted in the damaged myocardium could actually differentiate and function as a cardiomyocyte. Another mechanism which has been proposed to explain the beneficial influence of stem cells in cardiac repair is the secretion of growth factors and cytokines by transplanted cells to induce myogenic differentiation of transplanted cells and/or mobilization, homing and differentiation of host stem cells into myocytes [28]. hUCMs secrete CXCR4, the receptor for stem cell-derived factor 1 [10]. Local injection of SCF improved myocardial homing of systemically derived bone marrow stem cells [29]. Although we could detect some viable troponin-I- and F-actin-positive cells adjacent to and between the injured myocardium, the small number of BrdU+/troponin-I+ cells may not explain the overt improvement of LVEF in cell-transplanted groups. Therefore, we suggest that mechanisms other than transdifferentiation to cardiomyocytes should have been involved in myocardial repair following hUCM and dhUCM cell transplantation.

Potentially, stem cells could alter collagenase activity or other enzymatic pathways responsible for pathologic heart wall thinning in injured myocardium, resulting in digestion of scar tissues and modification of extracellular matrix proteins [30]. Overexpression of collagen type I and III may protect infarcted myocardium from remodeling and dilation, but the increase in collagen synthesis in myocardium could result in stiffness and dysfunction of the damaged heart. In our study, the number of fibroblasts and also the scar tissue decreased in the cell-transplanted groups in comparison with those in the MI and PBS groups. Consequently, the functional improvement in cell-treated hearts could also be explained by the potential of the transplanted hUCM cells to reduce scar tissue and to increase more viable functional tissues to support damaged heart function. These results are supported by a recent study in which doxorubicin-induced cardiac hypertrophy was corrected after transplantation of human UCB cells in mice [31].

Mesenchymal stromal cells have a relatively immunoprivileged phenotype; lack of HLA-class-II antigens enables them to survive and reside in an inflammatory milieu without activating the immune system. Similarly to MSCs, hUCM cells express HLA-class-I and do not express HLA-class-II surface markers [32]. A recent study has shown that when a single dose of umbilical cord cells from 4 MGH MHC-defined miniature swine leukocyte antigen (SLA)dd was injected to MHC-mismatched SLAcc and SLAac animals, the cell-treated animals failed to demonstrate any detectable level of serum antibody production of IgG directed towards SLAdd cells after one month [33]. We used cyclosporine A to suppress probable immune responses in the host animals. However, in one animal engrafted with hUCM cells and without cyclosporine A injection, no histopathologic sign of tissue rejection was detected (data not shown). Other researchers have implanted hUCM cells as xenografts and reported that such a procedure did not alleviate immune response in the host animals [18]. Nonimmunogenic nature of these cells proposes them as an alternative and valuable candidate for cell therapy in degenerative heart diseases. However, some debates remain to be elucidated before these cells could be utilized in clinical studies. The safety of hUCM cells as a homograft tissue, long-term survival, optimal cell dose, the most effective route of delivery, potentiality of hUCM cells in the treatment of different types of heart failure and the ability of hUCM cells in the treatment of ischemic myocardium compared with other cell types are yet unclear.

Conclusion

The hUCM cells may be considered an alternative cell source to repair the damaged myocardium after MI. These cells could differentiate into myocyte-like cells under influence of 5-azacytidine. Transplantation of hUCM cells clearly improves left ventricular function 30 days after transplantation at a similar rate to dhUCM cells. Furthermore, there is convincing evidence that hUCM cells are involved in the scar tissue remodeling during healing process after MI.

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Conflict of Interest

The authors have no potential conflict of interest to report.

References

1 Abdel-Latif A, et al: Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. Arch Intern Med 2007;167:989–997.
2 Thompson RB, et al: Intracardiac transplantation of a mixed population of bone marrow cells improves both regional systolic contractility and diastolic relaxation. J Heart Lung Transplant 2005;24:205–214.
3 Jiang Y, et al: Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–49.
4 Meluzin J, et al: Intracoronary delivery of bone marrow cells to the acutely infarcted myocardium. Optimization of the delivery technique. Cardiology 2009;112:98–106.
5 Martin-Rendon E, et al: 5-Azacytidine-treated human mesenchymal stem/progenitor cells derived from umbilical cord, cord blood and bone marrow do not generate cardiomyocytes in vitro at high frequencies. Vox Sang 2008;95:137–148.
6 Wei HM, et al: Human bone marrow-derived adult stem cells for post-myocardial infarction cardiac repair: current status and future directions. Singapore Med J 2009;50:935–942.
7 Govaert JA, et al: Poor functional recovery after transplantation of diabetic bone marrow stem cells in ischemic myocardium. J Heart Lung Transplant 2009;28:1158–1165 e1.
8 Wang S, et al: Intracoronary autologous CD34+ stem cell therapy for intractable angina. Cardiology 2010;117:140–147.
9 Gopinath S, et al.: Human umbilical cord blood derived stem cells repair doxorubicin-induced pathological cardiac hypertrophy in mice. Biochem Biophys Res Commun 2010; 395:367–372.
10 Weiss ML, et al: Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells 2006;24:781–792.
11 Kaviani A, et al: Fetal tissue engineering from amniotic fluid. J Am Coll Surg 2003; 196:592–597.
12 Schlechta B, et al: Ex-vivo expanded umbilical cord blood stem cells retain capacity for myocardial regeneration. Circ J 2010;74:188–194.
13 Iwasaki H, et al: Therapeutic potential of unrestricted somatic stem cells isolated from placental cord blood for cardiac repair post myocardial infarction. Arterioscler Thromb Vasc Biol 2009;29:1830–1835.
14 Ghodsizad A, et al: Transplanted human cord blood-derived unrestricted somatic stem cells improve left-ventricular function and prevent left-ventricular dilation and scar formation after acute myocardial infarction. Heart 2009;95:27–35.
15 Moelker AD, et al: Intracoronary delivery of umbilical cord blood derived unrestricted somatic stem cells is not suitable to improve LV function after myocardial infarction in swine. J Mol Cell Cardiol 2007;42:735–745.
16 Rabald S, et al: Cord blood cell therapy alters LV remodeling and cytokine expression but does not improve heart function after myocardial infarction in rats. Cell Physiol Biochem 2008;21:395–408.
17 Can A, Karahuseyinoglu S: Concise review: human umbilical cord stroma with regard to the source of fetus-derived stem cells. Stem Cells 2007;25:2886–2895.
18 Weiss ML, et al: Transplantation of porcine umbilical cord matrix cells into the rat brain. Exp Neurol 2003;182:288–299.
19 Wang HS, et al: Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 2004;22:1330–1337.
20 Aghaee-afshar M, et al: Potential of human umbilical cord matrix and rabbit bone marrow-derived mesenchymal stem cells in repair of surgically incised rabbit external anal sphincter. Dis Colon Rectum 2009;52:1753–1761.
21 Kadivar M, et al: In vitro cardiomyogenic potential of human umbilical vein-derived mesenchymal stem cells. Biochem Biophys Res Commun 2006;340:639–647.
22 Tomita S, et al: Autologous transplantation of bone marrow cells improves damaged heart function. Circulation 1999;100(19 suppl):II247–II256.
23 Wang JS, et al: Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 2000;120:999–1005.
24 Baksh DYR, Tuan RS: Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 2007;25:1384–1392.
25 Boheler KR, et al: Differentiation of pluripotent embryonic stem cells into cardiomyocytes. Circ Res 2002;91:189–201.
26 Hou M, et al: Transplantation of mesenchymal stem cells from human bone marrow improves damaged heart function in rats. Int J Cardiol 2007;115:220–228.
27 Zhang S, et al: Fusion of human hematopoietic progenitor cells and murine cardiomyocytes is mediated by alpha 4 beta 1 integrin/vascular cell adhesion molecule-1 interaction. Circ Res 2007;100:693–702.
28 Kuang D, et al: Stem cell factor/c-kit signaling mediated cardiac stem cell migration via activation of p38 MAPK. Basic Res Cardiol 2008;103:265–273.
29 Lutz M, et al: Local injection of stem cell factor (SCF) improves myocardial homing of systemically delivered c-kit + bone marrow-derived stem cells. Cardiovasc Res 2008;77: 143–150.
30 Aupperle H, et al: Effects of autologous stem cells on immunohistochemical patterns and gene expression of metalloproteinases and their tissue inhibitors in doxorubicin cardiomyopathy in a rabbit model. Vet Pathol 2007; 44:494–503.
31 Gopinath S, et al: Human umbilical cord blood derived stem cells repair doxorubicin-induced pathological cardiac hypertrophy in mice. BBRC 2010;395:367–372.
32 Weiss ML, et al: Immune properties of human umbilical cord Wharton's jelly-derived cells. Stem Cells 2008;26:2865–2874.
33 Cho PS, et al: Immunogenicity of umbilical cord tissue derived cells. Blood 2008;111: 430–438.