Original Research

Direct intracardiac injection of umbilical cord-derived stromal cells and umbilical cord blood-derived endothelial cells for the treatment of ischemic cardiomyopathy

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Abstract

The development of new therapeutic strategies is necessary to reduce the worldwide social and economic impact of cardiovascular disease, which produces high rates of morbidity and mortality. A therapeutic option that has emerged in the last decade is cell therapy. The aim of this study was to compare the effect of transplanting human umbilical cord-derived stromal cells (UCSCs), human umbilical cord blood-derived endothelial cells (UCBECs) or a combination of these two cell types for the treatment of ischemic cardiomyopathy (IC) in a Wistar rat model. IC was induced by left coronary artery ligation, and baseline echocardiography was performed seven days later. Animals with a left ventricular ejection fraction (LVEF) of $\leq$40% were selected for the study. On the ninth day after IC was induced, the animals were randomized into the following experimental groups: UCSCs, UCBECs, UCSCs plus UCBECs, or vehicle (control). Thirty days after treatment, an echocardiographic analysis was performed, followed by euthanasia. The animals in all of the cell therapy groups, regardless of the cell type transplanted, had less collagen deposition in their heart tissue and demonstrated a significant improvement in myocardial function after IC. Furthermore, there was a trend of increasing numbers of blood vessels in the infarcted area. The median value of LVEF increased by 7.19% to 11.77%, whereas the control group decreased by 0.24%. These results suggest that UCSCs and UCBECs are promising cells for cellular cardiomyoplasty and can be an effective therapy for improving cardiac function following IC.

Keywords: Human umbilical cord-derived stromal cells, human umbilical cord blood-derived endothelial cells, cell therapy, ischemic cardiomyopathy

Introduction

Cardiovascular disease constitutes a major cause of morbidity and mortality worldwide,[1–3] and it is important to develop new therapeutic strategies to reduce its immense social and economic impact. Ischemic cardiomyopathy (IC) involves changes in the cardiac structure, including thinning of the infarcted wall, cardiac dilatation, remodeling due to apoptosis and necrosis of cardiac myocytes, hypertrophy, fibrosis, and the infiltration of inflammatory cells.[4,5]

Cell therapy has shown promising results in several preclinical studies[6–10] and clinical trials[11–14] that have reported the safety and efficacy of cell transplantation for a wide range of therapeutic applications. Stem/progenitor cell-based therapies have been demonstrated to be effective for tissue engineering and have potential applicability for therapeutic neovascularization, vascular repair, and tissue engineering.[15–18]

Human umbilical cord blood (HUCB)[19,20] and human umbilical cord (HUC)[21,22] have been considered as potential cell sources for cell therapy, and they were recently studied as an option to treat/prevent cardiac diseases.[23,24]

The aim of this work was to evaluate the efficacy of umbilical cord-derived stromal cells (UCSCs), human umbilical cord blood-derived endothelial cells (UCBECs), and a combination of these two cell types to improve cardiac function in a rat model of IC. The transplantation of UCSCs and UCBECs was expected to not only improve cardiac function but also to facilitate the reduction of infarct size, attenuate the progression of left ventricular dysfunction in the ischemic tissue, and increase contractile function.

Materials and methods

Isolation and culture of UCSCs

Tissue collection for research was approved by the ethics committee of Pontifícia Universidade Católica do Paraná (number 2788/08). Under sterile conditions, the UC ($n = 23$) was washed with phosphate-buffered saline (PBS) three times to remove the blood. Then, the UC was cut open, and the Wharton's jelly (WJ) tissue was excised and minced into fine pieces that were 0.5–1 mm^2 in size. The WJ was immersed in an enzymatic solution, 1.49 µg/mL of collagenase type I (Invitrogen, Grand Island, NY, USA), incubated for 16 h at 37°C, and then washed with PBS and treated with 0.25% trypsin/EDTA (Invitrogen, Grand Island, NY, USA) for 30 min at 37°C. Finally, the cells were passed through a 40 µm nylon mesh (BD Falcon™, Franklin Lakes, NJ, USA), resuspended in Iscove's modified Dulbecco's medium (IMDM) (Invitrogen, Grand Island, NY, USA) supplemented with 1% penicillin/streptomycin (Invitrogen, Grand Island, NY, USA) and 15% fetal calf serum (FCS) (Invitrogen, Grand Island, NY, USA), and plated in 75 cm^2 flasks for culturing at 37°C with 5% CO_2 in a humidified atmosphere.

Isolation of mononuclear cells from HUCB

The UCB ($n = 23$) was diluted 1:3 with IMDM and carefully loaded onto a density gradient of Ficoll-Histopaque (1.077 g/mL) (Sigma-Aldrich, Saint Louis, MO, USA) to isolate the mononuclear cells (MNCs). The MNCs were isolated by centrifugation ($400 \times$ g, 30 min, room temperature) and washed twice with IMDM. The MNCs were carefully removed and transferred to a new conical tube, washed twice with IMDM, and resuspended in IMDM supplemented with 1% penicillin/streptomycin and 15% FCS.

Immunomagnetic selection of CD133$^+$ cells from UCB and culture of UCBECs

UCB MNCs expressing the CD133 antigen were immunomagnetically selected using the human CD133 MicroBeads and Magnetic Cell Sorting (MACS) system (Miltenyi Biotec®, Bergisch-Gladbach, Germany), as previously described by Senegaglia et al.[25] Briefly, the MNCs were incubated with a blocking solution and anti-CD133 antibody (Miltenyi Biotech, Bergisch-Gladbach, Germany) linked to magnetic MicroBeads for 30 min at 4°C. The magnetically labeled CD133$^+$ cells were retained in the column and were subsequently eluted as the positively selected cell fraction after removing the column from the magnetic field. The purity of the MACS-separated subpopulations was confirmed by flow cytometry with monoclonal antibodies (CD34, CD45, AC133). The isolated CD133$^+$ cells were plated in 25 cm^2 flasks in Endothelial Basal Medium 2 (EBM-2) medium supplemented with 10% FCS and Endothelial Cell Growth Medium 2 (EGM-2) MV Single Quots (Cambrex, Walkersville, MD, USA) at 37°C with 5% CO_2 in a humidified atmosphere.

Adipogenic, osteogenic, and chondrogenic differentiation

Adipogenic, osteogenic, and chondrogenic differentiation were conducted as previously described by Rebelatto et al.[26] Briefly, the cells were seeded on glass coverslips in 24-well plates, and lineage-specific differentiation was induced by adipogenic or osteogenic medium (Invitrogen, Grand Island, NY, USA). For chondrogenic differentiation, 5×10^5 cells were centrifuged in a 15 mL conical tube at $400 \times$ g for 10 min, and lineage-specific differentiation was induced by chondrogenic medium (Invitrogen, Grand Island, NY, USA). Adipogenesis was assessed by Oil Red O staining, osteogenesis was assessed by Alizarin Red S staining, and chondrogenesis was assessed by toluidine blue staining.

Flow cytometry

The UCSCs were incubated with the following monoclonal antibodies to determine their typical cell surface epitope profiles: anti-CD 14, anti-CD 19, anti-CD 29, anti-CD 31, anti-CD 34, anti-CD 44, anti-CD 45, anti-CD 73, anti-CD 90, anti-CD 117, HLA-DR (all from BD-Pharmingen™ San Jose, CA, USA), and anti-CD 105 (eBioscience Inc., San Diego, CA, USA). The UCBECs were incubated with the following monoclonal antibodies: anti-CD 29, anti-CD 31, anti-CD 34, anti-CD 45, anti-CD 106, anti-CD 117, anti-CD 144, anti-CD 146, anti-CD 166, anti-CD 309 (all from BD-Pharmingen™ San Jose, CA, USA), anti-CD 105 (eBioscience Inc., San Diego, CA, USA), and anti-CD 133 (Miltenyi Biotec®). Their viability was assessed by 7AAD (BD-Pharmingen™) staining. PE-, FITC-, APC-, and PerCP-conjugated anti-mouse IgG1 antibodies (all from BD-Pharmingen™ San Jose, CA, USA) were used as isotype controls. The data for cell staining were acquired using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) and analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

Immunofluorescence of UCSCs and UCBECs

In brief, UCSCs and UCBECs were washed with PBS, fixed with 2% paraformaldehyde and permeabilized with 0.2% Triton X-100. After blocking with 1% Bovine serum albumin , the UCBECs were incubated for 3 h at room temperature with polyclonal mouse anti-von Willebrand factor (vWF) antibody (Dako, Glostrup, Denmark; diluted 1:200) followed by FITC labeled anti-rabbit IgG secondary antibody (Sigma-Aldrich, Saint Louis, MO, USA; diluted 1:80), as well as mouse anti-human CD 31 (BD-Pharmingen™ San Jose, CA, USA; diluted 1:100) followed by Texas red X labeled goat anti-mouse secondary antibody (Santa Cruz Biotechnology, CA, USA; diluted 1:1500). The UCSCs were stained with mouse anti-human CD 105 (BD-Pharmingen™; diluted 1:100) followed by Texas red X labeled goat anti-mouse secondary antibody (Santa Cruz Biotechnology; diluted 1:1500), as well as goat anti-vimentin (Santa Cruz Biotechnology; diluted 1:50) followed by Alexa Fluor 488 labeled rabbit anti-goat secondary antibody (Molecular Probes, Oregon, USA; diluted 1:1500). Nuclear staining was performed with 4'-6-diamidino-2-phenylindole

(DAPI). The coverslips were mounted with mounting medium and observed by fluorescent microscopy (Leica).

Double positive staining with acetylated low-density lipoprotein and lectin

To confirm their EC phenotype, the UCBECs were examined for the ability to bind acetylated low-density lipoprotein (AcLDL) (Biomedical Technologies Inc., Stoughton, MA, USA) and lectin (Sigma). The cells were incubated with 2 mg/mL of AcLDL for 1 h at 37°C, fixed in 4% paraformaldehyde and counterstained with 50 mg/mL of lectin for 1 h at 37°C in the dark. Nuclear staining was performed with DAPI. The cells displaying double-positive fluorescence for AcLDL and lectin were considered to be ECs. After staining, the samples were viewed with an inverted fluorescent microscope (Leica, Solms, Germany).

Animal model

The animal studies were approved by the Ethics Committee in Research of the Pontifícia Universidade Católica do Paraná (no. 401). Male adult Wistar rats (*Rattus norvegicus*) were used for the study. The animals were housed in groups of four rats/cage at room temperature (18–21°C) in a humidity-controlled (55–65% relative humidity) environment with a 12-h light-dark cycle and *ad libitum* access to standard rodent chow and water.

Induction of IC

IC was produced as previously described.[27] Briefly, the rats received intramuscular injections of 5 mg/kg of meperidine with 0.04 mg/kg atropine. After 10 min, they were anesthetized with ≈4% halothane in an anesthesia chamber. A left thoracotomy was performed between the 4th and the 5th intercostal spaces. The thorax was opened, the left anterior descending coronary artery was occluded at 2 mm from its origin by ligating the artery between the pulmonary artery and the left atrial auricle with 4-0 silk thread. Then, the heart was rapidly returned to its normal position in the thorax, and the surgical incision was closed. The rat was placed in a recovery cage with a supply of oxygen for approximately 30 min. Analgesia (morphine 1 mg/kg/SC; flunixin meglumine 2.5 mg/kg) and antibiotic therapy (enrofloxacin 10 mg/kg/IM) were scheduled for up to 72 h.

Echocardiographic analysis

Baseline echocardiographs were performed seven days after IC induction using an echocardiographic system equipped with a 7.5-MHz phased-array transducer (Hewlett-Packard, Andover, MA, USA). The animals were anesthetized with intramuscular injections of ketamine chlorhydrate (50 mg/kg) and xylazine (5 mg/kg). All of the measurements were averaged from three consecutive cardiac cycles and were analyzed by one independent observer who was blinded to the treatment status of the animals. Animals with a LVEF of ≤40% were selected for the study.

Cell transplantation

The rats were first premedicated by intraperitoneal injections of 1.25 mg/kg diazepam and 12.5 mg/kg ketamine, as well as an intramuscular injection of 5 mg/kg of meperidine. Anesthesia was induced by ≈4% halothane in 100% oxygen in a glass induction chamber. Each rat was then endotracheally intubated, and anesthesia was maintained by ≈2% halothane vaporized in 100% oxygen (≈150 mL/min) in a semi-closed breathing circuit. Each rat was mechanically ventilated using a ventilator (Harvard Apparatus, South Natick, MA, USA), which was set to 70–80 breaths/min and 175–200 mL/min. The heart was exposed through a thoracotomy of the breastbone. The cells in IMDM or medium alone were administrated intramyocardially in three separated equivolumetric injections in the infarct border zone, totalizing 200 µL. The recovery and postsurgical care were identical to the procedures after surgical induction of IC.

Histology

The hearts were sectioned from the apex to the base into four transverse sections. Histological sections from formalin-fixed and paraffin-embedded tissues were cut at 4 mm thickness and stained with Masson trichrome. For each section, 10 randomly selected fields of view were captured using a microscope coupled to a video camera (Leica, Solms, Germany), which sent digital images to a computer, and were analyzed using Image Pro-plus 6.0 image analysis software (Media Cybernetics®, Silver Spring, MD, USA). To identify the effects of cells on the myocardial capillary density, the heart sections were stained with a monoclonal anti-laminin antibody (Dako, Glostrup, Denmark). For quantification, microscopic fields of view were selected from the infarct region, and the positively stained capillaries were counted. The capillary density was assessed by counting the number of capillaries in fields of view from tissue sections, and the data are expressed as the number of capillaries/field.

Statistical analysis

The results obtained from the study are expressed as the mean ± SD, the median, and the minimum and maximum values. A one-way analysis of variance (ANOVA) was used to compare the groups with respect to the quantitative variables that were assessed pretransplantation. An analysis of co-variance (ANCOVA) was used to compare the groups in relation to the posttransplant evaluations as well as to compare the differences between the pre- and post-transplant values, and the baseline values were used as the covariate. The nonparametric Kruskal-Wallis test was used to compare the groups in terms of the percentage of collagen and the number of capillaries. Student's *t*-test was used to compare the pre- and post-transplant values for paired samples. *P* values <0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS v.20.0 software.
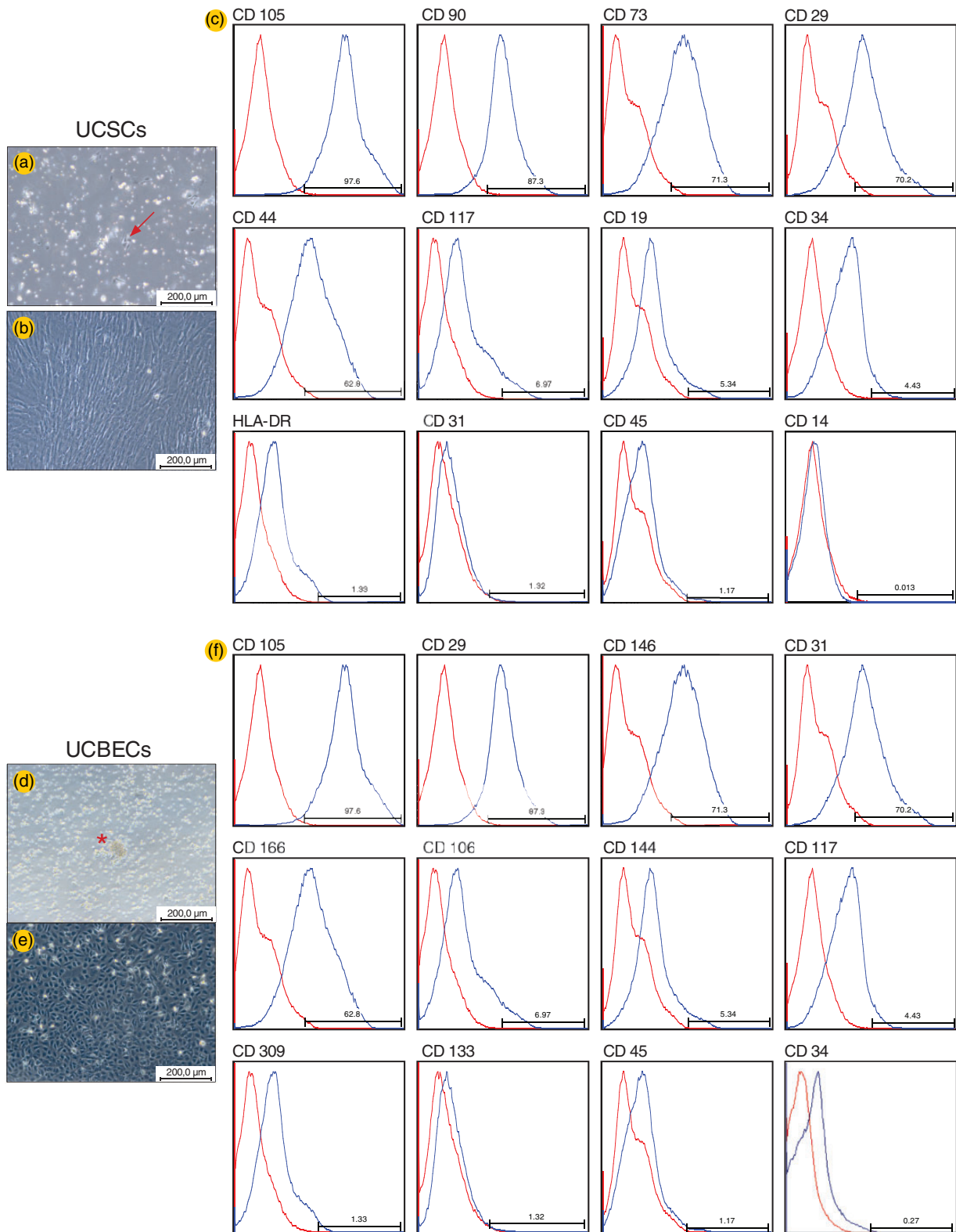
Figure 1 Characterization of UCSCs and UCBECs by morphology and flow cytometry assays. UCSCs displayed a typical spindle-shaped morphology (a and b), and UCBECs demonstrated a typical endothelial cobblestone morphology (d and e), as observed by phase microscopy. Flow cytometry histograms of UCSCs (c) and UCBECs (f). The blue line indicates the positively staining cells, whereas the red line indicates the isotype-matched monoclonal antibody control. The values are representative of three independent experiments.
UCSC: umbilical cord-derived stromal cells; UCBEC: umbilical cord blood-derived endothelial cells. (A color version of this figure is available in the online journal.)
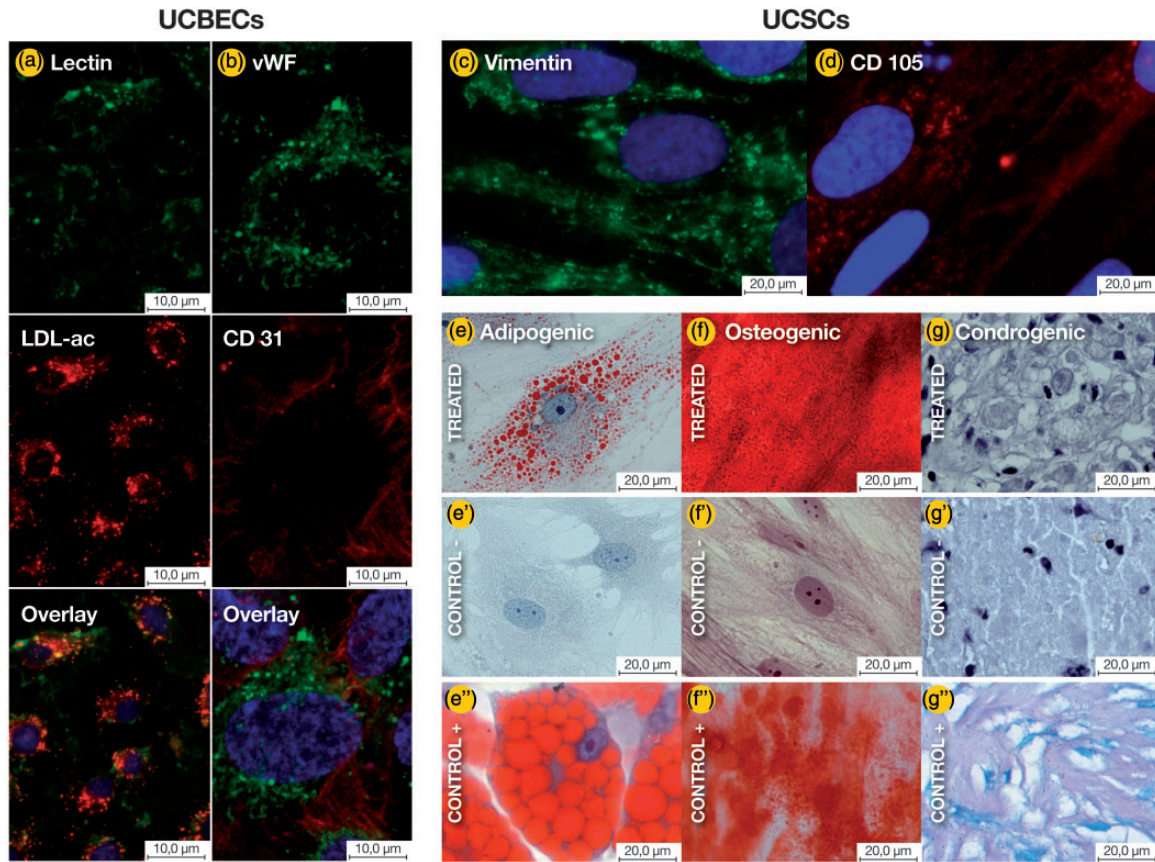
Figure 2 Detection of endothelial and stromal cell marker expression by immunofluorescence analysis. The UCBECs were shown to simultaneously bind fluorescein isothiocyanate UEA-1 (lectin) (a) and engulf DiI-acLDL (a'). (a'') Overlay of lectin+DiI-acLDL+DAPI. The UCBECs were labeled with vWF/FITC (b) and CD31/TexasRed (b'). (b'') Overlay of vWF+CD31+DAPI. The UCSCs were labeled with vimentin/FITC (c) and endoglin (CD105)/TexasRed (d). The nuclei were stained with DAPI (a'', b'', c and d). Characterization of MSC-like differentiation potential toward the osteogenic, chondrogenic, and adipogenic lineages. After three weeks in the respective induction media, the UCSCs stained positively for lipid vacuoles with Oil Red O (e), demonstrated the formation of mineralized matrix, as assessed by alizarin staining (f), and were positive for intracellular matrix mucopolysaccharides by toluidine blue staining (g). Cells cultured in growth medium without inductive factors served as negative controls (e', f', g'). BM–derived MSCs were utilized as a positive control (e'', f'', g''). Scale bars are specified in each image.
UCSC: umbilical cord-derived stromal cells; UCBEC: umbilical cord blood-derived endothelial cells; LDL: low-density lipoprotein; vWF: von Willebrand factor. (A color version of this figure is available in the online journal.)
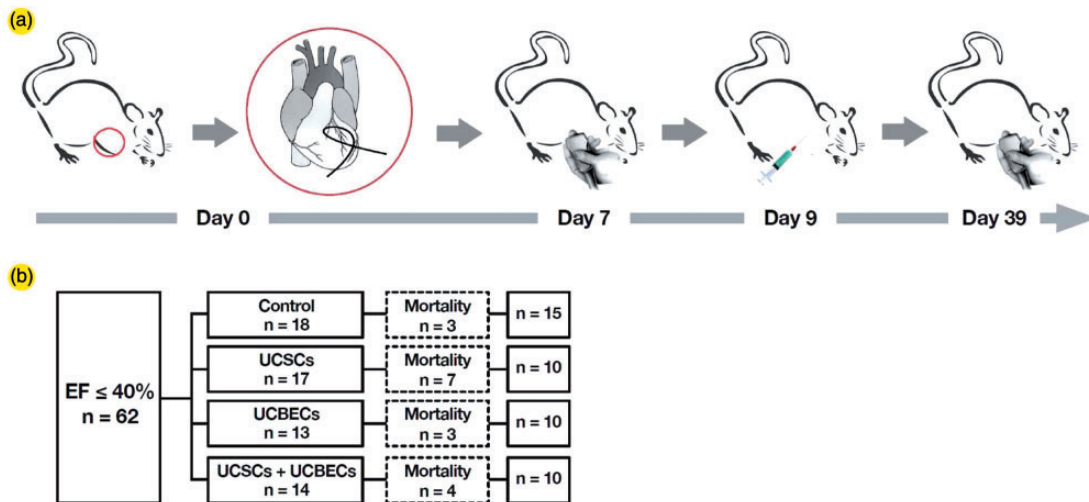
Figure 3 (a) Flow chart of the study design. Day 0: Exteriorization of the heart for coronary artery ligation. Day 7: The animals were subjected to echocardiography. Day 9: Animals with an LVEF of less than 40% were randomized and received a transplant. Day 39: A second ECHO was performed on the 30th day post-transplantation. (b) Mortality/survival rate for each group. Dashed boxes indicate the number of animals dead after the treatment (cell transplantation or medium injection)
UCSC: umbilical cord-derived stromal cells; UCBEC: umbilical cord blood-derived endothelial cells. (A color version of this figure is available in the online journal)

Results

Isolation of UCSCs, MNCs and purification of HUCB-derived CD133+ cells

A total of $5.26 \times 10^6 \pm 6.32$ cells were isolated from HUC. The mean volume of HUCB was 94.4 ± 42.30 mL, and the number of MNCs after isolation was $26.74 \times 10^6 \pm 19$. Flow cytometry analysis demonstrated that 68.15% (± 8.17) of the MNCs were CD45+, 1.66% (± 0.43) were CD133+, and 3.93% (± 5.19) were CD34+. After MACS-separation $17.26 \times 10^5 \pm 19.67$ cells were isolated. Flow cytometry analysis demonstrated that 10.9% (± 9.57) of the MACS-separated cells were CD45+, 84.4% (± 10.24) were CD133+, and 68.6% (± 21.44) were CD34+.

Characterization of UCSCs and UCBECs by morphology and flow cytometry assays

The cell cultures were examined daily under phase contrast microscopy, and a nearly homogeneous population of UCSCs with a fibroblast-like spindle-shaped morphology was observed. The UCBEC cultures contained multiple thin, flat cells that emanated from central clusters of rounded cells. After 15 days in culture, the UCBECs displayed a typical endothelial-like cobblestone morphology (Figure 1). Flow cytometry analysis was performed to characterize the UCSCs and UCBECs. The staining profiles of the cells for specific markers are provided in Figure 1.

Characterization of UCSCs and UCBECs by immuno-fluorescence and differentiation assays

The cultured UCBECs stained positive for CD31, vWF, and vascular endothelial growth factor, indicating an appropriately enriched culture of ECs. The UCSCs stained positive for vimentin and CD105. The negative control did not produce a positive signal. UCBECs in the third passage were positive for DiI-AcLDL uptake and lectin binding. The UCSCs differentiated into osteoblasts, adipocytes, and
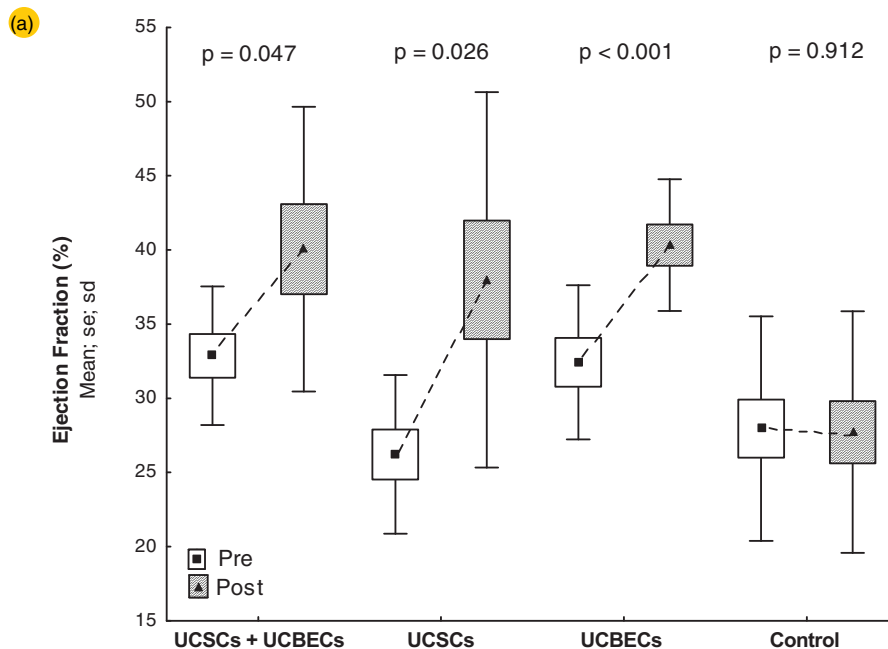
Group	EF pre	p-value*	EF post	p-value**	Dif EF	p-value**
UCSCs + UCBECs	32.86 ± 4.67	0.053	40.06 ± 9.59	0.002	7.19 ± 9.86	0.048
UCSCS	26.21 ± 5.35	0.484	37.98 ± 12.66	0.007	11.77 ± 14.05	0.002
UCBECs	32.42 ± 5.20	0.078	40.33 ± 4.43	0.001	7.91 ± 4.52	0.031
Control	27.95 ± 7.56		27.72 ± 8.15		-0.24 ± 8.18	

*ANOVA, p<0.05; ** ANCOVA adjusted to EF pre, p<0.05

Figure 4 Evaluation of cardiac function. (a) The left ventricular ejection fractions measured by echocardiography on the seventh day post-AMI (open boxes) and the 30th day post-transplantation (solid boxes) were significantly different for all transplantation groups (i.e. UCSCs + UCBECs, UCSCs, and UCBECs; *P* values are shown by each pair of boxes). The control group did not show a significant difference before and after transplantation. (b) LVEF pre transplant, post transplant, and the difference between pre-and post-transplant, for each cell transplantation group compared to the control group. The data are presented as the means. The *P* value was calculated by a ANOVA for the pre-transplant values and an ANCOVA for the post-transplant values and the comparisons between the cell groups and the control group (pre-transplant as a covariate; *P* < 0.05 was considered statistically significant). EF = ejection fraction. The boxes are the means ± standard error, and the bars are the means ± SD.
UCSC: umbilical cord-derived stromal cells; UCBEC: umbilical cord blood-derived endothelial cells; ANOVA: one-way analysis of variance; ANCOVA: analysis of co-variance. (A color version of this figure is available in the online journal.)
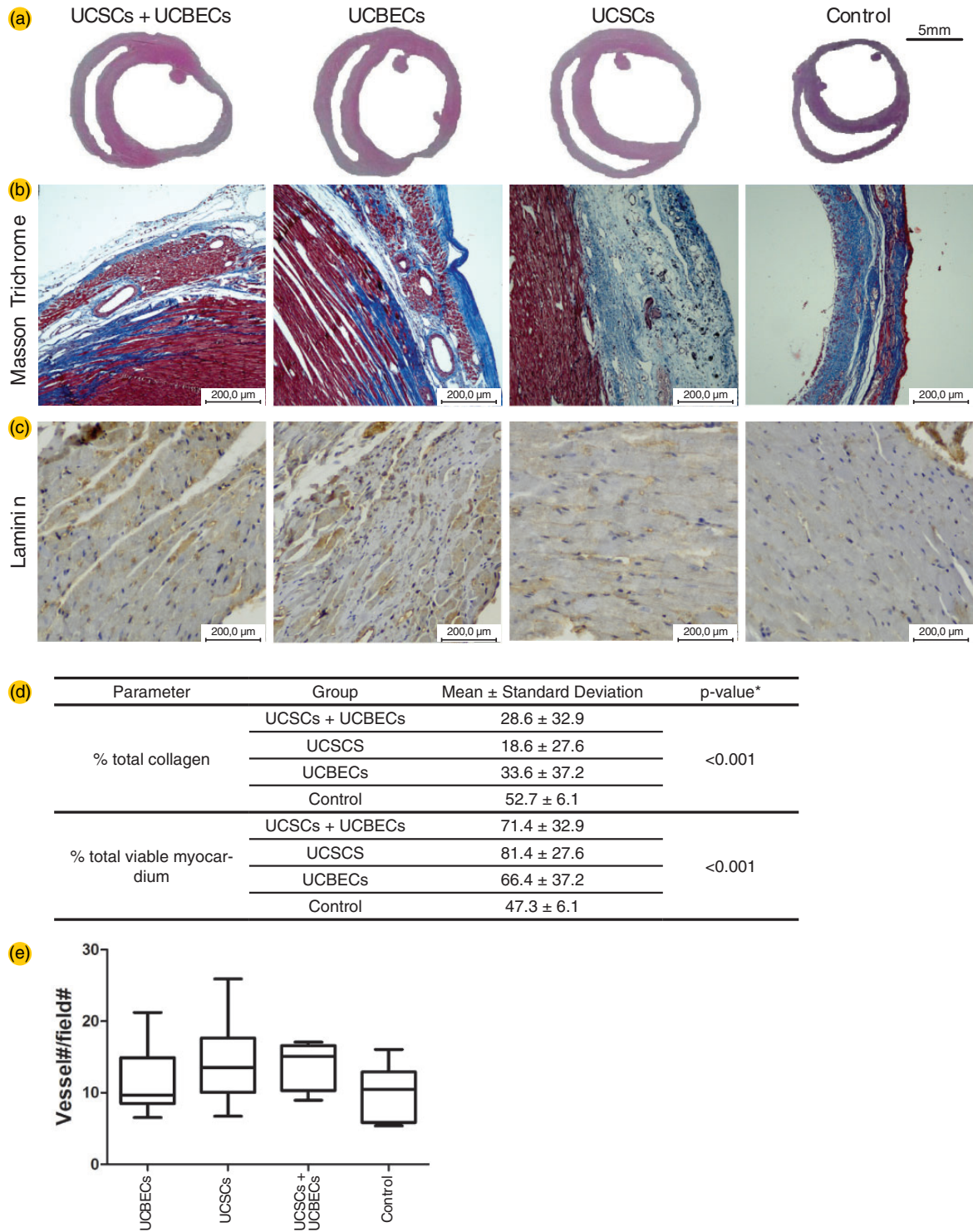
Figure 5 Histological analysis of the total collagen content, evaluated using Masson trichrome staining to detect myocardial fibrosis (collagen stained blue, viable myocardium stained red) (a), and capillary density analysis using an anti-laminin antibody (b). Quantification of the percentage of total collagen and the total viable myocardium. The total area of necrosis was significantly reduced in all three treatment groups compared to the control group (c). Quantification of the total capillary density in the myocardium. A trend of increased capillary density was observed in all the cell transplantation groups. However, the differences were not significant (d). UCSC: umbilical cord-derived stromal cells; UCBEC: umbilical cord blood-derived endothelial cells. (A color version of this figure is available in the online journal.)

chondrocyte-like cells, demonstrating their multipotency (Figure 2 and Figure S1).

Effect of UCSC, UCBEC, and UCSC + UCBEC transplantation on cardiac function

A total of 62 male Wistar rats with a left ventricular ejection fraction (LVEF) of less than or equal to 40% were randomized into four groups: Control ($n = 18$), UCSC ($n = 17$), UCBEC ($n = 13$) and UCSC + UCBEC ($n = 14$; Figure 3). After transplantation, a total of 17 animals died remaining 10 animals in each of the cell transplantation groups and 15 in the control group. The results from one month posttransplantation showed that there were significant differences between the pre- and post-transplant LVEF in the treatment and control groups ($P = 0.006$). However, the differences among the cell transplant groups were not significant ($P = 0.980$). LVEF improved by $7.19 \pm 9.86\%$ from the pre-transplant value in the UCSC+UCBEC group, $7.91 \pm 4.52\%$ in the UCBEC group, and $11.77 \pm 14.05\%$ in the UCSC group. In contrast, LVEF decreased by $0.24 \pm 8.18\%$ in the control group (Figure 4).

Reduction of myocardial fibrosis and increase in capillary density by UCSC, UCBEC, and UCSC + UCBEC transplantation

The transplantation of UCSCs, UCBECs, and UCSCs + UCBECs significantly attenuated the development of myocardial fibrosis. In addition, a trend of increased capillary density was observed in all the cell transplantation groups. However, the differences were not significant. Masson trichrome staining revealed that the areas containing collagen deposits were significantly smaller in the treated groups than in the control group. To evaluate the effects of the transplanted cells on cardiac fibrosis, we quantified the fibrotic area and the area of viable myocardium in the myocardial sections using image analysis software (Image-pro plus 6.0). The number of capillaries, which was counted using the technique described by Zhao et al.,[28] with modifications, showed a trend of increased numbers of blood vessel in the infarct area in the UCSC and UCSC + UCBEC groups (Figure 5).

Discussion

In the present study, we evaluated the effects of UCSCs, UCBECs, and a combination of these two cell types for the treatment of IC in a rat model of permanent ligation of the left coronary artery. Studies have shown that the transplantation of stem/progenitor cells improves cardiac function in experimental models of ischemic heart disease.[29–33] However, studies comparing the regenerative capacity of UCSCs and UCBECs are scarce, if not absent, for IC. Our study demonstrates, for the first time, the effects of UCSCs and UCBECs in animals with LVEF less than or equal to 40%, which corresponds to significant cardiac dysfunction. In humans, the effect of bone marrow-derived stem cells on LVEF was also greater and more statistically significant when participants had LVEF ≤40% of baseline compared to participants with LVEF >40% of baseline.[34]

The major findings of our study demonstrated that in an animal model of IC induced by ligation of the left coronary artery, the transplantation of 2×10^6 UCSCs, UCBECs, or UCSCs + UCBECs significantly improved heart function compared to the control group. However, there was not a significant difference among the cell therapy groups in global LVEF 30 days after the procedure, as revealed by ECHO analysis. The improved global heart function and decreased cardiac fibrosis in rats with IC imply the potential benefits of the direct transplantation of UCSCs, UCBECs, or UCSCs + UCBECs to the damaged area. Henning et al.[35] reported that directly injecting HUCB-derived cells into the myocardium was effective in reducing infarct size and improving ventricular function in non-immunosuppressed rats. The heterogeneity between the findings of clinical trials and experimental research can be attributed to differences in the disease context, patient population, cell isolation protocols, cell dose/type, timing of cell infusion, and the route of delivery.[34,36]

Another important result of this study was that UCSCs, UCBECs, and UCSCs + UCBECs significantly attenuated collagen deposition, which indicates that they inhibited the formation of a collagenous scar. This result suggests that the primary benefit may be reducing the number of apoptotic/necrotic cells in the border zone or favorably modulating the matricellular environment. We conducted a direct comparison of different cell types for the stimulation of angiogenesis in the same IC Wistar rat model. Injections of UCSCs, UCBECs, and UCSCs + UCBECs into infarcted rat hearts do not induced angiogenesis significantly, although a tendency was observed when treated with UCSCs and with both cell types together. This observation is at least partially discordant with the increased capillary density reported in other studies.[37–39] Nonetheless, differences in the procedures to generate heart ischemia, in the pathways of cell injection, cell number, cell sources/isolation, and even in the assays to quantify vessels might account for the conflicting results.

The mechanisms by which UCB and UC cells protect the heart and improve cardiac function appear to be complex and multifactorial.[40] Some studies suggest that the cells induce neovascularization in the necrotic area,[37–39] modulate the inflammatory reaction induced by ischemic cascades[41] and secrete growth factors[42,43] in animal models of IC.

In summary, these results demonstrate that the transplantation of ex vivo expanded UCSCs, UCBECs, and UCSCs + UCBECs promotes cardiac repair in IC animal models and could be used to improve the recovery of cardiac function after IC. Thus, these cells are promising tools for cardiovascular regenerative medicine, confirming their value for banking purposes and future therapeutic use.

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