

Comparison of biological characteristics of mesenchymal stem cells derived from the human umbilical cord and decidua parietalis

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Abstract. Mesenchymal stem cells (MSCs) are derived from the mesoderm and have the self-renewal capacity and multi-directional differentiation potential of adult stem cells. Stem cells from different sources have different molecular and growth characteristics; therefore, the mechanisms and effects of stem cell-mediated repair and tissue regeneration may be different. The aim of the present study was to compare the biological characteristics of MSCs derived from the umbilical cord (UC-MSCs) and MSCs derived from the decidua parietalis (DP-MSCs), and to provide new evidence for the selection of seed cells in regenerative medicine. Growth curves, cell doubling times, colony formation rates, immunophenotypes, differentiation capacities and secretion-factor levels of MSCs derived from the two sources were analysed. UC-MSCs and DP-MSCs exhibited similar properties with regards to morphology, spiral growth, immunophenotype, and potential to differentiate into osteoblasts and adipocytes. For each cell type, the positive rates of the cell surface markers CD73, CD90 and CD105 were >95%, whereas CD34 and CD45 positive rates were <1%. Analyses of *in vitro* growth kinetics revealed shorter cell-doubling times, and higher proliferative rates and colony formation rates of UC-MSCs compared with DP-MSCs ($P<0.05$). The concentration of basic fibroblast growth factor

in the supernatant from UC-MSCs was higher compared with that from DP-MSCs ($P<0.05$). However, UC-MSC supernatants exhibited lower levels of keratinocyte growth factor, vascular endothelial growth factor and stem cell factor compared with DP-MSCs ($P<0.05$). In conclusion, *in vitro* characterization of MSCs from these tissue sources revealed a number of common biological properties. However, the results also demonstrated clear biological distinctions and suggested that UC-MSCs may have more effective application prospects.

Introduction

Mesenchymal stem cells (MSCs), which are derived from the mesoderm and have the self-renewal capacity and multi-directional differentiation potential of adult stem cells, can be differentiated into cartilage, bone, skeletal muscle and other cell types under certain conditions (1-4). MSCs can be isolated from a number of diverse sources and exhibit multi-directional differentiation *in vitro*. Recent studies have demonstrated that MSCs repair damaged tissue (5,6). MSCs can not only function as seed cells of engineered myocardial tissue, bone and cartilage constructs, and important carrier cells in gene therapy, they can also repair damaged endometrium and inhibit graft vs. host responses (7). Stem cells have wide application prospects in the repair of damaged tissue (8-10) and may be used as a potential treatment for patients who suffer from infertility caused by intrauterine adhesions (11,12). MSC therapy is an effective treatment for myocardial necrosis of myocardial infarction, osteoporosis, bone cysts, lupus nephritis, diabetes, liver cirrhosis, liver failure, spinal cord injury and Parkinson's disease (13,14).

MSCs can be derived from a wide range of tissues, including the bone marrow, umbilical cord, placenta, amniotic fluid and dental pulp tissue. Stem cells from different sources have different molecular and growth characteristics; therefore, the mechanisms and effects of treatment may be different (15,16). In addition, the method of MSC culture differs in different laboratories, including the tissue adherent method (14) and the enzyme digestion method (11); therefore, the extent of cell amplification and cell quality is different, as well as clinical application solutions. Currently, there is no consensus on

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Abbreviations: MSCs, mesenchymal stem cells; KGF, keratinocyte growth factor; bFGF, basic fibroblast growth factor; DMEM/F12, Dulbecco's modified Eagle's medium/F12; EGF, epidermal growth factor; SCF, stem cell factor; VEGF, vascular endothelial growth factor; TGF- β , transforming growth factor- β

Key words: mesenchymal stem cells, umbilical cord, decidua parietalis, biological characteristics

the markers that identify or distinguish MSCs derived from different tissues; an internationally recognized standard for MSC culture has not been reached (17). The aim of the present study was to compare the biological characteristics of MSCs derived from the umbilical cord (UC-MSCs) MSCs derived from the decidua parietalis (DP-MSCs), such as proliferation, immunophenotype and differentiation potential under the same conditions. The results of the present study may provide novel evidence for the selection of seed cells in regenerative medicine.

Materials and methods

Sample collection and group allocation. All procedures in the present study were approved by the Ethical Committee of The First People's Hospital of Foshan and written informed consent was obtained from all donors. Foetal umbilical cord and decidua parietalis samples were obtained from 12 patients who underwent routine caesarean delivery for dystocia between January and April 2016 at the Department of Obstetrics, The First People's Hospital of Foshan. Samples were collected under sterile conditions and transported to the laboratory in Dulbecco's modified Eagle's medium (DMEM)/F-12 (Gibco; Thermo Fisher Scientific, Inc.) in an icebox. Donors were negative for hepatitis virus markers, syphilis and HIV.

Isolation and culture of UC-MSCs. Tissue samples comprising ~5 cm of the umbilical cord were washed three times with PBS. Amniotic membrane and blood vessels surrounding the umbilical cord were removed. The Wharton's jelly from the umbilical cord was cleaned by PBS and mechanically fragmented into 1 mm³ sections with ophthalmic scissors. Sections of Wharton's jelly were then suspended in DMEM containing 10% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 1% penicillin and 1% streptomycin (Gibco; Thermo Fisher Scientific, Inc.), and were incubated at 37°C in a humidified atmosphere containing 5% CO₂. The medium was replaced every 3 days, and tissue block cells were passaged upon reaching 80% confluence. The morphology of UC-MSCs was observed under an inverted optical microscope (Olympus Corporation). For further study, UC-MSCs were resuspended in DMEM/F12 at the required density and subjected to various assays.

Isolation and culture of DP-MSCs. Complete placenta tissue samples were washed three times with sterile PBS. The coarse surface of the decidua parietalis tissues close to the maternal side were scraped using surgical instruments and soaked in PBS. A total of 2 g decidua parietalis tissue was transferred to a 50-ml centrifuge tube and mechanically fragmented into 1-mm³ sections with ophthalmic scissors. Subsequently, tissue fragments were digested with 0.1% (m/v) collagenase I (Sigma-Aldrich; Merck KGaA) for 1.5 h at 37°C. Primary cells were obtained by filtering through cell strainers (pore size, 70 μm), resuspended in DMEM/F12 supplemented with 10% FBS, and seeded in 25 cm² flasks at a density of 1x10⁵/ml. Cells were cultured in an incubator at 37°C in a humidified atmosphere containing 5% CO₂ and passaged upon reaching 80% confluence. The medium was replaced every 3 days and the morphology was observed under an inverted

optical microscope (Olympus Corporation). For further study, DP-MSCs were resuspended in DMEM at the required density and subjected to various assays.

Determination of growth curves and population doubling times. The second and fifth generation of UC-MSCs and DP-MSCs were digested by trypsin and cultured in 24-well plates (1x10⁴ cells/ml) at 37°C in a humidified atmosphere containing 5% CO₂. Randomly selected wells (n=3) were digested with trypsin and counted daily using a Countess automatic cell counter (Invitrogen; Thermo Fisher Scientific, Inc.). Each well was counted three times, and the daily average was calculated for 7 days. The population doubling time during the logarithmic growth phase was calculated using the Patterson formula. Cell population doubling time (Td) was calculated using the following equation: $Td = t \times \lg 2 / (\lg N_t - \lg N_0)$, where T indicates incubation time; N₀ refers to cell number after inoculation and N_t refers to cell number at T hour culture.

Colony formation assay. The second and fifth generations of UC-MSCs and DP-MSCs were resuspended as single-cell suspensions following trypsin digestion and added in triplicate to 60-mm Petri dishes (100 cells/dish). Cells were cultured for 7 days, fixed with 4% paraformaldehyde for 5 min and stained with 0.1% crystal violet for 15 min at room temperature. After three washes with triple-distilled water, colonies that consisted of >30 nucleated cells were counted under an inverted optical microscope (Olympus Corporation) and were washed with ddH₂O. The number of colonies was determined using a camera, and the colony formation rate was calculated using the following formula: Colony formation rate = colony number/cell number x100%.

Immunophenotype analysis. Flow cytometric analysis was performed using an FC 500 flow cytometry instrument (Beckman Coulter, Inc.). Cell surface markers CD34, CD45, CD73, CD90 and CD105, which are considered as standard by the International Society For Cellular Therapy, were used to identify the MSC phenotype (18). Briefly, 2x10⁵ cells from the fifth generation of UC-MSCs and DP-MSCs were stained for 10 min with FITC-conjugated or phycoerythrin (PE)-conjugated monoclonal antibodies at 37°C. The following monoclonal antibodies were used: Mouse FITC anti-CD34 antibody (cat. no. 555821; 1:200); mouse FITC anti-CD45 antibody (cat. no. 555482; 1:200); mouse PE anti-CD73 antibody (cat. no. 550257; 1:200); mouse PE anti-CD90 antibody (cat. no. 561970; 1:200); and mouse FITC anti-CD105 antibody (cat. no. 561443; 1:200) (all from BD Biosciences).

Adipogenic differentiation. UC-MSCs and DP-MSCs at passage 5 were seeded in 6-well plates (2x10³ cells/ml, 3 ml). Adipogenic medium A (Cyanogen, Inc.) was added when cells reached 90% confluence and was replaced by adipogenic medium B (Cyanogen, Inc.) after 3 days. At 24 h, adipogenic medium B was replaced with adipogenic medium A, and this cycle was repeated three times. The adipogenic induction was then maintained by incubation in adipogenic medium A. Oil red-O staining (Sigma-Aldrich; Merck KGaA) was performed for 15 min at 37°C following a 14 day-induction and cells were observed under an inverted optical microscope (Olympus Corporation).

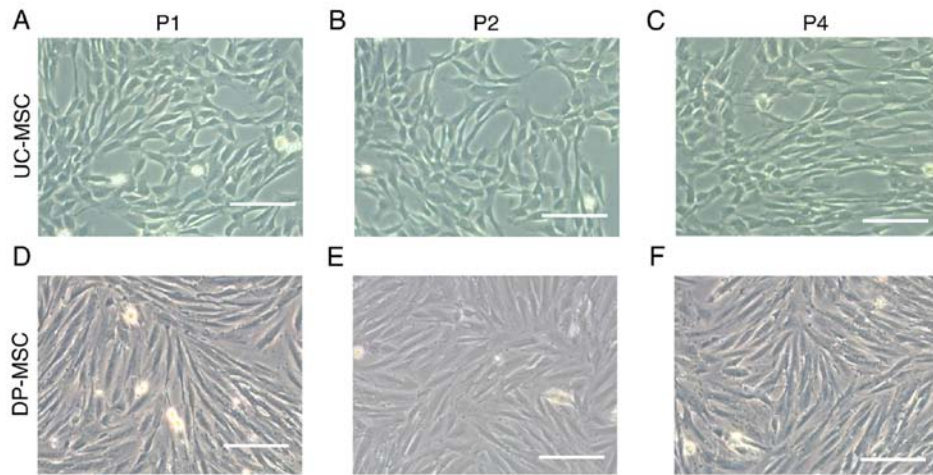


Figure 1. Morphological observations of UC-MSCs and DP-MSCs in different generations. (A-C) Morphology of UC-MSCs in the first, second and fourth generation, respectively. (D-F) Morphology of DP-MSCs in the first, second and fourth generation, respectively. Scale bar, 200 μm . DP-MSCs, mesenchymal stem cells derived from the decidua parietalis; P1, first generation; P2, second generation; P4, fourth generation; UC-MSCs, mesenchymal stem cells derived from the umbilical cord.

Osteogenic differentiation. UC-MSCs and DP-MSCs at passage 5 were resuspended at a density of 5×10^3 cells/ml (3 ml) and seeded in 6-well plates. Osteogenic medium (Cyanogen, Inc.) was added when cells reached 60% confluence and replaced every 3 days. After 14 days, the culture medium was removed, and cells were fixed with 4% paraformaldehyde for 15 min at room temperature. Fixed MSCs were then stained with alizarin red for 15 min at 37°C (Sigma-Aldrich; Merck KGaA) and observed under an inverted optical microscope (Olympus Corporation).

Detection of secreted factors. The double antibody sandwich method was used according to the ELISA kit manufacturer's protocol (ExCell Biology) to detect the content of basic fibroblast growth factor (bFGF; cat. no. EH022-48), epidermal growth factor (EGF; cat. no. EH016-96), keratinocyte growth factor (KGF; cat. no. EH122-96), stem cell factor (SCF; cat. no. EH231-48), transforming growth factor- β (TGF- β ; cat. no. EH010-96) and vascular endothelial growth factor (VEGF; cat. no. EH015-96) in culture supernatants of UC-MSCs and of DP-MSCs. Supernatants were obtained from MSCs cultured for 3 days and were centrifuged at $1,000 \times g$ for 10 min. Briefly, 200 μl standard substance and samples were added to coated wells and incubated for 2 h at room temperature. After three washes, 200 μl enzyme-labelled antibody was added to the wells and incubated for 1 h at room temperature. After a further three washes, 200 μl substrate was added to the wells and incubated for 20 min at room temperature. Stop buffer was added (50 μl /well) to terminate the reaction. The absorbance value at 450 nm was measured by automatic microplate reader within 30 min. The values of three replicates were averaged for each sample. CurveExpert 1.4 software (Hyams Development) was used to fit the curves and select the most appropriate equation to calculate the amounts of cytokines.

Statistical analysis. SPSS 17.0 software (SPSS, Inc.) was used for statistical analysis. Values are expressed as the means \pm standard deviation. Statistical analysis was performed

using one-way analysis of variance with the least-significant difference post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Isolated culture of UC-MSCs and DP-MSCs. The morphology of the two types of MSCs was compared and the results revealed that the umbilical cord and the placental wall decidua parietalis produced MSCs, which were spindle fibroblast-like cells with spiral growth (Fig. 1). The cell bodies of DP-MSCs were elongated compared with UC-MSCs.

UC-MSCs have a higher proliferative rate and shorter cell doubling time compared with DP-MSCs. The proliferative rate of MSCs was used to assess cell growth. The growth curves of the two types of MSCs exhibited a similar pattern. During the same time period, the number of UC-MSCs in the second and fifth generation was higher compared with the number of DP-MSCs (Fig. 2A). The cell doubling time in the second generation was 17.73 ± 0.51 h for the UC-MSC group and 21.93 ± 0.72 h for the DP-MSC group, and the difference between the two groups was significant ($P < 0.05$). The cell doubling time in the fifth generation was 14.50 ± 0.81 h for the UC-MSC group and 19.63 ± 1.1 h for the DP-MSC group, and the difference between the two groups was significant ($P < 0.05$) (Fig. 2B). The cell doubling time of MSCs was evaluated after culturing the two groups (Fig. 2B).

UC-MSCs exhibit higher colony forming efficiency compared with DP-MSCs. Colony forming efficiency of the two groups of MSCs was evaluated following culture (Fig. 2C). The colony forming efficiency of the UC-MSCs was significantly higher ($16.00 \pm 1.41\%$) compared with that of DP-MSCs ($13.60 \pm 1.50\%$) in the second generation ($P < 0.05$); the colony forming efficiency was $12.80 \pm 0.75\%$ in the UC-MSC group and $8.60 \pm 1.85\%$ in the DP-MSC group in the fifth generation, and the difference between the two groups was significant ($P < 0.05$) (Fig. 2C).

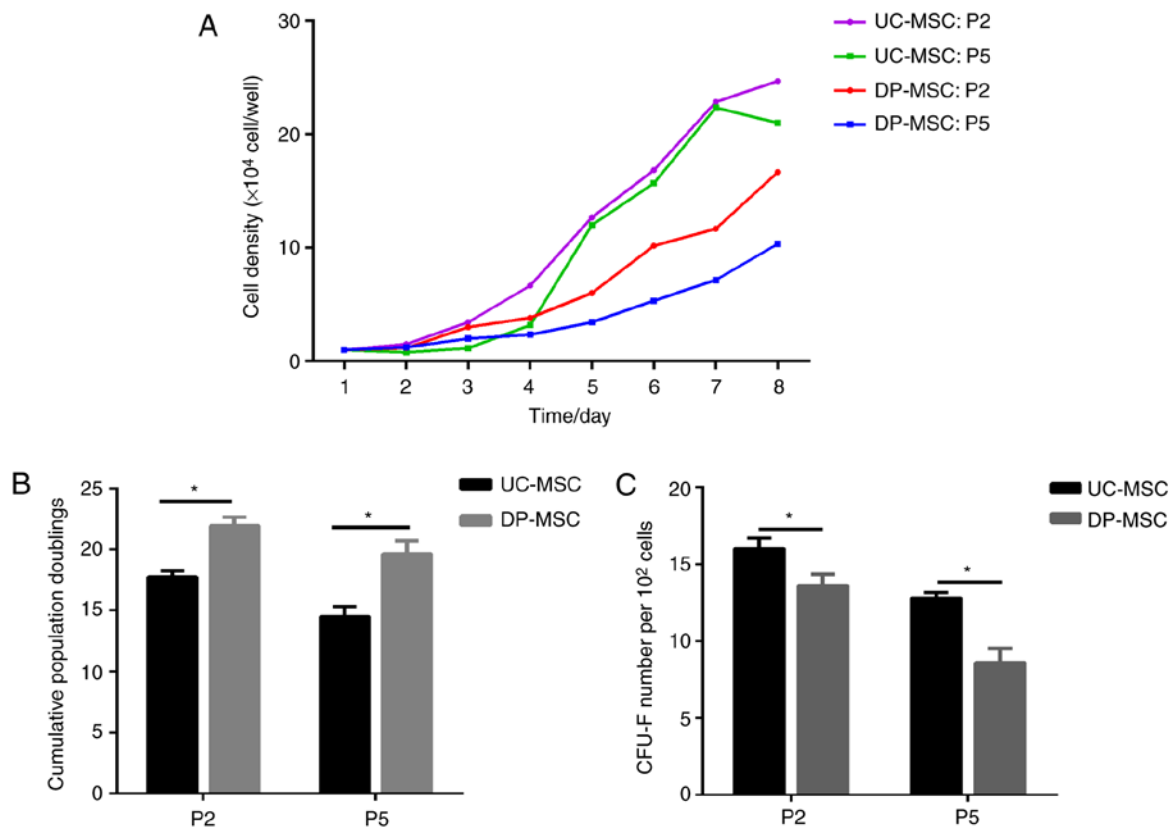


Figure 2. Comparison of growth characteristics of UC-MSCs and DP-MSCs in the second and fifth generations. (A) Growth curves, (B) cumulative population doubling (n=6) and (C) colony-forming abilities (n=6) of UC-MSCs and DP-MSCs. *P<0.05. CFU-F, colony forming unit frequency; DP-MSCs, MSCs derived from the decidua parietalis; MSCs, mesenchymal stem cells; P2, second generation; P5, fifth generation; UC-MSCs, MSCs derived from the umbilical cord.

Stem cell marker expression on UC- and DP-MSCs. MSCs from the UC and DP highly expressed the characteristic cell surface markers of MSCs CD73, CD90 and CD105, but did not express CD45 and CD34, which are characteristic cell surface markers of haematopoietic stem cells. The positive expression rate for CD73, CD90 and CD105 in UC-MSCs and DP-MSCs was >95%, and the positive expression rate for CD45 and CD34 was <1% (Table I; Fig. 3).

Differentiation of UC-MSCs and DP-MSCs. After induction with adipogenic medium, UC-MSCs and DP-MSCs gradually changed from fibroblast-like cells to flattened cells, and lipid droplets accumulated within them. The adipogenic differentiated MSCs were visualized by staining with Oil red-O on day 15; cellular staining was positive and the multiple lipid vacuoles in differentiated cells were stained red. After incubation with osteogenic medium for 15 days, MSCs exhibited obvious morphological alterations. Tightly packed colonies forming nodule-like structures were observed and deposition of calcium in these cells was observed by staining with alizarin red (Fig. 4).

Detection of cytokines secreted by UC-MSCs and DP-MSCs. MSC-secreted cytokines were assessed using ELISA. The results demonstrated that the bFGF content in cell supernatants of UC-MSCs was significantly higher compared with that in DP-MSCs (P<0.05; Fig. 5). The KGF, VEGF and SCF contents in cell supernatants of UC-MSCs were significantly lower compared with those of DP-MSCs (P<0.05). However, the

differences in EGF and TGF- β expression between UC-MSC and DP-MSC supernatants were not significant (Fig. 5).

Discussion

MSCs are important sources of stem cells for regenerative medicine due to their high self-renewal, proliferation and differentiation potential (19). MSCs are pluripotent stem cells derived from mesodermal interstitial tissue of early development (18), which are used for tissue repair and regeneration during individual ontogenesis and autoimmune diseases (20,21). Due to their high proliferation rates and multifunctional differentiation ability, MSCs can differentiate into endometrial cells, osteoblasts, chondrocytes and cardiomyocytes under certain conditions (22-25). MSCs have been successfully isolated from a number of adult tissues (26), such as adipose tissue (27), bone marrow (28) and umbilical tissue (29). However, MSCs from various tissue sources may have different biological characteristics. In the present study, the characteristics of UC-MSCs and DP-MSCs were analysed. The results revealed that the MSCs from the two sources demonstrated some biological distinctions with regards to growth characteristics and cytokine secretion, which may provide profound understanding of their biological functions.

In the present study, established methods (30) were used to isolate and culture MSCs from the umbilical cord and the placenta decidua parietalis tissue. UC-MSCs and DP-MSCs exhibited similar cell growth characteristics, surface markers, multipotent differentiation capabilities and paracrine ability.

Table I. Stem cell marker expression on MSCs.

Surface marker	Expression (%)	
	UC-MSCs (n=5)	DP-MSCs (n=5)
CD34	0.22±0.08	0.77±0.26
CD45	0.17±0.10	0.34±0.12
CD73	99.12±0.17	95.50±0.83
CD90	98.83±0.86	98.63±1.04
CD105	98.42±0.84	98.33±0.91

DP-MSCs, mesenchymal stem cells derived from the decidua parietalis; UC-MSCs, mesenchymal stem cells derived from the umbilical cord.

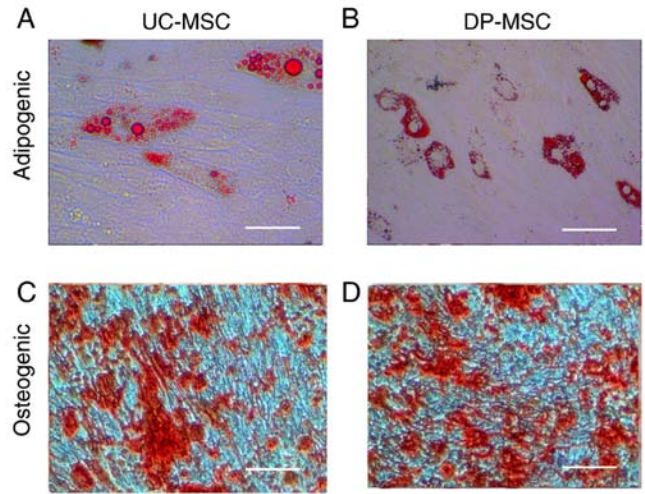


Figure 4. Cellular multipotent differentiation of UC-MSCs and DP-MSCs. (A and B) Following adipogenic induction for 14 days, UC-MSCs and DP-MSCs were positive for Oil red-O staining and contained an abundance of lipid droplets. (C and D) Following osteogenic induction for 14 days, UC-MSCs and DP-MSCs were positive for alizarin red staining. Scale bars, 50 μm. DP-MSCs, mesenchymal stem cells derived from the decidua parietalis; UC-MSCs, mesenchymal stem cells derived from the umbilical cord.

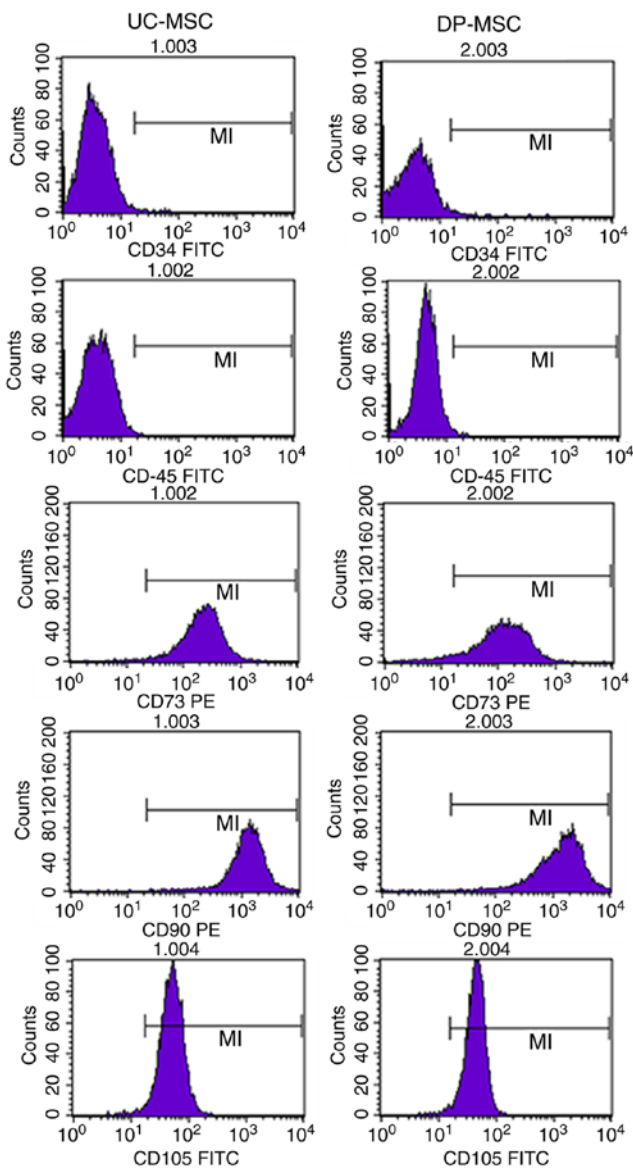


Figure 3. Surface markers of fifth generation UC-MSCs and DP-MSCs. Based on flow cytometric analysis, surface molecule markers CD73, CD90 and CD105 were highly expressed on UC-MSCs and DP-MSCs, whereas the expression of hematopoietic stem cell markers CD34 and CD45 <1%. DP-MSCs, mesenchymal stem cells derived from the decidua parietalis; PE, phycoerythrin; UC-MSCs, mesenchymal stem cells derived from the umbilical cord.

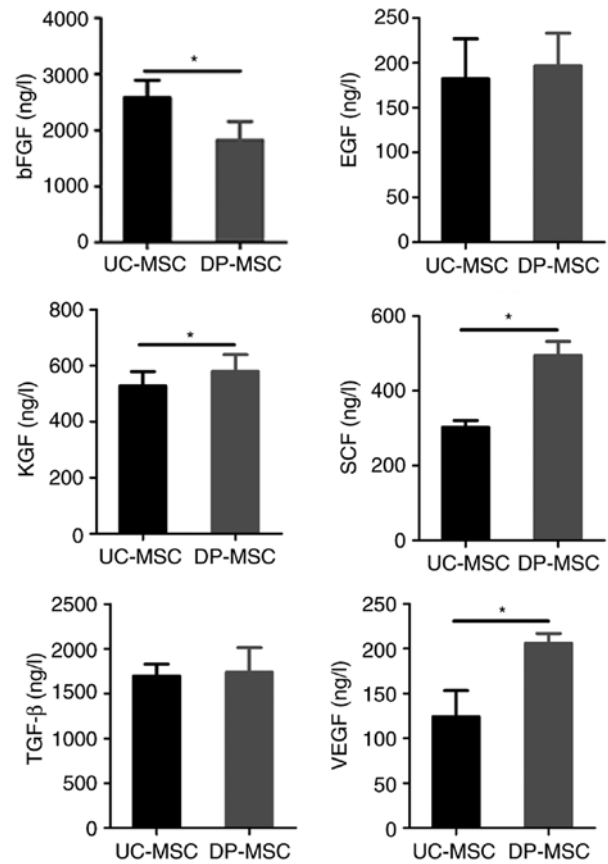


Figure 5. Comparison of bFGF, EGF, KGF, SCF, TGF-β and VEGF levels in UC-MSCs and DP-MSCs. Secretion of bFGF in UC-MSCs was higher compared with that in DP-MSCs. Secretion levels of KGF, SCF and VEGF in UC-MSCs were lower compared with those in DP-MSCs. No significant differences were observed between EGF and TGF-β secretion in UC-MSCs and DP-MSCs. *P<0.05. bFGF, basic fibroblast growth factor; DP-MSCs, mesenchymal stem cells derived from the decidua parietalis; EGF, epidermal growth factor; KGF, keratinocyte growth factor; SCF, stem cell factor; TGF-β, transforming growth factor-β; UC-MSCs, mesenchymal stem cells derived from the umbilical cord; VEGF, vascular endothelial growth factor.

UC-MSCs and DP-MSCs were similar morphologically and had a long fusiform shape and spiral growth, being grown under the same conditions. However, the doubling time of UC-MSCs was shorter compared with that of DP-MSCs, and the colony formation rate of UC-MSCs was higher compared with that of DP-MSCs at the same generation, possibly owing to a stronger proliferative capacity of UC-MSCs compared with that of DP-MSCs. Therefore, UC-MSCs may be used as a source of stem cells for the treatment of a number of diseases. Additionally, UC-MSCs and DP-MSCs had similar cell surface markers. The data revealed that the positive rates of CD73, CD90 and CD105 in each of the two MSCs groups surpassed 95%, whereas those of CD34 and CD45 were <1%. Both UC-MSCs and DP-MSCs had similar multilineage differentiation potential capacity towards osteogenesis and adipogenesis. These results indicated the feasibility of the isolation and culture of MSCs from umbilical cord and placenta wall decidual tissue.

A number of cytokines and signalling factors are secreted by MSCs, and MSCs from different species and sources produce different factors (31). MSC-secreted cytokines, such as bFGF, KGF, VEGF and SCF, serve important roles in cell proliferation, differentiation, growth and tissue repair (32-34). However, the characteristics of proteins secreted by UC-MSCs and DP-MSCs have not been extensively studied. Transplanted neural stem cells with high expression of bFGF have been demonstrated to promote cell migration and functional recovery following transient ischaemic stroke in rats (35). The results of the present study indicated that UC-MSCs and DP-MSCs secreted different cytokines; UC-MSCs exhibited a higher expression level of bFGF, and lower levels of KGF, VEGF and SCF compared with DP-MSCs, which suggested that these two types of tissue have different capacities for the secretion of signalling factors. These results may provide a theoretical basis for the study of stem cell therapy.

In summary, the present study compared the morphological and molecular characteristics of DC-MSCs and UC-MSCs. The results demonstrated clear biological distinctions between UC-MSCs and DP-MSCs, and revealed that UC-MSCs had a higher proliferative rate and colony forming efficiency compared with DP-MSCs. Therefore, UC-MSCs may have better application prospects. However, only two different sources of MSCs and several biological parameters were studied and compared in the present study. Nevertheless, the data extend the characterization of MSCs derived from different tissue types. As stem cell research develops, more potential applications of stem cells are being discovered, including injury repair (36). As cell therapy products are used in the human body, it is necessary to establish quality control and quality assurance systems, which will ensure product safety, efficacy and stability. The present study laid the foundation for the performance of clinical trials focussing on stem cell therapy. More extensive studies are required to facilitate clinical applications of MSCs.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

YTG and GW designed the study. DSL, YYZ, YPC and LJJ performed the experiments and analysed the data. YX and PFL collected the samples. XLZ and YLF performed flow cytometry and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures in the present study were approved by the Ethical Committee of The First People's Hospital of Foshan and written informed consent was obtained from all donors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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