A new method for the isolation of human umbilical cord mesenchymal stem cells

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Abstract

Mesenchymal stem cells (MSCs) are ideal candidates for regenerative medicine due to their multipotency. It is not easy to isolate MSCs from umbilical cord by traditional methods, MSCs are easily detached from plates after adding growth medium in the process of umbilical cord MSCs (UC-MSCs) isolation. In addition, UC-MSCs are easily susceptible to bacterial contamination. In this study, a new method for the isolation of human UC-MSCs was performed. The umbilical cord pieces was kept upside down for 3 hours before ading growth medium containing 100 U/ml penicillin and 100 mg/ml streptomycin. Being kept upside down for 3 hours was advantageous to UC-MSCs adherence. In order to identify UC-MSCs isolated by this new method, we performed multi-lineage differentiation experiments to confirm the multipotency of the isolated UC-MSCs. Through the adipogenic and osteogenic differentiation induction, the isolated MSCs were successfully differentiated into the adipocytes and osteocytes respectively. Besides, we also performed Flow Cytometer analysis to detect the expression of both positive and negative MSCs surface marker proteins. Results showed that >95% of the isolated MSCs could express the positive MSCs specific surface markers, but only <2% of the isolated MSCs could express the negative surface markers, further confirming that the isolated cells were indeed UC-MSCs.

Keywords

Umbilical cord; Mesenchymal stem cells (MSCs); Isolation.

1. Introduction

Mesenchymal stem cells (MSCs), an adult multipotent progenitor cells, was proved to be attractive seeding cells for tissue engineering [1]. They can be easily obtained from many different tissues, including bone marrow, adipose tissue, umbilical cord, and umbilical cord blood [2, 3]. They can be cultured for many passages in vitro as undifferentiated cells and provide a large number of cells required for tissue engineering. They are multipotent cells and can differentiate into many different cells under suitable conditions, including osteoblasts, chondrocytes, adipocytes, endothelial cells, and neurons [4-6]. MSCs were widely tested for tissue engineering in vitro, in animals and clinical trials [7]. They were recently isolated from human umbilical cord (UC-MSCs) [8]. UC-MSCs are more primitive than those isolated from other tissues [9]. The impressing proliferation capacity, differentiation potential and immunoregulatory functions as well as in vivo tropism confer UC-MSCs outstanding versatility in clinical applications. For example, UC-MSCs can function as candidate seeding cells in cellular transplant and regenerative medicine [10]. Previous studies showed that UC-MSCs were less immunogenic and were still viable and not rejected 4 months after transplantation as xenografts without the need of use of immunosuppressive drugs [11]. UC-MSCs can also play a promising immune-modulatory agents in immune-mediated disease or serve as target-delivering

vehicles in tumor or inflammation therapies [12]. In addition, umbilical cord can be easily obtained and provides a noncontroversial source of MSCs. Therefore, UC-MSCs represent a promising cell source for tissue engineering.

2. Materials and Methods

2.1 Materials

Dulbecco`s modified Eagle`s medium-low glucose (DMEM-LG) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). Penicillin/streptomycin and dimethyl sulphoxide (DMSO) were purchased from Sigma (St Louis, MO, USA). Ethanol, phosphate buffered saline (PBS), SDS and sodium chloride (NaCl) were purchased from Chemical Regent (Guangzhou, China). Basic fibroblast growth factor (bFGF) were purchased from Invitrogen (USA), Six-well plates were purchased from Corning (Acton, MA, USA).

2.2 Isolation and culture of UC-MSCs

Human umbilical cord was aseptically collected from full-term caesarean section patients with their consent at the First Affiliated Hospital of Jinan University, Guangzhou, China. Umbilical cord Wharton's jelly was cut into 2–3 mm^3 pieces, then was kept upside down for 3 hours in six-well plates in a 37 ℃ incubator, then, adding growth medium prepared from DMEM-LG, supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 5 ng/ml basic fibroblast growth factor, 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were harvested using 0.25% trypsin, and growth medium was changed every 3 days.

2.3 low cytometry analysis

To determine the MSC-specific marker expression, cells were harvested with trypsin for flow cytometry analysis as previously described with some modifications. After being fixed for 30 min with 4% paraformaldehyde at room temperature, the cells were incubated at 4℃ for 30 min with the following mouse anti-human antibodies, which were conjugated with phycoerythrin (PE): CD105-PE, CD73-PE, CD90-PE, CD45-PE, CD34-PE, CD14-PE,CD19-PE and HLA-DR-PE (Biolegend). PE-conjugated IgG2a (Biolegend) was used as isotype control for CD14-PE and HLA-DR-PE, and PE-conjugated IgG1 (Biolegend) for the others. After washing with phosphate-buffered saline (PBS), 1×10^6 cells were analyzed using FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

2.4 Adipogenic differentiation of UC-MSCs

Adipogenic differentiation was performed as previously described with minor modifications [10]. UC-MSCs were cultured in growth medium in a 24-well plate at a density of 1×10^4 cells/cm^2. After cell density reached at 70% confluence, the growth medium was changed to adipogenic differentiation medium consisting of DMEM-LG supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 60 μM Indomethacin, 1 μM dexamethasone, 0.5 mM IBMX and 5 μg/ml insulin solution (Sigma, St Louis, MO, USA). Cells were incubated for 21 days, and the medium was changed every 3 days. The generation of neutral lipid vacuoles was visualized by Oil Red O staining (Sigma).

2.5 Osteogenic differentiation of UC-MSCs

Osteogenic differentiation was performed as previously described [13]. UC-MSCs were cultured in growth medium in a 24-well plate at a density of 1×10^4 cells/cm^2. After cell density reached at 70% confluence, the growth medium was changed to the osteogenic differentiation medium consisting of DMEM-LG supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 100 nM dexamethasone, 0.2 mM L-ascorbate, and 10 mM β-glycerophosphate (Sigma). Cells were incubated for 21 days, and the medium was changed every 3 days. Osteogenic differentiation was examined by Alizarin red staining.

3. Results and discussion

3.1 Isolation of human umbilical cords MSCs

We isolated MSCs from human umbilical cords using a new method, and the umbilical cord pieces was kept upside down for 3 hours before adding growth medium containing 100 U/ml penicillin and 100 mg/ml streptomycin. Being kept upside down for 3 hours was advantageous to MSCs adherence. Results showed the representative phase-contrast images of UC-MSCs of primary cells (Fig. 1A), passage 1 (Fig. 1B), passage 2 (Fig. 1C), passage 3 (Fig. 1D), and the cells showed a fibroblast-like phenotype.

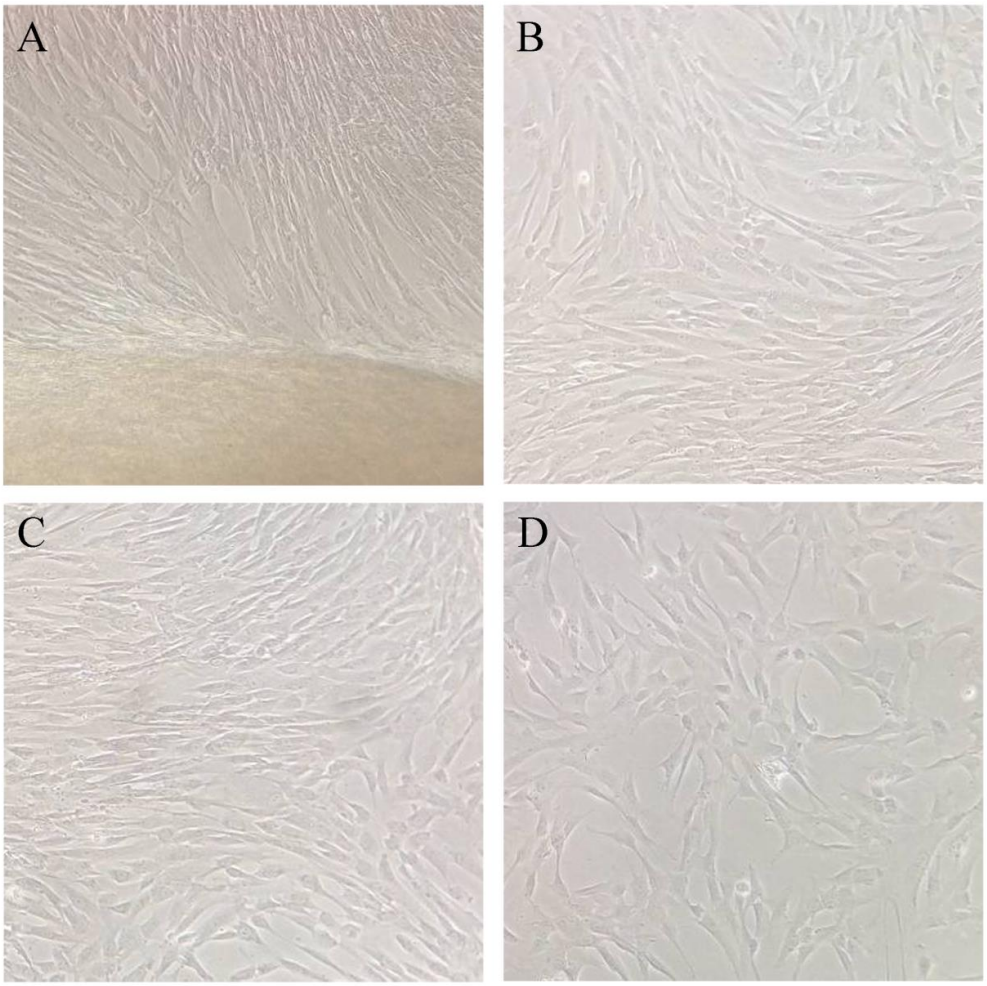

Fig. 1. Isolation and culture of UC-MSCs. These figures showed the representative phase-contrast images of UC-MSCs of primary cells (A), passage 1 (B), passage 2 (C), passage 3 (D). The cells showed a fibroblast-like phenotype.

3.2 Analysis of the typical MSCs marker expression by flow cytometry.

MSCs are highly heterogeneous, and genetically diversified and complicated. Thus, lacking the reliable identification of the MSCs used can compromise the accuracy and reliability of the experimental conclusions. Flow cytometry analysis showed that UC-MSCs were positive for typical MSCs marker CD105, CD73 and CD90, and negative for control markers CD45, CD34, CD14, CD19 and HLA-DR as expected (Fig. 2). Results showed that >95% of the isolated MSCs could express the positive MSCs specific surface markers, but only <2% of the isolated MSCs could express the negative surface markers, further confirming that the isolated cells were indeed MSCs.

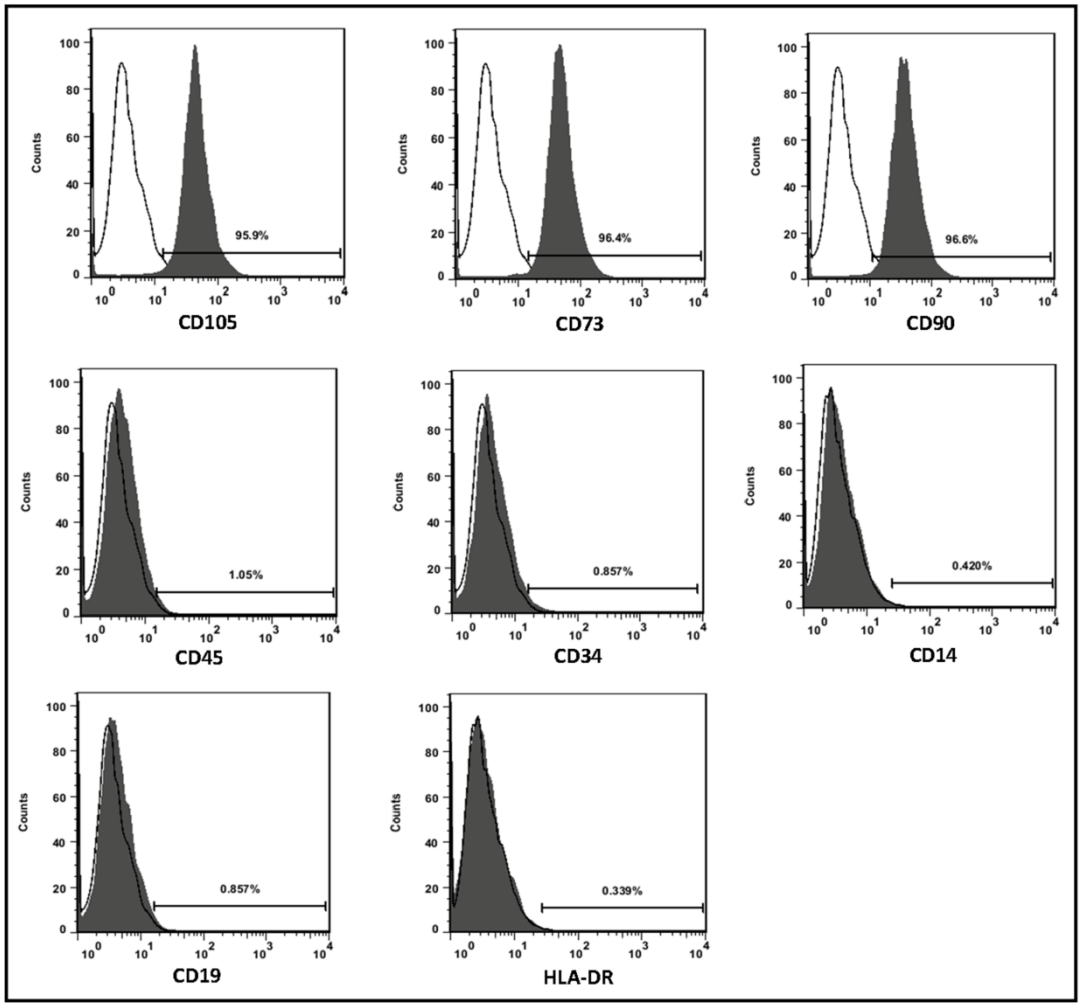

Fig. 2. Analysis of the MSC marker expression by flow cytometry. UC-MSCs isolated in this study were stained with phycoerythrin (PE)-conjugated antibodies. The open areas represent antibody isotype controls for background fluorescence, and the shade areas show the expression of the different MSCs markers.

3.3 Multi-potent differentiation potential of UC-MSCs.

UC-MSCs were induced into adipocytes and osteoblasts to examine their multi-lineage differentiation potential. Cells were cultured in the adipogenic differentiation medium and induced to differentiate into adipocytes. Oil Red O staining showed positive staining in the differentiated cells and no staining in the control cells (Fig 3). After culturing in the osteogenic differentiation medium for 21 days, UC-MSCs showed positive for Alizarin Red staining, demonstrating that UC-MSCs were able to differentiate into osteoblasts. Alizarin Red staining were negative in cells cultured in the growth medium as negative controls (Fig. 4). The results showed that UC-MSCs isolated in this study had multi-potent differentiation potential.

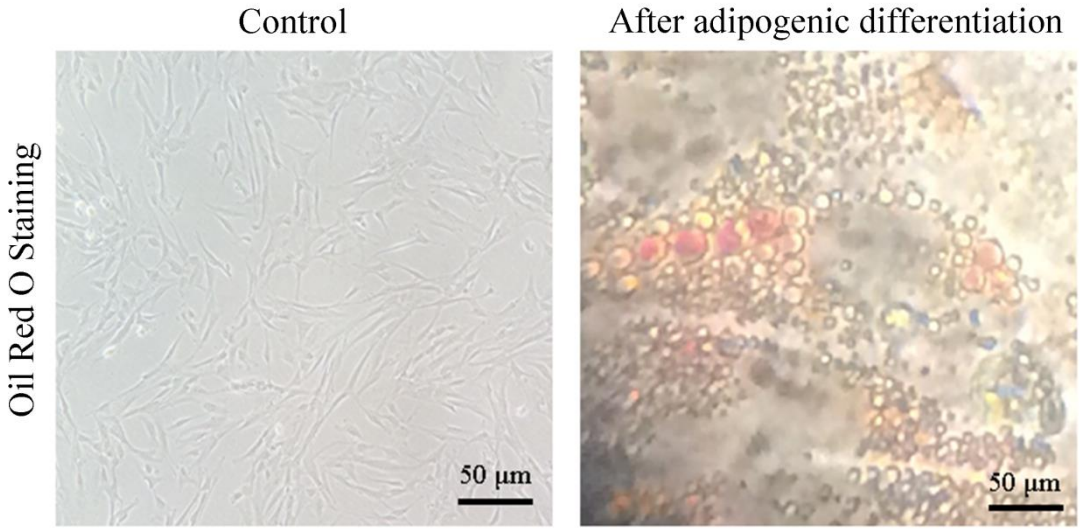

Fig. 3. Adipogenic differentiation of UC-MSCs. Adipogenic differentiation was examined by Oil red O staining after cells were cultured for 19 days in the adipogenic differentiation medium or in the growth medium as a control.

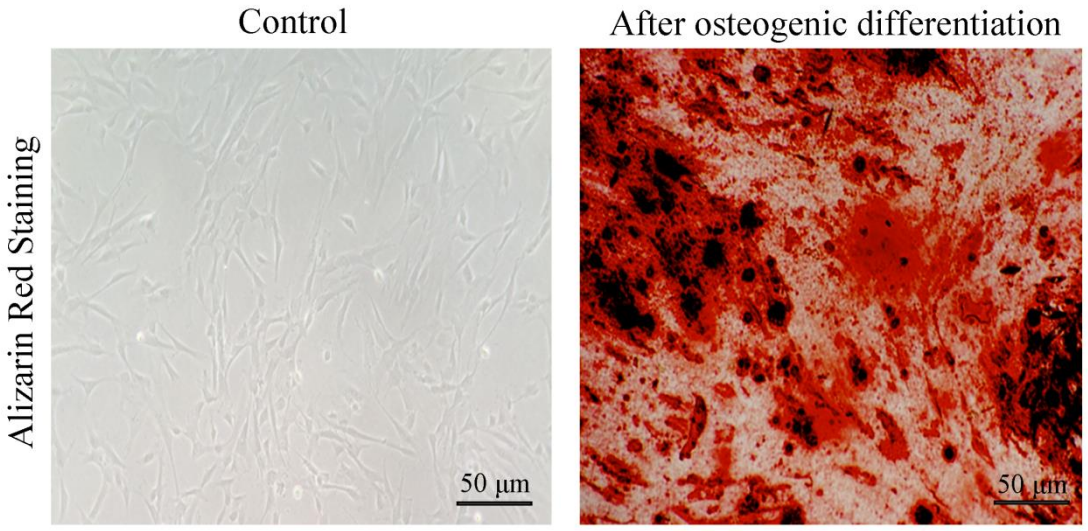

Fig. 4. Osteogenic differentiation of UC-MSCs. Osteogenic differentiation was examined by Alizarin Red staining after cells were cultured for 21 days in the osteogenic differentiation medium or in the growth medium as a control.

4. Conclusion

In this study, a new method for the isolation of human UC-MSCs was performed. The umbilical cord pieces was kept upside down for 3 hours before adding growth medium containing 100 U/ml penicillin and 100 mg/ml streptomycin. Being kept upside down for 3 hours was advantageous to MSCs adherence. We successfully isolated MSCs from human umbilical cord. Through the adipogenic and osteogenic differentiation induction, the isolated MSCs were successfully differentiated into the adipocytes and osteocytes respectively. Flow Cytometer analysis showed that isolated MSCs could express the positive MSCs specific surface markers, but could not express the negative surface markers, further confirming that the isolated cells were indeed MSCs.

Acknowledgements

The authors are grateful for the financial support of the Science and Technology Program of Guangdong No. 2017A020211014 and 2013B090500109 , the Science and Technology Program of Guangzhou No. 1563000464 and No. 201300000041. The authors declare no conflict of interest.

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