Wharton's Jelly Derived-Mesenchymal Stem Cells: Isolation and Characterization

Hossein Ranjbaran[1], Saeid Abediankenari[1], Masoud Mohammadi[1], Narjes Jafari[2], Alireza Khalilian[2], Zahra Rahmani[3], Marzei Momeninezhad Amiri[4], and Pirooz Ebrahimi[4,5]

[1] *Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Department of Biostatistics and Community Medicine, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[3] *Department of Obstetrics, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran*

[4] *School of Traditional Medicine, Iran University of Medical Sciences, Tehran, Iran*

[5] *Department of Molecular Medicine, Sapienza University, Rome, Italy*

Received: 28 Jun. 2017; Accepted: 21 Oct. 2017

Abstract- Wharton`s jelly-derived mesenchymal stem cells (WJ-MSCs), have a high proliferation valency and they do not produce teratogen or carcinogen after subsequent transplantation. They are known as regenerative medicine. Thus more research is needed on the isolation and characterization of mesenchymal stem cells. In this experimental study, we obtained Wharton's jelly tissues from mothers during normal vaginal delivery, after obtaining their informed consent. Mesenchymal stem cells were isolated from cultured Wharton`s jelly, cultured, and were then examined for their proliferation, immunophenotypes, and differentiation capacities. The immunophenotypes of WJ-MSCs were analyzed by flow cytometry. Differentiation was performed resulting in osteogenic, chondrogenic and adipogenic cells. WJ-MSCs formed a homogenous monolayer of adherent spindle-shaped cells. Our results showed the high capacity of the proliferation of WJ-MSCs. Immunophenotyping further confirmed the purity of the isolated cells; their surface antigen expression showed the phenotypical properties like those of WJ-MSCs. The expanded cells were positive for CD 90, CD105, and CD44; they were negative for CD34 and HLA-DR surface markers. The cells had the adipocytic, osteocytic and chondrogenic differentiation capacity. The isolation and characterization of WJ-MSCs with high purity had been conducted, and the results were obtained in a short span. The present study has revealed the feasibility of the culture medium with high glucose and 15% FBS in isolation and proliferation of WJ-MSCs. When Wharton`s jelly pieces were put in the dry bottom of the flask, very effective separation of the MSCs was achieved.

Acta Med Iran 2018;56(1):28-33.

Keywords: Mesenchymal stem cells; Wharton`s jelly; Flow cytometry; Immunophenotyping

Introduction

Mesenchymal stem cells (MSCs) are cells with a special self-renewal capacity and have the ability to get differentiated into ectoderm, endoderm, and mesoderm lineages. They also have immune-modulatory and anti-inflammatory effects (1-3) and are popular known as a regenerative medicine (4-6).

MSCs are identified using three characteristics: (1) adherence to the flask bottom, (2) differentiation into osteoblasts, chondroblasts and adipocytes and (3) expression of specific surface markers of CD90, CD105, CD73 and CD44, as well as the lack of expression of several markers including CD34 and HLA-DR (4,7,8).

In the past bone marrow was usually used for obtaining mesenchymal stem cells. But now numerous articles have been published about using embryonic cells, the umbilical cord, umbilical cord blood, and Wharton's jelly. MSCs from the umbilical cord or Wharton`s Jelly, are easily accessed and obtained compared to bone marrow and embryonic stem cells (8).

Wharton`s jelly-derived MSCs (WJ-MSCs) have a high proliferation valency; they do not turn into teratogenic or carcinogenic cells in case of transplantation (8). The bone marrow and adipose tissue, among others, are used as sources of MSCs (9-10),

Corresponding Author: S. Abediankenari
Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
Tel: +98 0912 1985667. Fax: +98 11 33247106, E-mail address: abedianlab@yahoo.co.uk

However, bone marrow as a source of MSC, comes with several disadvantages. First, only a small portion (0.05-0.001%) of the body`s bone marrow cells were included MSCs (9). Second, bone marrow gathering is the invasive and very painful procedure. On the one hand, although that adipose tissue is abundant in nature, its gathering requires an invasive procedure (10). Besides umbilical cord and Wharton`s jelly are better sources for MSCs because they are globally accessible tissues and their gathering creates no ethical harms since they are ordinarily discarded as human waste (11-17). MSC is the most important candidate for cellular therapy. This matter is well documented by the researches carried out in vitro (18), preclinical (19-21), and clinical conditions (22-25).

The aim of this study was to present the relevant methods for the isolation and characterization of Wharton`s jelly-derived MScs. It also aimed to pay special attention to their morphology and the differentiation into specific cell types including adipocytes, chondrocytes, and osteocytes. Furthermore, the immunophenotypes (positive and negative CD markers) of the isolated MSCs have been determined.

Materials and Methods

WJ-MSCs culture and expansion

Wharton`s jelly samples were procured from the Obstetrical Department of the Imam Hospital, Mazandaran University of Medical Sciences, Sari, Iran. The university's Ethics Committee approved our study plan, and the informed consents were obtained from all donors. Wharton`s Jelly has been collected from healthy full-term women, who underwent an elective cesarean section.

One hour after the collection, the Wharton`s jelly was transferred to the laboratory where it was disinfected and cut into pieces of 0.5-1$mm^2$.The Pieces were transferred to 10$cm^2$ plates, which contained High Glucose-Dulbecco`s Modified Eagle Medium-F12 (HG-DMEM-F12). HG-DMEM-F12 was supplemented with 15% FBS (Foetal Bovine Serum), Penicillin-100U/ml, Streptomycin-100µg/ml and 2mMol/L L-glutamine, then it was incubated in a humidified atmosphere containing 5%CO2 at 37° C.

It should be mentioned that the primary Wharton`s jelly was rinsed in phosphate –buffered saline (PBS) to eliminate blood clots. The medium was replaced every two days. The cells migrated from the explant to medium margins. After the cells reached a confluency of 90% in the second changing medium, the adherent cells were harvested with 0.25% trypsin-Ethylene Diamine Tetra Acetic acid (EDTA) (Gibco, Germany). Single-cell suspension was used for subsequent experiments and reseeded at a density of 5×10^5 of cells per 25cm^2 in culture flask.

Mesodermal lineage differentiation

The WJ-MSCs were induced to be differentiated into adipocytes, osteocytes, and chondrocytes. The differentiation protocol has been described previously (26). In this way, WJ-MSCs at 1.6 × 105 cells/ml were cultured in HG-DMEM-F12 complemented with 15% FBS and 2mMol/ L- glutamine and Penicillin-Streptomycin for 6 days. Then the cells were fixed with 0.4% paraformaldehyde (PFA) and stained with oil-red-o (Sigma, USA) to confirm the adipocyte.

For osteoblastic differentiation, the WJ-MSCs were cultured in HG-DMEM-F12 complemented with 10 nM b-glycerol phosphate (Sigma U.S.A) and 50mg/ml ascorbic acid-2 phosphate (Sigma, U.S.A). After fixation, the differentiated cells were stained with Alizarin red then the calcium deposition was confirmed. For chondrogenesis differentiation, WJ-MSCs were seeded in an HG-DMEM-F12 complete culture media at 1.6×105 cells/ml with 5 µl of chondrogenesis culture media for 14 days. The micromass cultures were then stained with Alcian blue.

Flow cytometry analysis

The adherent cells were treated by trypsin-EDTA and were collected by centrifugation at 300 g for 5 minutes. The cells were incubated for ~30 min in 100 µl of PBS supplemented with 1% bovine serum albumin with the following antibodies: anti-CD44-FITC (eBioscience), anti-CD105-PE (eBioscience), anti-CD34-PerCP (eBioscience), anti-CD90-FITC (eBioscience), and anti-HLA-DR-PE (eBioscience). The cells were then washed and centrifuged for 5 min at 300 g. The samples were analyzed by a Partec flow cytometer. The percentage of CD44-, CD105-, CD90-, CD-34-, and HLA-DR- positive or negative cell populations was calculated using the FlowJo FACS analysis software.

Results

MSCs morphology

Spindle-shaped MSCs and fibroblast cells were observed clearly under an inverted phase microscope. The WJ-MSCs created a homogenous monolayer of adherent spindle-shaped cells (Figure 1).

The MSCs were cultured and developed to the seventh passage. In primary passages, the cells proliferated quickly in the form of small spindle-shaped fibroblasts. The features of the cells were gradually changed as elaborated in the later passages (Figure1).

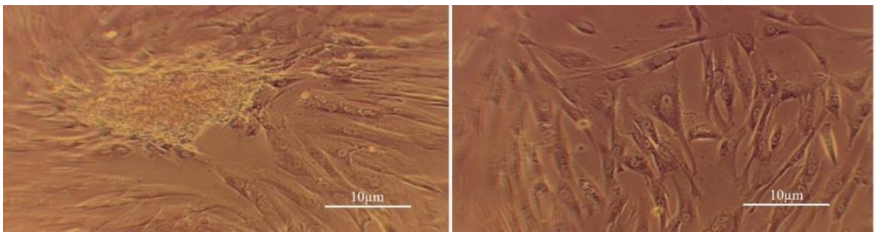

Figure 1. The Wharton`s jelly mesenchymal cells were grown from the edge of tissue explants and homogenous population of fibroblast-like MSCs at passage2-3 (× 100)

Multilineage differentiation

The differentiation capacity of the MSCs towards the three lineages (adipogenic, osteogenic, and chondrogenic) was confirmed (Figure 2).

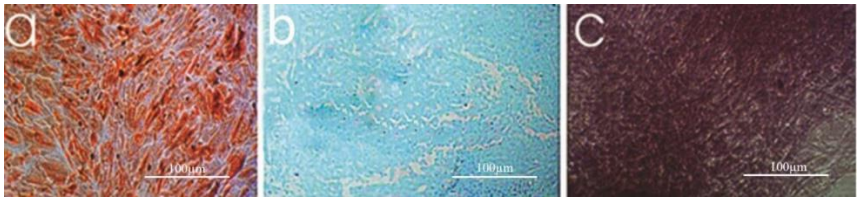

Figure 2. Multilineage differentiation of WJ-MSCs

(a) Adipogenesis was detected using oil-red-o staining. (b) Chondrogenesis was detected using Alcian blue. (c) Osteogenesis was detected using Alizarin-red staining (×100)

Immunophenotyping of WJ-MSCs

The immunophenotypes of WJ-MSCs were analyzed by flow cytometry. The results showed that WJ-MSCs were positive for CD90 (99.30%), CD105 (96.37%), and CD44 (83.11%); and were negative for hematopoietic marker CD34 (0.72%) and HLA-DR (0.85%) (Figures 3, 4).

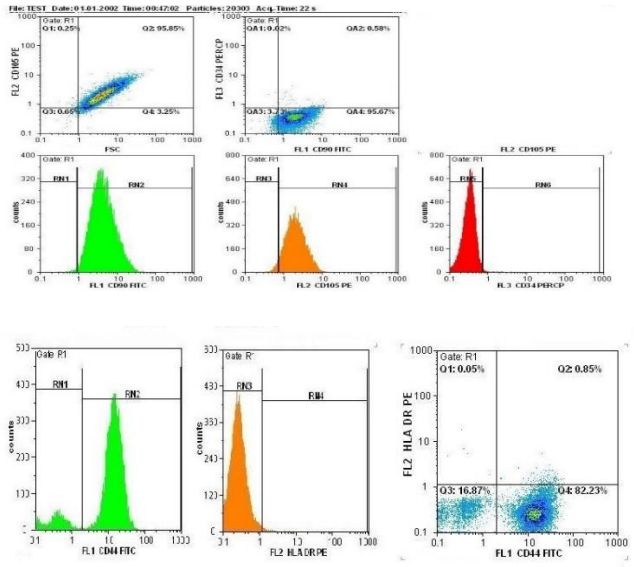

Figure 3. Flow cytometry analysis of mesenchymal stem cells derived from Wharton`s jelly

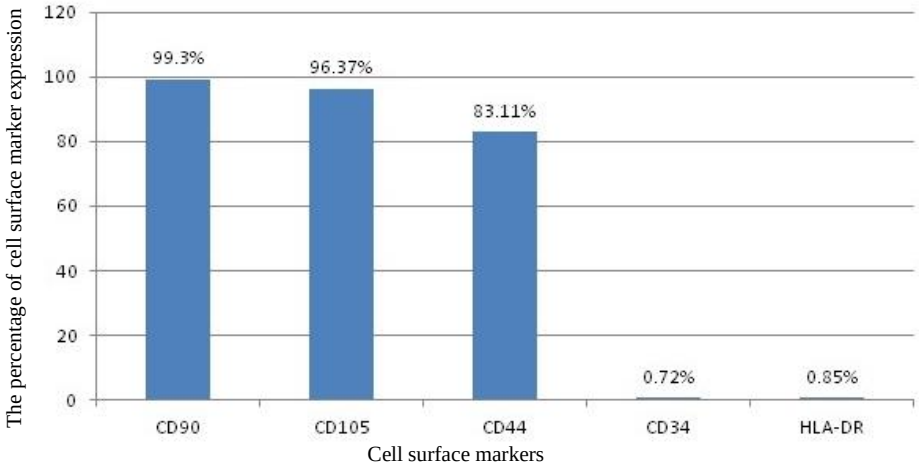

Figure 4. The percentage of WJ-MSCs surface marker expression
These cells expressed mesenchymal markers (CD90, CD105, CD44) and didn't express blood markers (CD34, HLA-DR)

In the present study, the MSCs were cultivated with HG and 15% FBS, which reached for a confluence of 90% in the second changing medium. However, in contrast, when the MSCs were cultivated with LG and 10% FBS, the confluence of 90% was obtained in the third changing medium. The medium was replaced every two days. Thereby, a shortened time span was achieved for obtaining WJ-MSCs (Table 1).

Table 1. Comparison of the numbers of changing medium in two different medium to reach a confluency of 90%, the medium was replaced every two days

The number of changing medium	Culture	Confluency
II	+15%FBS +HG	Confluency 90%
III	+10%FBS + LG	Confluency 90%

(HG=High Glucose ; LG=Low Glucose)

Discussion

Since mesenchymal stem cells isolated from Fetal tissues are discarded after delivery, and they possess similar phenotypes to bone marrow mesenchymal cells, so it has been tried to use embryonic tissue an alternative to bone marrow (26-27).

WJ-MSCs are not embryonic stem cells (ESCs) or adult stem cells (ASCs). These cells have the properties of embryonic stem cells and mature stem cells, which provide both embryonic stem cells and adult stem cells properties; thus their properties can be a useful source for the therapeutic use of stem cells (28).

In the present study, for the WJ-MSCs, we used flow cytometry to determine the positive and negative surface CD markers. The results of flow cytometry analysis showed that the mesenchymal stem cells derived from Wharton`s jelly were positive for CD90 (99.30%), CD105 (96.37%), and CD44 (83.11%); while they were negative for hematopoietic CD34 (0.72%) and HLA-DR (0.85%).

For the growth of WJ-MSCs, Chenphop *et al*., (2014) applied 10% FBS and Low Glucose (29); Nekoei *et al.,* (2015) applied 10% FBS and HG- DMEM (30); Fong *et al*., (2007) used HG-DMEM medium supplemented with 15% FBS (8).

In this study, more high-quality the MSCs with desired purity and adequate flow cytometry results were obtained by following several techniques and methods that were employed. Complying with the cooling chain requirements is important in terms of sample transfer. On the other hand, use of smaller Wharton's jelly caused the easier separation of MSCs. The important point is that in the first step for separating, millimeter-sized Wharton's jelly placed a dry flask bottom followed by, the addition of culture medium. If we place a piece of the Wharton's jelly into the flask containing the medium, it will not get strongly attached to the bottom of flask, and hence cell separation does not take place easily. In our study, HG-DMEM -F12 +15% FBS+L-glutamin+Penicillin/Streptomycin was used instead of Low Glucose DMEM-F12 and 10% FBS. Finally, we

obtained more appropriate mesenchymal stem cells within a shorter time.

We suggest further works to obtain a quick access to the WJ-MSCs with high purity percentages in a culture medium using different qualitative and quantitative methods.

In this study, the isolation and characterization of WJ-MSCs was achieved with high purity and obtained at a more suitable time. This research showed that the culture with high glucose and 15%FBS with putting pieces of Wharton`s Jelly to the dried bottom flask achieved a very effective separation of the MSCs.

Acknowledgments

This study was supported by a study Grant (no. 93.1462) from Immunogenetics Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran. Here by we thank Imam Hospital Sari, for their cooperation regarding procurement of umbilical cord samples.

References

1. El-Jawhari JJ, El-Sherbiny YM, Jones EA, McGonagle D. Mesenchymal stem cells, Autoimmunity and rheumatoid arthritis. QJM 2014; 107:505-14.
2. Abediankenari, Janbabaei Molla G, Ghasemi M, Yousefzadeh Y, Bahrami M, Alimoghaddam K. Vaccination of diffuse B cell large lymphoma with antigen-primed dendritic cells. Acta Med Iran 2013;51:284-8.
3. Wang D, Zhang H, Liang J, Li X, Feng X, Wang H, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. Cell Transplant 2013; 22:2267-77.
4. Kim MJ, Shin KS, Jeon JH, Lee DR, Shim SH, Kim JK et al. Human chorionic- plate-derived mesenchymal stem cells and Whar-ton's jelly-derived mesenchymal stem cells: a com-parative analysis of their potential as placenta-derived stem cells. Cell Tissue Res 2011;346:53-64.
5. Taghizadeh RR, Cetrulo KJ, Cetrulo CL. Wharton's Jelly stem cells: future clinical applications. Placenta 2011;32:S311-5.
6. Witkowska-Zimny M, Wrobel E. Perinatal sources of mesenchymal stem cells: Wharton's jelly, am-nion, and chorion. Cell Mol Biol Lett 2011;16:493-514.
7. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, et al.Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for cellularTherapy position statement. Cytotherapy 2006;8:315-7.
8. Fong CY, Richards M, Manasi N, Biswas A, Bongso A. Comparative growth behaviour and characterization of stem cells from human Wharton's jelly. Reprod Biomed Online 2007;15:708-18.
9. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
10. Abediankenari S, Ghasemi M. Generation of immune inhibitory dendritic cells and CD4+T regulatory cells inducing by TGF- beta. Iran J Allergy Asthma Immunol 2009 8:25-30.
11. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cell 2004; 22:1330-7.
12. Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. Stem Cell 2005; 23:220-9.
13. Weiss ML, Medicetty S, Bledsoe AR, Rachakatla RS, Choi M, Merchav S, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cell 2006; 24:781-92.
14. 14. Lu L-L, Liu Y-J, Yang S-G, Zhao QJ, Wang X, Gong W, et al. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis supportive function and other potentials. Hematological 2006; 91:1017-26.
15. Karahuseyinoglu S, Cinar O, Kilic E, et al. Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys. Stem Cells 2007; 25: 319-31.
16. Lund RD, Wang S, Lu B, Girman S, Holmes T, Sauvé Y, et al. Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. Stem Cells 2007; 25:602-11.
17. Rocca GL, Anzalone R, Corrao S, Magno F, Loria T, Lo Iacono M, et al. Isolation and characterization of Oct-4+/HLA-G+ mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. Histochem Cell Biol 2009;131:267-82.
18. Gnecchi M, He H, Liang OD, Melo LG, Morello F, Mu H, et al. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 2005;11:367-8.
19. Schuleri KH, Feigenbaum GS, Centola M, Weiss ES, Zimmet JM, Turney J, et al. Autologous mesenchymal stem cells produce reverse remodelling in chronic ischemic cardiomyopathy. Eur Heart J 2009;30:2722-32.

20. Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, et al. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res 2010;107:913-22.
21. Amado LC, Schuleri KH, Saliaris AP, Boyle AJ, Helm R, Oskouei B, et al. Multimodality noninvasive imaging demonstrates in vivo cardiac regeneration after mesenchymal stem cell therapy. J Am Coll Cardiol 2006;48:2116-24.
22. Karantalis V, DiFede DL, Gerstenblith G, Pham S, Symes J, Zambrano JP, et al. Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: the prospective randomized study of mesenchymal stem cell therapy in patients undergoing cardiac surgery (PROMETHEUS) trial. Circ Res 2014;114:1302-10.
23. Heldman AW, DiFede DL, Fishman JE, Zambrano JP, Trachtenberg BH, Karantalis V, et al. Trans endocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. JAMA 2014;311:62-73.
24. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009;54:2277-86.
25. Hare JM, DiFede DL, Castellanos AM, Florae V, Landin AM, El-Khorazaty J, et al. Randomized Comparison of Allogeneic Vs. Autologous mesenchymal stem cells for Non-ischemic Dilated Cardiomyopathy: POSEIDON-DCM Trial. J Am Coll Cardiol 2017;69:526-37.
26. Vater C, Kasten P, Stiehler M. Culture media for the differentiation of mesenchymal stromal cells. Acta Biomater 2011;7:463-77.
27. Battula VL, Treml S, Abele H, Bühring HJ. Prospective isolation and characterization of mesenchymal stem cells from human placenta using a frizzled-9-specific monoclonal antibody. Differentiation 2008; 76(4): 326-36.
28. Carlin R, Davis D, Weiss M, Schultz B, Troyer D. Expression of early transcription factors Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells. Reprod Biol Endocrinal 2006;4:8.
29. Sawangmake C, Osathanon T, Nowwarote N, Pavasant P. Neurogenic differentiation of human dental pulp stem cells using different induction protocols. Oral Disease 2014;20:352-8.
30. Nekoei M, Azarpira N, Kamalifar S, Kamalifar S. In vitro differentiation of human umbilical cord Wharton`s jelly mesenchymal stromal cells to insulin producing clusters. World J Clin Cases 2015;3:640-9.