Original Article

Comparative Analysis of Mesenchymal Stem Cells Isolated from Human Bone Marrow and Wharton's Jelly

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Abstract

Introduction: Bone marrow (BM) is a known source of mesenchymal stem cells (MSCs) that are used for cell therapy. This study attempts to identify if the Wharton's Jelly (WJ) is a suitable substitute for BM as a source for MSCs.
Materials and Methods: A population of human WJ and BM stem cells were isolated and incubated with fluorescein conjugated antibodies for five specific MSC markers. Cell populations were subsequently analyzed by flow cytometry.
Results: Most of the stem cells isolated from WJ and BM were positive for MSC markers. No significant differences existed between the expression of typical markers of MSCs in WJ isolated cells and that of BM.
Conclusion: WJ is a potential replacement for BM as a source of MSCs and can be used for cell therapy in clinical applications.
Keywords: Wharton's jelly ,Bone marrow, Mesenchymal stem cells

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Introduction

Mesenchymal stem cells (MSCs) are considered to be a population of multi-potent stem cells that have the capability to proliferate and differentiate into numerous types of mesodermal tissues including BM, cartilage and skeletal muscle [1]. MSCs are an attractive source of stem cells for regeneration of damaged tissues in clinical applications [2] due to their characteristics as undifferentiated cells, their ability to self-renew with a high proliferative capacity, and their potential to differentiate into mesoderm [3].

Known sources of MSCs are BM, umbilical cord (UC), cord blood, and placenta [1]. Although BM has been considered a main source for isolation of multipotent MSCs, [4], the limitations in using BM for stem cell therapy have mandated that researchers search for an alternative source for isolation of MSCs [5].

Wharton's Jelly (WJ), a part of the UC, contains mucoid connective tissue, fibroblast-like cells and multipotent cells such as MSCs [2]. With regards to the abundance of MSCs in the WJ and ease of their isolation, WJ can be investigated as a good replacement for BM.

In this study we compared the expressions of markers in MSCs isolated from WJ to those isolated from BM. We sought to determine if WJ was a suitable substitute for BM for the isolation of MSCs.

Materials and Methods

Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffered saline (PBS), penicillin, Ficoll and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The alkaline phosphatase kit was purchased from Chemicon (Billerica, MA, USA). Fluorescein isothiocyanate (FITC) conjugated antibody for CD73 and phycoerythrin (PE) conjugated antibodies for CD90, CD105, CD13, and CD49e were purchased from Beckman Coulter Company (Nyon, Switzerland).

Mesenchymal stem cell (MSC) isolation from human bone marrow (BM) and Wharton's Jelly (WJ)

A sample of human BM was obtained from the iliac crest according to standard protocol. was approved by the Ethics Committee at Tehran University of Medical Sciences. The sample used for isolation of BM stromal cells was provided from young, healthy adults, who were an average of 30 to 35 years of age with no signs or symptoms of any illnesses or diseases. BM samples were transferred on ice from the hospital to the laboratory in a heparin tube. Next, cells were isolated by density gradient centrifugation at 1700 rpm for 5 min using Ficoll-Hypaque Plus Solution. Cells were counted and seeded at a density of $2x10^6$ in 25 cm^2 flasks that contained 10 ml of DMEM supplemented with 1 ml FBS and 100 U/ml penicillin, then incubated at 37ºC in a 5% CO_2 incubator. After 72 h, non-adherent cells were removed and the medium renewed daily until cells reached 80% confluency.

In order to isolate stem cells from WJ, newborn UC was obtained from Milad Hospital, thoroughly rinsed with PBS, and then transferred to the laboratory under sterile conditions. The UC was dissected into 3-5 cm pieces; its vessels were removed and incubated with 3 ml of collagenase for 1 h. The homogenate was then centrifuged at 1000 rpm for 5 min. After discarding the liquid, the pellet was suspended in culture medium containing and maintained at 37ºC in a 5% CO_2 incubator. After three days, any non-adherent cells were removed and the medium was renewed daily until cells reached 80% confluency.

To show the existence of embryonic stem cells in WJ, we performed the alkaline phosphatase test

using an Alkaline Phosphatase Detection Kit. Media was aspirated on the fifth day and MSCs were fixed with 4% paraformaldehyde in PBS for 1-2 min, then rinsed with rinse buffer. Cells were incubated with a stain solution that contained a mixture of fast red violet (FRV) with naphthol AS-BI phosphate solution and water (FRV:naphthol: water) in the dark at room temperature for 15 min. Staining solution was aspirated and the wells were rinsed with rinse buffer. Wells were filled with PBS to prevent cell drying, then the numbers of colonies were counted with a light microscope (Olympus, Tokyo, Japan).

Flow cytometry

To investigate the presence of MSCs, we used flow cytometry to analyze the fourth passage cultured cells for the presence of MSC markers.

Cells isolated from BM and WJ were trypsinized and washed with PBS. Then, cells at a density of $5x10^6$ were incubated with 20 µl FITC conjugated monoclonal antibody for CD73 or PE conjugated monoclonal antibodies for CD90, CD105, CD13, and CD49e for 1 h at room temperature. Cells were washed with PBS and centrifuged at 1000 rpm for 5 min. Isotype control antibodies were used for eliminating any non-specific reaction. Analysis was performed with a FACS caliber flow cytometer (Becton Dickinson).

Statistical analysis

Data were expressed as mean±SD. Statistical comparisons between BM and WJ were performed with the independent t-test using SPSS software version 18 (Chicago, IL, USA). Differences were considered statistically significant at $P<0.05$.

Results

BM and WJ stem cell populations showed morphological similarity to that of fibroblasts (Figure 1). The population of stem cells isolated from BM was multipotent adult stem cells whereas the WJ stem cell population was pluripotent embryonic stem cells.

More than 80% of the MSCs derived from the BM and WJ stem cell populations expressed the typical MSCs marker proteins CD90, CD73, CD13, CD49e and CD105 (Figure 2).

Expressions of MSCs markers in the BM and WJ stem cell populations are listed in Table 1. There were no significant differences between BM and WJ in the MSC surface markers ($P>0.05$) despite the fact that CD73, CD90 and CD105 expressed more in WJ [89±0.6 (CD73), 93.6±0.6 (CD90) and 90.7±0.7 (CD105)] compared with BM [74.1±0.3 (CD73), 76.7±0.8 (CD90) and 83.8±0.7 (CD105)]. Expressions of CD13 and CD 49e were higher in BM, but were not significantly different ($P>0.05$).

Discussion

BM and UC tissue are good sources for MSCs and both have potential for multidifferentiation [6]. In this study we have compared the morphologies and surface markers of MSCs from different sources, BM and WJ tissue. Our results showed no significant differences between MSCs marker expressions in cells isolated from BM and WJ. Most cells isolated from BM and WJ exhibited typical MSCs characteristics that included fibroblastic morphology and the expression of a set of specific surface proteins.

However, several studies have shown UC to be a suitable source for MSCs isolation in cell therapy [3,7]. These investigations showed that WJ had therapeutic potential, possibly as a substitute cell for BM-derived MSCs for therapeutic purposes [8]. There was no comparative analysis between the cell populations isolated from WJ and BM for MSCs. Our study, for the first time, introduced WJ as a source for MSCs by comparing specific marker expressions and characteristics of MSCs to those derived from BM.

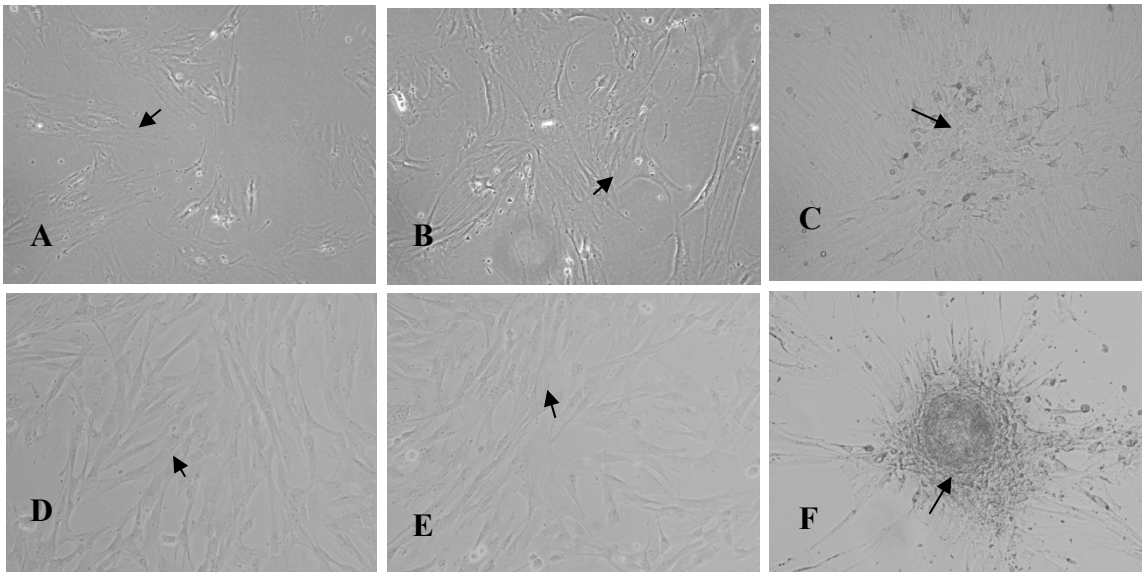

Figure 1. Digital images at 40x magnification of bone marrow (BM) and Wharton's Jelly (WJ) stem cell population. A, B and C show morphology of the cells isolated from BM during the first, second and third passages, respectively. D, E and F represent the first, second and third passage morphology of WJ-isolated cells, respectively.
Arrow indicate stem cells BM and WJ.

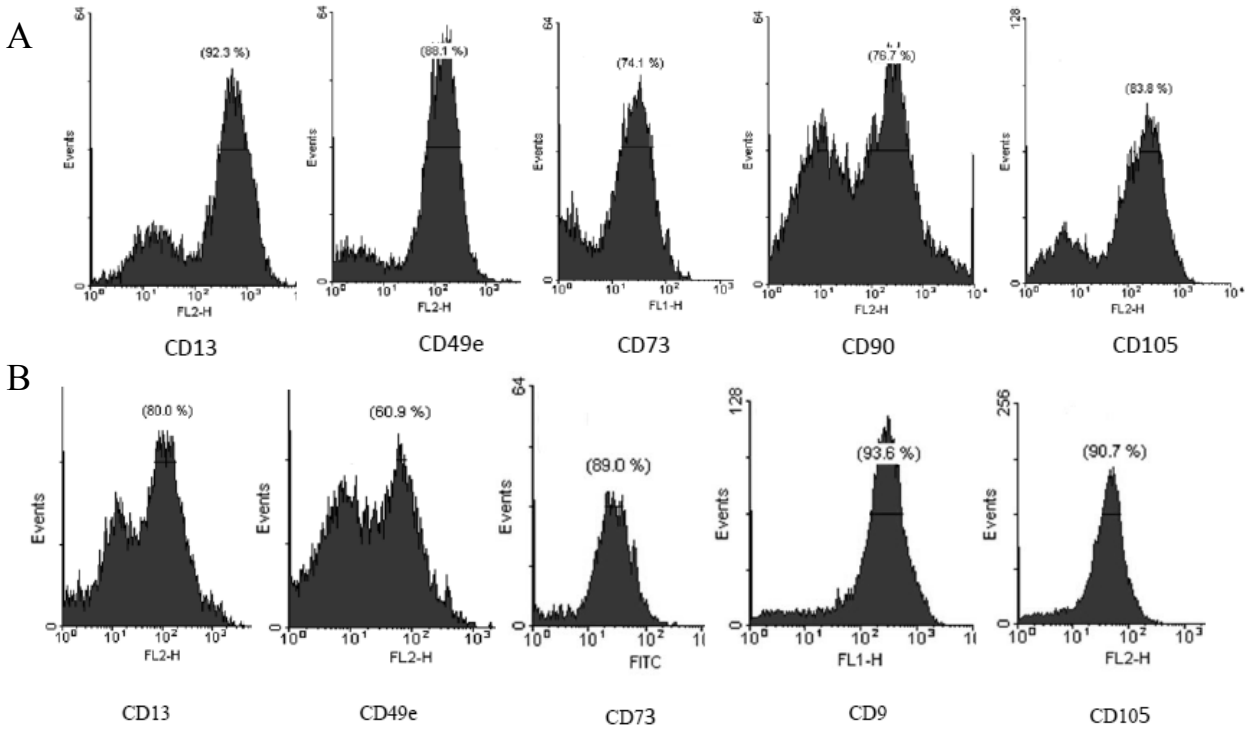

Figure 2. Flow cytometry histogram of the immunophenotype of bone marrow (BM) and Wharton's Jelly (WJ) mesenchymal stem cell (MSCs) population. Expressions of five markers (CD13, CD 49e, CD73, CD90 and CD105) are shown. A and B represent expressions of markers in BM and WJ isolated cells, respectively.

Table 1. Expression of markers on mesenchymal stem cells (MSCs) isolated from bone marrow (BM) and Wharton's Jelly (WJ) as analyzed by flow cytometry.

Maker	BM(%)	UCM(%)
CD13	92.3±0.3	80±0.4
CD49e	88.1±0.1	60.9±0.6
CD73	74.1±0.3	89±0.6
CD90	76.7±0.8	93.6±0.6
CD105	83.8±0.7	90.7±0.7

Numbers show mean values of the percentag
e of positive cells±standard deviation (SD) of the total number of cells analyzed.
Significant differences were not observed between WJ compared with BM (P>0.05).
Abbreviations: BM, bone marrow; WJ, Wharton's Jelly

In this study we selected five specific MSC markers to increase the specificity of investigating the difference between these cell populations in BM and WJ. All the markers (CD13, CD49e, CD73, CD90 and CD105) highly expressed in MSCs isolated from both sources [9-14].

It has been shown that cells isolated from UC could differentiate into osteocytes, chondro-cytes and adipocytes [2]. Results from a previous study have clearly suggested that WJSC (wharton's jelly stem cells) possessed superior proliferation capacity and grew more than adult tissue [10].

This study introduced WJ as a source for MSCs that had similar cell characteristics to BM. These results might enable the use of WJ as an alternative source for MSCs in emergency situations.

We did not investigate the potential of MSCs isolated from WJ to differentiate into other cells. Thus, in order to confirm WJ as a suitable substitute for BM in stem cell therapy, an investigation of the function and potential of MSCs for differentiating into organic cells in WJ in comparison with BM is recommended.

Our findings indicate that WJ is an important source of MSCs that can be used in cell therapy. Therefore, we can introduce WJ as an alternative source for human MSCs for experimental and clinical applications in comparison with BM.

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