CLINICAL PROSPECTS OF SCALE-UP FOETAL WHARTONS JELLY DERIVED MULTIPOTENT STROMAL CELLS TO FULFIL THE THERAPEUTIC DEMANDS

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ABSTRACT

Increasing clinical application of multipotent stromal cells has led to the development of large-scale manufactured of MSCs. In this context, the final cellular product should meet the various regulatory criteria, such as safety, integrity, strength, purity and quality that have been ascribed to cell therapy products. In this study, we have optimized large scale expansion of WJ-MSCs for clinical and therapeutic applications. The biologic properties of UC-MSCs, such as plastic adherence, morphology, specific surface antigens, and multipotent differentiation potential, were retained after clinical-large scale expansion. The cells formed a monolayer of homogenous spindle-like cells. Cell size underwent no obvious change, as evidenced by the consistent harvested cell density after clinical-scale expansion. We successfully up-scaled up to P2 to get as many as 5.22x10^9 cells from a single UC and also observed up to P10 with no significant change in viability, stemness and karyotyping. The growth rate were rate of WJ-MSCs as seen by CFU analysis and PD experiments. A set of typical surface phenotypic characteristics by flow cytometry confirmed that more than 95% of the cells reacted with antibodies against CD73, CD90, and CD105 and less than 1% reacted with CD45, CD34, CD79, and HLA-DR. These cells could be induced to differentiation potential of osteogenic, chondrogenic and adipogenic lineages was proved for their exploitation as a potential therapeutic tool to meet increasing therapeutic demands.

KEYWORDS: Multipotent Stromal Cells (MSCs), Umbilical Cord (UC), Wharton’s jelly (WJ), Cumulative Population Doubling Level (CPDL), Population doubling (PD), Passage (P), Cluster of Differentiation (CD).

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INTRODUCTION

Regenerative properties and therapeutic benefits of multipotent stromal cells are well understood and progressed rapidly since the pioneering work of Friedenstein et al.\(^1\). Multipotent stromal cells (MSCs) can be described as robust, highly proliferative and display typical mesenchymal stemness characteristics such as plastic-adherence, the expression of markers such as CD 73, CD 90 and CD 105 and the non-expression of hematopoietic stem cell markers such as CD 34, CD 45. In addition to this they also have known to possess immunomodulatory and trophic properties were allowing them to be transplanted even against xenogenic barriers\(^2\-\(^3\). Recent research on these stem cells helps us to understand that they are able to migrate to the targeted area upon administration (homing) where they provided healthy microenvironment enabling engraftment of differentiated cells. MSCs have been isolated from the bone marrow, umbilical cord tissue matrix\(^4\), umbilical cord blood\(^4\), amniotic membrane\(^5\), menstrual fluid, adipose tissue, pulp of deciduous teeth etc. While MSCs can be obtained from any of these sources only MSCs from the umbilical cord tissue matrix and umbilical cord blood are fetal in nature. Among all fetal tissues, human Umbilical Cord (hUC) is a hassle free source of MSCs. Fetal umbilical cord is about 60-65cm in length and weighs approximately 40 grams\(^6\). From a single donor \(\sim 1 \times 10^{17}\) umbilical cord tissue cells could be obtained and these cells showed no phenotypic or karyotypic alterations\(^7\). This makes them very unique and lucrative candidate for cell based therapies. Since stem cells are being used in number of human and animal clinical trials of regenerative medicine and tissue engineering, scale-up of these stem cells is the current market demand. Even though bone marrow derived stem cells were well established source for MSCs their lack of numbers and anguish surgical procedures makes fetal derived MSCs an ideal choice due to their abundance, ease of isolation, lack of ethical issues and enhanced immunosuppressive properties\(^8\)-\(^9\). We here in the current investigation have tried to isolate, optimize, phenotypically characterize and clinically expand stromal cells at minimum passage level for the successful therapeutic application. We could isolate large number of WJ-MSCs at Passage 2 \([P(2)]; sufficient for clinical application. We carried out different quality analysis to check the efficacy of isolated cells at various stages of expansion. We observed that WJ-MSCs isolated can be expanded up to \(P(2)\) to get as many as \(5.22 \times 10^9\) cells from a single umbilical cord and also observed up to passage 10 with no significant change in viability, stemness, karyotyping and sterility. We focused on MSCs of the Wharton’s jelly (WJ) from situated at the interface between the mother and the fetus; they have a high immunological tolerance, which makes them easy to be used in therapeutic application\(^10\)-\(^12\). In summary these primitive cells have greater ability to expand in culture and have different physiology relatively because of their naive status.

MATERIALS AND METHODS

Isolation and culture of WJ-MSCs:

(i) Umbilical cord Collection and Transportation

Umbilical cords (n=5) irrespective of the sex of baby were collected from full-term births after cesarean section was obtained from the C section delivery process with parental permission. The cord was clamped and approximately 25-40cms of it was cut and transferred into a labeled tube containing collection medium with antibiotics. In temperature controlled container (2–15°C), the umbilical cord was collected for standardization were transported and processed within 36hrs. Collection medium used was Dulbecco’s modified Eagle medium -Nutrient mixture Ham’s F-12 (1:1) with L-Glutamine (1X); 2.438g/L Sodium Bicarbonate (DMEM/F12; Gibco, USA) and 0.125mg/ml Cefoperazone sodium\(^{13}\) (MP Biomedicals, LLC).

(ii) Separation of Wharton’s jelly

The cord was washed twice with Dulbecco’s Phosphate-Buffered saline (DPBS); decontaminated thoroughly with 70% alcohol
for 2 minutes and again washed twice with DPBS to remove all traces of blood samples. The blood vessels are removed from cord and Wharton’s jelly was separated and cut into small pieces. Approximately 35-40 jelly explants of about 0.5mm size each were plated in tissue-culture-grade T-75 flask (Nunc, Denmark) containing culture medium. The culture medium used was Dulbecco’s modified Eagle medium -Nutrient mixture Ham’s F-12 (1:1) with Glutamax (1X); 2.438g/L Sodium Bicarbonate; Sodium Pyruvate (DMEM/F12+; Gibco, USA) and 10% fetal bovine serum (FBS; Gibco, US) supplemented with 3ng/mL bFGF (Sigma, USA) was used for all the experiments. All flasks were left undisturbed in a 5% CO₂ incubator maintained at 37°C for 15–18 days by changing media at an interval of 4 days. Cells were harvested at 80% confluency using 0.125% trypsin (Gibco); a cell count was performed and seeded into T-175 tissue culture flasks for further expansion.

(iii) Up scaling experiments
The isolated Wharton’s jelly were plated at P(0) in T-75 tissue culture flasks (Corning, NY, USA) at seeding density of approximately 0.5 to 0.7 grams. Harvested cells at P0 were seeded at a density of 3000 cells/cm² for P(1) in T-175 tissue culture flasks (Corning, NY, USA) and for P(2) in hyper flasks (Corning, NY, USA). Similarly, 3000 cells/cm² were plated at P(3) to P(10) in T-175 tissue culture flasks. The harvested cells were suspended in a cryoprotectant solution composed of 90% complete media and 10% dimethyl sulfoxide (DMSO) (Origen Biomedical, USA) and stored in the vapor phase of a liquid nitrogen tank until further use.

(iv) Cell Proliferation assay
The proliferation was measured as previously described with some modifications. Estimated growth efficiency and proliferation potential of the amniotic membrane MSCs were determined based on the total CPDL using the formula CPDL = ln (Nf / Ni) ln2, where Ni is the initial number of cells seeded, Nf is the final number of harvested cells, and ln is the natural log. Cells (3000 cells/cm²) were plated in Culture flask and sub cultured for 4-5 days. The cells were then counted and 3000 cells/cm² were re-plated. To determine the CPDL, the population doubling for each passage was calculated and then added to the population doubling levels of the previous passages.

(v) Colony forming unit (CFU) assay
About 100 MSCs were plated in duplicates on a 100 mm² cell culture dish with Culture Medium and incubated for 15 days. The cultures were replenished every 3 days using fresh complete medium. Cultures were stopped and assessed for CFU determination on day 15 by staining with 5% crystal violet for 10 minutes. The cells were rinsed twice with distilled water, all visible colonies were counted and the images were captured using Nikon Eclipse 90i microscope.

Characterization of WJ-MSCs
(vi) Phenotype and Purity
Harvested cells were analyzed for immuno phenotypic characterization with 1x10⁵ cells were incubated with specific mouse anti-human primary antibodies conjugated to flurochromes. Cells were stained with antibodies CD90-FITC (2µl), CD73-APC (5µl), CD105-PE (20µl), CD45-FITC (20µl), CD34-PE (20µl), CD79a-APC (20µl) and HLA-DR (5µl) (BD Pharmingen, NJ). Cells incubated with identical concentrations of FITC- (5µl), PE- (5µl), PerpCyc.5.5 (5µl), APC- (5µl) conjugated mouse IgG isotype secondary antibodies (BD Pharmingen, NJ) served as isotype controls. After incubation for 10mins at 37°C cells were acquired by flowcytometer (FACSCalibur, BD Biosciences, USA). Approximately 10,000 events were detected and data analysis was done using the CellQuest Pro software (BD Biosciences, USA).

(vii) Tri-lineage Differentiation
Osteoblast differentiation was induced by culturing WJ-MSCs in DMEM/F12+ supplemented with 10% FBS, 10-8 M dexamethasone (Sigma-Aldrich), 30 µg/ml ascorbic acid (Sigma-Aldrich) and 10 mM β-glycerophosphate (Sigma-Aldrich) for 3 weeks. Fresh medium was replenished every 3 days. Calcium accumulation was assessed by Von Kossa Staining. The differentiated cells were washed with PBS and fixed with 10% formalin.
for 30 minutes. The fixed cells were incubated with 5% silver nitrate for 60 minutes under UV light and then treated with 2.5% sodium thiosulphate for 5 minutes. To induce adipogenic differentiation, WJ-MSCs were cultured for 21 days in DMEM/F12+ supplemented with 10% FBS, 1 µM dexamethasone, 0.5 mM isobutyl methyl xanthine, 1µg/ml insulin and 100 µM indomethacin (Sigma-Aldrich). Inducing factors were added to the replenished medium every 3 days. Cells were fixed in 10% formalin for 20 minutes. 200 µl of oil red O staining solution was added and incubated for 10 minutes at room temperature. The cells were rinsed five times with distilled water. To induce chondrogenic differentiation, WJ-MSCs were cultured in STEMPRO (Invitrogen) chondrogenesis differentiation medium (chondrocyte differentiation basal medium with chondrogenesis supplement). Chondrogenesis differentiation medium was replenished every 3 days. For staining, cells were fixed with 4% formalin for 30 minutes. Safranine O staining solution was added and incubated for 5 minutes. The cells were rinsed with distilled water and images were captured using Nikon Eclipse 90i microscope.

(viii) Chromosomal analysis
Karyotyping of final harvested cells was carried out using a standard Giemsa banding procedure. 1 x 10^5 cells were seeded in T-25 flask containing culture media. Once 90 % confluency was achieved, cells were arrested used 5 drops of colchicine (Karyomax Colcemid solution, USA) and incubated for 3 hrs. At least 20 metaphase spreads were analyzed and the presences of sex chromosomes, acrocentric and metacentric phases were analyzed. Images were acquired using Olympus BX51 Bright field microscope using karyovision software (Olympus, Japan).

(ix) Endotoxin Assay
Endotoxin levels were determined by the gel clot Limulus Amebocyte Lysate test method (LAL). Endotoxin produces a gelation reaction with amebocytes or circulating blood of Limulus polyphemus (American Horseshoe crab). Limulus Amebocyte Lysate was reconstituted with reagent water and mixed in equal parts of the sample (Lonza, USA). After incubation in the presence of endotoxin; gelation occurs but in the absence of endotoxin, gelation does not occur. The LAL test requires serial dilutions with neutral pH. LAL is limited to aqueous solutions or extracts of test specimen hence endo toxin testing was performed on the cell supernatant obtained at the time of P(2). The lysate sensitivity used in the assay was 0.125EU/ml.

(x) Cell Counts and Viability
Cell numbers were determined by using a hemocytometer and viability was assessed by 0.4% trypan blue dye (Gibco, USA) exclusion method.

(xi) Sterility and Mycoplasma Assay
Sterility (aerobic, anaerobic, fungal) and mycoplasma assays were performed before cryo preservation. The sample was injected into aerobic and anaerobic BacT Alert bottle (Biomerieux, INC, Derham) and incubated for 7 days. Mycoplasma detection assay was done by Lucetta Luminometer method; the commercially available kit, MycoAlert (Lonza, USA) was used for the detection of mycoplasma in the cell culture supernatant.

RESULTS

1. Expansion of WJ-MSCs
The growths of the cells were observed for the first 24 hours, at which time the cells were mostly round and oval in shape. After 24 hour incubation, the most of the cells were adherent to the flask and became spindle in shape. Approximately 11.55-14.92 x10^6 cells were isolated primarily from the Wharton’s jelly (n=5) at the end of Passage 0 after 14-18 days. Adherent cells with fibroblastic morphology could be observed as early as 24h after seeding (Figure1). Cells were trypsinized and were seeded into 3000cells/cm^2 in T-175 flasks. Once confluency achieved 1.72 x10^8-2.62 x10^8 adherent cells were harvested in 4-6 days. After successive passages with a 1:3 split ratio, 2.53 x10^9. 5.22x 10^9 P(2) MSCs were harvested in 6-7 days and cryopreserved at the end of the
first expansion period. Viability of the freshly harvested cells was greater than 95% in all cases. The harvested cells from isolated Wharton’s jelly from one cord using current method yields $1.93-2.46 \times 10^4$ cells/cm$^2$ at P(0), $4.46-5.37 \times 10^4$ cells/cm$^2$ at P1, $4.42-5.93 \times 10^4$ cells/cm$^2$ at P(2) and $4.74-5.66 \times 10^4$ cells/cm$^2$ at P(10) accordingly (Table 1).

Morphology of Wharton’s jelly MSCs from Phase-Contrast Microscope.

**Figure 1**
Morphology of WJ-MSCs of one representative donor at passage 0 cultured at different days of interval: WJ-MSCs form a monolayer of adherent fibroblat-like cells by day 4, day 8 and day 16 at Scale bar of 4x (UPPER) and at scale bar of 10x (LOWER) respectively.

**Table 1**
Basic Data of Wharton’s jelly Expansion MSCs.

<table>
<thead>
<tr>
<th>Passage Level</th>
<th>Wharton’s jelly</th>
<th>WJ1</th>
<th>WJ2</th>
<th>WJ3</th>
<th>WJ4</th>
<th>WJ5</th>
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<tr>
<td>P(0)</td>
<td>Length of Umbilical Cord (cm)</td>
<td>38</td>
<td>29</td>
<td>34</td>
<td>32</td>
<td>26</td>
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<td>Total Weight of WJ separated (g)</td>
<td>5.86</td>
<td>4.4</td>
<td>5.63</td>
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<td>Seeding density (g)</td>
<td>0.73</td>
<td>0.55</td>
<td>0.70</td>
<td>0.64</td>
<td>0.73</td>
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<td></td>
<td>Culture period (Days)</td>
<td>13</td>
<td>18</td>
<td>15</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Harvested Cells [$\times 10^6$ cells]/8T-75</td>
<td>12.35</td>
<td>11.55</td>
<td>14.92</td>
<td>14.75</td>
<td>14.7</td>
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<td>Harvested Cells ($\times 10^4$ cells/cm$^2$)</td>
<td>2.06</td>
<td>1.93</td>
<td>2.47</td>
<td>2.46</td>
<td>2.45</td>
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<td>P(1)</td>
<td>No of flasks Used (T175) at P1</td>
<td>24</td>
<td>22</td>
<td>28</td>
<td>28</td>
<td>28</td>
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<td></td>
<td>Total Yield of cells at P1 ($\times 10^8$)</td>
<td>1.88</td>
<td>1.72</td>
<td>2.62</td>
<td>2.64</td>
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<td>Harvested cells ($\times 10^5$ cells/cm$^2$)</td>
<td>4.57</td>
<td>4.46</td>
<td>5.30</td>
<td>5.37</td>
<td>5.20</td>
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<td>Duration of P(1) culture (Days)</td>
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<td>5</td>
<td>6</td>
<td>5</td>
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<td>P(2)</td>
<td>No of flasks Used at P2 (Hyper flask)</td>
<td>36</td>
<td>33</td>
<td>51</td>
<td>51</td>
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<td></td>
<td>Total Yield of cells at P2 ($\times 10^8$)</td>
<td>2.92</td>
<td>2.53</td>
<td>4.83</td>
<td>5.22</td>
<td>4.44</td>
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<td>Harvested cells ($\times 10^5$ cells/cm$^2$)</td>
<td>4.65</td>
<td>4.42</td>
<td>5.52</td>
<td>5.93</td>
<td>5.23</td>
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<td>Duration of P2 culture (Days)</td>
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<td>6</td>
<td>6</td>
<td>7</td>
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<td>P(10)</td>
<td>No of T175 flasks Used at P10</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total Yield of cells at P10 ($\times 10^8$)</td>
<td>17</td>
<td>16.6</td>
<td>19.6</td>
<td>19.8</td>
<td>19.2</td>
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<td>Harvested cells ($\times 10^5$ cells/cm$^2$)</td>
<td>4.86</td>
<td>4.74</td>
<td>5.60</td>
<td>5.66</td>
<td>5.49</td>
</tr>
<tr>
<td></td>
<td>Duration of P10 Culture (Days)</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
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</table>
2. Cumulative Population Doubling Level Analysis

To measure the proliferation potential of the isolated WJ-MSCs, we calculated the CPDL. WJ-MSCs (3000 cells/cm²) were seeded in a culture plate and sub cultured 4-5 days later. The procedure was repeated until passage 10 to measuring the CPDL. The stable increased graph of cell growth was observed (Graph 1).

Graph 1

Cell Growth Curve of Wharton’s jelly MSCs.

The CPDL was measured from passage 2 to passage 10, and evaluated as described in the materials and methods section. Cells grew consistently until passage 10.

3. Colony forming unit (CFU)

Data from five different samples in duplicates showed that WJ-MSCs cultured in DMEM/F12+ supplemented with 10% FBS (Fig 2 and Graph 2). The CFU potential of WJ-MSCs under optimized conditions indicates that the efficiency of WJ-MSCs, which were analyzed with each individual donor WJ-MSCs proven with distinct colony forming unit ability in Graph 2.

CFU formation of Wharton’s jelly MSCs.

Figure 2

CFU formation was examined DMEM/F-12 and FBS as supplements and the culture plates were stained with crystal violet. The images of five different cord plates (UPPER) WJ1-WJ5 along with replica (LOWER) WJ1.R–WJ5.R is shown respectively.
Cells grew consistently until passage 10. The CFU analysis was depletes that clonogenic capacity of WJ-MSCs of five donors at P 10, and as described in the materials and methods section.

4. Phenotype and Purity
A set of typical flow cytometry analysis of WJ-MSCs after expansion when labeled with antibodies against human antigens CD34(PE), HLA-DR(PerCP-Cy5.5), CD45(FITC), CD79(APC) as negative markers and CD105(PE), CD73(APC), CD90(FITC) as Mesenchymal specific markers. From passage 1 onwards, flow cytometry showed homogeneous reactivity of consistently more than 95% of cells with antibodies were against CD73, CD90, CD105, and less than 1% reactivity with CD45, CD34, CD79, and HLA-DR. This demonstrated that MSCs were phenotype purity and the level of MSC purity was fairly stable between P3, P7, and P10 cell population. From passage 1 onwards, flow cytometry showed homogeneous reactivity of consistently more than 95% of cells with antibodies against CD73, CD90, CD105, and less than 1% reactivity with CD45, CD34, CD79, and HLA-DR. This level of purity was not changed even at the end of passage 10 (Graph.3).
Immunophenotype of Wharton’s Jelly derived MSCs.

**Graph 3**
A set of typical surface phenotypes that define Wharton’s jelly Mesenchymal stromal cells and demonstrate the level of MSCs purity was detected by flow cytometric analysis.

5. **Trilineage Differentiation**
The expanded MSCs were subjected to Adipogenic, Osteogenic and Chondrogenic differentiation. Induction of Osteogenesis in the expanded MSCs resulted in extracellular matrix mineralization and calcium deposition which was confirmed by Von-Kossa staining (Fig 3A). Adipogenic differentiation was confirmed when accumulated lipid vacuoles stained positive with Oil Red O staining (Fig 3B). Positive staining of collagen fibers with Safranine O stained patches indicated that MSCs were successfully differentiated to chondrocytes (Fig 3C). No changes were observed in undifferentiated or control cells.
Comparative in vitro Trilineage differentiation potential of Wharton’s Jelly derived MSCs.

**Figure 3A**

**Osteogenic lineages.** Induction of Osteogenesis in the expanded MSCs resulted in Calcium accumulation which was confirmed by Von-kossa were observed with each individual donor samples (WJ1-WJ5), Corresponding uninduced control cultures (Control) for comparision. Scale bar = 40µm.

**Figure 3B**

**Adipogenic lineages.** WJ-MSCs derived after expansion were induced to adipogenic (oil red O staining), adipogenic differentiation were confirmed by accumulation of lipid vacuoles stained positive with Oil Red O staining were observed with each individual donor samples (WJ1-WJ5), corresponding uninduced cultures (Control) for comparision and no changes were observed in undifferentiated or control cells. Scale bar = 40µm.
Chondrogenic lineages. Positive staining of collagen fibers with Safranine O stained patches indicated that MSCs were successfully differentiated to chondrocytes with each individual donor samples (WJ1-WJ5), corresponding uninduced cultures (Control) for comparison and no changes were observed in undifferentiated or control cells. Scale bar = 40µm.

6. Karyotype analysis
The Metaphase chromosome spreads were analyzed in different batches of passaged 10 MSC’s wherein their karyotyping pattern was observed to be normal. The Genetic stability was analyzed for chromosome after conventional Giemsa banding for expanded MSCs was also observed to be normal.

7. Microbiological tests
Cultures were observed to be absolutely sterile and free of any aerobic, anaerobic and fungal contamination when checked periodically on the supernatants. Endotoxin levels of the final products were lower than 0.2EU/ml.

DISCUSSION
All studies were conducted in accordance with the criteria proposed by the International Society for Cellular Therapy (ISCT) to define human MSCs. Stem cell has the therapeutic potential on the replacement of dead cells or tissues lost due to injury or disease or aging. Two kinds of somatic stem cells viz., hematopoietic and mesenchymal (MSCs) have been extensively studied and used in stem cell therapies. Cord blood stem cells are primarily hematopoietic and thus can be utilized for blood related diseases. MSCs are unlike hematopoietic stem cells they do not give rise to blood cells but the repertoire of cell types they can differentiate into includes chondrocytes, osteoblasts, adipocytes, myocytes and neural cells. The nomenclatures of UC derived MSCs have not been unified yet and the cells have been referred to in many ways, mainly depending on from which compartment of the cord the cells have been isolated. The UC usually comprises two arteries and a vein, which are immersed within a mucoid connective tissue, the so called Wharton's jelly, and are enclosed by a simple amniotic epithelium. At least four separate regions were found to contain mesenchymal cells. Thus, MSCs could be isolated from the subendothelial\textsuperscript{15-17}, the WJ\textsuperscript{18-20}, the perivascular region\textsuperscript{21-22}, and the umbilical cord blood\textsuperscript{23-25}. Compared to UC derived MSCs, bone marrow derived MSCs percentage is as
low as 1: $10^5$ of mononucleated cells. It has also been reported that the number of colony forming units-fibroblast cells is 20-fold higher in umbilical cord tissue when compared with the MSCs from bone marrow. MSCs from bone marrow could be obtained from only 10% of the samples but umbilical cord tissue always gave sufficient numbers of MSCs. MSCs can also be obtained from adipose tissue but this involves an invasive procedure such as liposuction. Further, as pointed out earlier only the MSCs from umbilical cord tissue are fetal in nature. In the present study, we successfully isolated and characterized MSCs from WJ. The collected UC-WJ was seeded in basal culture medium and sub cultured until passage 10; with a seeding density of 3000 cells/cm$^2$. We could expand these cells to a clinical quantity of $5.22 \times 10^9$ cells at passage 2 within 28 days. Apart from these we also observed that this source allowed rapid initial isolation of a large number of cells avoided the necessity of extensive multiplication and potential epigenetic changes. Using this procedure, we obtained cells from five umbilical cord samples showed similar morphologies. A consistent level of cell proliferation is a distinct characteristic of stem cells. These cells have a self-renewal ability related to continuous and steady proliferation. Our results demonstrated that the isolated MSCs are capable of self-renewal, a typical feature of Mesenchymal cells. Furthermore, the cells were fibroblast-like, adhered to the plastic culture dish surface, and possessed a normal karyotype. Due to their robust proliferation, the MSCs may be sufficient for in vivo applications. Immuno-phenotype characterization indicated that the MSCs were positive for MSCs markers such as CD73 (96.2-99.99%), CD90 (98-99.5%), and CD105 (97.3-99.8%). MSCs display a distinctive pattern of cell surface antigens such as CD73, CD90, and CD105. Antigens that are not typically found on MSCs include CD79 (0.58-0.45%), CD34 (0.07-0.02%), CD45 (0.63-0.47%), and HLA-DR ($0.86-0.62\%$). These cells were found to have an immunophenotype characteristic of MSCs and are capable of self-renewal.

CONCLUSION

With their ability to differentiate into multiple lineages, secrete factors related to immune regulation, and migrate toward sites of inflammation, makes MSCs a good source for many clinical implications in various fields. The results of multiple clinical trials used MSCs have been promising but also highlight the critical challenges that must be addressed in
the future. More research is needed to determine the mechanisms and biological properties of MSCs to enhance their therapeutic efficacy in various diseases. Furthermore, the heterogeneity of the MSC population presents a challenge for generalized findings. Therefore, it is important to standardize the generation protocols, including cell culture conditions, source, passage, and cell density, as they may impact MSCs phenotype as well as functions. Further randomized, controlled, multicenter clinical trials are necessary to determine the optimal conditions for MSCs therapy. With further advances, MSCs will play an important role in managing many disorders that lack effective standard treatment. Furthermore, an animal toxicity study would determine the clinical toxicity associated with the therapeutic product, adequate safety margin, dosage limitations and route of administration to ensure systemic dissemination of MSCs for intend therapeutic usage warrants further investigation.

CONFLICT OF INTEREST
Conflict of interest declared none.

REFERENCES

14. Park SB, Seo MS, et al. Isolation and characterization of amniotic fluid-derived...