Srp Arh Celok Lek. 2013 Mar-Apr;141(3-4):178-186
ОРИГИНАЛНИ РАД / ORIGINAL ARTICLE
DOI: 10.2298/SARH1304178T
UDC: 602.9

Mesenchymal Stem Cells Isolated from Peripheral Blood and Umbilical Cord Wharton's Jelly

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SUMMARY

Introduction Mesenchymal stem cells (MSCs) are a promising tool for regenerative medicine, but due to the heterogeneity of their populations, different sources and isolation techniques, the characteristics defining MSCs are inconsistent.

Objective The aim of this study was to compare the characteristics of MSCs derived from two different human tissues: peripheral blood (PB-MSCs) and umbilical cord Wharton's Jelly (UC-MSCs).

Methods The PB-MSC and UC-MSC were isolated by adherence to plastic after gradient-density separation or an explant culture method, respectively, and compared regarding their morphology, clonogenic efficiency, proliferating rates, immunophenotype and differentiation potential.

Results MSCs derived from both sources exhibit similar morphology, proliferation capacity and multilineage (osteogenic, chondrogenic, adipogenic and myogenic) differentiation potential. Differences were observed in the clonogenic capacity and the immunophenotype, since UC-MSCs showed higher CFU-F (colony-forming units-fibroblastic) cloning efficiency, as well as higher embryonic markers (Nanog, Sox2, SSEA4) expression. When additional surface antigens were analyzed by flow cytometry (CD44, CD90, CD105, CD33, CD34, CD45, CD11b, CD235a) or immunofluorescent labeling (vimentin, STRO-1 and α-smooth muscle actin), most appeared to have similar epitope profiles irrespective of MSC source.

Conclusion The results obtained demonstrated that both MSCs represent good alternative sources of adult MSCs that could be used in cell therapy applications.

Keywords: mesenchymal stem cells; peripheral blood; umbilical cord; characterization

INTRODUCTION

Adult mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, are undifferentiated multipotent cells with the ability both to self-renew [1, 2] and to differentiate into various cell phenotypes, either of mesenchymal origin (osteoblasts, chondrocytes, adipocytes, stromal cells, fibroblasts and tendons) [3, 4], or non-mesodermal origin (hepatocytes, neural cells and epithelial cells) [5]. MSCs reside primarily in the bone marrow, but were also shown to be present, with similar but not identical features, in diverse host tissues including cord blood and umbilical cord, adult peripheral blood, adipose tissue, trabecular bone and dental pulp [6-11]. In the human body, MSCs are regarded as readily available reservoirs of reparative cells able to mobilize, proliferate, and differentiate to the appropriate cell type in response to specific signals [3, 4]. Due to their indispensable regenerative, reparative, angiogenic and immunosuppressive properties, all of which collectively point out their therapeutic potential, MSCs have generated increasing interest in a variety of biomedical disciplines and several areas of ongoing clinical applications. However, because of the heterogeneity of the stem cell populations, as well as different sources of their origin and different isolation techniques used among laboratories, the characteristics defining MSCs are inconsistent. Whether these cell populations isolated from diverse sources represent intrinsically similar or different cell types is still largely under debate.

OBJECTIVE

The objective of this study was to compare biological characteristics of MSCs derived from two different human tissues with a common feature that both are discarded after routine medical interventions and therefore are readily available source for MSCs isolation. We chose to compare MSCs derived from one adult tissue, such as peripheral blood, and one perinatal tissue, umbilical cord Wharton's Jelly. The comparison was made to describe MSCs behavior in cell culture, which could be useful for their future use in potential medical procedures. After isolation and establishment of long-term cultures, cells were further characterized regarding their morphology, clonogenic efficiency, proliferating rates, immunophenotype and differentiation potential.

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MATERIAL AND METHODS

MSCs isolation

All samples were obtained in accordance with the local ethical committee standards and the Declaration of Helsinki and after the study subjects provided informed consent.

The peripheral blood-derived MSCs (PB-MSCs) were obtained from mononuclear cells of 6 healthy donors by density gradient centrifugation, plated at a density of $4\times10^5/cm^2$ in $25cm^2$ flasks in growth medium (GM) containing 10% fetal bovine serum (FBS) and 100 units/ml Penicillin/Streptomycin (Pen/Strep) (all from PAA Laboratories, Linz, Austria) in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich, St Luis, MO, USA) and cultured in a humidified atmosphere at 37°C with 5% CO_2. The medium was replaced twice a week and non adherent cells were discarded. After two weeks one colony of adherent fibroblast-like cells was noticed in one of the seeded specimens. When the colony reached the approximate size of 5 cm^2, cells were detached and seeded in a new flask in GM. Cells reached confluence after 10 days. Following first confluence, cells were passaged regularly.

Umbilical cord-derived MSCs (UC-MSCs) were isolated from umbilical cords obtained after normal full-term delivery. Six umbilical cords, app. length 20 cm, were stored aseptically in cold PBS with 100 μg/ml Pen/Strep, and 2.5 μg/ml Amphotericin B (PAA Laboratories), within 6-24 hours from partum. The umbilical vein and arteries were dissected from the tissue, which was then cut into fragments (2-3 mm^3). Several pieces of tissue were placed in a Petri dish with a low amount of GM to allow attachment to the plastic and later on were covered completely with culture medium. GM was changed every second day. After 7 days, when the cells grew out of the explanted tissue, the cord fragments were removed and attached cells were cultured until reaching confluence. MSCs were successfully isolated from two out of six specimens.

Cell culturing and passaging

After reaching confluence, cells were subcultured routinely in GM, using Trypsin/EDTA solution (PAA Laboratories). The populations of MSCs were expanded for up to 40 passages for PB-MSCs, and 20 passages for UC-MSCs. For long-term storage of cells, deep-freezing medium consisting of 90% FBS with 10% DMSO was used.

Cell growth assays

Cell growth assays were performed as described [12]. Short-term cell growth assays were performed by seeding MSCs, passage (P) 4 to 6, in 6 well plates at 4×10^4 cells/well, and incubating them in GM at standard culture conditions. At days 2, 4, and 7, the cells were detached, counted and their viability determined by Trypan blue exclusion test (Invitrogen, Carlsbad, CA, USA). These assays were repeated at least 3 times for each MSC type. To determine the long-term population doubling times MSCs were plated in duplicate in 6 well plates at 1×10^4 and 2×10^5 cells/well. At confluence, the cells were detached, counted and reseeded at the initial cell density. This procedure was repeated at every passage for 24 days. The population doubling times (PDT) were calculated according to the formula PDT = (T–T0) lg2/(lgNt–lgN0), where T0 and T are starting and ending time of cell culture, respectively, while N0 and Nt represent the cell number at the start and the end of each culture.

Colony-Forming Units-Fibroblastic (CFU-F) assay

CFU-F assays were performed by plating MSCs (P3 to P6) in 6 well plates at 10, 50 and 100 cells/well in GM, in two replicas. After 14 days under standard culture conditions, the cells were fixed with ice-cold methanol and stained with 0.3% crystal violet. The number of visible colonies (more than 50 cells) was counted.

Flow cytometry analysis

To examine the mesenchymal phenotype, cells (P3 to P6) were subjected to flow cytometry analysis. The cells, harvested by 1mM EDTA, were washed in cold phosphate-buffered saline (PBS) with 0.5% bovine serum albumin (BSA) from Sigma-Aldrich, and aliquots of 2×10^5 cells were labeled with following monoclonal mouse anti-human antibodies: anti-CD34-PE (Dako Cytomation, Glostrup, Denmark), anti-CD11b-FITC (Biosource, Camarillo, CA), anti-CD105-R-PE (Invitrogen), anti-CD45-FITC, anti-CD33-Fluorescein, anti-CD235a-PE, anti-CD90-PE, CD44H-PE (all from R&D Systems, Minneapolis, MN, USA). For the embryonic stem cell (ESC) markers expression analyses, MSCs were fixed in formaldehyde and permeabilized in 90% methanol. Afterwards, aliquots of 2×10^5 cells were labeled with primary antibody for SOX2, SSEA4 and NANOG and with secondary FITC conjugated antibody (all purchased from R&D). To determine the level of nonspecific binding, isotype control antibodies were used. Flow cytometry was performed using a CyFlow CL (Partec, Münster, Germany).

Immunofluorescent labeling

MSCs were seeded over rounded coverslips in GM and cultured for 24 hours. After being fixed with 4% formaldehyde in PBS, cell monolayers were permeabilized with 0.1% TritonX-100 (AppliChem, Darmstadt, Germany) in PBS and incubated with mouse anti-Vimentin, anti-STRO-1 and anti-α-SMA (alpha-Smooth Muscle Actin) antibody (Sigma-Aldrich), followed by incubation with anti-mouse-FITC or anti-mouse-TRITC secondary antibody and 1 μg/ml DAPI (all from Sigma-Aldrich). The samples were observed and photographed by an epi-fluorescence microscope.

RT-PCR

Two micrograms of total RNA isolated from PB-MSCs and UC-MSCs were reverse transcribed using Superscript II (Invitrogen, Carlsbad, CA). PCRs were performed using One-step PCR (Invitrogen). The primer sets used were as follows: 5'-GTGATGGCGAAGCGAGTGAA-3' and 5'-CCGAGCCCGAACACACAGAA-3' for eNOS; 5'-GCAGGAACCCAGACAACCG-3' and 5'-GACCCAGGTAGACGATGTAG-3' for uPA; 5'-GGGACTATCCACCTGCAAGA-3' and 5'-CCTCCTTGGCGTAGTAGTCG-3' for TGF-β; and 5'-ACCACAGTCCATGCCATCAC-3', 5'-TCCACCACCCTGTTGCTGTA-3' for GAPDH. Amplicons were resolved in 1.5% agarose gel stained with ethidium bromide.

Multilineage differentiation

To determine the differentiation potential of MSCs, third to fifth passage cells were seeded in 24 well plates at 4000 cells/cm^2 and cultured in GM. After the cells reached subconfluence, GM was replaced with specific osteogenic, chondrogenic, adipogenic or myogenic medium as previously described [13]. As control, cultures with GM only were used. To further confirm the identity of differentiated cells, they were stained for alkaline-phosphatase (ALP) by BICP/NBT, proteoglycans by safranin-O, intracytoplasmatic lipid droplets by Oil Red O or myotubes formation by crystal violet as previously reported [13].

Statistical analysis

To evaluate the probability of significant differences among the samples Student's t-test was performed using the Origin PC Program with a $p<0.05$ considered significant.

RESULTS

Isolation and culture of different MSCs

Depending on the starting amount and type of tissue used to establish the primary cultures, cells that morphologically resembled MSCs could be seen as early as 7 days post-plating for the UC-MSCs up to 15 days post-plating for the PB-MSCs, and the confluence could be reached within 10-15 days. Although during the initial culture period some morphological heterogeneity in the adherent fraction could be seen, as the cultures were passaged, morphological homogeneity was gradually achieved, cells were fibroblast-like and no spontaneous differentiation was noticed (Figure 1). However, to minimize eventual differences due to various starting conditions, the comparative analyses presented here were mainly focused on cells expanded under similar conditions, i.e. passages later than three.

Growth characteristics of different MSC populations

When the clonogenic capacity of MSCs was analyzed using CFU-F assay, results demonstrated the presence of clonogenic cell populations with fibroblast-like morphology in both MSCs examined, but with different frequencies, ranging from 25% up to 95%. Common feature among tested cells was that MSC populations showed decreases in CFU-F frequencies as the initial plating density was increased, with the lower cell plating density having the highest CFU-F numbers (Graph 1). However, when the colony-forming efficiency, defined as the ratio of the number of colonies to the number of cells seeded, was compared, UC-MSCs had up to 2-fold higher CFU-F capacity than the PB-MSCs.

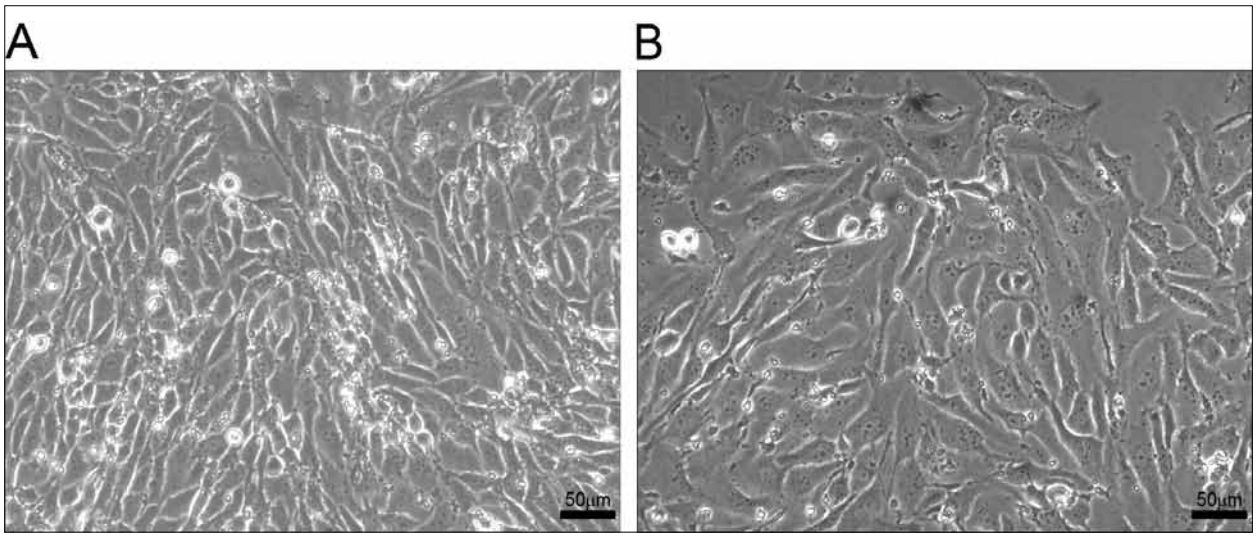
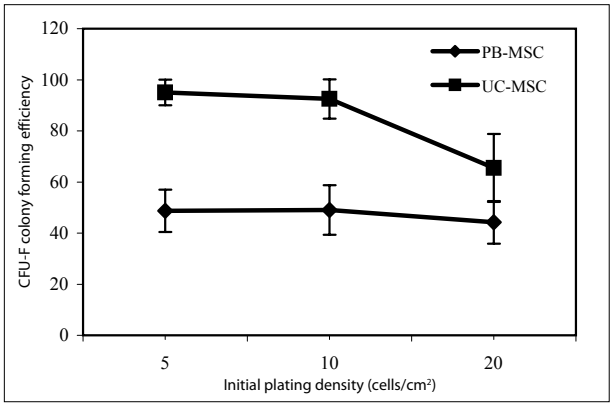


Figure 1. Morphology of PB-MSCs and UC-MSCs cultures. Phase contrast micrographs of PB-MSCs (A) and UC-MSCs (B) monolayer cultures at passage 4. In monolayer culture, the cells assumed a polymorphic, fibroblast-like morphology, which was maintained throughout the passaging process.

doi: 10.2298/SARH1304178T

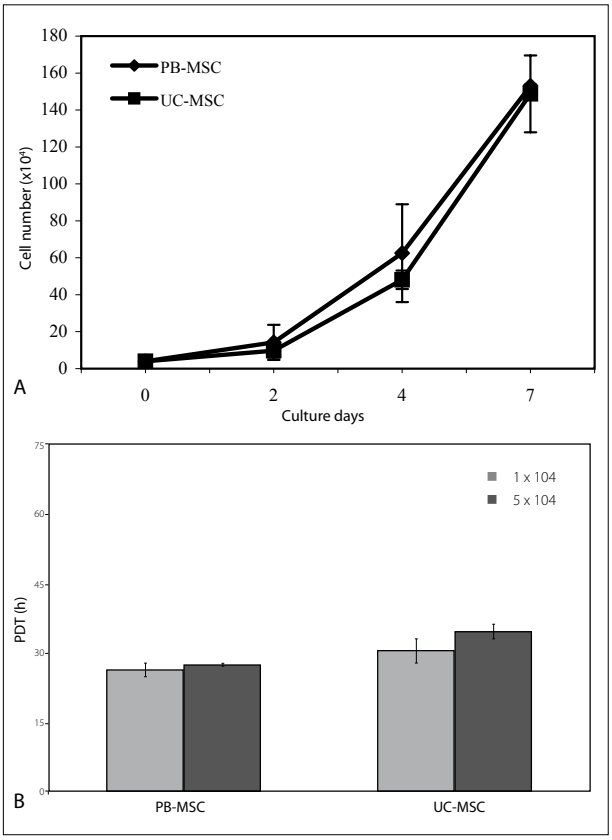


Graph 1. Colony-forming efficiency after expanding MSCs at different initial plating densities

MSCs were cultivated at low density (10, 50 or 100 cells/well) in growth medium for 14 days and stained for CFU-F with crystal violet. Colony-forming efficiency (CFU-F) is defined as the ratio of the number of colonies to the number of cells seeded. The results are presented as mean±SD for three experiments, each performed in duplicate.

Culture kinetics of different MSCs are shown in Graph 2. When the growth kinetics of MSCs was compared by seeding 4th to 6th cell passage, the differences related to the tissue of origin could not be observed. As shown in Graph 2A, short-term proliferation assay demonstrated similar and extremely high proliferation rate of both PB-MSCs and UC-MSCs, as the cell number of these MSC populations increased almost 40-fold at day 7 after plating.

To confirm expandability of the cells, we evaluated the long-term proliferation ability, in which the cells were repeatedly seeded at different plating densities to minimize any effect of density-dependent cell growth. The PDT values obtained for both MSC populations were consistent with the results obtained within the initial expandability studies, since PB-MSCs and UC-MSCs had short PDTs of app. 27 h and 35 h for 5×10^4 cells plated, respectively (Graph 2B).



Graph 2. Comparative growth kinetics of PB-MSCs and UC-MSCs cultures. (A) Short-term proliferation of MSCs (growth curves of representative MSC populations from each source are plotted); (B) Population doubling times of representative MSCs in long-term cultures (the results are expressed as the mean±SD of three experiments each performed in duplicate).

Immunophenotyping of different MSC populations

The specific markers for MSCs identification were analyzed by flow cytometry. As expected, both MSCs types were negative for hematopoietic markers, such as CD11a, CD33, CD45 and Glycophorin-CD235a (Figure 2). However, 40% of either PB-MSC or UC-MSC was positive for CD34. The rate of positivity for the typical mesenchymal marker proteins in both PB-MSCs and UC-MSCs was low, since both MSCs types were negative for CD90, while only 6% of PB-MSC and 10% of UC-MSC expressed CD105. The presence of another mesenchymal marker CD44H was approximately 30% for PB-MSC, and ranged 11-22% in UC-MSC cells. Additional immunocytochemical labeling demonstrated that both PB-MSCs and UC-MSCs showed positive signal for mesenchymal cell marker Vimentin, but were negative for the α-SMA and STRO-1 (Figure 3).

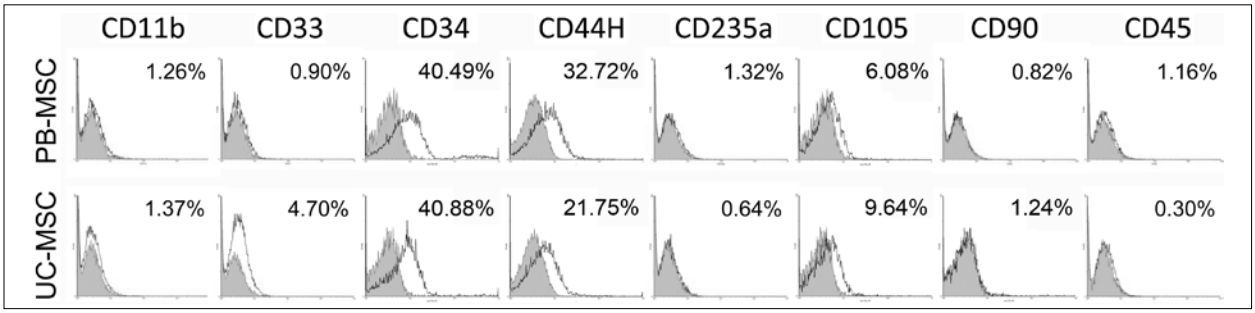


Figure 2. Immunophenotypic profile of PB-MSCs and UC-MSCs. Representative flow cytometry histograms show the expression (unsheded peaks) of selected molecules (CD11b, CD33, CD34, CD44H, CD235a, CD105, CD90, CD45) by different MSC populations compared with isotype controls (shaded).

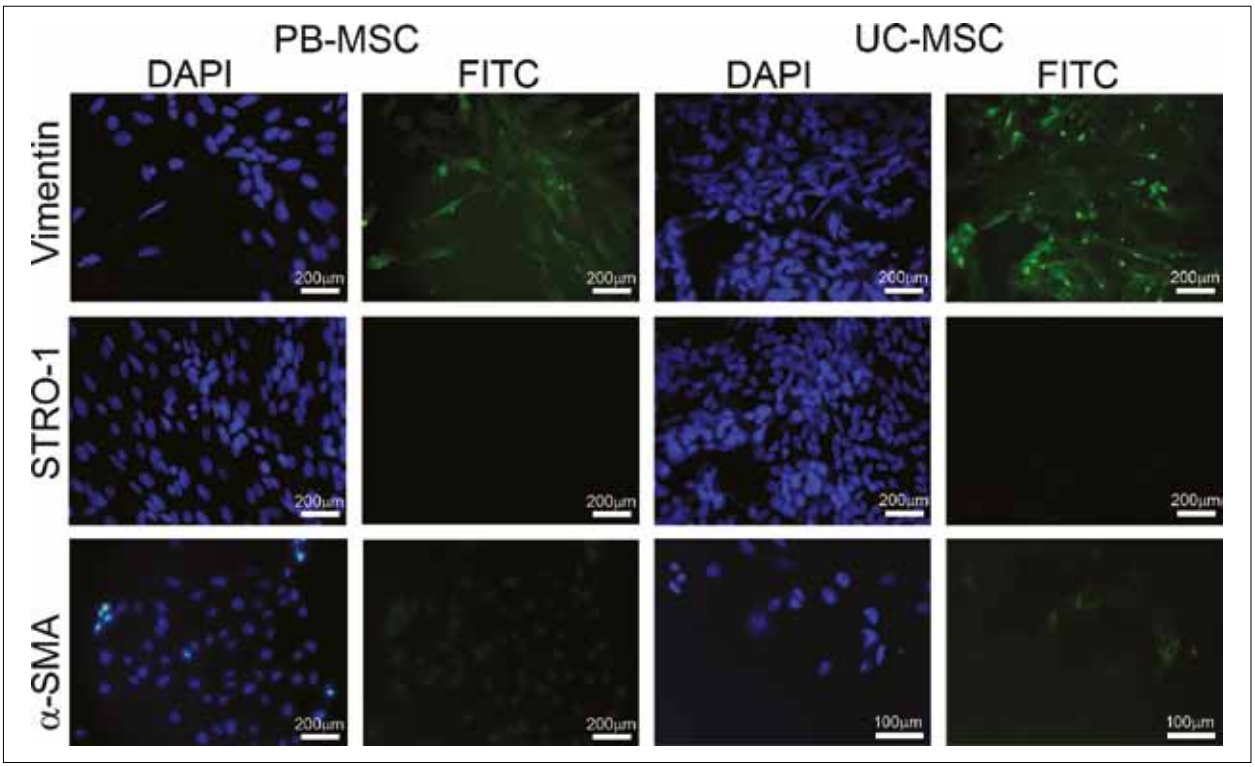


Figure 3. Immunofluorescent labeling of PB-MSCs and UC-MSCs. Representative panels of immunocytochemical detection of mesenchymal markers vimentin, STRO-1 and α-SMA on PB-MSCs and UC-MSCs. Positive cytoplasmic staining of MSCs for intracellular MSCs markers by indirect immunofluorescence with mouse anti-vimentin, mouse anti-STRO-1 and mouse anti-α-SMA antibody and anti-mouse FITC (green) or TRIC (red) secondary antibody, while all nuclei were counterstained with DAPI (blue). Cells were examined using an immunofluorescence microscope.

To further define PB-MSCs and UC-MSCs phenotype we analyzed the expression of ESC markers, such as Nanog, Sox2 and SSEA4. The results presented in Figure 4, evidenced that both MSC types expressed these markers. However, UC-MSCs showed higher expression of all three analyzed ESC markers, since 50-72% of UC-MSCs and 37% of PB-MSCs positively expressed Nanog, while 40-47% of UC-MSCs and 20% of PB-MSCs positively expressed Sox2. Percentage of cells expressing SSEA4 was 21% in PB-MSCs populations, whereas it ranged from 33 to 83% within UC-MSCs.

The expression of several genes known to be associated with MSCs functions, such as endothelial nitric oxide synthase (eNOS), urokinase plasminogen activator (uPA) and transforming growth factor beta (TGFb) were analyzed by RT-PCR. Obtained results demonstrated that all three genes were expressed in both PB-MSC and UC-MSCs, but with different expression levels (Figure 5).

Differentiation potential of different MSC populations

As a functional assay to confirm MSCs identity, we next examined the differentiation potential of MSC populations from different sources. Although isolation procedures, as well as the immunophenotype profiles of MSCs differ, their mesenchymal lineage differentiation capacity remained conserved, as MSCs of both populations studied exhibited osteogenic (Figure 6A), chondrogenic (Figure 6B), adipogenic (Figure 6C) and myogenic (Figure 6D) differentiation potential.

DISCUSSION

The issue of ready available sources for MSCs isolation has growing importance, since these cells can be beneficial agents in regenerative medicine. In this study we performed a comparative analysis of MSCs derived from peripheral blood and umbilical cords Wharton's Jelly, normally discarded as medical waste. The two MSCs types showed similar morphology, proliferative rates, differentiation capacity, but differed in clonogenic capacities and, in part, in immunophenotype.

Plastic adherence is a well-described property of MSCs and one of the three criteria suggested by the International Society for Cellular Therapy (ISCT) [14] to define MSCs. Although we succeeded to isolate plastic adherent cells, from both types of tissue, the amounts of MSCs obtained were rather variable. MSCs were obtained from some of the umbilical cords, while only one colony of adherent fibroblast-like cells was isolated out of all peripheral blood samples. This observation is in agreement with the reported data that peripheral blood MSCs are extremely low in frequency [15, 16]. Additionally, the elapsed time

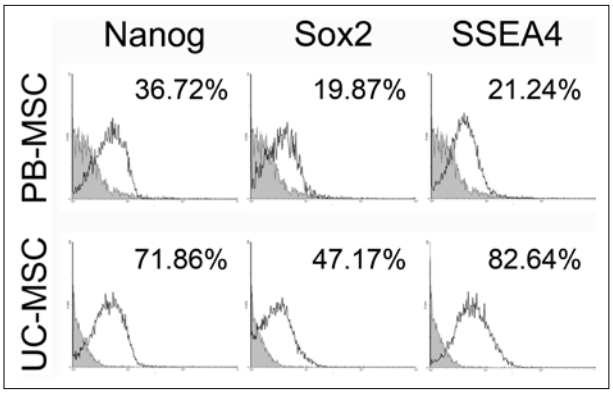


Figure 4. Embryonic stem cell markers expression in PB-MSCs and UC-MSCs. Representative flow cytometry histograms show the expression (unshaded peaks) of selected molecules (SOX2, NANOG and SSEA4) by different MSC populations compared to isotype controls (shaded).

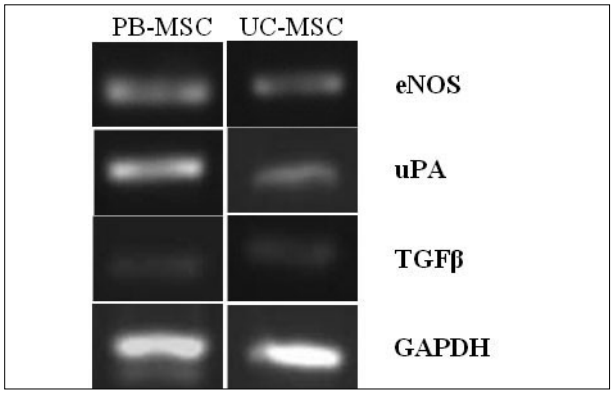


Figure 5. RT-PCR analysis of eNOS, uPA, TGF-beta mRNA expression in PB-MSCs and UC-MSCs. The gel from the representative experiment is presented. GAPDH was used as gel loading control.

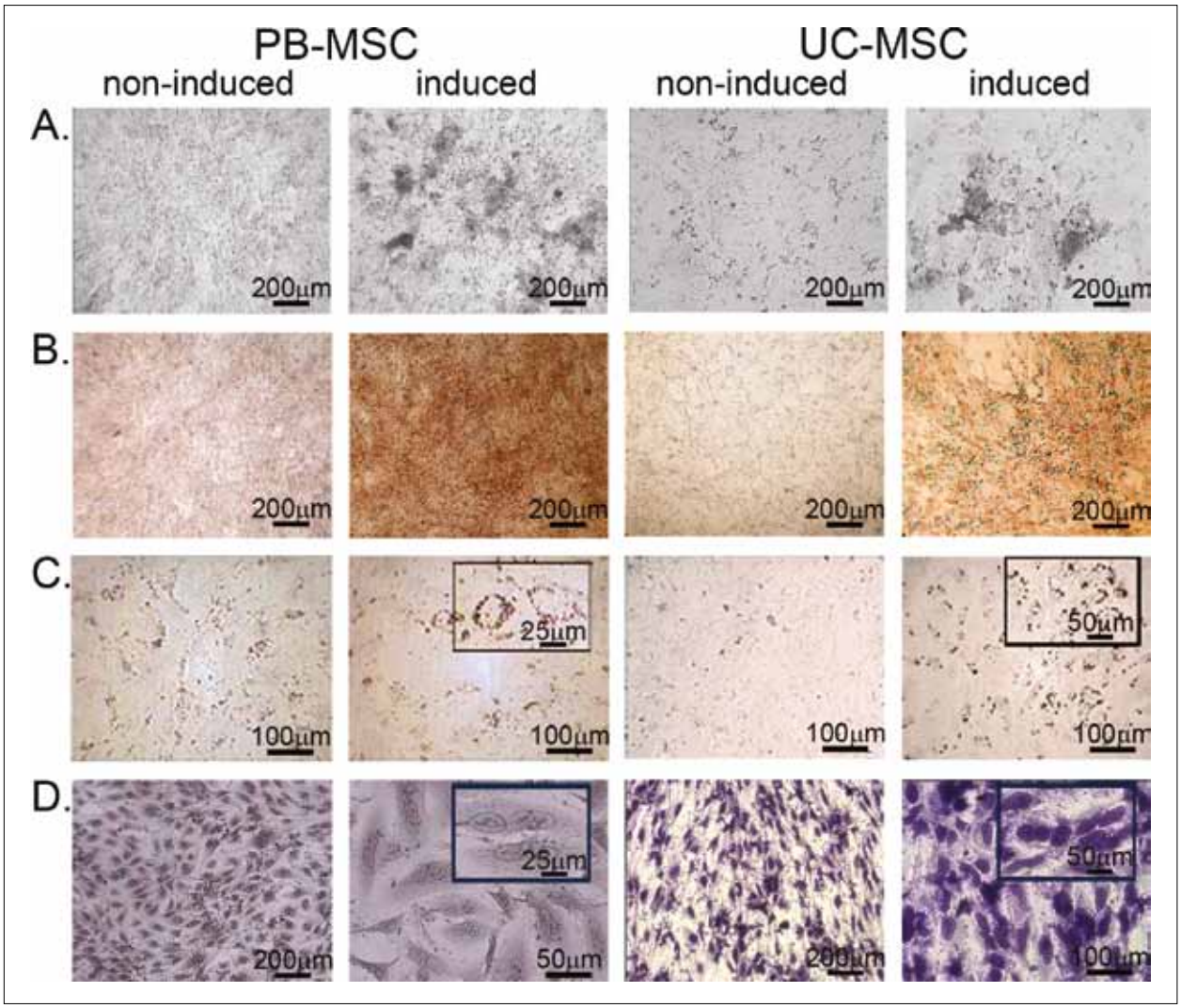


Figure 6. Differentiation of PB-MSCs and UC-MSCs. (A) Osteogenic differentiation was proved by positive staining for ALP activity; (B) Chondrogenic differentiation of MSCs with positive staining of proteoglycans by safranin-O; (C) Adipogenic differentiation was confirmed by Oil Red O staining of intracytoplasmatic lipid droplets; (D) Myogenic differentiation characterized by the formation of myotubes stained with crystal violet.

between the tissue samples removal and processing was an important factor for the successful isolation of MSCs, particularly for the umbilical cord samples.

The clonogenic capacities of MSCs derived from these two sources also differed, as UC-MSCs possessed 2-fold higher colony forming efficiency, similar to previous reports demonstrating higher proliferation capacities of neonatal tissue-derived MSCs in comparison to the adult tissue-derived MSCs [15,16, 17]. The greater expansion capability of UC-MSCs was confirmed, as the mean doubling time of UC-MSCs was about 30 hours. Although the colony forming efficiency of PB-MSC was lower, these cells proliferate in the same range as UC-MSCs. The short doubling time of PB-MSCs, along with the fact that these cells could be expanded up to 40 passages indicated their high self-renewal capacity. These characteristics are important for cell therapy and tissue engineering, since it is likely that large amounts of MSCs would be needed for potential application.

Next criterion proposed by ISCT to define human MSCs is the combination of expressed, mesenchymal, and not-expressed hematopoietic cell surface molecules. The flow cytometry analysis of MSCs confirmed the absence of hematopoietic markers, such as CD11a, CD33, CD45 and Glycophorin-CD235a, on both types of MSCs. Considerable proportion (40%) of either PB-MSCs or UC-MSCs were positive for hematopoietic progenitor cell antigen CD34. Similar findings were previously reported for MSCs derived from adipose tissue [18, 19]. PB-MSCs and UC-MSCs showed low expression of mesenchymal markers, as they were negative for CD90, and slightly positive for CD105 (6-10%) and CD44H (10-30%). Immunocytochemical staining showed that both MSCs were positive for Vimentin, while α-SMA and STRO-1 were not expressed, which is in agreement with previous findings [20]. The expression of Vimentin confirmed mesenchymal origin of the cells and distinguished them from epithelial and endothelial cells. The disparity observed in the expression of mesenchymal markers, could be due to various reasons. The most important one is the lack of unique MSC markers, which along with the recent reports demonstrating that CD105, CD90 and CD44 molecules are also expressed on human skin or lung fibroblasts [21, 22], point out that additional markers should be used to define MSCs. The phenotypic features can be mediated by different tissue sources, the extraction methods employed, as well as the *in vitro* culture conditions that might cause the loss of cell surface proteins [17, 23].

Expression of ESC markers, Nanog, Sox2 and SSEA4, in PB-MSCs and UC-MSCs, is in agreement with findings that human adult MSCs derived from different sources may express ESC markers [24, 25]. Although these results indicate that both MSCs types are highly multipotent, higher percentage of cells positive for ESC markers in UC-MSCs population indicated that postnatal UC-MSCs are more primitive than adult PB-MSCs. Further support that these cells are MSCs are the results showing positive expression of eNOS, uPA and TGFb genes, as it has been shown that proliferating human BM-MSCs accumulate eNOS in the nuclear compartment [26], as well as that TGFb is the well known growth factor involved in the cell stemness and differentiation [27]. Also, recent studies have suggested that MSCs regulatory roles in tissue regeneration involve induction of angiogenesis through uPA production [28].

Due to differences in marker expression, the conserved mesodermal differentiation capacity within the investigated MSCs seems to be more relevant for their quality at present, as the multilineage differentiation potential is the hallmark of MSCs. As both MSC populations gave rise to four distinct mesenchymal cell lineages, the multipotent nature of these MSCs was successfully demonstrated.

A large number of publications on MSCs derived from a variety of sources, reported by different groups, demonstrated variability between MSCs. The influence of the tissue of origin and the MSC "age" on their biological properties cannot be negated. Of particular interest are the differences between the MSCs derived from adult and neonatal tissues, since MSCs from birth-associated tissues are considered more primitive than those obtained from other tissues, with higher proliferative and expansion potential, as well as greater accessibility of clinical samples [16]. As for PB-MSCs, human MSCs that circulate in the bloodstream have been reported, but the real tissue origin has been debated, as beside the suspected contamination during sample collection and the perivascular location of the MSCs, there are speculations that these cells are migrants from the bone marrow or other organs [15, 16, 29, 30]. Although it seems that the number of circulating MSCs in human blood is low under steady-state conditions, the possibility that MSCs circulate due to specific organism requirements, e.g. during tissue injury, cannot be excluded. The ability of hematopoietic stem cells (HSCs) to egress from the marrow and home to other sites indicates that MSCs might possess this feature, too.

Beside the migratory capabilities, it is possible that MSCs can share other characteristics with HSCs, such as their hierarchical organization. Namely, HSCs are today widely recognized as heterogeneous population with hierarchical structure in which the multipotency is progressively restricted. Therefore, the population diversity observed between the MSCs described previously, as well as in our study, could be due to the isolation of cells representing stem/progenitor cells of different maturity. The lack of specific MSC markers makes their identification and study more difficult, but until this issue is resolved, the ease of accessibility for isolation, high expansion potential in culture, presumptive plasticity, immunosuppressive properties, homing to sites of tissue injury and ethical considerations are supporting the use of adult MSCs for clinical application.

CONCLUSION

In summary, our findings demonstrate that umbilical cord matrix and peripheral blood can be used as alternative

sources of adult MSCs. While MSCs therapy is very promising, the lack of unique phenotypic markers and inefficient extraction are factors currently limiting their use. Further research is necessary to improve our understanding of cells behavior during *ex vivo* expansion for their safe and effective use.

ACKNOWLEDGEMENTS

This work was supported by Grant No 175062 from the Ministry of Education and Science of the Republic of Serbia. The authors are thankful to technician Mrs. Snežana Marković for help during the experiments processing.

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Особине мезенхимских матичних ћелија изолованих из периферне крви и Вартонове слузи пупчане врпце

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КРАТАК САДРЖАЈ

Увод Мезенхимске матичне ћелије (ММЋ) су посебно значајне за регенеративну медицину. С обзиром на хетерогеност њихових популација, разноврсност извора и техника изолације, не постоје јединствена обележја која одређују ММЋ.

Циљ рада Циљ ове студије била је упоредна анализа биолошких карактеристика ММЋ изолованих из периферне крви (ПК-ММЋ) и Вартонове (*Wharton*) слузи пупчане врпце (ВСП-ММЋ).

Методе рада ПК-ММЋ и ВСП-ММЋ су изоловане на основу особине да приањају на пластичну подлогу, а упоређиване су њихове морфолошке одлике, клоногени и пролиферативни капацитет, имунофенотип и потенцијал диференцијације.

Резултати Добијени резултати показали су да оба типа ММЋ имају сличне морфолошке особине, сличан пролиферативни капацитет и мултипотентни потенцијал диференцијације у ћелије различитих мезенхимских ткива (коштаног, масног, хрскавичавог и мишићног). Разлике су примећене у клоногеном капацитету и имунофенотипу, будући да су ВСП-ММЋ испољиле већи капацитет за формирање *CFU-F* (енгл. *colony-forming unit-fibroblasts*), као и већу експресију маркера типичних за ембрионалне матичне ћелије (*Nanog*, *Sox2*, *SSEA4*). Испитивање површинских антигена типичних за ММЋ (*CD44, CD90, CD105, CD33, CD34, CD45, CD11b, CD235a*) проточном цитометријом и имунофлуоресцентно обележавање додатних мезенхимских маркера (*Vimentin*, *STRO-1* и алфа-актина глатких мишићних ћелија) није указало на разлике у експресији ових маркера.

Закључак Оба типа ММЋ су погодан алтернативни извор адултних ММЋ које би се могле користити у ћелијској терапији.

Кључне речи: мезенхимске матичне ћелије; периферна крв; пупчаник; карактеризација

Примљен • Received: 01/06/2012 **Прихваћен • Accepted:** 03/09/2012