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# Comparison of Characteristics of Mesenchymal Stem Cells Obtained Mechanically and Enzymatically from Placenta and Umbilical Cord

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## Abstract

Mesenchymal stem cells (MSC) are a good source for the cell therapy thanks to their abilities. MSCs exert very important immunomodulatory effects: they suppress T- and B-cell proliferation and natural killer cell function, and they also limit the expression of the Major Histocompatibility Complex II. Placenta and umbilical cord are the most convenient sources of MSCs, which do not entail ethical problems. Additionally, placental delivery and isolation of umbilical cord is not connected with a difficult or invasive method. MSCs from Wharton's jelly and placenta (the amnion, the chorion, the villi, the *Decidua basalis*) were isolated with the protocol: mechanically and enzymatically (collagenase digestion). The proliferation potential was evaluated with a PDT analysis. For multi-differentiation of MSCs, cells were incubated in different differentiation media for 2-3 weeks at 37°C in an atmosphere with 5% $CO_2$ and 90% humidity.

We successfully isolated MSCs from placenta and umbilical cord. Mechanical isolation was possible for all types of tissues. In the case of enzymatic digestion of the umbilical cord, we were able to obtain MSCs in a limited number only, insufficient for further analysis.

All the isolated MSCs had typical fibroblastic morphology, expressed cell-surface markers (CD73, CD90, CD105), did not express hematopoietic and endothelial markers and differentiated into osteocytes, chondrocytes, and adipocytes, which is a set of possibilities recommended by the International Society of Cell Therapy. We didn't notice significant differences in morphology or differentiation between cells due to tissue of origin and method of isolation. Both: collagenase digestion and mechanical cutting resulted in high amount of cells harvested, but usage of enzyme makes this process faster.

**Keywords:** Mesenchymal stem cells differentiation; Stem cell therapy

## Introduction

Mesenchymal stem cells (MSCs) are a good source for the cell therapy thanks to their features. MSCs are multipotent, have the ability of self-renewal and can differentiate into different types of cells, such as chondrocytes, adipocytes, osteocytes, cardiomyocytes, neuronal cells and other [1]. The minimal criteria for MSCs from the International Society for Cellular Therapy include: adherence to plastic, positive expression of CD73, CD90 and CD105, and negative expression of CD45, CD34 and other hematopoietic markers. MSCs exert very important immunomodulatory effects: they suppress T- and B-cell proliferation and natural killer cell function, and they also limit the expression of the major histocompatibility complex II (MHC II) [2,3]. The immunomodulatory potential of MSCs is well known. They produce high levels of CD200 [4], a cell surface molecule that is involved in regulation of immune cells, such as macrophages. Therefore, MSCs can be used to effectively treat the graft-versus-host disease (GVHD) and a number of other diseases [5,6].

It was determined that MSCs reside in many tissues. Fried stein was the first to isolate and identify MSCs from bone marrow [7]. Later, MSCs were successfully isolated from a range of tissues and organs: bone marrow, adipose tissue, peripheral blood, amniotic fluid, placenta and umbilical cord [8,9]. Recently, scientists have shown a greater interest in the placenta and umbilical cord as a source of MSCs, which do not entail ethical problems. Additionally, placental delivery and isolation of umbilical cord does not require a difficult or invasive method.

Several protocols described two different methods of isolation: mechanical separation and enzyme digestion [10,11]. In our experiment, we isolated MSCs by mechanical cut and collagenase digestion to compare cell populations from a given tissue isolated with different methods. With the enzymatic method, cells can be obtained faster.

## Material and Methods

### Term placenta and umbilical cord sample collection

Human full-term placentas and umbilical cords were obtained from healthy women aged 19-33 years, at the time of a routine caesarian section or vaginal delivery in an affiliated hospital in Cracow. The mean gestational age was 39 weeks. Samples were collected in accordance with a protocol approved by a committee from the Ministry of Health in Poland. Placentas were obtained from 10 donors: two from caesarian section and eight from vaginal delivery. We used two methods to isolate MSCs from different parts of the placenta: the amnion, the chorion, the villi and the *decidua basalis*, and from Wharton's jelly from umbilical cord.

### Cell isolation

MSCs from Wharton's jelly and placenta were isolated with the same protocol: mechanically and enzymatically, using collagenase IV (Gibco), as previously described [12]. All tissue samples were washed in sterile 0.9% NaCl with antibiotics/antimicotic (Gibco). The samples from placenta and umbilical cord were cut into small pieces, washed with 0.9% NaCl twice and incubated at 37°C, 90% Rh, 5% $CO_2$ in 75 $cm^2$ flasks in Mesencult (STEMCELL Technologies) with a supplement in the MC case, or with gentle rotation at room temperature with 1% collagenase IV (Gibco, Life Technologies) for 1.5 hours (CD). After CD, cells were collected by centrifugation, resuspended in medium and cultured using the Mesencult medium at 37°C in an atmosphere with 5% $CO_2$ and 90% humidity.

### Immunophenotype analysis by flow cytometry

At third passage, MSCs from all tissues were harvested using trypsin (Thermo Fisher). For phenotypic analysis, all the MSCs extracted from culture were incubated for 30 min with phycoerythrin-conjugated antibodies against the human antigens: CD73 (BD Biosciences), CD90 (BD Pharmingen, BD Biosciences) and CD105 (BD Pharmingen, BD Biosciences), and with fluorescein isothiocyanate-conjugated antibodies against the human antigens: HLA-DR, CD34, CD45, CD 19 and CD14 (BD Bioscience). Surface staining was analyzed in a FACS Calibur machine (BD Bioscience).

### Population Doubling Time (PDT)

The proliferation potential was evaluated with a PDT analysis. We used cells from the third (P3) and fifth passage (P5), from all samples. MSCs were seeded on a T25 dish and cells were then harvested and counted until they reached about 100% confluency.

The PDT was calculated using the following formula:

$PDT=(CT*Ln^2)/ln(Nf/Ni)$

CT-cell-culture time, Nf-final number of MSCs, Ni-initial number of MSCs [13].

### *In vitro* multi-differentiation of MSCs isolated mechanically and enzymatically

Cells from the third passage were seeded on a $5*10^3/cm^2$ 24-well plate (BD Falcon) to obtain all the types of differentiation and were grown to confluence and then cultured in appropriate medium. For osteogenic differentiation, cells were incubated in StemPro Osteogenesis Differentiation Medium (Gibco), for chondrogenic differentiation in StemPro Chondrogenesis Differentiation Medium (Gibco) and for adipogenic differentiation in StemPro Adipogenesis Differentiation Medium (Gibco), for 2-3 weeks at 37°C in an atmosphere with 5% $CO_2$ and 90% humidity.

After a fixed period of incubation we evaluated differentiation using differential staining. To evaluate calcium deposits, we used staining with alizarin red (Sigma), to assess synthesis of proteoglycans by chondrocytes we applied Alcain Blue staining (Sigma), and for lipid droplets we used Oil Red O(Sigma) staining.

### The evaluation of immunomodulatory potential of MSCs

To assess immunomodulatory potential of MSCs from different sources we used flow cytometer FACS Calibur (BD Bioscience). The cells were harvested, incubated with phycoerythrin-conjugated antibodies against the human antigens CD200 40 min.

## Results

### Characterization of MSCs from placenta and umbilical cord

We used two different methods to isolate cells from the collected material and successfully isolated MSCs from placenta and umbilical cord. Morphologically, MSCs were fibroblast-like, plastic adherent cells. Mechanical isolation was possible for all types of tissues (Figure 1).

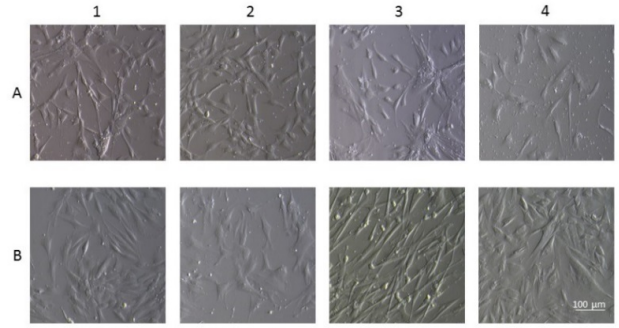


**Figure 1**: The cells isolated enzymatically (A) and mechanically (B), 1-chorion, 2-amnion, 3-villi, 4-*Decidua basalis*. The scale is 100 µm.

In the case of enzymatic digestion of the umbilical cord, we were able to obtain a limited amount of MSCs that was insufficient for further analysis (Figure 2).

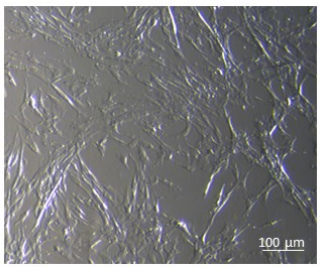


**Figure 2**: The cells isolated mechanically from umbilical cord. The scale is 100 µm.

### Flow cytometry

We identified the following cell surface phenotypes of MSCs: $CD73^+$, $CD90^+$, $CD105^+$, $CD14^-$, $CD19^-$, $CD34^-$, $CD45^-$ and $HLA\text{-}DR^-$ (Figure 3).

### PDT of MSCs

MSCs from different tissues demonstrated different proliferative potential (Figure 4). Obviously, cells isolated from the third passage (P3) showed higher proliferative capacities than MSCs from the fifth passage (P5).

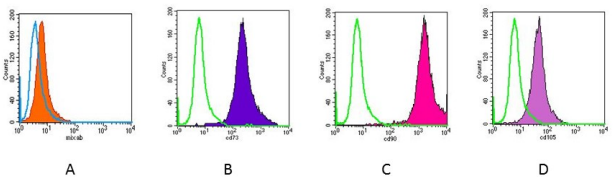


**Figure 3**: Exemplary differentiation of UCF-derived MSCs. Transparent histograms represents cells population stain with polyclonal isotype control antibodies conjugated to fluorescein (FITC) or phycoerythrin (PE) (green or red, respectively). Filled histograms represent cell population labelled with monoclonal antibodies against the mix of hematopoietic and endothelial markers: CD14, CD19, CD34, CD45 and HLA-DR, CD73 (B), CD90 (C), and CD105 (D) conjugated with fluorescein or phycoerythrin (green or red, respectively).

The level of proliferative potential of MSCs from amnion (P3, P5) isolated by collagenase was higher than the potential of the cells from amnion isolated mechanically. The same held for the P5 cells from the *decidua basalis* and chorion. The opposite was observed for villi P3 and *decidua basalis* P3: MSCs isolated enzymatically demonstrated a lower potential for proliferation than cells isolated mechanically.

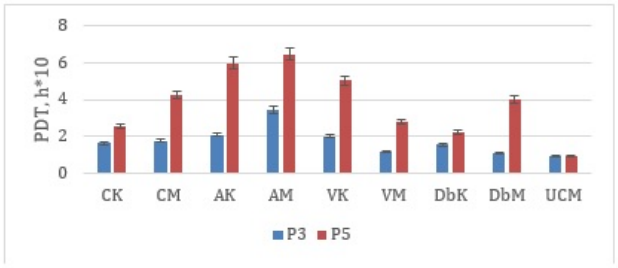


**Figure 4**: Analysis of the mean proliferative capacities of MSCs from different samples, where CK, AK, VK, DbK-chorion, amnion, villi, *decidua basalis*, respectively, isolated enzymatically, CM, AM, VM, DbM, UCM-as above, isolated mechanically. P3 and P5-passage three and five, respectively.

## Multi-differentiation

To analyze the multi-differentiation potential of cells isolated mechanically and enzymatically we used kits from Gibco by Life Technologies. We obtained osteogenic, chondrogenic and adipogenic tissues from all samples (Figures 5 and 6).

## Immunomodulatory potential of MSCs from placenta and umbilical cord

The immunomodulatory potential of MSCs from passages 3 (P3) was very similar across the sampled material, being the highest in the MSCs from villi (Figure 7). At passages 5 (P5) CD200 expression was lowered in all of the sampled organs, with the exception of *decidua basalis* isolated mechanically. The high expression of CD200 at P0 could be explained contamination with blood cells.

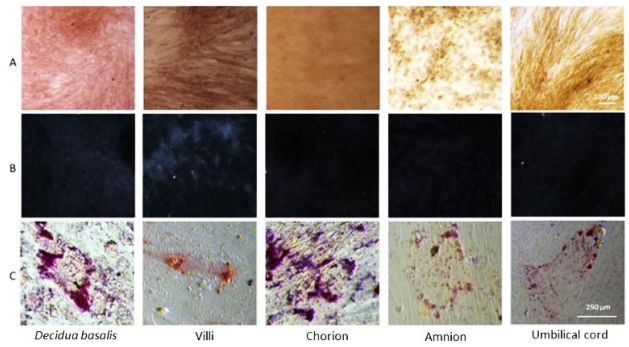


**Figure 5**: Multi-differentiation potential of MSCs isolated mechanically. A-osteogenic, B-chondrogenic, C-adipogenic differentiation.

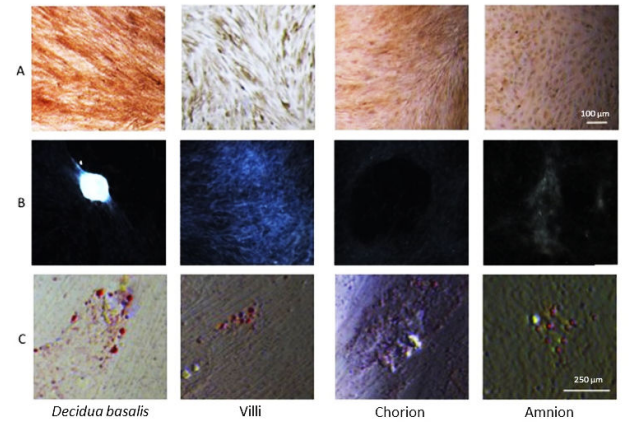


**Figure 6**: Multi-differentiation potential of MSCs isolated enzymatically. A-osteogenic, B-chodrogenic and C-adipogenic differentiation.

## Discussion

In the present study, we compared the basic characteristics of a population of stem cells isolated from different parts of the placenta and Wharton's jelly from umbilical cord using two methods: collagenase digestion and simple mechanical cut. In the case of enzymatic digestion of the umbilical cord, we were able to obtain only a limited number of MSCs, which was insufficient for further analysis, probably due to a negative effect of the collagenase. Using another enzyme, the STEMzyme II (animal origin free mix of enzymes) (Worthington), we obtained a sufficient amount of cells, which also fulfilled the minimal criteria, were plastic- adherent, and had cell surface markers and differentiation potential into mesodermal lineage (data not shown). All the isolated MSCs had typical fibroblastic morphology, expressed cell-surface markers (CD73, CD90, CD105), did not express hematopoietic and endothelial markers and differentiated into osteocytes, chondrocytes and adipocytes, which is a set of criteria recommended by the International Society of Cell Therapy (ISCT). We did not notice significant differences in morphology or differentiation between cells due to tissue of origin and

method of isolation. Both collagenase digestion and mechanical cutting resulted in a high amount of cells harvested but using an enzyme makes this process faster. All cells can be cryopreserved, banked, thawed and successfully cultured (data not shown).

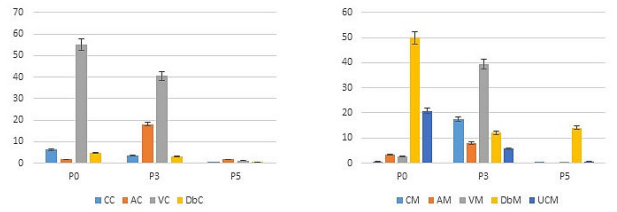


**Figure 7**: Comparison of the mean CD200 expression in MSCs from chorion, amnion, villi, *Decidua basalis* and umbilical cord isolated using collagenase (CC, AC, VC, DbC) and mechanically (CM, AM, VM, DbM, UCM). P0, P3, P5-passages 0, 3, 5.

The therapeutic application of MSCs may be more promising due to their unique possibilities. The capacity of MSCs to differentiate into other types of cells and their immunomodulatory effects make these cells very important in future clinical research. The ability of MSCs to modulate immune response can be used in an inflammatory context: MSCs secrete cytokines which can inhibit inflammatory process. Using MSCs in GVHD disease [14,15], multiple sclerosis [16], joint diseases [17] and various inflammatory diseases [18] has proved successful.

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