

Dataset Paper

Heterogeneity of Umbilical Cords as a Source for Mesenchymal Stem Cells

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The object of the paper is to show the heterogeneity of 300 cord samples processed in the current research. The differences in effectiveness of mesenchymal stem cell (MSC) isolation are shown. Moreover, the recommendations for choosing the method of MSC isolation depending on the value of stromal-vascular rate are given. The data can be useful for selecting the optimal conditions to obtain MSC and for further cryopreservation of umbilical cord tissue.

1. Introduction

Mesenchymal stem cells (MSCs) from umbilical cord matrix are extremely promising for use in regenerative medicine. Accessibility and safety of material selection, absence of ethical conflicts, simplicity of cells isolation, relative homogeneity of the material, and presence of the specific populations of MSCs are the main advantages of umbilical cord matrix as the source of MSCs. Early ontogenetic population of cells can be isolated from properly processed umbilical cord matrix [1–4].

According to a generous amount of modern papers, obtaining MSCs from umbilical cord matrix is considered to be a methodologically easy task [3–6]. Many variations of enzymatic way of umbilical cords' processing with collagenases of different types, hyaluronidase, trypsin, and other enzymes are proposed by different authors. Moreover the efficiency of mechanical way of processing and various combinations of these approaches are shown [3–9]. The possibility of obtaining the big number of cells from each specimen regardless of any cord characteristic is demonstrated.

However, the cord may have individual peculiarities in a case of each organism. MSCs as the part of cord stroma can be more or less available for obtaining depending on organometric characteristics of the cord. Presumably the health of mother and child and the external factors' influence may have an impact on efficacy of MSC isolation. But that statement needs to be proven. Most of the researches that substantiate the efficiency of different methods of MSC acquiring and claim extremely high amount of obtained cells were made with a low number of samples and thus may not fully reflect the cords' heterogeneity.

After processing 300 samples within 3 years, we have demonstrated that under similar conditions the possibility of cells' obtaining from different cords is not the same as well as the viability of the resulting cultures. In addition, we have demonstrated that the method for obtaining cells should also be chosen individually based on the organometric characteristics of the cord. We propose to use the stromal-vascular rate (SVR) that can be easily calculated using simple formulas [10]. Enzymatic, mechanical, or both of

these approaches should be chosen depending on the values of this index. This paper demonstrates the difference of obtaining cells from distinct types of umbilical cords using different methodological approaches as well as the cases when successful obtaining is not possible.

This data is extremely valuable because cryopreservation of cord matrix and even whole cords are in great request. Perhaps the individual peculiarities of mother and child and the organometric characteristics of the cord should be considered for storage.

2. Methodology

Human umbilical cords ($n = 300$) from both sexes were collected from full-term births after normal vaginal delivery with informed consent using the guidelines approved by the Institutional Ethics Committee (IEC) at the Municipal Maternity Hospital No. 5, Kyiv, Ukraine.

The UC samples (length range 19–32 cm) were collected in a phosphate-buffered saline (PBS) containing antibiotic-antimycotic solution 1 mL/100 mL (PAA, Austria) and then processed within 5 hours after birth. After transferring under sterile conditions, the cross-sectional areas of all three vessels and whole cord were estimated. The UC was washed twice in PBS to remove blood. The following formulas for evaluating of SVR were implemented: $S = \pi r^2 = \pi(d/2)^2$, and $SVR = S_{str}/S_{vess}$.

Then the sample was cut into two pieces: one of which underwent enzymatic treatment and another was processed with mechanical approach. Vessels were removed from both pieces.

For enzymatic treatment the sample was minced into small fragments (5–10 mm) and put into Petri dish with enzyme solution (mixture of 0.1% collagenase IV (Sigma, Germany) and hyaluronidase (Sigma, Germany) in PBS). Then the dish was placed into 5% CO₂ environment at 37° for 45 min. After the incubation, the solution was collected and centrifuged at 200 ×g, resuspended in PBS, and centrifuged again at 200 ×g. Then the cells were seeded on two 75 cm² culture flasks (PAA, Austria).

Mechanical approach was performed by mincing the sample with scissors into as small fragments as possible and placing the squash into three 75 cm² culture flasks (PAA, Austria).

Low-glucose DMEM (Sigma, Germany) was used for culturing. Medium was supplemented with 10% FBS (PAA, Austria), 2 mM L-glutamine, and 2 ng/mL bFGF. The first change of medium was carried out at the third day, but only half of the medium was changed. After 3–5 days full medium change was performed. Next medium changes took place twice a week.

The passaging was performed using 0.25% trypsin-EDTA solution. Two hundred cells per cm² were seeded on 75 cm² culture flasks (PAA, Austria) at every reseeding. Each passage has been cultured for 14–21 days, until the 75% confluence was observed.

The phenotype of MSCs was investigated by FACS analysis at the Laboratory of Cell and Tissue Cultures (State

Institute of Genetic and Regenerative Medicine National Academy of Medical Sciences of Ukraine). FITC-labeled primary antibodies CD90, CD105, CD34, and PE-labeled CD73 (Beckton Dickinson) were applied according to the manufacturer's protocol. BD FACSAria cell sorter and BD FACSDiva software with Data File Structure Flow Cytometry Standard (FCS) were used.

Chondrogenic induction was performed according to standard recommendations. Results were estimated using Alcian blue (PAA, Austria) staining.

Statistical calculations were made using Microsoft Excel 2003 and 2007 software.

Thirty samples were contaminated with fungal or bacterial infections. For 46 samples, obtaining of cell cultures was not successful for unclarified reasons. Seventy-five samples were able to generate primary cell cultures, but they were discarded at early passages (0–2) because of premature senescence and apoptosis or/and atypical morphology or low (<75%) expression of positive markers. Successful obtaining of MSCs for long-term culturing was observed in 149 samples. The gender had no crucial effect on the efficacy of MSC obtaining (Table 1).

The range of SVR from 2.4 to 6.5 was observed. The average value of SVR was 4.743, standard deviation, 1.01; min, 2.4 ($n = 2$); and max, 6.5 ($n = 2$). The most effective method of MSC obtaining from cords with low SVR was enzymatic. Mechanical treatment was most efficient for cords with high SVR. Both approaches can be used for obtaining cells from cords with average SVR. In addition, the frequencies of occurrence of distinct SVR values among male and female samples are given in Table 2.

Successfully obtained MSC cultures met the minimal criteria for defining MSCs [11]. Plastic-adherent cells in cultures exhibited typical spindle-shaped fibroblast-like morphology and were able to form colonies (Figure 1). FACS analysis demonstrated that cell populations were positive for the standard surface markers CD90, CD73, and CD105 (>75%) and were negative for CD34 (<1%). Examples of phenotypes of successfully obtained MSC cultures are shown (Figure 2). Cells were able to differentiate into chondrogenic and adipogenic lineage under certain conditions (the example of chondrogenesis in pellet is given in Figure 3).

3. Dataset Description

The dataset associated with this Dataset Paper consists of one item which is described as follows.

Dataset Item 1 (Table). There were processed 141 female and 159 male cords. In the table, “C+” indicates effective MSC culturing; “C+/-”, culture death at 1-2 passages; “*”, contamination; and “C-”, no cultures obtained.

Column 1: Number

Column 2: SVR

Column 3: Gender

Column 4: Result

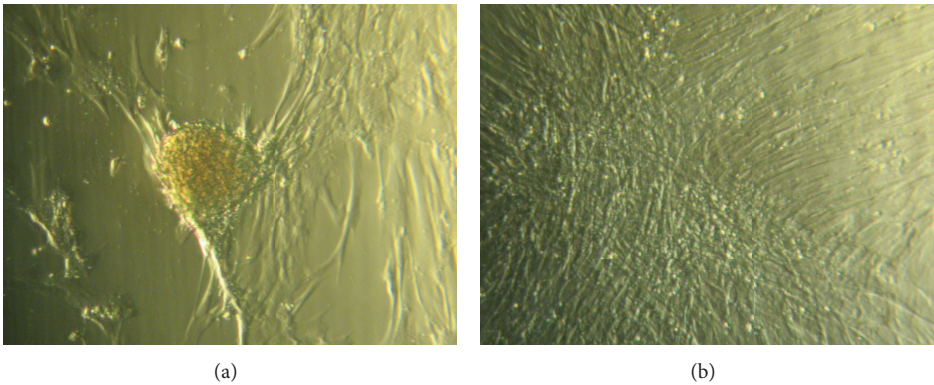


FIGURE 1: P1 and P3, vital cultures, $\times 100$, Leica DMIL, Cannon PowerShot 640A.

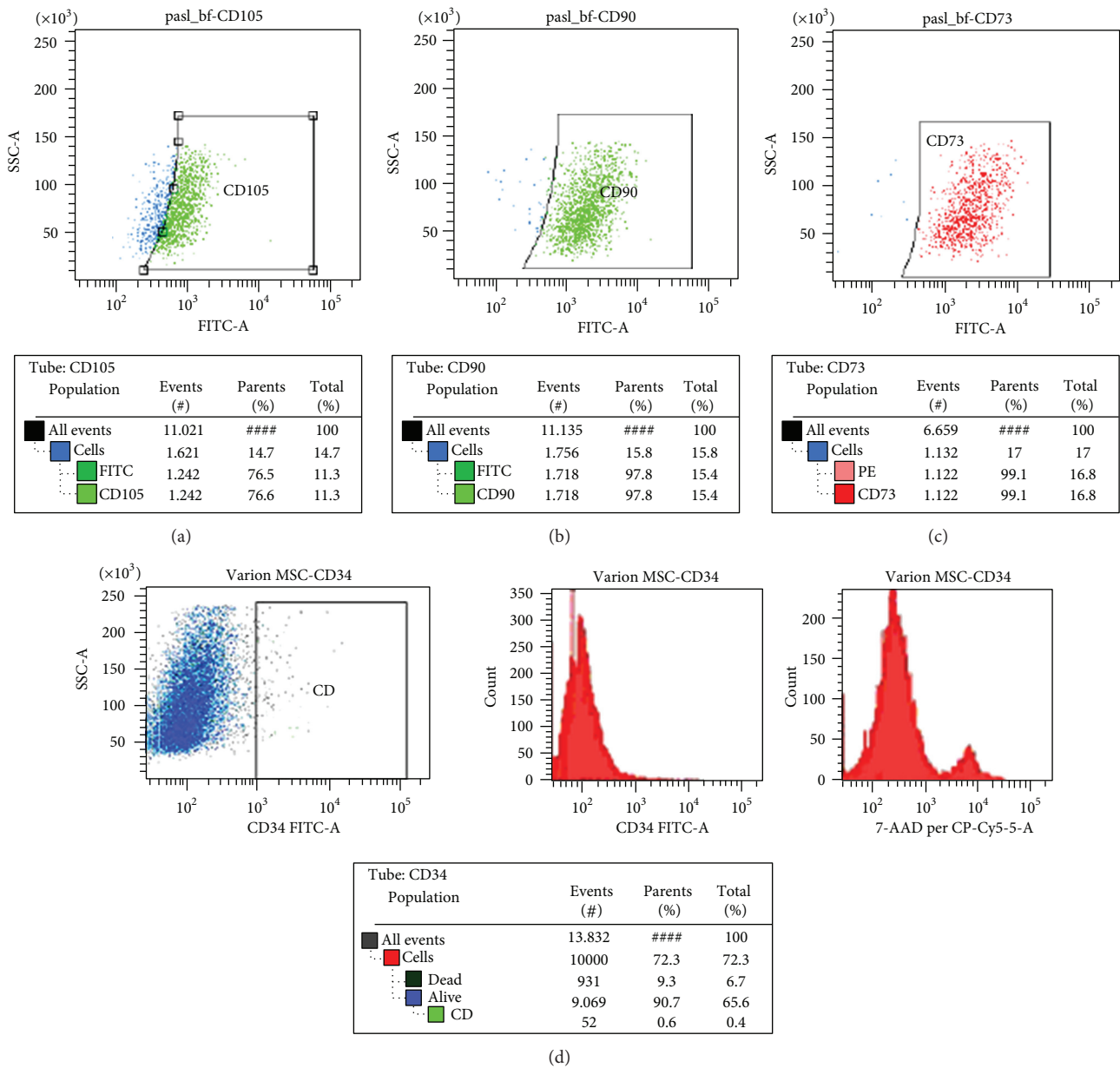


FIGURE 2: Examples of FACS analysis results are provided: CD105, 76.6%; CD90, 97.8%; CD73, 99.1%; and CD34, 0.6%.

TABLE 1

Male				Female			
C+	C−	C+/-	*	C+	C−	C+/-	*
69 (46.31% from 149)	28 (60.87% from 46)	43 (57.33% from 75)	19 (63.33% from 30)	80 (53.69% from 149)	18 (39.13% from 46)	32 (42.67% from 75)	11 (36.67% from 30)

TABLE 2

SVR	n	Gender		C+ (% from 149)	C− (% from 46)	C+/- (% from 75)	* (% from 30)
		Male	Female				
2.4–4 (low)	57	30	27	16.78	23.91	18.67	23.33
4.1–5.4 (average)	177	92	85	61.07	56.52	64	40
5.5–6.5 (high)	66	37	29	22.15	19.57	17.33	36.67

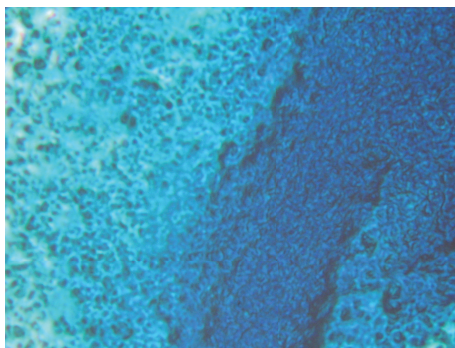


FIGURE 3: Positive Alcian blue staining of the pellet after 21 days of chondrogenic induction, $\times 100$, Leica DMIL, Cannon PowerShot 640A.

4. Concluding Remarks

After three years of working with umbilical cords taken from Ukrainian full-term deliveries, we consider that it is not possible to obtain MSCs from each sample. We assume that it can be explained with the differences in peculiarities of mothers and newborns. It must be taken into account when the cryopreservation of cord tissue is planned. Moreover we recommend choosing the most convenient method of processing the umbilical cords depending on their organometric parameters. Following the evaluation of the value of stromal-vascular rate (SVR), it should be decided whether to use the enzymatic or mechanical approaches or to combine them. Each cord sample has its own architectonics; so it is impossible to use one common method to isolate cells. Therefore cords with low SVR should be treated with the predominance of enzymatic approaches in contrast to cords with high SVR that should be treated using mechanical approaches.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at <http://dx.doi.org/10.7167/2013/370103/dataset>.

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