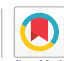
2026. Volume 1

Research Paper



Improved Cryopreservation of Umbilical Cord Blood MSCs with Low-dose DMSO

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Citation Vahidi M, Mozayyeni H, Mohajeri Iravani M, Emamie A, Ghorbani M. Improved Cryopreservation of Umbilical Cord Blood MSCs with Low-dose DMSO. Journal of Translational Regenerative Medicine. 2025; 1:E1006. http://dx.doi.org/10.32598/JTRM.1.1006

Article info:
Received: 10 Jan 2026
Accepted: 25 Jan 2026
Publish: 25 Mar 2026

ABSTRACT

Background: Given the challenges in isolating mesenchymal stem cells (MSCs) from bone marrow (BM), umbilical cord blood (UCB) can be a promising source of MSCs. On the other hand, cryopreservation of adherent MSCs is a solution for long-term storage of these cells and subsequent experimental use of them. In the present study, we investigated the isolation and identification of UCB-MSCs and the effect of cell freezing with dimethyl sulfoxide (DMSO).

Methods: The successfully isolated UCB-MSCs were cultured in DMEM containing 15% fetal bovine serum and 1% penicillin-streptomycin. After flow cytometric analysis of the cells, we investigated the differentiation potential of MSCs. Finally, the CFU-F assay was performed before and after freezing with 5% and 10% DMSO.

Results: Results showed that the average number of mononuclear cells obtained from 12 UCB samples was $58.2 \pm 10.7 \times 10^6$ with a viability rate of 90±3%. MSCs were successfully isolated with a 33% recovery rate. These cells had fibroblastic-like morphology and were immunophenotypic, with adipogenic, osteogenic, chondrogenic, and neural differentiation capacities.

Conclusion: Based on the results, the use of 5% DMSO for UCB-MSC cryopreservation is recommended as an alternative to the conventional 10% DMSO. The UCB should be considered a promising alternative to BM as a source of MSCs. The use of the slow-freezing method with two concentrations of DMSO is effective in retaining the proliferation, cell-surface markers, and differentiation ability of human UCB-MSCs.

Keywords: Umbilical cord blood, Mesenchymal stem cells, Dimethyl sulfoxide (DMSO), Differentiation, Freezing

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Highlights

- UCB-MSCs preserved their characteristic immunophenotype and multilineage differentiation potential after cryopreservation.

- Cryopreservation with 5% DMSO showed higher post-thaw cell viability compared with the conventional 10% DMSO method.

- Slow-freezing protocols effectively maintained colony-forming ability, surface marker expression, and osteogenic, adipogenic, chondrogenic, and neural differentiation capacities.

- The findings support the use of umbilical cord blood as a promising alternative source of mesenchymal stem cells for regenerative medicine applications.

Plain Language Summary

Mesenchymal stem cells (MSCs) are special cells that can repair and regenerate damaged tissues. Bone marrow is commonly used to obtain these cells, but collecting bone marrow is invasive and painful. Umbilical cord blood is a safer and easier alternative source. In this study, researchers isolated MSCs from human umbilical cord blood and evaluated how well these cells survived after freezing and thawing. The researchers compared two concentrations of dimethyl sulfoxide (DMSO), a chemical commonly used to protect cells during freezing: 5% and 10%. The results showed that MSCs frozen with 5% DMSO had better survival and maintained their important biological properties more effectively than cells frozen with 10% DMSO. The cells were still able to grow, form colonies, and differentiate into bone, fat, cartilage, and nerve-like cells after thawing. These findings suggest that umbilical cord blood is a valuable source of MSCs and that using lower concentrations of DMSO may improve the safety and quality of cryopreserved stem cells. This approach may help support future regenerative medicine and cell therapy applications.

Introduction

Mesenchymal stem cells (MSCs) are obtained from different tissues of the body, and have several potential uses [1]. They are capable of differentiating into different mesodermal and endodermal lineages, such as bone, fat, and cartilage. While debates are still ongoing, research has shown that MSCs have the capacity to reproduce and regenerate damaged tissues as stem cells [2]. Although bone marrow (BM) is the main source of MSCs, these cells are suitable for every situation due to high viral expression and attenuation of cell aging [3]. In addition, collecting a BM sample is a painful, invasive procedure that requires finding alternative sources of MSCs with less pronounced clinical effects and a lower risk of infection. Umbilical cord blood (USB) was introduced in 1988 as an alternative source rich in progenitor, pre-hematopoietic, and non-hematopoietic stem cells [4]. Endothelial cells, MSCs, and unrestricted somatic stem cells are among stem cell sources [5]. UCB-MSCs are more primitive than MSCs from BM and other sources, because the former have a lower recovery rate compared to the latter [6]. Unlike BM, the differentiation and reproductive power of UCB-MSCs do not change during frequent passages [7].

Due to challenges with recovery methods, standard freezing remains a persistent challenge for researchers and medical centers. Among the advantages of cryopreservation methods are the saving of time and culture medium, and protection against contamination and genetic drift that may result in immune rejection [8]. Cryopreservation is a method for long-term storage; cells and tissues are frozen during the process. However, the dehydration of compressed cells should be taken into account. Internal morphology and integrity of cells may change, depending on temperature and additives, and may lead to undesirable results and even cell death. To avoid damage caused by dehydration or the formation of ice crystals inside the cell body during freezing, special chemicals are added, which preserve the biological traits of frozen cells and tissues [9]. Dimethyl sulfoxide (DMSO) is one of the most widely used chemicals for this purpose.

The present study aims to examine the recovery of UCB-MSCs and the freezing process using two concentrations of DMSO. Viability of cells, preservation of cell

surface markers, potential of the cells to differentiate into bone, cartilage, and fat lineages, using the colony-forming unit-fibroblast (CFU-F) assay in previous samples before/after storage in liquid nitrogen, were also studied.

Materials and Methods

Materials

The materials that used in this study included: phosphate-buffered saline (PBS; Gibco-BRL, USA), Ficoll-Hypaque low-density mononuclear cells (MNCs) <1.077 g/mL (Cedar Lane, Canada), Trypan-blue (Sigma Aldrich, USA), Dulbecco's modified Eagle's medium (DMEM-low glucose; Gibco-BRL, USA), fetal bovine serum (FBS; Gibco-BRL, USA), penicillin-streptomycin (Gibco-BRL, USA), Trypsin-EDTA (Gibco-BRL, USA), paraformaldehyde (Merck, Germany), Dexamethasone (Sigma Aldrich, USA), β-glycerophosphate (Sigma Aldrich, USA), ascorbate (Sigma Aldrich, USA), 3-isobuthyl-1-methyl-xanthin (IBMX; Sigma Aldrich, USA), indomethacin (Sigma Aldrich, USA), transforming growth factor (TGF)-β (Sigma Aldrich, USA), Insulin-transferrin-selenium (ITS) natrium (Gibco, USA), retinoic acid (Sigma Aldrich, USA), Giemsa (Merck, Germany).

Clinical samples

As an experimental study, UCB samples were collected from healthy mothers (20-33 years) who had successfully passed a full-term pregnancy period. Samples were collected in special bags (Beassat, Iran) containing citrate-phosphate dextrose-adenine as an anticoagulant.

Cell processing and culturing UCB-MSCs

After transferring UCB samples to the laboratory at 22-25 °C, they were diluted in a 1:1 ratio with PBS and were carefully overlaid on Ficoll-Hypaque low-density MNCs <1.077 g/mL. After separation, the samples were centrifuged at 430×g for 30 min. Afterwards, the interphase layer between plasma and Ficoll-Hypaque, which contained MNCs, was collected slowly and transferred to a new tube, diluted with PBS. Recovered cells in PBS were centrifuged at 1300 rpm for 5 min.

After counting and evaluating viability by 0.05% Trypan-blue, MNCs were cultured in 25 cm^2 tissue culture flasks (Nunc, USA) containing DMEM-low glucose, supplemented with 15% FBS and 1% penicillin-streptomycin. At confluency of 55-56%, the cells were harvested by 0.05% trypsin-EDTA. Then, 2×10^5 cells/cm^2 were seeded in 25 cm^2 tissue culture flasks and incubated at 37 °C in a humidified ambient containing 5% CO_2. To remove floating cells, fresh medium was added to each tissue culture flask every 3-4 days for 14 days of culture. After this period, the MSCs began to reproduce, and homogeneous cell populations were gradually observed.

Flow cytometric analysis of UCB-MSCs

Cells from the third passage were evaluated by flow cytometry (PAS and CyFlow Space cytometers, Partec, Germany) to analyze immunophenotypic markers of the MSCs. Trypsinized cells were centrifuged at 1300 rpm for 5 min, and the plate was washed twice with PBS containing 2% FBS. After washing, the cells were counted and resuspended at a concentration of 1×10^4 cells/antibody test. For this purpose, the samples were stained with 10 µL phycoerythrin (PE)-conjugated mouse anti-human CD14, CD29, CD44, CD45, CD73, CD105, CD106, CD271, and HLA-DR, fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD34 and peridinin chlorophyll protein (PerCP)/cyanin (CY-5.5)-conjugated mouse anti-human CD90 (BD Biosciences, USA; except for monoclonal antibody against human CD34, which was from DAKO, Denmark). Negative control staining was performed using an FITC/PE/ PerCP-CY5.5-conjugated mouse IgG1 isotype antibody. After 45-min incubation at 4 °C in the dark to remove unlabeled antibodies, the cells were washed with PBS containing 2% FBS (stain buffer) through centrifugation at 1300 rpm for 5 min. Before flow cytometric analysis, the cell plates were suspended and fixed with paraformaldehyde containing 1% FBS.

Osteogenic differentiation

Third-passage UCB-MSCs were cultured under previously described culture conditions until they reached 70% confluence. After seeding and incubating 3×10^4 cells in 12-well plates, osteogenic differentiation was induced using osteogenic induction medium containing 0.1 µM dexamethasone, 10 mM β-glycerophosphate, 0.05 mM ascorbate, and 10% FBS for 19 days. Culture medium with no differentiation factors was used as a control. Alizarin Red S staining was performed to quantify mineralisation after 19 days of culture.

Adipogenic differentiation

For adipogenic differentiation, 3×10^4 cells/mL from the third passage were first seeded in 12-well plates and incubated in culture medium containing serum until 70% confluence was reached. After adding adipogenic induction medium containing 1 µM dexamethasone, 0.5 mM

IBMX, 10 ng/mL insulin (Sigma-Aldrich, USA), 0.2 mM indomethacin, and 10% FBS, the cells were incubated for 21 days. Then, the cells were fixed with 4% paraformaldehyde and stained with Oil Red-O. Lipid-rich vacuoles were considered a sign of differentiation.

Chondrogenic differentiation

For chondrogenic differentiation, 2×10^5 cells from the third passage were first seeded in the wells containing 500 µL of culture medium supplemented with serum and incubated for 24-48 h until they reached 70% confluency. Then, cells were incubated with chondrogenic induction medium containing DMEM, 10 ng/mL TGF-β, 50 µg/mL Ascorbic acid, Dexamethasone 10^{-7} M, and ITS 100X for 28 days. Afterwards, the cells were harvested, embedded in paraffin, and stained with Alcian blue after deparaffinization and washing with different ethanol concentrations.

Neural differentiation

For neural differentiation, 2×10^4 cells from the third passage were first seeded in 12-well plates. After reaching 50% confluency, the cells were induced by the neural induction medium containing DMEM, 0.5 mM IBMX, Retinoic acid 10^{-6} M, and 2% FBS for 7 days. Expression of β-tubulin and neuron-specific enolase (NSE) markers was analyzed by immunocytochemistry (ICC). Neuron marker expression and cell morphology were investigated by fluorescent microscopy. Cell culture and incubation were performed according to the protocols of Hung et al. [10] and Levy et al. [11].

CFU-F assay

Cells from the third passage were used for the CFU-F assay. Trypsinization was performed when the cells reached 70-80% confluence. Three 10 cm^2 cell culture plates containing 100, 500, and 1000 cells were used. The medium used for the CFU-F assay was DMEM containing 10% FBS. The culture medium was renewed every 3-4 days. More than 50 cells with a diameter of 2 mm were counted by marking fibroblast colonies with Giemsa on the 14th day of culture. The cells were fixed in methanol for 10 min, then incubated with Giemsa stain for 20 min. Cellular colonies were counted after rinsing them.

Freezing and defreezing MSCs

After trypsinization, the cells were rinsed twice with DMEM containing 10% FBS, and the cell count for each vial was set to 7105 after determining cell viability. 200 µL cell suspension was transferred to a 1.5 mL cryovial with two densities of DMSO as follows: 5% DMSO containing 95% FBS and 10% DMSO containing 90% FBS, and kept on a freezing-controlled container (-1 ˚C/min; Nalgene, USA) in a freezer (-80 ˚C) for 24 hours. Afterwards, the sample was transferred to a nitrogen tank. For defreezing the sample, the frozen cells on cryovials were immediately transferred to a water bath at 37 ˚C. Then, the floating cells in DMEM containing 10% FBS were centrifuged at 1300 rpm for 5 min. After determining cell viability, they were transferred to tissue culture flasks and incubated at 37 ˚C.

Statistical analysis

Data were presented as Mean±SD. The paired t-test was used to compare the mean values of cell percentages and numbers between wells coated with FBS and those left uncoated. Also, the paired t-test and Friedman's test were used to analyze the flow cytometry (performed in Flomax v.2.4e and Cyflogic v.1.1.0), CFU-F assay, and cell viability percentage data. All data were analyzed in SPSS software, version 27.

Results

Number of isolated mononuclear stem cells (MNCs) and their viability rate

MNCs were recovered from 12 samples of UCS cells using the Ficoll-Paque centrifugation. Along with counting, the viability of the cells was also determined. On average, 67.2±14.2 mL was collected from each sample, and the average number of MNCs obtained from samples was $58.2\pm10.7\times10^6$ with a viability rate of 90±3%. Table 1 presents the results for the number and volume of the samples, the number of the cells, and their viability rate. To study the effect of coating on the removal of undesired adhesive cells, the bottoms of some flasks were coated with FBS at room temperature for 45 min. After removing FBS, the cells were seeded. On average, as shown in Figure 1, coating the primary culture oflasks accelerated confluency by 22.8±2.6%, while the total rate inflasks lacking FBS was 26.5±3.8%. This difference was statistically significant ($P<0.05$).

Isolation of UCB-MSCs

The isolated MNCs from UCB cells were cultured, with the first medium renewal at day 5. This period gave the adhesive cells enough time to attach to the bottom of the tissue culture flask. The presence of red blood cells and platelets, along with MNCs, prevented the attachment of

Table 1. Number and volume of UCB samples, number of isolated MNCs and viability rate after isolation

No.	Volume of UCBs (mL)	MNCs (10^6)	Viability (%)
1	51	54	90
2	72	70	93
3	75	72	90
4	102	70	92
5	62	60	95
6	67	62	90
7	72	53	84
8	62	48	86
9	77	70	92
10	52	45	88
11	62	52	90
12	52	42	90
Mean±SD	67.2±14.2	58.2±10.7	90±3

adhesive MNCs. Hence, only half of the culture medium was refreshed to enable adhesion of other MNCs. Since a wide range of cells is found in UCB, other adhesive cells also had a chance to grow. The cell population in the primary passage was highly heterogeneous, and cells of different sizes were observed in the primary culture flask. In later cell passages, the MSC population grew uniformly.

Viability of MSCs before and after cellular freezing with DMSO

A Nalgene freezing container was used to control freezing time at a rate of -1 °C/min. In addition, cells from the third phase cultured in logarithmic space were used for freezing. Viability rate before freezing, after freezing with 5% DMSO, and after freezing with 10% DMSO

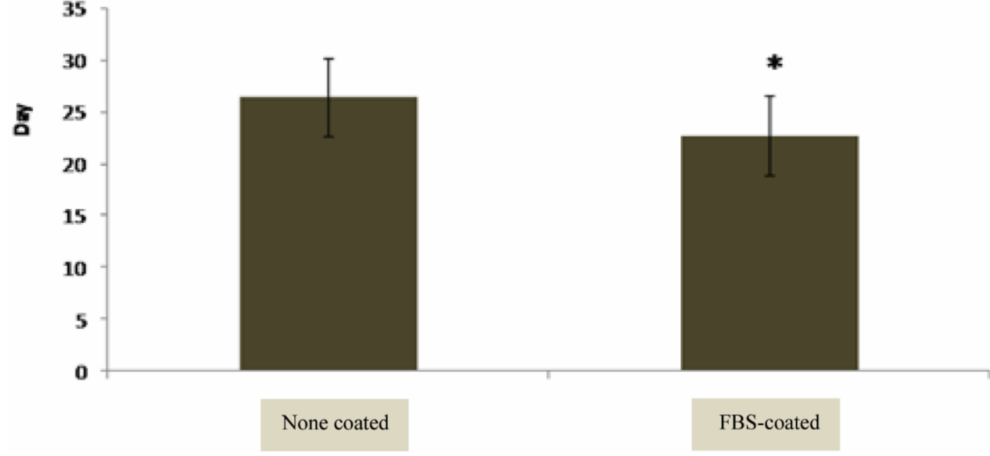

Figure 1. Comparison of average time (days) required to achieve confluency in primary passage in coated and non-coated tissue culture flasks

*P<0.05.

Note: The average time in FBS-coated flasks was 22.8±2.6 days, which is a significant time-saving compared to that of non-coated flasks (26.5±3.8 days).

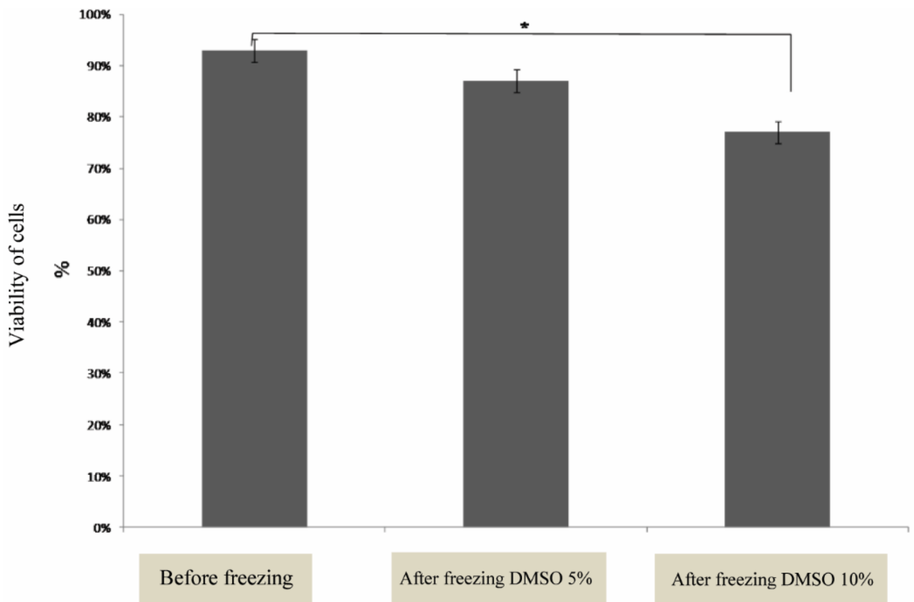

Figure 2. Comparison of the average cell viability rate before and after cellular freezing at two concentrations of DMSO

*P<0.05 (n=4).

was 93±2%, 87±2% and 77±2%, respectively. A significant decrease in viability was observed between the pre-freezing and post-freezing rates with 10% DMSO (Figure 2).

Flow cytometry results before and after cellular freezing

The phenotype and morphology of MSCs derived from UCS cells were confirmed by flow cytometry. According to the results, positive markers for recovered UCB-MSCs, including CD44, CD29, CD106, CD90, and CD73, were observed. The cells expressed CD 271 marker, but not the hematopoietic markers (i.e. CD14, CD45, CD34, and HLA-DR). To assess the effect of cellular freezing on the preservation of cell surface antigens, all markers were again analyzed by flow cytometry after freezing MSCs with 5% and 10% DMSO. As shown in Figure 3, cellular markers (CD29, CD44, CD73, CD90, CD105, and CD106) were positive before freezing and remained positive after freezing. On the other hand, the cells with negative marker expression before freezing (CD14, CD34, CD45, and HLA-DR) had no expression after freezing. Moreover, the marker CD271 was negative before and after freezing.

Osteogenic differentiation before freezing and after cellular freezing

As an index of cell differentiation, the mineralization capacity of differentiated cells was assessed by Alizarin Red S staining. Control samples showed no inorganic sediments and were negative for this marker. To assess and compare the sensitivity of cellular potential to freezing, MSCs were cultured after freezing in 12-well plates under the same conditions as before freezing and osteogenic induction medium was then added. Alizarin Red-S staining was repeated. Morphology of the cells and special markers showed preservation of the differentiation potential of MSCs for developing osteocytes after freezing (Figure 4).

Adipogenic differentiation before and after cellular freezing

Fat vesicles in differentiated cells, as an index of cell differentiation, were stained with Oil Red O. Control samples in the absence of differentiation medium showed no fat vesicles, and they were negative for this marker. To assess and compare the sensitivity of cellular potential after freezing, MSCs were cultured in 12-well plates after freezing under the same conditions as pre-freezing, and then adipogenic induction medium was added. Morphology of the cells and special staining showed preservation of the differentiation potential of MSCs into adipocytes after freezing (Figure 5).

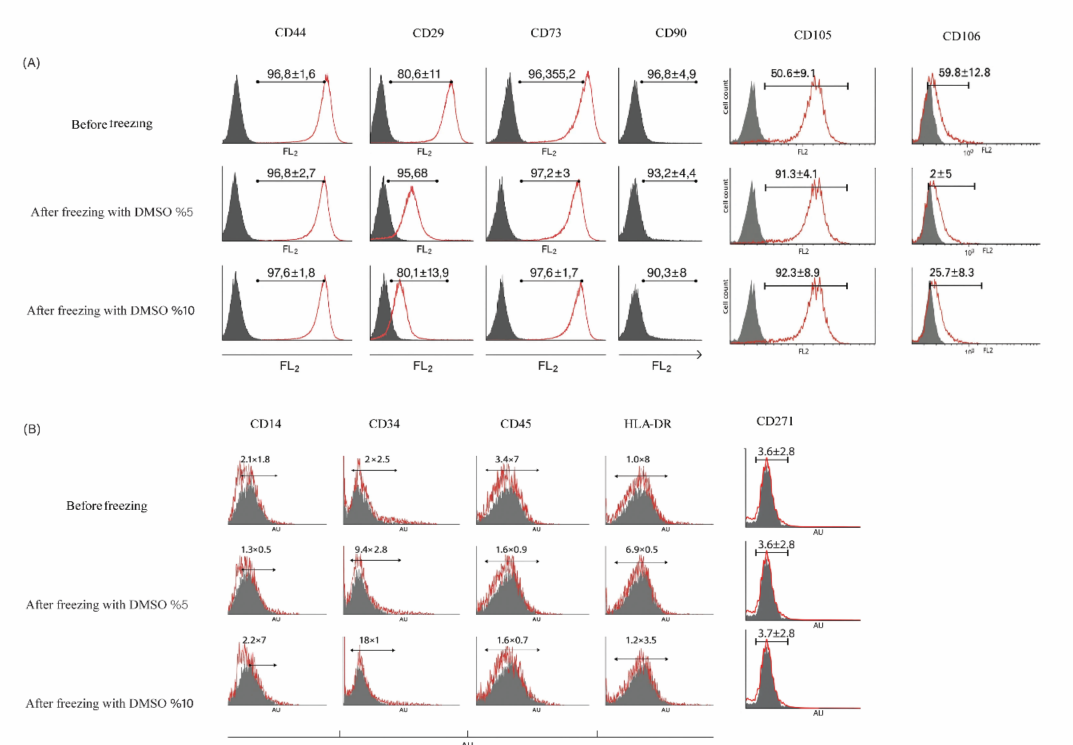

Figure 3. Phenotype analyses of UCB-MSCs by flow cytometry

A) Stromal cells and positive markers CD44, CD29, CD73, CD90, CD105 and CD106, B) Hematopoietic and negative markers CD14, CD34, CD45, CD271, and HLADR

Note: Flow cytometry was performed to assess cell-surface protein expression before and after freezing with two DMSO concentrations.

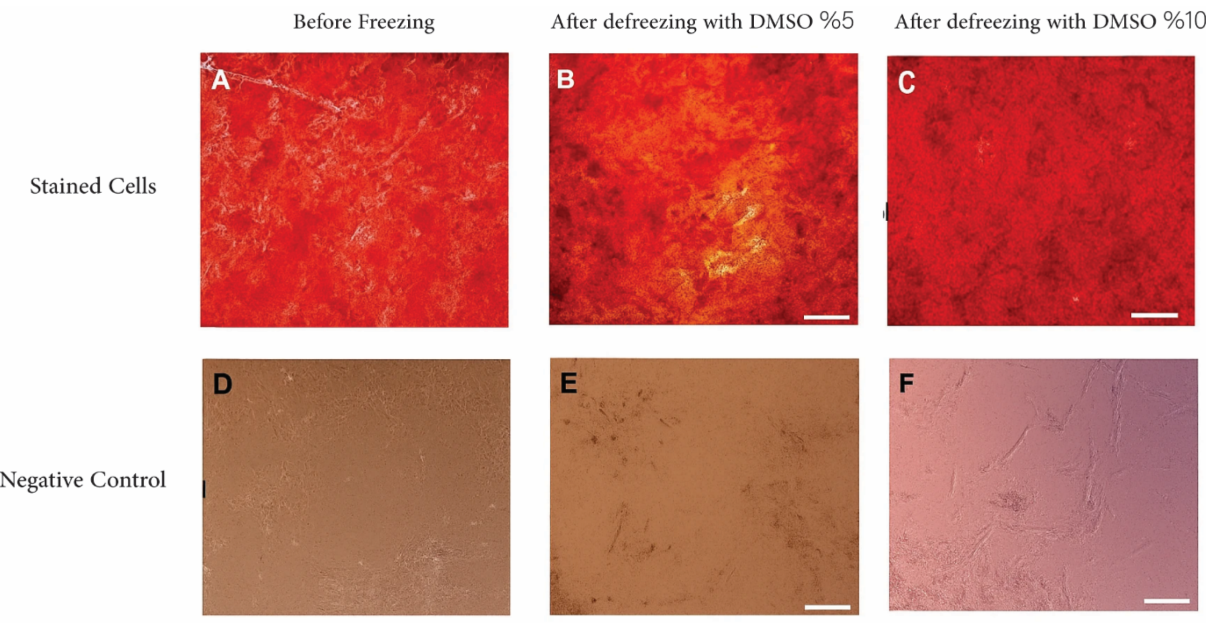

Figure 4. Osteogenic differentiation of MSCs before and after freezing (Alizarin red S staining; magnification ×100)

A) Before freezing, B) After freezing with 5% DMSO, C) After freezing with 10% DMSO, D, E, F) Non-differentiated MSCs (negative controls)

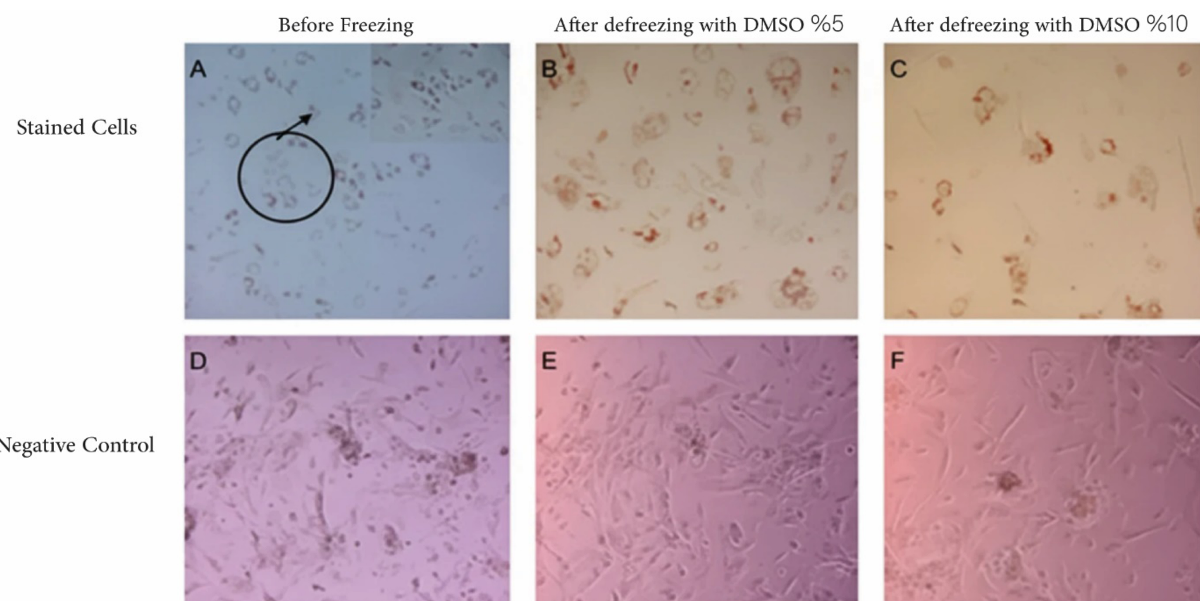

Figure 5. Adipogenic differentiation of MSCs before and after freezing (Oil Red O staining; magnification ×100)

A) Before freezing, B) After freezing with 5% DMSO, C) After freezing with 10% DMSO, D, E, F) Non-differentiated MSCs (negative controls)

Chondrogenic differentiation before and after freezing

By fixing the differentiated samples at the end of the differentiation process, Alcian Blue staining confirmed chondrogenic differentiation. To assess and compare the sensitivity of cellular potential after freezing, MSCs were cultured in plates after freezing under the same conditions as pre-freezing, and chondrogenic induction medium was then added. Different morphologies and cell densities were clearly evident upon Alcian Blue staining. The sample preserved the potential for differentiation to chondrocytes after freezing (Figure 6).

Neural differentiation before and after cellular freezing

Neural induction medium supplemented with 2% FBS was used for neural differentiation. More serum was used to compensate for the role of unknown and different factors, which may disturb the differentiation process. The samples were examined under a micro-

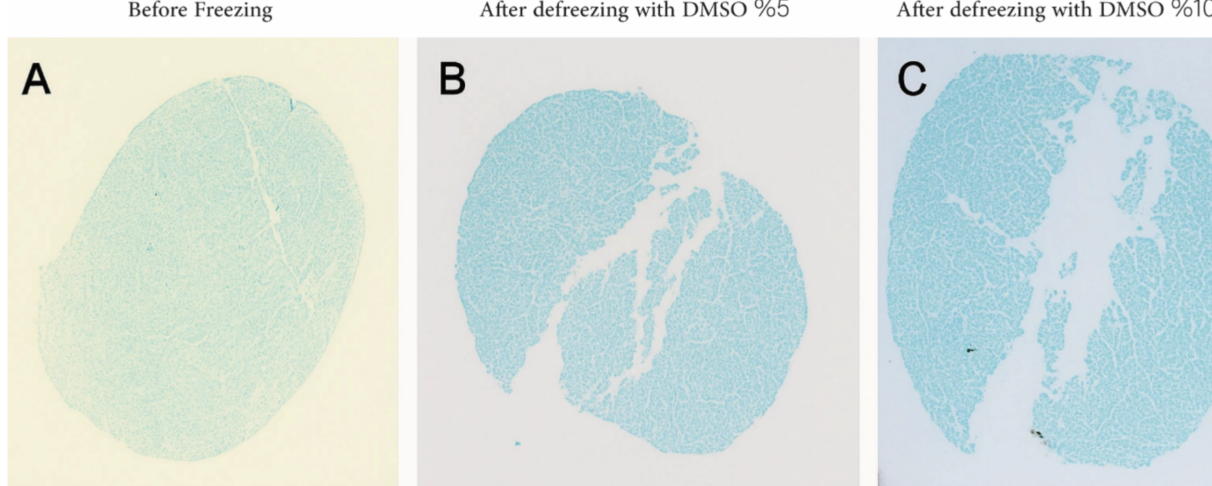

Figure 6. Chondrogenic differentiation of MSCs before and after defreezing (Alcian Blue staining; magnification ×100)

(A) Before freezing, B) After freezing with 5% DMSO, C) After freezing with 10% DMSO

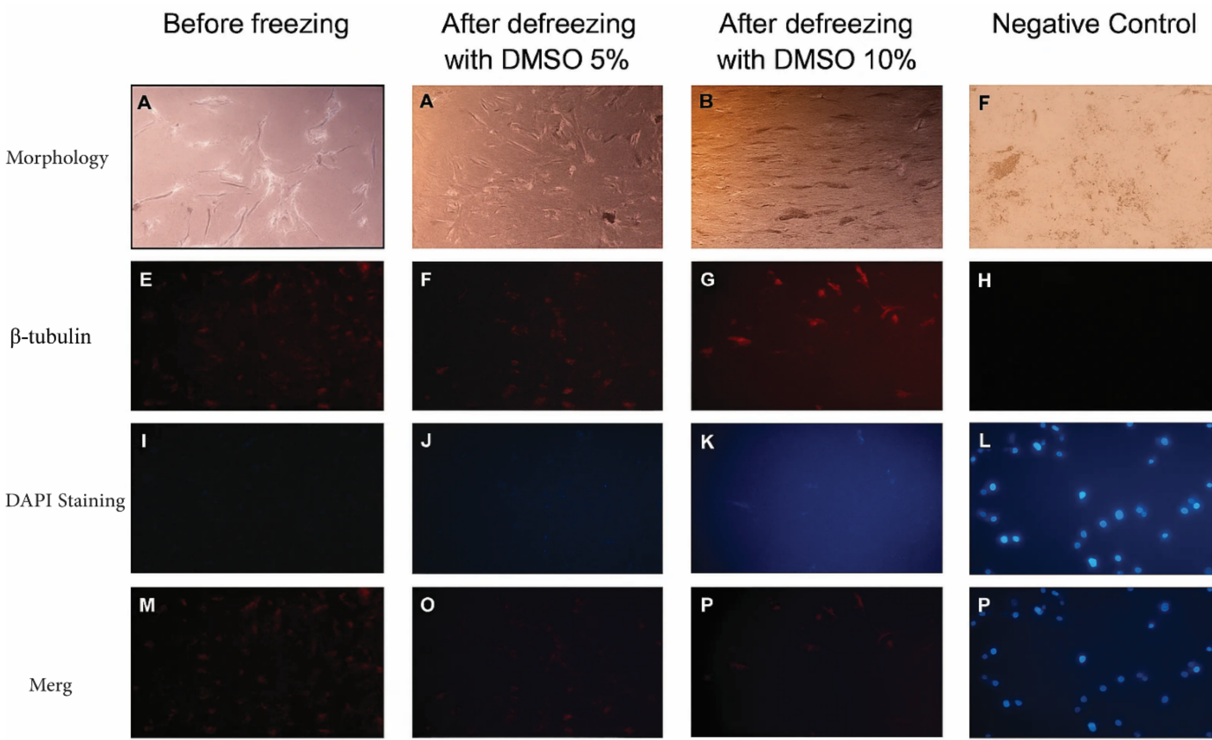

Figure 7. Neural differentiation of UCB-MSCs before and after freezing with two concentrations of DMSO

B) β-tubulin expression (DAPI staining; magnification ×200), C) B Tubulin expression before and after defreezing (magnification ×100),D) DAPI staining before and after defreezing (magnification ×100); Merged B, C) (magnification ×100); E, F, G, H) Negative control to morphology, neural expression, DAPI staining and merge them

scope on day 7 of culture. Along with expression of neuron markers (>70%), some cells were stretched in one direction. The control samples showed no change in neuronal marker shape or expression, and no marker absorption was observed. By fixing the differentiated sample at the end of the differentiation process, staining of β-tubulin and NSE confirmed neural differentiation. To assess and compare the sensitivity of cellular potential after freezing, MSCs were cultured in 12-well plates after freezing under identical conditions to pre-freezing, and neural induction medium was then added. The morphology changes and neuron differentiation markers were surveyed on day 7 of culture. With any concentrations of DMSO, there was no difference in morphological changes and in β-tubulin and NSE expressions compared to the samples before freezing (Figures 7 and 8).

CFU-F assay before freezing and after cellular freezing

Primary stem traits of the MSCs were investigated using this CFU-F assay. As the method required, 100, 500, and 1000 cells were cultured in large plates (10 cm^2), and the number of separate colonies was counted on day 14 of culture. Colonies with a diameter of more than 2 mm or 50 cells were counted. Recovered UCB-MSCs were collected to examine the effect of cellular freezing. The test was carried out before and after freezing with two DMSO concentrations. Figure 9 displays the average of colony production before and after freezing with DMSO. As can be seen, the formation of colonies decreased after freezing, and the difference in reduction was significant for 10% DMSO ($P<0.05$).

Discussion

Several studies have reported low success of recovery and reproduction of UCB-MSCs. For example, Mareschi et al. [12], and Yu et al. [13] concluded that recovery from UCB-MSCs was not feasible. In this study, we focused on the culture of UCB-MSCs and the method improved the recovery rate of MSCs. Our results confirmed that 4 of 12 UCB samples contained MSCs, with a 33% recovery rate. This is consistent with the results of Wagner et al. Rebelatto et al. and Biebeck et al. who reported a successful recovery rate of 25-40% [14, 15].

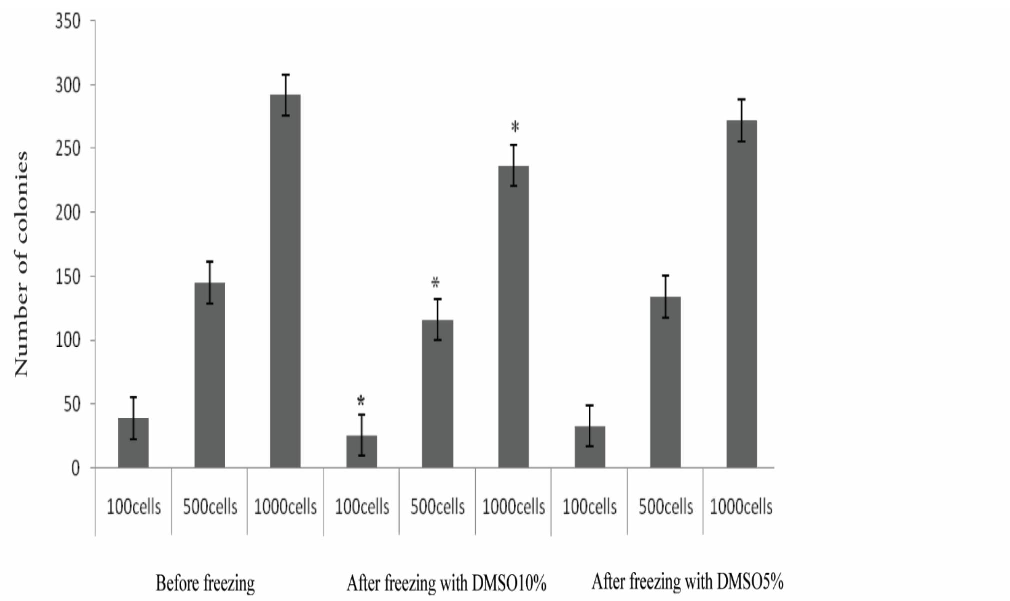

Figure 9. Comparison of the average number of colonies before and after freezing with two DMSO concentrations for three different numbers of cells

*P<0.05.

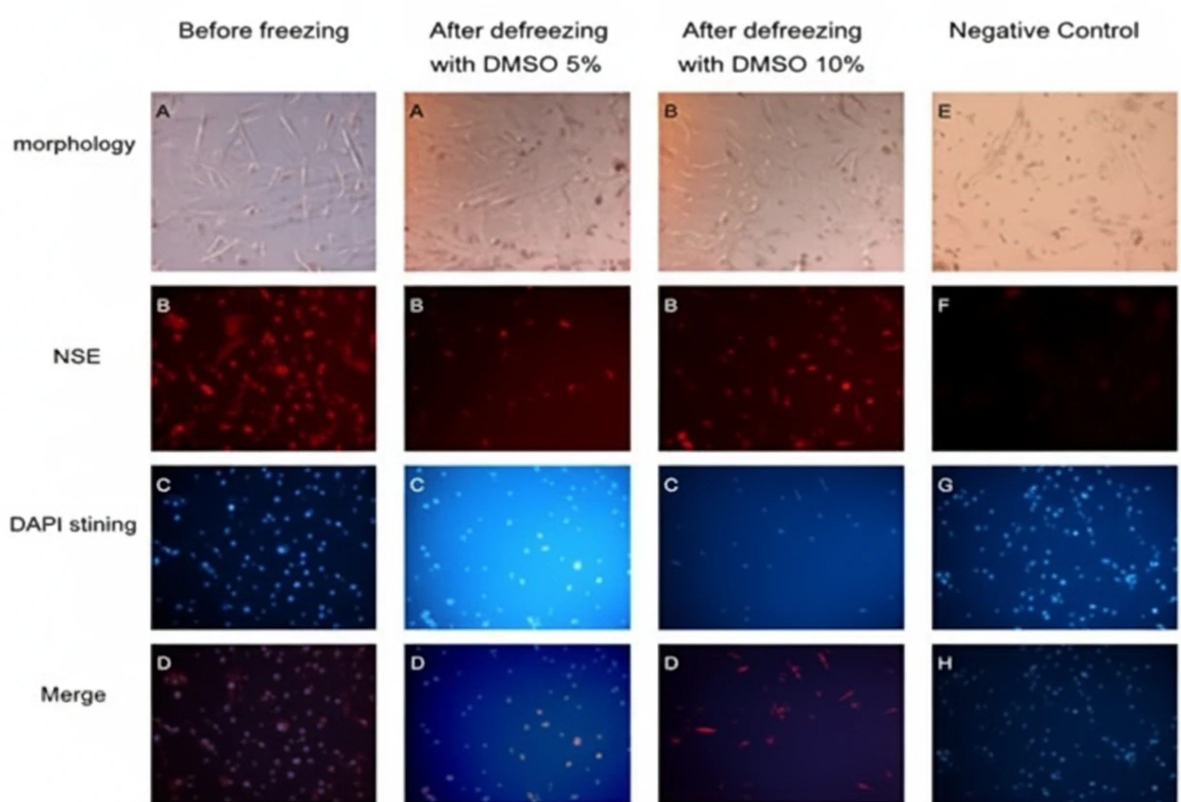

Figure 8. Neural differentiation of UCB-MSCs before and after freezing with two concentrations of DMSO

B) NSE expression (DAPI staining; magnification ×200), C) NSE expression before and after defreezing (magnification ×100), D) DAPI staining before and after defreezing (magnification ×100), Merged B, C) (magnification ×100); E, F, G, H) Negative control to morphology, neural expression, DAPI staining and merge them

The minimum required volume for recovery was 51 mL (average: 67.2±14.2 mL). This value was a key factor for the recovery rate of MSCs. However, it is against the results of two studies that suggested a minimum volume of 33 mL and 45 mL [17, 18], and the results of Rebelatto et al. who argued that sample volume was not effective [16]. The CFU-F assay was performed to assess the reproduction ability of the MSCs according to MSC protocols [19]. The results showed that CFU-F for the cells from passage 4 (n=3) was 32.4%. In the study by Vishnubalaji et al. this percent for BM-derived and adipose tissue-derived MSCs in passage 4 was 8.5% and 24.2%, respectively [20]. No similar report was found for UCS cells

The results of flow cytometric analysis of cell-surface markers for UCB-MSCs were consistent with other studies [16-21], except for CD106. Similar to BM, markers including CD29, CD44, CD73, CD90, and CD105 were positive, while markers such as CD14, CD34, and HLA-DR were negative. The expression level of CD106 was 74.2±43.2 in Biebeck et al.'s study [17] and was negative in Tais Sibov et al.'s study [22]. The value reported in our study was 26.8±12.8%. The osteogenic differentiation potential of isolated MSCs was confirmed by Alizarin Red S staining of inorganic calcium sediments. This result is consistent with the results of other studies [16, 17, 23]. The observation of lipid-rich vacuoles is consistent with previous reports for MSCs, suggesting similar adipogenic capacities [16, 17, 24], but the differentiation results differed from those of Kern et al. 2006, which showed the inability of UCS-MSC differentiation into adipocytes compared with BM cells [15]. The results regarding chondrocyte differentiation, including differentiation medium, differentiation potential, and markers, are consistent with the results of other studies [16, 17, 27].

In order to investigate the neural differentiation of MSCs, we used the protocol that was also used in other studies [28, 10, 11], which proved retinoic acid and IBMX as the main elements to promote neural differentiation of MSCs, in addition to 2% reduction in FBS level. The differentiation evaluation period was 7 days. The results of differentiation, in terms of morphological changes, along with the expression levels of NSE and β-tubulin neuron markers, were assessed by ICC.

Cell viability after freezing was assessed to evaluate the effect of DMSO on freezing. The results showed an extreme decline in viability when 10% DMSO was used. In comparison with the pre- freezing rate, this decline was significant for freezing with 10% DMSO. Cellular freezing with 5% DMSO also led to a decrease in viability, but the reduction was not significant in comparison with the pre- freezing rate. Comparison of differentiation potential after freezing showed that the potential of UCB-MSCs to differentiate into bone, fat, cartilage, and neuronal cells was not affected by 5% or 10% DMSO concentration. Flow cytometry analysis for cell surface markers of UCB-MSCs, both before and after freezing, was negative. No significant difference was found in the expression of antigens (CD14, CD34, CD45, HLA-DR) before and after freezing. Evaluation of the effects of two different concentrations of DMSO on the stemness of UCB-MSCs was done using the CFU-F assay in three cellular densities before and after freezing, showing that cellular freezing resulted in a reduction in the number of colonies after freezing, where the reduction was significant when 10% DMSO was used. For 5% DMSO, colony formation rate was not significant in comparison with pre-freezing numbers. Our results are consistent with the findings of Sorensen-Haack et al. and Xiang et al. on BM-MSCs [25, 26].

Conclusion

The use of a slow-freezing medium supplemented with 5% DMSO results in better recovery of UCB-MSCs than with 10% DMSO. The use of a slow-freezing method increases the capacity for preserving reproduction ability, cell differentiation, and surface antigen expression compared with the situation before freezing. Therefore, the freezing method can be used for freezing MSCs in larger scales. The technique is also a good manufacturing practice for laboratories.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This study was financially supported by the Research Center of the Iranian Blood Transfusion Organization, Tehran, Iran.

Authors' contributions

Conceptualization: Mahdi Ghorbani; Methodology: Mahmoud Vahidi, and Hossein Mozayyeni; Software: Hossein Mozayyeni; Validation: Hossein Mozayyeni and Mojgan Mohajeri Iravani; Formal analysis: Hossein Mozayyeni and Mahdi Ghorbani; Investigation: Mahmoud Vahidi, Mojgan Mohajeri Iravani, and Amir Emamie; Resources: Amir Emamie; Data curation: Mojgan Mohajeri Iravani

and Mahdi Ghorbani; Writing: All authors; Visualization: Mahmoud Vahidi; Project administration: Mahdi Ghorbani; Funding acquisition: Amir Emamie.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank Saeideh Sohrabi for her assistance in collecting samples.

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