

Dovgalyuk A.I., Redko O.S., Petrenko N.V., Lavrenchuk H.Y.¹, Kyryk V.M.², Korda M.M.
 I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine,
 Ternopil, Ukraine; ¹State Institution "National Research Center for Radiation Medicine of
 National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine;
²State Institution "National Scientific Center "The M.D. Strazhesko Institute of Cardiology,
 Clinical and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine",
 Kyiv, Ukraine

OPTIMIZATION OF ISOLATION, LOW-SERUM CULTURING, AND IMMUNOPHENOTYPING OF HUMAN UMBILICAL CORD MESENCHYMAL STEM CELLS

e-mail: dovgaluk@tdmu.edu.ua

Mesenchymal stem cells from the human umbilical cord are a promising resource for cell therapy due to low immunogenicity and non-invasive harvesting. This study optimized an isolation protocol using collagenase I and evaluated cell properties during expansion. Umbilical cord explants were digested with 0.1 % collagenase I and cultured in Advanced DMEM/F12 with 2–10 % fetal bovine serum for 10 passages. Immunophenotypic analysis was performed via flow cytometry. Primary cells exhibited typical adherent fibroblast-like morphology. Low-serum maintenance (2 % fetal bovine serum) supported robust expansion without spontaneous differentiation. Flow cytometry confirmed high expression of positive markers (CD73, CD90, CD105) and complete absence of negative markers (CD34, CD45, HLA-DR). This protocol allows efficient isolation and expansion of human umbilical cord mesenchymal stem cells while maintaining their canonical immunophenotype under low-serum conditions, minimizing foreign protein exposure for future clinical applications.

Key words: mesenchymal stem cells, umbilical cord, cell culture, immunophenotyping, low-serum media, flow cytometry.

Довгальук А.І., Редько О.С., Петренко Н.В., Лавренчук Г.Й., Кирик В.М., Корда М.М.

ОПТИМІЗАЦІЯ ПРОТОКОЛУ ВИДІЛЕННЯ, КУЛЬТИВУВАННЯ В НИЗЬКОСИРОВАТКОВОМУ СЕРЕДОВИЩІ ТА ІМУНОФЕНОТИПУВАННЯ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН ПУПОВИНИ ЛЮДИНИ

Мезенхімальні стовбурові клітини пуповини людини є перспективним ресурсом для клітинної терапії завдяки низькій імуногенності та неінвазивному отриманню. Це дослідження оптимізувало протокол виділення за допомогою колагенази I та оцінило властивості клітин під час їх розмноження. Експлантати пуповини піддавали ферментативному розщепленню 0,1 % розчином колагенази I та культивували у середовищі Advanced DMEM/F12 з додаванням 2–10 % ембріональної сироватки телят протягом 10 пасажів. Імунофенотиповий аналіз проводили методом проточної цитометрії. Первинні клітини демонстрували типову адгезивну фібробластоподібну морфологію. Підтримання життєдіяльності в умовах низького вмісту сироватки (2 % ембріональної сироватки телят) забезпечувало стабільне розмноження клітин без спонтанного диференціювання. Проточна цитометрія підтвердила високу експресію позитивних маркерів (CD73, CD90, CD105) і повну відсутність негативних маркерів (CD34, CD45, HLA-DR). Цей протокол дозволяє ефективно виділяти та розмножувати мезенхімальні стовбурові клітини пуповини людини із збереженням їхнього канонічного імунофенотипу в умовах низького вмісту сироватки, що мінімізує вплив чужорідних білків для майбутнього застосування у клініці.

Ключові слова: мезенхімальні стовбурові клітини, пуповина, культура клітин, імунофенотипування, низькосироваткове середовище, проточна цитометрія.

The study is a fragment of the research projects "Investigation of the regenerative potential of cellular therapy agents in acute respiratory distress syndrome", state registration No. 0121U100159, and "The use of regenerative medicine technologies for the chronic wounds treatment", state registration No. 0124U001099.

Mesenchymal stem cells (MSCs) are currently widely used in regenerative medicine as promising agents for the renewal of damaged tissues due to their chemotactic, paracrine, and immunomodulatory properties [9, 15]. MSCs can be isolated from various sources, including bone marrow, adipose tissue, and perinatal derivatives such as the umbilical cord [6; 21]. Human umbilical cord-derived MSCs (hUC-MSCs) present a superior alternative to adult sources as they are harvested non-invasively, exhibit low immunogenicity, and do not show tumorigenicity [6, 11, 22].

HUC-MSCs secrete a wide range of bioactive molecules (growth factors, cytokines, micro RNAs, defensins, etc) that exert anti-inflammatory, anti-apoptotic, antioxidant angiogenic, and antimicrobial effects [3, 4, 8, 20]. Various protocols have been developed for the isolation and cultivation of hUC-

MSCs, differing in the segment of the cord used (Wharton's jelly, perivascular region, or whole cord), enzymatic digestion methods, culture media composition, and passaging techniques [2, 5, 9, 19]. These variations significantly influence the initial cell yield, proliferation rate, and biological characteristics of the resulting MSCs [3, 9, 19]. A primary translational goal is to minimize animal-derived components, such as fetal bovine serum (FBS), to reduce the risk of clinical immune reactions and xeno-contaminants [13].

The purpose of the study was to optimize a low-serum culture protocol using Advanced DMEM/F12 and characterize the morphology, adhesion, and canonical surface marker expression of hUC-MSCs during expansion.

Materials and methods. The studies were conducted at the Cell Culture Division of the

Interdepartmental Educational and Research Laboratory of I. Horbachevsky Ternopil National Medical University during 2021–2025. Immunophenotyping by flow cytometry was performed at the Cell and Tissue Technologies Department of the State Institution “National Scientific Center “The M.D. Strazhesko Institute of Cardiology, Clinical and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine” in 2024. Biological material was obtained exclusively accompanied by the written informed consent of the parturient women. Prior to the study, ethical approval from the Bioethics Commission of I. Horbachevsky Ternopil National Medical University was obtained, as indicated in protocol No. 60, dated September 1, 2020.

The umbilical cords were obtained under aseptic conditions from female newborns with informed consent of their mothers after a full-term pregnancy that proceeded without complications and ended with a cesarean section. For transporting the biological material to the laboratory, the umbilical cord was placed in a transport medium containing 200 mg of ciprofloxacin, 10,000 units of penicillin, 10,000 µg of streptomycin, and 25 µg of Gibco Amphotericin B, diluted in 100 ml of PBS and immediately delivered to the lab at 4 °C [7].

For MSCs isolation, only 5–7 cm of the umbilical cord from the placental side was used. Under sterile conditions, the umbilical cord was cut into small fragments measuring 0.5–2 mm³, transferred into centrifuge tubes, and subjected to enzymatic digestion using 0.1 % collagenase I (Sigma-Aldrich, USA), diluted in 2 ml of DMEM/F12 Advanced culture medium (Gibco, USA), avoiding flotation.

The enzymatic digestion was carried out for 1 hour at 37 °C in a water bath, with thorough mixing of the tube contents every 15 minutes. Afterwards the resulting suspension was pipetted and centrifuged for 5 minutes at 1610×g. The pellet which included partially digested tissue pieces (explants) was resuspended in 7 ml of Advanced DMEM/F12 enriched with 10 % fetal bovine serum (FBS) (Gibco, USA), seeded into culture flasks with a surface area of 25 cm² containing Advanced DMEM/F12 medium supplemented with 10 % FBS, 1 % L-Glutamine-Penicillin-Streptomycin solution (Sigma, USA) and 240 µg/L Heparin solution (Sigma, USA) and kept inside incubators at 37 °C and 5 % CO₂.

The resulting primary culture was assigned passage zero (P0). The first visual assessment of cell morphology and population density was performed using an inverted microscope (Delta Optical NIB-100) 48 hours after seeding to allow cells to spread out and attach themselves to the bottom of the flasks.

To remove dead or damaged cells and unattached blood elements, the culture medium was completely replaced 72 hours after the cultivation start, maintaining the FBS concentration at 10 %.

Further analysis of the primary hUC-MSC culture was conducted 5 days after seeding. At this stage of cultivation, cell population density

(confluence) was approximately 50 %. At this point, the growth medium was changed again, reducing the FBS concentration to 2 % and preserving the previous concentrations of L-Glutamine-Penicillin-Streptomycin Heparin supplements. Following this, every 3 days a complete medium change or replacement of ½ or ¼ volume of medium was performed while maintaining the FBS concentration.

Upon reaching 80 % confluence, passaging was performed by trypsinization using TrypLE Express Enzyme (Gibco, USA). The flask bottoms were rinsed with HBSS solution, TrypLE enzyme was added, and the flasks were placed in a CO₂ incubator for 5 minutes to detach the cells from the culture plastic surface. The enzyme was neutralized using conditioned medium. The collected cell suspension was centrifuged for 8 minutes at 530×g, after which the cells were seeded into new flasks at a density of 50,000 cells per 1 ml of medium, and a new passage was assigned. Cell counting in the suspension was performed using a hemocytometer after staining with the vital dye trypan blue.

Cryopreservation of the cells was performed at various passages. 1 ml of a cell suspension with a concentration of 2–4 million cells per ml was added into 2 ml cryovials, followed by the addition of 1 ml of the prepared freezing medium (30 % DMEM/F12 Advanced, 40 % FBS, 20 % conditioned medium, and 10 % dimethyl sulfoxide (Sigma, USA)). After filling, the cryovials were immediately subjected to gradual cooling at a rate of 1 °C per minute using a ZPM-1 programmable mobile freezer (Kharkiv, Ukraine). Samples were then stored in liquid nitrogen at -196 °C.

After thawing, MSCs were cultured for 24 hours in a growth medium consisting of Advanced DMEM/F12 supplemented with 10 % FBS, 1 % L-glutamine–penicillin–streptomycin solution, and 240 µg/L heparin solution. Afterward, the concentration of FBS was reduced to 2 %.

For the analysis of MSC-specific cell surface marker expression cells cryopreserved at passage 5 and subsequently thawed were used. Immunophenotyping was performed by flow cytometry using mouse anti-human monoclonal antibodies CD90 FITC, CD73 PE, CD105 PerCP-Cy5.5, CD45 FITC, CD34 PE, and HLA-DR APC-Cy7 (all from BD Biosciences, USA). To compensate for spectral overlap of fluorochromes in multicolor analysis, control samples were used: unstained, single-stained, and fluorescence minus one (FMO) controls. A total of 1×10⁶ thawed and washed cells were resuspended in 100 µL of DPBS supplemented with 1 % FBS, incubated with antibodies for 30 minutes at +4 °C in the dark, then washed with 1 mL of CellWash buffer (BD Biosciences, USA) by centrifugation at 300×g for 5 minutes, and finally resuspended in 350 µL of DPBS for analysis. Immediately before acquisition, the cell suspension was filtered through a 70 µm cell strainer to remove aggregates. Measurements were performed on a BD FACS Aria cell sorter using BD FACSDiva software v.6.1.3. At least 30,000 events were recorded per sample for analysis.

Results of the study. Microscopic analysis 48 hours post-seeding revealed cell migration out of tissue explants with typical adherent, fibroblast-like morphology (Figure 1A). A shift in the medium's pH indicator (phenol red) towards

orange/acidic was noted due to cellular activity.

Cells expanded consistently across 10 passages while retaining their characteristic morphology without signs of spontaneous differentiation (Figure 1 B–F).

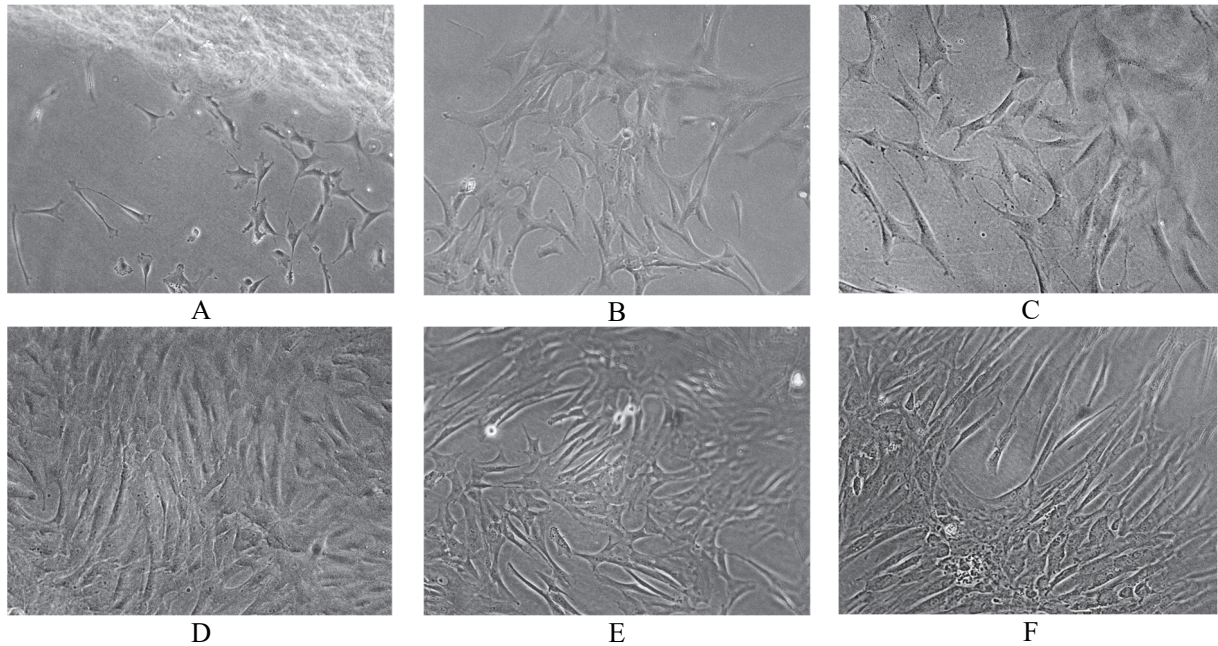


Fig. 1. A–F. Morphological characteristics of hUC-MSCs at 100× magnification.

Flow cytometric analysis demonstrated that hUC-MSCs expressed high levels of positive mesenchymal markers: CD73 (99.1 %), CD90 (99.5 %), and CD105 (99.3 %). Conversely, hematopoietic and immune markers CD34, CD45,

and HLA-DR were negative (<0.5 %) (Fig. 2). This immunophenotypic profile is consistent with the characteristics of mesenchymal stem cells as defined by the International Society for Cellular Therapy.

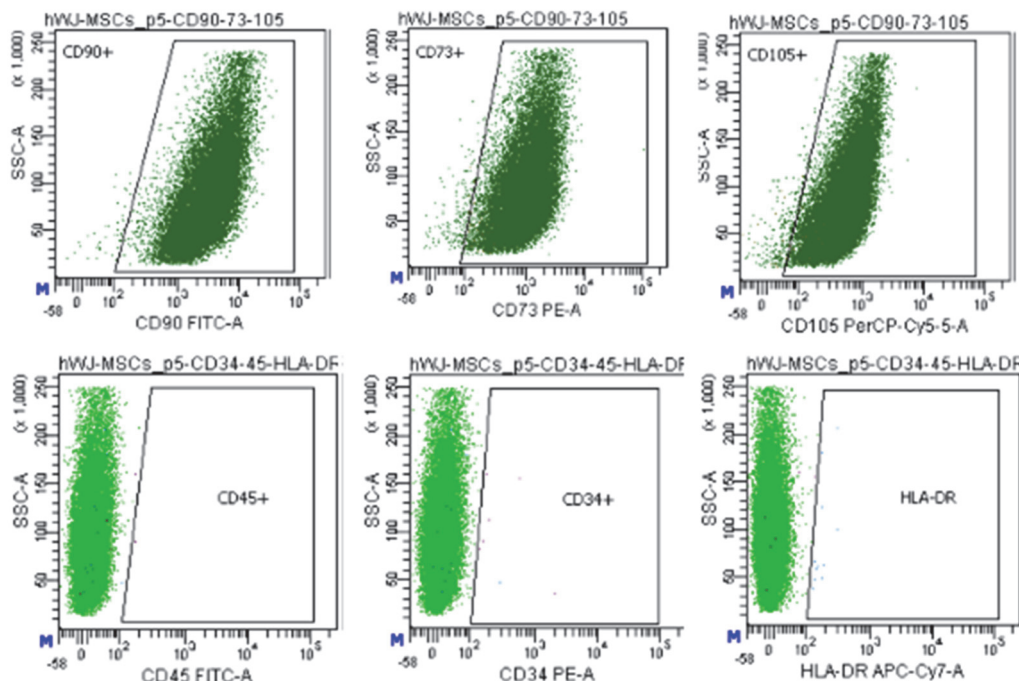


Fig. 2. Dot-plot histograms showing the expression of CD90, CD73, CD105, CD45, CD34, and HLA-DR markers on human umbilical cord-derived MSCs.

(A) hUC-MSCs migrating out from an umbilical cord tissue explant during initial culture establishment (P0). (B–F) hUC-MSCs at successive passages (P1, 2, 5, 7 and 10 respectively), displaying typical fibroblast-like morphology consistent with mesenchymal lineage. Cells

remained adherent and morphologically stable across passages, with no signs of spontaneous differentiation or abnormal growth patterns.

Discussion. Our transport medium formulation builds upon standard antibiotic-antimycotic protocols

described by De Francesco et al. [7], but is further optimized with Ciprofloxacin to prevent hospital-acquired bacterial contamination during delivery.

Numerous studies have shown that human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) offer distinct advantages over alternative cell sources. Specifically, they exhibit higher proliferation rates and greater expansion potential compared to bone marrow MSCs. Consequently, hUC-MSCs represent an ideal candidate for cell therapy, bypassing the limitations associated with bone marrow harvesting and the ethical constraints of embryonic stem cells [8, 16, 21].

The choice of culture medium is critical for efficient hUC-MSC processing. Alpha-MEM (α -MEM) is a more basic formulation containing nonessential amino acids, nucleosides, and vitamins, but lacking growth factors. To sustain MSC proliferation, laboratories routinely supplement α -MEM with 10–20 % fetal bovine serum (FBS). Because α -MEM lacks built-in nutrient supplements to substitute for serum, high FBS levels are mandatory to support cell attachment and growth during explant seeding [19].

In contrast, commercial kits such as StemPro™ MSC SFM and MesenCult™-ACF provide defined, serum-free formulations optimized for high-density expansion. While these media can rival or exceed the proliferation rates of serum-based cultures, recent evidence suggests that completely serum-free environments can induce subtle phenotypic shifts. For instance, Kang et al. (2024) demonstrated that MSCs expanded in StemPro™ and MesenCult™ differed in morphology, surface-marker profiles, and differentiation capacity compared to standard FBS cultures [12]. Therefore, while serum-free media successfully eliminate animal-derived components – a major goal in translational medicine – researchers must rigorously verify that key therapeutic properties are retained [19].

Advanced DMEM/F12 strikes an effective balance by enriching the basal formulation with extra nutrients and antioxidants, allowing MSCs to maintain rapid growth with significantly reduced serum concentrations. Although FBS is a potent mitogen source supplying essential hormones, attachment factors (fibronectin/vitronectin), and growth factors (PDGF, FGF, TGF- β), high serum levels also introduce differentiation signals (e.g., insulin, BMPs) that can compromise stemness [13]. Consequently, serum concentration acts as a double-edged sword: higher levels accelerate growth but elevate the risk of spontaneous lineage commitment [13].

The primary advantage of Advanced DMEM/F12 is its ability to decouple proliferation from excessive serum dependency. Enhanced nutrient

buffering allows hUC-MSCs to thrive at just 2 % FBS, minimizing serum-driven differentiation signals, reducing costs, and mitigating batch-to-batch variability. Our protocol capitalizes on this by utilizing 10 % FBS during the initial days to maximize cell yield, followed by a transition to 2 % FBS in Advanced DMEM/F12 to maintain the cells in an undifferentiated state. This approach complies with regulatory guidelines that increasingly favor low-serum expansion systems to minimize xeno-contaminants and clinical immune reactions [12].

To confirm the identity of the expanded cells, we evaluated them against the minimal criteria proposed by the Mesenchymal Stromal Cell Committee of the International Society for Cellular Therapy [16]: plastic adherence, specific surface antigen expression, and multipotent differentiation potential.

Our immunophenotypic analysis confirmed strong expression of the canonical markers CD73, CD90, and CD105. CD73 (5'-nucleotidase) modulates purinergic signaling by converting extracellular AMP to adenosine, mediating anti-inflammatory and immunomodulatory effects that are vital for treating inflammatory diseases [10]. CD90 (Thy-1) is involved in cell adhesion, migration, and interaction with the extracellular matrix, serving as a key indicator of retained self-renewal capacity [18]. CD105 (endoglin) functions within the TGF- β receptor complex, linking it to both immunomodulation and pro-angiogenic properties beneficial for wound healing and ischemic conditions [17].

Crucially, the cells completely lacked the hematopoietic markers CD34 and CD45. The absence of CD34 excludes contamination with hematopoietic stem and endothelial progenitor cells [14], while the lack of CD45 confirms the absence of leukocytes, monocytes, and granulocytes [1]. Ensuring a CD45-negative profile is essential for clinical safety, as it verifies that the cell product will not trigger adverse immune responses or graft-versus-host disease upon administration [22].

Ultimately, the primary challenge in MSC expansion for therapeutic applications is establishing culture conditions that balance rapid growth with phenotypic retention while minimizing xeno-derived serum. Conventional protocols use 10–20 % FBS, which increases the risk of viral transmission, high batch-to-batch variability, and undesirable immune responses in recipients. The established protocol using Advanced DMEM/F12 addresses these challenges by offering a reproducible, low-serum system that ensures robust cell expansion while firmly preserving the canonical MSC immunophenotype and therapeutic potential

Conclusion

Enzymatic disintegration with 0.1 % collagenase I combined with a low-serum Advanced DMEM/F12 culture system (2 % FBS) provides an efficient, reproducible method for isolating and expanding human umbilical cord-derived mesenchymal stem cells.

The isolated cells exhibit a classic fibroblast-like morphology, maintain robust plastic adherence, and support stable expansion across 10 passages without spontaneous lineage differentiation.

Flow cytometry confirms high expression of positive markers (CD73, CD90, CD105 >99.1 %) and the complete absence of hematopoietic and immunological markers (CD34, CD45, HLA-DR), fully satisfying the International Society for Cellular Therapy minimal characterization criteria.

Thus, the established low-serum protocol successfully preserves the canonical immunophenotype and multipotent potential of human umbilical cord mesenchymal stem cells while minimizing foreign protein exposure, making them highly valuable and safe candidates for future clinical applications in regenerative medicine.

References

1. Al Barashdi MA, Ali A, McMullin MF, Mills K. Protein tyrosine phosphatase receptor type C (PTPRC or CD45). *Journal of Clinical Pathology*. 2021;74(9):548-552. doi: 10.1136/jclinpath-2020-206927
2. Abouelnaga H, El-Khateeb D, Moemen Y, El-Fert A, Elgazzar M, Khalil A. Characterization of mesenchymal stem cells isolated from Wharton's jelly of the human umbilical cord. *Egyptian Liver Journal*. 2022;12:1-9. doi: 10.1186/s43066-022-00216-x
3. Cai C, Li H, Tian Z, Liang Q, Shen R, Wu Z, et al. HGF secreted by hUC-MSCs mitigates neuronal apoptosis to repair the injured spinal cord via phosphorylation of Akt/FoxO3a pathway. *Biochemical and Biophysical Research Communications*. 2024;692:149321. doi: 10.1016/j.bbrc.2023.149321
4. Castro Ramos A, Widjaja Lomanto MY, Vuong CK, Ohneda O, Fukushige M. Antibacterial effects of human mesenchymal stem cells and their derivatives: A systematic review. *Frontiers in Microbiology*. 2024;15:1430650. doi: 10.3389/fmicb.2024.1430650
5. Celikkan FT, Mungan C, Sucu M, Ulus AT, Cinar O, Ili EG, et al. Optimizing the transport and storage conditions of current Good Manufacturing Practice grade human umbilical cord mesenchymal stromal cells for transplantation. *Cytherapy*. 2019;21(1):64-75. doi: 10.1016/j.jcyt.2018.10.007
6. Choudhery MS. Perinatal tissue-derived stem cells: An emerging source for regenerative medicine. *Cells*. 2021;10(12):3278. doi: 10.3390/cells10123278
7. De Francesco F, Sagnella N, Riccio V, Riccio M. Procurement, Processing, and Storage of Human Amniotic Membrane: A Comprehensive Review. *Diagnostics*. 2025;15(3):144-159. doi: 10.3390/diagnostics15030144
8. Dovgalyuk A, Levkiv M, Dudkevych O, Pokryshko O. Antimicrobial properties of mesenchymal stem cells: prospects for application against antibiotic-resistant infections. *Cell Organ Transpl*. 2025; 13(2):e2025132185. doi: 10.22494/cot.v13-2.185
9. Fu Q, Han M, Dai X, Lu R, Deng E, Shen X, et al. Therapeutic effect of three-dimensional hanging drop cultured human umbilical cord mesenchymal stem cells on osteoarthritis in rabbits. *Stem Cell Research & Therapy*. 2024;15(1):311. doi: 10.1186/s13287-024-03905-y
10. Galgaro BC, Beckenkamp LR, Nunnenkamp M, Korb VG, Naasani LI, Roszek K, et al. The adenosinergic pathway in mesenchymal stem cell fate and functions. *Medicinal Research Reviews*. 2021;41(4):2316-2349. doi: 10.1002/med.21796
11. He J, Yao X, Mo P, Wang K, Yang ZL, Tian NN, et al. Lack of tumorigenesis and protumorigenic activity of human umbilical cord mesenchymal stem cells in NOD SCID mice. *BMC Cancer*. 2022;22(1):307. doi: 10.1186/s12885-022-09431-5
12. Kang M, Yang Y, Zhang H, Zhang Y, Wu Y, Denslin V, et al. Comparative Analysis of Serum and Serum-Free Medium Cultured Mesenchymal Stromal Cells for Cartilage Repair. *International Journal of Molecular Sciences*. 2024;25(19):10627. doi: 10.3390/ijms251910627
13. Pilgrim CR, McCahill KA, Rops JG, Dufour JM, Russell KA, Koch TG. A review of fetal bovine serum in the culture of mesenchymal stromal cells and potential alternatives for veterinary medicine. *Frontiers in Veterinary Science*. 2022;9:859025. doi: 10.3389/fvets.2022.859025
14. Radu P, Zurzu M, Paic V, Bratucu M, Garofil D, Tigora A, et al. CD34 Structure, functions and relationship with cancer stem cells. *Medicina*. 2023;59(5):938. doi: 10.3390/medicina59050938
15. Redko O, Dovgalyuk A, Kramar S, Ohinska N, Nebesna Z, Korda M. Mesenchymal stem cell therapy modulates macrophage dynamics in ARDS-associated liver injury in rats. *Cell Organ Transpl*. 2023; 11(2):114-121. Available from: <https://doi.org/10.22494/cot.v11i2.157>
16. Renesme L, Pierro M, Cobey KD, Mital R, Nangle K, Shorr R, et al. Definition and characteristics of mesenchymal stromal cells in preclinical and clinical studies: a scoping review. *Stem Cells Translational Medicine*. 2022;11(1):44-54. doi: 10.1093/stcltm/szab011
17. Rossi E, Bernabeu C. Novel vascular roles of human endoglin in pathophysiology. *Journal of Thrombosis and Haemostasis*. 2023;21(9):2327-2338. doi: 10.1016/j.jth.2023.05.023
18. Saalbach A, Anderegg U. Thy-1: more than a marker for mesenchymal stromal cells. *The FASEB Journal*. 2019;33(6):6689-6696. doi: 10.1096/fj.201802134R
19. Todtenhaupt P, Franken LA, Groene SG, van Hoolwerff M, van der Meeren LE, van Klink JM, et al. A robust and standardized method to isolate and expand mesenchymal stromal cells from human umbilical cord. *Cytherapy*. 2023;25(10):1057-1068. doi: 10.1016/j.jcyt.2023.06.004
20. Trigo CM, Rodrigues JS, Camões SP, Solà S, Miranda JP. Mesenchymal stem cell secretome for regenerative medicine: Where do we stand? *Journal of Advanced Research*. 2024;65:120-135. doi: 10.1016/j.jare.2023.12.019
21. Xie Q, Liu R, Jiang J, Peng J, Yang C, Zhang W, et al. What is the impact of human umbilical cord mesenchymal stem cell transplantation on clinical treatment? *Stem Cell Research & Therapy*. 2020;11:1-13. doi: 10.1186/s13287-020-02011-x
22. Zhao Q, Zhang L, Wei Y, Yu H, Zou L, Huo J, et al. Systematic comparison of hUC-MSCs at various passages reveals the variations of signatures and therapeutic effect on acute graft-versus-host disease. *Stem Cell Research & Therapy*. 2019;10:1-14. doi: 10.1186/s13287-019-1478-4

Conflict of interest. The authors have no conflicts of interest to declare.

ORCID: Dovgalyuk A.I. <https://orcid.org/0000-0003-3976-0245>, Redko O.S. <https://orcid.org/0000-0002-9860-9003>, Petrenko N.V. <https://orcid.org/0000-0003-1780-6951>, Lavrenchuk H.Y. <https://orcid.org/0000-0001-7200-712X>, Kyryk V.M. <https://orcid.org/0000-0001-7636-1302>, Korda M.M. <https://orcid.org/0000-0003-0676-336X>.

Article received: 11.07.2025