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Problematic issues in the isolation of adipose-derived mesenchymal stromal cells: new methodological aspects (pilot research)

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Isolation of mesenchymal stromal cells (MSCs) from adipose tissue is a relevant topic in regenerative medicine and cell therapy. MSCs have significant potential for treating various diseases due to their ability to differentiate, self-renew, and secrete biologically active molecules. However, the isolation process faces challenges like variations in tissue collection methods, quality, sample transportation, laboratory sterility, and incubation stability. Insufficient coverage of these issues in scientific publications complicates their resolution, particularly under the conditions in Ukraine.

The aim is to identify and discuss the key problems associated with the isolation of adipose-derived mesenchymal stromal cells, as well as the experimental search for potential solutions to overcome these obstacles for the optimization of the isolation methodology.

Materials and methods. A literature review was conducted using PubMed and Google Scholar, selecting key publications on isolation methods, MSCs characteristics, and good manufacturing practice (GMP) principles. Inclusion criteria: full-text articles on adipose MSCs isolation, comparison of enzymatic methods of isolation, GMP standardization, and cell characteristics. Following this, a pilot study was conducted using lipoaspirate and fragments of subcutaneous adipose tissue (SAT) with the patients' consent. Processing in Zaporizhzhia State Medical and Pharmaceutical University's GMP-compliant lab: mechanical grinding (for SAT), DPBS washing with antibiotics, enzymatic digestion (collagenase or trypsin) at 37 °C, 600 g centrifugation, filtration, DMEM / FBS cultivation. Viability was assessed in Goryaev chamber with trypan blue; passaging at 60–70 % confluency.

Results. Abdominal lipoaspirate yielded larger MSCs volumes with higher proliferation than thigh or excised SAT. Collagenase is considered the "gold standard" in terms of efficiency, but trypsin has been shown to be a cost-effective alternative with similar viability, adhesion, and differentiation (chondro-, osteo-, adipogenic). Cultivation with medium changes supported growth; passaging prevented senescence.

Conclusions. Optimization of the methodology for isolating mesenchymal stromal cells of adipogenic origin under GMP-compliant conditions can be achieved by using lipoaspirate from the abdominal area, using trypsinization as an effective and cost-effective alternative to collagenase for enzymatic isolation, and strictly adhering to cultivation, passage, and quality control protocols.

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Проблемні питання виділення мезенхімальних стромальних клітин жирового походження: нові методологічні аспекти (пілотне дослідження)

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Виділення мезенхімальних стромальних клітин (МСК) із жирової тканини є актуальною темою в регенеративній медицині та клітинній терапії. МСК мають значний потенціал для лікування різних захворювань завдяки здатності до диференціації, самооновлення та секреції біологічно активних молекул. Однак процес виділення супроводжується численними викликами, такими як варіації в методах збору тканини, її якості, транспортуванні зразків, стерильності лабораторії та стабільності умов інкубації. Недостатнє висвітлення цих проблем у наукових публікаціях ускладнює їх вирішення, особливо в умовах України.

Мета роботи – виявлення та обговорення ключових проблем, пов'язаних із виділенням мезенхімальних стромальних клітин із жирової тканини, а також експериментальний пошук потенційних рішень для подолання цих перешкод для оптимізації методології виділення.

Матеріали і методи. Здійснили комплексний огляд наукової літератури, що індексується в базах PubMed і Google Scholar. Обирали ключові публікації щодо методів виділення, характеристик МСК і принципів належної виробничої практики (GMP). До аналізу залучали повнотекстові статті щодо виділення МСК із жирової тканини, порівняння ензиматичних методів, стандартизації протоколів (GMP) і характеристики клітин. Після цього здійснили пілотне дослідження з використанням ліпоаспірату та фрагментів підшкірної

жирової тканини за згодою пацієнтів. Обробка, яку здійснили в лабораторії Запорізького державного медико-фармацевтичного університету, що відповідає вимогам GMP: механічне подрібнення, промивання в DPBS з антибіотиками, ензиматична дигестія (колагеназа або трипсин) при 37 °C, центрифугування (600 g), фільтрація, культивування в DMEM із FBS. Життєздатність оцінювали в камері Горяєва з трипановим синім; пасажування при 60–70 % конфлюентності.

Результати. Ліпоаспірат з абдомінальних ділянок давав більший об'єм МСК із вищою проліферацією, ніж зі стегон чи ексцизованих фрагментів. Колагеназа – «золотий стандарт» за ефективністю, але трипсин визначено як економічну альтернативу з подібною життєздатністю, адгезією та диференціацією (хондро-, остео-, адипогенна). Культивування з заміною середовища забезпечувало ріст, а пасажування запобігало сенесценції.

Висновки. Оптимізація методології виділення мезенхімальних стромальних клітин адипогенного походження в умовах, що відповідають вимогам GMP, може бути досягнута шляхом використання ліпоаспірату з черевної порожнини, застосування трипсинізації як ефективної та економічно вигідної альтернативи колагеназі для ферментативного виділення, а також чіткого дотримання протоколів культивування, пасажування та контролю якості.

Сучасні медичні технології. 2026. Т. 18, № 2(69). С. 112-121

The isolation of mesenchymal stromal cells (MSCs) has become a relevant topic in the field of regenerative medicine and cell therapy. MSCs possess significant potential for the treatment of various diseases due to their ability to differentiate, self-renew, and secrete biologically active molecules [1].

MSCs are defined by their ability to self-renewal and differentiation into various cell lineages [2]. In this regard, MSCs have attracted significant attention due to their ability to differentiate into various cell types, including osteoblasts, chondrocytes, myocytes, and adipocytes [3]. These cells can be isolated from various tissues, such as bone marrow, adipose tissue, synovial membrane, and perinatal tissues, each of which demonstrates unique regenerative properties [4]. To date, abdominal fat is considered a reliable source of MSCs due to the technical and methodological ease of identifying them, high cell concentration and homogeneity, as well as high differentiation potential and secretory activity [5]. However, the process of their isolation is accompanied by numerous challenges that may affect the quantity, quality, and subsequent viability of the obtained cells.

The main problems encountered by researchers include variations in methods of adipose tissue collection, its quality and quantity, as well as external influences on cell culture (sample transportation, laboratory sterility, stability of incubation conditions and reagent storage) [6]. Furthermore, the insufficient coverage of specific difficulties associated with MSCs isolation in scientific communities and publications complicates the resolution of these issues, particularly under the conditions in which Ukraine finds itself.

In this article, we will examine the main methodological challenges in isolating adipose-derived MSCs, as well as possible solutions for overcoming them. The analysis of these issues and the proposed approaches may contribute to the improvement of the methodology, which, in turn, will impact on the efficiency of further research and clinical applications of MSCs.

Aim

The aim of the research is to identify and discuss the key problems associated with the isolation of adipose-derived mesenchymal stromal cells, as well as the experimental search for potential solutions to overcome these obstacles for the optimization of the isolation methodology.

Materials and methods

In this work, a comprehensive literature review was conducted regarding the current state of knowledge on the isolation of adipose-derived MSCs, and these protocols and recommendations were compared with our own experience. The analysis was based on searches of databases PubMed and Google Scholar. Keywords used: mesenchymal stem/stromal cells, lipoaspirate, subcutaneous adipose tissue, abdominal fat, protocol, incubation. The search period was 5 years; however, key historical publications (2006, 2011, 2014, 2016, and 2017) were also included in the final analysis, due to fundamental importance for understanding the methods of isolation and characterization of adipose-derived MSCs and the principles of good manufacturing practice (GMP). Inclusion criteria: full-text articles and protocols focused on the isolation of MSCs from adipose tissue; comparison of enzymatic and non-enzymatic methods of their isolation; study of protocol standardization (GMP) and cell characteristics (phenotype, proliferation, viability). Exclusion criteria: documents that were focused on the isolation of MSCs from sources other than adipose tissue (for example, bone marrow or placenta); did not contain original data regarding isolation protocols or GMP; duplicates or conference abstracts.

All found publications initially underwent screening by title and abstract to assess their compliance with the above-described criteria, after which a full text review was conducted for final inclusion. To ensure objectivity and comparability of data, results from different articles were summarized, with particular attention paid to quantitative indicators. The results of our own research published in the article were approved by the local bioethics commission (Protocol No. 15 dated December 10, 2025).

Pilot experiment. For MSCs isolation, lipoaspirate or a fragment of subcutaneous adipose tissue (SAT) of abdominal region and lateral surface of the thigh were obtained during surgeries in healthy patients who provided written informed consent prior to the surgical intervention for the use of biomaterial for experimental purposes. Groups for the pilot study were formed with 5 individuals each, aged from 23 to 55 years, with a body mass index (BMI)

Fig. 1. Laboratory of "Cell Cultures and Bioengineering of Zaporizhzhia State Medical and Pharmaceutical University" (original picture).

corresponding to the first degree of obesity Each patient sample was divided into two equal parts for collagenase- and trypsin-based isolation, with three replicates performed for each method (n = 15 in each group).

After the surgery, the lipoaspirate or SAT fragment was delivered within 1 hour for *in vitro* research to the specially equipped laboratory "Cell Cultures and Bioengineering of Zaporizhzhia State Medical and Pharmaceutical University", which is a structural subdivision of the Center for Collective Use Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University under conditions of compliance with high standards of equipment quality, asepsis conditions, sterility, and biological safety *(Fig. 1)*.

Statistical analysis was performed using one-way analysis of variance (ANOVA) for normally distributed data or the Kruskal–Wallis test for non-normal distributions. Normality was verified using the Shapiro–Wilk test. All parameters were compared using one-way ANOVA, followed by Tukey's post-hoc test (for ANOVA) or Dunn's test (for the Kruskal–Wallis test) for multiple comparisons where significance was detected. A two-tailed p-value of <0.05 was considered statistically significant for all tests. All calculations were performed using Statistica software (License No. JPZ804I382130ARCN10-J).

Results

The primary challenge for all cell culture laboratories worldwide is adequate technical equipment and adherence to ISO 14644-1 cleanliness standards [7]. Any laboratory must comply with the requirements of GMP, which encompasses validation and calibration of equipment, clear standard operating procedures, personnel control (requirements for attire (special gowns, caps, masks, shoe covers) and staff training to ensure aseptic technique), and environmental monitoring (regular sampling of air, surfaces, and water to control for the presence of microorganisms and mycoplasmas) [8].

These standards serve to ensure that the cells used in research or for clinical applications are safe, sterile, and unaltered. Therefore, the Zaporizhzhia State Medical and Pharmaceutical University laboratory is equipped with all necessary licensed equipment, including a biological safety laminar cabinet HR40-IIB2 (Haier Biomedical, China); CO_2 incubator HCP-80 (Haier Biomedical, China); inverted microscope ZEISS Primovert with the capability to switch from phase contrast to fluorescent mode (Zeiss, Germany); water bath thermostat WB-4MS (Biosan, Latvia); benchtop centrifuge with interchangeable rotors of various volumes (LMC Biosan, Latvia); magnetic stirrer MS-3000 (Biosan, Latvia); pH meter LAQUA-PH2000-SR (Horiba, Japan); pharmaceutical refrigerator HYC-390/F (Haier Biomedical, China); ultra-low temperature freezer. All equipment is certified and undergoes annual metrological control.

Sterility and biosafety conditions are maintained through trained personnel, adherence to restricted laboratory access, use of special clothing, stability of temperature conditions and filtration of incoming air to the premises (presence of a ventilation system, temperature relay, and controlled temperature and airflow), maintenance of sterile conditions (treatment of all surfaces with 70 % ethyl alcohol, constant UV sterilization, autoclaving), and proper disposal of consumable materials and biological fluids [9].

The collection of lipoaspirate / fragment of subcutaneous adipose tissue is a crucial stage for obtaining MSCs and requires adherence to sterility and technical standards. The proper collection methodology ensures the quality of the obtained material and increases the likelihood of effective cell seeding and survival [10]. However, some researchers have expressed concerns regarding the reliability and consistency of methods for collecting lipoaspirate or SAT fragments. Variations in liposuction techniques, adipose tissue quality, and external influences on cell culture may affect the quantity and quality of the obtained MSCs [11]. Without standardized protocols and stringent quality control measures, the lipoaspirate collection process may not always yield high-quality cells suitable for clinical application [12].

Table 1. Results of MSCs isolation from different sites using different enzymes, Me [Q1; Q3]

Parameter, units of measurement	Abdominal lipoaspirate, n = 5		Abdominal fragment, n = 5		Thigh fragment, n = 5	
Patient's age, years	27 [25; 42]		27 [27; 51]		27 [25; 52]	
Patient's BMI, kg/m^2	31 [31; 32]		32 [32; 33]		31 [31; 32]	
Indicators	**Abdominal lipoaspirate, n = 15**		**Abdominal fragment, n = 15**		**Thigh fragment, n = 15**	
	Collagenase	**Trypsin**	**Collagenase**	**Trypsin**	**Collagenase**	**Trypsin**
Total cell count, cells in 5 squares	156 [153; 160]	155 [152; 159]	101 [98; 105]*	100 [97; 104]*	65 [62; 69]*$	65 [62; 68]*$
Live cells, count	148 [145; 152]	146 [143; 150]	91 [88; 94]*	90 [87; 93]*	55 [52; 57]*$	54 [51; 57]*$
Dead cells, count	8 [8; 8]	8 [8; 9]	9 [9; 10]*	10 [9; 10]*	10 [10; 12]*$	11 [11; 12]*$
Cell concentration, ×10^6 cells/mL	0.624 [0.610; 0.638]	0.620 [0.606; 0.636]	0.404 [0.390; 0.418]*	0.400 [0.386; 0.414]*	0.260 [0.246; 0.274]*$	0.260 [0.246; 0.270]*$
Cell viability, %	94.9 [94.8; 95.0]	94.7 [94.5 94.8]	90.7 [90.5; 91.0]*	90.2 [89.7; 90.8]#*	83.9 [83.5; 84.5]*$	82.9 [82.6; 83.4]*$

#: statistically significant difference between trypsin-based and collagenase-based isolation; ***:** statistically significant difference in indicators of lipoaspirate compared to the abdominal SAT fragment; **$:** statistically significant difference in indicators of thigh SAT fragment compared to the abdominal SAT fragment.

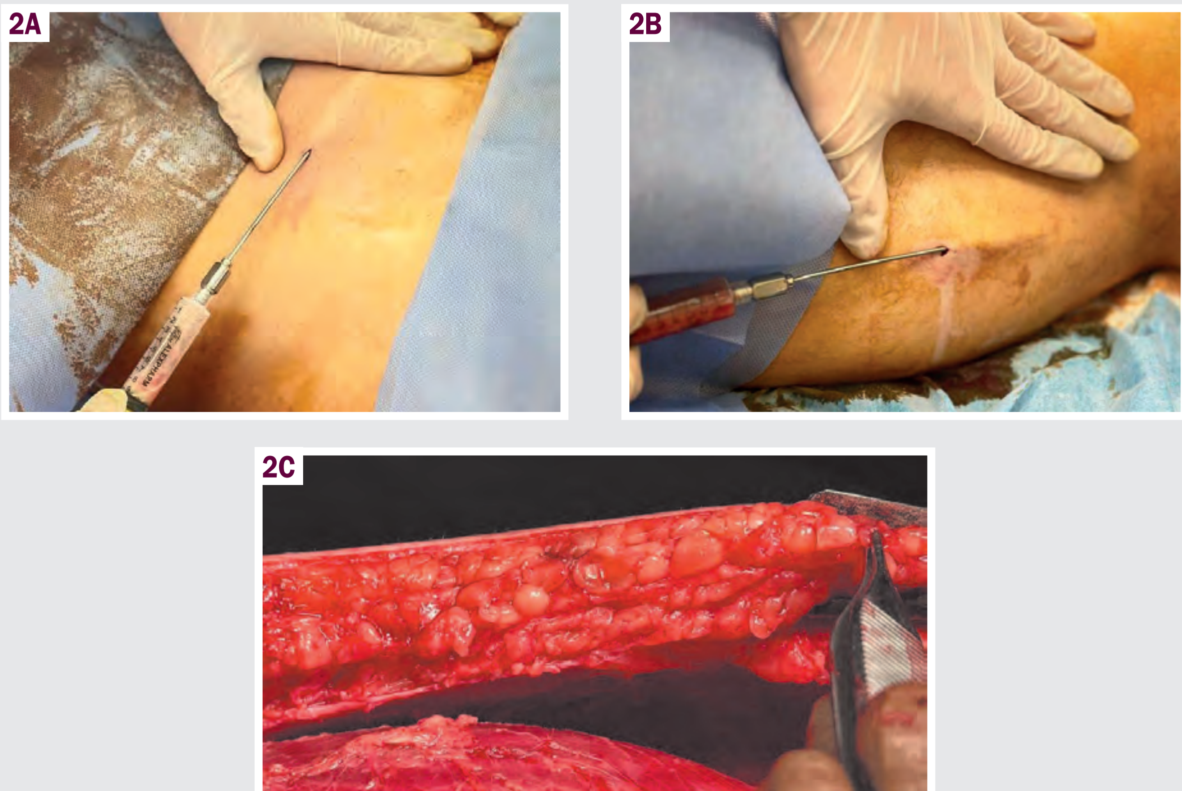

Fig. 2. Collection of lipoaspirate from various anatomical sites of SAT (original pictures). **A:** sites on the anterior abdominal wall; **B:** sites on the lateral surface of the thigh; **C:** collection of a fragment of adipose tissue from the surgical wound site.

Therefore, based on the literature data [5,6,11,12,13,14,16] and our own experience, various anatomical sites of SAT and various methods of obtaining MSCs (from lipoaspirate or SAT fragments) were selected *(Fig. 2, Table 1).*

The advantages and disadvantages of each method are determined by its invasiveness, quantitative / qualitative cell composition, viability, and purity of the final product.

Lipoaspiration is considered a less invasive method than surgical excision and allows for obtaining a significantly larger volume of lipoaspirate, which means a greater number of MSCs; however, one of the drawbacks is the mechanical damage to cells caused by high negative pressure during aspiration and turbulence, which reduces their viability [13]. Cells obtained from a fragment of subcutaneous adipose tissue by excision experi-

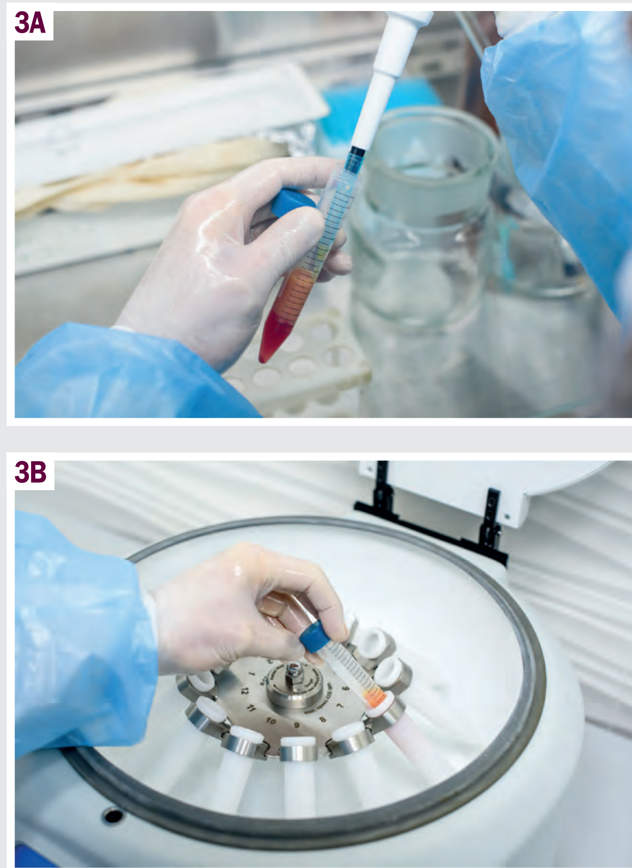

Fig. 3. Washing (**A**) and centrifugation (**B**) of the sample (original pictures).

ence minimal mechanical stress. Scientific sources highlight a higher yield of viable MSCs per unit weight of tissue compared to lipoaspirate, and these cells are also characterized by a better proliferative potential [14].

However, the main drawback is the limited volume of tissue that can be obtained and, consequently, an insufficient amount of cell mass for scaling up the research as observed in our study. Therefore, we subsequently focused on articles concerning lipoaspiration. Zhu R. et al. in their study demonstrated that MSCs isolated from abdominal fat exhibited higher proliferative capacity and better cell growth parameters compared to cells isolated from other depots [15]. As noted above, the quality of cellular material is influenced not only by the method of adipose tissue harvesting, but also by the anatomical site, which has been established to hold significant importance. Thigh adipose tissue, particularly in women, differs in its hormonal profile and is less metabolically active. This may impair its suitability for certain regenerative purposes; moreover, from a practical standpoint, the abdomen is usually easier to access and allows for obtaining a larger volume of lipoaspirate with less discomfort to the donor, especially in the context of cosmetic surgery [16].

The main stages of the protocol for isolating MSCs of adipogenic origin, common to all the studied sources [5,6,11,12,13,14, 15,16,17,18,19,20,21,22], are material harvesting, mechanical washing, enzymatic isolation, and cell culturing. We present the main provisions of these protocols that were used by us in the study.

The obtained fragment is mechanically minced using a sterile scalpel under conditions of a biological safety cabinet, followed by removal of blood and other components to isolate adipose tissue. Subsequently, washing is performed in buffer (DPBS, Dulbecco's phosphate-buffered saline, without Ca^{2+} and Mg^{2+} ions; Sigma Aldrich, USA) with the addition of 200 µL of antibiotic-antimycotic solution (×100: 10,000 U penicillin, 10 mg streptomycin, 25 µg amphotericin B in 1 mL; Sigma Aldrich, USA) until the sediment wash waters are clear and until the supernatant exhibits a "pearly sheen" *(Fig. 3A)*.

In case of lipoaspirate, the procedure is the same, except without mechanical mincing, which significantly simplifies the process and positively affects cell viability. After visual confirmation of obtaining the desired color of the material, centrifugation is initiated in DPBS buffer (volume ratio of buffer to lipoaspirate 1:1) at 600 g for 5 min, which helps to separate the adipose cells from liquids and other components *(Fig. 3B)*.

The next stage is enzymatic isolation, which remains controversial and involves various enzymatic treatment options, including trypsin or collagenase. Thus, experimental studies have shown that cells isolated by the trypsinization method retain the classical immunoprofile, are capable of pluripotent differentiation, and demonstrate higher proliferative activity compared to cells obtained using collagenase [17]. The use of type I collagenase, although widespread, has a number of limitations: high cost, variable duration of enzymatic digestion,

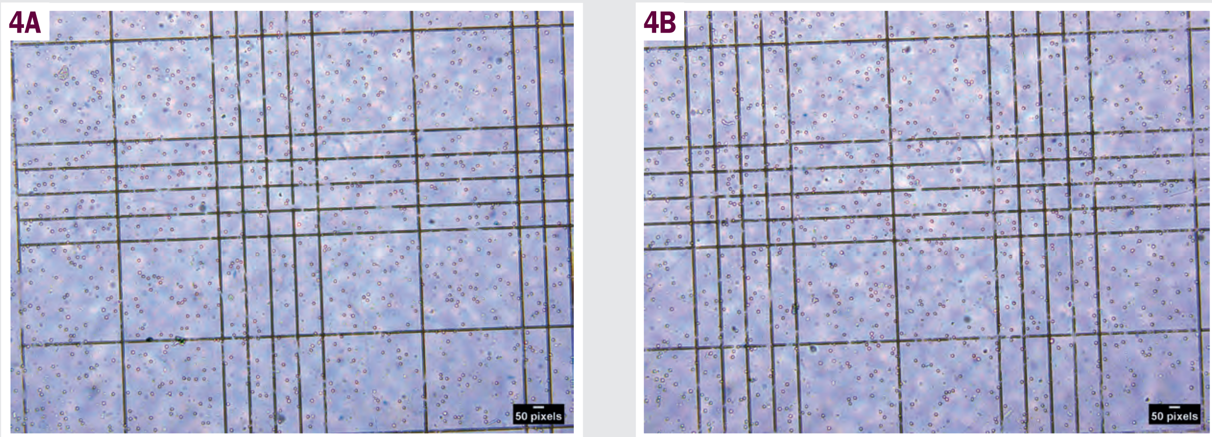

Fig. 4. Cell counting in the Goryaev chamber, magn. ×10. **A:** cells isolated with trypsin; **B:** – cells isolated with collagenase.

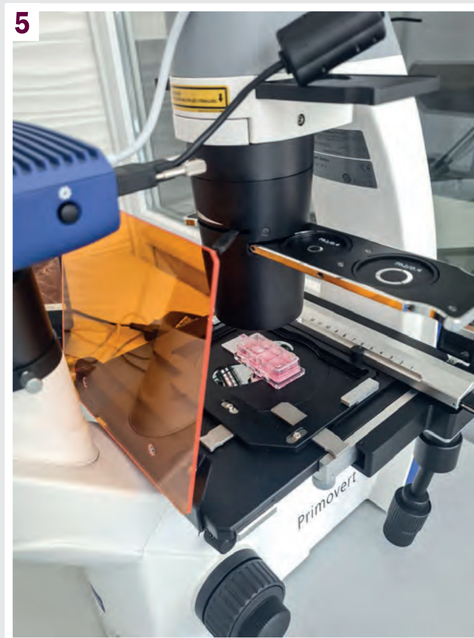

Fig. 5. Visual assessment of culture condition on PrimoVert (original picture).

potential damage to cellular structures and functions, which complicates the reproducibility of results and their clinical application [15]. An additional practical obstacle is the fact that collagenase is primarily supplied by manufacturers in powder form; therefore, diluting it to very low concentrations require the preparation of a stock solution followed by the application of the serial dilution method (aliquots). This appears impractical due to the need to store the enzyme solutions at -20 °C, necessitating thawing of the solutions each time they are used and is further complicated by the fact that the collagenase solution loses its enzymatic activity over time [23].

We performed two methods of enzymatic isolation to determine the more suitable approach for further study *(Table 1)*. According to the standard procedure, cell washing is conducted, during which the supernatant after centrifugation is aspirated, and the pellet is resuspended in a solution of type I collagenase (SCR103, Sigma Aldrich, USA) or a 0.25 % trypsin solution containing 2.5 g of porcine trypsin and 0.2 g of EDTA-4Na per liter of Hank's Balanced Salt Solution (HBSS; Sigma Aldrich, USA) in a 1:1 ratio with the volume of the lipoaspirate. Incubation is performed in a water bath at a temperature of 37.0 °C with regular gentle shaking of the tube every 10 minutes for 40 minutes. The preparation of working solutions of trypsin / collagenase is crucial, involving the dilution of a 0.25 % trypsin solution in DPBS at a ratio of 1:99 with the addition of a 10 % antibiotic-antimycotic solution, and the same method for collagenase (0.5–2.0 mg/mL HBSS).

After incubation, the enzyme was inactivated by adding an equal volume of complete medium (CM), and the suspension was centrifuged at 600 g for 10 min. Subsequently, the fat fraction was removed, and the liquid portion was resuspended and passed through Biofill cell filters (China) with pore diameters of 100 μm and 70 μm to remove microfragments of adipose tissue and erythrocyte conglomerates. The resulting pure cell

suspension was centrifuged (600 g × 10 min). The liquid phase was removed using a dispenser with a sterile tip, and the pellet was resuspended in 1 mL of complete nutrient medium CM, which consisted of 1 part FBS (fetal bovine serum, Sigma Aldrich, USA), 9 parts DMEM (Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/L glucose, sodium bicarbonate, L-glutamine, sodium pyruvate, Sigma Aldrich, USA), and 0.1 part antibiotic-antimycotic solution.

Cell viability assessment and counting were performed using a Goryaev chamber and 0.4 % trypan blue (Sigma Aldrich, USA), based on a volume ratio of the dye to the cell suspension of 1:1 *(Table 1).* Cells stained blue were considered non-viable *(Fig. 4)*.

The "5 squares' method" was used with the calculation of cell count (C) and viability (V) according to the formulas:

$C = (N \times 10000 \times D) / 5$ (1),

where (N) – the sum of counted cells in 5 squares; (D) – dilution factor with trypan blue (2); 10,000 – the volumetric coefficient of the Goryaev chamber.

$V = (\text{Live cells} / (\text{Live cells} + \text{Dead cells})) \times 100\ \%$. (2)

The results of observations on MSCs cultures isolated using different enzymes revealed no significant differences between their studied parameters in our study *(Table 1)*.

Trypsin-isolated cells showed good viability and adhesive capacity, which enables the formation of a sufficient cell population for its optimal growth in a culture flask; however, cells isolated with collagenase showed the same parameters. In both cases, the MSCs property for chondro-, osteo-, and adipogenic differentiation was preserved. In most studies collagenase is the "gold standard" for dissociation of adipose tissue, due to the similarity of key characteristics, it exhibits higher efficiency and better osteogenic potential (which is important for our further research) compared to trypsin isolation [18]. Nevertheless, the obtained results demonstrate that trypsin is an effective and more economical alternative method.

The next stage of our isolation experience was the seeding of MSCs into culture flasks with an area of 25 cm^2 to 1 mL of the cell suspension, such an amount of CM was added that approximately $1–2 \times 10^6$ cells were present in 5 mL of it. Exactly this number of cells in a volume of 5 mL of CM was seeded into a sterile flask, placed in a CO_2 incubator, and cultured under standard conditions (5 % CO_2, 95 % humidity, and 37 °C). After 24 hours, the culture medium was replaced with fresh medium to remove non-adherent elements, with subsequent replacement twice a week. Visual monitoring of the culture status was performed using an inverted microscope PrimoVert ("Carl Zeiss", Germany) with an AxioCam 208 color camera ("Carl Zeiss", Germany) *(Fig. 5).* The described stage is fully regulated by all the studied protocols [5,6,11–22].

Although the described methodology for MSCs isolation is widely used, it is not without limitations [24]. The stages of mechanical and enzymatic processing may potentially damage or alter cell properties, leading to an inconsistent quantitative and qualitative composition of the isolated population, and moreover, it does not guarantee a "pure line" without the risk of contamination [25]. Additionally, the use of fetal bovine serum in the culture medium raises concerns regarding the introduction of xenogeneic components, which may elicit an immune response or infection risks in clinical applications [26]. Further research is needed to develop more standardized protocols that can reliably isolate high-quality therapeutic-grade MSCs [27].

Special attention should be paid to the mandatory periodic replacement of the medium in cell cultures, which is crucial for maintaining cell viability and functionality. Regular medium replacement provides essential nutrients and removes metabolic waste, promoting successful proliferation of the cell culture [28]. It is important to adhere to sterile conditions and to monitor the stability of reagent storage, as well as their expiration dates [29]. However, excessive or untimely replacement of the medium may also harm cell cultures [30]. Frequent medium changes can disrupt cell attachment, induce stress, and lead to undesirable differentiation or loss of stem cell properties [31]. Furthermore, over-reliance on monitoring reagent expiration dates may overlook other important factors that can affect the quality of the cell culture, such as batch-to-batch variability or the impact of long-term storage conditions [32]. A balanced approach that considers the individual needs of the cell line is essential for maintaining optimal cell viability and functionality [19].

A significant stage in the MSCs isolation is the control of cells quality. After medium replacement, cells are regularly checked for signs of stress, or contamination. Proper documentation is important, which involves recording the date and time of medium replacement, volumes of the used medium, and dates of package opening. An equally important aspect is monitoring the progress of cell growth and proliferation *(Fig. 6)*.

The process of maintaining the cell population in the MSCs culture involves their passage (reseeding cells into new culture flasks), which was performed upon reaching 60–70 % confluence of the monolayer. This is an important stage in working with cell cultures that helps maintain cell viability. Before starting the passaging, it is necessary to check the readiness of the cells, which involves examining the flask with the culture under a microscope for morphological changes, signs of contamination, or cell death *(Fig. 6)*.

It should be noted that passaging is critical for maintaining the viability of cell cultures. Proper execution of this procedure ensures optimal conditions for their growth, which in turn contributes to obtaining reliable results in research [20]. Although passaging is an important stage for maintaining cell viability, the methodology is not without drawbacks [21]. Repeated passages and subculturing may lead to genetic and epigenetic changes in cell cultures, potentially altering their properties [22]. Furthermore, excessive passaging may cause senescence and reduce the scalability of the population, which will affect their therapeutic potential [33].

Based on the above, in our study, the passaging procedure was performed as follows: the nutrient medium was drained from the flask, the cells were washed twice with warm (37 °C) DPBS containing a 10 % antibiotic-antimycotic solution, and then 1.5 mL of trypsin solution with EDTA (37 °C) was added to the flask. The solution was distributed evenly across the flask with careful circular motions, and after 1.0–1.5 min, the degree of cell rounding was assessed under an inverted microscope as an indicator of their disaggregation from the flask bottom. When the majority of cells detached (but no more than 1.5–2.0 min after the addition of trypsin), the enzyme was neutralized with an equivalent volume of CM (37 °C). The resulting cell suspension was centrifuged (600 g

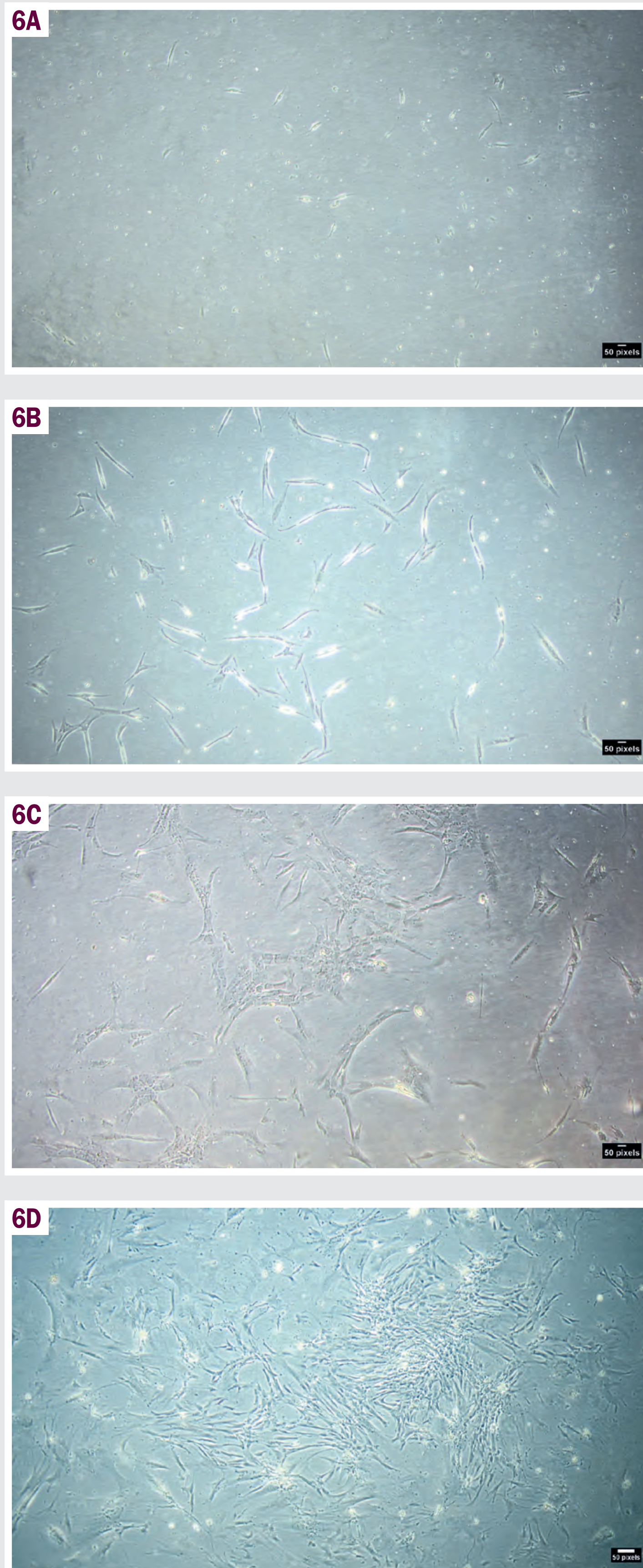

Fig. 6. MSCs passage 1 on different days. **A:** 3 days, magn. ×10; **B:** 5 days, magn. ×10; **C:** 9 days, magn. ×10; **D:** 13 days, magn. ×4.

for 10 min). Cell counting was performed in a Goryaev chamber, with simultaneous assessment of their viability using trypan blue. Following this, the liquid phase was aspirated and replaced with such a volume of CM that 5 mL contained 1–2 × 10^6 cells. Cell seeding was carried out onto adhesive microscope slides Millicell EZ Slide 8-well glass (Sigma Aldrich, USA) at a density of 20,000 cells per well *(Fig. 5)*.

Discussion

The aim of this study was to identify key methodological challenges in the process of isolating adipose-derived MSCs and to optimize the isolation protocol for our laboratory. The relevance of the work is underscored by the growing potential of MSCs in regenerative medicine, particularly considering the advantages of abdominal fat as a cell source that is easily obtainable, has a high concentration of MSCs, and possesses a potent differentiation potential. Our results confirm that successful isolation and cultivation of MSCs critically depend on adherence to high quality standards regulated by GMP, which includes laboratory technical equipment, equipment validation, and strict aseptic control [8]. These measures are essential for minimizing contamination risks and maintaining cell viability, which is one of the primary challenges in MSC culture.

The choice of biomaterial source (lipoaspirate versus a fragment of SAT) and anatomical site is a key factor affecting the yield and quality of MSCs [13,14,16]. Literature data and our experience indicate the advantages of lipoaspirate as a less invasive method for the cell population, allowing for the extraction of a larger amount of cell mass, despite the potential for mechanical damage, in contrast to surgical excision of a SAT fragment, which limits the amount of material for scaling [13,14,16].

The enzymatic isolation step is the second critical stage of the protocol. Although collagenase type I is widely used due to its high efficiency in adipose tissue dissociation and better osteogenic potential, we also investigated trypsinization as an alternative approach. Our observations showed that trypsin-isolated cells exhibited viability, high adhesive capacity, and pluripotent differentiation potential (chondro-, osteo- and adipogenic differentiation) compared to those isolated with collagenase. Given the high cost, instability during storage and difficulties in preparing working solutions of collagenase, trypsin appears to be an effective, more economical and practical alternative, that simplifies protocol standardization, particularly under conditions of limited funding or logistical challenges [17,15,23]. We have confirmed that standard cultivation conditions, regular medium changes, and the use of an antibiotic-antimycotic are effective for maintaining cell culture. The passaging step is critically important to prevent cell aging and loss of their properties [20].

Despite the detailed protocol for isolating MSCs from adipose tissue, commercially available ready-made MSC cultures represent an alternative approach. The advantages of this approach are obvious: time savings, guaranteed high initial viability and standardized quality. However, commercial cultures are financially expensive and limit the researcher's control over the origin of the cells and the number of passages performed, which introduces additional variability [34].

A detailed analysis of the scientific literature showed that the established standard protocols for working in cell culture laboratories with MSCs vary, the stages of implementation depend on the direction of researchers' activities, equipment, type of MSCs used (bone marrow, adipose tissue, umbilical cord blood), and the purpose of cultivation (for differentiation, expansion, or cryopreservation).

Conclusions

Optimization of the methodology for isolating mesenchymal stromal cells of adipogenic origin under GMP-compliant conditions can be achieved by using lipoaspirate from the abdominal area, using trypsinization as an effective and cost-effective alternative to collagenase for enzymatic isolation, and strictly adhering to cultivation, passage, and quality control protocols.

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