SURGERY

APPLICATION OF MEMBRANE-PROTECTANT IN THE PROCESS OF INCREASING THE VIABILITY OF AUTOLOGOUS FAT GRAFT

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RESUME

Background. *Autologous adipose tissue transplantation is becoming increasingly popular in reconstructive surgery, but the main unsolved problem at the moment is the high percentage of partial volume loss due to autograft resorption.*
The aim. *Evaluation of the viability of adipocytes under incubation in solutions of different biochemical compositions, and clinical testing of the effectiveness of an optimized fat graft.*
Materials and methods. *The comparative spectral analysis of the content of ions (mainly oxygen) in the cytoplasm of fat cells grown from solution samples on a solid substrate using a scanning electron microscope in low vacuum was performed. The composition in 3 samples that spent 6 h in artificial solutions was investigated. The EDAX TEAM program was used to analyze the energy dispersive X-ray spectroscopy data.*
Results. *Statistical and morphological analysis of the obtained results revealed differences in the composition of viable cells in the studied samples, varying up to 50 %. The most effective was the solution with dimethyloxobutylphosphonyldimethylate, which demonstrated an optimal level of oxygen ion content (O), as well as pronounced integrity of the cell membrane compared to other samples during electron microscopy and histological examination.*
Conclusion. *One of the key factors is the medication support of the autograft during the initial stages of engraftment after transplantation. By measuring the ionic content of the intracellular matrix, we were able to examine in vitro the effect of solutions of different substances to achieve this goal. For autograft preservation, the best option is a solution with a membrane protector dimethyloxobutylphosphonyldimethylate for its ability to preserve cell homeostasis.*

Keywords: *reconstructive surgery, ionic composition, electron microscopy, hypoxia, lipofilling*

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ПРИМЕНЕНИЕ МЕМБРАНОПРОТЕКТОРОВ В ПРОЦЕССЕ ПОВЫШЕНИЯ ЖИЗНЕСПОСОБНОСТИ АУТОЛОГИЧНОГО ЖИРОВОГО ТРАНСПЛАНТАТА

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РЕЗЮМЕ

Обоснование. *Трансплантация аутологичной жировой ткани становится все более популярна в реконструктивной хирургии, однако основной нерешенной проблемой на текущий момент является высокий процент частичной потери объема из-за резорбции аутотрансплантата.*

Цель исследования. *Оценка жизнеспособности адипоцитов в условиях инкубации в растворах различного биохимического состава и клиническая апробация эффективности оптимизированного жирового трансплантата.*

Методы. *Производился сравнительный спектральный анализ содержания ионов (преимущественно кислорода) в цитоплазме жировых клеток, высаженных из образцов растворов на твердую подложку при помощи растрового электронного микроскопа в низком вакууме. Исследовался состав в 3 образцах, которые провели 6 часов в искусственных растворах. Для анализа данных энергодисперсионной рентгеновской спектроскопии использовалась программа EDAX TEAM.*

Результаты. *Статистический и морфологический анализ полученных результатов выявил различия в составе жизнеспособных клеток в исследуемых образцах, варьирующиеся в пределах до 50 %. Наиболее эффективным оказался раствор с диметилоксобутилфосфонилдиметилатом, продемонстрировавший оптимальный уровень содержания ионов кислорода (O), а также выраженную целостность клеточной мембраны по сравнению с другими образцами в ходе проведения электронной микроскопии, а также гистологического исследования.*

Заключение. *Одним из ключевых факторов является медикаментозная поддержка жировой ткани на начальных этапах приживления после трансплантации. Путем измерения содержания ионов во внутриклеточном матриксе мы смогли рассмотреть в лабораторных условиях влияние растворов различных веществ для достижения данной цели. Для сохранения аутотрансплантата лучшим вариантом является раствор с мембранопротектором диметилоксобутилфосфонилдиметилата по способности сохранения гомеостаза клетки.*

Ключевые слова: *реконструктивная хирургия, ионный состав, электронная микроскопия, гипоксия, липофилинг*

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BACKGROUND

Autologous fat grafting is becoming increasingly popular, with statistics indicating a 10 % annual rise in the number of lipofilling procedures in Russia [1]. In addition to aesthetic corrections, this tissue is increasingly being used in various reconstructive techniques, particularly as an alternative to synthetic implants or volume-enhancing agents (e.g., those based on hyaluronic acid) [2, 3, 4], or in combination with implants for enhanced results [5].

Special attention is given to the use of autologous fat grafts for closing deep soft tissue defects. This method is preferred for chronic non-healing wounds with a bone bed, offering advantages such as ease of application and good aesthetic results following subsequent dermoplasty [6].

Consequently, we can observe the increasing popularity of adipose tissue utilization. Unfortunately, as with any method, lipofilling has its own drawbacks. The key complications of lipofilling include ischemia, hypoxia, and necrosis of the transplanted fat tissue. These conditions develop due to insufficient neoangiogenesis and limited diffusion of oxygen and nutrients to the graft. Impaired microcirculation leads to a cascade of ischemic changes, manifested by progressive cellular hypoxia and adipocyte death. In clinical practice, this results in partial graft resorption, the formation of cystic cavities, and foci of fat necrosis, which significantly reduces the predictability and stability of the outcome. Resorption of fat tissue after transplantation can sometimes reach 80 % [7]. Currently, research is being conducted to increase cell viability by incorporating autologous mesenchymal stem cells or treating the lipoaspirate with radiofrequency helium plasma [8, 9].

Therefore, this study explored solutions that improve graft cell survival. Establishing an optimal microenvironment is essential to enhance the antioxidant effect, accelerate engraftment, shorten revascularization time, and improve cell membrane stability through cytoprotective properties.

This requires an understanding of the nutritional mechanisms of fat tissue after transplantation, including the sources of substances and the pathways for their delivery to cellular structures.

Initially, it was believed that the transplanted tissue rapidly revascularizes through the formation of vascular anastomoses between the capillaries of the fat graft and the vascular network of the recipient site [10]. That is, survival was determined by their revascularization and the provision of adequate blood supply from the recipient site's vascular network. It has been shown that only the superficial layer of the lipograft, approximately 300 μm thick, is accessible to microcirculation from the recipient site's capillaries, with subsequent blood flow restoration through neoangiogenesis. In this regard, small fat conglomerates show better survival than large volumetric areas of transplanted tissue introduced into the recipient site as a single mass without uniform distribution [11]. According to genetic studies, free fat grafts undergo metabolic reprogramming towards the glycolytic pathway [12]. The shift from aerobic energy metabolism to glycolysis allows cells to survive under oxygen-deficient conditions. As a result of glycolytic glucose utilization, 2 ATP molecules are formed, instead of 36 as in the aerobic pathway. With insufficient blood supply, this leads to rapid depletion of the cells' own energy stores. Also, under oxygen deficiency, the conversion of lactate to pyruvate is interrupted, leading to lactate accumulation and the subsequent development of local tissue acidosis [13].

Optimizing the graft microenvironment through the use of specialized solutions that ensure cellular homeostasis and metabolic activity (antioxidants (glutathione, mannitol), membrane protectors (dimethyloxobutylphosphonyldimethylate) [14], energy metabolism substrates (adenosine and others) is a key approach to preventing or compensatorily correcting these pathological mechanisms.

THE AIM OF THE STUDY

A comprehensive assessment of adipocyte viability under incubation in solutions of various biochemical compositions and clinical testing of the effectiveness of an optimized fat graft in soft tissue defect reconstruction.

MATERIALS AND METHODS

Within the framework of this study, a quantitative analysis of the intracellular ionic composition, particularly the concentration of oxygen and other elements, in adipose tissue cell cytoplasm exposed to various storage media was conducted. Adipose tissue was obtained from 3 patients (female, aged 30–35 years, without chronic diseases) undergoing elective upper blepharoplasty for aesthetic purposes and used as biological material. All samples were collected under sterile conditions and not subjected to any additional processing. Upon collection, the material was divided into three equal-volume and equal-mass portions (Samples 1–3), each exposed to a different storage medium for analysis.

Characteristics of experimental groups:

Sample 1 (control): Stored in a standard injection solution without any additional ionic supplements. This sample was used as a control medium with minimal ionic exposure.

Sample 2: Incubated in 0.9 % sodium chloride solution (saline), which is the most commonly used medium in clinical practice.

Sample 3: Placed in a 15 % dimethyloxobutylphosphonyldimethylate solution, which has potential antioxidant and membrane-stabilizing properties.

All samples were stored at a temperature of +22 ± 1 °C for a strictly fixed period of 6 hours.

After the completion of the specified period, each specimen was analyzed using low-vacuum scanning electron microscopy (LVSEM). For quantitative analysis of the ionic composition, energy-dispersive X-ray spectrometry (EDX) analysis was employed, utilizing EDAX TEAM™ software. Through the quantitative elemental analysis conducted by energy-dispersive X-ray spectroscopy (EDX), using the eZAF algorithm (ZAF-correction), verification was carried out for the presence of specific peaks in the spectral graph, which may indicate an increase in the concentration of additional ions that were not included in the scope of this study.

Quantitative data are presented as the mean (M) ± the standard error of the mean (SEM), as well as the median (Me) and the interquartile range (Q1–Q3). The normality of distribution was tested using the Shapiro – Wilk test. For comparisons, the Student's *t*-test or Mann – Whitney U-test was used (depending on the distribution). If more than two groups were analyzed, analysis of variance (ANOVA) with Tukey's post hoc test was applied. Differences were considered statistically significant at $p < 0.05$.

Measurements were performed on representative areas of the cytoplasm with a diameter of 25 micrometers. Repeated scans ($n = 3$ per sample) were used to determine the mean values of key ions, including oxygen, sodium, potassium, and chlorine. The obtained data were compared to identify changes in ionic profiles depending on the storage medium composition.

A total of 103 foreign and domestic literature sources indexed in RSCI, PubMed, Scopus, and SSCI were analyzed for this study. Of these, 22 sources were specifically used for the article, with 18 published within the past 5 years.

Prior to the study, informed consent was obtained from the patient regarding the use of their data in the form of photographs and publication of the clinical case. The patient was informed about possible risks and complications associated with the study, and a clinical study was conducted.

RESULTS AND DISCUSSION

The primary aim of the study was to assess the impact of the chemical composition of the medium on the maintenance of the intracellular ionic composition, which can serve as an indicator of cellular viability in transplanted adipose tissue and may change significantly during their storage, processing, and transplantation [15, 16]. Oxygen levels can serve as a diagnostic marker when assessing cell viability [17, 18], which can be explored using electron microscopy in conjunction with spectral analysis of adipocytes.

In each specimen, visually intact cells were selected (Fig. 1):

Cell surface contours: The cell has a regular round or oval shape, the contour is clear, without ruptures, folds, or deformation.

Cell membrane: Smooth or with a uniform, fine-grained structure, without any defects such as cracks, pores, or areas of damage.

Plasmolysis/damage artifacts: no signs of wrinkled and collapsed cells, or membrane "blurring" or fragmentation, are observed.

Surface uniformity: intact cells exhibit a homogeneous surface.

In the photo, the following features can be observed:

1. Substrate fibers (cellulose);
2. Connective tissue remnants;
3. Solution droplets;
4. Adipocyte.

Visual analysis by electron microscopy was used as a method to assess morphology [19, 20]. The results showed that Sample 3, which had been incubated for 6 hours in a solution containing dimethyloxobutylphosphonyldimethylate, demonstrated the best preservation of cellular structure (Fig. 1C). Scanning electron microscopy examination revealed uniformity and integrity of cell surface contours, as well as the absence of significant signs of plasmolysis and membrane damage, which may indicate a membrane-stabilizing effect of the solution component.

In contrast, Samples 1 and 2, which were stored in an injection solution and 0.9 % sodium chloride solution (saline), respectively, showed focal changes in the cell membrane morphology, with localized areas of deformation and irregularities in the perimeter. These changes may indicate a decrease in the structural integrity of cell membranes under conditions without the specific membrane protective properties presumably possessed by dimethyloxobutylphosphonyldimethylate.

Subsequently, the study conducted a quantitative analysis of ion content (Fig. 2) and a spectral analysis (Table) of the samples. The obtained data allowed for a comparison of the effects of different solutions on the level of adipocyte ionic homeostasis and the identification of a preferred medium for short-term storage of adipose tissue for transplantation purposes.

Analysis of the spectral composition of the cellular environment revealed a significant increase in the oxygen ion concentration in Samples 2 and 3 compared to Sample 1 (control). This increase in O concentration, according to EDX data, may reflect increased metabolic activity or improved tissue respiration conditions as a result of exposure to the components of the studied solutions, particularly physiological saline (NaCl) and dimethyloxobutylphosphonyldimethylate. However, simultaneously, there was an increase in the sodium ion concentration, especially in the NaCl solution. This finding requires particular attention, as sodium is the main extracellular cation and its intracellular accumulation may serve as a marker of impaired ionic homeostasis. An excessive Na^+ influx into the cell can lead to an osmotic imbalance, destabilization of membrane potential, and activation of pathways associated with cellular dysfunction and death [21, 22, 23]. This emphasizes the importance of choosing a medium that

ensures both membrane stability and a physiologically balanced ionic environment for preserving the viability of transplanted cells.

During the study, while searching for ion "peaks" that are not included in the studied structure and may potentially affect viability, we only observed the presence of phosphorus ions in the sample containing dimethyloxobutylphosphonyldimethylate. This occurrence is due to the specific composition of the preparation.

In the context of a clinical study (approved by the Local Ethics Committee of the Russian Biotechnological University, Moscow, Volokolamskoye Shosse, 11, Protocol No. 9/4-6 dated April 28, 2025), we performed an additional autologous adipose tissue graft transplantation to verify the laboratory results (Fig. 3).

A soft tissue defect measuring 5 × 3 cm, with exposed muscle fibers and moderate signs of inflammation is present. There is peripheral edema and skin hyperemia, as well as the presence of fibrin. The general blood parameters are within the range of a moderate inflammatory response.

Treatment course: After surgical debridement of the wound, a fat graft was harvested from the anterior abdominal wall region, following the standard lipoaspiration technique using an infiltration solution (0.9 % NaCl with lidocaine and epinephrine). The extracted lipoaspirate was divided into two equal portions: one portion was washed three times with standard isotonic solution and the other with a solution containing 15 % dimethyloxobutylphosphonyldimethylate.

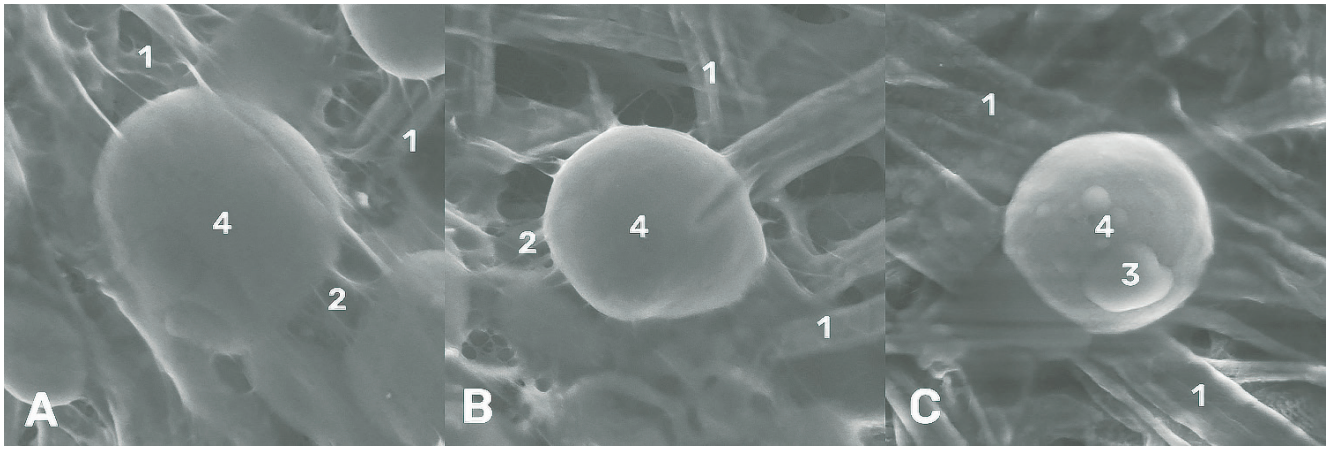


FIG. 1.
Study of adipocytes by electron microscopy (A – solution for injection, B – 0.9 % NaCl, C – 15 % Dimethyloxobutylphosphonyldimethylate)

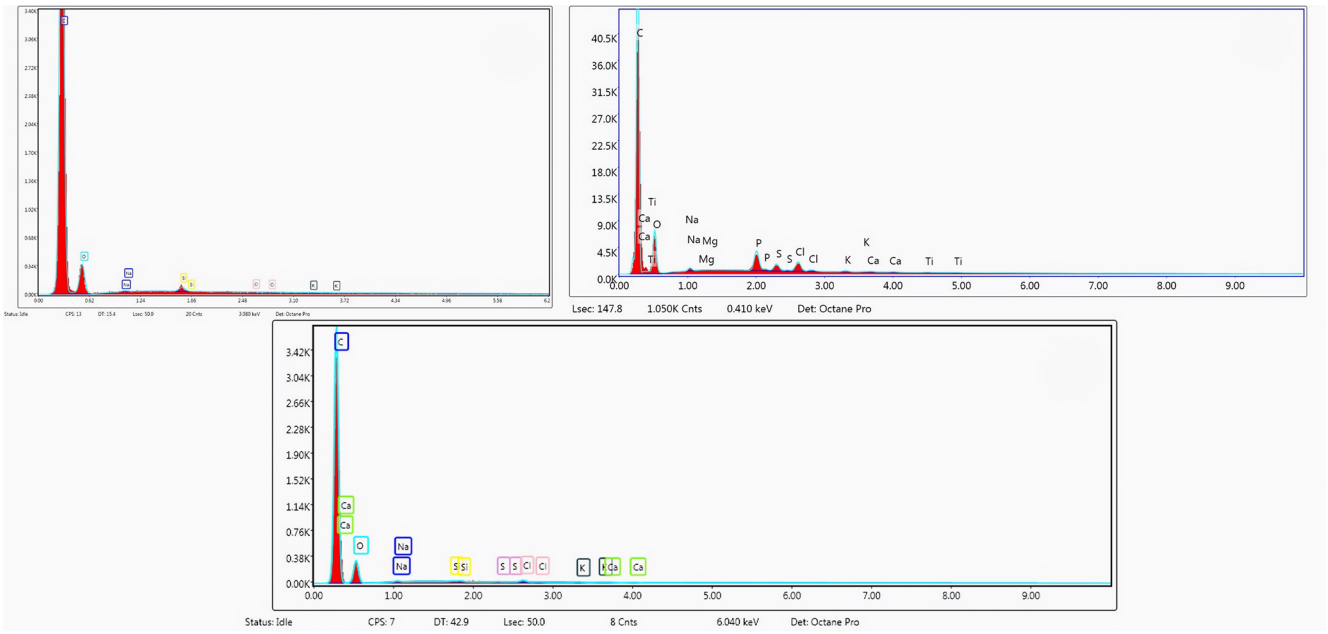


FIG. 2.
eZAF Smart Quant Results. (A – injectable solution, B – 15 % dimethyloxobutylphosphonyldimethylate, C – 0.9 % NaCl)

Subsequently, these solutions were mixed in a 1:10 ratio (1 part of the solution to 10 parts of washed lipoaspirate and added to the samples that had been previously washed with the same solution. After preparation, the fat grafts were placed throughout the entire depth of the defect, and both areas were covered with mesh aseptic dressings using Voskopran wound coverings, followed by application of a vacuum therapy (VAC) system to enhance graft viability and create a controlled healing environment.

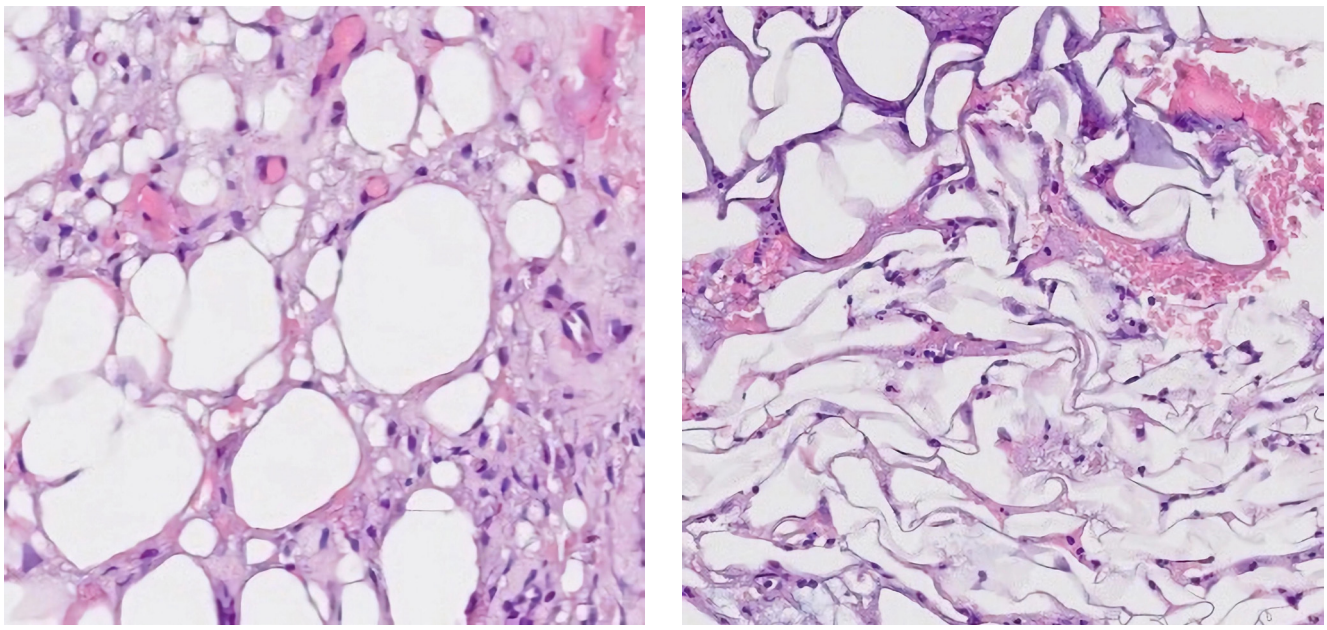
On day 5, a dressing change was performed, followed by a visual assessment of the condition of the graft and the healing process. Tissue samples were also taken for histological examination (Fig. 3).

The histological analysis revealed the presence of mature adipocytes with preserved morphological structure in the area treated with Dimephosphon. In the control group (0.9 % NaCl), there was fragmentation of cellular structures and signs of partial membrane destruction.

TABLE

QUANTITATIVE ION CONTENT (SUM SPECTRUM)

Element	Sample	Repeats (*n* = 3)	M ± m	Me (Q1–Q3)
C	1	83.9; 84.7; 85.2	84.60±0.38	84.70 (83.90–85.20)
C	2	79.1; 80.2; 81.0	80.10±0.48	80.20 (79.10–81.00)
C	3	75.5; 76.4; 77.5	76.47±0.59	76.40 (75.50–77.50)
O	1	14.3; 14.9; 15.4	14.87±0.33	14.90 (14.30–15.40)
O	2	18.5; 19.2; 20.0	19.23±0.37	19.20 (18.50–20.00)
O	3	21.8; 22.5; 23.0	22.43±0.33	22.50 (21.80–23.00)
Na	1	0.10; 0.13; 0.16	0.13±0.03	0.13 (0.10–0.16)
Na	2	0.22; 0.28; 0.34	0.28±0.06	0.28 (0.22–0.34)
Na	3	0.15; 0.19; 0.23	0.19±0.04	0.19 (0.15–0.23)
S	1	0.08; 0.11; 0.16	0.12±0.04	0.11 (0.08–0.16)
S	2	0.02; 0.05; 0.08	0.05±0.02	0.05 (0.02–0.08)
S	3	0.45; 0.55; 0.65	0.55±0.10	0.55 (0.45–0.65)
Cl	1	0.01; 0.03; 0.05	0.03±0.02	0.03 (0.01–0.05)
Cl	2	0.15; 0.24; 0.32	0.24±0.09	0.24 (0.15–0.32)
Cl	3	0.18; 0.21; 0.25	0.21±0.04	0.21 (0.18–0.25)
K	1	0.02; 0.04; 0.06	0.04±0.02	0.04 (0.02–0.06)
K	2	0.05; 0.07; 0.09	0.07±0.02	0.07 (0.05–0.09)
K	3	0.10; 0.16; 0.18	0.15±0.04	0.16 (0.10–0.18)
Ca	1	0.04; 0.05; 0.06	0.05±0.01	0.05 (0.04–0.06)
Ca	2	0.04; 0.05; 0.06	0.05±0.02	0.05 (0.03–0.08)
Ca	3	0.07; 0.10; 0.11	0.09±0.02	0.10 (0.07–0.11)



A B

FIG. 3.
Histological examination. Hematoxylin and eosin staining. Magnification x20. (A – lipoaspirate treated with 15 % dimethyloxobutylphosphonyldimethylate, B – lipoaspirate treated with 0.9 % NaCl)

CONCLUSION

In addition to the technical aspects of adipose tissue transplantation, such as injection volume, anatomical characteristics of the recipient area, and high injection pressure, a key factor in the success of the procedure is the preparation of the autologous graft. This study investigated the effect of various processing methods of harvested tissue on the viability of adipocytes.

Sample 3 showed the most favorable results after being incubated in a solution containing dimethyloxobutylphosphonyldimethylate. This was evident from both the high level of oxygen saturation and the preserved cellular membrane integrity. Sample 2 showed satisfactory oxygenation parameters, but it had elevated intracellular sodium and chloride concentrations and decreased membrane stability when maintained in isotonic sodium chloride (NaCl) solution.

Histological analysis confirmed the benefit of using a specialized solution. In the group treated with dimethyloxobutylphosphonyldimethylate, mature adipocytes with an intact morphological structure and minimal signs of liponecrosis were predominant. In contrast, in the NaCl group, there was significant cell fragmentation and extensive areas of necrosis, indicating reduced graft viability.

The obtained data confirm the positive effect of specialized solutions on adipocyte viability, and emphasize the need for further research into various compositions, including clinical assessment of a solution containing dimethyloxobutylphosphonyldimethylate as a potential membrane-stabilizing agent.

Conflicts of interest
The authors declare no conflicts of interest.

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