SMJ Saratov Medical Journal
ISSN 2712-8253, Saratov Medical Journal
2026. Volume 7. Issue 2. Article CID e0202
DOI: 10.15275/sarmj.2026.0202

Original article

Reprint

Morphological changes in surrounding tissues during lipofilling modeling and the use of hyaluronic acid fillers

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Received 17 February 2026, Accepted 27 May 2026

© This article is an open access publication. Russian text published in the *Saratov Journal of Medical Scientific Research*, 2026; 22(1): 111-116. https://doi.org/10.15275/ssmj2201111. ISSN 1995-0039.

Abstract:
Objective: To evaluate the morphological changes in the surrounding tissues at the recipient site during lipofilling modeling and the use of hyaluronic acid fillers.
Materials and Methods: The study was performed on 50 sexually mature Wistar rats. Group 1 (the negative control) included intact animals (n=5). In Group 2 (n=15), rats were subjected to a single injection of 0.9% sodium chloride solution into the withers area. In Group 3 (n=15), milled fat was injected intradermally into the withers area, while hyaluronic acid filler was injected into the withers area in Group 4 (n=15). At 30, 90, and 180 days after the experiment, 5 animals in each of groups 2, 3, and 4 were euthanized, followed by an assessment of changes in the recipient tissues. Sections were stained using the Mallory's method, as well as hematoxylin and eosin.
Results: The thickness of the epidermis was lower in Group 3 than in Group 4 on Days 30 and 180 (p<0.001 and p=0.018, correspondingly). The thickness of the dermis was higher on Day 30 in Group 3 (injection of milled fat) and on day 180 in Group 4 (hyaluronic acid filler; p<0.001). The area size of the sebaceous glands in Group 4 increased on Days 90 and 180, compared with Day 30 (p=0.02). The thickness of the fat graft decreased on Day 90 (p=0.041). In Group 4, after 90 days, integration of the gel with the surrounding tissues was noted, and after 180 days, the thickness of the implanted hyaluronic gel varied, since it was not always distributed evenly (cavities were formed).
Conclusion: The use of lipofilling, compared to hyaluronic acid filler, leads to a more uniform distribution of adipose tissue and a reduction in the area size of the sebaceous glands, which contributes to an increase in the thickness of both epidermis and dermis.

Keywords: lipofilling, hyaluronic acid, fat transplantation, inflammation.

Cite as: Lavrentieva EA, Korolev AG, Tsymbal AA, Kastyro IV, Barannik MI, Kartasheva AF, Sarygin PV, Ganshin IB. Morphological changes in surrounding tissues during lipofilling modeling and the use of hyaluronic acid fillers. *Saratov Medical Journal* 2026; 7(2): e0202. https://doi.org/10.15275/sarmj.2026.0202.

Background

Fillers of various types and structures are widely used for aesthetic or therapeutic correction of facial and body contours. Hyaluronic acid fillers are the most studied and natural [1]. However, the use of hyaluronic acid fillers in clinical practice can lead to many critical complications, such as vascular embolism, thoracic outlet syndrome, tissue necrosis, etc. [2]. In this regard, some authors prefer autologous fat grafting [3]. Meanwhile, the consequences of surgical interventions (surgical trauma) can lead to the development of aseptic local inflammation and trigger mechanisms of general adaptation syndrome, both of which harmfully affect wound healing and implant survival [1].

Currently, most studies focus on the expression of immunohistochemical markers, such as platelet and endothelial cell adhesion molecule 1 (also known as CD31), vascular endothelial growth factor (VEGF), and stromal vascular fraction (SVF) at the site of autologous fat transfer (lipofilling) [4], and on assessing the toxicity and safety of various types of hyaluronic acid fillers [5]. However, there are virtually no experimental studies on histological evaluation of surrounding tissues, in particular the skin and its appendages, when exposed to both methods of tissue deficiency repair [6]. Such studies could more clearly define the indications for the use of certain lipografting and/or lipofilling methods and improve the clinical effect during body contouring.

Objective: To evaluate morphological changes in surrounding tissues at the recipient site during simulated lipofilling and the use of hyaluronic acid fillers.

Materials and Methods

Our study was performed on 50 mature male Wistar rats. Group 1 (the negative control) included intact animals (n=5).

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Group 2 (the positive control) animals received a single injection of 0.1 mL of 0.9% sodium chloride solution into the withers (n=15). In Group 3 rats (n=15), fat was harvested from the groin under general anesthesia using Zoletil 100 (Virbac, France). Milled fat measuring 1×2×1 mm was injected intradermally into the withers of Group 3 animals.

In Group 4, rats (n=15) were implanted with a biodegradable hyaluronic acid-based preparation at a concentration of 24 mg/mL, phosphate buffer, and 2% lidocaine solution. The preparation had a gel-like consistency. In the experimental groups, autologous fat graft and filler were injected subcutaneously into the withers area under general anesthesia with Zoletil 100 solution. After 30, 90, and 180 days, animals in groups 2, 3, and 4 were euthanized, and the skin and subcutaneous structures of the withers in the injection area were assessed histologically. Sections were stained, according to standard protocols, with hematoxylin and eosin, and also using the Mallory's method. The preparations were scanned on a KFBIO 400 device (Konfoong Bioinformation Tech Co., Ltd., China). Scanned sections were analyzed using Aperio ImageScope v.12.2.2.5015 software (Leica Microsystems, France). Fat graft survival, filler distribution, and the response of surrounding tissues at the recipient site were examined.

Statistical processing of collected data was performed using Statistica 8.0 (StatSoft, USA). The mean (M) and its standard error (SE) were calculated for the sample at each time point for each group. The median (Me) and the 25th and 75th percentiles were estimated for fat graft thickness. The Kolmogorov-Smirnov test was employed to assess normality of data distribution. Intergroup differences were assessed using the nonparametric Mann-Whitney U test for skewed distributions or the Student's t-test for uniform distributions; the critical significance level was set at 0.05.

The study was approved by the Ethics Committee of the Medical Institute at the Patrice Lumumba Peoples' Friendship University of Russia (protocol No. 02-24 of February 20, 2024).

Results

The thickness of the epidermis in animals of Group 1 was similar to Group 2 rats. After lipofilling (Group 3) at 30 days (p=0.003) and injection of the hyaluronic acid filler (Group 4) at 30 (p=0.005), 90 (p=0.002), and 180 (p=0.005) days, we noted that in Group 4, the thickness of the epidermis was higher than in both control groups (*Figure* 1A, *Table* 1). On Days 30 (p<0.001) and 180 (p<0.001), the thickness of the epidermis in Group 3 was statistically significantly lower than in Group 4 (*Figure* 2A).

There was no difference in the thickness of the granular layer between two control groups. According to the Mann–Whitney test, after 30 (p=0.02) and 180 (p=0.012) days in Group 3, and after 30 (p=0.01) and 180 (p=0.004) days in Group 4, the granular layer was thicker than in the control groups (*Figure* 1B, *Table* 1). This parameter value was lower in Group 3 vs. Group 4 on Day 90 (p<0.001). In the fat graft group, the thickness of the granular layer on Day 90 decreased compared to Day 30, but increased again on Day 180 after surgery (p<0.001). In the hyaluronic acid filler group, the granular layer was significantly thinner at 30 days postoperatively than at 90 and 180 days (p<0.001).

The thickness of the cornified layer did not differ between the control groups. According to the Mann-Whitney test, the thickness of this layer, compared with the negative and positive control groups, was higher only in Group 3 at 30 days postoperatively (p=0.005). At 90 days, the cornified layer was thicker in the hyaluronic acid filler group than in the lipofilling group (p=0.003). In Group 3, the thickness of this layer significantly decreased at 90 and 180 days vs. Day 30 (p<0.001; *Figure* 1C, *Table* 1).

The thickness of the dermis did not differ between the control groups. According to the Mann-Whitney test, it was higher on day 30 in Group 3 (*Figure* 2B) and on day 180 in Group 4 (p<0.001; *Figure* 2C), as well as at 90 and 180 days in Group 3 (p=0.018), and at 30 (p=0.014) and 180 (p<0.001) days in Group 4 (*Figure* 1D, *Table* 2).

Sebaceous gland area size did not differ between the control groups. According to the Mann-Whitney test, this parameter value significantly decreased vs. to the control group only on Day 30 postoperatively (p<0.001). In Group 4, on the contrary, it increased after 90 and 180 days after the introduction of a hyaluronic acid filler compared to the control data (p=0.02). In Group 3, an increase in the value of this parameter was noted on Day 90 vs. Day 30 (p<0.001), but after 180 days, the sebaceous gland area size did not differ from that on Day 30. In Group 4, a stable increase in the sebaceous gland area size was noted after 90 and 180 days compared to Day 30 after the introduction of a hyaluronic acid-based product (p=0.002; *Figure* 1E, *Table* 2).

According to the Mann-Whitney test, fat graft thickness significantly decreased on Day 90 postoperatively compared to Day 30 (p=0.041; Figure 1D, 1E). Gel thickness in Group 4 was greater than in Group 3 on days 30 (p=0.038), 90 (p=0.007), and 180 (p<0.001; *Figure* 1D).

In Group 4, 90 days after hyaluronic filler implantation, integration of the gel with the surrounding tissue was observed (*Figure* 2F). The implanted gel often had a bubbly or layered consistency. After 180 days, the thickness of the implanted hyaluronic acid gel in this group varied, as the gel was often unevenly distributed, forming patches (*Figure* 2C). Cavities frequently appeared after gel implantation (*Figure* 2D). They were not the result of gel degradation, as they were visible even early after implantation. After 180 days of hyaluronic acid filler implantation, no noticeable reduction in the thickness of the injected filler was observed vs. earlier measurements (*Figure* 1F).

Discussion

The epidermis of control animals was thinner than in experimental animals, and higher mitotic activity was observed in the basal keratinocytes of experimental rats. The number of rows of granular keratinocytes and the size of keratohyalin granules in their cytoplasm are indicators of epidermal keratinization activity [7]. In our study, epidermal thickening in the experimental groups was due to the stimulation of tissue proliferation by growth factors from the SVF cells, which was confirmed by an increase in Ki-67 protein expression at the recipient site after fat grafting [8].

It should be noted that all animals in the control group lacked subcutaneous adipose tissue. In rats of Group 3, a subcutaneous fat base formed, which was likely caused by the activation of pericytes in the hypodermis and their differentiation into adipocytes [9]. Moreover, only some individuals in Group 4 exhibited foci of adipose tissue in the hypodermis. According to experimental data by some authors, hyaluronic acid fillers can stimulate the formation of

adipose tissue [10], which is also confirmed by the present study. A hyaluronic acid filler was detected at all times after the experiment, but it was not always distributed evenly in the skin after 30 and 90 days, making it difficult to measure its thickness; this finding is confirmed by the data collected by other authors [11]. Furthermore, a hyaluronic acid filler can inhibit lipolysis [12].

Milled autologous fat and hyaluronic filler integrate well with surrounding skin tissues, thus promoting the formation of new microvessels. Our recent study demonstrated that autologous fat grafting stimulates the expression of VEGF A, which, in turn, promotes better fat engraftment and stimulation of neoangiogenesis and adipogenesis [13]. According to many authors, milled fat contains SVF [12], from which future endothelial cells (fibroblast-like precursors capable of accumulating lipids and acquiring the morphology of fat cells) differentiate [3, 14]. In the autologous fat transfer group in this study, the preservation of adipose tissue by Day 30 after the experiment can be explained by the release of inflammatory mediators that initiate both the activity of the SVF [14] and stress-related mechanisms. In contrast, hyaluronic acid is not foreign to the skin, as it is part of the extracellular matrix (glycosaminoglycans) and does not cause immune responses upon implantation [15, 16], although it sometimes stimulated the formation of adipose tissue foci in some animals of Group 4, which is supported by the data of some studies [10].

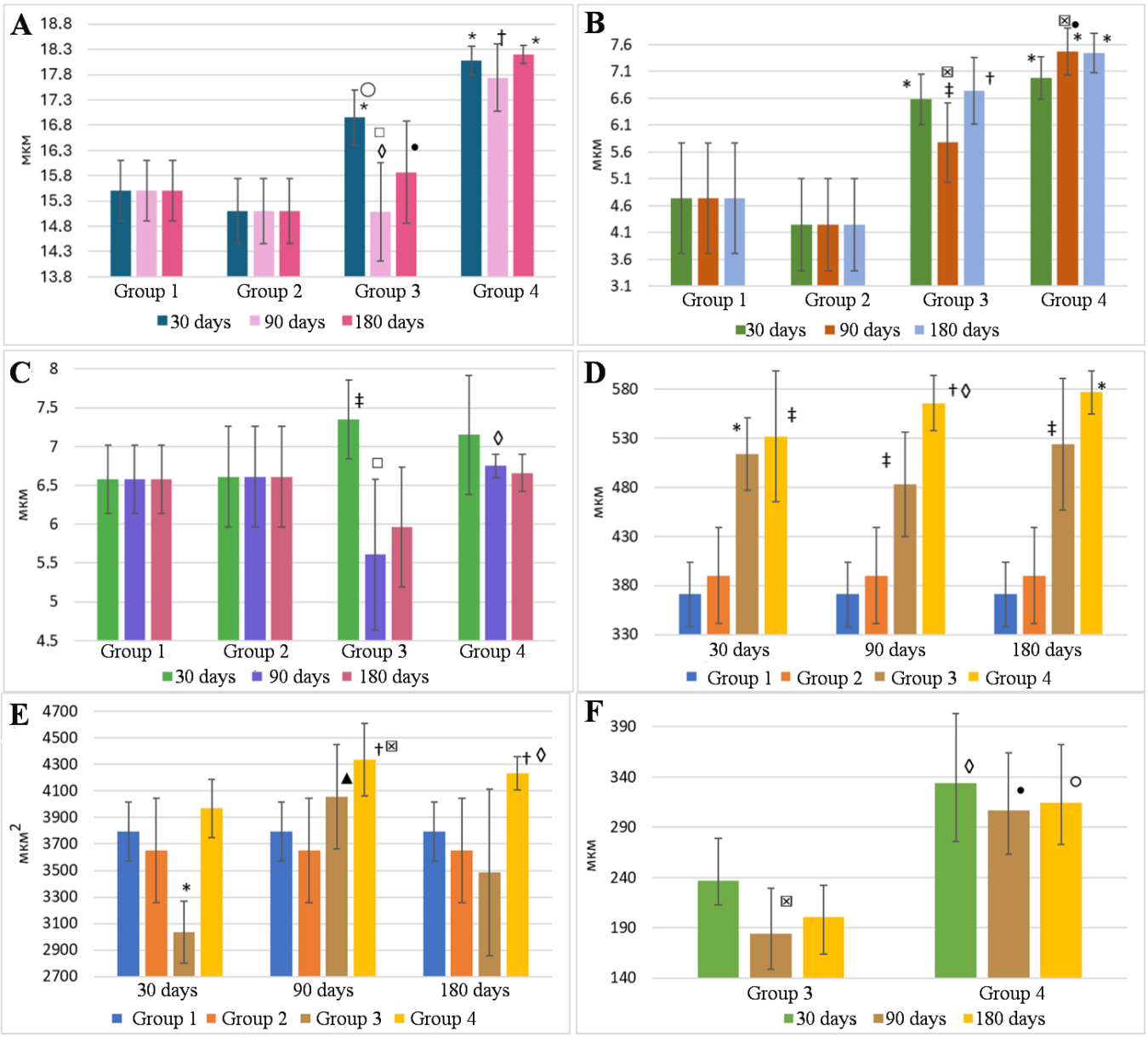


Figure 1. Thickness of the epidermis (A), granular layer (B), cornified layer (C), and dermis (D); area size of the sebaceous glands (E) after implantation of fat grafts and administration of a hyaluronic acid-based filler in rats; fat graft thickness (F). Differences between the experimental and control groups: *p<0.001; †p<0.01; ‡p<0.05; differences between the experimental groups at each time point: ○p<0.001; •p<0.01; ◊p<0.05; intragroup differences between time points: ▲p<0.001; □p<0.01; ⊠p<0.05.

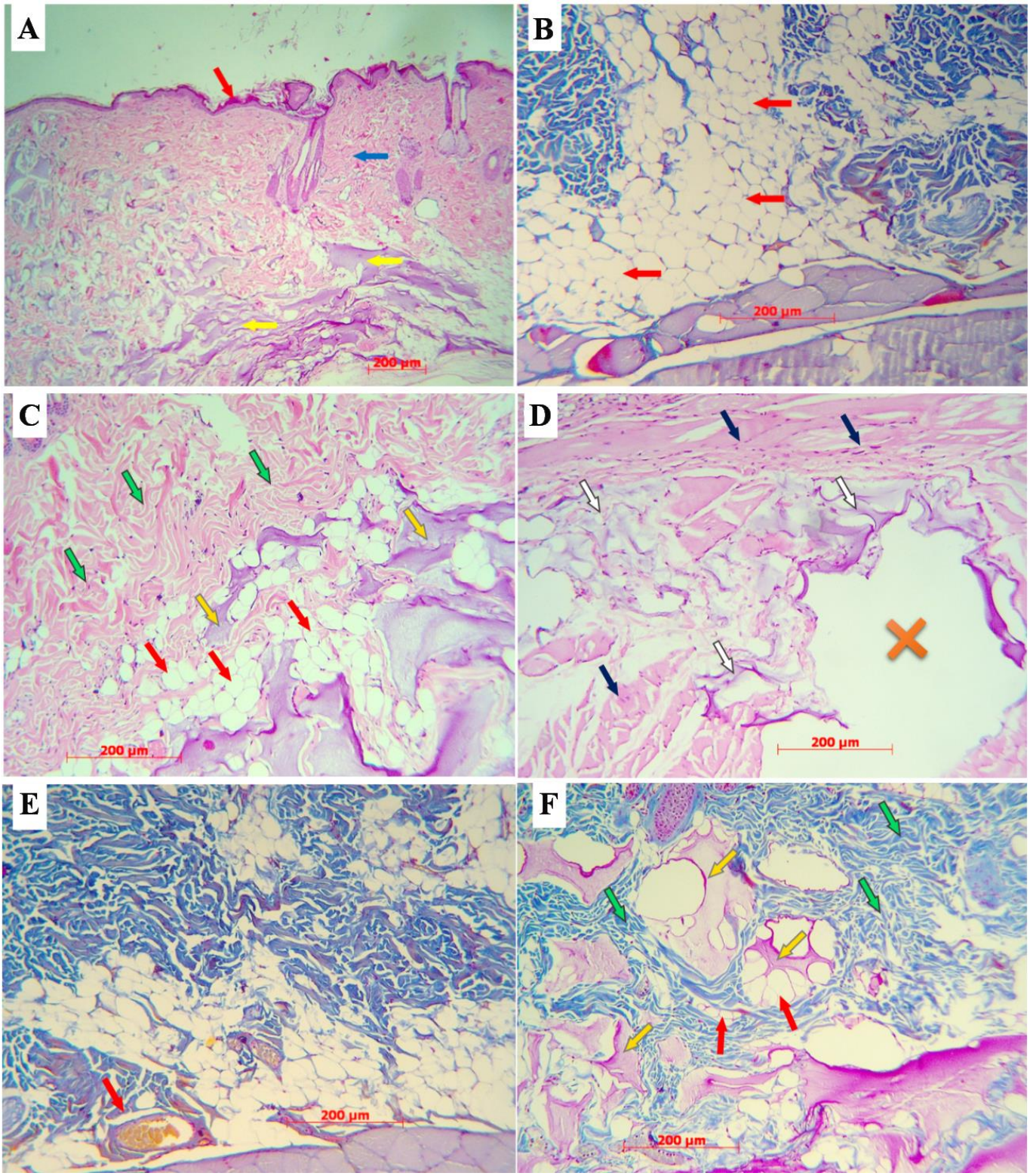


Figure 2. Histological sections. A – Skin of Group 4 animal on Day 30; the epidermis is shown by the red arrow, the dermis is pointed to by the blue arrow, the gel is indicated by the yellow arrows, stained with hematoxylin and eosin. B – dermis and hypodermis of Group 3 animal on Day 30; diffuse distribution of adipose tissue (red arrows), stained according to Mallory's trichrome method. C – uneven distribution of the gel in Group 4 animal (Day 180), dermal fibers (green arrows), fat cells (red arrows), gel (filler) (yellow arrows); stained with hematoxylin and eosin. D – cavities in the dermis after filler implantation (Day 180): cavity (marked with ×), dermal fibers (blue arrows) and gel (white arrows); stained with hematoxylin and eosin. E – dermis and hypodermis in a Group 3 rat (after 90 days): diffuse distribution of adipose tissue (red arrows), microvessels (red arrow); Mallory's trichrome stain. F – integration of gel (yellow arrows) with surrounding tissues in Group 4 (after 90 days): dermal fibers (green arrows), fat cells (red arrows), Mallory's trichrome stain.

Table 1. Changes in tissue thickness at the recipient site after fat graft implantation and administration of a hyaluronic acid filler, M±SE

Day#	Group			
	1	2	3	4
Epidermis thickness, μm				
30	15.5±0.6	15.1±0.6	16.9±0.5	18.1±0.2
90			15.1±0.9	17.7±0.6
180			15.8±1	18.2±0.1
Granular layer thickness, μm				
30	4.7±1.1	4.2±0.8	6.5±0.4	6.9±0.3
90			5.7±0.7	7.4±0.4
180			6.7±0.6	7.4±0.3
Cornified layer thickness, μm				
30	6.5±0.4	6.6±0.6	7.3±0.5	7.1±0.7
90			5.6±0.9	6.75±0.1
180			5.9±0.7	6.6±0.2

Table 2. Changes in the thickness of the dermis and sebaceous gland area size at the recipient site after fat graft implantation and hyaluronic acid filler injection, M±SE

Group	Day#		
	30	90	180
Dermis thickness, μm			
1	371±33		
2	390±49		
3	514±37	483±53	524±67
4	532±67	566±28	577±22
Sebaceous gland area size, μm²			
1	3,794±224		
2	3,653±394		
3	3,036±235	4,055±395	3,486±628
4	3,968±218	4,336±274	4,234±125

The preference for using a particular implant largely depends on the specified clinical goals. Implanted fat successfully takes root in the recipient area and can provide long-term favorable outcomes in soft tissue volume restoration. However, there is evidence of a deforming effect of fat grafts when they are used to correct protruding scars, which can lead to visible skin deformation [2]. Despite its effectiveness, lipofilling in visually accessible skin zones should be performed with caution [17], including due to possible local complications such as erythema, edema, and hematoma at the fat harvesting area or fat grafting site [18].

Hyaluronic gel implantation is characterized by a more predictable outcome [19], since hyaluronic acid (as a component of the extracellular matrix) is distributed evenly, without causing inflammatory responses or complications. Some authors pointed out that the duration of its clinical and aesthetic effects is much shorter than that of transplanted fat grafts [20].

Conclusion

The need to evaluate morphological changes in surrounding tissues at the recipient site during lipofilling simulations and the use of hyaluronic acid fillers is pathogenetically substantiated in this article. For instance, in an experiment using autologous fat transfer, we observed a more uniform distribution of adipose tissue and a reduction in the area size of sebaceous glands, compared with hyaluronic acid fillers. The latter contribute to an increase in the thickness of the epidermis (including due to the growth of the granular layer), the thickness of the dermis, and the filler per se in the subcutaneous space, compared to lipofilling.

It should be noted that the choice of a specific type of implant is determined primarily by the specific clinical needs, while it is recommended to consider the detected changes in the surrounding tissues and the mechanisms that caused them as well.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

I.V. Kastyro, A.G. Korolev, and A.A. Tsymbal designed the study; E.A. Lavrentyeva and A.G. Korolev conducted the experiment; A.G. Korolev and E.A. Lavrentyeva performed statistical analysis; E.A. Lavrentyeva, A.G. Korolev, and I.V. Kastyro wrote the article; I.B. Ganshin, M.V. Barannik, P.V. Sarygin, and A.A. Tsymbal edited the text.

Funding

The authors declare no external funding for the study or publication of this article.

Bioethics compliance

The study was conducted in accordance with the ethical standards for the treatment of animals adopted by the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes*.

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