Article

Morphological Changes of the Recipient Site During Autotranplantation of Fat in Rats after Various Methods of Its Mechanical Processing

Margarita Kostyaeva[1], Svetlana Moroz[2], Elina Lavrenteva[2], Maxim Khlystalov[2], Svetlana Ibadullaeva[2], Yuliya Ivanova[3].

[1] Department of histology, embryology & cytology (Head of the Department – MD, Associate Professor T.H. Fatkhudinov, Peoples' Friendship University of Russia named after Patrice Lumumba, Moscow, Russia;
[2] Department of plastic surgery (Head of the Department – MD, Associate Professor I.B. Ganshin), Peoples' Friendship University of Russia named after Patrice Lumumba, Moscow, Russia;
[3] Department of clinical physiology and non-medicinal methods of treatment (Head of the Department – MD I.V. Kastyro), Peoples' Friendship University of Russia named after Patrice Lumumba, Moscow, Russia;

* Correspondence: ivanovaulia2005@mail.ru;
kostyaeva_mg@rudn.university , https://orcid.org/0000-0001-5182-0373 (M.K.);
moroz.svetlana@yandex.ru, https://orcid.org/0000-0002-3892-0596 (S.M.);
laveelina@ya.ru, https://orcid.org/0000-0002-1641-5863 (E.L.);
hlystalov@inbox.ru, https://orcid.org/0009-0002-6766-8323 (M.K.);
ibadullayeva00@gmail.com, https://orcid.org/0009-0006-9651-949X (S.I.);
ivanovaulia2005@mail.ru, https://orcid.org/0009-0000-7869-1823 (Y.I.).

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Abstract: Introduction. Autologous fat transplantation is a widely used technique in aesthetic and reconstructive surgery, employed for correcting atrophic changes and scar deformities.

Materials and methods. The animals were divided into 5 groups, with 6 individuals in each group. Group 1 – intact animals (without exposure); group 2 – control (saline solution); group 3 consisted of animals implanted subdermally, through an incision with a diameter of 1 mm, a fragment of fat previously extracted from a skin incision in the groin area. In group 4, fat crushed with a scalpel was injected under the skin with a syringe, once. In group 5, animals were implanted with fat crushed using a shredder nozzle in a Luer Lock syringe. 4 implantations were performed, with a volume of 0.05 ml. The sections were stained using the Mallory method, hematoxylin and eosin, methylene blue.

Results. Morphometric parameters of the dermis indicate an increase in its thickness, mainly of the mesh layer, as well as, physiologically significant, an increase in the proportion of microcirculatory vessels in the skin of animals of the experimental groups. The number of sebaceous gland profiles in the experimental groups was slightly higher than in the control groups ($p<0.05$). The thickness of the fat autograft was significantly greater in the third group ($p<0.001$), where a large fat fragment was implanted, however, in this group, almost all rats had pathological reactions in the form of leukocyte, mainly lymphocytic, infiltrations and necrosis of varying severity. Infiltrations containing lymphocytes and fibroblast-like cells were also observed in animals of the fourth and fifth groups, but were significantly less pronounced than in the third. In animals of the fifth group, relatively large fat fragments surrounded by lymphocytic infiltrates were detected only in the skin of two rats, in other animals, fat fragments of microscopic size were found, which, as a rule, were integrated with the adipose tissue of the transplant site.

Conclusion. The introduction of autologous fat can not only lead to its gradual degradation, but also stimulate the formation of new adipose tissue in the injection area. The components of the fat graft (lipids) are incorporated into the newly formed adipose tissue. This process is more effective the smaller the size of the injected graft particles. A significant factor, largely determining the effectiveness of the process, is the stimulation of angiogenesis, which is evidenced by an increase in the number of microvessel profiles in the section and their greater volumetric proportion in the newly formed connective tissue

Keywords: autofat, inflammation, fat graft, fat graft modeling.

1. Introduction

Autologous fat transplantation is a widely used technique in aesthetic and reconstructive surgery, employed for correcting atrophic changes and scar deformities [1]. The main disadvantage of artificial fillers and implants is that they are non-biological substrates and often provoke implant rejection, contour deformities, implant migration, and capsular contractures [2]. Adipose tissue is a biologically active substance, and its function extends far beyond fat storage. It, or rather its stromal-vascular fraction, is one of the main sources of mesenchymal stem cells (MSCs). The multipotency of these cells manifests, in particular, in their ability to differentiate into adipocytes, osteocytes, chondrocytes, and other cells of mesenchymal origin [3]. Adipose tissue is a more accessible source of cellular material than red bone marrow cells [4]. Currently, the effect of MSCs on the stimulation of angiogenesis, remodeling of fibrous tissue, and the possibility of implementing these biological effects in clinical practice through autologous fat transplantation for regenerative purposes in both plastic surgery and related specialties are being actively studied [5].

The aim of this study was to investigate the histological changes in autologous fat grafts and surrounding tissues in rats after various fat processing methods, 30 days after surgical interventions.

2. Patients and Methods

A comparative histological study was conducted on male inbred Wistar rats, 3-4 months old, weighing 195±25 g. The animals were divided into 5 groups, with 6 individuals in each group. Group 1 – intact animals (without any intervention); Group 2 – control group (physiological saline); Group 3 consisted of animals that received a subdermal implant, through a 1 mm incision, of a fat fragment previously extracted from a skin incision in the inguinal region. In Group 4, fat minced with a scalpel was injected under the skin using a syringe, in a single dose. In Group 5, animals were implanted with fat minced using a mincing nozzle in a Luer Lock syringe. Four implantations were performed, with a volume of 0.05 ml each. The material was injected into the interscapular region of the back, slightly lifting the skin for subdermal administration in a 1 cm2 area. After 1 month, the animals were euthanized using a toxic dose of Zoletil 100 solution. For histological examination, a 4 cm2 skin flap was taken and fixed in 10% neutral formalin. Fixation, preparation, and staining of the samples were carried out according to classical methods. The sections were stained using Mallory's method, hematoxylin and eosin, and methylene blue. An Axiostar light microscope (Carl Zeiss) was used in the study. During histological examination, the characteristics of the epidermis, dermis, and subcutaneous fat layer were taken into account, and the specific proportion of microcirculatory vessels was assessed. One section from each animal in the group was examined. Measurements were performed at a maximum magnification of 400, in fields of view where vessels were present. The total area of microcirculatory vessels in the dermis was measured, and then the total area of the dermis in the field of view was measured. The calculation was performed using the formula:

$P = \Delta Sc/Sd.$

P - the specific proportion of vessels (%), Sc - the total area of vessels in the field of view, Sd - the area of the dermis in the field of view.

Morphometric studies were performed using an ocular micrometer. In each sample, 10 measurements of the epidermis and 10 measurements of the dermis were made. Then the average value was calculated. The epidermis was measured from the stratum corneum to the basement membrane, excluding areas with a hair follicle funnel.

The results were processed using the "Microsoft Excel" version 2010 statistical software package. To assess the statistical significance of differences between groups, the following values were calculated: sample mean ( ), difference of sample means ( ), standard error of the difference of sample means ( ), and a 95% confidence interval (95% CI) for the difference of means. A critical significance level of p=0.05 was considered.

Animal studies were conducted in accordance with the requirements of the Order of the Ministry of Higher and Secondary Special Education of the USSR No. 742 of November 13, 1984, "Rules for Conducting Work Using Experimental Animals".

3. Results

Table 1 data indicate the state of the epidermis, where an increase in the thickness of the cellular epidermal layers occurs, mainly due to an increase in the rows of cells in the granular layer. This is particularly evident in the skin of rats in the fourth group ($p<0.01$). During the study, an increase in the rows of cells in the spinous layer and mitotic figures (metaphase plates) ($p<0.01$) were also noted in the skin samples of the experimental animals.

Table 1. Values of cellular epidermis layer thickness 30 days after fat tissue transplantation.

Parameter (microns)	1 group	2 group	3 group	4 group	5 group
Thickness of the cellular epidermis	16,6±0,54	16,2±0,63	17,9±0,45	18,2±0,67	17,6±0,33
Thickness of the granular layer	5,2±0,47	5,0±0,33	6,5±0,38	7,2±0,41	6,9±0,33

Morphometric parameters of the dermis (Table 2) indicate an increase in its thickness, mainly in the reticular layer, as well as, which is physiologically significant, an increase in the proportion of microcirculatory vessels in the skin of animals in the experimental groups ($p<0.001$). However, no significant differences were observed between the groups regarding the area of sebaceous glands. The number of sebaceous gland profiles in the experimental groups was slightly higher than in the control group ($p<0.05$). The thickness of the fat autograft was significantly greater in the third group ($p<0.001$), where a large fat fragment was implanted, but in this group, almost all rats showed pathological reactions in the form of leukocyte, mainly lymphocytic, infiltrations and necrosis of varying severity (Fig. 1).

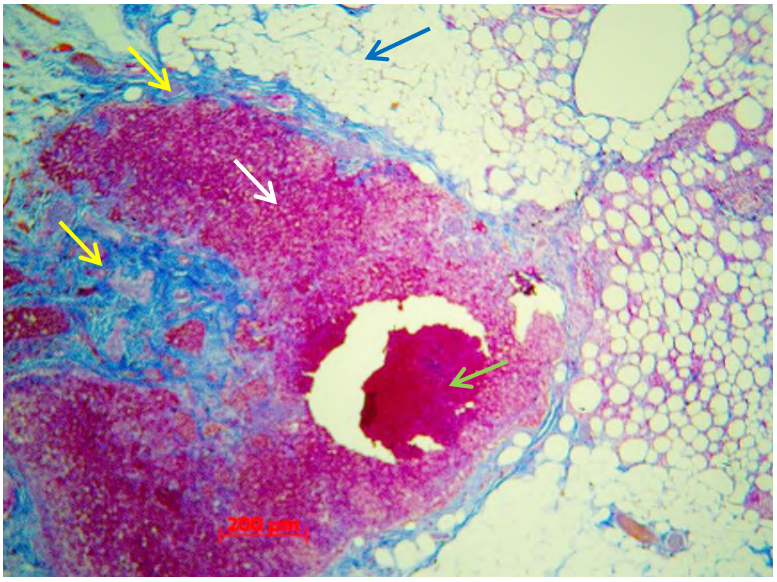

Figure 1. Changes in the fat graft area in group 3. Okr. according to Mallory, uv. x 100. Note: necrosis site (green arrow), lymphocytic shaft (white arrow), connective tissue (yellow arrows).

Table 2. Morphometric parameters of dermis thickness, fat graft thickness, and dermis structure status 30 days after fat tissue transplantation.

Parameter	Thickness of the dermis, microns	Thickness of the fat implant, microns	Sebaceous gland area, mm2	The proportion of vessels of the microcirculatory bed in %	Parameter
Group 1	348±32	0	4965,6±459	5,06±1,14	Group 1

Group 2	380±41	0	5120,5±637	6,05±1,22	Group 2
Group 3	510±56	700±86	4945,6±417	8,05±0,99	Group 3
Group 4	630±44	490±33	5642,6±233	9,45±1,03	Group 4
Group 5	590±22	450±27	5324,9±146	8,65±1,07	Group 5

In animals of the fourth and fifth groups, infiltrations containing lymphocytes and fibroblast-like cells were also observed, but were significantly less pronounced than in the third group ($p<0.01$). This may be due to the significant size of the fat graft, which mechanically damages the surrounding tissues, and a longer period of its breakdown into smaller fragments. In the animals of the fifth group, which received an implant of fat minced using a special nozzle, relatively large fat fragments surrounded by lymphocytic infiltrates were detected only in the skin of two rats; in the remaining animals, microscopic fat fragments were found, which, as a rule, were integrated with the adipose tissue at the transplantation site.

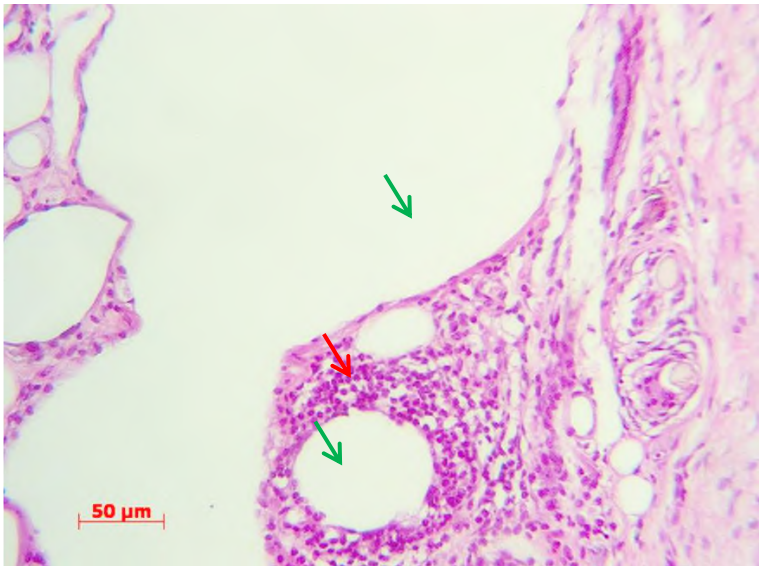

Figure 2. Changes in the area of homogenized fat transplantation in group 5. Ocd. hematoxylin and eosin, uv. x 100. Note: fat fragments (green arrows), infiltration (red arrow).

In the fourth group, where fat minced with a scalpel was implanted once, similar to the fifth group, relatively large rounded fat fragments were found in only two animals. These fragments, as in the previous group, were accompanied by moderate lymphocytic infiltration and limited focal tissue necrosis. In the remaining four rats, fragments of the fat graft were not found in the skin sections at all. However, in the area where the subcutaneous tissue is usually located, between the reticular layer of the dermis and the subcutaneous muscle, rather extensive accumulations of adipose tissue cells were regularly found.

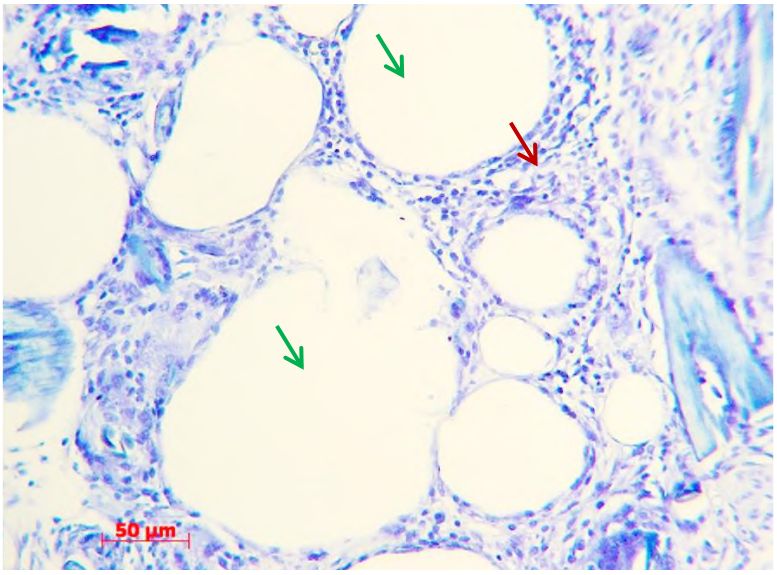

Figure 3. Changes in the area of the fat graft in group 4. Okr. methylene blue, uv. x200. Note: fragments of fat (green arrows), infiltrate (red arrow).

4. Discussion

In accordance with the aim and objectives of this work and the data obtained from the experimental study, it is advisable to discuss issues concerning changes in the morphological parameters of the skin of rats that underwent autologous fat transplantation in the form of fat fragments (grafts) of various sizes. The conditions of the study were standardized as much as possible in terms of the type, age, and weight of the experimental animals, their housing method, and their distribution into groups. To study the effects of autologous fat grafts of different sizes, the animals were divided into five groups.

Numerous studies have shown that adipose tissue is a highly biologically active type of connective tissue. Its stromal-vascular fraction contains multipotent MSCs and a large population of progenitor cells, including adipocyte precursors [6]. The capabilities of MSCs in adipose tissue extend beyond local effects – stimulation of angiogenesis, remodeling of fibrous tissue, stimulation of wound healing, modulation of inflammatory and immune responses, and others.

5. Conclusions

Our studies indicate that the introduction of autologous fat can not only lead to its gradual degradation, but also stimulate the formation of new adipose tissue in the injection area. The components of the fat graft (lipids) are incorporated into the newly formed adipose tissue. This process is more effective the smaller the size of the injected graft particles.

A significant factor, largely determining the effectiveness of the process, is the stimulation of angiogenesis, which is evidenced by an increase in the number of microvessel profiles in the section and their greater volumetric proportion in the newly formed connective tissue.

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Application of artificial intelligence: The review is written without the use of artificial intelligence technologies.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Peng Z, Tang P, Zhao L, Wu L, Xu X, Lei H et al. Advances in biomaterials for adipose tissue reconstruction in plastic surgery. Nanotechnology Reviews. 2020;9(1):385-95.
2. Cheng C, Wang W, Pu LLQ. Targeted fat grafting: the concept of anatomic restoration for safe and optimal facial rejuvenation. Aesthetic Surgery Journal. 2020;40(12):705-7.

3. Sinno S, Mehta K, Reavey PL, Simmons C, Stuzin JM. Current trends in facial rejuvenation: An assessment of ASPS members' use of fat grafting during face lifting. Plastic and Reconstructive Surgery. 2015;136(1):20-30.
4. Negenborn VL, Groen JW, Smit JM, Niessen FB, Mullender MG. The use of autologous fat grafting for treatment of scar tissue and scar-related conditions: A systematic review. Plastic and Reconstructive Surgery. 2016;137(1):31-43.
5. Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M, Sbarbati A. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. Plastic and Reconstructive Surgery. 2007;119(5):1409-22.
6. Khazaei S, Keshavarz G, Bozorgi A, Nazari H, Khazaei M. Adipose tissue-derived stem cells: a comparative review on isolation, culture, and differentiation methods. Cell Tissue Bank. 2022;23(1):1-16.
7. Khouri RK Jr, Khouri RK. Current Clinical Applications of Fat Grafting. Plastic and Reconstructive Surgery. 2017;140(3):466-86.