




# Are Preadipocytes More Advantageous Than Adipocytes in Soft Tissue Augmentation?

Emine Burcu Cigsar Kuzu <sup>1,\*</sup>, Sule Oztan<sup>2</sup>, Kubilay Dogan Kilic<sup>3</sup>, Eray Copcu<sup>2</sup>

<sup>1</sup> Izmir State Hospital, Izmir, Turkey

<sup>2</sup> MEST Plastic, Reconstructive and Aesthetic Surgery Practice

<sup>3</sup> Department of Histology and Embryology, Medical Faculty, Ege University, Izmir, Turkiye

\*Corresponding Author: Izmir State Hospital, Izmir, Turkey. Email: ebcigsar@gmail.com

Received: 29 April, 2024; Revised: 16 September, 2024; Accepted: 24 September, 2024

## Abstract

**Background:** Autologous fat grafting in pediatric patients is limited due to restricted fat tissue reserves. Preadipocytes, derived from adipose tissue, offer a promising alternative for soft tissue augmentation in children.

**Objectives:** This study aims to compare the effectiveness of preadipocyte fat grafting with conventional adipose grafting, particularly in cases where adipose tissue availability is limited.

**Methods:** In this experimental study, 28 male Wistar albino rats were used. Fat was harvested from periepididymal fat pads to prepare preadipocyte and adipocyte suspensions. These suspensions were injected subcutaneously into the right and left scapular areas, respectively. The rats were divided into four groups based on post-injection excision timing. Volumes of excised tissue were calculated using a hemi-ellipse formula. Histopathological examinations were conducted, comparing necrosis, fibrosis, vascularity changes, cyst formation, and inflammatory cell density.

**Results:** Statistical analysis was performed using GraphPad Instat 3.0 and SPSS 17.0 software. Histopathologically, preadipocyte grafts showed better scores for necrosis, fibrosis, cyst formation, vascularity, and inflammation across all groups. Although no significant volumetric differences were observed in the other groups, in the fourth group (22 weeks), volumetric loss of adipocyte grafts was significantly greater than that of the preadipocyte grafts ( $0.09 \pm 0.01$  vs.  $0.05 \pm 0.02$ ,  $P < 0.05$ ).

**Conclusions:** The findings suggest that preadipocyte grafting holds promising potential for soft tissue augmentation, particularly in pediatric cases where limited fat tissue availability presents challenges. Preadipocytes' inherent characteristics, including resistance to hypoxia and enhanced promotion of angiogenesis, may contribute to their sustained volume retention and improved histopathological outcomes. The significant reduction in adipocyte graft volumes may be attributed to their susceptibility to hypoxia and graft resorption. The ability of preadipocytes to withstand ischemic conditions and promote angiogenesis could contribute to their enhanced viability. Further research and clinical investigations are warranted to establish preadipocyte grafting as a viable solution for soft tissue augmentation, especially in pediatric patients with limited adipose tissue availability. A comprehensive assessment of volumetric changes and histopathological outcomes provides deeper insight into the potential benefits of preadipocyte grafting in pediatric healthcare.

**Keywords:** Fat Graft, Preadipocyte, Adipocyte, Child

## 1. Background

Autologous fat grafting is widely used to address reconstructive and aesthetic issues in both adult and pediatric populations (1). A wide range of conditions, including congenital anomalies like hemifacial atrophy, pectus excavatum, cleft lip, subcutaneous soft tissue defects post-resection, and post-traumatic defects of the

face and body such as burn injuries or postoperative scars, are treated with adipose tissue grafts (1). While autologous fat grafts are extensively utilized in adults, their application in pediatric patients is limited, primarily due to children's relatively restricted fat tissue reserves. Extensive research on the use of autologous fat grafting in children and adolescents remains crucial to

fully understanding its potential benefits in pediatric healthcare.

Adipose tissue, a rich source of mesenchymal stem cells, primarily consists of mature adipocytes, extracellular matrix, and stromal vascular fraction, which contains adipose tissue-derived stem cells, preadipocytes, fibroblasts, smooth muscle cells, and immune cells (2). Preadipocytes are the precursors of mature adipocyte cells. Unlike mature fat cells, preadipocytes differentiated from mesenchymal stem cells within the stromal-vascular section of adipose tissue retain the potential to proliferate and differentiate throughout their lifespan (3). With these characteristics, preadipocytes present a promising cell source for soft tissue augmentation.

## 2. Objectives

This study aims to compare the volumetric and histopathologic changes of preadipocyte fat grafting with conventional adipose grafting, highlighting the feasibility of autologous preadipocyte tissue transplantation, even in cases with limited adipose tissue supply.

## 3. Methods

### 3.1. Experimental Design

A total of 28 male Wistar albino rats weighing 250 - 300 g were included in the study. All procedures were carried out according to the guidelines for the care and use of laboratory animals at Adnan Menderes University. The animals were housed in standard cages, subjected to a 12-hour light/12-hour dark cycle, provided ad libitum access to water and rat chow, and monitored closely throughout the study period. The rats were divided into four groups according to the timing of excision: Group 1 (n = 7) at 3 weeks post-grafting, group 2 (n = 7) at 6 weeks post-grafting, group 3 (n = 7) at 10 weeks post-grafting, and group 4 (n = 7) at 22 weeks post-grafting.

### 3.2. Adipose Tissue Harvesting

For the procedure, all rats were anesthetized with an intramuscular injection of 5 mg/kg xylazine and 50 mg/kg ketamine. Following abdominal shaving and application of a 5% iodine solution, a 2 g fat sample was harvested from the right epididymal fat pad through an

abdominal incision in each rat. The adipose tissue samples were promptly transported to the laboratory in sterile plastic containers containing basal medium.

### 3.3. Preparation of the Preadipocytes Suspensions

The isolation and primary culture of preadipocytes were conducted following the method proposed by van Harmelen et al. (4). Fat tissue samples were placed in Petri dishes containing phosphate-buffered balanced salt solution (PBS) within a laminar flow cabinet and finely dissected with scissors. Visible connective tissue, debris, and blood vessels were removed. The dissected adipose tissue was transferred to tubes, and collagenase solution (3 mL solution per 1 mL fat tissue) was added. The sealed tubes were placed horizontally in a shaker bath at 37°C for approximately 90 minutes to facilitate digestion. After digestion, the tubes were centrifuged at 200 g for 10 minutes at room temperature. The supernatant, containing mature adipocytes and collagenase solution, was separated from the pellet containing the preadipocytes. A 1 mL solution was left at the bottom to prevent the pellet from drying out. To remove erythrocytes, an erythrocyte lysis buffer [155 mmol NH<sub>4</sub>Cl, 5.7 mmol K<sub>2</sub>HPO<sub>4</sub>, 0.1 mmol ethylenediaminetetraacetic acid (EDTA)] was added to the tube and incubated. The suspension was then filtered through a 150-µm filter and re-centrifuged. The supernatant was discarded, and 10 - 20 mL of basal medium, consisting of an equal volume of Dulbecco's Modified Eagle Medium (DMEM) and F12 solution, plus 15 mmol 4-(2-hydroxy-ethyl)-1-piperazine ethane sulfonic acid (HEPES), 14 mmol NaHCO<sub>3</sub>, 33 µM biotin, and 17 mmol D-pantothenate, was added to the pellet. After filtering through a 70-µm filter, cell counts were determined using a Neubauer hemocytometer, and cell viability was assessed with trypan blue staining.

An inoculation medium of 1 million cells/mL was prepared by adding gentamicin (50 mg/mL) and 10% fetal bovine serum (FBS) to the basal medium. The cells were transferred to culture plates at an inoculation density of 30,000 - 50,000 cells/cm<sup>2</sup> and incubated in the inoculation medium at 37°C and 5% CO<sub>2</sub> for 16 - 24 hours for optimal cell adhesion. Once cells adhered, they were washed twice with warm PBS to eliminate non-adherent cells. A preadipocyte basal culture medium was prepared by adding insulin (66 nmol), triiodo-L-tyrosine (1 nmol), transferrin (10 mg/mL), and

gentamicin (50 mg/mL) to the basal medium to promote preadipocyte production. The cells in the culture plates were observed daily under an inverted microscope. Spindle-shaped preadipocytes reached confluence in the culture plate within 7 - 9 days, at which point the cells were detached from the plates using trypsin/EDTA solution. The cells were recounted, and the solution was diluted with PBS to a cell density of 10,000 - 30,000 cells/mL. Cell suspensions were then incubated with 5 mL of immunofluorescent dye PKH26 (Sigma, St. Louis, MO, USA) for five minutes. Residual dye was removed by washing the cells three times with PBS solution.

#### 3.4. Preparation of the Adipocyte Suspensions

The isolation and primary culture of preadipocytes were conducted following the method proposed by van Harmelen et al. (4). Fat tissue samples were placed in Petri dishes containing PBS within a laminar flow cabinet and finely dissected with scissors. Visible connective tissue, debris, and blood vessels were removed. The adipose tissue fragments were transferred to tubes, and collagenase solution was added. The sealed tubes were placed horizontally in a shaker bath at 37°C for approximately 90 minutes to facilitate digestion. At the end of this process, a homogeneous adipocyte solution was obtained. The cells were counted using a Neubauer hemocytometer and diluted with PBS to achieve a cell density of 10,000 - 30,000 cells/mL. The cell suspension was incubated with 5 mL of immunofluorescent dye PKH26 for five minutes. Residual dye was removed by washing the cells three times with PBS solution.

#### 3.5. Graft Implantation

After shaving the scapular region and marking each injection site, a 1 mL preadipocyte suspension was introduced subcutaneously on the right side of the scapular area using a 21-gauge needle attached to a 10 mL syringe. A vacuum effect was applied to the tissue by pulling the syringe plunger upwards, and 1 mL of the preadipocyte suspension was slowly injected into the right scapular area. The vacuum application was maintained for 3 minutes to prevent the spread of the cell suspensions from the subcutaneous tissue. Using the same method, 1 mL of adipocyte suspension was injected into the left scapular area with an 18-gauge

needle. The injection sites on the right and left scapular areas were excised at 3, 6, 10, and 22 weeks, according to each group's timeline.

#### 3.6. Evaluation

##### 3.6.1. Volumetric Evaluation

Right and left scapular fat tissue corresponding to the injection sites was excised under general anesthesia, and the width, length, and height were measured. The hemi-ellipse formula  $[(2/3\pi) \times (a) \times (b) \times (c)]$  was used to evaluate the volume of the excised tissue, where a, b, and c represent the width, length, and height radius of the excised tissue, respectively. The volumes of the excised fat tissues, where preadipocyte cell suspensions and adipocyte cell suspensions were grafted, were calculated in each group and compared.

##### 3.6.2. Histologic Evaluation

Tissue samples were divided into two portions. One portion was frozen at -40°C, and 4 µm thick sections were prepared for assessment with the immunofluorescent dye PKH26 (Sigma, St. Louis, MO, USA). Stained specimens were evaluated under fluorescence microscopy.

The remaining tissue samples were immersed in 10% neutral buffered formalin, subsequently embedded in paraffin, and sectioned into 5 µm slices. Slides were processed for histological staining with hematoxylin and eosin. After staining, an experienced histologist, blinded to the experimental conditions, evaluated the slides using an Olympus IX71 microscope (Olympus, Center Valley, PA). The samples were assessed for indicators such as necrosis, fibrosis, vascularity changes, cyst formation, and inflammatory cell density, with scores ranging from 0 to 2 assigned for each parameter, as outlined in Table 1. Total histopathology scores were calculated for both preadipocyte and adipocyte grafts within each experimental group.

#### 3.7. Statistical Analysis

All results were expressed as mean ± SD and median (minimum-maximum). Statistical analysis was performed using GraphPad InStat 3.0 (San Diego, CA, USA) and SPSS 17.0 statistical software. One-way analysis of variance (ANOVA) and Tukey's multiple comparison

**Table 1.** Histopathological Scoring System Scale

Criteria	Score		
	0	1	2
Necrosis	None	Mild	Severe
Fibrosis	None	Mild	Severe
Vascularity	Increased	Normal	Mild
Cyst formation	None	Visible	-
Inflammatory cell density	Mild	Normal	Increased

post hoc test were used for intra-group comparisons, while Wilcoxon's rank-sum test was employed for comparing adipocyte and preadipocyte groups across the different weeks. Significance was accepted at  $P < 0.05$ .

## 4. Results

### 4.1. Assessment of Graft Volume Retention

Animals were sacrificed at designated weeks according to their groups, and grafts were dissected. When the volumes of preadipocyte and adipocyte grafts were compared, a noticeable decline in volume was observed over time in both tissue types. This volumetric reduction was more pronounced in adipocyte grafts; however, despite these differences, they were not statistically significant in group 1 (3 weeks), group 2 (6 weeks), and group 3 (10 weeks). In group 4 (22 weeks), however, preadipocytes retained their size, and the volumetric loss in adipocyte grafts was significantly more substantial compared to the preadipocyte grafts ( $0.09 \pm 0.01$  vs.  $0.05 \pm 0.02$ ,  $P < 0.05$ ). A comprehensive overview of graft volumes for both preadipocytes and adipocytes is provided in [Table 2](#).

### 4.2. Histopathological Results

Immunologic evaluation using PKH26 (Sigma, St. Louis, MO, USA) confirmed that the grafted preadipocytes and adipocytes remained viable and well-preserved at the recipient site. Histopathological examination provided crucial insights into the immunologic response of preadipocytes and adipocytes following grafting, revealing that preadipocytes exhibited significantly lower total histological scores compared to adipocytes across all experimental groups ([Table 3](#)). These findings indicate a notable discrepancy in graft outcomes between the two cell types ([Figure 1](#)).

## 5. Discussion

The term "soft tissue defect" refers to a deficiency in connective tissue at any level, often leading to disruption of the natural tissue contour (5). Despite advancements in medical knowledge and technological innovations in correcting soft tissue defects, these remain challenging for surgeons, with complexity heightened in pediatric patients. The limited amount of adipose tissue, combined with concerns about altering the contour of the donor site to achieve better cosmesis elsewhere, makes autologous adipose grafting a significant dilemma, especially in the pediatric population.

Adipose tissue-derived stem cells have gained widespread use in cellular therapy due to their remarkable ability to differentiate into diverse cell types and their significant anti-inflammatory, anti-fibrotic, and pro-angiogenic effects (6). Both in vivo and in vitro studies have demonstrated preadipocytes' capacity to differentiate into mature adipose cells (7). In contrast to the limited regenerative capabilities of adipocytes, preadipocytes offer greater potential for soft tissue augmentation due to their ability to differentiate and proliferate throughout their entire lifespan (8).

The ultimate success of fat grafting depends on efficient revascularization and histological harmony between donor and recipient sites. Post-graft inflammation, fibrosis, and cyst formation are extensively studied in the literature. Trotzier et al. assert that the inflammatory response at the donor site reduces angiogenesis and blood flow to the graft, ultimately diminishing graft survival (9). Conversely, preadipocytes do not typically elicit a host reaction or provoke an inflammatory response, focal cell necrosis, cyst formation, or fibrosis in experimental studies (10, 11). Bellini et al. attribute preadipocytes' resistance to

**Table 2.** Volumetric Analysis and Preadipocyte and Adipocyte Grafts <sup>a</sup>

Groups and Categories	Mean ± SD	Median	Minimum-Maximum
<b>I</b>			
Preadipocyte	0.13 ± 0.05	0.12	0.06 - 0.21
Adipocyte	0.12 ± 0.06	0.12	0.01 - 0.21
<b>II</b>			
Preadipocyte	0.12 ± 0.06	0.13	0.06 - 0.21
Adipocyte	0.11 ± 0.06	0.11	0.03 - 0.21
<b>III</b>			
Preadipocyte	0.09 ± 0.05	0.08	0.03 - 0.20
Adipocyte	0.08 ± 0.02	0.09	0.03 - 0.10
<b>IV</b>			
Preadipocyte	0.09 ± 0.01 <sup>b</sup>	0.10	0.07 - 0.12
Adipocyte	0.05 ± 0.02 <sup>b</sup>	0.05	0.03 - 0.09

<sup>a</sup> Values are presented as number (cm<sup>3</sup>).

<sup>b</sup> P < 0.05.

**Table 3.** Histopathological Total Scores of Preadipocytes and Adipocytes Within Groups

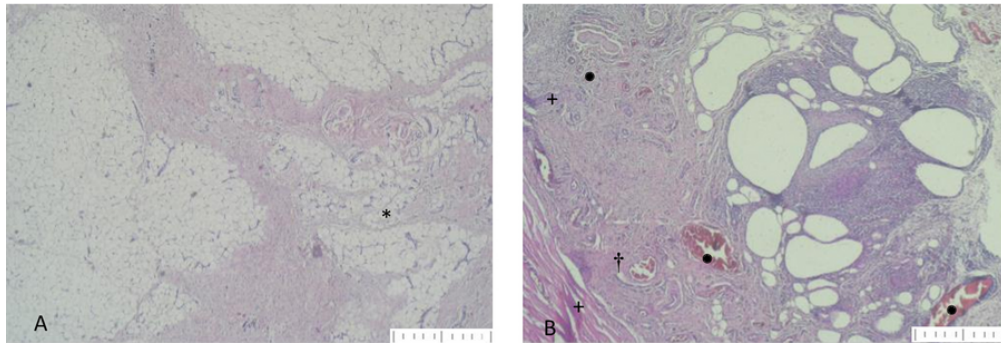
Groups and Categories	Mean ± SD	Median	Minimum-Maximum
<b>I</b>			
Preadipocyte	4.14 ± 1.46	4.0	2.0 - 6.0
Adipocyte	7.00 ± 2.16	8.0	3.0 - 9.0
<b>II</b>			
Preadipocyte	3.28 ± 0.48	3.0	3.0 - 4.0
Adipocyte	5.75 ± 1.58	6.0	3.0 - 8.0
<b>III</b>			
Preadipocyte	2.75 ± 0.89	2.5	2.0 - 4.0
Adipocyte	5.42 ± 1.39	6.0	3.0 - 7.0
<b>IV</b>			
Preadipocyte	2.85 ± 0.76	3.0	2.0 - 4.0
Adipocyte	4.71 ± 1.53	4.0	3.0 - 8.0

hypoxia to the inherent characteristics of progenitor cells (12). Their minimal metabolic activity and lower oxygen consumption rates enable them to endure extended periods without nutritional supply.

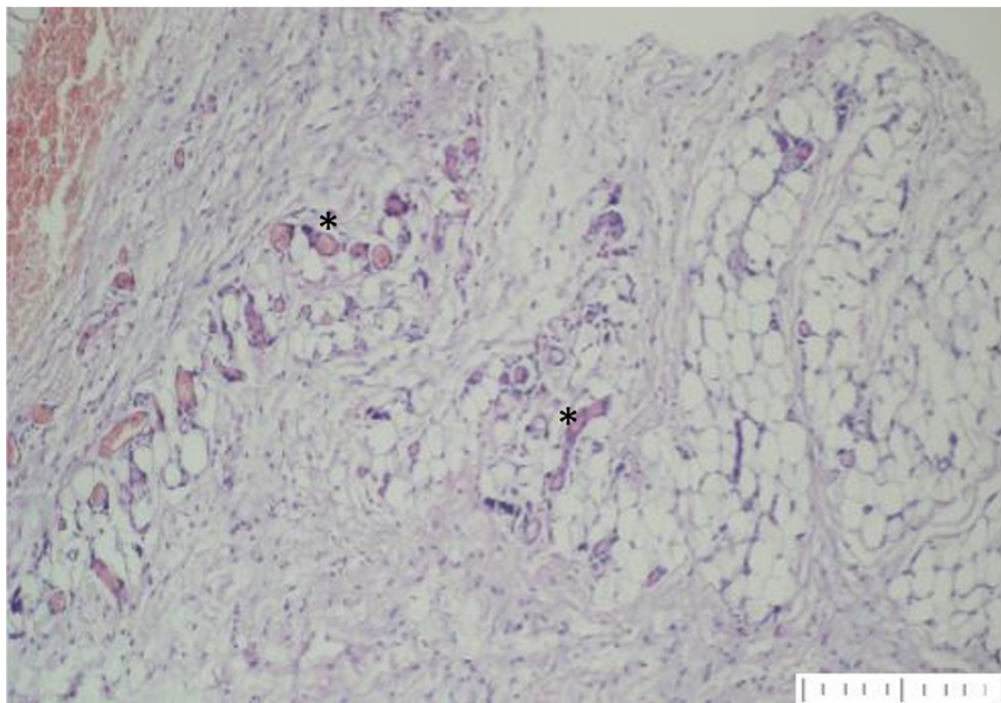
In this study, histopathologic assessment was conducted using a total scoring system consisting of necrosis, fibrosis, cyst formation, vascularity, and inflammatory cell density. Notably, the scores for preadipocyte groups were significantly better than those for adipocyte groups across all cohorts (P < 0.05). PKH26 evaluation played a pivotal role in reaffirming the viability and robust protection of grafted preadipocytes and adipocytes within the recipient site. Our comprehensive histopathological examination

provided valuable insights into the subsequent immunologic responses of these cell types following grafting. It was consistently observed that preadipocytes exhibited significantly lower total histological scores than adipocytes across all experimental groups. This significant difference highlights a substantial variance in graft outcomes between these two cell populations, likely influenced by variations in immune system interactions, cellular behaviors, or microenvironmental factors.

Since the initial use of fat grafting by Neuber, graft resorption has remained a significant concern (13). Numerous volumetric analyses consistently reveal a relatively modest fat graft survival rate, ranging from 50



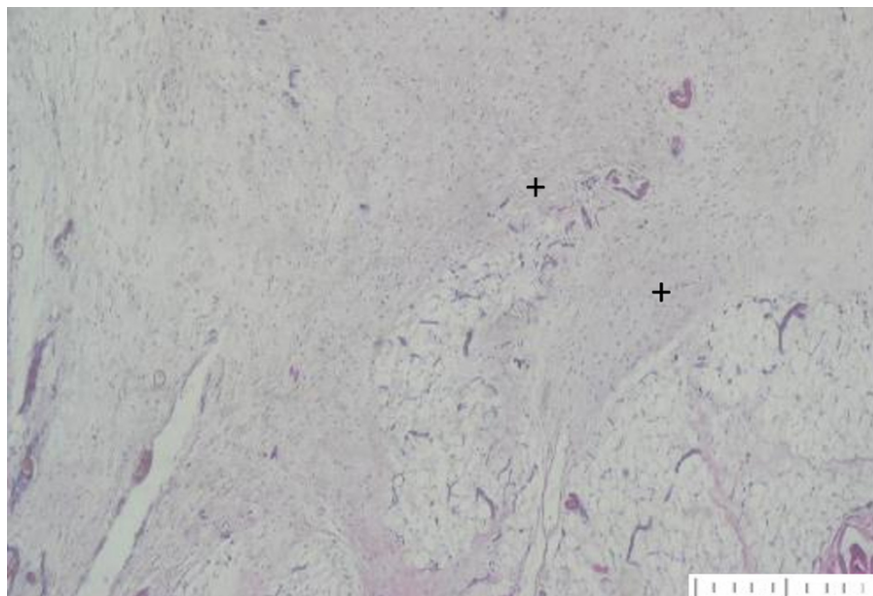
**Figure 1.** A, \*: Fat tissue structure formation from the preadipocyte grafts in group 4 (H&E stain, × 40 magnification); B, cyst formation from adipose grafts from group 4. †: Infiltration of inflammatory cells around the cystic areas; +: Necrosis formation; •: Vascular structures. (H&E stain, × 40 magnification)



**Figure 2.** The sample obtained from the preadipocyte grafts in group 3, \*: Vascular structures around the fat cells (H&E stain, × 40 magnification)

to 65% (14, 15). In clinical practice, Coleman applied his self-developed technique of structural fat grafting through facial infiltration in a cohort of 400 patients, reporting that grafts were effectively maintained over 6 years; however, graft preservation was assessed photographically (16).

In an experimental study, Schoeller et al. implemented 1 mL of preadipocytes into a fibrous capsule within the rectus muscles of rats and evaluated fat grafts at 1, 3, 6, and 12 months. He observed no fibrosis or abscess formation, and grafted fat volumes were sustained in all groups (17). Various theories, such



**Figure 3.** The sample obtained from the adipocyte graft in group 3, +: The area of fibrosis adjacent to adipose tissue (H&E stain, × 40 magnification)

as apoptosis due to insufficient vascularization of centrally located fat cells and trauma-induced damage to the grafts, have been proposed to explain graft resorption (18, 19). After adipose tissue transplantation, a hypoxic state persists until capillary circulation is established, with nutrition provided by diffusion from adjacent tissue during this period.

Yoshimura et al. found that while adipocytes are highly susceptible to hypoxia and die within 24 hours, adipose tissue-derived stem cells remain functional for up to 72 hours. Similarly, Zhu et al. determined that preadipocytes enhance graft retention and quality by promoting angiogenesis and preventing apoptosis through growth factor expression (20, 21). Our histologic evaluation confirmed the presence of well-organized vascularization around preadipocyte grafts (Figure 2) and fibrosis formation around adipocyte grafts (Figure 3).

### 5.1. Conclusions

Our study highlights the significant differences in the behavior of preadipocytes and adipocytes in grafting procedures, emphasizing the need for

thorough assessment in cellular therapies. The findings reveal that grafted preadipocytes can sustain their volume over the duration of this experiment, whereas volumetric loss increased in adipocyte grafts. This suggests promising potential for autologous preadipocyte grafts in enhancing wound healing, addressing postoperative scars, and correcting congenital defects, including cleft palates, chest wall, and breast deformities (22, 23).

Given the challenges associated with fat grafts, particularly in pediatric patients with limited donor tissue, the use of adipose tissue-derived stem cells presents a transformative opportunity for soft tissue augmentation. Our results support further exploration into the application of preadipocytes in clinical practice, which may ultimately enhance efficacy and safety in regenerative medicine.

### Footnotes

**Authors' Contribution:** E. B. C. K. and S. O.: Designed the study; K. D. K.: Performed histopathologic studies; E. B. C. K. and S. O.: Collected and analyzed data; E. B. C. K.: Wrote the manuscript; E. C.: Provided technical support

and conceptual advice. All authors read and approved the final version of the manuscript.

**Conflict of Interests Statement:** The authors declared no conflict of interests.

**Data Availability:** The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to ethical considerations.

**Ethical Approval:** This study was approved by Adnan Menderes University Ethical Committee (NO:B.30.2.ADU.0.06.00.00/124-HEK/2008/047) the paperwork in uploaded as pdf file.

**Funding/Support:** This study did not received any funding.

## References

- Mughal M, Sindali K, Man J, Roblin P. 'Fat chance': a review of adipose tissue engineering and its role in plastic and reconstructive surgery. *Ann R Coll Surg Engl*. 2021;**103**(4):245-9. [PubMed ID: 33682428]. [PubMed Central ID: PMC10335005]. <https://doi.org/10.1308/rcsann.2020.7031>.
- Cypess AM. Reassessing Human Adipose Tissue. *N Engl J Med*. 2022;**386**(8):768-79. [PubMed ID: 35196429]. <https://doi.org/10.1056/NEJMra2032804>.
- Thomaz FM, de Jesus Simao J, da Silva VS, Machado MMF, Oyama LM, Ribeiro EB, et al. Ginkgo biloba Extract Stimulates Adipogenesis in 3T3-L1 Preadipocytes. *Pharmaceutic (Basel)*. 2022;**15**(10). [PubMed ID: 36297406]. [PubMed Central ID: PMC9610090]. <https://doi.org/10.3390/ph15101294>.
- van Harmelen V, Skurk T, Hauner H. Primary Culture and Differentiation of Human Adipocyte Precursor Cells. *Human Cell Cult Protocol*. **107**. New Jersey, US: Humana Press; 2004. p. 125-36. <https://doi.org/10.1385/j-59259-861-7:125>.
- Gentile P, Garcovich S. Systematic Review: Adipose-Derived Mesenchymal Stem Cells, Platelet-Rich Plasma and Biomaterials as New Regenerative Strategies in Chronic Skin Wounds and Soft Tissue Defects. *Int J Mol Sci*. 2021;**22**(4). [PubMed ID: 33546464]. [PubMed Central ID: PMC7913648]. <https://doi.org/10.3390/ijms22041538>.
- Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, Slepicka P, et al. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. *Biotechnol Adv*. 2018;**36**(4):1111-26. [PubMed ID: 29563048]. <https://doi.org/10.1016/j.biotechadv.2018.03.011>.
- Nic-Can GI, Rodas-Junco BA, Carrillo-Cocom LM, Zepeda-Pedreguera A, Penalzoa-Cuevas R, Aguilar-Ayala FJ, et al. Epigenetic Regulation of Adipogenic Differentiation by Histone Lysine Demethylation. *Int J Mol Sci*. 2019;**20**(16). [PubMed ID: 31408999]. [PubMed Central ID: PMC6719019]. <https://doi.org/10.3390/ijms20163918>.
- Ambele MA, Dhanraj P, Giles R, Pepper MS. Adipogenesis: A Complex Interplay of Multiple Molecular Determinants and Pathways. *Int J Mol Sci*. 2020;**21**(12). [PubMed ID: 32560163]. [PubMed Central ID: PMC7349855]. <https://doi.org/10.3390/ijms21124283>.
- Trotzier C, Sequeira I, Auxenfans C, Mojallal AA. Fat Graft Retention: Adipose Tissue, Adipose-Derived Stem Cells, and Aging. *Plast Reconstr Surg*. 2023;**151**(3):420-31. [PubMed ID: 36730531]. <https://doi.org/10.1097/PRS.00000000000009918>.
- Surowiecka A, Struzyna J. Adipose-Derived Stem Cells for Facial Rejuvenation. *J Pers Med*. 2022;**12**(1). [PubMed ID: 35055432]. [PubMed Central ID: PMC8781097]. <https://doi.org/10.3390/jpm12010117>.
- Zhang Y, Cai J, Zhou T, Yao Y, Dong Z, Lu F. Improved Long-Term Volume Retention of Stromal Vascular Fraction Gel Grafting with Enhanced Angiogenesis and Adipogenesis. *Plast Reconstr Surg*. 2018;**141**(5):676-86. [PubMed ID: 29334574]. <https://doi.org/10.1097/PRS.0000000000004312>.
- Bellini E, Grieco MP, Raposio E. The science behind autologous fat grafting. *Ann Med Surg (Lond)*. 2017;**24**:65-73. [PubMed ID: 29188051]. [PubMed Central ID: PMC5694962]. <https://doi.org/10.1016/j.amsu.2017.11.001>.
- Shih L, Davis MJ, Winocour SJ. The Science of Fat Grafting. *Semin Plast Surg*. 2020;**34**(1):5-10. [PubMed ID: 32071573]. [PubMed Central ID: PMC7023968]. <https://doi.org/10.1055/s-0039-3402073>.
- Huang RL, Xie Y, Wang W, Tan P, Li Q. Long-term Outcomes of Temporal Hollowing Augmentation by Targeted Volume Restoration of Fat Compartments in Chinese Adults. *JAMA Facial Plast Surg*. 2018;**20**(5):387-93. [PubMed ID: 29710230]. [PubMed Central ID: PMC6233616]. <https://doi.org/10.1001/jamafacial.2018.0165>.
- Wang W, Xie Y, Huang RL, Zhou J, Tanja H, Zhao P, et al. Facial Contouring by Targeted Restoration of Facial Fat Compartment Volume: The Midface. *Plast Reconstr Surg*. 2017;**139**(3):563-72. [PubMed ID: 28234822]. <https://doi.org/10.1097/PRS.0000000000003160>.
- Coleman SR. Facial augmentation with structural fat grafting. *Clin Plast Surg*. 2006;**33**(4):567-77. [PubMed ID: 17085224]. <https://doi.org/10.1016/j.cps.2006.09.002>.
- Schoeller T, Lille S, Wechselberger G, Otto A, Mowlavi A, Piza-Katzer H. Histomorphologic and volumetric analysis of implanted autologous preadipocyte cultures suspended in fibrin glue: a potential new source for tissue augmentation. *Aesthetic Plast Surg*. 2001;**25**(1):57-63. [PubMed ID: 11322400]. <https://doi.org/10.1007/s002660010096>.
- Stillaert F, Depypere B, Doornaert M, Creyten D, De Clercq H, Cornelissen R, et al. Autologous plasma and its supporting role in fat graft survival: A relevant vector to counteract resorption in lipofilling. *J Plastic Reconstr Aesthetic Surg*. 2016;**69**(7):952-8. <https://doi.org/10.1016/j.bjps.2016.03.014>.
- Gao S, Lu B, Zhou R, Gao W. Research progress of mechanisms of fat necrosis after autologous fat grafting: A review. *Med (Baltimore)*. 2023;**102**(10). e33220. [PubMed ID: 36897702]. [PubMed Central ID: PMC9997804]. <https://doi.org/10.1097/MD.00000000000033220>.
- Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: roles in adipose tissue remodeling and potential use for soft tissue augmentation. *Regen Med*. 2009;**4**(2):265-73. [PubMed ID: 19317645]. <https://doi.org/10.2217/17460751.4.2.265>.
- Zhu M, Zhou Z, Chen Y, Schreiber R, Ransom JT, Fraser JK, et al. Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention. *Ann Plast Surg*. 2010;**64**(2):222-8. [PubMed ID: 20098110]. <https://doi.org/10.1097/SAP.0b013e31819ae05c>.



22. Shih L, Abu-Ghname A, Davis MJ, Xue AS, Dempsey RF, Buchanan EP. Applications of Fat Grafting in Pediatric Patients. *Semin Plast Surg.* 2020;**34**(1):53-8. [PubMed ID: [32071580](#)]. [PubMed Central ID: [PMC7023971](#)]. <https://doi.org/10.1055/s-0039-3402517>.
23. Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. *Ann Med Surg (Lond).* 2017;**20**:49-60. [PubMed ID: [28702187](#)]. [PubMed Central ID: [PMC5491488](#)]. <https://doi.org/10.1016/j.amsu.2017.06.059>.