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Original Article

The effect of host tissue and radiation on fat-graft survival: A comparative experimental study ☆

Antonios Tsimponis [a,*], Dimitrios Dionyssiou [a], Theodora Papamitsou [b], Efterpi Demiri [a]

[a] Department of Plastic Surgery, Aristotle University of Thessaloniki, Papageorgiou General Hospital, Thessaloniki Greece
[b] Laboratory of Histology-Embryology, Aristotle University of Thessaloniki, Greece

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ABSTRACT

Because lipofilling is often associated with various reconstructive procedures, especially breast reconstructions, improving fat-graft retention remains a major concern for plastic surgeons. We conducted an experimental protocol in a rat model simulating an autologous breast reconstruction method using the fat-augmented latissimus dorsi myocutaneous (LDM) flap. This study aimed to compare the survival rates of autologous adipocytes when injected subcutaneously and intramuscularly and to evaluate the role of recipient host tissue, volume of the injected fat, and postoperative radiation on fat-graft retention.

Thirty rats were divided into five groups (A, B, C, D, and E), of six rats each. All animals underwent a pedicled LDM flap transfer to the anterior thoracic wall, and different volumes of autologous fat were injected into three recipient areas, namely, the pectoralis major and latissimus dorsi muscles and the subcutaneous tissue of the flap's skin island, as follows: 1 mL of fat was injected in total in group A, 2 mL in groups B and D, and 5 mL in group C. Group D animals received postoperative radiation (24 Gy), whereas group E

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* Correspondence to: Antonios TSIMPONIS, Plastic Surgeon, Department of Plastic Surgery, Aristotle University of Thessaloniki, Papageorgiou General Hospital, Periferiaki Odos Thessaloniki, 56403, Greece, Tel: +306948 466179
E-mail address: atsimponis@gmail.com (A. Tsimponis).

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animals (controls) did not undergo any fat grafting procedure. Eight weeks after surgery, adipocyte survival was assessed in all groups using histological and immunochemistry techniques.

The results showed that the pectoralis major muscle was the substrate with the highest adipocyte survival rates, which were proportional to the amount of fat injected, followed by the latissimus dorsi muscle and the subcutaneous tissue. Increased volumes of transplanted fat into the subcutaneous tissue did not correspond to increased adipocyte survival. Irradiation of host tissues resulted in a statistically significant decrease in surviving adipocytes in all three recipient sites ($p<0.001$). Our study strongly suggests that muscle ensures optimal fat-graft retention, whereas postoperative radiation negatively affects adipocyte survival following fat transplantation.

Introduction

Adipose tissue, as a tool of regenerative medicine, reconstructive surgery, and tissue engineering, has dominated medical and biotechnology developments during recent decades.[1] Autologous fat transfer is one of the most popular procedures in both reconstructive and aesthetic surgery. In 2009, fat grafting accounted for 5.9% of plastic surgery operations, and in 2020, liposuction associated with or without fat grafting ranked fourth among all procedures performed by plastic surgeons according to statistics from the American Society of Plastic Surgeons.[2,3]

The applications of fat transplantation in the past four decades have significantly increased, and adipose tissue is now considered an important tool both for its use in replacing and filling volume defects and for its regenerative properties as it promotes healing and tissue regeneration.[4-6]

Autologous fat transplantation is a field of constantly evolving research aimed at establishing the ideal method, which ensures maximum efficiency and stable long-term results.[7,8] However, despite increasing knowledge on fat harvesting, processing, and transfer, little is known about fat uptake at the microscopic level and the role of host tissue in the overall fat-graft retention, as well as the role of radiation in the areas that receive fat grafting.

The aim of our experimental study is to evaluate and compare the survival rates of autologous fat graft injected subcutaneously and into muscle tissues in irradiated and non-irradiated rats, an experimental model that simulates a breast reconstruction method with the autologous LDM flap augmented with fat.

Materials and methods

Our experimental study was carried out at the Experimental and Research Center of Papageorgiou Hospital in Thessaloniki and was compliant with the national regulations for animal experimental studies (ethical approval No. 13/9658). A total of 30 adult female Albino Wistar rats, weighing 500-600 g, were included in the protocol and were randomly divided into five groups, A, B, C, D, and E, each containing six animals.

In all groups, a latissimus dorsi (LD) myocutaneous flap was raised and transferred to the anterior thoracic wall, simulating a breast reconstruction model. Different volumes of autologous fat (group A=1 mL, group B=2 mL, group C=5 mL, group D=2 mL) were harvested and equally injected into the LD muscle, the subcutaneous tissue of the LD skin island, and the pectoralis major (PM) muscle;

group D rats were irradiated with a single dose of 24 Gy, 1 week after surgery. Group E rats (control group) did not undergo the lipotransfer procedure.

Surgical technique

All animals were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). In groups A, B, C, and D, fat was harvested from both inguinal areas through 2-cm skin incisions, under aseptic conditions[9]; a total of 2-2.5 mL of subcutaneous inguinal fat was removed, depending on the group of each animal (Fig. 1). The fat was minced using a No. 11 blade and then liquefied by successive passes through two 1-mL Luer-lock syringes using a three-tube system. The processed fat was then washed with sterile saline to remove any remaining blood clots and/or fibrous elements. Group E rats were not subjected to the fat harvesting procedure.

On the right anterior thoracic wall of animals of all groups, a 2 × 1 cm skin spindle was excised with a No. 15 scalpel. This was followed by the removal of the underlying subcutaneous tissue and preparation of the superficial surface of the PM muscle at the level of the muscle fascia.

By changing the position of the rat from supine to prone, a 2 × 1 cm fusiform skin island was drawn on the lower right dorsal region, over the LD muscle, with a slight downward and medial direction. The LDM flap was elevated according to the standard technique, with complete detachment from the underlying fascia and surrounding muscles (Fig. 2). After raising the flap on its vascular pedicle, a subcutaneous tunnel was prepared, and the flap was transferred through the tunnel into the anterior thoracic area.

Using 1 mL syringes with an 18G metal cannula, the harvested autologous fat was equally injected in linear deposition into (a) the subcutaneous tissue of the skin island of the LD flap, (b) the LD muscle, and (c) the PM muscle (Fig. 3). Depending on the animal group, fat was injected as follows:

Group A: 0.33 mL subcutaneously, 0.33 mL into the LD muscle, 0.33 mL into the PM muscle (total 1 mL)

Figure 1. Autologous fat harvesting of from both inguinal areas

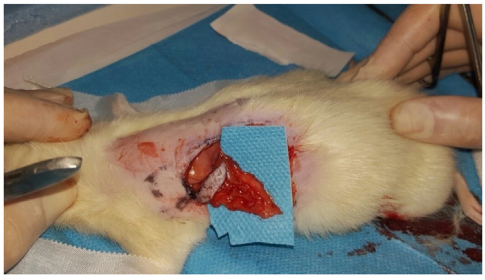


Figure 2. Dissection of the LD myocutaneous flap

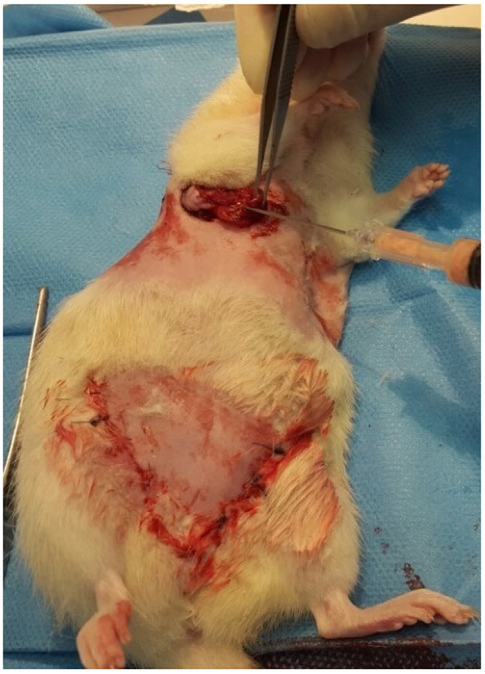


Figure 3. Injection of autologous fat into the LD muscle, after transfer of the flap to the anterior chest

Group B: 0.66 mL subcutaneously, 0.66 mL into the LD muscle, 0.66 mL into the PM muscle (total 2 mL)
Group C: 1.66 mL subcutaneously, 1.66 mL into the LD muscle, 1.66 mL into the PM muscle (total 5 mL)
Group D: 0.66 mL subcutaneously, 0.66 mL into the LD muscle, 0.66 mL into the PM muscle (total 2 mL)

After the procedure was completed, the skin incisions were stitched intradermally with 4-0 absorbable sutures. All animals were individually housed, with ad libitum access to food and water.

On the 8th postoperative day, the animals of group D were put under general anesthesia and transferred to the Radiotherapy department where a single radiation dose of 24 Gy was administered to the anterior thoracic wall (Fig. 4). During the irradiation procedure, special parameters were set, and care was taken to irradiate only the superficial tissues of the chest.[10,11]

All animals were sacrificed at postoperative week 8, and samples from the subcutaneous tissue, LD, and PM muscles were collected for histological and immunohistochemical assessment for fat-graft survival. Table 1 illustrates the flow diagram of our study.

Histological Study

All samples were fixed in 10% buffered formalin, embedded in paraffin, and sectioned at 4 μm. Hematoxylin and eosin (H&E) and immunohistochemical staining (anti-perilipin monoclonal antibody) were performed.

In H&E stained sections, the presence of viable fat cells was evaluated and scored on a scale of 0 to 3: 0 for absence, 1 for minimal presence, 2 for moderate presence, and 3 for extensive presence.

In immunohistochemical sections, cells taking up the perilipin antibodies were considered viable in contrast to those that did not take up or weakly took up the antibodies. Thus, three categories of cells were distinguished: the strongly positive round-shaped cells that were considered alive, the weakly

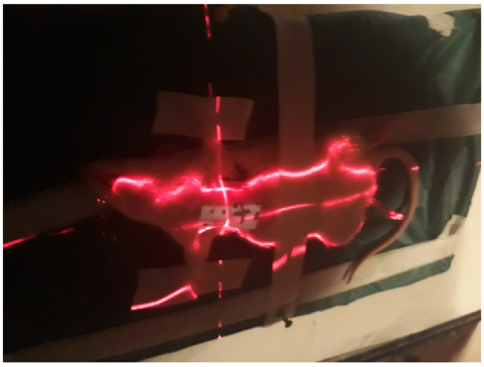
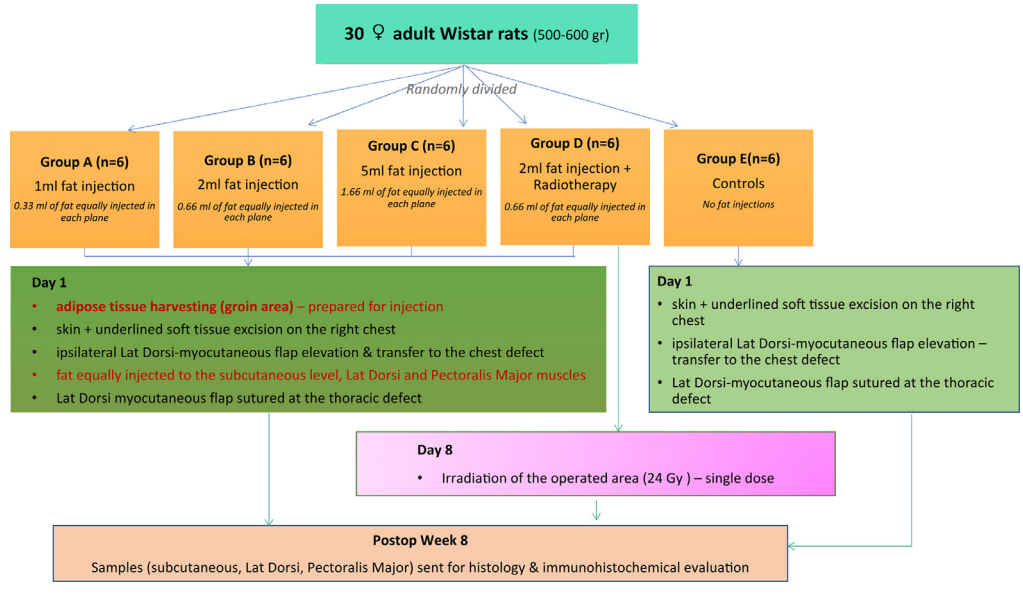


Figure 4. Postoperative administration of radiotherapy on the superficial tissues of the anterior thoracic area

Table 1
Flow chart of our experimental study.



positive or positive cells with an irregular shape that formed the group of adipocytes undergoing apoptosis, and the cells negative for perilipin antibody binding that were characterized as dead cells. For our study, only strongly perilipin antibody-positive cells were included in the study as viable cells.

Fat cell counting methodology

Images of H&E and perilipin immunochemistry sections were viewed and analyzed with Image J software.[12] Three separate optical microscope images (× 40 magnification) were randomly taken for each transplanted area; the subcutaneous tissue of the skin island of the LD flap, the LD muscle, and the PM muscle were imported into Image J open-source software, and the mean viable adipocytes were recorded and statistically analyzed.

Statistical Analysis

We used SPSS data package (version 26) to analyze results. The quantitative variables studied were the mean viable adipocytes for each of the transplanted sites and for each group of animals. All quantitative variables were initially tested for normality with the Shapiro–Wilk test. Comparisons of variables were made according to their parametricity with either the independent Student's t-test or with the Mann-Whitney U test. For multiple comparisons of quantitative variables, comparisons were made with the ANOVA and Bonferroni tests. In all comparisons, the level of statistical significance was set at $p<0.05$.

Results

Mean viable adipocytes per field of view were calculated using the open-source Image J software for each group (A, B, C, D, and E) and for each different recipient site, i.e. LD muscle, PM muscle, and subcutaneous tissue (SUB).

According to our results, group C animals (5 mL injected fat) were found to have the highest mean number of viable adipocytes (139.11±87.70) per field including all recipient layers, followed by group B (2 mL injected fat) with 122.44±64.34 viable adipocytes, group A (1 mL injected fat) with 88.06±35.28, and finally, group D (2 mL injected fat) animals, which received postoperative radiation and had a mean number of 79.83±43.27 viable adipocytes per field; a statistically significant difference was observed only in the comparison of groups C and D ($p=0.029$). Table 2 summarizes the mean values per group, including all three recipient sites. In group E animals (no fat injected), no evidence of transplanted adipocytes was documented.

In group A animals (1 mL injected fat), survival of transplanted adipocytes in the LD and the PM were found to be similar (mean values for LD=109 and PM=109.83, $p=1.000$); adipocyte survival when injected into the subcutaneous tissues of the LD-flap skin island (mean value for SUB=45.33) was significantly lower compared to both the LD ($p<0.001$) and PM muscle ($p<0.001$), as shown in Table 3.

In group B (2 mL injected fat), the PM muscle was found to have the highest survival values (Fig. 5) of the transplanted adipocytes (mean value for PM=198.16), followed by the LD muscle (LD=122.5), and the subcutaneous tissue (SUB=46.67), as shown in Table 4. Differences were statistically signif-

Table 2
Summary of the mean values of viable adipocytes per group, including all three recipient sites.

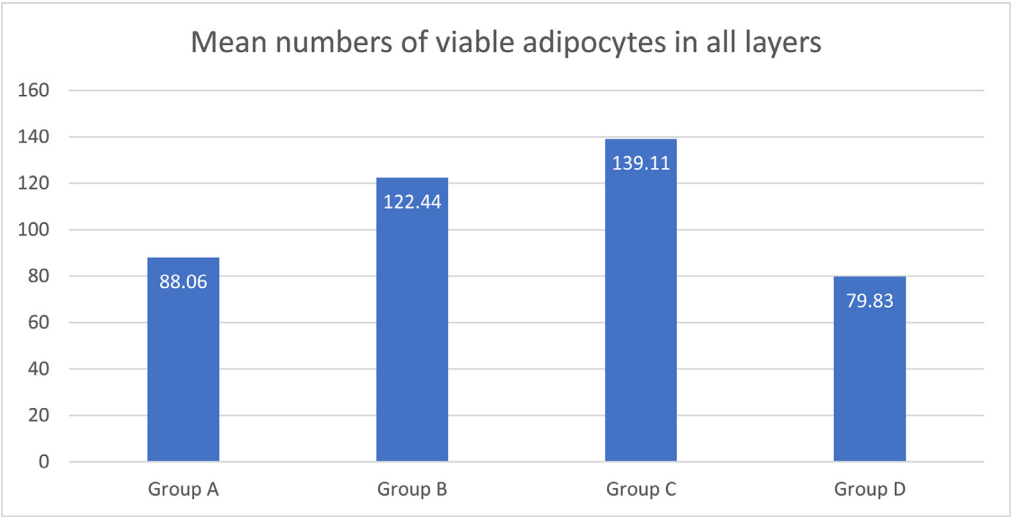
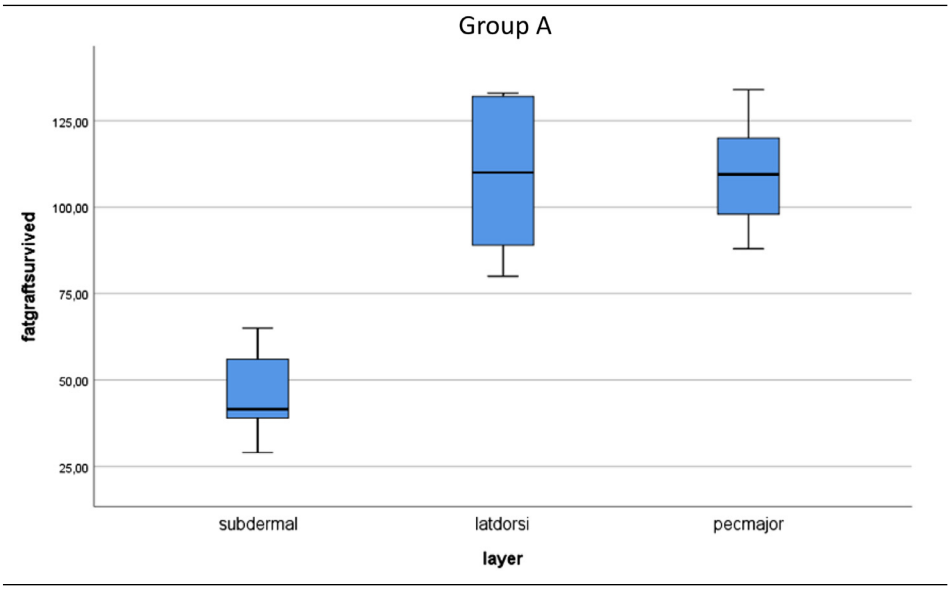


Table 3
Mean values of viable adipocytes per recipient site in Group A.



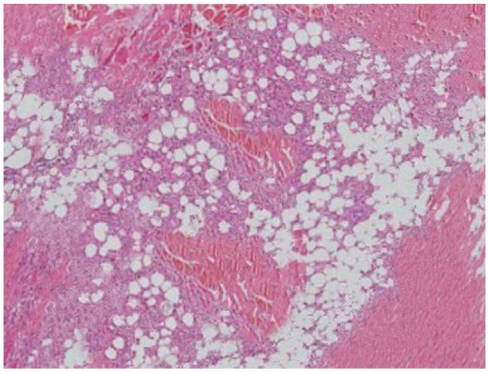
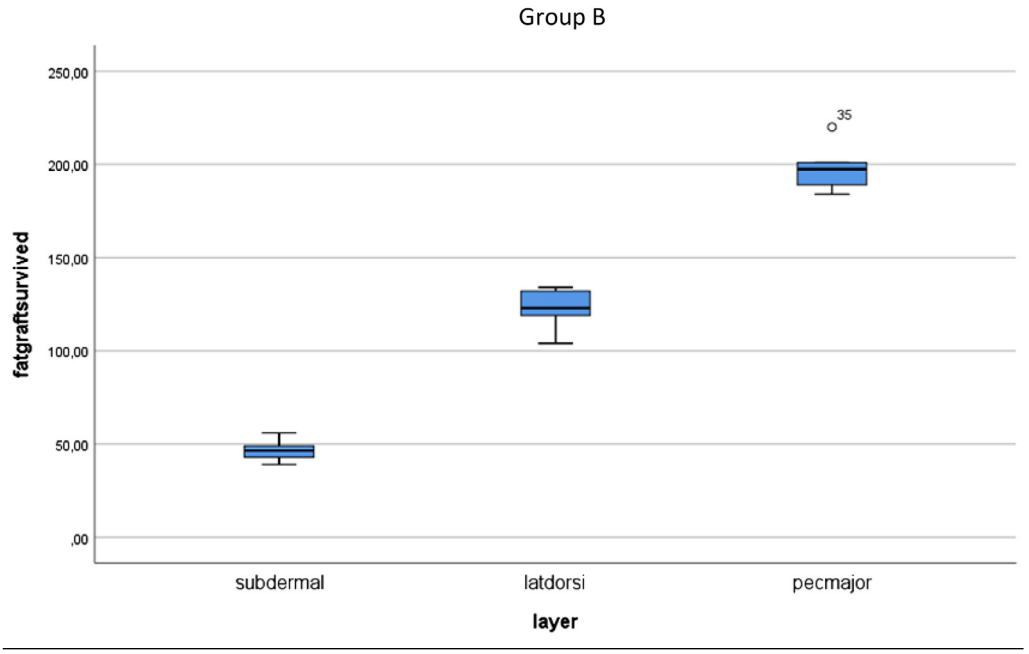


Figure 5. Fat cells present from a PM muscle section in a group B animal (hematoxylin and eosin; magnification × 40)

icant for all individual comparisons (pSUB vs LD <0.001, pSUB vs PM <0.001, pLD vs PM <0.001). Similar to group B were the results in group C (5 mL injected fat); adipocyte survival in the PM muscle was statistically significantly higher (Fig. 6) compared with that of the LD muscle and the subcutaneous tissue (PM=246 vs LD=128.33 vs SUB=43) (p<0.001) (Table 5).

In group D (2 mL injected fat followed by postoperative radiation), the PM muscle provided a better substrate for adipocyte survival (Fig. 7) compared with the LD muscle and the subcutaneous tissue of the flap's skin island (PM=123 vs LD=90.16 vs SUB=26.33) (p<0.001) (Table 6). Mean values of viable adipocytes in group D were significantly lower compared with those in group B (2 mL injected fat without radiation) in all three recipient sites (p<0.001) (Fig. 8).

Table 4
Mean values of viable adipocytes per recipient site in Group B.



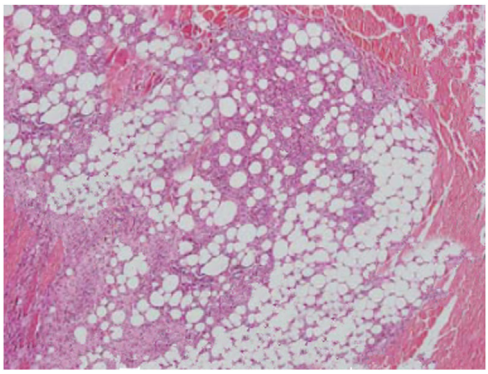


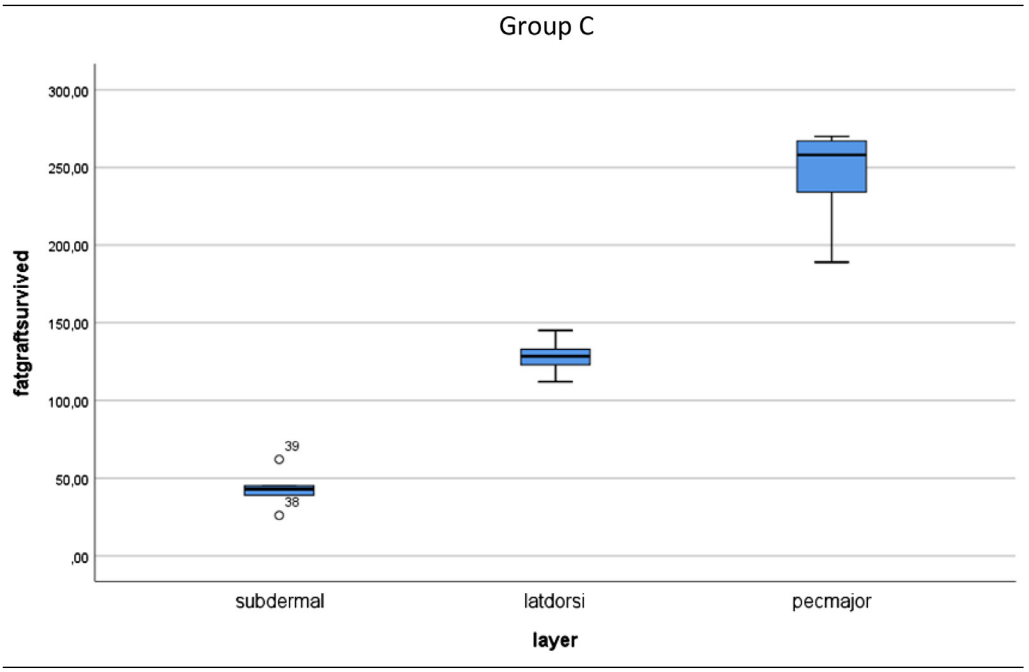
Figure 6. Viable fat cells from a PM muscle section in a group C animal (hematoxylin and eosin; magnification × 40)

Discussion

Improving survival rates and retention of autologous fat grafts after transplantation continues to be a major concern for plastic surgeons, as an increasing number of lipofilling procedures are currently performed in various aesthetic and reconstructive cases. Although increased blood supply to muscle tissue suggests that intramuscular fat injections may be optimal,[13] fat transfer into the muscle has recently been implicated in serious complications, particularly when associated with gluteal augmentation surgeries.[14,15]

Published data have reported variable results regarding the survival of adipose cells injected into the muscle or into various tissue layers in animal models. Guerrerosantos et al[16] were among the first to report increased viability of intramuscularly injected autologous fat grafts at long-term follow-up;

Table 5
Mean values of viable adipocytes per recipient site in Group C.



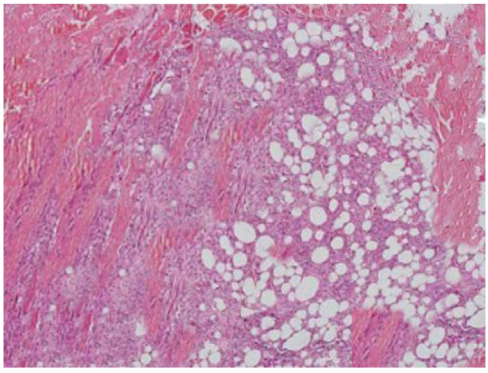
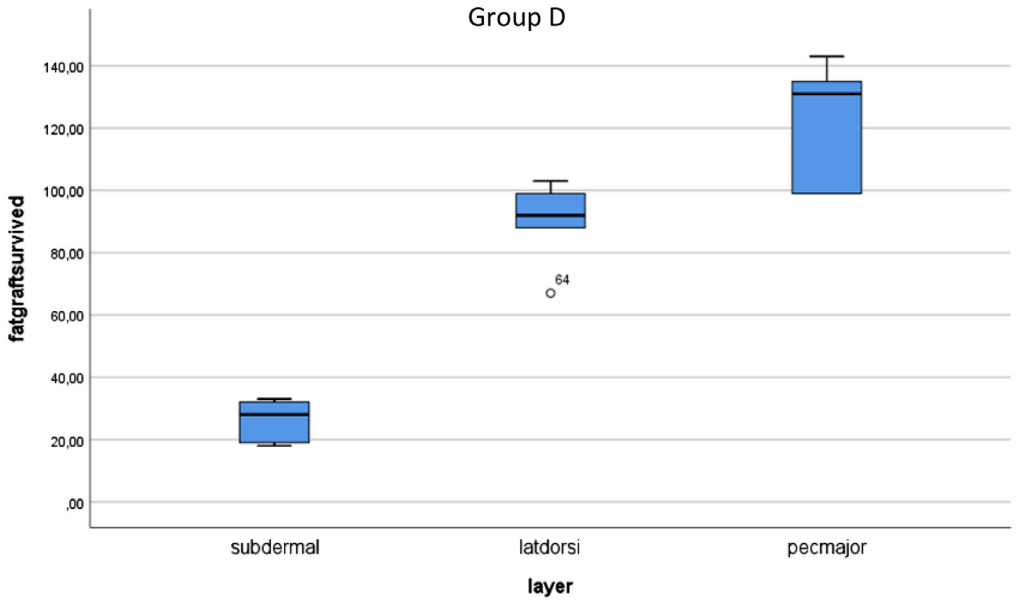


Figure 7. Presence of viable fat cells and fibrosis from a PM muscle section in a group D animal (hematoxylin and eosin; magnification × 40)

the results of this experimental study revealed higher fat-graft survival rates in the muscle compared with subcutaneous tissues, and the results of clinical cases with intramuscular lipofilling for body contouring were also promising.[17] Aygit et al,[18] in an experimental study designed to evaluate the viability and revascularization of intramuscularly injected autologous fat using scintigraphic imaging and histopathological examination, showed adipose tissue uptake greater than 50%. Karacaoglu et al[19] compared the volume retention of fat grafts placed in subcutaneous, supramuscular, and submuscular levels of rabbit facial tissues; their analysis revealed significantly increased fat-graft survival rates in the supramuscular layer (81.95% ±4.40%) compared with subcutaneous (41.62% ± 3.2%) and submuscular (37.31% ±5.77%) ($p<0.01$) levels, which was attributed to the increased vascularization of the

Table 6
Mean values of viable adipocytes per recipient site in Group D.



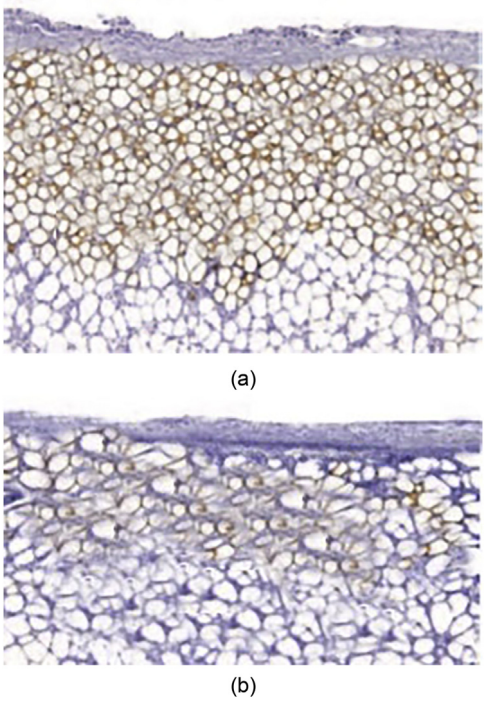


Figure 8. Comparative images demonstrating the significant impact of irradiation on the presence of perilipin-positive adipocytes in the subcutaneous layer in a group B (a) versus a group D animal (b) (magnification × 40).

supramuscular layers. On the other hand, in a comparative experimental study evaluating the fate of fat grafts transplanted into different recipient sites, Shi et al[13] reported that although survival rates were higher for adipocytes injected intramuscularly compared with subcutaneously and fat pad injected fat, fat pads showed the highest graft retention rates. According to the authors, although the survival rate for intramuscularly injected fat cells was higher, muscle movement negatively affected fat-graft retention. Since mechanical forces induced by muscle movement have been implicated in the inhibition of the adipogenic differentiation of stem/progenitor cells, injection of free fat plus botulinum toxin A or muscle denervation and immobilization was found to significantly reduce the level of fat-graft resorption.[20]

Histopathological and imaging techniques have both been used to assess fat-graft retention in the published literature. In most animal studies, H&E staining and quantitative evaluation of sections were used to determine the amount of fat graft that survived.[13,19,20] Additional immunohistochemical methods such as staining with antibodies to perilipin, S100, and a-SMA may contribute significantly to distinguishing viable from apoptotic adipocytes.[21-23] Among the imaging techniques that have been used to evaluate the volume of fat retained after transplantation, Technetium-99m scintigraphy,[18] micro Magnetic Resonance Imaging (MRI)[19] and Computed Tomography (CT) scans[24] have been most frequently used, although their utility in evaluating living adipocytes still remains questionable.

In the present study, the experimental model was designed to simulate an autologous breast reconstruction method, namely the fat-augmented LD myocutaneous flap, with the aim of comparing fat-graft viability after subcutaneous and intramuscular injections of different fat volumes and using two recipient muscles, i.e., the transposed pedicled LD muscle and the PM muscle. In addition, we evaluated the effect of postoperative radiation on fat-graft retention. A combination of histological examination with H&E and immunochemistry (with anti-perilipin antibodies) was used to assess the viability of transplanted adipocytes. Our evaluation methodology was improved using Image J software, which ensures more accurate and objective measurements.[12]

According to our results, the PM muscle was found to be the substrate that provided the highest adipocyte survival rates regardless of the amount of fat transplanted, followed by the LD muscle and, finally, the subcutaneous tissue, which showed the lowest numbers of viable fat cells. Differences in adipocyte survival between recipient tissues were found to be statistically significant for all individual comparisons in all animal groups ($p<0.001$). Mean values of viable fat cells transplanted into the muscle were proportional to the volumes of fat grafts injected; on the contrary, increased volumes of transplanted fat in the subcutaneous tissues did not result in increased adipocyte incorporation. Postoperative irradiation of the fat-graft recipient sites resulted in a statistically significant decrease in adipocyte survival in all three host tissues ($p<0.001$).

Although the effectiveness of fat grafting in reversing the post-radiation effects and complications on the target tissues has been documented in numerous experimental and clinical studies,[25–28] there is very limited published data reporting on the effect of radiation on transplanted fat grafts.[23] To our knowledge, the present experimental study reports the first attempt to compare fat-graft survival rates in different tissues exposed to radiation and documents the negative effect of radiotherapy in autologous fat-graft retention.

Although the histological analysis in our protocol did not focus on the vascular status of the examined specimens, our results suggest that reduced vascularity of the host tissue, caused either by the elevation and transposition of the LDmuscle compared with the PM muscle that was left in situ, or from the adverse vascular effects of postoperative irradiation, may adversely affect fat-graft viability with statistical significance.

A limitation of our study is that we did not use an imaging investigation to document long-term fat-graft retention; however, the histological and immunohistochemical examinations that were used clearly showed statistically significant differences in adipocyte survival rates between the host tissues and animal groups that were analyzed.

The conclusions of our study may have important implications for clinical fat grafting procedures, especially in cases of breast reconstruction. Since the PM muscle has been shown to provide the highest survival rates of transplanted fat cells, plastic surgeons performing fat-augmented LD-based autologous breast reconstructions,[29,30] should consider injecting large volumes of fat not only into the LD muscle and skin but also into the PM tissue to optimize final results. Although further studies on fat-

graft retention after radiation are needed, our results showed that radiation therapy adversely affects adipocyte survival after transplantation.

Conflict of interest

None.

Ethical Approval

Our experimental study was carried out at the Experimental and Research Center of Papageorgiou Hospital in Thessaloniki, and was compliant with the national regulations for animal experimental studies (ethical approval No. 13/9658).

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None.

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