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Effect of adipose tissue-derived stromal vascular fraction on the viability of random-pattern skin flaps: an experimental study

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ABSTRACT

Aims: Random pattern skin flaps are still widely used in plastic surgery. However, necrosis in distal flap sections resulting from ischemia is a serious problem, increasing the cost of treatment and hospitalization. The aim of this study was to test the effects of adipose tissue derived stromal vascular fraction (SVF) on random pattern skin flaps in rats.

Methods: In this experimental study conducted on Wistar rats, three groups were formed using the simple random sampling technique. Group 1 consisted of rats with a raised flap and a subcutaneous SVF injection. Group 2 comprised rats that underwent a flap operation with no additional treatment. Group 3 included rats with a raised flap and a subcutaneous saline injection. Tissue necrosis and level of survival area was detected with planimetric method and histopathologic examination was performed for detect of level of vascular density. On the 7th day post-operation, the viability assessment was calculated based on the ratio of the living flap area to the total flap area.

Results: The mean viability rate in Group 1 was higher compared to other groups, while there was no significant difference between Group 2 and Group 3 (Group 1=79.2±4.9 % vs. Group 2=41.0±5.9 % vs. Group 3= 39.2±2.7 %, p<0.001). The mean vascular densities of the flaps were higher in Group 1 compared to the other groups, while it were similar between Group 2 and Group 3 (Group 1=29.8±1.2 vs. Group 2=8.4±4.6 vs. Group 3=9.3±1.8, p<0.001).

Conclusion: The use of fat tissue-derived SVF injections has been found to be advantageous in improving the survival of random pattern flaps, frequently utilized in plastic and reconstructive surgery.

Keywords: Adipose tissue, flap viability, rat, stromal vascular fraction

INTRODUCTION

Random pattern skin flaps are a cornerstone in reconstructive plastic surgery, offering the ability to cover defects and improve aesthetic outcomes. However, their effectiveness is frequently marred by the occurrence of necrosis, particularly in the distal sections, which is primarily a consequence of inadequate blood supply or ischemia.[1] This ischemic necrosis not only affects the success of surgical outcomes but also elevates healthcare costs and extends hospital stays.[2] Thus, finding solutions to enhance the survival of these flaps is of paramount importance in the field of plastic surgery.

Recent studies have demonstrated that (SVF) derived from adipose tissue possesses significant potential in tissue regeneration and repair.[3,4] SVF, is particularly rich in mesenchymal stem cells (MSCs), but also includes a variety of other cell types such as endothelial progenitor cells, pericytes, fibroblasts, and immune cells. The diversity of cell types within SVF contributes to its broad range of regenerative capabilities.[5] In the context of plastic surgery, the utility of SVF in enhancing the viability of skin flaps is of significant interest. The ability of SVF to promote angiogenesis could potentially address the critical issue of ischemia in flap surgeries. Enhanced blood supply facilitated by the growth factors within SVF could improve the survival rates of skin flaps, thereby reducing the incidence of necrosis.[6,7]

We hypothesized that the injection of SVF obtained from adipose tissue could enhance the viability of random pattern skin flaps in Wistar rats. This study aimed to investigate the effect of injecting adipose tissue-derived SVF, containing stem cells, endothelial cells, and preadipocytes, on the viability of random pattern skin flaps in rats.

METHODS

This experimental study was conducted on Wistar rats in February 2006 at the Vakıf Gureba Training and Research Hospital's Plastic, Reconstructive and Aesthetic Surgery Clinic. The study protocol received approval from the İstanbul University Experimental Medicine Research Center Ethics Committee (Date: 08.08.2001, Decision No: 0530-63-01/264).

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Thirty female Wistar rats, each weighing between 300-350 grams on average, were utilized. During the experiment, these animals were kept under identical laboratory conditions (standard room temperature and natural daylight) and were nourished with rat feed and tap water. All surgical procedures were performed under general anesthesia. Anesthesia was achieved with an intramuscular injection of ketamine hydrochloride (65 mg/kg) and xylazine hydrochloride (0.65 mg/kg).

Formation of the SVF

Through an incision made parallel to each of the inguinal creases, two samples of fat tissue, each with dimensions of 1×1×1 cm, were surgically excised from the inguinal fat pads. The excised fat tissue was then transferred into a sterile container containing 1 cc of physiological saline. As previously described in the literature,[8] the fat tissue, which was converted into a suspension by manual crushing, was transferred to a centrifuge tube and centrifuged at 1500 rpm for 5 minutes. After centrifugation, the triglycerides collected in the upper layer and the mature fat cells in the middle layer were removed from the tube. The stromal vascular fraction accumulated at the bottom of the suspension was then aspirated into 1 cc insulin syringes, and a 30 gauge needle was used for injection.

Surgery Procedure

Using an electric shaver, the animals' backs were shaved, followed by a cleaning with Betadine solution. Subsequently, 3×10 cm caudal-based dorsal skin flaps were removed using the Khouri-modified McFarlane flap model (Figure 1).[9] Once the flap had been raised, it was re-sutured to the area where it was removed (since no other suturing was performed beforehand), using a 4/0 silk suture with a sharp needle.

Figure 1. The McFarlane flap was planned to be 3x10 cm-sized with a caudal base.

Experimental Groups

Using a simple random sampling technique, the animals were divided into three groups, each consisting of 10 rats. Group 1 consisted of rats with a raised flap and a subcutaneous SVF injection. Group 2 comprised rats that underwent a flap operation with no additional treatment. Group 3 included rats with a raised flap and a subcutaneous saline injection.

In Group 1, a suspension containing 1 cc of SVF was injected subcutaneously at six quadrants on each flap. Flaps in Group 2 were sutured directly to the area from which they were lifted without any injection. In Group 3, a total of 1 cc of saline solution was injected into the subcutaneous tissue of the flaps in 6 quadrants (Figure 2). At the end of the operation, to prevent the animals from damaging each other's flaps, each animal was placed in a separate cage and monitored for 7 days.

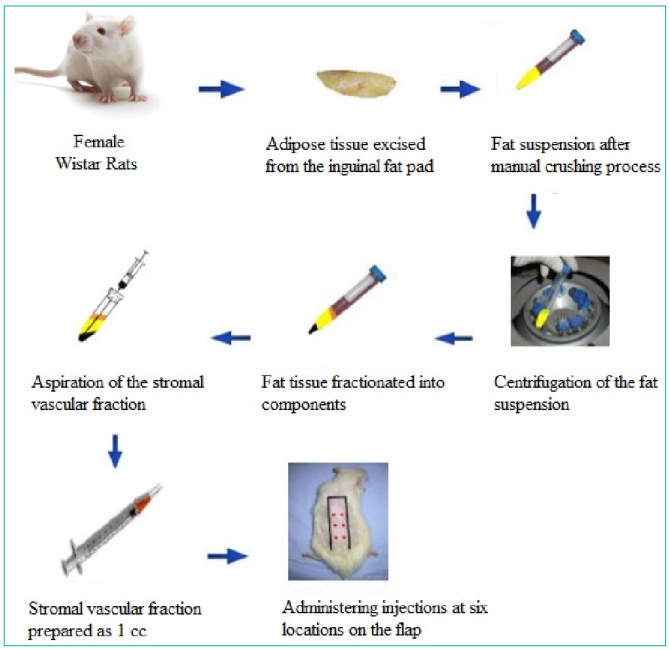

Figure 2. Schematic summary of the experimental stages

Viability Assessment

On the 7th postoperative day, flap viability percentages were calculated using Sasaki's paper template method.[10] For this purpose, the animals were re-anesthetized, and the flap sizes and necrosis lines were copied onto transparent acetate paper using a colored acetate pen. From the obtained shapes, the area of the entire flap was calculated first, followed by the area representing only the living skin. The resulting values were then ratioed and expressed as a percentage of viability. Additionally, the flap areas of all animals were photographed from an equal distance using an M-307 Hp-photosmart digital camera, these images were then graphically processed in Microsoft Paint version 5.1.

Measurement of Capillary Density

On the 10th postoperative day, full-thickness tissue samples measuring 0.5×0.5 cm were taken from the ischemic transition zones of the flaps in all three groups. After staining with Hematoxylin Eosin, the capillary count within a 1 mm^2 area was identified under a light microscope.

Statistical Analysis

All analyses were conducted using IBM SPSS Statistics for Windows 14.0 (SPSS Inc., Chigaco, IL, USA) software. The normal distribution of numerical variables was assessed using the Kolmogorov-Smirnov test and they presented as mean±standard deviation. Comparisons between groups were conducted using the Kruskal-Wallis test (post-hoc test: Mann-Whitney U with Bonferroni correction). Significance was accepted at P-value<0.05 (*) for all statistical analyses.

RESULTS

On the 7th day post-operation, the viability assessment was calculated based on the ratio of the living flap area to the total flap area. Accordingly, the mean viability rate in Group 1 was higher compared to other groups, while there was no significant difference between Group 2 and Group 3 (Group 1=79.2±4.9% vs. Group 2=41.0±5.9% vs. Group 3=39.2±2.7%, p<0.001). **Figure 3A** illustrates the necrosis and viable parts of the McFarlane flap's distal portions in the groups, while **Figure 3B** presents the viability rates of these flaps. Additionally, **Figure 3C** depicts the digital images of the necrotic and surviving areas within the groups' flaps.

The mean vascular densities of the flaps were higher in Group 1 compared to the other groups, while it were similar between Group 2 and Group 3 (Group 1=29.8±1.2 vs. Group 2=8.4±4.6 vs. Group 3=9.3±1.8, p<0.001) (**Figure 4A**). The microscopic examination of the tissue samples taken from the ischemic transition zones of the flaps belonging to the groups, in terms of capillary density, is shown in **Figure 4B**.

DISCUSSION

Random pattern skin flaps are widely used in plastic surgery. When the length of the flap is excessive, necrosis can develop at the distal end of the flap, which poses a significant problem for both the patient and the surgeon. The viability of these types of flaps primarily depends on the flap maintaining sufficient blood flow. Previous studies have shown that vasoconstriction, edema formation, and leukocyte activation and accumulation play significant roles in flap necrosis.[11-13] Eliminating or reducing these factors can positively impact the viability of the flap.[12,14] Numerous studies have shown that stem or endothelial cell precursors in the peripheral circulation contribute to neovascularization in ischemic tissues.[15-17] Endothelial progenitor cells, also known as angioblasts, are abundantly present in the adipose tissue-derived SVF, and these cells are precursors for new blood vessel formation.[18] In the process of new blood vessel formation, VEGF is recognized as a key cofactor. In vitro models have indicated that the viability of ischemic skin flaps is directly related to the formation of new endothelial cells and that VEGF is the primary mediator in this process.[19]

Previous studies have demonstrated that neovascularization in ischemic tissues can be achieved through the intravenous infusion of endothelial progenitor cells.[20] However, local injection has certain advantages over intravenous infusion. Firstly, it can increase the local density of endothelial progenitor cells in the target tissue. Secondly, it guards against the systemic adverse effects of the cells, including their role in promoting angiogenic diseases.[21,22] Hence, our study employed the local injection approach.

In a study conducted on athymic rats, a limited amount of stem cells was harvested from the bone marrow, and the allo-injection method was used to increase the number of cells to be injected into the vein.[23] Histological examinations of this mentioned study have shown that the increased granulation tissue in the injected flaps contained a dense microvascular network newly formed due to the effect of vascular endothelial progenitor cells.[23] In a study conducted on Sprague Dawley rats with a pedicled fascial flap combined

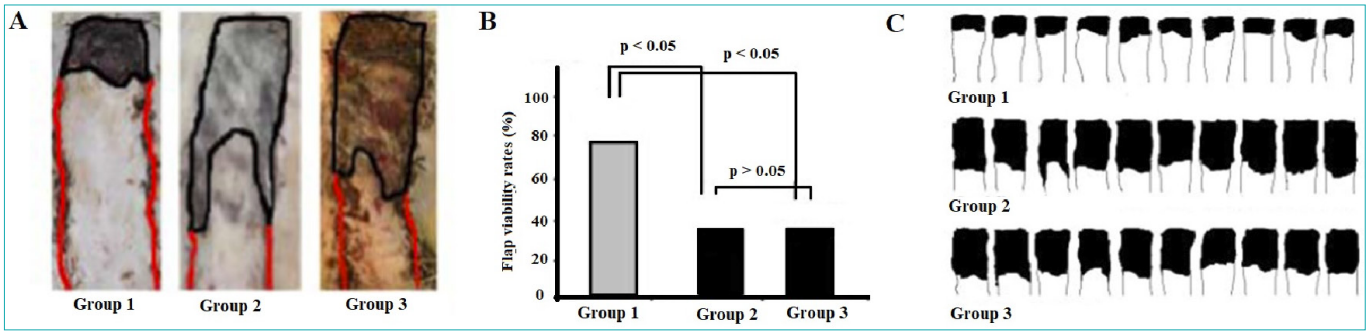

Figure 3. A) Necrosis and viable flap parts observed in the McFarlane flap distal parts of the groups (Viability rate, Group 1: 79.2±4.9% vs. Group 2: 41.0±5.9% vs. Group 3: 39.2±2.7%, p<0.001). **B)** Comparison of flap viability rates. **C)** The digital images of the necrotic and surviving areas within the groups' flaps

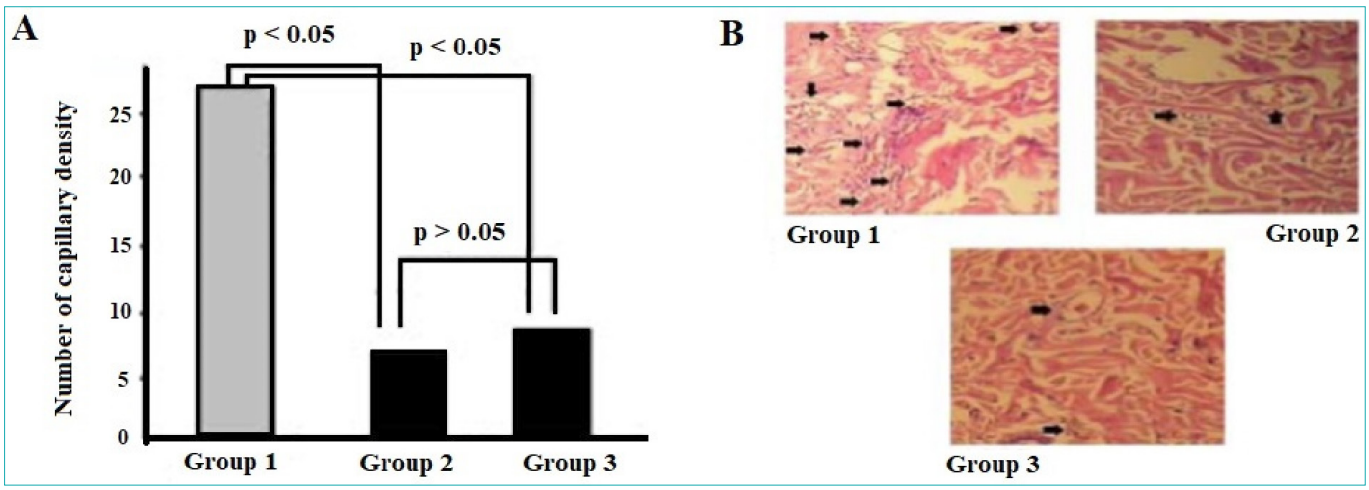

Figure 4. A) Comparison of vascular densities between groups B) The microscopic examination of the tissue samples taken from the ischemic transition zones of the flaps belonging to the groups, in terms of capillary density

with a free skin model, it was found that the SVF-treated group exhibited higher blood perfusion and flap survival rates compared to the control group. Moreover, this group not only exhibited increased angiogenesis but also improved wound healing through enhanced intercellular adhesion, cell migration, and a favorable immune response.[24] In a study involving rats exposed to freeze injury via a liquid nitrogen-cooled cryoprobe, findings demonstrated that SVF-treated rats exhibited markedly decreased acute inflammation and fibrosis on day 14, compared to the control group. Furthermore, this group demonstrated increased granulation tissue, improved re-epithelialization, and heightened neovascularization.[25] In the current study, on the 7th day post-operation, the SVF-treated group observed a higher flap viability rate and vascular density compared to other groups. Aligned with existing literature, these results demonstrate the substantial contribution of SVF to wound healing. However, the impact of SVF can differ based on the region of its extraction.

In a study conducted on female Sprague-Dawley rats, SVF were derived from fat tissues obtained from peri-ovarian, peri-renal, mesenteric, and omental regions. It was reported that the omental fat tissue, which had the least amount of fat, possessed the highest fraction of SVF cells and viable cells.[26] Regardless of the source of SVF, among its potential mechanisms for enhancing flap survival rates is the positive effect on flap circulation through direct vasodilation caused by growth factors produced by these cells, the enhancement of new blood vessel formation driven by the angiogenic action of growth factors, and the direct involvement of endothelial progenitor cells in the formation of new vessels.[23,27] An experimental study, which observed enhanced flap viability after the administration of bone marrow-derived progenitor cells, demonstrated that these injected cells differentiated into vascular endothelial cells.[23] Consistent with this study, the quantitative increase in vascular density observed in flaps treated with SVF injections in our study may have originated from new capillary formation.

The results of this study suggest that the SVF can increase flap viability, decrease the risk of necrosis, and thereby improve healing processes. The preliminary findings of this study may aid in devising novel therapeutic strategies in plastic and reconstructive surgery, and pave the way for the exploration of adipose tissue-derived cells in wider biomedical applications. To confirm and expand the findings of this study, more prospective randomized controlled trials are needed to investigate the effects of SVF obtained from different adipose tissue sources or in different animal models on flap viability.

Limitations

This study has some significant limitations. In this study, SVF was solely obtained from inguinal fat pads. Variability in sourcing SVF from various fat tissues and changes in the dosage and technique of application might result in substantial differences in outcomes. Additionally, our study's reliance on a mouse model, while advantageous for conducting controlled experiments, limits the direct application of our findings to human clinical contexts. Variations in skin physiology and wound healing processes between mice and humans might influence the relevance of our outcomes.

CONCLUSION

The use of fat tissue-derived SVF injections has been found to be advantageous in improving the survival of random pattern flaps, frequently utilized in plastic and reconstructive surgery. Fat tissue harvested from common plastic surgery procedures such as liposuction or dermolipectomy may become increasingly valuable in the future, particularly in tissue engineering applications.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was performed in accordance with the Declaration of Helsinki, and was approved by the İstanbul University Experimental Medicine Research Center Ethics Committee (Date: 08.08.2001, Decision No: 0530-63-01/264).

Informed Consent

The need for informed consent was waived under the approval of the İstanbul University Experimental Medicine Research Center Ethics Committee due to the design of the study.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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