Cerrahpaşa Med J 2022; 46(3): 215-222

ORIGINAL ARTICLE

Using Reverse-Flow Prefabricated Vascular Structures for Removing Cutaneous Island Flap: An Experimental Study

Güncel Öztürk[1], Anıl Demiröz[2], Yağmur Aydın[2]

[1]Department of Aesthetic and Plastic Surgery, Private Practice, İstanbul, Turkey
[2]Department of Aesthetic and Plastic Surgery, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey

Cite this article as: Öztürk G, Demiröz A, Aydın Y. Using reverse-flow prefabricated vascular structures for removing cutaneous island flap: An experimental study. *Cerrahpaşa Med J.* 2022;46(3):215-222.

Abstract

Objective: While there are studies in the literature on island flap removal using prefabricated vascular structures, there are no experimental studies on island flap removal using reverse-flow prefabricated vascular structures. An island flap using a femoral–saphenous vascular nerve package as a reverse-flow prefabricated vascular carrier experiment was planned for the study.

Methods: Twenty-four male Wistar-Albino rats weighing between 250 and 350 g were divided into 4 equal groups. The experiment was carried out in 2 stages. In the first stage, femoral artery (group II) and vein (group III) were ligated separately or both were ligated (group I) to create a reverse-flow flap. Consecutively, the femoral–saphenous vascular nerve package was dissected and prepared to serve as a prefabricated vascular carrier with the aid of the microscope, and the rear area was then transferred under the skin.

Results: After the macroscopic observation, the areas living on the skin island were shown. In scintigraphy and in angiographic and histopathological assessments, the presence of blood flow and areas living in the skin island were shown in prefabricated vascular structures. No statistically significant difference was observed in comparisons between the groups (analysis of variance, $P > .05$). Post-hoc Tukey's test revealed high similarities between the sham group and group III as well as between group I and group II.

Conclusions: The island flap model lifted using the reverse-flow prefabricated vascular structures described for the first time in this experimental study can be accepted as an unusual flap model.

Keywords: Experimental, flap, island, reconstruction, reverse

Introduction

Although there are many flap options defined in reconstructive surgery, the diversity of defects and growing expectations in functionality result in continuous further research on the subject. In fact, serious discussion of the different flap patterns started as a result of a detailed examination of blood circulation of the skin and the vascular anatomy of superficial tissues.[1,2] Thus, it has been shown that flaps can be prepared without adhering to the vascular anatomy in classical flap design, and these new flap types can be applied in cases of some difficult conditions in the clinic.

The reverse-flow flap is a flap model in which the blood flowing into the feeding main artery fills the vascular bed "proximal-to-distal," that is, from the distal to the proximal (i.e., not the orthograde way but the retrograde way). When considered from this point of view, it is not possible to include all flaps with a "pedicle distal" in the reverse-flow class. Because "orthograde" is filled with blood flow, the "route" of the vessels in the flap pedicle can be viewed from the bottom-up, that is, "ascendant" according to the posture of the body.[3]

Flap prefabrication can be interpreted as creation of a flap, which is not available at hand. The "flap retardation" process has been used for a long time. It is "tissue expansion," which is a more developed form of flap retarding. However, the attempt to add a new tissue and create a new composition before transferring it to an existing flap attracted attention to the concept of "prefabrication." The process based on enrichment with grafts without removing an existing flap and transferring it in the next session is defined as "prelamination," which can be considered as a subtype of prefabrication.[4] The step after prelamination is differentiating a new flap with "vascular carrier induction." A pedicle consisting of an artery and vein and a thin flap tissue that serves as a capillary bed is prepared as a "vascular carrier." The vascular carrier can be placed under or between the tissues that need to be prepared as a flap, and composite tissues or thin skin flaps can be prepared with the desired properties.[5]

It is known that island flaps are lifted by using straight-flow prefabricated vascular carriers. It is not known whether the opposite is possible in the experimental setting. In other words, if the flat-flow prefabricated main vein feeding the island flap is damaged proximally for any reason, it is not known whether the reverse-flow island flap is fed.

This question led to the idea of designing a new flap, and this new flap model was "island flap removed using reverse-flow prefabricated vascular structures."

Methods

Experimental Study Plan

The present study was approved by the İstanbul University-Cerrahpaşa University Animal Ethics Committee (Date: August 27, 2008, Number: 118). The procedures were compatible with the Guide for the Care and Use of Laboratory Animals.

Received: June 2, 2022 **Accepted:** August 29, 2022 **Publication Date:** October 4, 2022
Corresponding author: Güncel Öztürk, Department of Aesthetic and Plastic Surgery, Private Practice, İstanbul, Turkey
e-mail: drgunceloz@gmail.com
DOI: 10.5152/cjm.2022.22036

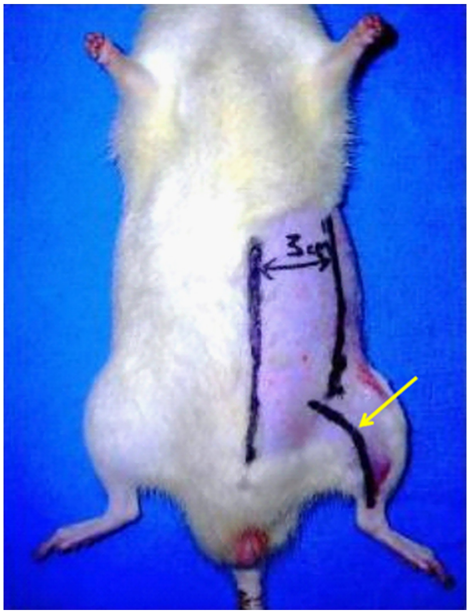

Figure 1. Marking the incision.

Twenty-four Wistar-Albino male rats, weighing between 250 and 350 g, were used as subjects. Rats were fed standard feed and water. During the study, the rats were kept under similar conditions that were determined for experimental animals in the laboratory, and 3 animals were housed per cage. For anesthesia, 75 mg/kg ketamine (Ketalar®) and 10 mg/kg xylazine (Rompun®) were injected intraperitoneally. Twenty-four male rats were divided into 4 groups. On the same side of the animals, the inner side of the thigh, abdomen, and rear areas were shaved, and the surgical field was cleaned. Prior to the surgery, additional care was taken to protect the medial branches of the superficial inferior epigastric vessels from the midline of the anterior abdominal wall lateral to the lateral. The incision to be made from the inside of the thigh was marked (Figure 1). The flap was planned in an area of 3 × 3 cm in the rear area on the outside of the protected zone. The dissection of the vascular nerve package from the Achilles medial to the inguinal ligament was made with the aid of a microscope, leaving a thin muscle sheath around the femoral–saphenous vascular bundle. The same incisions and dissections were performed at room temperature in all groups to rule out errors that may occur between the groups due to incision and dissections. In this study, six Wistar-Albino male rats were used in each group: sham group and 3 experimental groups. In all the groups, the femoral artery was connected in a different way than the vein and the proximal of the superficial inferior epigastric artery and vein.

The experiment was planned in two stages:

a. Prefabrication of femoral–saphenous vein nerve bundle to the subcutaneous rear area;
b. Removal of prefabricated island flaps.

Prefabrication of the Femoral–Saphenous Vascular Nerve Bundle to the Subcutaneous Border Area

After a skin incision was made from the inguinal ligament midline to the Achilles medial, the femoral–saphenous vascular nerve bundle was revealed (Figure 2a). The point where the distal part of the vascular nerve bundle reaches the rear area after the femoral–saphenous vein was dissected and removed with the help of a microscope until the inguinal ligament, leaving a thin muscle sheath (Figure 2b).

The distal part of the vascular carrier was transferred through a subcutaneous tunnel and fixed with a 5/0 Prolene stitch to the middle of the 3 × 3 cm area in the rear area (Figure 2c). The incision on the inner side of the thigh was sutured with 5/0 Prolene sutures (Figure 2d).

Sham Group

The femoral artery and vein were not ligated through the proximal of the superficial inferior epigastric vessels.

Experimental Groups

Group I

The femoral artery and vein were ligated through the proximal of the superficial inferior epigastric vessels (Figure 3a and b).

Group II

Only the femoral artery was ligated through the proximal of the superficial inferior epigastric vessels. The femoral vein was left unligated (Figure 4a and b).

Group III

Only the femoral vein was ligated through the proximal of the superficial inferior epigastric vessels. The femoral artery was left unligated.

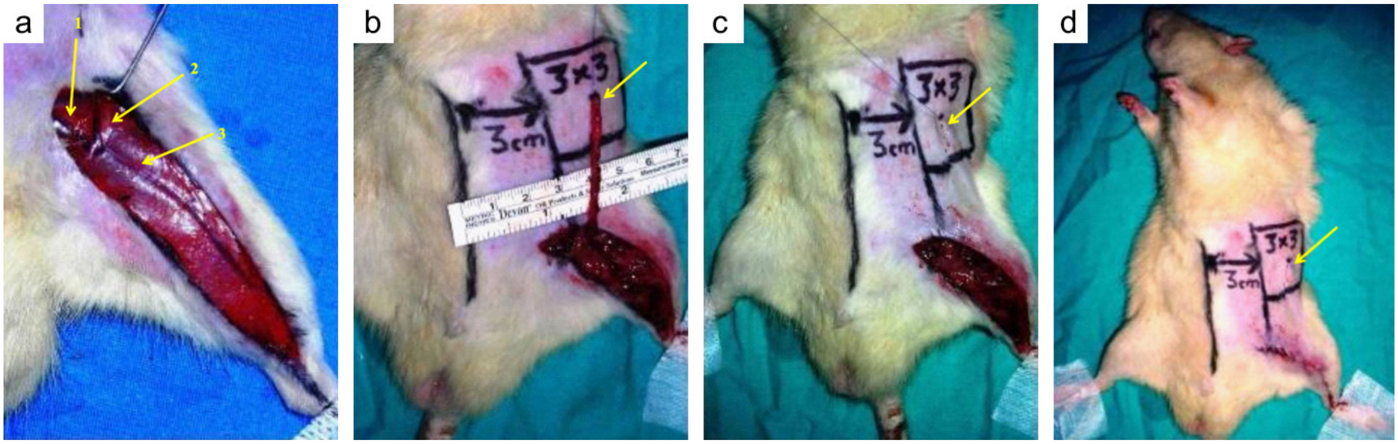

Figure 2. (a). Femoral–saphenous vascular nerve bundle was revealed. (b). Reaching to the rear area. (c). Fixation phase (d). Suturing stage.

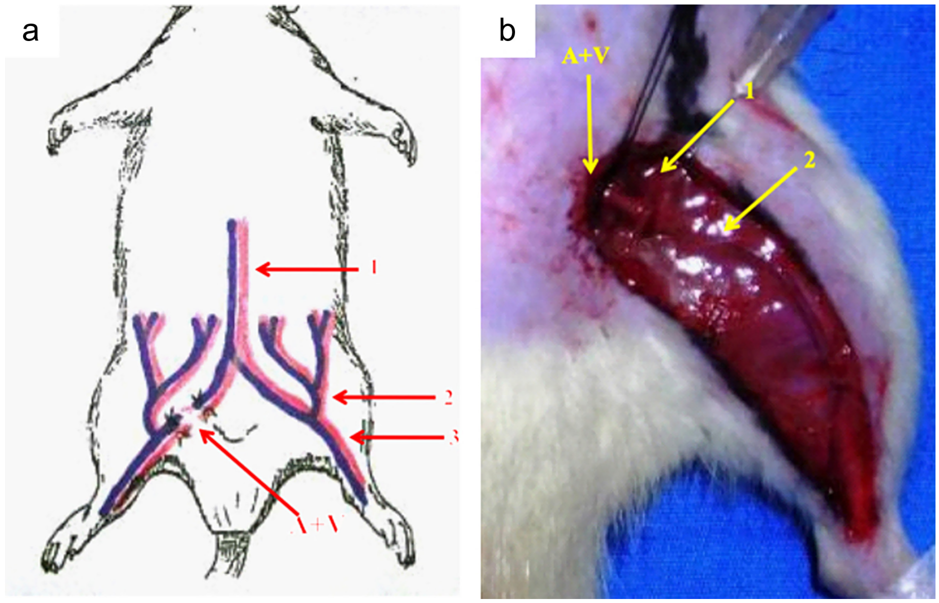

Figure 3. a and b. The femoral artery and vein were ligated from the proximal of superficial inferior epigastric vessels.

Removal of Prefabricated Island Flaps

Sham Group

Femoral artery is not ligated through the proximal of the superficial epigastric vessels. An elevation of the 3 × 3 cm of island flap which included prefabricated femoral–saphenous vascular nerve was perfromed.

Experimental Group

Group I

The femoral artery and vein, ligated through the proximal of the superficial epigastric vessels, were removed from the 3 × 3 cm island flap with a prefabricated femoral–saphenous vein nerve pedicle after 3 weeks and then sutured in place (Figure 5a and b).

Group II

The femoral artery, ligated through the proximal of the superficial epigastric vessels, was removed from the 3 × 3 cm island flap with a prefabricated femoral–saphenous vascular nerve pedicle after 8 weeks and then sutured in place (Figure 6a and b).

Group III

Femoral artery is not ligated through the proximal of the superficial epigastric vessels. An elevation of the 3 × 3 cm of island flap which included prefabricated femoral–saphenous vascular nerve was perfromed pedicle after 8 weeks and then sutured in place (Figure 7a and b).

Assessment of the Living of Island Flaps

Observation of the Flap Skin Island

Instead of having all the flaps removed, they were sutured and followed up daily until the seventh day. At the end of the seventh day, the skin island area living in prefabricated flaps was evaluated macroscopically and documented with a digital camera.

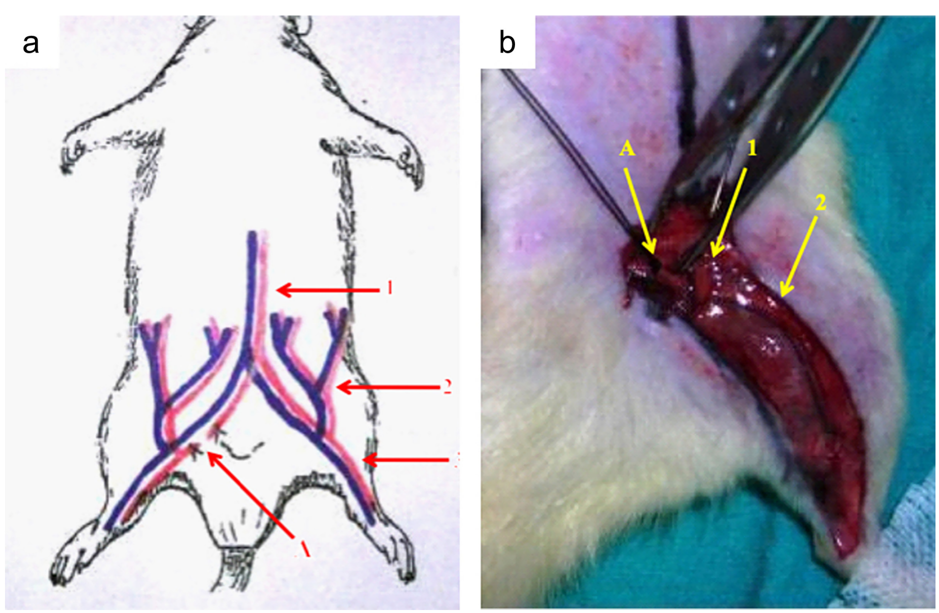

Figure 4. a and b. The femoral artery was ligated proximal to the superficial inferior epigastric vessels.

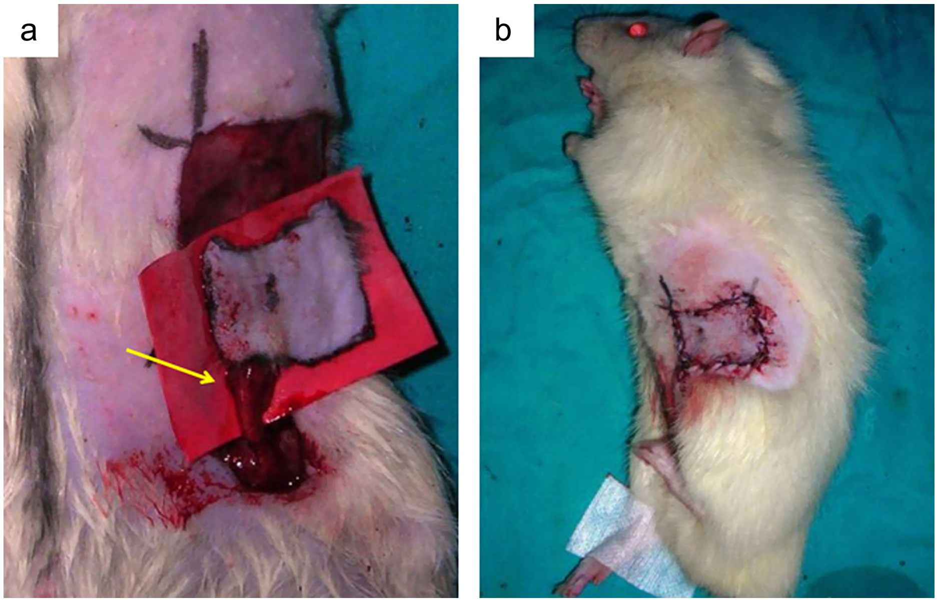

Figure 5. a and b. The femoral artery and vein were removed after 3 weeks of the prefabricated femoral–saphenous vein nerve pedicle, the 3 × 3 cm island flap, and then sutured in place.

Scintigraphic Assessment

Two rats from each group were included in this study. Methoxyisobutyl isonitrile (MIBI) compound was used for scintigraphic imaging.

While the subjects were under anesthesia in the nuclear medicine scintigraphy room, prefabricated pedicled island flaps were removed and an elastic layer was placed. The reason for the placement of the elastic layer was due to a concern that the radionuclide material might pass through the pedicle and incur involvement in the skin island, resulting in contamination of the skin island.

A 0.2 MIBI injection was given for each flap from the tail vein with a 30 G injector. After 30 minutes, the flap was cut from the proximal of the prefabricated pedicle and imaging was performed. Only prefabricated flaps were included in the imaging area. Static and dynamic images were taken on the 15th minute. ADAC Medical Systems Vertexplus's Epic Dual-Head Gamma Camera and Pegasys Computer were used for scintigraphic examination.

Angiographic Assessment

The left and ventricular–aortic and right atrium–vena cava junction for groups I and II (since femoral arteries are connected and blood supply may be based on the superior superficial epigastric artery) was cannulated. After the arterial bed was cannulated with 18-22 G and venous side with 14-18 G, both cannulae were fixed tightly with silk ligaments. Rats were killed with 2 mL of 2% lidocaine injected slowly. A mixture of barium sulfate (diluted 1 : 1 with saline), 3 mL of castor ink, and 5% gelatin (fully dissolved in a microwave oven by heating up to 50°C) was prepared. The

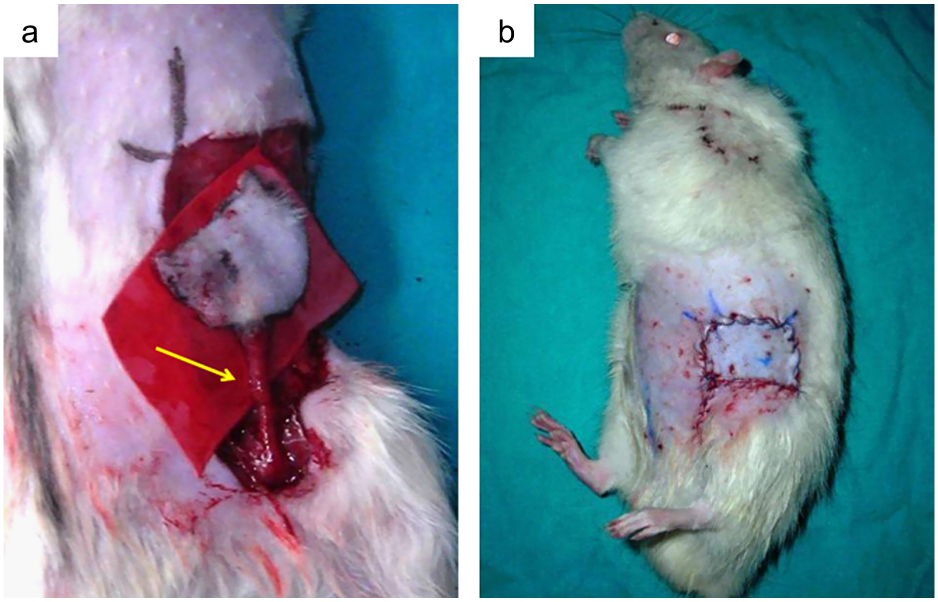

Figure 6. a and b. The femoral artery, ligated through the proximal of the superficial epigastric vessels, was removed from the 3 × 3 cm island flap with a prefabricated femoral–saphenous vascular nerve pedicle after 8 weeks and then sutured in place.

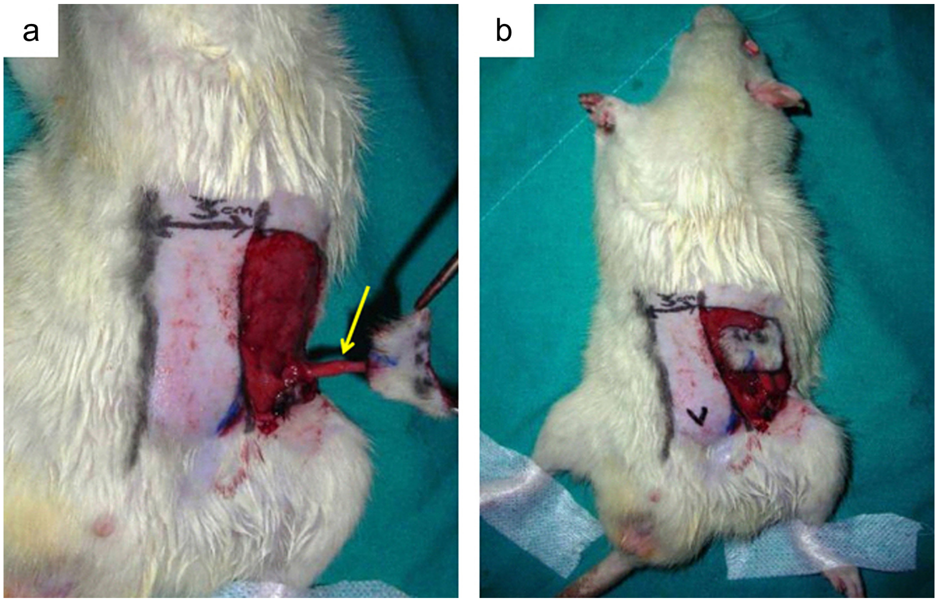

Figure 7. a and b. The femoral vein was removed after 8 weeks of the prefabricated femoral–saphenous vein nerve pedicular 3 × 3 cm island flap and then sutured in place.

prepared barium sulfate plus 5% gelatin mixture was administered slowly from the catheter under low pressure. When the mixture started to leak from the wound lips the injection was terminated, the vessel was attached, the flap was separated and left overnight in the refrigerator, and the contrast material was removed. The "Siemens 3000 Nova Mammomat" mammography device was used to visualize the vascular structure.

Histopathological Assessment

In this study, a total of 12 rats, 3 of them from each of the 4 groups, were used. In all 4 prefabricated island flap models, the flaps were dissected and removed under the microscope as described to show the presence of flow in the pedicle and the viability of the skin island the abdominal aorta and inferior vena cava–aortic junction was cannulated for groups I and II. A mixture of barium sulfate (diluted with 1 : 1 saline) was prepared for microangiography, 3 mL of castor ink, and 5% gelatin was prepared. The prepared mixture was given slowly from the catheter under low pressure. The injection was stopped when it started to leak from the edges. After separating the vein stem, cross sections were taken from prefabricated pedicles and the skin island and embedded in paraffin blocks. It was stained with the hematoxylin–eosin dye method and examined under a light microscope.

Statistical Methods

Seven days after the island flap was removed with a prefabricated vascular carrier pedicle and sutured in place, the living area of the 9 cm^2 skin island was evaluated among the groups as a percentage. To compare mean values, the normality assumption of parametric tests was verified by applying the Shapiro–Wilk test. Analysis of variance test was used for determining whether there is a difference between groups. If the results were statistically significant, post hoc Tukey's tests were used for comparing the groups. *P* values of .01 and .05 were regarded as significant. The statistical analyses were performed using the Statistical Package for the Social Sciences (version 17.0).

Results

Evaluation of Flap Skin Island

Island flaps, which were removed, were instead sutured with prefabricated vascular carriers in all groups. After the surgery, the living skin island area on the seventh day was macroscopically taken and viewed with a digital camera (Figures 8-10). There was no necrosis in the flap in the sham group and group III (Figures 8 and 9), while partial necrosis areas were seen in the flap in group I and group II (Figures 8 and 9).

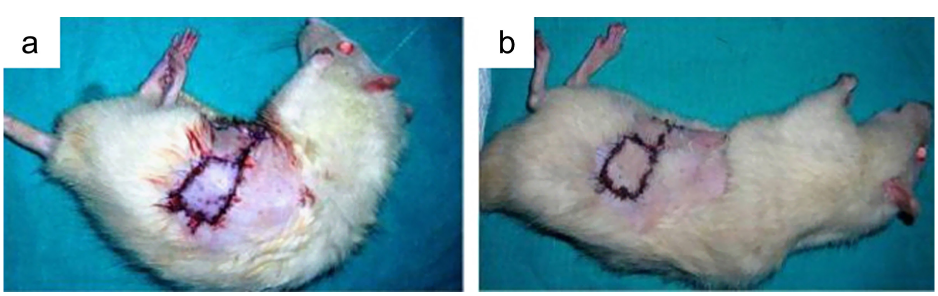

Figure 8. a and b. After the surgery, the living skin island area on the 7th day was macroscopically taken and viewed with a digital camera in the island flap are seen.

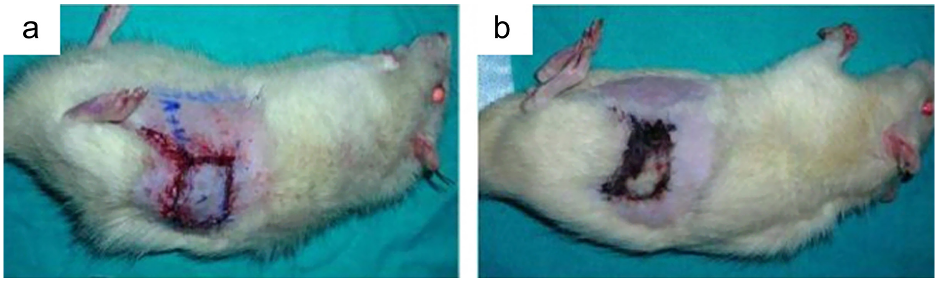

Figure 9. a and b. After the surgery, the living skin island area on the seventh day was macroscopically taken and viewed with a digital camera in the island flap.

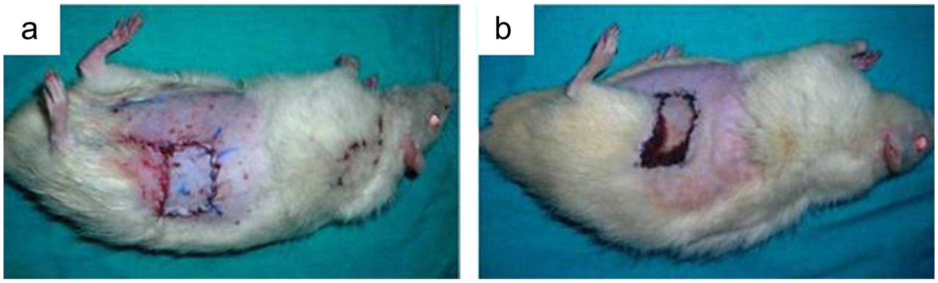

Figure 10. a and b. After the surgery, the living skin island area on the seventh day was macroscopically taken and viewed with a digital camera in the island flap.

Scintigraphic Assessments

Islands created with eight prefabricated vascular carriers were included in this study. It was observed that the radioactive substance injected into all flap models was retained.

Angiographic Assessments

In the sham group, thin vascular connections are clearly selected in the prefabricated vascular carrier and island flap.

In group I, reverse-flow prefabricated vascular carrier and thin vascular connections are seen in the island flap.

In group II, fine vascular connections are clearly selected in the prefabricated vascular carrier and island flap.

In group III, prefabricated vascular carrier and thin vascular connections in the island flap are seen.

Histopathological Assessments

Cross sections taken from the pedicle and skin island of prefabricated island flap models painted with hematoxylin–eosin were examined under a light microscope. Epidermis and dermis layers were observed to be alive in the skin island sections. In prefabricated pedicle sections, a mixture of barium sulfate, gelatin, and castor ink was seen in all groups within the artery and vein.

Comparison of Groups

Island flaps of area 9 cm^2 created with six prefabricated vascular carriers in each of the 4 groups were removed and sutured in place. On the seventh day after the surgery, the percentage of living areas of the skin island was revealed (Figure 11). Statistical

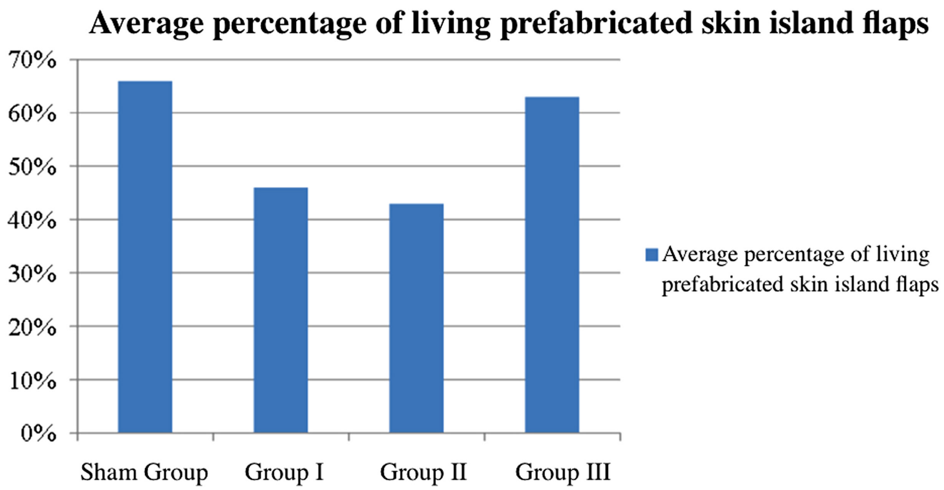

Figure 11. Percentage of living areas of prefabricated island flaps that were lifted and sutured in place via pedicles in every group on the seventh day.

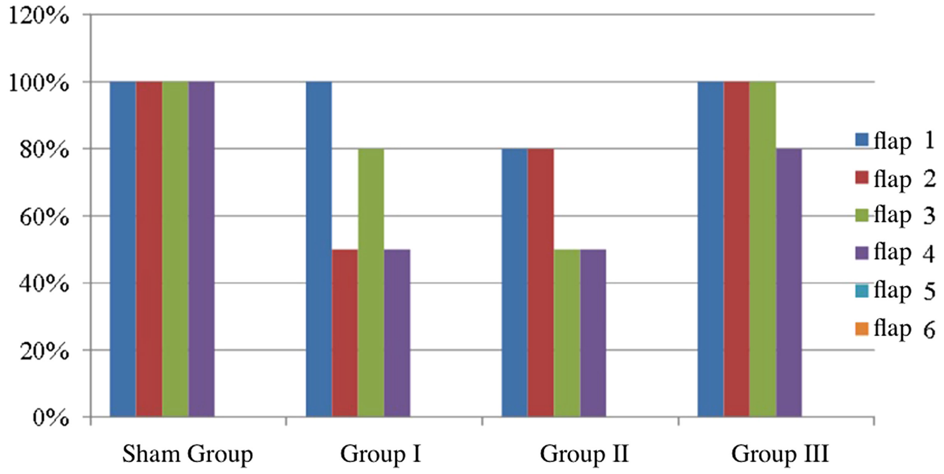

Figure 12. Comparison of average values of areas with the living prefabricated skin island flaps in percentages seven days after the operation.

analysis revealed it to be 66.67% (SD: ±51.64) in sham group, 46.67% (SD: ±40.82) in group I, 43.33% (SD: ±36.15) in group II and 63.33% (SD: ±49.67) in group III. According to the proportion of living area in the skin island among the groups, the average percentage is the highest in the sham group and the lowest in group II (Figure 12). Seven days after the island flap was removed with a prefabricated vascular carrier pedicle and sutured in place, there was no statistically significant difference in island flaps in the sham group compared to the average percentage of living area, group I, group II and group III ($P > .05$). In comparisons between the groups, no statistically significant difference was observed in the average percentage of living areas in island flaps ($P > .05$).

Discussion

Successful results of flap prefabrication in experimental environment may not be fully met in a clinic. In experimental studies, especially which include rats, the process of "new vessel formation" (neovascularization) is faster. Regardless of the prefabrication method, many compound tissue flaps can be prepared successfully. However, the reliable neovascularization process is longer and open to complications even in proportionally smaller flaps in human prefabrication trials.[6,7]

In our study, the femoral–saphenous vein was dissected around the nerve bundle with the help of a microscope until the inguinal ligament, leaving a thin muscle sheath (vascular carrier). The distal part of the vascular carrier was transferred under the skin to the rear area and fixed with 5/0 Prolene sutures. Instead of the island flaps removed with prefabricated vascular carrier after 8 weeks, 3 × 3 cm island flaps with reverse-flow prefabricated femoral–saphenous vascular nerve pedicle were inserted. The comitant vein system accompanying the artery, the advanced veins in the vein system, and the connections between the vein systems are some of the anatomical features that stand out in humans. In contrast, the presence of vein valves has not been demonstrated in objective research in rats. Despite the belief that there should be no valves in the rat veins, microscopic dissections and histological examinations do not support this. In addition, the rat does not have a distinct deep and superficial vein system distinction as in humans. Another difference is in the "comictan" vein system that accompanies the arteries. It is not possible to exemplify the twin-vein system (except for rattail), which travels on either side of the artery and has connections between them, as in humans. The general order encountered in rats is the only vein structure that accompanies the artery.

Reverse-flow flap models were also used in other studies.[8–10] In our study, prefabricated island flaps were prepared. The dimensions of the living flaps of the island flaps formed with direct-flow prefabricated vascular carrier and reverse-flow prefabricated vascular carrier were compared on average. As a result of the study, it was observed that the average island flap dimensions formed by reverse-flow prefabricated vascular carrier was smaller than the average island flap dimensions created by the "direct"-flow prefabricated vascular carrier.

In our study, before the femoral–saphenous vascular nerve pedicle was prefabricated, the femoral artery and vein were ligated from the proximal of the superficial epigastric vessels. After 8 weeks, reverse- and straight-flow prefabricated femoral–saphenous vascular nerve pedicle, 3 × 3 cm island flaps were removed. Areas living on the skin island were inspected for 7 days with macroscopic observation. It was observed that the MIBI radionuclide material given in scintigraphy was retained in all of the island flap models. Angiographic study showed the presence of blood flow in pedicles of island flap models created with a prefabricated vascular carrier. Histopathologically, cross sections taken using hematoxylin–eosin staining method showed the presence of blood flow in prefabricated vascular structures and areas living on the skin island. In the comparisons made between the groups, no statistically significant difference was observed in the average percentage of island flap living areas. The findings were interpreted in favor of the presence of blood flow in the island flap removed using reverse-flow prefabricated vascular structures. In this case, the reverse-flow prefabricated island flap was supplemented with blood by the inferior superficial epigastric artery, with the superior superficial epigastric and deep epigastric artery in the "reverse" direction. In the inferior superficial epigastric vein, the superior superficial epigastric and deep epigastric vein system was provided by reverse flow.

The island flap model lifted using the reverse-flow prefabricated vascular structures described in this study can be considered an unusual flap model.

It is thought that this method can be helpful in problematic cases where repetitive reconstructions are required and in cases where the feeding artery is injured. However, further clinical studies are needed to confirm using this new flap model in the clinical settings.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İstanbul University-Cerrahpaşa University Animal Ethics Committee (Date: August 27, 2008, Number: 118).

Informed Consent: Informed consent is not obtained since this study is a animal experiment.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – G.Ö., Y.A.; Design – G.Ö., Y.A.; Supervision – G.Ö., A.D.; Funding – G.Ö., Y.A.; Materials – G.Ö., Y.A.; Data Collection and/or Processing – G.Ö., Y.A.; Analysis and/or Interpretation – G.Ö., A.D.; Literature Review – G.Ö., A.D.; Writing – G.Ö., A.D.; Critical Review – G.Ö., Y.A.

Declaration of Interests: The authors declare that they have no competing interest.

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

References

1. Hong JP. The superficial circumflex iliac artery perforator flap in lower extremity reconstruction. *Clin Plast Surg*. 2021;48(2):225-233. [CrossRef]
2. Zheng YH, Yin LQ, Xu HK, Gong X. Non-invasive physical therapy as salvage measure for ischemic skin flap: A literature review. *World J Clin Cases*. 2021;9(14):3227-3237. [CrossRef]
3. Tan YY, Liaw F, Warner R, Myers S, Ghanem A. Enhanced recovery pathways for flap-based reconstruction: systematic review and meta-analysis. *Aesthet Plast Surg*. 2021;45(5):2096-2115. [CrossRef]
4. Pribaz JJ, Fine NA. Prelamination: defining the prefabricated flap—a case report and review. *Microsurgery*. 1994;15(9):618-623. [CrossRef]
5. Erk Y, Rose FA, Spira M. Vascular augmentation of skin and musculocutaneous flaps. *Ann Plast Surg*. 1983;10(5):341-348. [CrossRef]
6. Huang X, Liu D, Gu S, et al. Augmentation of perforator flap blood supply with vascular supercharge or flap prefabrication: evaluation in a rat model. *Plast Reconstr Surg*. 2021;147(5):1105-1115. [CrossRef]
7. Xu H, Steinberger Z, Wo Y, et al. Supercharging strategies for prefabricated flaps in a rat model. *J Reconstr Microsurg*. 2019;35(8):568-574. [CrossRef]
8. Lombardo GAG, Tamburino S, Tarico MS, Perrotta RE. Reverse flow shunt restricted arterialized venous free flap. *J Hand Surg Am*. 2018;43(5):492.e1-492.e5. [CrossRef]
9. Chen C, Tang P, Zhao G. Direct and reversed dorsal digital island flaps: a review of 65 cases. *Injury*. 2014;45(12):2013-2017. [CrossRef]
10. Regmi S, Gu JX, Zhang NC, Liu HJ. A systematic review of outcomes and complications of primary fingertip reconstruction using reverse-flow Homodigital Island flaps. *Aesthet Plast Surg*. 2016;40(2):277-283. [CrossRef]