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Fat prefabrication using a fascial flap in the rat model

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SUMMARY. Prefabrication of fat tissue using a fascial flap based on the superficial inferior epigastric artery was studied in rats. First, the superficial inferior epigastric fascia was transposed over the inguinal fat pad. Two weeks later fascia and fat were elevated together as a prefabricated composite flap. At this stage, a pilot study was done in ten rats and perfusion of the flaps was tested with fluorescein. After confirming fluorescein staining of the prefabricated flaps, the study continued with experimental and control groups of rats. In the experimental group, prefabricated flaps were transposed to the subcostal area. In the control group, the pedicles of the flaps were severed, creating composite grafts. These grafts were transferred to the subcostal area in the same manner as in the experimental group. One week later the flaps were re-elevated and grafts were exposed. Fluorescein tests and Indian ink microangiography were carried out. In the experimental group, the flaps were stained, while grafts in the control group were not stained. Fat and fascia were found to be viable in the experimental group, while they were necrotic in the control group on histopathological examination. Based on these findings, we can conclude that the prefabrication of fat by vascular fascia is successful and may have application in plastic surgery.

Keywords: fascial flap, fat prefabrication.

Fat is an essential tissue for the plastic surgeon. Since first used as a free graft by Neuber in 1893, fat has been variously used in soft tissue augmentation.[1–8] In experimental and clinical researches on free fat grafts, unpredictable resorption has always been reported as a problem.[9–12] This is due to low tolerance of the fat cells to ischaemia. For this reason, interest has shifted from the use of autogenous fat grafts to alloplastic materials such as silicone, collagen and hydroxyapatite.[13–15] The problem of ischaemia will be overcome if fat tissue can be transferred as a flap.

Introduction of the concept of flap prefabrication into plastic surgery, made it possible to use the tissues which are closest to the ideal for reconstruction, by transforming them into flaps.

In this research the possibility of using fascial flaps as vascular carriers to create vascularised free fat flaps was studied in a rat model.

Materials and methods

Forty Sprague-Dawley male rats weighing 350–400 g were used. The anaesthetic regimen consisted of an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Each rat was put into a separate cage and standard care was applied during the experiment. Animals were sacrificed with the administration of a high dose of pentothal. Five animals which died during the experiment were excluded from the groups.

After depilation of abdominal skin, a 5–6 cm long median incision was made and extended to the right inguinal area in a reverse 'L' shape (Fig. 1). On the right side, the inguinal fat pad was exposed without disturbing its abdominal wall connection (Fig. 2). On the left side, the superficial inferior epigastric fascia (SIEF) was dissected as a 2.5 × 6 cm flap preserving its supplying superficial inferior epigastric (SIE) artery and vein (Fig. 3). The vascularisation of the fascia was checked by microangiography in five rats in a preliminary study (Fig. 4).

The superficial inferior epigastric fascia flap was transferred over the right inguinal fat pad (Fig. 5). The edges of the transferred flap were secured to the abdominal wall with 6/0 polypropylene (prolene, ©Ethicon Ltd, UK). The skin was closed with 4/0 polypropylene (prolene, ©Ethicon Ltd, UK).

Figure 1—Preoperative planning. On the right side SIEF flap and on the left side inguinal fat pad were marked. Arrow indicates the rotational arc of SIEF flap.

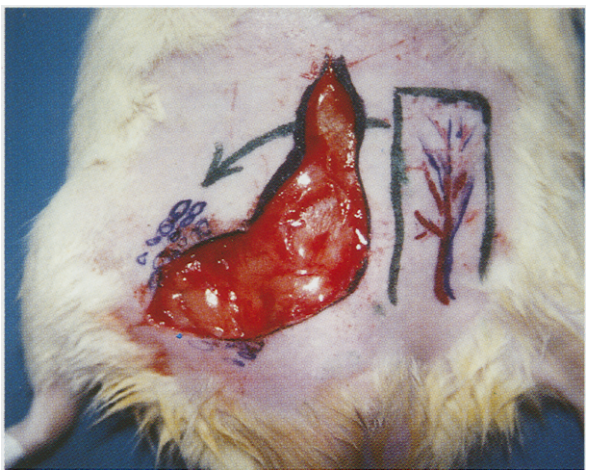

Figure 2—The right inguinal fat pad was exposed by reverse 'L' shape incision.

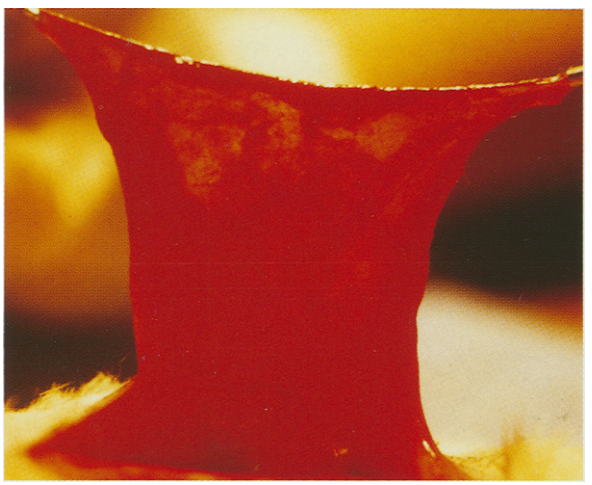

Figure 3—SIEF flap. Note the rich vascular network.

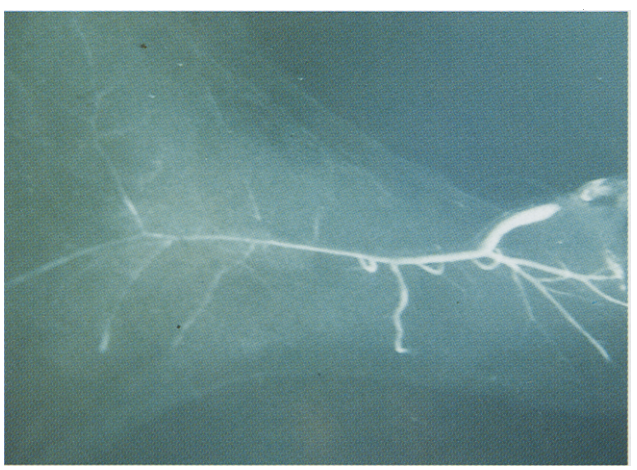

Figure 4—Rich vascular network of SIEF flap was visualised in microangiography.

After 2 weeks the SIEF flap was exposed extending from the left to the right inguinal area. A 2 × 2 cm area of fascia together with the underlying right inguinal fat pad was elevated from the abdominal wall whilst preserving the SIE vessels (Figs 6, 7). Thus a composite island flap consisting of fascia and fat based on the SIE vessels was obtained and then the animals studied in three groups.

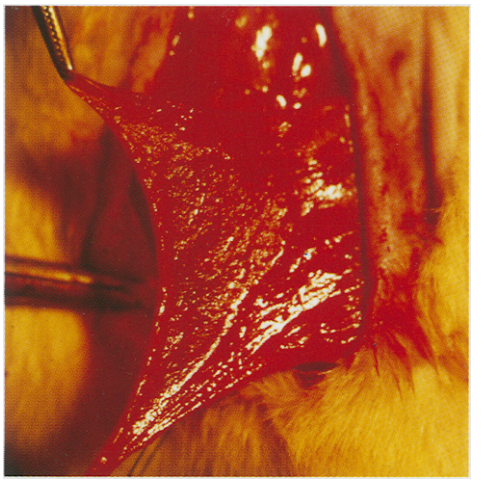

Figure 5—SIEF flap transposing to the right inguinal fat pad.

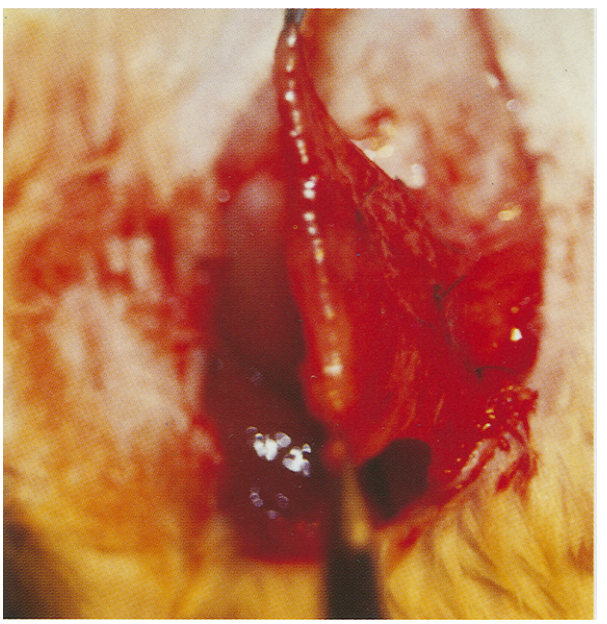

Figure 6—At the end of the 2-week staging period, fascia and fat were re-elevated together as a prefabricated fascia–fat flap.

1. Pilot group

Perfusion of the composite island flaps was checked in 10 rats. The fluorescein test was carried out by the injection of 0.2 ml 10% Na fluorescein into the external jugular vein and observation of staining of fascia and fat when illuminated by Wood's lamp after 20 min. During this procedure the flap was isolated from the body in order to avoid accidental staining. Then the flap was harvested and prepared for histopathologic examination.

2. Experimental group

In 15 rats, the composite island flap consisting of fat and fascia was transferred to a subcutaneous pocket created by blunt dissection in the left subcostal area. The flap was re-elevated 1 week later. In five rats, a fluorescein test was carried out. In ten rats Indian ink microangiography was carried out and specimens were prepared for histopathologic examination. The

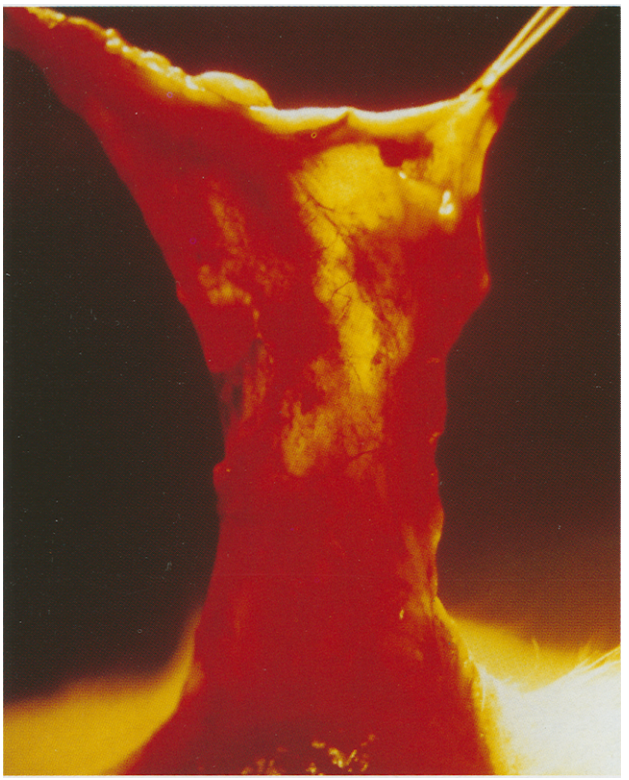

Figure 7—Prefabricated fascia–fat flap.

technique for Indian ink microangiography was as follows. After cannulation of the aorta and inferior vena cava, heparinised saline was injected into the aorta, until it came back out of the inferior vena cava. Then Indian ink was injected in the same way. Vascular staining was observed both directly and with the help of transillumination. The pedicle of the flap was ligated and the specimen was prepared for histopathologic examination.

3. *Control group*

In 15 rats the vascular pedicle was severed, thus creating a composite fascia–fat graft. This was transferred to the subcostal pocket in a similar manner to that in the experimental group. The subcostal pocket was opened 1 week later. Fluorescein tests in five rats and Indian ink injection studies in ten rats were carried out and the composite grafts harvested for histopathologic examination.

All specimens were fixed in 10% formalin for 24 h. The paraffin sections were cut parallel to the vascular axis. The slices were stained with H&E.

Results

The highly vascular network of the SIEF flap was clearly demonstrated after its elevation (Fig. 3). In five rats SIE vessels and their branches were visualised by microangiography, which revealed that the SIE vessels were the nutrient vessels of the elevated fascia (Fig. 4).

Fascia and the fat tissue were found to be well integrated and stuck to each other at the end of a 2-week staging period. There were bleeding spots in both tissues (Fig. 7).

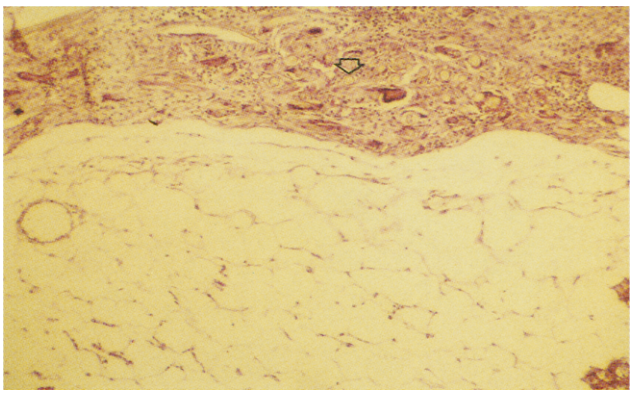

Figure 8—Histological section of fascia–fat flap in pilot group. Fascia was integrated with fat. Arrow indicates the blood vessel filled with fluorescein material (H&E; × 20).

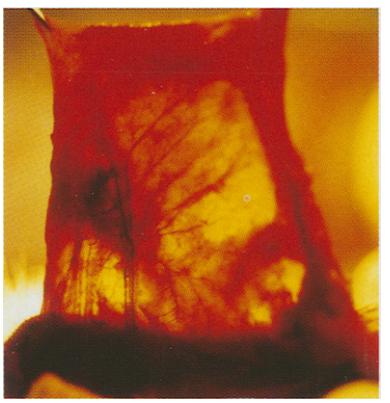

Figure 9—By transillumination, vascular network filled with Indian ink is observed.

1. *Pilot group*

In the pilot study, both fat and fascia were stained after the fluorescein test. The fascia and the fat tissue were found to be integrated in the histopathologic examination (Fig. 8). Also, fibroblastic proliferation and increased vascularisation were observed. Fluorescein was visible in some blood vessels. There was a diffuse inflammatory infiltration in all areas. Adipocytes and fibroblasts are presumed to be healthy due to their intact cellular membranes and nuclear staining propensity. All these findings showed that both the fat and fascia remained viable after a staging period of 2 weeks.

2. *Experimental group*

Bleeding from both the fat and the fascia was observed after re-elevation of the flaps in the experimental group. The fat and fascia were adherent to each other and both tissues were stained with the fluorescein test. The Indian ink reached to the periphery of the fat tissue of the flaps in all the animals on which microangiography was performed (Fig. 9). Histopathological findings of integrity of the membrane of fibroblasts and adipocytes, and staining capacity of their nuclei indicated the survival of these tissues (Fig. 10). Blood

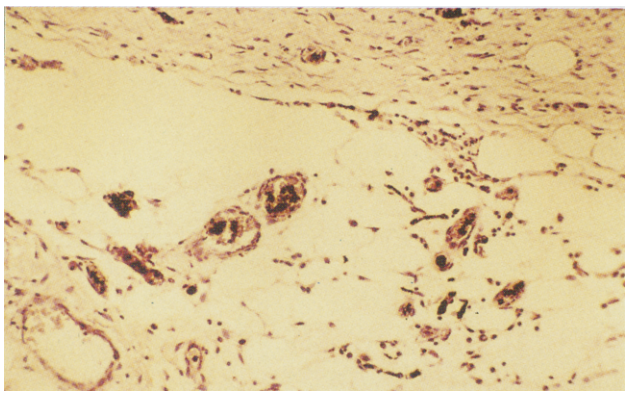

Figure 10—Vessels stained with Indian ink are seen in both fascia and fat tissue (H&E; × 50).

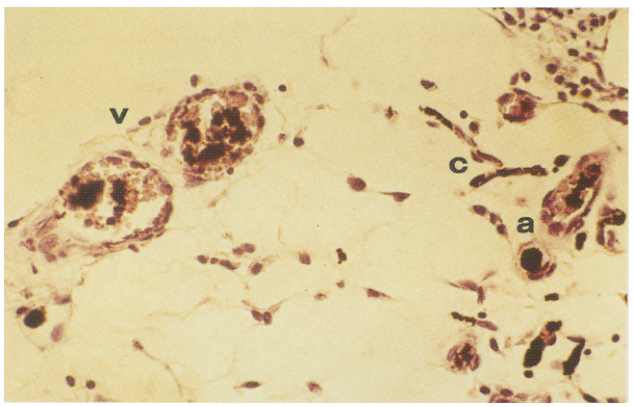

Figure 11—High power magnification shows arterioles (a), capillaries (c) and venules (v) containing Indian ink within fat tissue indicating that there is a true circulation (H&E; × 100).

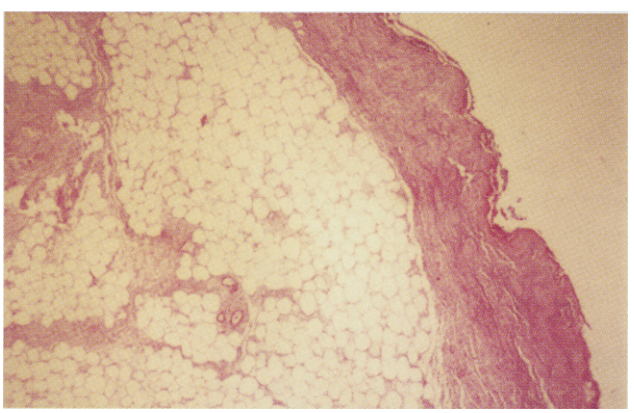

Figure 12—Necrotic fascia and fat. Diffuse, homogeneous eosinophilic staining of the cytoplasms in both tissues (H&E; × 20).

vessels (arterioles, capillaries and venules) containing black stain (Indian ink) were observed in both fascia and fat tissue indicating angioneogenesis from the fascia to the fat tissue (Fig. 11).

3. *Control group*

In the control group, the composite fascia–fat grafts were found to be dark brown and shrunken at the end of 2 weeks. These tissues were very fragile. There was no staining of the composite grafts in either the fluorescein tests or the Indian ink microangiography. On

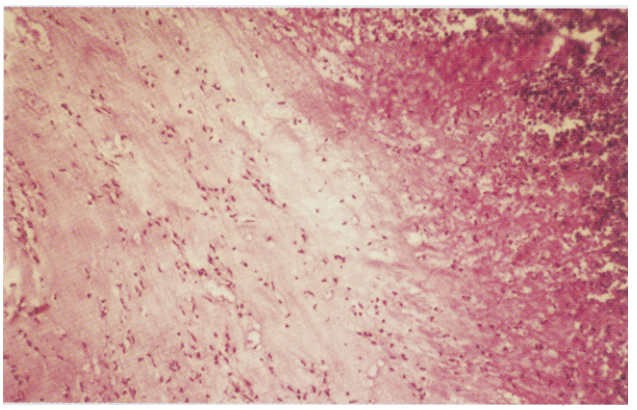

Figure 13—A distinct demarcation line separates fibrous proliferation (left) from extensive liquefactive necrosis (right) (H&E; × 50).

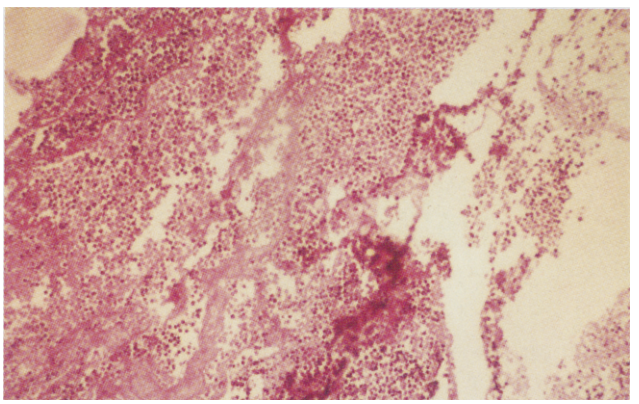

Figure 14—Extensive inflammatory infiltration consisting of PMN leukocytes destroying the integrity of fatty tissue (H&E; × 50).

microscopic examination, fascia showed signs of coagulation necrosis, with a diffuse homogeneous eosinophilic background appearance and lack of nuclear and cell membrane staining (Fig. 12). Some parts of the necrotic fat tissue adjacent to the fascia liquefied (Fig. 13). Moreover, extensive lymphocyte-rich inflammatory infiltration was observed in fat tissue (Fig. 14). There were no blood vessels containing stain.

Discussion

Several investigators have reported that the weight and volume of free fat grafts reduces by 40–50%.[16] These unsatisfactory results of free fat autotransplantation are thought to be due to the fat cells having a low tolerance to ischaemia and the slow rate of revascularisation of the fat.[17] Fat grafts have been transferred to richly vascular areas in order to try and decrease the revascularisation period and the ischaemia. For example, in an experimental study by Smahel et al[18] adipose tissue was grafted around vessels but this technique had no effect on the initial ischaemic phase. Von Heimburg et al[16] suggested that expanded fat grafts survived better. He also indicated the importance of early revascularisation in achieving fat graft survival.

Of course, fat transferred as a vascularised flap does not require neovascularisation for survival.

When fat tissue is transferred as a vascularised flap the resorption should be minimal as has been shown experimentally by Eppley et al.[14] Free groin fat flaps have been used clinically in hemifacial atrophy by Anderl[19] and others, but the pedicle (superficial circumflex iliac artery) is very short and variable.[20] In the human body there is no fat flap which has a constant and reliable pedicle. Prefabrication of a fat tissue flap using a vascular carrier could enhance pedicle reliability and allow choice of a fat rich donor area. The choice of vascular carrier for fat tissue prefabrication is the key to success for this procedure. Several vascular carriers for different tissues have been defined to date.[21] Washio[22] used a segment of intestine for abdominal skin and subcutaneous tissue prefabrication. Erol and Spira[23,24] revascularised abdominal panniculus by omentum and transferred it to the breast defect successfully. Rich vascular network, large surface area and pliability makes omentum a good vascular carrier but it has an important disadvantage in that laparotomy is necessary.

The opposite approach of transferring fat to a chosen carrier has also been attempted. For example, in an experimental study Hirase et al[25] transferred a pedicled flap of abdominal fat that had been dissected based along the inguinal ligament and transferred it over the epigastric vessels. There are two problems with this model. First, fat tissue is traumatised during the initial transfer. The second problem is to find reliable vessels in the area where the fat tissue is to be transferred, in clinical practice. Of course, transferring the vascular carrier towards the fat tissue is preferred instead of transferring the fat tissue towards the carrier. This concept is the cornerstone of our investigation.

Recently, Adams et al[26] prefabricated fat tissue by muscle flap in rabbits, named 'myoadipose flap'. They elevated the parascapular fat pad with its proximal blood supply intact and then used latissimus dorsi muscle for prefabrication of this fat. Here there is a problem similar to the one seen in the study of Hirase et al, in that the fat tissue is traumatised during the prefabrication. Besides this problem, they used a muscle flap for prefabrication, when there was no need for prefabrication of fat tissue in order to transfer it by muscle since vascular fat transfer was already realised. The latissimus dorsi flap and TRAM flap are examples of flaps which combine fat and muscle. Recently Barnett and Gianoutsos[27] described a technique for adding more fat to a latissimus dorsi flap for the purpose of obviating the need for implants. If the point is the transfer of fat tissue, another important thing that must be paid attention to is the minimisation of the effects of the carrier regarding the volume and pliability of the fat tissue. Of course the muscle will affect both. And donor morbidity is to be taken into consideration while using muscle as a carrier.

Khouri et al[28–30] used a vascularised fascia flap as a vascular carrier for prefabrication. They successfully used superficial inferior epigastric fascia for knee joint prefabrication in rats. They also transferred this experimental work into clinical practice when they used temporalis fascia as a vascular carrier for a second toe PIP joint transfer. They maintained that fascia was the most effective vascular carrier on account of its high vascularity, large surface contact, thin architecture and good vascular pedicle. In addition to all these features easy dissection and minimal donor morbidity make fascia (such as the forearm and superficial temporal fascia) an excellent vascular carrier.

In our study, the superficial inferior epigastric fascia was used as a carrier. After a 2-week staging period, fascia and the right inguinal fat pad were harvested, as a composite island flap. The flaps showed bleeding positive staining on fluorescein test and stained nuclei and intact cell membranes on histopathological examination. This showed the viability of the fat and fascia but was not sufficient to prove that the fat was nourished by the vascularised fascia. To do this a prefabricated composite island flap was transferred to a subcutaneous pocket in the subcostal area where there was no fat. One week later, the flap was re-elevated to evaluate the viability of the fat. To address the question of whether the fat tissue of this flap was maintaining its viability by its fasciovascular pedicle or whether it was surviving as a fat graft, comparison was made with a control group. In the control group a composite fascia–fat graft was created by dividing the fasciovascular pedicle, and this graft was transferred into a subcutaneous pocket. The viability of the graft was evaluated 1 week later, and all the graft tissues were found necrotic. These tissues stained neither in fluorescein test nor in Indian ink angiography. On histopathology, lack of nuclear and cellular membrane staining of both adipocytes and fibroblasts was accepted as evidence of both fascia and fat necrosis.

In the transferred composite prefabricated vascularised island flaps bleeding points were observed from the fat of the re-elevated flaps. Staining was observed in the fat tissue on fluorescein test and in Indian ink angiography. Histopathologic examination also verified that the fascia and fat tissue were viable, and the filling of arterioles, capillaries and venules with black stain (Indian ink) in fat tissue indicated that there was a true circulation in this tissue. All of the findings of this study lead to the conclusion that the fat tissue was nourished by the fascia and prove that prefabrication of fat by fascial flap was successful.

This model suggests that the potential to create prefabricated fat flaps may increase the choice of donor sites for vascularised fat transfer in clinical practice.

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