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Blood flow through prefabricated flaps—an experimental study in rabbits

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SUMMARY. **Prefabricated flaps were created by femoral vessel implantation beneath the abdominal skin in rabbits. The area of survival and blood flow through the prefabricated flap were measured after 1, 2, 4, 6, 8, and 12-week intervals. The parameters at 6, 8 and 12-week intervals were significantly larger than those for random pattern flaps ($p < 0.01$), but were significantly smaller than those for axial pattern flaps ($p < 0.01$). The minimum interval at which the prefabricated flap could be transferred successfully was between 6 and 8 weeks in this model. Microangiography demonstrated that neovascularisation began at the distal end of the implanted vascular bundle and spread throughout the flap by 8 weeks.**

Subsequent to the report by McGregor and Morgan,[1] axial pattern island flap transplantation has become popular among reconstructive surgeons world-wide. However, this method has several disadvantages, including limited availability of donor sites, loss of function and donor site deformity. In addition, axial pattern flaps are usually too thick to apply to the face or the hand since the nutrient vascular pedicle enters the deep layer of subdermal tissue. To overcome problems associated with axial pattern flaps, prefabricated flaps prepared by vascular carrier implantation into the donor tissue have been produced by several investigators. The feasibility of this technique in the transfers of bone, joint, muscle, and skin using the implantation technique of various vascular carriers, for example, blood vessels, omentum, vascularised muscle, and vascularised fascia, has been demonstrated experimentally and clinically by many authors.[2–11] All these studies were qualitative analyses demonstrating the feasibility of prefabrication of many types of tissue. Recently, a few quantitative studies of prefabrication have also been performed,[12–14] but the minimum period after which the prefabricated flap could be transferred successfully was determined mainly by the extent of survival area and microangiographic findings. The most important factor to consider when attempting to determine flap viability is capillary blood flow through the flap. To date, there have been no experimental studies performed in which capillary blood flow through prefabricated flaps has been measured using a microsphere technique. This study was therefore designed to determine the viability of the prefabricated flaps by measurement of the area of survival and blood flow, and by microangiographic and histological observation of neovascularisation, after various intervals of time in a rabbit model.

Materials and methods

One hundred and twelve Japanese white male rabbits, weighing 2.0–2.5 kg each, were used. They were divided into 8 groups (14 animals per group). For all operative procedures, rabbits were anaesthetised with nitrous oxide (1.0 l/min), oxygen (1.0 l/min) and halothane (1.0–2.5%). Intravenous infusions of isotonic saline were administered continuously through an ear vein during all surgical procedures. The left lower limb and left abdominal regions were shaved, washed with soap, and sterilised with povidone-iodine solution in all cases.

Experimental groups

Group 1: Random pattern flap (*Fig. 1A*). On the left anterior abdominal wall, a medially based rectangular random pattern flap, 6 × 4 cm in size, was constructed; it excluded the epigastric neurovascular bundle and was immediately sutured back to the original bed.

Group 2: Prefabricated flap (*1-week interval*) (*Fig. 1B*). First stage: vascular bundle implantation. An incision was made over the left femoral vessels from the inguinal region to below the knee. The vascular bundles with surrounding areolar tissue were identified and carefully dissected, preserving a 7 cm segment from the femoral vessels to the saphenous vessels, using a microscope. The femoral nerve was divided in the inguinal region. All the vascular branches, including the inferior epigastric vessels and the distal ends of the femoral vessels, were ligated and divided. The vascular pedicle, connected proximally to the iliac vessels, was then brought proximally and implanted into a subcutaneous pocket, 1 cm in breadth and 6 cm in length, beneath the skin of the left abdomen. The distal end of the vascular pedicle was sutured to the subcutaneous tissue.

Second stage: prefabricated flap isolation. The rectangular cutaneous flap (6 × 4 cm), which was centred over the course of the implanted vessels and included the panniculus carnosus muscle, was elevated on a pedicle of the implanted vascular bundle as shown in Figure 1B′, and then sutured back into place 1 week after the first stage of preparation.

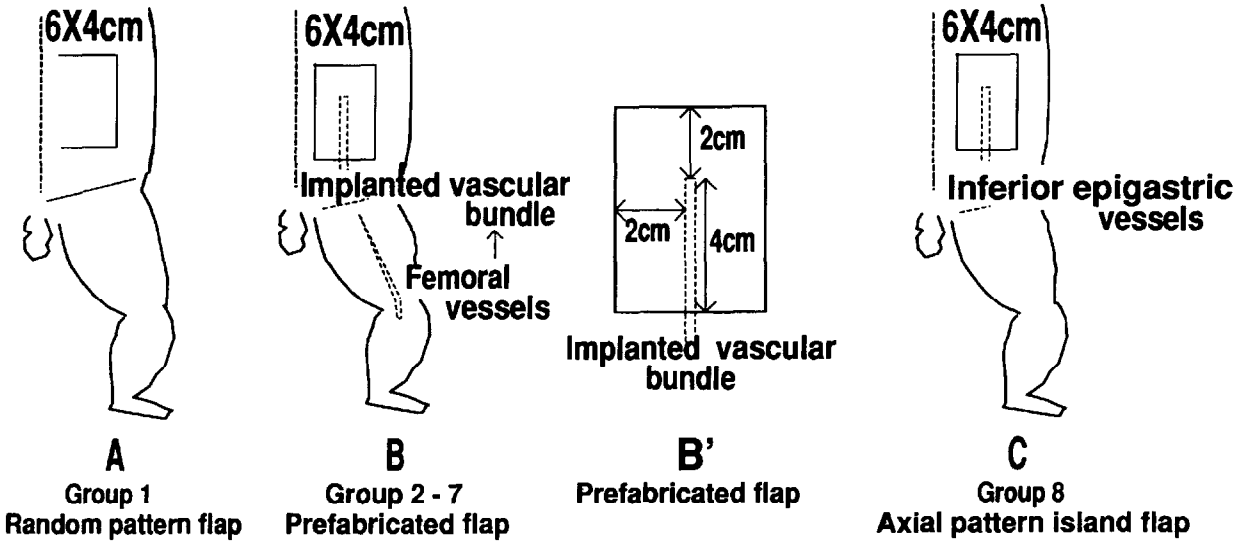

Fig. 1

Figure 1—Creation of flap models. (A) Random pattern flap. Medially based rectangular random pattern flap, 6 × 4 cm in size, was elevated. (B) Prefabricated flap. Left femoral vessels were implanted beneath the abdominal skin and the flap, 6 × 4 cm in size, was elevated after a 1-, 2-, 4-, 6-, 8- or 12-week interval. (B') shows relationship between the implanted vascular bundle and the flap. (C) Axial pattern island flap. Left abdominal flap, 6 × 4 cm in size, was isolated on a pedicle of inferior epigastric vessels.

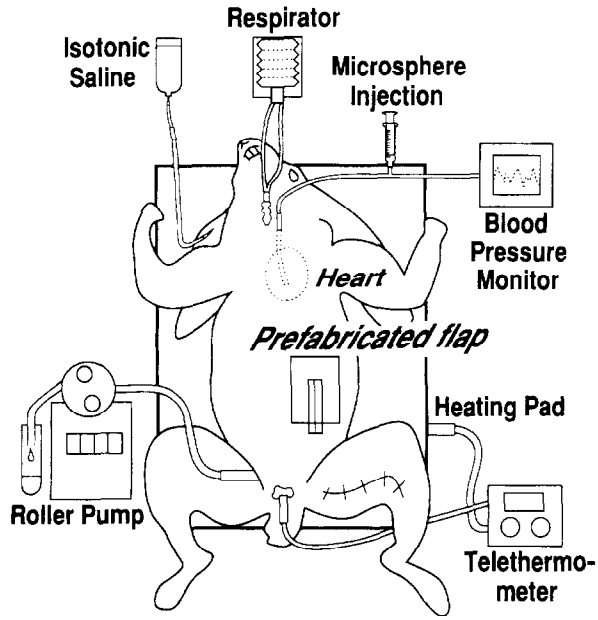

Fig. 2

Figure 2—Blood flow measurement. Blood flow to the flaps was measured at the third day after flap elevation using a coloured microsphere technique under constant circulatory conditions. Two million coloured microspheres, 15 μm in diameter, were injected into the heart through the carotid artery.

Group 3: Prefabricated flap (2-week interval). The prefabricated flap was isolated 2 weeks after the first stage of preparation.

Group 4: Prefabricated flap (4-week interval). The interval between the first and second stages was 4 weeks.

Group 5: Prefabricated flap (6-week interval). The interval was 6 weeks.

Group 6: Prefabricated flap (8-week interval). The interval was 8 weeks.

Group 7: Prefabricated flap (12-week interval). The interval was 12 weeks.

Group 8: Axial pattern island flap (Fig. 1C). A rectangular island flap in the left anterior abdominal wall, 6 × 4 cm in size, was isolated on a pedicle of the inferior epigastric vessels, and immediately resutured to its original bed. The epigastric nerve was divided at a point where it bifurcated from the femoral nerve.

Evaluations

1. Measurement of flap survival area. All the flaps were observed to evaluate circulatory viability 3 days after elevation (second stage). To determine the area of survival of the flaps, they were photographed along with a scale. These pictures were then input into a personal computer (PC-9801VX, NEC, Tokyo, Japan) using a colour image processor (SPICCA-2, Nippon Avionics, Tokyo, Japan). Surviving regions appeared pink, while regions of necrosis appeared dry, shrunken and dark.

2. Measurement of blood flow (Fig. 2). Blood flow through the flap was measured in 10 rabbits in each group, thus for a total of 80 rabbits, following determination of viability of the flap. Each animal was anaesthetised and laid in the supine position. Following intravenous injection of tubocurarine chloride (Amelizol®, Yoshitomi Pharmaceuticals, Osaka, Japan) at 3 mg/kg, each animal was immediately intubated by tracheotomy and mechanically ventilated with a respirator (EVM-50 type, Aika, Tokyo, Japan). The tidal volume and the respiratory rate were set at 30 ml and 30 cycles/min, respectively. Body tem-

perature was measured by a telethermometer placed in the rectum, and a heating pad (KN-474, Shibaura Electro, Tokyo, Japan) was used to maintain body temperature at 38.5±0.5°C.

To measure arterial pressure, a polyethylene catheter (internal diameter, 0.2 mm, external diameter, 0.5 mm; Natume Co. Ltd, Tokyo, Japan) was placed in the left carotid artery and calibrated manometrically at the start of each experiment. The tip of this catheter, which was also used to inject microspheres, was advanced gently into the left ventricle. Its position in the heart was determined by changes in the recorded blood pressure. Another polyethylene catheter, connected through a constant flow roller pump (Minipuls 2, Gilson, Meddleton, WI, USA) to a centrifuge tube, was placed in the right femoral artery in order to obtain arterial blood samples at the rate of 2.5 ml/min.

Microsphere injection was delayed for a short period (10–15 min) to permit the circulation to reach a steady state. Coloured microspheres (E–Z Trac, Los Angeles, CA, USA) 15.3±0.17 μm (mean±SD) in diameter were used. The microspheres were vortexed for approximately 1 min just prior to injection. A total of 0.2 ml of suspension containing 2 million spheres in an aqueous solution of 0.05% Tween 80 and 0.01% Thimersal was flushed into the left ventricle for each measurement. Following microsphere injection, 2.0 ml of isotonic saline was flushed through the catheter to the left ventricle to ensure adequate delivery of the microspheres. The total time of injection was 30 s.

Blood sampling started 30 s before microsphere injection and continued until 60 s after injection. The total collection time was 2 min, and a total of 5 ml was sampled from each animal. All animals were then killed by intravenous overdose of pentobarbital sodium. The flap, normal skin (3×4 cm) in the right abdominal wall, and a 3 g portion of right kidney were removed from each animal and weighed. All tissue and blood samples were digested by alkaline hydrolysis, and microspheres were collected by centrifugation. Blood flow in each tissue was calculated by the ratio of the number of microspheres included in the sampled blood to that in each tissue, using the procedures described by Hale *et al.*[15]

3. Microangiography. Following observation of flap viability, microangiography was performed in the 4 animals in each group not tested for blood flow. Each animal was heparinised intravenously. The abdominal aorta was cannulated with a polyethylene tube, and the vascular system of the lower limbs was irrigated with heparinised saline. A 25% suspension of micropaque with 5% gelatin in a saline solution, which was kept mixed during the experiment, was injected into the abdominal aorta at a constant injection pressure of 100 mmHg until the contrast medium drained from the inferior vena cava. Following injection, all animals were killed by an overdose of pentobarbital sodium. The flap was excised, pinned on cardboard and immediately frozen. Fifteen minutes later, microangiographic X-rays of the whole flaps were obtained using a Softex X-ray machine (CSM-21, SOFTEX, Kanagawa, Japan) and Fuji FR film (FUJI Photo Film Co. Ltd, Tokyo, Japan).

4. Histological examination. All the flaps were immediately fixed following microangiography in 10% formal saline for histological examination, and 1 mm slices were cut free-hand, perpendicular to the long axis of the vascular pedicle at the centre of the flap, embedded in paraffin, and stained with haematoxylin and eosin.

Statistical analysis

Area of survival and blood flow data are expressed as means±SEM. Statistical analyses were performed on a personal computer (PC-9801VX). To compare values between groups, the Kruskal–Wallis test was used. Apparent differences were tested for statistical significance using the multiple comparisons test (Wilcoxon type), with p values of 0.05 or less taken to indicate statistical significance.

Results

1. Area of survival (Fig. 3)

In group 1, the medial half of the flap survived, and its area of survival was 7.65±1.06 cm^2.

In group 2, all the prefabricated flaps showed necrosis over their entire surface. Thus no areas of survival were measured in any of the 1-week interval group specimens.

The area of survival of the prefabricated flap increased as the interval between implantation and elevation grew longer.

In group 3, only a small region of survival was

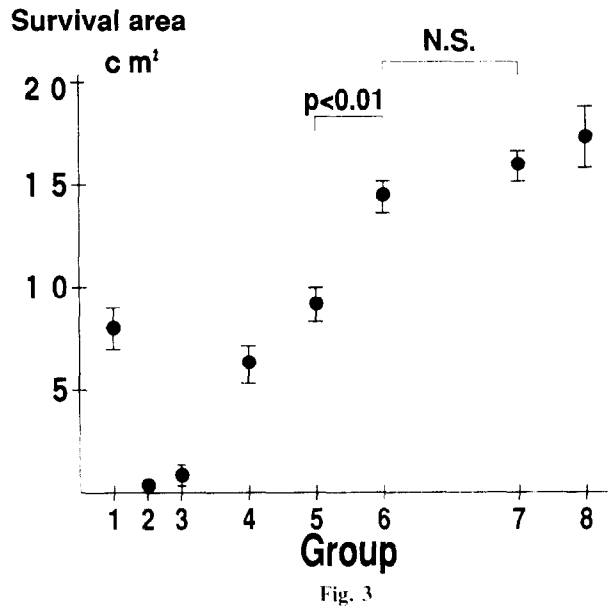

Fig. 3

Figure 3 Results of study of area of survival. Group 1, the random pattern flap; group 2, 1-week interval; group 3, 2-week; group 4, 4-week; group 5, 6-week; group 6, 8-week; group 7, 12-week interval prefabricated flap; and group 8, the axial pattern flap. The area of survival increased as the time interval increased, but plateaued at 8 weeks. There were significant differences in area of survival between the 6- and 8- week interval groups ($p < 0.01$). NS: no significant difference.

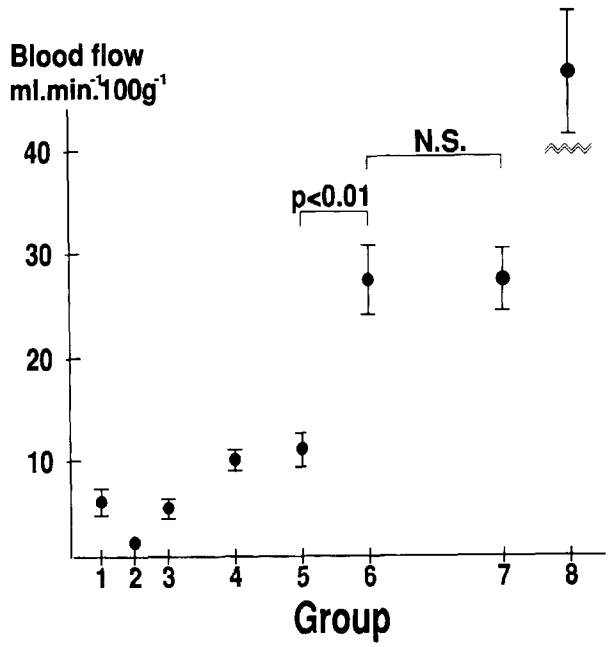

Fig. 4

Figure 4—Results of study of blood flow. Group 1, the random pattern flap; group 2, 1-week interval; group 3, 2-week; group 4, 4-week; group 5, 6-week; group 6, 8-week; group 7, 12-week interval prefabricated flap; and group 8, the axial pattern flap. Blood flow through prefabricated flap increased as the time interval increased, but plateaued at 8 weeks. There were significant differences in blood flow between the 6- and 8-week interval groups ($p < 0.01$). NS; no significant difference.

observed at the distal end of the implanted vascular bundle. The area of survival was 0.97 ± 0.30 cm^2.

In group 4, the area of survival became wider and bell-shaped, and was 6.62 ± 0.95 cm^2.

In group 5 flaps, the area of survival was wider than that of the group 4 flaps, with the area of survival in this group 8.78 ± 0.91 cm^2.

In group 6, most regions of the flap survived, but small areas of necrosis were present at the corner of the rectangular flaps. The area of survival was 14.61 ± 0.72 cm^2.

In group 7, almost all of each of the flaps survived, and the area of survival was 15.99 ± 0.69 cm^2.

In group 8, all of each of the flaps survived, and the area of survival was 17.35 ± 1.78 cm^2.

Statistical analyses of the area of survival between groups demonstrated that values for group 1 were significantly larger than the corresponding values for both group 2 and group 3 ($p < 0.01$), but were not significantly different from the corresponding values of either group 4 or group 5. Areas of survival in groups 6 and 7 were significantly larger than the corresponding values for group 1 ($p < 0.01$), but were not significantly different from the values of group 8. Statistical significance was noted between each of groups 2, 3, 4 and 5 on the one hand, and groups 6 and 7 on the other ($p < 0.01$). However, the differences in values between groups 6 and 7 were not significant.

2. *Blood flow*

There were no significant changes in arterial blood pressure, heart rate, respiratory condition and body temperature due to microsphere injection in any animal.

Blood flow of normal skin and kidney. The blood flow through normal right abdominal skin and kidney of the 80 rabbits tested was 10.39 ± 3.80 ml·min^{-1}·100 g^{-1} and 460.3 ± 36.5 ml·min^{-1}·100 g^{-1}, respectively.

Blood flow through the flaps: (Fig. 4). Blood flow through group 1 flaps was 5.54 ± 2.09 ml·min^{-1}·100 g^{-1}. The corresponding values for groups 2, 3, 4, 5, 6 and 7 were 0.38 ± 0.14, 4.24 ± 0.52, 9.88 ± 0.59, 10.83 ± 1.62, 27.39 ± 3.36, and 27.60 ± 2.77 ml·min^{-1}·100 g^{-1} respectively. Blood flow through group 8 flaps was 58.96 ± 4.92 ml·min^{-1}·100 g^{-1}.

Statistical analyses of these blood flow values demonstrated that blood flow in group 1 flaps was significantly larger than that in group 2 flaps ($p < 0.05$), but not significantly different from that in groups 3, 4, or 5; it was significantly less than that in the flaps of groups 6, 7 and 8 ($p < 0.01$). Blood flow in group 8 flaps was significantly larger than that through the prefabricated flaps of the other groups (groups 1, 2, 3, 4; $p < 0.01$, groups 5, 6, 7; $p < 0.05$). A statistically significant difference was noted between each of groups 2, 3, 4 and 5 on the one hand and groups 6 and 7 on the other ($p < 0.01$). The difference in flap blood flow between groups 6 and 7 was not significant.

3. *Microangiographical findings*

In group 1, pre-existing straight vessels were filled with contrast medium in the medial half of the flap, while the lateral half was avascular.

In group 2, neither newly formed nor pre-existing vessels were evident; the implanted vascular bundle alone was dilated and filled with contrast medium (Fig. 5). In group 3, some pre-existing vessels had become connected to the implanted vascular bundle through small, meandering vessels, which were newly formed only in the region of the distal end of the implanted vascular bundle (Fig. 6). In group 4 (Fig. 7) and group 5 (Fig. 8), newly formed vessels were larger in size and more numerous. A larger number of pre-existing vessels was filled with contrast medium, due to wider connections through the newly formed vessels. No contrast medium was observed along the margins of the flap. A dense vascular network was observed in all regions of the flap in group 6 (Fig. 9). Group 7 flap microangiograms appeared similar to those of flaps in group 6, but the newly formed vascular network was even more dense than that in group 6 flaps (Fig. 10).

Numerous pre-existing vessels were observed across the entire surface of the flap in group 8.

4. *Histological findings*

Surviving regions of flaps in group 1 had nearly normal skin and muscle architectures, but necrotic regions showed a remarkable degree of degeneration. The flaps in groups 2 and 3 showed a similarly great degree of degeneration to the necrotic regions of the flaps in group 1. The epidermis, dermis, muscle layer,

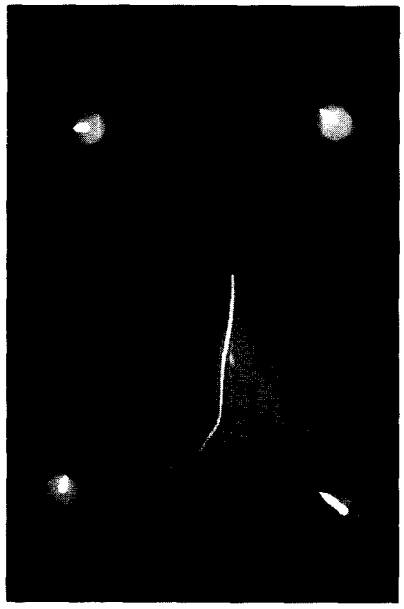

Fig. 5

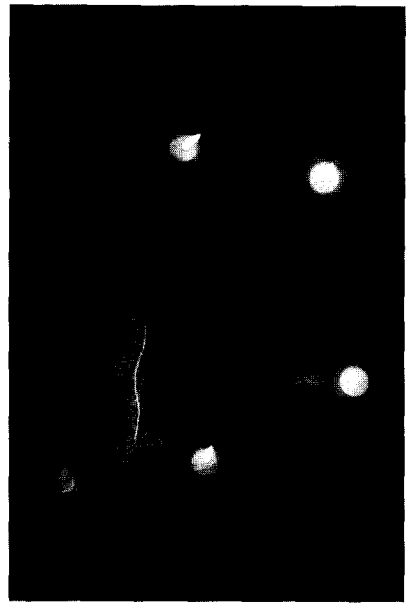

Fig. 6

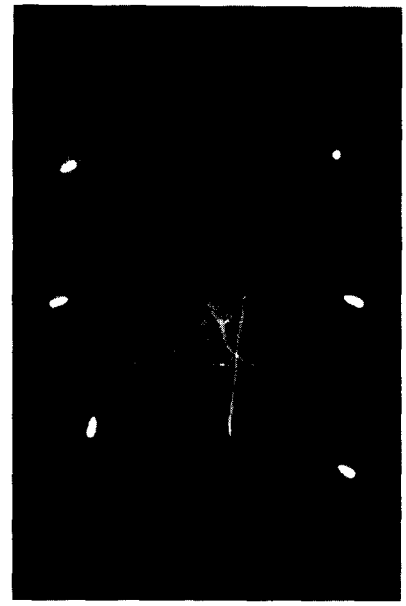

Fig. 7

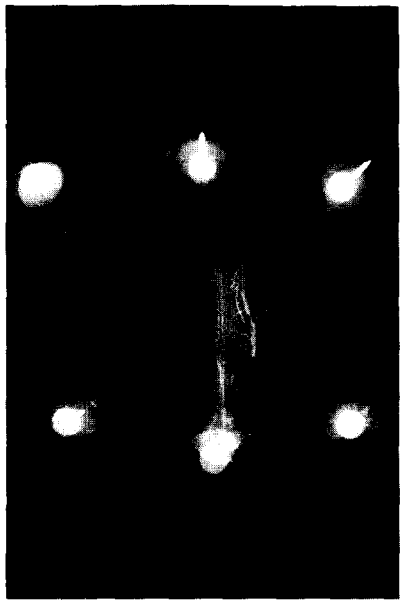

Fig. 8

Fig. 9

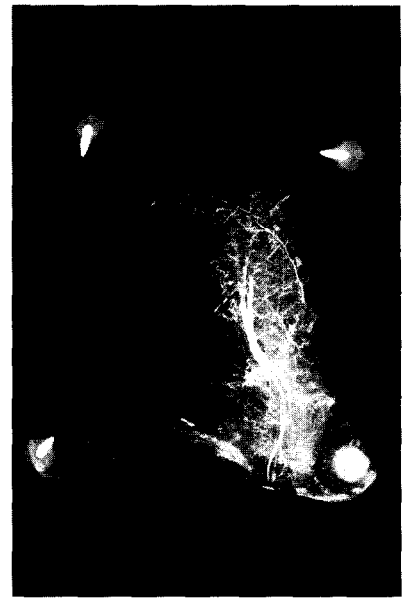

Fig. 10

Figure 5—Microangiogram of a prefabricated flap after 1-week interval. Only the implanted vascular bundle is shown. Neither newly formed nor pre-existing vessels are evident. **Figure 6**—Microangiogram of a prefabricated flap after 2-week interval. Some pre-existing vessels have connected with the implanted vascular bundle through small, meandering, newly formed vessels. Newly formed vessels are present only in the region of the distal end of the implanted vascular bundle. **Figure 7**—Microangiogram of a prefabricated flap after 4-week interval. Newly formed vessels are larger in size and more numerous, but do not reach the margin of the flap. **Figure 8**—Microangiogram of a prefabricated flap after 6-week interval. Newly formed vessels are more numerous than those in prefabricated flaps after 4-week interval. **Figure 9**—Microangiogram of a prefabricated flap after 8-week interval. Newly formed vessels and a vascular network are present in all portions of the flap. **Figure 10**—Microangiogram of a prefabricated flap after 12-week interval. The network of newly formed vessels is even more dense than that in the 8-week interval flaps.

sweat glands, and hair follicles of these flaps were severely atrophic or necrotic, and micropaque did not fill the vessel lumina within the flaps (Fig. 11). Surviving regions of the flaps in groups 4 and 5 had nearly normal structures of the epidermis, dermis, muscle layer, sweat glands, and hair follicles. However, the necrotic regions of the flaps in groups 4 and 5 were similar to those in flaps in groups 2 and 3. The flaps in groups 6 and 7 had nearly normal skin and muscle architecture, but were slightly more atrophic than the flaps in group 8. Micropaque injection resulted in dye uptake into the dermal capillary network and an engorged subdermal plexus (Fig. 12). The flaps in group 8 had nearly normal skin and muscle architectures, and more copious dye uptake than did the flaps in groups 6 and 7.

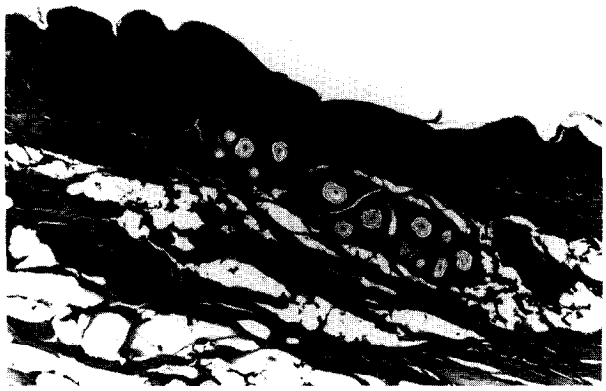

Fig. 11

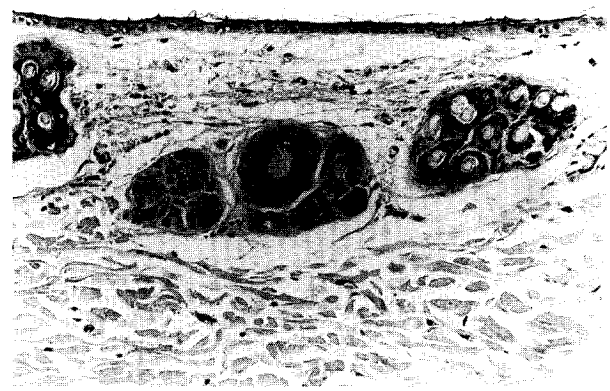

Fig. 12

Figure 11—Light micrographs (HE stain, × 100) of a prefabricated flap after 1-week interval. The epidermis, dermis, connective tissue, muscle layer, sweat glands and hair follicles of these flaps show severe atrophy or necrotic changes. **Figure 12**—Light micrographs (HE stain, × 100) of a prefabricated flap after 12-week interval. The prefabricated flaps after a 12-week interval maintained nearly normal skin and muscle architecture. Structure of the epidermis, dermis, muscle layer, sweat glands and hair follicles was preserved.

Discussion

Capillary blood flow is the principal factor determining flap viability.[16] Guba *et al.*[17] and Pang *et al.*[18] reported measurements of experimental flap blood flow using the radioactive microsphere technique. Disadvantages of this technique include its requirement for special equipment, inconvenient disposal of animals, and the potentially dangerous use of radiation. The use of non-radioactive coloured microspheres as described by Shell *et al.*[19] provides an effective alternative method that overcomes these disadvantages. This method has already been tested by the authors[20] in a comparative study involving measurement of blood flow through skin flap and kidney in rabbits; a good correlation with results obtained using radioactive microspheres was demonstrated. In the present experimental study, blood flow through the normal skin and kidney was also measured using the coloured microsphere technique. Rates of blood flow through the normal right abdominal skin and kidney of 80 rabbits were 10.39 ± 3.80 ml·min^{-1}·100 g^{-1} and 460.3 ± 36.5 ml·min^{-1}·100 g^{-1}, respectively. These values are almost the same as those obtained by Boom and Saxena[21], 12 ± 1 ml·min^{-1}·100 g^{-1}, and 462 ± 32 ml·min·$^{-1}$ 100 g^{-1} (mean ± SEM), for blood flow through normal skin and kidney in rabbits using the radioactive microsphere technique. We thus confirmed that the coloured microsphere technique is a very safe, useful, and reliable method for the measurement of flap blood flow in this rabbit experimental model.

In our model, both the area of survival and blood flow were measured on the third day after flap elevation. There were two main reasons why both the areas of survival and blood flow were measured at this time. First, both surviving and necrotic regions of the flaps became clearly observable 3 days after flap elevation. Second, as reported by Erdogan[22] in his experimental study of the prefabricated flap, revascularisation from the surrounding skin and the recipient bed to the prefabricated flap occurs subsequent to the fourth day after flap elevation; hence, until 3 days after flap elevation, blood flow from surrounding tissue to the prefabricated flap can be ignored.

The original flap area was 24 cm^2 (6 × 4 cm in size), but 100% flap area survival actually corresponded to between 15 cm^2 and 20 cm^2. This difference was due to flap contraction following elevation and exclusion of the sutured area from calculations of the area of survival.

In the present experiments, the medial half of the random pattern flap survived, and a survival area of 7.65 cm^2 was obtained. Blood flow through the random pattern flap was 5.54 ml·min^{-1}·100 g^{-1}. In order to create a prefabricated flap, the femoral vessels were implanted into this tissue. Chronological observations of formation of a neovascular network in this flap demonstrated that the area of survival and blood flow increased with time. Four weeks after implantation, both the area of survival and blood flow in prefabricated flaps were comparable to those in random pattern flaps. From the 8th week on, the values of these two parameters were significantly higher in prefabricated flaps than in random pattern flaps ($p < 0.01$). However, blood flow through prefabricated flaps was significantly lower than that through equal-sized axial pattern flaps. Even when the time permitted for neovascularisation was prolonged, blood flow through prefabricated flaps did not surpass that through axial pattern flaps. Tark *et al.*[14] compared prefabricated flap and axial pattern flap surface perfusion, using fluorometry in a rat model. They also reported that skin surface perfusion of prefabricated flaps did not become as high as that of axial pattern flaps. These findings indicate that a prefabricated flap is intermediate between a random pattern flap and an axial pattern flap in blood flow, and that it is more effective in its viable size than a random pattern flap, if used in tissues where elevation of an axial pattern flap is impossible.

Microangiographic study demonstrated that neovascularisation began at the distal end of the implanted vascular bundle and spread across the implant as the interval of time since implantation increased. Six weeks or longer was required for the formation of a vascular

network in prefabricated flaps which was better than that in random pattern flaps.

Based on these results, it therefore seems that a prefabricated flap can serve more effectively than a random pattern flap if the neovascularisation time is longer than 8 weeks.

A question that remains related to the clinical use of prefabricated flaps is the size of the region of viability that can be obtained at various times of neovascularisation. In the past, the timing of elevation of a prefabricated flap was determined empirically by trial and error. In our study, both blood flow and the area of survival plateaued in value at 8 weeks. The values for the 8-week interval group were significantly larger than those for the 6-week interval group ($p < 0.01$), while there was no significant difference in values between the 8- and 12-week interval groups. Microangiograms also demonstrated that the neovascular network had spread throughout the flap in animals of the 8-week interval group. Histologically, flaps in the 8-week interval group retained nearly normal skin and muscle architecture throughout their extent; 8-week interval flaps were, however, slightly more atrophic than the axial pattern flaps. These results obtained with our rabbit model indicated that the earliest interval at which a viable prefabricated flap (6×4 cm) could be raised was between 6 and 8 weeks after vascular bundle implantation. This finding is similar to that reported by Martinot *et al.*[12] and Morrison *et al.*[13] Martinot reported that 8–10 weeks was required for neovascularisation to occur in a 6×7 cm prefabricated flap in rats. Morrison reported that in his rabbit model the difference in rate of survival between flaps elevated either 2 or 4 weeks after implantation and those elevated 8 or 12 weeks after implantation was quite significant. The similarity of values obtained for this parameter in the present study and those in the studies of Martinot *et al.* and Morrison *et al.* is attributable to the fact that approximately the same size of flap was used in each of these three studies. On the other hand, Shen[7] reported that a prefabricated flap prepared by implantation of the central auricular vessels into the frontal skin of the head became sufficiently viable to permit elevation in 2 weeks. The shorter period to elevation in Shen's study was probably the consequence of use of a smaller flap size (4×2 cm) than that in other studies. Thus, the time required for neovascularisation varies depending upon the size of the prefabricated flap to be elevated.

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