Prefabricated Osteocutaneous Neural Island Flap Model

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Abstract: Neural-based flaps are an interesting clinical choice particularly in difficult cases that may not be reconstructed with known techniques. Their popularity is gradually increasing because these flaps offer the advantage of preservation of major extremity arteries and avoidance of microsurgical techniques. Our aim was to explore the feasibility of prefabrication of an osteocutaneous neural island flap model in this study. A peripheral nerve of the rat was implanted into the subcutaneous tissue of a skin flap that was connected to a segment of bone by a soft-tissue bridge, to prefabricate an osteocutaneous flap that was supplied only by the intrinsic vasculature of that nerve after a preliminary delay period. At the end of this study, based on direct observation, microangiographic findings, and additionally, a detailed histologic analysis consisting of both qualitative and quantitative assessments, we have proved that it was possible to prefabricate an osteocutaneous composite flap based on the vascularity of a peripheral nerve after a 2-step delay period. We believe that the clinical application of this new flap will gradually develop based on further experimental studies.

Key Words: neural island flap, neurocutaneous flap, peripheral nerve implantation, vascular implantation

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Masquelet et al[1] were the first to introduce the concept of neuroskin flaps in 1992. The constant association between the superficial sensory nerves and a rich arterial axis, along with the role of this network in the profusion of the skin was the basis for this flap type. These researchers proved that skin island flaps can be effectively supplied by the vascular axis of superficial nerves in the leg, and since then, these flaps have been popularized by many reconstructive surgeons.

This flap type is particularly useful in the reconstructive surgery of the distal part of the extremities. Additionally, its popularity is gradually increasing because of offering the advantage of preservation of major extremity arteries and avoidance of microsurgical techniques.

In a recent study, we have introduced a reliable skin flap prefabrication model that was supplied by the intrinsic vasculature of a peripheral nerve, which was implanted into the subcutaneous tissue of that flap. We termed this as the "prefabricated neural island flap."[2] On the basis of same principle, in this study our aim was to explore the feasibility of prefabrication of osteocutaneous neural island flap model. In brief, a peripheral nerve of the rat was implanted into the subcutaneous tissue of a skin flap that was connected to a segment of bone by a soft-tissue bridge, to prefabricate an osteocutaneous flap that was supplied only by the intrinsic vasculature of that nerve after a preliminary delay period.

MATERIALS AND METHODS

A total of 25 adult Wistar rats, weighing 200 to 250 g were used in this study. The study approval and consent were obtained from the Animal Care and Use Committee of Hacettepe University (Number, 2004/59–7). All animals were kept nil per os the night before the procedure. No prophylactic antibiotics were administered. Each rat was anesthetized with an intramuscular injection of ketamine hydrochloride (40 mg/kg), with supplemental doses administered as necessary. In the postoperative period, all rats were housed in standard environmental conditions, kept in individual cages, fed with standard rat chow and tap water, which was available ad libitum.

An operating microscope (Zeiss OPMI, 6SD; Carl Zeiss, Gottingen, Germany) was used during dissections. To provide analgesia, 300 mg/kg per oral acetaminophen was used for the first 2 days postoperatively.

The study was divided into following 2 parts: Part I: Anatomic Dissections (n = 5); Part II: Flap Study (n = 20).

Part I: Anatomic Dissections (n = 5)

Anatomic dissections were performed in a total of 5 animals to discover the anatomy of the rat and the potential nerve to be used for vascularization of an osteocutaneous flap, following euthanasia of the rats by an overdose of pentobarbital. At the end of dissections, we decided to use the sciatic nerve and the lumbar region skin connected to a segment of femur for part II of the study.

Part II: Flap Study (n = 20)

The 20 rats in this part of the study were divided into 2 groups.

Group I (n = 10): Prefabricated Osteocutaneous Neural Island Flap Group

Dissection of the Sciatic Nerve

A longitudinal incision was made for the exposure of the sciatic nerve of one of the lower extremities of the rat. The dissection of the sciatic nerve was performed under the operating microscope (under ×6 magnification), starting from the sciatic notch to the bifurcation of the nerve that was approximately at the distal part of the femur. At the end of the dissection, the sciatic nerve was transected from this region, and freed proximally until the sciatic notch. The total length of the dissected nerve stump was about 23 mm at the end of this dissection, which was long enough to reach the lumbar region of the rat (Fig. 1).

Preparation of the Osteocutaneous Flap

Based on our experience obtained from "prefabricated neural island flap model," a skin flap with the dimensions of 20 mm width and 30 mm length was planned on the lumbar region of the rat (Fig. 2). Additionally, a segment of femur about 1 cm in length was cut and a soft-tissue bridge containing an arterial branch was left intact between the skin flap and the segment of femur during the flap harvesting. This flap was harvested at subpannicular level as a

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FIGURE 1. Exposed sciatic nerve after the dissection.

FIGURE 2. The design of the osteocutaneous flap for prefabrication.

peninsular random pattern flap based on medial dermal pedicle. Afterward, the dissected sciatic nerve stump was passed through the gluteus superficialis muscle to reach underneath the skin flap in the lumbar region. Next, the nerve stump was sutured to the subcutaneous tissue of the peninsular flap with 8–0 nylon sutures (Ethilon monofilament, polyamide 66 suture, W2808, Ethicon Ltd., Edinburg, United Kingdom) and the flap was replaced in situ with 4-0 absorbable sutures (Vicryl J&J.497G, Ethicon Ltd., Edinburg, United Kingdom) (Figs. 3 and 4). The total length of the delay process was planned as 3 weeks that was divided into 2 steps (2 weeks + 1 week). The medial dermal pedicle of the peninsular flap was cut and primarily closed with 4–0 absorbable sutures at the end of the second week of the delay period (Fig. 5). After an additional 1 week, the osteocutaneous flap was harvested as an island flap based on only the sciatic nerve following the transection of all musculocutaneous perforators and the dermal pedicles once more and replaced in situ. The dilated perineural vasculature of the neural pedicle was obvious under an operating microscope at this time (×15) (Figs. 6–8).

Group II (n = 10): Graft Group

The same procedures as those in Group I were performed in all the rats of this group and additionally, the sciatic nerve supplying the island flap was transected and ligated with 8–0 nylon sutures at the end of the third week of the delay period. Afterward, the

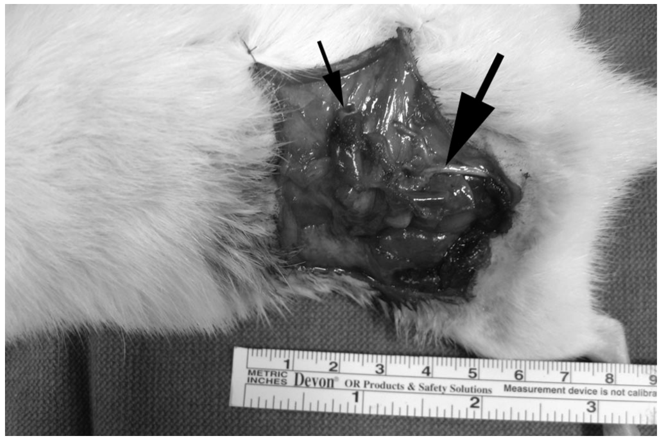

FIGURE 3. The dissected sciatic nerve stump was passed through the gluteus superficialis muscle to reach underneath the skin flap in the lumbar region and was sutured to the subcutaneous tissue of the flap (marked with thick arrow). The bone segment that was harvested with the skin flap is marked with thin arrow.

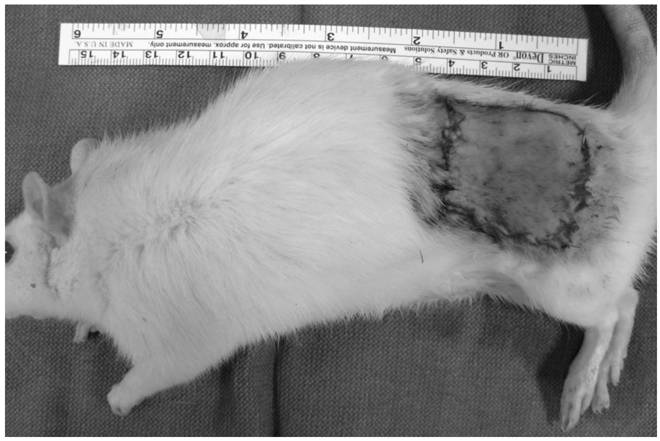

FIGURE 4. The osteocutaneous flap was replaced in situ. Note that the medial part of the flap was left intact.

osteocutaneous flap was replaced in situ as a graft with 4-0 absorbable sutures.

Flap Viability Evaluation

All the flaps and grafts were evaluated on a daily basis until postoperative day 7. The final decision was made based on direct observation, histologic analysis, and microangiography. The survival areas of the cutaneous part of the flaps were determined and traced onto acetate sheets. The surviving flap was calculated as a percentage of total flap dimensions with the paper template technique.[3]

The flaps were then submitted for microangiography to delineate the vascular pattern of the composite flaps. The detailed technique of the angiography was described elsewhere.[4] Briefly, after catheterization of the carotid artery of the rats, the composite flap was harvested based solely on its neural pedicle. Following the euthanasia of the rats with an overdose of pentobarbital, the rats were infused with the mixture of lead oxide and gelatin. Next, the composite flap ex vivo underwent radiography with a soft x-ray machine at the settings of 22kV and 5mA (Mammo Diagnost UC, Philips, Hamburg, Germany).

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FIGURE 5. The medial dermal pedicle of the peninsular flap was cut at the end of the second week of the delay period.

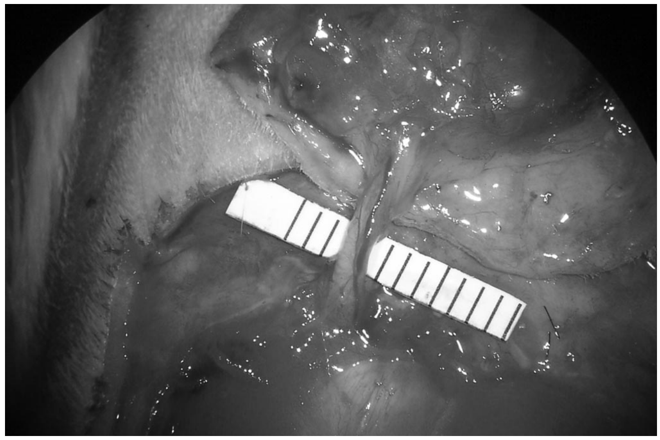

FIGURE 7. The dilated perineural vasculature of the neural pedicle was obvious under an operating microscope at the end of the third week of the delay period (×15).

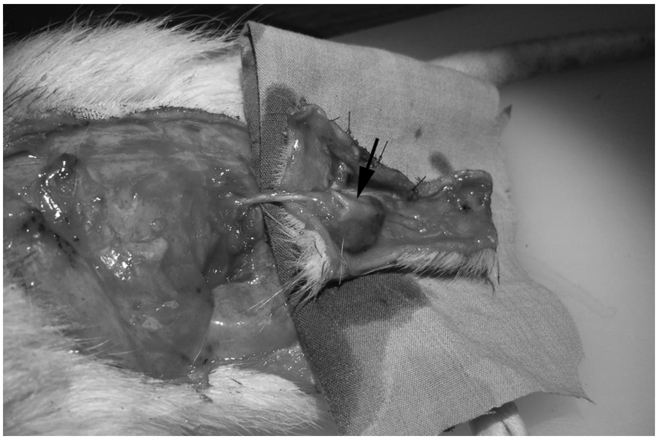

FIGURE 6. The osteocutaneous flap was harvested based on only the sciatic nerve at the end of the third week of the delay period. The bone segment connected to the skin flap is marked with thin arrow.

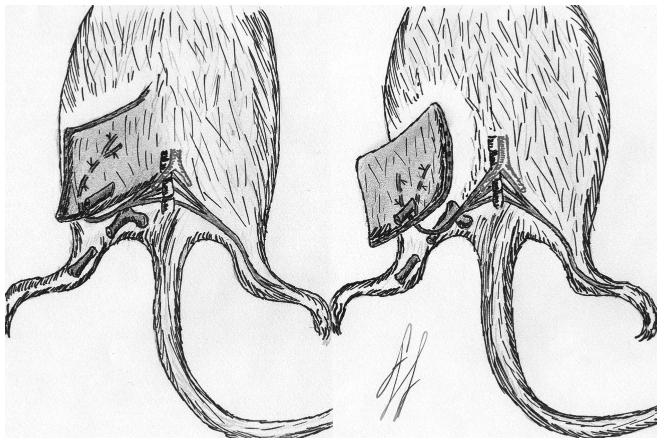

FIGURE 8. The drawing of the design of the delay procedure (left) and the prefabricated osteocutaneous flap that was harvested based on only the sciatic nerve at the end of the third week of the delay period (right).

Following the microangiographic evaluation, the composite flaps ex vivo were submitted for histologic evaluation. The specimens were placed in 10% phosphate buffered formalin (pH 7.0) at room temperature for fixation. Samples were decalcified by immersion in De Castro solution for 5 to 10 days before dehydration. They were rinsed in buffer, and dehydrated in a graded series of ethanol before embedding in paraffin. Five micrometer thick serial sections were cut with a sliding microtome (Leica, Germany). Hematoxylin and Eosin, and Masson trichrome stained sections were evaluated for overall morphology and bone graft viability. The histologic findings were evaluated by 2 blinded investigators (Petek Korkusuz and Rumeysa Hekimoglu) by using Leica DMR microscope (Germany). The interobserver reliability (r) for their evaluation was 1.00. The images were captured using the Leica DC500 digital camera (Germany). Quantitative analysis was carried out on serial sections of bone graft by using Leica Qwin Plus computer image analysis system (Germany) by modifying the literature.[5] A 10-mm bone graft was divided into 5 equally spaced regions. From each region, 20 sections were randomly taken, giving a total of 20 × 5 measurements from each sample. At this stage, 2 parameters were quantified. Initially, the number of total lacuna was counted and osteocyte-containing lacuna per total lacuna was calculated at 200× magnification. Later, a green to red stained bone area ratio was calculated on Masson trichrome-stained sections to measure the active/remodeled bony surface.

Statistical Analysis

The histomorphometric data were analyzed by nonparametric Mann-Whitney *U* test to assess statistical significance. Descriptive statistical values were expressed as minimum, maximum, median, mean, and standard deviation.

RESULTS

Part I: Anatomic Dissections (n = 5)

On the basis of our previous experience, sciatic nerve was selected to be used for vascularization of the prefabricated composite flap. The dissected sciatic nerve could easily reach the dorsolumbar region, which was preferred to prevent autocannibalization. Besides, during anatomic dissections, we realized that it was possible to harvest the skin flap on the dorsolumbar region connected to a segment of femur via a soft-tissue bridge containing an arterial branch. A meticulous dissection enabled us to harvest the osteocu-

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FIGURE 9. Prefabricated osteocutaneous neural island flap group (Group I) on day 7, depicting total survival of the skin of the composite flap.

FIGURE 10. The graft group (Group II) on day 7, depicting total necrosis of the skin of the composite flap.

taneous flap with minimal destruction of the vasculature within this soft-tissue bridge between the skin flap and the femoral bone segment.

Part II: Flap Study (n = 20)

The mean survival rate in the cutaneous parts of the prefabricated osteocutaneous neural island flap group (Group I) was 98.4% ± 6.42%, demonstrating a much greater survival than the graft group, which was 3.5% ± 2.76% (Figs. 9, 10).

Vascularity of the prefabricated osteocutaneous neural island flap and the course of the lead oxide-gelatin mixture passing through the dilated intraneural vessels of the neural pedicle to the flap were demonstrated by microangiographic evaluation. Increased vascularity was evident particularly around the osseous portion of the composite flap (Fig. 11).

Osseous part of the osteocutaneous composite flap viability was quantitatively assessed by the new or actively remodeled bony surface area and the ratio of osteocyte-containing lacunae. The active and/or new-formed green-stained bone proportion was significantly higher in prefabricated osteocutaneous flap group (Group I) samples comparing with that of the graft group (Group II) (P = 0.00). The osteocyte-containing lacuna density was also significantly higher in prefabricated osteocutaneous flap group (Group I) compared with that of graft group (Group II) (P = 0.00) (Tables 1, 2) . The nerve containing pedicle stimulated new bone formation within its surrounding by stimulating sprouting of new patent small blood vessels within that capsule. A highly vascular and cellular connective tissue surrounding the neural pedicle initiated the intramembranous formation starting from the endosteal and periosteal edges of the cortical bone (Fig. 12).

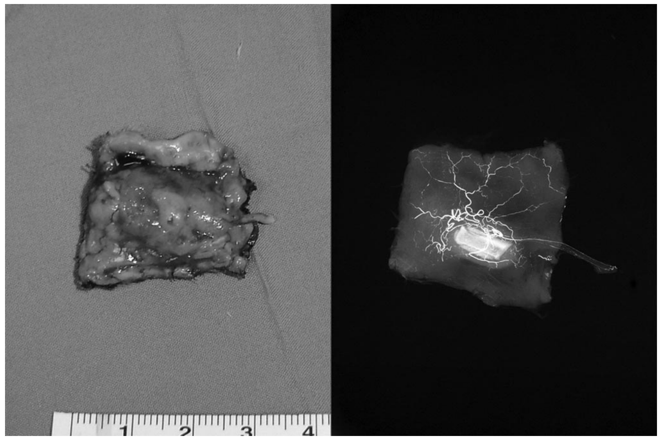

FIGURE 11. The flap ex vivo (left) and the microangiographic appearance of the flap specimen (right). Note the increased vascularity around the bone segment.

TABLE 1. The Active and/or New Formed Green-Stained Bone Proportion (gb_rb) and the Osteocyte-Containing Lacuna Density (osl_total) of the Groups I and II Are Expressed as Minimum, Maximum, Median, Mean, and Standard Deviation

Group	gb_rb	osl_total
Graft (group II)		
N		
Valid	20	20
Missing	0	0
Mean	0.00449498	0.04195036
Median	0.00193776	0.03845294
Standard deviation	0.004664087	0.021177459
Minimum	0.000178	0.014981
Maximum	0.014681	0.102811
Flap (group I)		
N		
Valid	20	20
Missing	0	0
Mean	0.36868767	0.32515403
Median	0.30522902	0.33195837
Standard deviation	0.208844600	0.064643218
Minimum	0.114771	0.217906
Maximum	0.892758	0.458863

DISCUSSION

Despite the major advances in free flap surgery and the revolutions in pedicled flap choices providing multiple surgical options for reconstruction of the different parts of the body, researchers are still seeking new reconstruction choices for the challenging cases. Neural-based flaps are one of the most interesting of these choices particularly in difficult cases that may not be reconstructed with known techniques.[6]

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TABLE 2. The Active and/or New Formed Green-Stained Bone Proportion (gb_rb) and the Osteocyte-Containing Lacuna Density (osl_total) Are Significantly Higher in Prefabricated Osteocutaneous Flap (Group I) Samples Comparing to That of Graft Group (Group II) ($P = 0.00$)

Mann-Whitney *U* Test
Ranks

	Group	N	Mean Rank	Sum of Ranks
gb_rb	Graft (group II)	20	10,50	210,00
	Flap (group I)	20	30,50	610,00
	Total	40		
osl_total	Graft (group II)	20	10,50	210,00
	Flap (group I)	20	30,50	610,00
	Total	40		

Test Statistics*

	gb_rb	osl_total
Mann-Whitney *U*	0,000	0,000
Wilcoxon W	210,000	210,000
Z	−5,410	−5,410
Asymp. Sig. (2-tailed)	0,000	0,000
Exact Sig. (2* [1-tailed Sig.])	0,000†	0,000†

*Grouping variable: group.
†Not corrected for ties.
Asymp. indicates asymptomatic; Sig., significance.

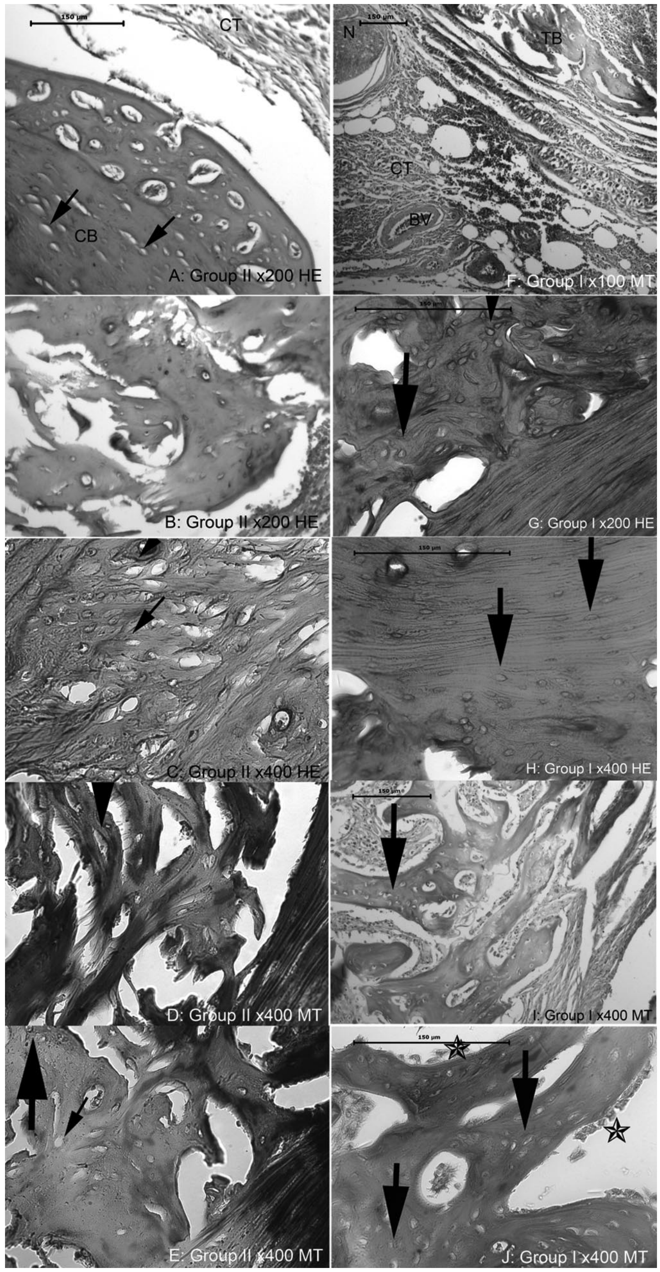

FIGURE 12. Histologic examination: left column belongs to graft group (Group II), right column to the prefabricated osteocutaneous neural-island flap group (Group I), respectively. Note that the osteocyte filled lacunae number is higher at the right column when compared with that of the left column. The new bone trabeculae are lined by active osteoblasts in (J). The compact bone undergoes healthy remodeling in (F) and (G) comparing to (C). Thin arrows indicate empty lacunae; thick arrows, osteoblast filled lacunae; CB indicates compact bone; TB, trabecular bone; Bv, blood vessel; N, nerve; CT, connective tissue; Star, osteoblasts; HE, Hematoxylin and eosin; MT, Masson trichrome.

"Neuroskin flap,"[1] "neurocutaneous flap,"[7–9] "neurofasciocutaneous flap,"[10–12] and "neuroadipofascial pedicled fasciocutaneous flap"[13–15] are the different flap names appearing in the literature. All of these describe the flaps based on the blood supply to the skin, which issues from arteries that accompany nerves. In the beginning, the skin flap was presented as based on a pedicle containing a wide subcutaneous tissue, a distinct perforating artery, and a cutaneous vein, in addition to a cutaneous nerve. Akyurek et al was the first to introduce the "neural island flap" design that designates a skin flap supplied only by the intrinsic vasculature of a cutaneous nerve without including any vein, fascia, and subcutaneous tissue into the pedicle after a preliminary delay procedure.[16] Subsequently, Ozkan et al[6] and our group[17] have described the neuromuscular and neuromusculocutaneous flap models based on the same principle. Next, we have introduced a constant and reliable skin flap model that was supplied solely by the intrinsic vasculature of an interfascicularly dissected nerve.[18] Recently, we have experimentally proved that the prefabrication of the neural island skin flap was possible as well.[2] On the basis of promising results of that study, our aim was to prove the feasibility of "prefabrication of the osteocutaneous neural island flap" in this study.

In the first part of the study, on the basis of our previous experiences, we decided to use the sciatic nerve as the neural pedicle of the prefabricated composite flap. This nerve was long enough to reach the dorsal region of the rat when properly dissected.

Our first choice was the dorsal region of the rat for the cutaneous part of the composite flap. A dorsally located flap is proved to be well protected from autocannibalization and flap loss caused by environmental factors. In addition, easy daily observation can be made without the need for manipulation of the rat.[19]

To create the osteocutaneous flap, we were initially planning to include a portion of the iliac bone for the osseous part of the flap. However, during the anatomic dissections in the first part of the study, we have realized that except a very small segment, most of the iliac crest was located far from the skin and there was a thick muscle layer between the iliac bone and the skin flap planned on the

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lumbar region of the rat. Besides, we could not demonstrate a constant septocutaneous or musculocutaneous perforator between the skin flap and the bone. Therefore, we thought that it was unpractical to harvest an osteocutaneous flap including the iliac bone with this skin flap.

However, during the anatomic dissections, we have found that a soft-tissue bridge containing a small arterial branch is found between the proximal third of the femur and the skin flap planned on the lumbar region of the rat. We could easily harvest the osteocutaneous flap with minimal destruction of the vasculature within this soft-tissue bridge between the skin flap and the femoral bone segment.

The selection of the "delay" periods was based on our previous experiences about the "nerve based flap models in the rat." A preliminary delay period of 2 weeks was required in these neural island flap models without nerve implantation.[16–18] In our recent study, we have proved that a reliable skin flap can be prefabricated that was supplied by the intrinsic vasculature of a cutaneous nerve, which was implanted into the subcutaneous tissue of that flap. The delay period was increased to 3 weeks in 2 steps as 2 weeks plus 1 week because of the implantation of the nerve into subcutaneous tissue. At the end of that study, we saw that this period was enough to prefabricate a neural island flap with the dimensions of 25 $\times$ 30 mm.[2] Based on promising results of that study, 3 weeks of delay period in 2 steps (2 weeks plus 1 week) was selected in this study. Presently, we are working on the effects of shorter delay periods, 1-step delay process, and skin flaps and bone segments in greater dimensions. The results of these studies will be published in the near future.

At the end of this study, based on direct observation, microangiographic findings and additionally, a detailed histologic analysis consisting of both qualitative and quantitative assessments, we have proved that it was possible to prefabricate an osteocutaneous composite flap based on the vascularity of a peripheral nerve after a delay period.

Nerve-based flaps are particularly useful in selected cases in reconstructive surgery of the distal part of the extremities, which are not good candidates for free tissue transfers. However, sometimes these tissue defects may also include bony defects. To the best of our knowledge, none of the nerve-based flaps described in the literature can be harvested as an osteocutaneous flap for the reconstruction of such a defect. The major clinical implication of our flap model introduced in this study is that an osteocutaneous composite flap can be prefabricated by using the cutaneous nerves of the neighborhood territories independently from the cutaneous nerve of its own territory. To give an example of the clinical implication of our model, in a selected case in which we do not want to sacrifice the peroneal artery of a lower extremity, the skin territory of the peroneal osteocutaneous flap connected to a segment of fibula can be prefabricated based on the sural nerve, after a preliminary delay process as described earlier in the text.

Prefabrication of an osteocutaneous composite flap by the method described in this study requires 2 or more surgical procedures with certain delay periods between them. This can be considered as a disadvantage of all prefabricated flaps. Additionally, the production of sensorial deficiency in the territory of a cutaneous nerve is the common disadvantage of all nerve-based flaps.

CONCLUSION

Based on detailed animal studies, this report presents a new flap prefabrication concept. We confirmed in this study that, prefabrication of an osteocutaneous composite flap is certainly possible based solely on the vascularity of a peripheral nerve, which was implanted into the subcutaneous tissue of that flap. We elected to name it the "prefabricated osteocutaneous neural island flap." We believe that the clinical application of this new flap will gradually become widespread based on further experimental studies.

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