# ORIGINAL ARTICLE

# Rat Dorsal Paired Island Skin Flaps: A Precise Model for Flap Survival Evaluation

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Abstract. Rectangular flaps on the rat or mouse dorsum are frequently used for flap survival elongation research. However, since rectangular flaps are purely random, the survival rate varies among individuals. A reliable animal flap model with low individual variation is desirable for flap survival research, especially in the angiogenic field. We investigated the survival rate of paired flaps in the rat dorsum containing 3 vascular territories in each flap, and determined their usefulness for flap elongation research.

Two symmetrical adjoining rectangular flaps  $(11 \times 3 \text{ cm each})$  were drawn on the rat dorsum. Two days after material injection, flaps were elevated with only the deep circumflex iliac vessels as the vascular pedicle. Flaps were immediately sutured back and the flap survival area was measured 7 days after the operation. The control group (n = 9) had saline solution injected in both flaps. The bone marrow group (n = 8) had bone marrow injected in the right flap, and saline solution injected in the left flap.

In the control group, the survival rate of the paired flaps was not statistically different (right flap,  $89.0\pm5.6\%$ ; left flap,  $89.3\pm4.5\%$ ). In the bone marrow group, the survival rate between the bone marrow injected flap ( $89.9\pm3.7\%$ ), and the saline flap ( $84.8\pm4.3\%$ ) was statistically different.

The rat dorsal paired island skin flap model shows low difference in flap survival rate and uses an internal control. This is a suitable model for flap survival elongation research. (Keio J Med 57 (4) : 211-216, December 2008)

Key words: flap model, vascular territory, axial pattern flap, bone marrow, flap survival evaluation

# Introduction

Much time and effort is spent on procedures to elongate flap survival areas. However, it remains difficult to completely prevent flap necrosis.<sup>1</sup> Although the delay procedure is a widely used method of improving flap survival, it requires multiple procedures,<sup>1,2</sup> and a more convenient procedure is necessary. In order to study flap survival, a flap model that can accurately and sensitively reflect the effects of drugs or cells is necessary. Many flap models have been developed, and MacFarlane's dorsal flap model is the one most frequently used.<sup>3</sup> However, this model utilizes a purely random flap, resulting in unstable blood circulation, unpredictable necrosis, and variations in skin vasculature.<sup>4</sup> Moreover, the single flaps require a large number of animals to obtain statistically significant result. Recently, with the advancements in vascular anatomy and surgical techniques, there has been greater demand clinically for axial pattern flaps over random pattern flaps, thus making the axial pattern flap model more suitable for the study of flap survival.<sup>5</sup>

Previously, Taylor and Minabe demonstrated that rat dorsal skin is supplied by 3 vessels; namely, the lateral thoracic (LT), posterior intercostal (IC), and deep circumflex iliac (DCI) vessels.<sup>6</sup> A rat dorsal axial pattern flap model utilizing these vessels was first described by Syed *et al.* in 1992.<sup>7</sup> Since that time, the model has undergone further developments.<sup>4,5,8,9</sup> These previous reports all presented variations of paired rectangular flaps on the dorsal skin, but did not investigate the creation of

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Fig. 1 Design of the rat dorsal paired island skin flap model. Two rectangular flaps  $(11 \times 3 \text{ cm})$  were marked on the dorsal area. The flaps included three vascular territories. LT, lateral thoracic vessels; IC, posterior intercostal vessels; DCI, deep circumflex iliac vessels.

paired axial pattern flaps. In this study, we investigated a rat dorsal paired island skin (DPIS) flap model covering the 3 vascular territories, and compared survival rates between the paired flaps.

#### **Materials and Methods**

# DPIS flap model design and elevation of flaps

Male Sprague-Dawley (SD) rats, weighing 350-400 g, were used in this study. Rats were anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital, and the dorsal skin hair was shaved. Next, a flap design, which was modified from previous reports,<sup>5,9</sup> was drawn on the skin. The margins of the flaps were as follows: the lateral borders were 3 cm to either side of midline; the medial border was dorsal to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular to midline over the apex of the scapula; and the proximal border was a line perpendicular. Two symmetrical adjoining rectangular flaps (11 × 3 cm each) were drawn on each rat (Fig. 1). Two days after injection of solution, the flaps were elevated under the panniculus carnosus. The LT and IC vessels were incised

with the skin margin, but the soft tissue was gently dissected in order to preserve the DCI vessel. Finally, the flap was elevated, retaining only the attachment to the DCI vessel as the vascular pedicle (Fig. 2). Flaps were then immediately sutured back to the donor sites with 5-0 nylon sutures, or stapled with a skin stapler. Seven days after flap elevation, rats were sacrificed by an overdose of pentobarbital, and the survival areas of the skin flaps were observed. The experimental protocol was approved by the Animal Research and Care Committee of the School of Medicine, Keio University.

# Bone marrow group and control group

To study elongation of flap survival, we used bone marrow, which has already been recognized to produce an angiogenesis effect.<sup>10,11</sup> To harvest bone marrow, SD rats were sacrificed and their femurs removed. Next, both edges of the epiphyses were cut and the marrow was flushed out repeatedly with heparinized saline. The bone marrow from 2 rats (4 femurs) was collected and mixed with saline for a total volume of 0.3 mL. For rats in the bone marrow group, 0.1 mL of the bone marrow/ saline mixture was injected into the right flap of each rat intradermally at 3 points on the midline along the long axis 1, 2, and 3 cm proximal to the distal end. The left flap was injected with saline at the equivalent 3 points as an internal control. Injections were performed in the same manner for the control group, but saline was used for both the right and left sides. In a preliminary experiment, the flap with the DCI pedicle always became necrotic about 2 cm proximal from the distal end (data not shown). Therefore, we chose this site as the injection point, along with additional injection sites proximal and distal to this point, i.e., at 1, 2, and 3 cm proximal to the distal end. A total of 20 rats were used for this study. The rats were randomly divided into two groups, with 10 rats in the bone marrow group and 10 rats in the control group.

### Measurement of survival rates of flaps

Seven days after flap elevation, the rats were sacrificed and the flap survival areas were observed. Distal areas of the skin flap that were hard or covered with crust were judged to be necrotic. The total area of the flaps and the survival area were traced on clear acetate sheets and scanned as digital images. The digital images were analyzed using Adobe Photoshop CS3 Extended software (Adobe Systems, Inc., San Jose, CA) to calculate the percentage of the survival area. Measurements were performed twice, and mean values were used for statistical analysis. The survival rate was expressed as the percentage of the survival rates was expressed as A

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**Fig. 2** (A) Angiogram showing normal angiographic anatomy of the rat dorsum. The broken lines indicate the vascular territories. The dotted line indicates the midline of the dorsum. (B) The flap elevated under the panniculus carnosus. Three vessels supply the dorsum skin. (C) The elevated left flap after dissection of the pedicle. LT, lateral thoracic vessels; IC, posterior intercostal vessels; DCI, deep circumflex iliac vessels.

the right flap survival rate minus the left flap survival rate.

#### Statistical analysis

All values were expressed as the mean  $\pm$  standard deviation. Statistical significance was determined using the paired t-test to compare paired flaps, and the Mann-Whitney *U*-test to examine differences between the groups. Statistical correlations between paired flaps were analyzed by Searman's correlation test. A *p*-value less than 0.05 was considered statistically significant. No correction was made for multiple testing.

# Angiographic evaluation

We performed angiography to investigate the relationship between survival area and vascular territory, and to compare changes in the vessels between paired flaps. Two rats were randomly chosen from each group. The rats were anesthetized, the carotid artery was cannulated, and the rats were euthanized by an overdose of pentobarbital. Next, 150 mL/kg of a lead oxide/gelatin mixture was injected into the cannula and the rats were incubated at 4°C. After 24 hours, the dorsal skin flap was harvested and placed on IX Envelopak FR film (Fuji Photo Film Co., Ltd., Tokyo, Japan) and radiographed (60 kVp, 5 mA, 15-second exposure) using a soft x-ray machine (ESM; Softex Co., Tokyo, Japan).

#### Results

We began with 10 rats in each group, but one rat in each group was excluded from the study because of the absence of intercostal (IC) vessels, and one rat in the bone marrow group died of unknown causes. All of the flaps showed congestion on the distal border on postoperative day 3, and demarcation was clearly observed at day 7. In the control group, the necrotic area was almost equal in size between the paired flaps in each individual, and interestingly, the shape of the necrotic areas was also nearly symmetrical (Fig. 3A). Angiography revealed flap necrosis in the LT vessel territory, the third territory (Fig. 3B). In many of the rats in the bone marrow group, the right flaps that received bone marrow showed longer survival than the contralateral flaps (Fig. 3C). In the angiogram, the necrotic area of the bone marrow injected flap was localized to the distal end, and vascularity was moderately increased in the third territory; however, the survival area could not be perfectly determined based on angiography because some contrast medium flowed backward into the vessel and the necrotic area (Fig. 3D).



**Fig. 3** (A) A representative rat from the control group. The survival areas appear equal on both sides and the areas of necrosis are nearly symmetrical. (B) Angiogram of the same rat from a control group rat. Two vascular territories, posterior intercostal vessels and deep circumflex iliac vessels, survived equally on both side. (C) A representative rat from the bone marrow group. The survival area in the right bone marrow flap is larger than in the control flap. (D) Angiogram of the same rat from a bone marrow group rat. The necrotic area of the right bone marrow flap was localized to the distal end, and vascularity was moderately increased in the third territory. Arrowheads indicate back flow of the contrast medium. The broken lines indicate the boundary of the necrotic area. IC, posterior intercostal vessels; DCI, deep circumflex iliac vessels.

There was no significant difference in the right flap survival rate (89.0  $\pm$  5.6%) and the left flap survival rate  $(89.3 \pm 4.5\%)$  in the control group (*p*=0.79) (Fig. 4A). The flap survival rate ranged from 80.5% to 98.9%, and the difference in survival rate ranged from 0.2% to 4.8%. In the bone marrow group, there was a significant difference in the right flap (bone marrow injected) survival rate (89.9  $\pm$  3.7%) and the left flap survival rate (84.8  $\pm$ 4.3%) (p=0.005) (Fig. 4A). The flap survival rate ranged from 78.9% to 92.6%, and the difference in survival rate ranged from 1.5% to 10.8%. The difference in survival rate was  $0.3 \pm 3.3\%$  for the control group and  $5.1 \pm 3.3\%$ for the bone marrow group, which was a significant difference (p=0.03) (Fig. 4B). There was also a positive correlation in survival rate between paired flaps in the control group ( $r_s=0.67$ , p=0.02), but no such correlation in the bone marrow group ( $r_s=0.36$ , p=0.19) (Fig. 4C). This correlation suggests that a change in survival rate is linked between paired flaps in the control group, i.e., if one of the DPIS flaps has a long survival length, then the other side will also have a long survival length.

#### Discussion

The DPIS flap model consists of paired symmetrical axial pattern flaps on the rat dorsum. The flaps include 3 territories and are attached only by the DCI vessel. There were no research about equality of the survival rate between paired axial pattern flaps in previous reports.<sup>4,5,8,9</sup> In this study, the difference in survival rates between the paired flaps was  $0.3 \pm 3.3\%$  in the control group, which was contrary to that seen in the bone marrow group at  $5.1 \pm 3.4\%$ . Thus, in the DPIS flap model one flap can be used as an internal control. The flap survival rate in our study ranged from 80.5% to 98.9% in the control group. There was an 18.4% difference in the range of each flap, even when using the same species, sex, body weight, technique, etc. We speculate that differences in the survival rate of each flap were due to individual differences between animals, especially with regard to vascularity, which can contribute to the extent of necrosis in the third territory. Moreover, subtle differences in technique, such as those that occur in the preparation around the pedicle and the position of the flap, will also make a difference. However, the difference in survival rate between paired flaps in each individual ranged from 0.2% to 4.8%, and the individual difference was reduced to as low as 3.6%.



**Fig. 4** (A) Comparison of the survival rates. There was no significant difference between paired flaps in the control group. In the bone marrow group, there was a significant difference between paired flaps. (B) Box plot comparing differences in survival rates between the control group and bone marrow group. There was a significant difference between the groups. (C) Survival rate correlation between paired flaps. The correlation in survival areas of the paired flaps in the control group suggests that the survival rate between paired flaps is linked; however, no correlation is seen in the bone marrow group.

The internal control also canceled out differences due to the influence of the experimental environment. Thus, the DPIS flap model can easily detect changes due to the injected materials by using an internal control.

One reason for the equality in the areas of necrosis in this model is the 3 territory system axial pattern, which uses the linking phenomenon.<sup>12,13</sup> The linking phenomenon occurs when the adjoining territory can be reliably captured by the dominant territory, and flap necrosis usually occurs in the third territory. In the DPIS flap model, DCI is the dominant territory, IC is the adjoining territory, and LT is the third territory. The extent of necrosis in the third territory varied between animals in the control group due to individual differences of the animals; however, between paired flaps, the extent of necrosis was similar, and symmetrical necrosis was seen in the control group.

We previously created a model with LT dominant double symmetrical flaps, but the dissection of the LT vessel was difficult since it arises from muscle, contrary to the DCI vessel, which arises from adipose tissue (Fig. 5).

The DPIS flap model has the same advantages of previous studies.<sup>5,9</sup> It is cost effective since extra animals are not needed for controls, easy to observe, prevents auto-cannibalism, and it is easy to create the flap. However, a problem with this model, although very rare, is the congenital absence of IC vessels. In this case, when elevating the flap, it will survive because the DCI and LT vessels expand into neighboring territories from the beginning (Fig. 6). The only way to prevent this problem is to directly observe the flap elevation and rule out cases where the IC vessel is absent.

The DPIS flap model uses two flaps on each rat, which have equal areas of necrosis. When introducing target drugs or cells to a flap, the contralateral flap will be the internal control used to detect the small change induced by the drugs or cells. In this study, we used bone marrow to demonstrate that the paired flaps can produce equal necroses in the control group. Bone marrow contains several kinds of stem cells, growth factors, and cytokines



**Fig. 5** (A) Magnification of the lateral thoracic vessels. The lateral thoracic vessel runs intramuscularly under the flap margin. Dissection of the lateral thoracic vessels is difficult for creating a lateral thoracic vessel dominant flap. (B) Magnification of the deep circumflex iliac vessels. The deep circumflex iliac vessel arising from adipose tissue. LT, lateral thoracic vessels; DCI, deep circumflex iliac vessels.

that induce angiogenesis. Although the details of the mechanism of action and contribution to angiogenesis require further exploration, bone marrow transplantation to the ischemic limb is already in clinical use.<sup>10,11,14</sup>

This model will be especially useful for detecting the effect of angiogenic agents such as pharmacologic drugs, cells, and cytokines.

#### Conclusion

We created a rat dorsal paired island skin flap model. The paired flaps have equal areas of necrosis, and the difference in the survival area was  $0.3 \pm 3.3\%$ . Due to this low variability in flap survival area, one flap can be successfully used as an internal control. This flap model is suitable for investigating flap survival elongation.

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**Fig. 6** The angiogram of the excluded rat from the bone marrow group. It shows the absence of posterior intercostal vessels in the left flap and no occurrence of necrosis. The right flap was injected into the bone marrow, and there was increase of vascularity in the distal end of flap. LT, lateral thoracic vessels; IC, posterior intercostal vessels; DCI, deep circumflex iliac vessels.

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