

Description of a new experimental model skin flap for studying skin viability in rats¹

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ABSTRACT

PURPOSE: To describe a new experimental flap for studying skin viability in rats.

METHODS: Twenty male Wistar rats weighing between 250-300g were divided into two groups: group A – McFarlane technique, a 4 x 10cm flap was used (McFarlane); and in group B modified McFarlane technique, a 3 x 10cm flap was used. Seven days later, the animals were sacrificed and the area of necrosis was evaluated in both groups.

RESULTS: Group A presented necrosis in 3% of the total area of the flap (CI: 0.01-0.05), Group B presented necrosis in 37% of the total area of the flap (CI: 0.29-0.46), (p<0.001).

CONCLUSION: The modified McFarlane flap presented a larger area of necrosis and could be an adequate experimental model of skin flap viability.

Key words: Surgical Flaps. Necrosis. Models, Animal. Rats.

Introduction

Flaps have been used in plastic surgery since 800b.C. Skin flaps are basically employed for the repair of large wound defects. The techniques of skin flaps are well established and they vary according to the location of the wound and the extension of the defect¹. The most feared complication of a skin flap is a significant area of necrosis causing the failure of the surgical procedure. The estimated frequency of necrosis of skin flaps varies between 2 and 20%^{1,2}.

Bearing this fact in mind, a reproducible and effective experimental model of necrosis of skin flaps would be useful to evaluate different substances and devices that might increase the viability of flaps^{3,4}.

McFarlane *et al.*⁵ described the dorsal cutaneous flap in rats. Since then this experimental model has been adopted as the gold standard in studies evaluating the viability of axial flaps. The original dimensions described in McFarlane's *et al.* study were of 4 cm across the base by 10 cm in length (i.e. a 1:2.5 ratio), taking the scapulae as the base of the flap. In the original study, the authors described necrosis of 25-50% of the flap length.

However, in the literature several studies have cited this technique as a reference, but with different dimensions. Flaps measuring 2 x 8cm⁶, 3 x 7cm⁷, and 3 x 9cm⁸ have been described and attributed to McFarlane, casting doubt on the results of the original description. In addition, the location of the base described as cranial (at the level of the scapulae) has also not been uniform⁶⁻⁸.

In the light of the lack of consensus, and because of the need for an effective and reproducible experimental model⁹ of skin flap necrosis, we decided to conduct a comparative study between the flap described by McFarlane and a skin flap with different dimensions.

Methods

This project was approved by the Ethics Committee, School of Medicine, University of Sao Paulo. All the experiments complied with regulations on animal experimentation described by the Council for international Organization of Medical Sciences (CIOMS).

Twenty male Wistar rats weighing between 250-300 g were divided into two groups:

- Group A (10 animals) – McFarlane flap (4 x 10cm);
- Group B (10 animals) – modified flap (3 x 10cm).

All the animals were housed in separate cages, with *ad libitum* offer of food and water, 25°C temperature and controlled day and night cycling.

The animals were anesthetized by inhalation of a mixture of 20% isoflurane and oxygen and positioned in ventral decubitus. The dorsal region was shaved. Aqueous 0.5% chlorhexidine was used for antiseptics of the surgical field.

Flap design

Group A – McFarlane

Starting from the rat's midline, two equidistant points were marked out using a marker pen, 2.0 cm on each side of the midline, at the level of the base of the scapulae. This was defined as the base of the flap. The length of the flap was marked out as two parallel lines 10 cm long, starting from the previously established base. Thus, the flap final dimensions were 4 x 10cm (Figure 1A).

Group B – Modified flap

Starting from the rat's midline, a flap was delimited by marking out points 1.5 cm on each side of the midline, at the level of the base of the scapulae, and this was taken as the base of the flap. The remaining markings were the same as described for group A (Figure 1B).

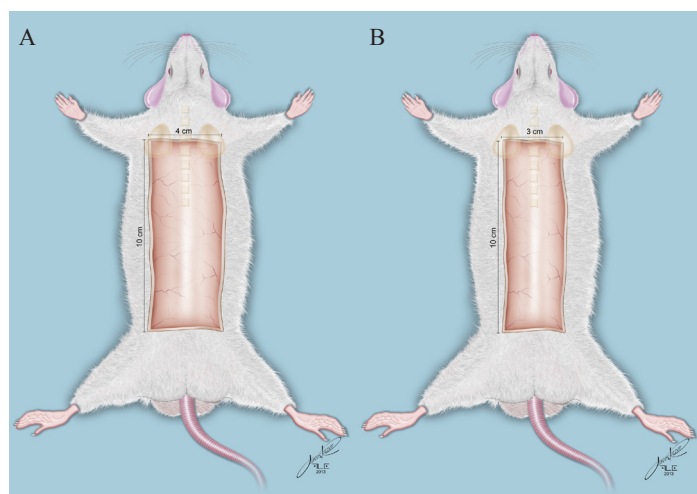


FIGURE 1 – **A.** McFarlane, with 4 x 10cm dimensions. **B.** Modified dorsal skin flap, with 3 x 10cm dimensions.

The flap dissection and suture

After demarcating the flap, the skin was incised. The flap was raised by dissecting the areolar tissue at the level of the panniculus carnosus and deep fascia of the rat's dorsal musculature, as far as the interscapular base.

The flap was repositioned in its original position and was sutured with 4-0 monofilament nylon single stitches at 0.5cm intervals.

After recovery from anesthesia, the animals were kept in separate cages with *ad libitum* offer of food and water for seven days. Paracetamol (40mg) was added to 100ml of water, in conformity with the recommendations for laboratory animal care. All the animals were observed daily.

Evaluation of skin flap viability

On the seventh postoperative day, the flaps were photographed with a ruler in centimeters that was placed on the animal back. This image was then digitalized using the Image J® software (NIH; <http://rsbweb.nih.gov/ij>). All the photographs were calibrated for readings in mm². By using the area function of the software it was possible to measure the total area of the flap and the area of necrosis.

Viable skin was defined as a pink, warm, soft on touch tissue (Figure 2A). Necrotic area was defined as a brown-black, cold and hard to manipulate skin (Figure 2B).

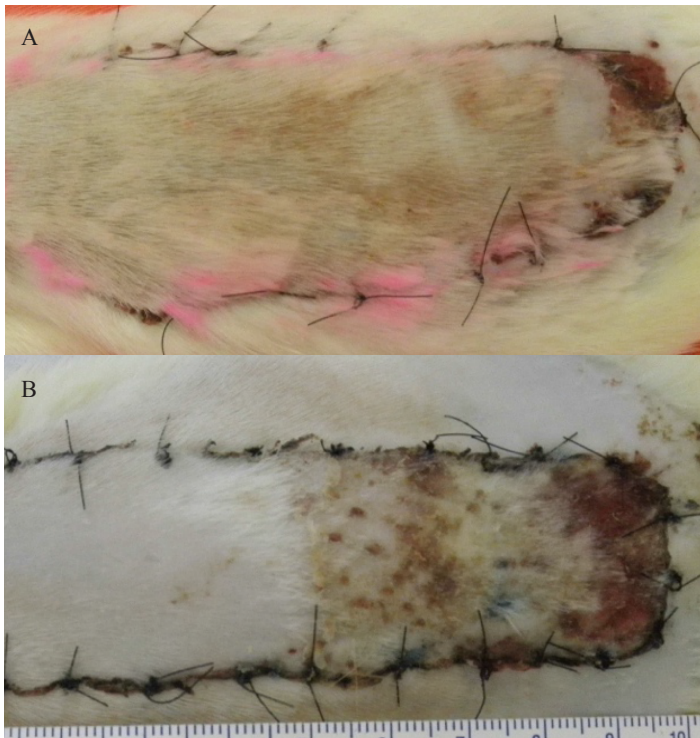


FIGURE 2 – A. Viable flap with a small area of necrosis (arrows); B. Flap with a significant area of necrosis (arrows).

Excel software (Windows7®) was employed to calculate the area of necrosis to the total area ratio.

Statistical analysis

The necrosis/total flap area ratio was compared between groups A and B by using the Mann-Whitney test. The tests were calculated using the IBM SPSS statistical software (Statistical

Package for the Social Sciences), version 21.0. p<0.05 was considered significant.

Results

After measuring the areas on the flap, the total area, area of necrosis and ratio of the area of necrosis/total area were calculated for both groups, as shown in Tables 1 and 2.

TABLE 1 - McFarlane flap (group A): total area, area of necrosis and ratio of the area of necrosis/total area.

Animal	Total Area	Area of Necrosis	Necrosis Area / Total Area
1	3632.507	167.851	0.046
2	3538.111	157.429	0.044
3	5220.246	245.206	0.047
4	3382.465	263.879	0.078
5	5129.246	0	0
6	5231.855	0	0
7	3290.583	143.975	0.044
8	4098.522	0	0
9	4223.094	0	0
10	4429.851	0	0

TABLE 2 - Modified flap (group B): total area, area of necrosis and ratio of the area of necrosis/total area.

Animal	Total Area	Necrosis Area	Epitheliolysis	Necrosis Area/ Total Area
1	3297.828	1102.385	0	0.335
2	2675.927	1039.013	0	0.388
3	2458.671	1427.141	0	0.580
4	2919.654	734.07	309.004	0.251
5	2717.011	932.382	598.465	0.343
6	2891.185	1423.789	0	0.492
7	2726.746	1057.967	0	0.388
8	3137.027	551.826	599.139	0.176
9	2756.083	1239.072	178.498	0.449
10	2717.011	932.382	598.465	0.343

The mean ratios of the area of necrosis/total area were 3% and 37% for the McFarlane and modified flap groups respectively (Figure 3). The comparison between groups is demonstrated in Table 3.

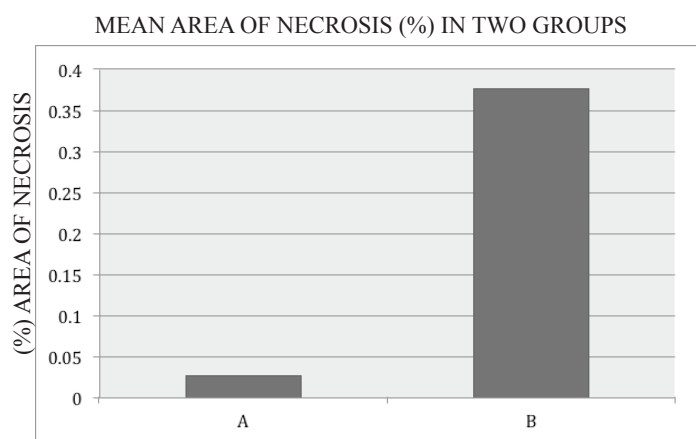


FIGURE 3 - Graphic showing McFarlane flap (A) and new flap (B).

TABLE 3 – Ratio between the area of flap necrosis and the total area, using the Mann-Whitney test.

	Mean	Standard deviation	Standard error	95% CI
Group A (n = 10)	0.03	0.003	0.001	0.01 – 0.05
Group B* (n = 10)	0.37	0.12	0.004	0.29 – 0.46

95% CI = 95% confidence interval

* A vs B = $p < 0.001$

Discussion

Although the flap described by McFarlane *et al.*⁵ is considered the gold standard for experimental studies on skin viability, it has been observed that there is not uniformity in this technique. Based on the literature, we noted that several studies cited this technique as a reference, but many of them had not used the dimensions established by McFarlane. In addition, it was seen that several authors who had used the original design (4x10cm) described areas of necrosis which varied from 10%¹⁰ to 60%¹¹.

Another point to be considered is that there was some variation in the location of the base among the flaps attributed to McFarlane *et al.*⁵. Some were elevated with the base in the caudal part of the animal's back⁶, which others had the base in the cranial part, at the level of the scapulae⁷.

In view of the lack of consensus, we decided to conduct a study on a new flap with 3x10cm dimension, with a cranial base, comparing the area of necrosis at the seventh postoperative day with the original flap proposed by McFarlane *et al.*⁵

The cranial base was chosen because the base of the animal's scapulae forms a rigid anatomical parameter. Moreover, in all dissections, it was noted that in this anatomical position there

is a small vascular bundle that follows along the length of this flap, which makes it an axial flap.

However, this positioning can be criticized. Briggs *et al.*¹² reported that the position of the forelimbs caused variations in the location of the flap when the base was positioned at the level of the scapulae. Depending on the angle formed between the forelimbs and trunk, the base of the flap could change because the scapulae could shift up to one centimeter. This was not confirmed in the present study because the animal was positioned on a wooden board that was appropriate for this purpose and the animal's forelimbs were kept fixed, i.e. always at the same angle, without changing the level of the scapulae.

Following these simple rules, we can say that all the flaps were drawn up and the incisions were made correctly and in a reproducible manner because rigid anatomical parameters were employed.

We utilized the same parameters as those describe by Gemperli *et al.*¹³ to define the macroscopic criteria for flap viability. Viable area was defined as a pink, warm and soft-to-touch skin on the 7th postoperative day. Necrotic area was defined as a brown-black, rigid-to-touch skin. And the area that was between these two presentations was classified as epithelysis. For statistical purpose, only necrotic and total areas were evaluated. In clinical practice, most of epithelysis areas regenerate with conservative measures such as.

In this study, necrosis was observed on almost 40% of the area of the modified flap, more than 10x when compared with the necrosis observed in the McFarlane group. These findings confirm that the flap described by McFarlane *et al.*⁵, in the form that was proposed, does not produce a significant area of necrosis. Therefore the McFarlane flap does not seem to be suitable for studying strategies that might change the viability of dorsal skin flaps. On the other hand, the modified flap with a shorter cranial base gave rise to an area of necrosis that was compatible with the expectations for an experimental model that would be suitable for studying skin viability.

Conclusion

The modified flap with dimensions of 3x10cm, with a cranial base, is more appropriate for experimental studies for the viability of skin flaps.

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