

## Original Article

# Comparison of subdermal and perforator delay techniques on a rat flap model

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### ABSTRACT

**Background:** In this study, we investigated the subdermal and perforator delay phenomena as a method to improve flap survival. **Materials and Methods:** In this experimental study, we used 24 rats in three groups. In the control group, the dorsal flaps were elevated and reinserted back to their place. In the experimental groups, we practiced the delay phenomena with two different techniques. In the first experimental group, cranial and lateral side incisions were performed; however, the flaps were not cut-off from the underlying fascia. In the second experimental group, we placed a silicon sheet under the planned flap to cut-off the circulation from the perforator vessels. Four weeks after the delay procedure, the flaps were raised completely and reinserted back to their place. **Results:** The average of necrotic area in the control group was 21.9% ( $\pm 7.70$ ). There was no necrosis in both experimental groups ( $P < 0.0001$ ). Histological examination revealed that collagen density in both of the experimental groups was increased in comparison to the control group, it has only been found a significant first experimental group ( $P = 0.0315$ ). We have not found any significant difference in lymphocyte density between the groups. Angiographic imaging has showed an increase in the vascular density in the flaps of the first experimental group. **Conclusion:** We believe that both of these delay techniques can be adapted to clinical applications and used safely to increase flap survival.

### KEY WORDS

Flap delay; rat; skin flap

### INTRODUCTION

Flap survival depends on many internal and external factors.<sup>[1-5]</sup> Understanding of flap physiology is crucial for the success in reconstructive surgery.

Flap microcirculation is composed of small vessels such as arterioles, capillaries and venules. These can be affected by many biochemical and neurological impulses. Flap circulation can be severed by the impairment of arterial

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or venous circulation.<sup>[6]</sup> In this regard, venous flaps have certain advantages to traditional arterial flaps among which are easier design and less donor-site morbidity.<sup>[7]</sup> Many factors have been shown to have effects on flap circulation such as topical applications,<sup>[8]</sup> pharmacological agents,<sup>[9]</sup> lasers,<sup>[10]</sup> photodynamic therapy,<sup>[11]</sup> oxygen pressure,<sup>[12]</sup> and scar penetration of neovascularisation.<sup>[13,14]</sup>

Delay procedure has been shown to have significant impact on flap survival.<sup>[15]</sup> Although the developments in the techniques of axial and free flaps have decreased the need for this procedure, many surgical and non-surgical delay procedures have been defined.

Delay procedure is frequently performed to increase the viability of flaps. Classically, this procedure is performed 2–3 weeks before the surgery. Axial pattern skin flaps can also benefit from the delay procedure. In previous studies, rat skin island flap models have been used as a model for the delay procedure.<sup>[16]</sup> As a result, the delay procedure has been shown to have positive effects on flap circulation and has been adopted by plastic surgeons.

The delay procedure performed on the TRAM flap is one of the best examples of this application. In the practice of plastic surgery, the delay procedure can be performed with surgical techniques (incisions, saturations and ligations), pharmacological agents (vasoconstrictor and vasodilator agents), lasers<sup>[17]</sup> and mechanical impulses (extracorporeal shock wave).<sup>[18]</sup>

Subdermal plexus from the adjacent skin and musculocutaneous/septocutaneous perforators are the two major blood supplies of skin flaps. In this study, our aim was to compare the effectiveness of subdermal and perforator delay procedures with clinical and histological findings in a rat skin flap model.

## MATERIALS AND METHODS

This study was conducted in the Istanbul University Cerrahpasa Medical School Animal Research Laboratory with the approval of Istanbul University local Ethical Committee. Twenty-four female Sprague-Dawley rats averaging at 225 g weight were used. 9 cm × 3 cm caudally based dorsal flaps were designed on the back skin of each animal [Figure 1].<sup>[19]</sup> The flaps were raised at the subcutaneous level, and subdermal plexus was preserved during this procedure. The deep fascia was not included in the flap to avoid any vascular supply from the subfascial and suprafascial plexuses.

Animals were divided into three groups with eight in each group.

### First experimental group (subdermal delay)

In this group, the first delay procedure was performed in which the flaps were only incised at the cranial edge. One week later, a second delay procedure involving the excision of the lateral edges was performed [Figure 2]. This procedure was performed in two stages to reduce the surgical stress and to enhance the new vessel formation. The connection of the flap with the underlying fascia was kept intact during these procedures. Four weeks later, the first delay procedure, the flaps were raised completely and sutured back to their location with 4.0 polypropylene sutures together with the control group.

### Second experimental group (perforator delay)

In this group, a delay procedure was performed in which the flaps were only incised at the cranial edge, and the flaps were dissected from the underlying fascia. A silicon sheet was placed under the flap. Lateral edges were kept intact [Figure 3]. Four weeks after the delay procedure, the flaps were raised completely and sutured back to their location with 4.0 polypropylene sutures together with the control group.

### Control group

Caudally based flaps were raised without any delay and sutured back to their location with 4.0 polypropylene sutures along with the other animals from the experimental groups [Figure 4].

Post-operative follow-up was performed by photographing of the flaps at 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> days following the final surgery. At the end of the follow-up period, flap areas

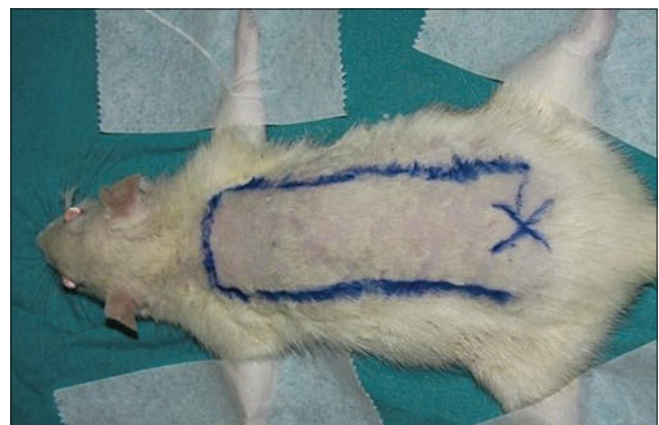


Figure 1: Mc Farlane dorsal skin flap model

were marked and photographed. The marked flap areas were copied to an X-ray film, were photographed. Flap area calculations were performed with the VistaMetrix software (© SkillCrest, LLC).

All the animals were sacrificed with high-dose barbiturate, and the tissue samples were taken for the histological examination. Samples from the proximal and distal portion of the flaps were prepared in 10% formaldehyde. Lymphocyte and collagen densities were evaluated under light microscopy. H and E staining and Masson staining were used, and scoring was performed with a cell density scale.

Kruskal–Wallis and Dunn’s multiple comparison tests were used for statistical analysis.

Angiography was performed on two rats on the 7<sup>th</sup> post-operative day before sacrifice.

Inferior vena cava and aorta were cannulated [Figure 5]. A mixture of 40 ml 40% barium sulphate and 10% gelatine was prepared inside saline solution.<sup>[20]</sup> This solution was injected into the vessels, and the animals were kept in – 20°C overnight. The flaps were removed from the animals on the next day. The flaps were stabilised on plastic plates from corners. The flaps were photographed on high-resolution mammography machines.

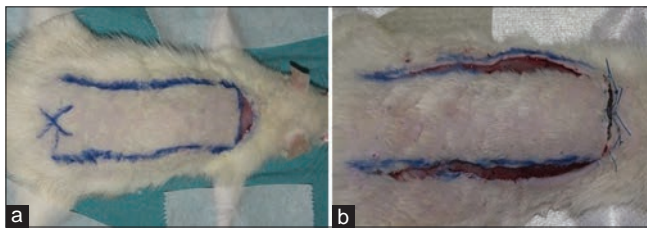


Figure 2: (a) Superior and (b) lateral incisions on the first experimental group



Figure 4: Skin flaps raised on caudal pedicle, 4 weeks after the delay

## RESULTS

During the follow-ups, the flaps of the control group started to show discoloration on the first post-operative day. Demarcation of the necrotic areas became visible between on 5<sup>th</sup> and the 7<sup>th</sup> day postoperatively. The average of the necrotic area on the flaps was 21.9% ( $\pm 7.70$ ).

In the experimental groups, two animals on Group 1 and three animals on Group 3 showed some discoloration on the first post-operative day. These findings were all confined in areas which were 1–2 cm in diameter. On the follow-ups, these areas recovered completely; hence, there was no necrosis at the end of the study on both of these groups. These results were significantly different than the control group ( $P < 0.0001$ ) [Figure 6].

### Evaluation of histological findings

Lymphocyte and collagen densities were evaluated under light microscopy [Figure 7]. Seven pairs of samples were taken from each group. Density scoring was performed in accordance with the following scale:

- 0: None
- 1+: Low density
- 2+: Medium density
- 3+: High density.

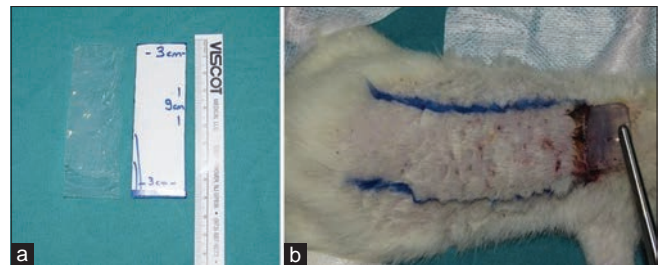


Figure 3: (a) Silicone sheet used in the second experimental group. (b) Silicone sheet is placed under the flap from a superior incision

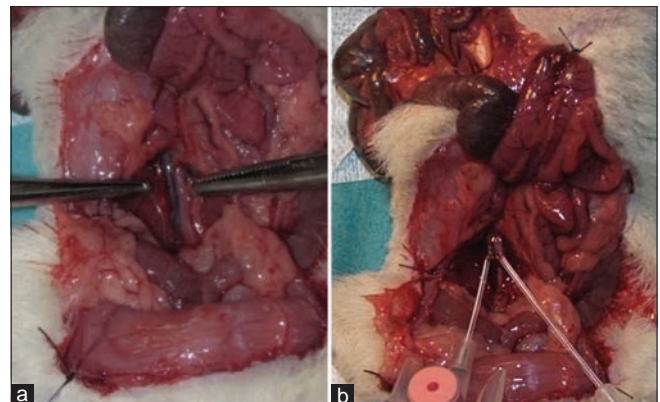
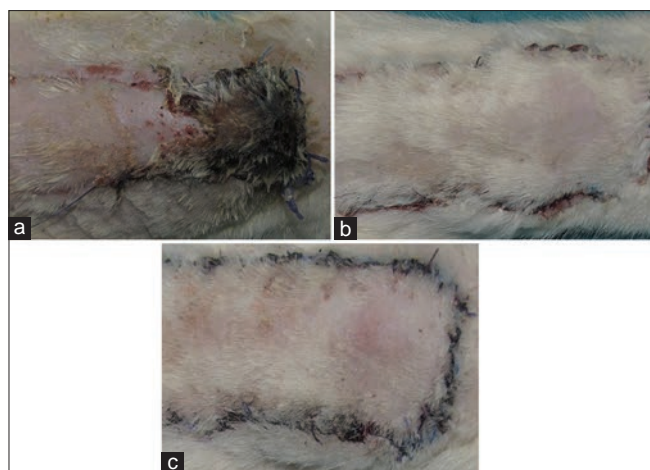


Figure 5: (a) Dissection (b) Cannulation for the angiographic studies



**Figure 6:** Photographs of the flaps at the end of the study from selected samples. (a) Control group. (b) First experimental group. (c) Second experimental group

There was only a statistically significant difference in the collagen density results of the control group and the proximal of the first experimental group ( $P = 0.0315$ ) [Figure 8].

In the samples taken from the distal flap portions of the experimental groups, there was a significant increase in the vascular density; however, this parameter has not been scored.

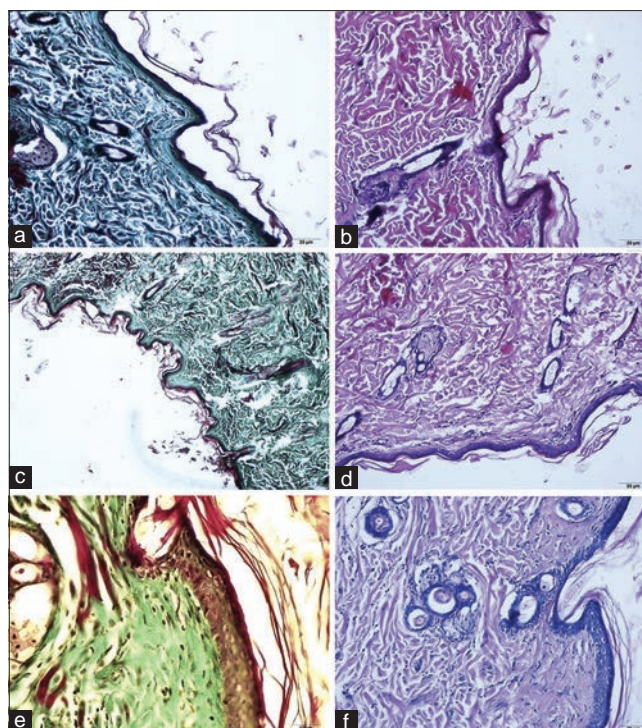
### Evaluation of angiography results

The angiography images were magnified and evaluated. In the subjective evaluation of the images, the experimental groups showed a slight increase in the amount of vascularisation when compared to the control group. This difference was especially visible in the first experimental group [Figure 9].

## DISCUSSION

Delay procedure on the skin flaps makes larger-sized flaps possible. The exact mechanisms behind this procedure are not clear. Many studies have been conducted to answer this question.<sup>[21-24]</sup> The amount of vascularity increases required for an adequate delay still remains as an important question. This will depend on many variables as well as the delay technique. Although we believe that it will not be possible to quantitatively measure this amount, the future studies involving more variables might give us valuable insight into this point.

At the end of the study, none of the experimental groups showed a permanent circulation problem or necrosis.



**Figure 7:** Collagen density shown with the Masson's trichrome staining. (a) Control group, (c) first experimental group, (e) second experimental group. Lymphocyte density shown with haematoxylin and eosin staining. (b) Control group, (d) first experimental group, (f) second experimental group

This finding can be further supported by the fact that no distortion on the epidermis was found microscopically. Under light microscopy, there was an increase in the vascular density and neovascularisation. The 4 weeks of delay time used in our study was probably effective in reducing the amount of necrosis for the perforator delay group. There was a significant amount of discoloration after the flap elevation in three animals in this group. This discoloration did not eventually result in permanent necrosis. We think that this might have been different if a shorter duration of delay time was chosen. Although 2 weeks of delay time was shown to be adequate for dermal delay techniques,<sup>[25]</sup> to the best of our knowledge, there was not any previous study on the adequate amount of delay time for the perforator technique. We thought that the dermal vascularity increase will be much slower in perforator delay group, which in principle will be similar to the expander techniques in which the perforator circulation is similarly interrupted.

In our study, there was not any significant increase in the lymphocyte density in the experimental groups. These results suggest that the flaps were not in an inflammation state. Collagen density has increased in both experimental groups. The collagen formation suggests an increase in

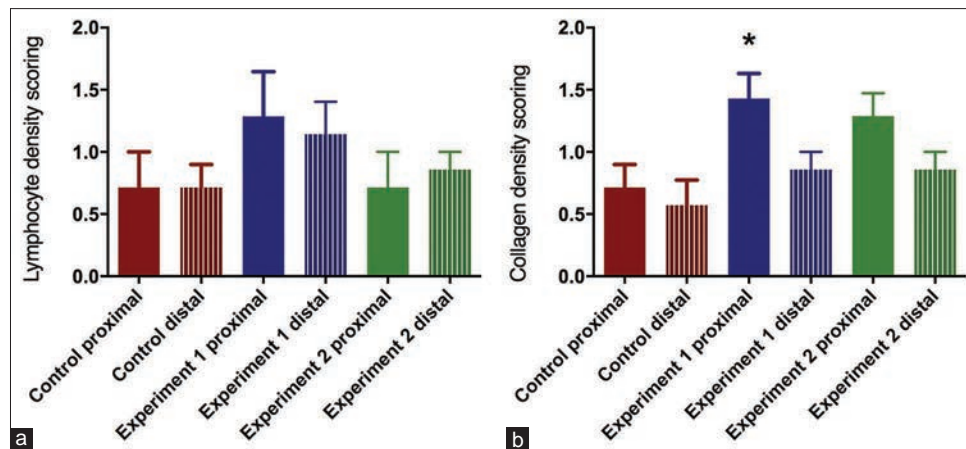


Figure 8: (a) Average of lymphocyte density scorings. (b) Average of collagen density scorings

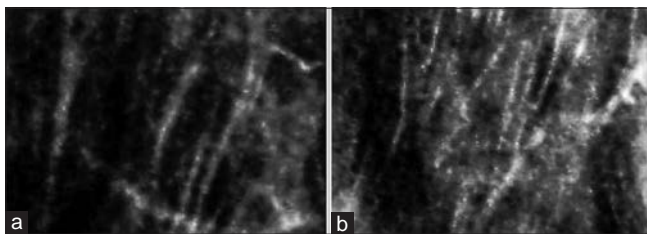


Figure 9: Angiography results from selected samples. (a) Control group. (b) First experimental group

neovascularisation and fibroblast activity. Zhou *et al.* have shown in a study that the injection of fibroblast growth factor and collagen matrix increases neovascularisation on rabbits.<sup>[26]</sup> Rao *et al.* have also shown an increase in angiogenesis with collagen matrix and endothelial and mesenchymal stem cells.<sup>[27]</sup> All of these studies indicate that the importance of collagen matrix for neovascularisation.

The angiographic imaging performed in our study was technically demanding. We believe that angiographic studies on small animals such rats will usually result in a low success rate and are difficult to interpret. A slight increase in vascularisation in experimental groups was detected with these studies. This increase in vascularisation has to be further investigated for venous and arterial components. Lymphatic vessels also seem to play an important role on flap viability. We believe that these can be further investigated with lymphoscintigraphic imaging on larger mammals such as pigs.

It is crucial to point out the fact that regional variations have to be taken into consideration. These results may turn out differently in other flap locations. Differences between human and rat physiologies are another

factor to be considered.<sup>[28]</sup> These differences might be particularly important in neovascularisation and delay durations. There are many unknowns in the equation of flap physiology. Although many of these factors have been clarified by previous studies, it should not be discouraging to see that similar studies reveal different results.

Histological results show that collagen and vascular densities were lower on the second experimental group. Although there was not any permanent necrosis in any of the experimental groups, these results might suggest that necrosis would be more likely in this second experimental group. The incisions that were performed in stages on the first experimental group might have positive effects on flap circulation by decreasing the amount of stress and ischaemia on the tissues. It seems that by gradually increasing the stress we can enhance the circulation of the flap. In a previous study, Callegari *et al.* have showed that choked vessels can enlarge with the ligation of blood vessels from the adjacent angiosomes.<sup>[29]</sup> This result is consistent with our findings. Lateral incisions performed in the first experimental group do have a similar effect described in the earlier study of George *et al.* In this study, an occlusion clamp was used instead of the lateral incisions performed in our study. We believe that both of these techniques would open adjacent choked vessels as described earlier which would yield to similar results regarding the flap survival.

## CONCLUSION

Our results support the idea that the delay procedure has a positive effect on flap viability. In conclusion, flap viability and histological data at the end of the study suggest that

either subdermal or perforator circulation can be interrupted for a successful delay procedure. The papers published in angiosomes,<sup>[30]</sup> and the perforasomes<sup>[31]</sup> have changed our understanding of the random flaps in a profound way. These theories actually made it clear that there are actually no random flaps. However, in practice when the surgeon is unable to identify the angiosomes and the perforasomes associated with the skin area where the flap is to be raised the term random flap is used. Circulation problems are common with these types of flaps since they are raised without the knowledge of their vascular supply. These problems usually limit the usage of these of flaps on smaller defects.

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### Conflicts of interest

There are no conflicts of interest.

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