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# The Effect of Topical Minoxidil Pretreatment on Nonsurgical Delay of Rat Cutaneous Flaps: Further Studies

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**Abstract** The purpose of this study was to determine the effectiveness of topically applied minoxidil in the pharmacological delay phenomenon and to demonstrate the comparable microscopic and macroscopic changes between minoxidil-pretreated flaps and surgically delayed flaps. A modified version of the McFarlane flap was used. Group 1 rats, in which a caudally based dorsal skin flap was raised and sutured back, were the control group. In group II, minoxidil solution was spread over the marked skin flap area for 7 days. On the 7th day, a caudally based dorsal skin flap was elevated and then sutured back. Group III rats underwent a surgical delay procedure alone. On the 7th day after flap elevation, evaluation was done by histologic examination and calculation of the flap survival areas in all groups. The lowest flap survival rate appeared in group I and was statistically different from groups II and III. The mean surviving skin flap area in the minoxidil-pretreated group was significantly larger than that in the control group. After histologic evaluation, moderate angiogenesis was also detected in group II. We also found that surgical delay significantly reduced flap necrosis when compared to the minoxidil pretreatment group. According to our study, minoxidil may be considered an effective vasoactive agent for the stimulation of angiogenesis in rat cutaneous flaps and capable of achieving pharmacological delay and increasing flap survival.

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**Keywords** Minoxidil · Surgical delay · Flap · Rat

## Introduction

Although distal area necrosis is a well-known complication of the random-pattern flap design, it is widely used for reconstructive purposes in the daily clinical practice of plastic surgery. Sufficient blood supply is crucial for survival of skin flaps, so any damage to flap vascularity or a risky flap design may result in distal flap necrosis. Researchers have focused on enhancing flap viability, especially in risky flap designs, high-risk patients, and extended axial pattern flaps, to avoid potential necrosis of the flaps. Skin flap viability may be enhanced by using various pharmacological and surgical delay procedures. The efficacy of those procedures in preventing skin flap ischemia has been proven in some clinical and experimental studies [1–5].

Surgical delay is known to be the most effective technique to enhance flap viability and is often used clinically to provide better flap survival. However, it has certain disadvantages such as bleeding, infection, pain, swelling, and scar formation. It also necessitates a two-stage procedure and long-term wound care. Many pharmacological agents such as sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors, anticoagulants, adenosine, monophosphoryl lipid A, growth hormone, and glucocorticoids have been

investigated experimentally to reveal their beneficial effects on flap survival [2, 3]. However, up to now, none of these pharmacological agents has been widely accepted as a safe and effective agent for use in daily clinical practice to enhance flap viability because they necessitate systemic application, resulting in an increased risk of potential systemic side effects.

In this study, we investigated a well-known vasoactive agent, minoxidil, via topical application on the rat dorsal skin to demonstrate its influence on the nonsurgical delay phenomenon by eliminating the need for systemic administration and systemic side effects. Survival of rat cutaneous flaps pretreated with minoxidil was compared with that of flaps raised after surgical delay.

## Materials and Methods

Twenty-four male Wistar rats, 110–120 days old, weighing between 280 and 300 g, were used in this study. The animals were randomly divided into three groups ($n = 8$). The rats were placed in separate cages and fed standard rat chow and water *ad libitum* upon completion of the experiments. They stayed in a temperature-controlled room on a 12 h light/12 h dark cycle with free access to food and water. This experiment was approved by the Ethical Committee of the University for Animal Research.

After the rats were anesthetized via intramuscular injections of ketamine 10 mg/kg and subcutaneous injections of xylazine hydrochloride 3 mg/kg, the dorsal skin was shaved with an electric clipper and then disinfected with povidone-iodine. During the surgical procedure, a local sterile environment and asepsis were provided. A supplemental dose of anesthetic was given when needed during the experiment. A modified version of the McFarlane flap model [6], described by Khouri et al. [7], was used. Group 1 rats, in which a caudally based, 9 cm × 3 cm dorsal skin flap was raised, including skin and panniculus carnosus, were chosen as the control group. After elevation of the flap, it were sutured back into it initial position (Fig. 1). In group II, a skin flap area, measuring 9 cm × 3 cm and extending from scapular tips to hip joints, was marked symmetrically on both sides of the midline of the rat dorsum. Then 20 mg of 5 % minoxidil solution, which was produced commercially, was spread uniformly with an applicator over this region twice a day for 7 days to create a pharmacological delay in this region. On the 7th day, a caudally based dorsal skin flap was elevated and then sutured back into place with polypropylene sutures. On the 7th postoperative day, the flaps were evaluated for necrosis (Fig. 2). Group III rats underwent a surgical delay procedure by incising two longitudinal borders of the outlined flap and undermining the flap completely, including the panniculus carnosus. A bipedicled dorsal skin flap, measuring 9 cm in length and 3 cm in width, was created. After the complete elevation of the flap, it was sutured back into its initial position. On the 7th postoperative day, the cranial pedicle was cut, the flap was re-elevated, and then sutured back into its resting position. Flap viability was evaluated 14 days after the first operation (Fig. 3).

In group I on the 7th day, in group II on the 7th day following flap elevation, and in group III on the 14th day after the first operation, the rats were reanesthetized for evaluation of flap viability. Total flap area and necrotic regions were drawn on a clear acetate template. After electronically scanning the templates, the average area of flap survival was calculated for each rat (Table 1). For the histologic evaluation, two full-thickness skin biopsies, 0.5 cm × 0.5 cm in size, were harvested from the midline of the flap, 1 cm away from the proximal side and 1 cm proximal to the necrotic area in the distal side. Animals were then killed via an intraperitoneal injection of sodium thiopental (150 mg/kg).

For statistical analysis of flap survival areas, $p < 0.05$ was regarded as indicating statistical significance and data were expressed as the mean ± standard deviation (SD). SPSS for Windows 16.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses of the data. ANOVA (post-hoc Tukey test) was used to detect differences among the groups.

Biopsy samples were fixed in 10 % formaldehyde solution for 24 h, embedded in paraffin, sectioned, and stained with routine hematoxylin & eosin (H&E) stain. Subdermal vascular architecture and angiogenesis were evaluated under a light microscope to investigate the influence of minoxidil. Slides were examined by a pathologist in a blinded fashion. Angiogenesis (newly formed capillaries) was evaluated semiquantitatively, so the intensity of neovascularization was scored in the areas where it was prominent by using 200× magnification. Scoring scale was 0 = no angiogenesis, 1 = minimal angiogenesis, 2 = moderate angiogenesis, and 3 = intense angiogenesis (Table 2). Angiogenesis scoring was performed for all slides in each experimental group.

Immunohistochemical staining for vascular endothelial growth factor (VEGF) was also performed on each section of tissue sample to evaluate newly formed capillaries. Determination of VEGF expression was performed by counting the newly formed capillaries in both proximal and distal sides of each skin flap specimen in ten high-power fields (HPF) and a mean value of the capillary number was obtained for each specimen. In the statistical analysis of immunohistochemical staining, the mean number of the newly formed vessels was compared among the groups

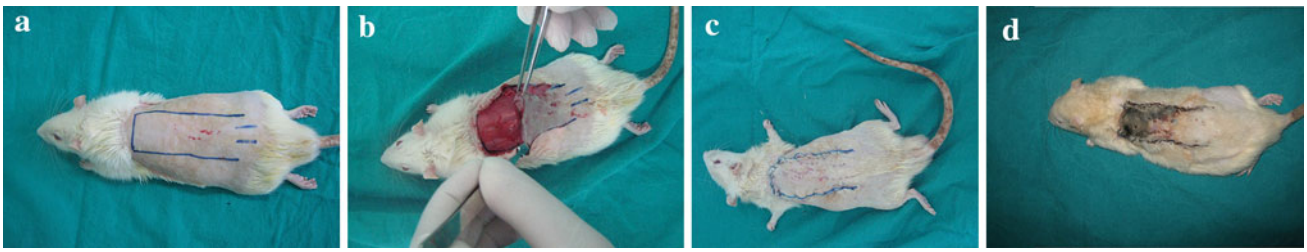


**Fig. 1** Design of the experiment in the control group. **a** Marking of the rat dorsal flap with the dimensions of 9 × 3 cm. **b** Elevation of the flap with the panniculus carnosus. **c** The flap is sutured back into its initial position. **d** Appearance of flap viability on the 7th day

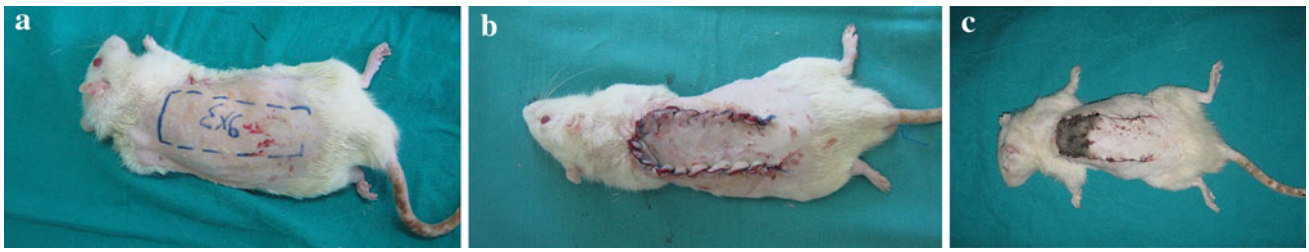


**Fig. 2** Steps of the experiment in group 2. **a** Planned flap area is drawn on the dorsal skin of the rat. **b** After the pretreatment with minoxidil for 7 days, a caudally based dorsal skin flap is elevated and then sutured back into its resting place. **c** Appearance of the flap on the 7th day after the surgery

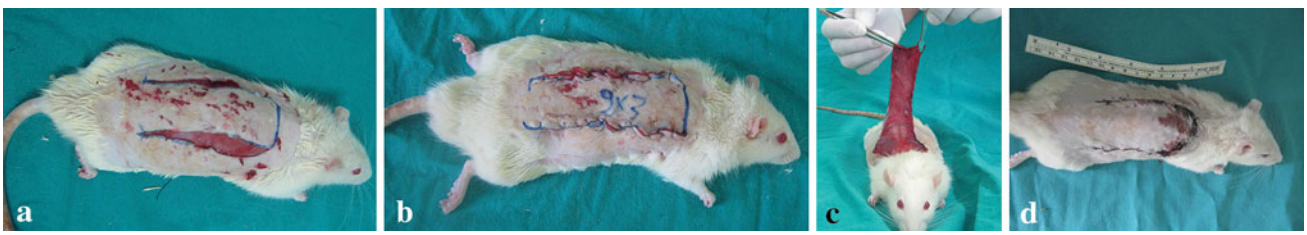


**Fig. 3** Experimental design of the surgical delay. **a** Elevation of bipedicled dorsal skin flap. **b** Flap is sutured back into its initial position. **c** On the 7th postoperative day, the cranial pedicle is cut, the flap is re-elevated, and then sutured back. **d** Appearance of flap viability on the 7th day

**Table 1** Survival areas of the flaps in the groups

| | Flap survival (%) | | |
|---|---|---|---|
| Rat No. | Group I | Group II | Group III |
| 1 | 57 | 71 | 72 |
| 2 | 58 | 68 | 78 |
| 3 | 63 | 69 | 80 |
| 4 | 62 | 75 | 81 |
| 5 | 61 | 69 | 73 |
| 6 | 59 | 66 | 70 |
| 7 | 54 | 71 | 74 |
| 8 | 65 | 73 | 76 |
| Mean ± SD | 59.8 ± 3.56* | 70.2 ± 2.8** | 75.5 ± 3.9*** |

* Significant difference versus groups II and III ($p < 0.05$)

** Significant difference versus groups I and III ($p < 0.05$)

*** Significant difference versus groups I and II ($p < 0.05$)

with the Kruskal–Wallis test, and proximal and distal side values within each group were compared with a Mann–Whitney *U* test.

**Table 2** Angiogenesis scores in the experiment groups

| | Score | | |
|---|---|---|---|
| Angiogenesis site of flap | Group I | Group II | Group III |
| Proximal zone | 1 | 2 | 3 |
| Distal zone | 0 | 2 | 3 |

Scoring was evaluated as 0 = no angiogenesis, 1 = minimal angiogenesis, 2 = moderate angiogenesis, 3 = intense angiogenesis

## Results

All animals that underwent surgery survived throughout the study and there were no signs of infection or hematoma in the flaps. The mean and SD percentages of surviving flap areas were determined for each group (Table 1). In group I (control group), the average survival area was 59.8 ± 3.56 %; in group II, it was 70.2 ± 2.8 %; and for the group III, it was 75.5 ± 3.9 %. The lowest flap survival rate appeared in group I, which was statistically different from

that of groups II and III (Fig. 4). Compared with groups I and II, group III had a significantly increased percentage of flap survival, which was statistically different from the other groups ($p < 0,05$). The mean surviving skin flap area in the minoxidil-pretreated group was significantly larger than that of the control group, which showed the efficacy of minoxidil in the pharmacological delay process, and a considerable amount of reduction in flap necrosis was obtained (Table 1). After histologic assessment, minimal angiogenesis was revealed at the proximal area of the flaps in the control group, which was scored as 1, and no angiogenesis was seen at the distal area, which was scored as 0. Moderate angiogenesis was also found in group II, which was scored as 2 on both sides, and intense angiogenesis was observed at both proximal and distal areas of the flaps in group III, which was scored as 3 (Fig. 5). Although some differences in the microscopic appearance of all slides obtained from each group were observed, all animals in each group had the same angiogenesis scores at the proximal and distal areas of the skin flaps. There was increased hair growth on the flaps after minoxidil treatment compared to the control group.

In the statistical analysis of immunohistochemical staining, the mean values of the number of newly formed capillaries were found to be significantly different among the groups ($p < 0.01$). The number of new capillaries was the highest in group III and was significantly higher in group II than in group I, supporting the results obtained from semiquantitative analysis of routine H&E staining (Table 3). When the distal and proximal capillary counts were compared statistically within each group, the proximal areas showed significantly higher numbers of capillaries than the distal sites of the flap ($p < 0.05$) (Fig. 6).

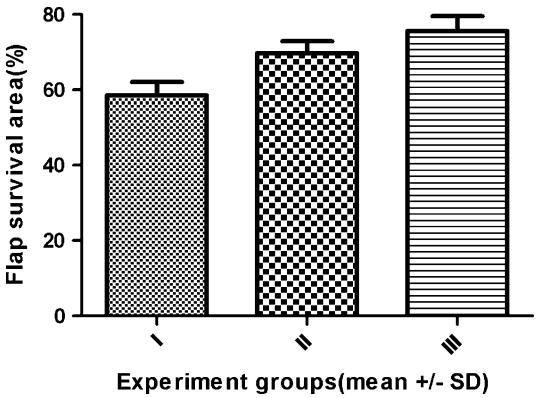


**Fig. 4** Comparison of mean survival areas (±SD) among the groups. Note that flap survival areas were significantly higher in *groups II* and *III* than in *group I* ($p < 0.05$), and a statistically significant difference was observed between the minoxidil-pretreated group and the other groups ($p < 0.05$)

## Discussion

After the elevation of a random-pattern skin flap in reconstructive surgery, flap necrosis does not frequently occur, but it results in severe morbidity related to the defect area. To overcome unpredicted flap loss in random-pattern skin flap design, various measures have been advocated, including both surgical and pharmacological methods. However, none of the methods, except for surgical delay, has been found to be effective in improving flap survival and suitable for clinical use extensively without any drawbacks to surgical methods or pharmacological agents. In this study, we used a topical agent, minoxidil, a well-known vasoactive agent, to demonstrate its influence on the delay phenomenon and to compare its efficacy with the surgical delay procedure. Topical application of minoxidil for pharmacological delay has the advantages of elimination of systemic side effects and time-consuming procedures such as the need for hospitalization and additional surgical intervention, and also is easy to use and is inexpensive.

In our study, minoxidil pretreatment increased the survival of rat dorsal flaps when compared to the results of the control group. It seems that it is a useful agent in for creating nonsurgical delay; however, it was not as effective as the surgical delay procedure in improving flap survival, at least with this posology, concentration, and time interval. The most significant increase in flap survival occurred in the surgically delayed flaps. After the minoxidil solution was sprayed over the flap surface, it was distributed over the flaps by spreading it with gentle fingertip massages. It was assumed that the minoxidil was absorbed through the skin and hair follicles. The absorbed minoxidil possibly acted as a stimulator of angiogenesis in the flaps, but no systemic reaction was observed during the experiments, during which the heart rate of the animals was monitored.

It seems that the efficacy of minoxidil arises from its local effect, not its systemic effect. The angiogenic effect of minoxidil may be due to its possible direct proangiogenic action or stimulation of the release of angiogenic factors; however, with the design and results of this experiment, it is difficult to see the exact mechanism of minoxidil that leads to angiogenesis. Stimulation of the release of angiogenic factors may be considered its main effect on angiogenesis of the flap, as its direct effect on vascular muscles causing vasodilation was well known. Minoxidil is known to be a vasoactive agent and to increase blood flow to the tissues when administered systemically [8–10], so it has been used as an antihypertensive agent. It has been shown that its mechanism of action is direct vasodilation of vascular smooth muscles induced by its active metabolite, minoxidil sulfate ($MxSO_4$), and it acts as a $K^+$ channel agonist to enhance $K^+$ permeability, leading

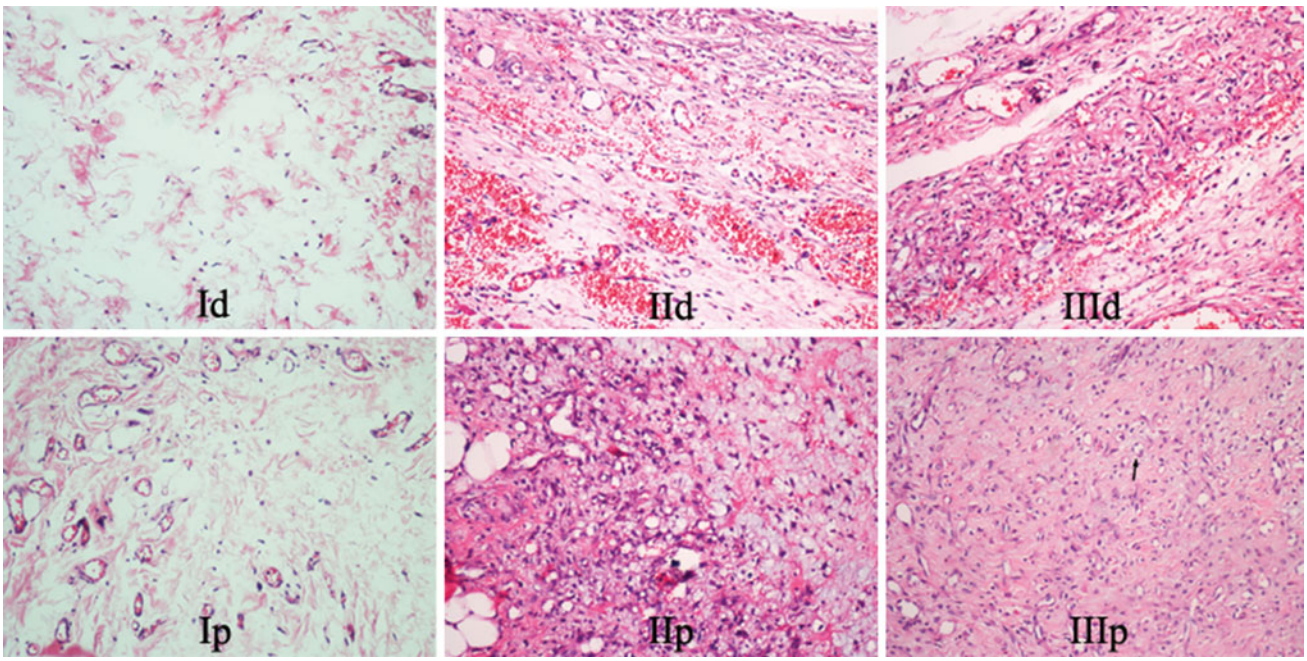


**Fig. 5** Histologic changes in the vascular structures of the flaps. **Id** Appearance of vascularity that occurred in the distal site of the flaps in group I, which was scored as 0, showing no increase in vascularization (HE, ×200). **Ip** Minimal angiogenesis in the proximal site of the flaps in group I, which was scored as 1 (HE, ×200). **IId, IIp** Moderate vascularity occurred at the proximal and distal parts of the flaps in group II, which was scored as 2 (HE, ×200). **IIId, IIIp** Intense vascularity occurred in both sides of the flaps in group III, which was scored as 3 (HE, ×200). d represents distal area of the flaps and p shows proximal site of the flaps. I, II, and III are the experiment groups

**Table 3** Mean number of newly formed capillaries in ten high-power fields

| Rat No. | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| 1 | 17 | 35 | 49 |
| 2 | 18 | 40 | 58 |
| 3 | 22 | 41 | 60 |
| 4 | 22 | 37 | 59 |
| 5 | 9 | 22 | 45 |
| 6 | 9 | 28 | 49 |
| 7 | 10 | 27 | 42 |
| 8 | 12 | 27 | 43 |
| Mean ± SD* | 14.8 ± 5.6 | 32.1 ± 7.0 | 50.6 ± 16.2 |

* There is a statistically significant difference among the groups ($p < 0.05$)

to relaxation in smooth muscle [11]. As it has a specific effect on hair follicles, stimulating hair growth, it is now widely used topically for the treatment of androgenic alopecia [8]. While the exact mechanism that causes hair growth remains unclear, minoxidil was shown to stimulate the cutaneous blood flow in human balding scalps [12]. Although it is a well-known vasoactive agent and its angiogenic and vasoactive properties have been reported in the literature, no studies had been performed to determine the influence of minoxidil on the nonsurgical delay process [8–16]. Pavlovitch [13] reported on two patients with angiogenic lesions of the scalp that occurred 2 and 3 months after topical application of minoxidil, suggesting that it led to angiogenesis in long-term use. In another study [14], the acute effect of topical minoxidil on digital blood flow was evaluated in cases with Raynaud's phenomenon. Topical 5 % minoxidil solution was applied to the subject's fingers on two separate occasions, and digital skin temperature, systolic blood pressure, and laser Doppler flow were measured. Results showed that minoxidil was not effective in improving digital blood flow.

The effect of minoxidil on skin flap survival had been previously explored [15, 16]. These initial studies used minoxidil as a vasoactive agent by means of topical or systemic administration. Smith and Dolan [15] investigated the ability of three vasoactive topical agents to improve the survival of random skin flaps in rats. After the application of prostaglandin $E_2$, minoxidil, or nitroglycerin to the rat dorsal flaps, improvement in viability was determined in adult Sprague–Dawley rats. The average flap survival areas of the nitroglycerin and minoxidil groups were found to be not significantly different from the control group. In this experiment, minoxidil 2 % was applied topically to the flaps during a 72 h period after flap elevation. In our experiment, minoxidil pretreatment using a 5 % concentration of minoxidil for 7 days prior to flap elevation was

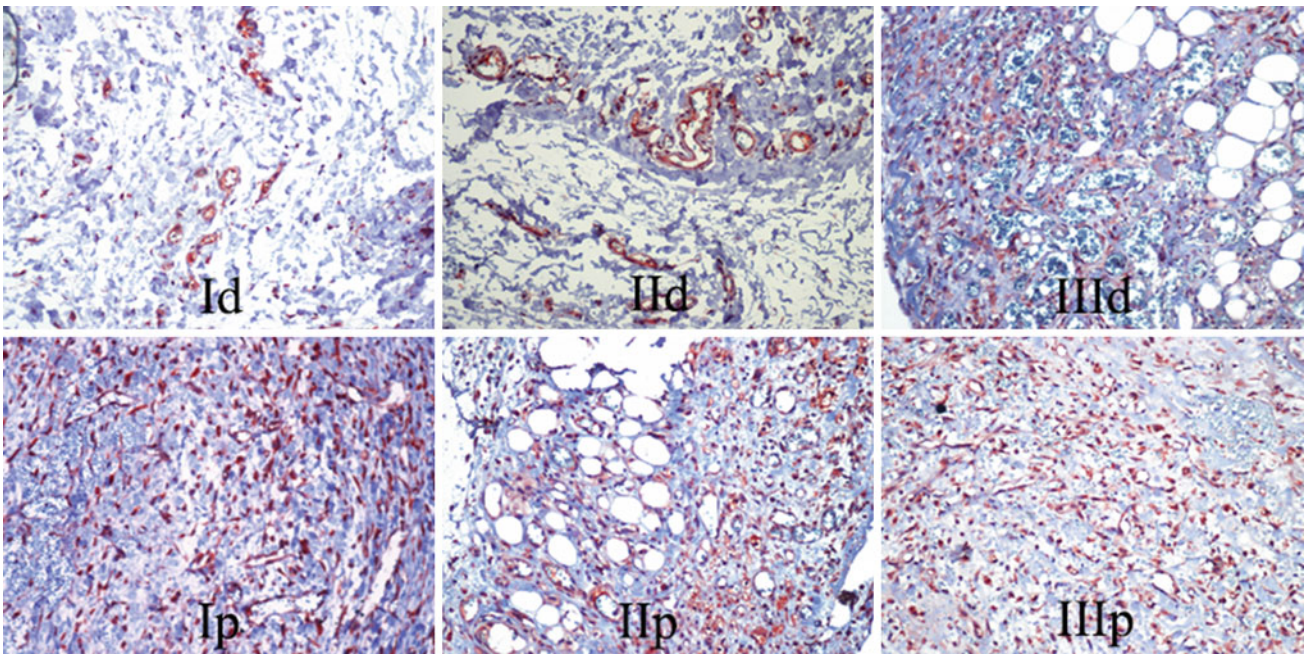


**Fig. 6** Histologic appearance of the newly formed vascular structures of the flaps after immunohistochemical staining for vascular endothelial growth factor (VEGF, ×200). Expression of VEGF correlated with the intensity of the newly formed capillaries, so it increased significantly from group I to group II and group III, respectively, and decreased from the proximal sites of the flaps to the distal sites. The number of newly formed capillaries was statistically different among the groups. **Id** represents distal sites of group I flaps and **Ip** represents proximal sites of group I flaps. **IId** shows distal area of group II flaps and **IIp** the proximal sites of group II flaps. **IIId** shows distal area of group III flaps and **IIIp** the proximal sites of group III flaps

performed to create a pharmacologic delay on the planned flap area.

In another study [16], a rat ventral cutaneous flap was elevated to evaluate the influence of minoxidil on ischemic flap necrosis prevention. In the experimental group, minoxidil sulfate was given by orogastric tube once a day (50 mg/kg day) and was started 24 h before surgery and continued until the 7th postoperative day. Although laser fluxometry showed increased blood flow in some points of the flaps, necrotic areas were not significantly different between the experimental and control groups. In our study, minoxidil was utilized only topically, not systemically, and pretreatment was done for nonsurgical delay in the flaps, so our design was quite different from that study.

Histologic assessments of minoxidil-pretreated flaps proved that minoxidil was capable of stimulating angiogenesis in the flaps, leading to a considerable increase in flap survival. However, the angiogenesis that appeared in the minoxidil-pretreated flaps, which was scored as 2, was not as much as that in the flaps that underwent the surgical delay procedure, which was scored as 3. These results, supported by immunohistochemical staining, suggest that the effect of minoxidil, when administered as proposed in this study was efficient in stimulating angiogenesis and increasing flap survival, but it did not obtain a similar result as the surgical delay. Flap survival of the minoxidil-pretreated group was significantly better than that of the control group but worse than that of the surgical delay group. Further studies are required to prove the effectiveness of topical minoxidil use in clinical settings by using various posology and application times.

When dealing with the outcomes of this experiment, minoxidil may be considered an effective vasoactive agent for the stimulation of angiogenesis in the rat cutaneous flaps, capable of pharmacologic delay and increasing flap survival.

**Conflicts of interest** The authors have no conflicts of interest or financial ties to disclose. Furthermore, this study was not supported by any external funding, nor were any special products, devices, or drugs used in the work presented.

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