Journal of Plastic Surgery and Hand Surgery

ISSN: 2000-656X (Print) 2000-6764 (Online) Journal homepage: www.tandfonline.com/journals/iphs20

Comparison of incisional delay patterns on a rat random flap model

Dincer Altınel, Merdan Serin, Havva Erdem, Burcu Biltekin, Onder Huseyinbas, Gaye Toplu & Sevgi Kurt Yazar

To cite this article: Dincer Altınel, Merdan Serin, Havva Erdem, Burcu Biltekin, Onder Huseyinbas, Gaye Toplu & Sevgi Kurt Yazar (2019) Comparison of incisional delay patterns on a rat random flap model, Journal of Plastic Surgery and Hand Surgery, 53:4, 247-253, DOI: 10.1080/2000656X.2019.1588740

To link to this article: https://doi.org/10.1080/2000656X.2019.1588740

Published online: 31 Mar 2019.

JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2019, VOL. 53, NO. 4, 247–253
https://doi.org/10.1080/2000656X.2019.1588740

ARTICLE

Comparison of incisional delay patterns on a rat random flap model

Dincer Altınel[a], Merdan Serin[a], Havva Erdem[b], Burcu Biltekin[c], Onder Huseyinbas[d], Gaye Toplu[a] and Sevgi Kurt Yazar[a]

[a]Department of Plastic and Reconstructive Surgery, University of Health Sciences, Istanbul Training and Research Hospital, Istanbul, Turkey; [b]Department of Pathology, Ordu University Training and Research Hospital, Ordu, Turkey; [c]Department of Histology and Embryology, Istanbul University, Cerrahpasa Medical School, Istanbul, Turkey; [d]Animal Research Laboratory, Bezmialem University Medical School, Istanbul, Turkey

ABSTRACT

One of the simplest form of surgical delay can be performed by placing an incision around the flap without undermining, prior to flap elevation. In this study, we have compared the efficiency of different patterns of skin incision to improve flap survival. Twenty-eight animals were used in four groups. Incisional delay was performed prior to flap elevation in the three experiment groups. Complete incision of the three flap edges was performed in the all experiment groups with the exception of an intact skin section on the middle 1/3rd of the bilateral edges in group 1 (bilateral skin edge preserved delay: BSEPD), of a unilateral edge in group 2 (unilateral skin edge preserved delay: USEPD) and of the superior edge in group 3 (superior skin edge preserved delay: SSEPD) without any undermining. Two weeks following the delay procedure, dorsal skin flaps were raised and reinserted back to their place. The results were evaluated with the measurement of necrotic flap area, microangiographic imaging and histological evaluation. The mean percentage of necrotic flap area to whole flap area was 16.94%, 7.54%, 23.34% and 50.6% in the BSEPD, USEPD, SSEPD and control groups, respectively. In selected microangiographic images, vessels were more prominent in the delay groups. The results of the study indicate that three sided incision with an intact skin on the superior edge is not effective in providing a sufficient delay and flap survival improvement when compared to incisions with intact skin on the unilateral and bilateral edges.

Abbreviations: BSEPD: Bilateral skin edge preserved delay; USEPD: Unilateral skin edge preserved delay; SSEPD: Superior skin edge preserved delay; H&E: Hematoxylin and Eosin; TRAM: Transverse Rectus Abdominis Myocutaneous flap

ARTICLE HISTORY
Received 29 November 2018
Revised 5 February 2019
Accepted 25 February 2019

KEYWORDS
Surgical flap delay; random flaps; rat

Introduction

Many factors are effective in determining the fate of skin flaps [1–5]. Flap physiology principles are essential for success in reconstructive surgery. The circulation of small vessels like arterioles, capillaries and venules, which are essential for flap survival, can be altered by many biochemical and neurological factors [6]. Skin flap necrosis can be a result of arterial or a venous insufficiency. Various drugs [7], external applications [8], oxygen pressure [9] and previous scars [10,11] can have effect on flap survival.

Previous studies have shown the significance of the delay procedure on flap survival [12]. Despite the developments in the reconstructive surgery techniques, delay procedure is still widely used in surgical practice. In the practice of plastic surgery, delay procedure can be performed with various surgical techniques, pharmacological agents and external physical effects [13,14].

Surgical delay aims to enhance the blood supply to the proposed flap area by disturbing one of its sources of blood supply. A well-known example for the delay procedure used in the transverse rectus abdominis flap with the ligation of the deep inferior epigastric vessels 2 weeks prior to flap elevation. In a random flap, surgical delay can be performed by the obstruction of blood flow to the superficial dermal plexus with a skin incision or to the deep dermal plexus with the ligation of perforator vessels. Dincer et al. have previously shown in their study that perforator delay with a superior incision was as effective as having a three-sided incision. Gersch et al. have similarly reported in their study that bilateral incision with undermining and a three-sided incision were both effective in providing delay. These results indicate the efficiency of incisional delay techniques without undermining. But the ideal incisional pattern for an efficient delay is currently not clearly established [15,16].

In this study, our aim was to compare effectiveness of different patterns of delay incisions combined with partially intact skin edges, on the basis of clinical and histological findings in a rat random skin flap model.

Materials and methods

This study was conducted with the approval of local ethical comity. Twenty-eight female wistar rats were used for the study. The average weight of the animals was 300 g. A common control group was used with another simultaneous study on random flap survival, in which exact flap design was used and was performed around the same time with same age rats, to prevent unnecessary sacrifice of additional animals.

Surgical anesthesia was performed with intraperitoneal injection of 90 mg/kg ketamine (Ketalar, Pfizer, New York, NY) and

CONTACT Merdan Serin merdanserin@gmail.com Department of Plastic and Reconstructive Surgery, University of Health Sciences, Istanbul Research and Teaching Hospital, Istanbul, Turkey

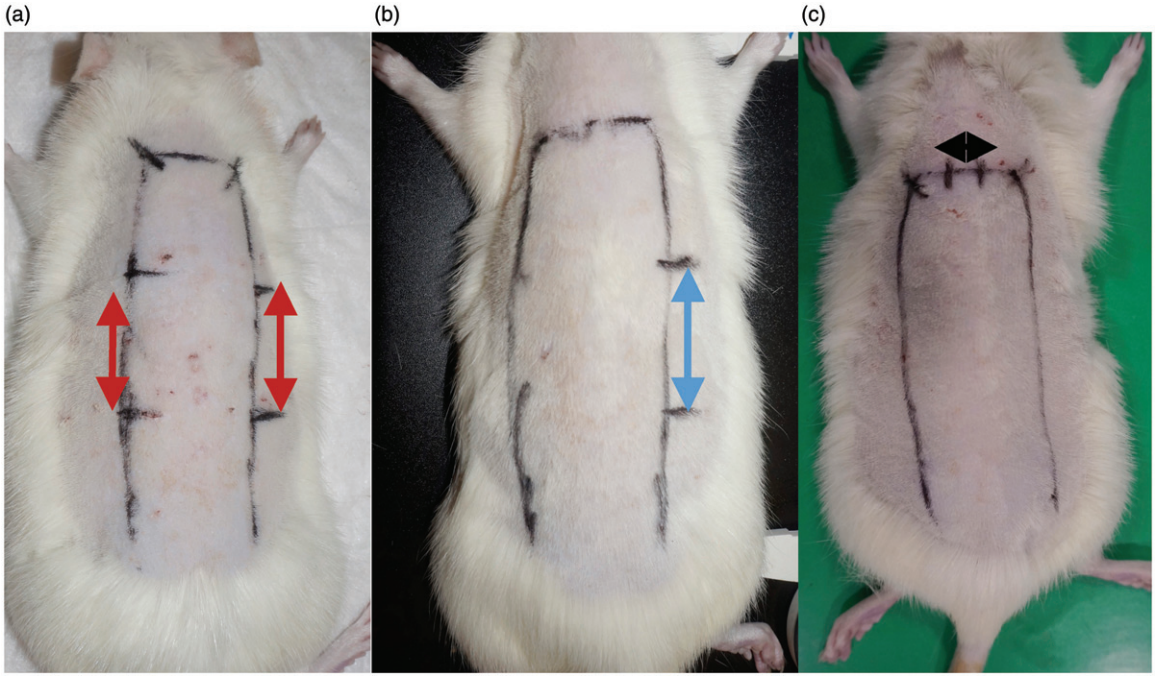

Figure 1. Delay group designs prior to dorsal skin flap after flap elevation. The intact skin edges are indicated with arrows. (a) BSEPD group. (b) USEPD group. (c) SSEPD group.

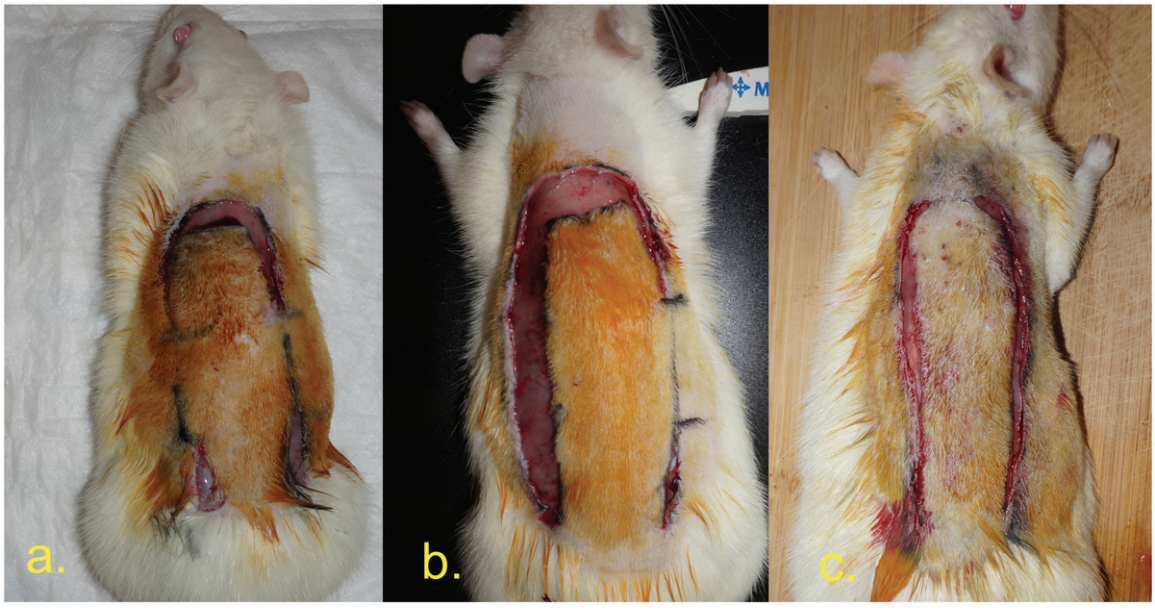

Figure 2. The postoperative view of the animals in delay groups following the completion of delay incisions. (a) BSEPD group. (b) USEPD group. (c) SSEPD group.

10 mg/kg xylazin (Rompun, Bayer, Leverkusen, Germany). Dorsal skin was shaved with an electric razor prior to surgery. Skin was cleaned with iodine solution. The rats were randomized into four groups with seven animals in each (Figures 1 and 2):

Group 1: bilateral skin edge preserved delay (BSEPD). Surgical delay with the incision of the three flap edges with the exception of a bilateral 3 cm long intact skin on the lateral edges was performed 14 days before the elevation of the flaps.

Group 2: unilateral skin edge preserved delay (USEPD). Surgical delay with the incision of the three flap edges with the exception of a unilateral 3 cm wide intact skin on a lateral edge was performed 14 days before the elevation of the flaps.

Group 3: superior skin edge preserved delay (SSEPD). Surgical delay with the incision of the three flap edges with the exception of a 1 cm wide intact skin on the superior edge was performed 14 days before the elevation of the flaps.

In all of the three experiment groups, skin incisions were performed carefully not to raise the flap from the underlying fascia. Incisions were sutured with 4.0 polypropylene sutures. Protective rat vests were used to prevent self-mutilation following the surgery.

Group 4: control group: The animals in this group were kept together in similar conditions with the animals in the other groups without any surgical intervention prior to flap elevation.

Following two-week period, Adamson modification of McFarlane dorsal skin flaps based on caudal pedicles was elevated in all of the animals [17,18]. The dimension of the dorsal flap was 9×3 cm. The flaps were raised at the level of deep to pannus carnosus (Figure 3). Following flap elevation, the flaps were reinserted and adapted back to their place with 4/0 polypropylene sutures. Protective rat vests were used to prevent self-mutilation following the surgery. Following the elevation of the flaps, daily digital photos were taken with a digital camera (Sony Nex-3n, Tokyo, Japan). VistaMetrix (Version 1.35.0, Skillcrest LLC) software was used to calculate necrotic and whole flap areas.

Seven days after the flap elevation histological samples were taken from the proximal and the distal ends of the flaps under surgical anesthesia. Afterwards abdominal cavity was exposed for angiographic study. Twenty-two-gauge and 20-gauge cannulas were used for cannulation of aorta and inferior vena cava. Pentothal Sodium IV (Pentothal Sodium flk.; Abbott®, Chicago, IL) was used to euthanize the animals. A mixture of 40% barium sulfate (40 cm^3) (Radyobarit Suspension®) and 10% gelatin (10 cm^3) was prepared and diluted in 100 cm^3 of saline solution. This mixture was injected from aorta and inferior vena cava. Following injection, the cannula caps were closed and the bodies were placed in −18 freezer overnight. Following day, the bodies were thawed, flaps were removed from the bodies and placed on cartoon board. These boards were placed in +4 freezer for another night. The next day, the flaps were placed in high resolution mammography machines for imaging (GE Mammography Machine).

The tissue samples collected for histological analysis were fixed in 10% formaldehyde and were embedded in paraffin blocks. Sectioned blocks were stained with hematoxylin and eosin for examination under light microscopy. The number of capillary formations were counted by the same pathologist under high magnification. Histological parameters such as inflammation, vascular congestion, extravascular erythrocytes, epidermal injury and dermal fibrosis were evaluated. The findings were placed into a scale from 0 to 3 (0: none, 1: low, 2: medium, 3: high).

Percentage of necrotic flap areas and histological results in group were compared using the Kruskal–Wallis, one-way ANOVA test and Dunn's multiple comparisons test. Statistical evaluation was performed with Prism 7 software (version 7.00 for Windows, GraphPad Software, La Jolla, CA).

Figure 3. Perioperative photograph following the elevation of the skin flap two weeks after the incisional delay procedure.

Results

Flap necrosis area percentage results

There was no necrosis present in the animals of the experiment groups at the end of the two-week period following the delay procedure, prior to flap elevation. The mean percentage of necrotic flap area to whole flap area seven days after the flap elevation was 16.94%, 7.54%, 23.34% and 50.6% in the BSEPD, USEPD, SSEPD and control group, respectively. Flap necrosis area percentages in each group were compared using the Kruskal–Wallis test. There was statistically significant difference between the BSEPD and control group ($p = .0178$). There was also statistically significant difference between the USEPD and the control group ($p = .0011$). There was no statistically significant difference between SSEPD and control groups (Figure 4; Table 1).

Evaluation of angiography images

The flaps were placed on cardboards to prevent folding and high resolution images were taken with the mammography machine were magnified in the computer to visualize the vessels. The vessels were marked in each image. In selected images, vessels were more prominent in the images from the delay groups (Figure 5) [19,20].

Evaluation of histological results

Mean number of capillary formations counted in superficial dermis was 11 ± 3.162, 13.67 ± 4.041, 14.5 ± 9.055 and 15.67 ± 6.658 in BSEPD, USEPD, SSEPD and control group, respectively. The differences between the groups were not significant ($p = .8287$). Mean

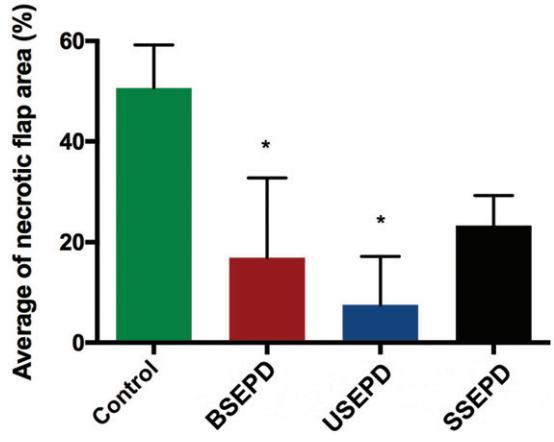

Figure 4. Average of necrotic flap area percentage for each group ($*p < .05$).

Table 1. Summary of statistical results of necrotic flap area measurements.

Dunn's multiple comparisons test	Mean rank diff.	Significant?	Summary	Adjusted p value
SSEPD vs. BSEPD	4.548	No	ns	>.9999
SSEPD vs. USEPD	7.833	No	ns	.3243
SSEPD vs. CONTROL	−8.167	No	ns	.3899
BSEPD vs. USEPD	3.286	No	ns	>.9999
BSEPD vs. CONTROL	−12.71	**Yes**	*	**.0178**
USEPD vs. CONTROL	−16	**Yes**	**	**.0011**

The bold values indicate statistical significance. ($*p < 0.05$, $**p < 0.01$, ns: not significant).

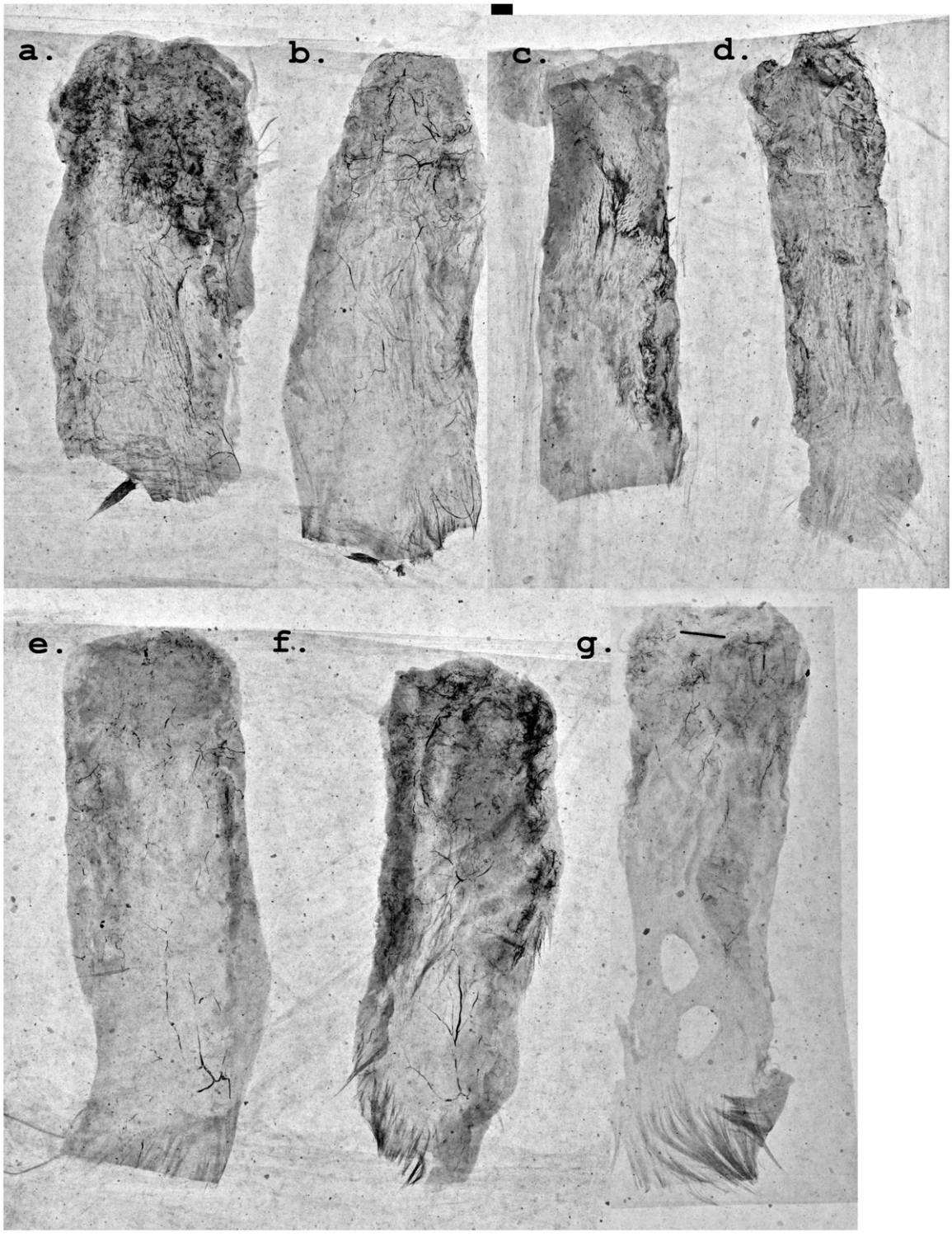

Figure 5. Angiography results from selected samples. (a, b) BSEPD group; (c, d) USEPD group; (e, f) SSEPD group and (g) control group.

number of capillary formations counted in deep dermis was 22.75 ± 10.31, 28.67 ± 8.963, 9.25 ± 12.12 and 21 ± 5.568 in BSEPD, USEPD, SSEPD and control group, respectively. The differences between the groups were not significant ($p = .6307$). Mean of the inflammation score was 0, 0, 0.25 ± 0.4629 and 0.5 ± 0.5774 in BSEPD, USEPD, SSEPD and control group, respectively. The differences between the groups were not significant ($p = .3668$). Mean of the vascular congestion score was 0.25 ± 0.5, 1 ± 0, 0.5 ± 0.7559 and 0.25 ± 0.5 in BSEPD, USEPD, SSEPD and control group, respectively. The differences between the groups were not significant ($p = .5136$). Mean of the epidermal injury score was 0, 0, 0.875 ± 0.8345 and 0.3333 ± 0.5774 in BSEPD, USEPD, SSEPD and control group, respectively. The difference between the USEPD, BSEPD and control groups was significant ($p = .0475$). Mean of the dermal fibrosis score was 0.5 ± 0.5774, 0.5 ± 0.7071, 1 ± 0.9258 and 2 ± 0 in BSEPD, USEPD, SSEPD and control group, respectively. The difference between the BSEPD, USEPD, SSEPD and the control was significant ($p = .0117$) (Figures 6 and 7).

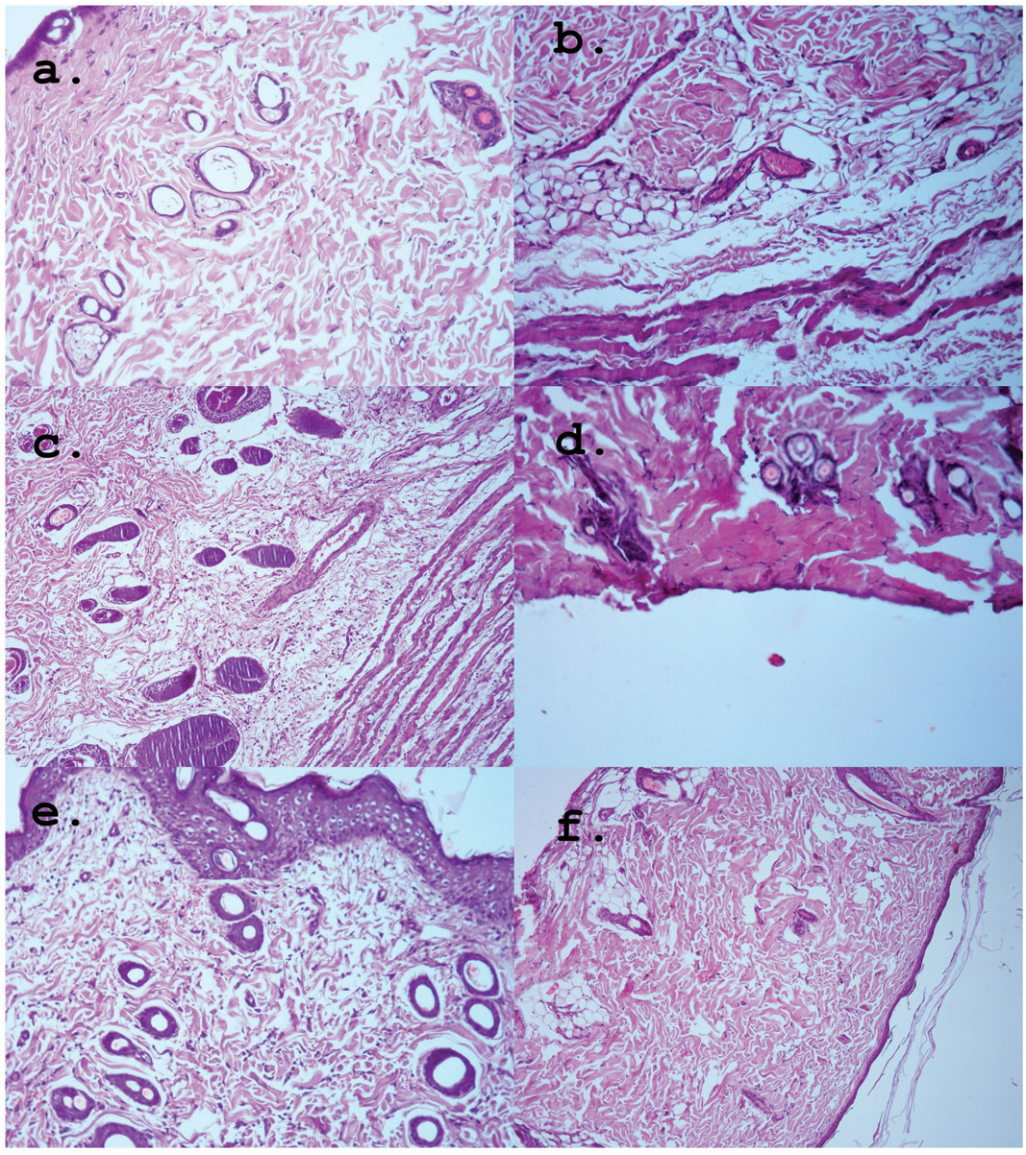

Figure 6. Photomicrographs of histological samples. (a) An image from BSEPD group showing signs of fibrosis (hematoxylin and eosin (H&E), ×200). (b) An image from BSEPD group showing signs of vascular congestion (H&E, ×200). (c) An image from USEPD group showing signs of inflammation (H&E, ×100). (d) An image from USEPD group showing signs of epidermal injury (H&E, ×200). (e) An image from SSEPD group showing signs of extravascular erythrocytes (H&E, ×200). (f) An image from control group (H&E, ×100).

Discussion

Delay procedure is commonly used in reconstructive surgery practice to increase the vascularization of the skin prior to flap elevation. This procedure is usually performed 2–3 weeks prior to the surgery. Rat skin island flap has been used as a model for the delay procedure in previous studies [21]. In various studies, delay procedure has been shown have positive effects on flap circulation and these results have been adopted by plastic surgeons. TRAM flap delay is one of the best examples of this application.

In our study, best flap survival results were achieved with the USEPD group. A considerable amount of incision on the one side of the skin edge was required to achieve a successful delay. It has been shown in previous studies that decrease in tissue oxygenation can induce neovascularization via various cytokines [22–26]. We believe that this decrease in tissue oxygenation has to reach a certain level for a successful delay effect.

On the other hand, the results from the SSEPD group support the idea that complete incision of the both lateral sides without any intact skin can completely diminish the positive effect of the delay procedure. The SSEPD group has resulted in the least amount of increase in the flap survival which was statistically not significant when compared to the control group. This shows that the increase in the excision length in this group has a negative impact on the delay procedure of random flaps. This can actually feel counter intuitive and contradicts with the previous idea that the decrease in the tissue oxygenation can increase neovascularization. We believe that this result shows that the neovascularization increase from the underlying fascia is not significant when compared to the increase from the subdermal plexus in two-week period. The SSEPD group had the greatest amount of vascular stress among other groups, but since the incisions covered the both lateral edges completely there was no physical space left for the neovascularization to move forward except the underlying fascia.

Since the SSEPD group was the least efficient, we also predict that the intact skin left on the edges for an efficient delay should be closer to the pedicle. The blood flow will become less efficient as the intact skin is placed away from the pedicle since the

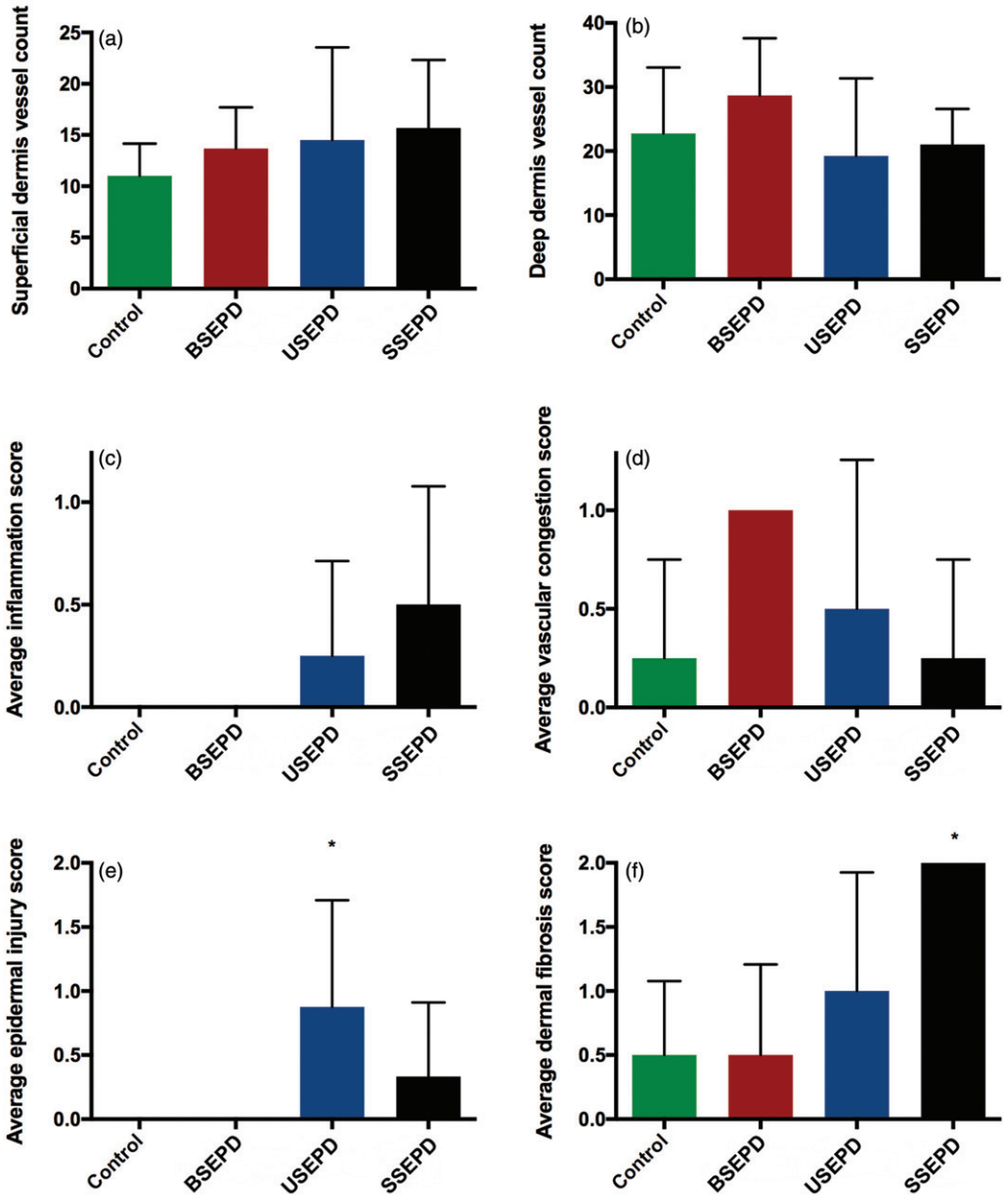

Figure 7. Average number of capillary formations in (a) superficial and (b) deep dermis and histological parameters, ((c) inflammation, (d) vascular congestion, (e) epidermal injury and (f) dermal fibrosis) calculated for each group ($^*p < .05$).

perfusion pressure will become less efficient The results also indicate that, despite statistically not significant, USEPD might be more efficient in providing a delay than a BSEPD. This might be due to the fact that asymmetrical perfusion of the proposed flap area could be more efficient to open the choke vessels. In general, the success of the delay will be correlated with the power and the direction of the perfusion. The results of this study also indicate that a previous linear scar in a flap edge should not be as problematic as previously thought. In contrast, it might prove beneficial to increase the blood flow to the area.

The histological evaluation did not reveal any difference in the number other the vessels in the dermis. These results indicate that the incisional delay procedure does not have an impact on the number of vessel but rather does effect the rate of blood flow from the existing vessels. More prominent vessels found in the angiographic images of the animals in the delay groups support this idea. Dermal fibrosis score was significantly higher in the SSEPD group. This result correlates with the length of the delay incision in this group. This could also be a contributing factor in the inefficiency of the delay in this group.

A balance must be maintained for an efficient delay procedure between impairment in the skin perfusion and leaving enough intact skin for the neovascularization to progress. Placement of too large and too many intact skin edges and keeping incisions short might result in less impairment of tissue vascularization which as a result might not be enough to induce neovascularization. On the other hand, absence of intact skin with complete incision of the edges might induce the neovascularization more, but this time there might not be enough intact skin left for the neovascularization to progress. Therefore, we conclude from the results of these studies that the complete incision of one of the lateral edges of a random flap is required for an efficient delay.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Zhang F, Oswald T, Lin S, et al. Vascular endothelial growth factor (VEGF) expression and the effect of exogenous VEGF on survival of a random flap in the rat. Br J Plast Surg. 2003;56:653–659.

[2] Wang LR, Cai LY, Lin DS, et al. Effect of electroacupuncture at The Zusanli Point (Stomach-36) on dorsal random pattern skin flap survival in a rat model. Dermatol Surg. 2017; 43:1213–1220.

[3] Ghavami A, Nutt MP, Hardy SP. Heat shock protein and high-dose aspirin: effects on random skin flap survival in a rat model. Ann Plast Surg. 2002;48:60–67.

[4] Davies BW, Lewis RD, Pennington G. The impact of vasodilators on random-pattern skin flap survival in the rat following mainstream smoke exposure. Ann Plast Surg. 1998; 40:630–636.

[5] Can A, Temel M, Dokuyucu R, et al. The effect of coenzyme Q10 (ubiquinone) on random pattern skin flap survival in rat model. Ann Plast Surg. 2016;77:e9–e14.

[6] Seyhan T, Deniz M, Borman H, et al. Comparison of two different vascular delay methods in a rat cranial epigastric perforator flap model. Ann Plast Surg. 2010;64:89–92.

[7] Karacaoglu E, Yuksel F, Turan SO, et al. Chemical delay: an alternative to surgical delay experimental study. Ann Plast Surg. 2002;49:73–80, discussion 2–1.

[8] Huemer GM, Froschauer SM, Pachinger T, et al. A comparison of pretreatment with a topical combination of nonivamide and nicoboxil and surgical delay in a random pattern skin flap model. J Plast Reconstr Aesthet Surg. 2009;62: 914–919.

[9] Stewart RJ, Moore T, Bennett B, et al. Effect of free-radical scavengers and hyperbaric oxygen on random-pattern skin flaps. Arch Surg. 1994;129:982–987, discussion 7–8.

[10] Serin M, Bayramicli M. Evaluation of scar penetrating neovascularisation in a rat epigastric flap model. J Plast Surg Hand Surg. 2015;49:295–299.

[11] Edstrom LE, Robson MC, Hopp D. Survival of skin flaps based on scar. Ann Plast Surg. 1981;7:44–47.

[12] Gillies H. The tubed pedicle in plastic surgery. New York Med J. 1920;111:1.

[13] Cohen M, Kattan A, Gat A, et al. Comparison of flash lamp pulsed-dye laser (585 nm) and conventional surgery in the delay of random dorsal rat flaps. Lasers Surg Med. 1999;25: 178–186.

[14] Reichenberger MA, Keil H, Mueller W, et al. Comparison of extracorporal shock wave pretreatment to classic surgical delay in a random pattern skin flap model. Plast Reconstr Surg. 2011;127:1830–1837.

[15] Gersch RP, Fourman MS, Dracea C, et al. The delay phenomenon: is one surgical delay technique superior? Plast Reconstr Surg Glob Open. 2017;5:e1519.

[16] Serin M, Altinel D, Biltekin B, et al. Comparison of subdermal and perforator delay techniques on a rat flap model. Indian J Plast Surg. 2018;51:196.

[17] Adamson JE, Horton CE, Crawford HH Jr, et al. The effects of dimethyl sulfoxide on the experimental pedicle flap: a preliminary report. Plast Reconstr Surg. 1966;37: 105–110.

[18] McFarlane RM, Heagy FC, Radin S, et al. A study of the delay phenomenon in experimental pedicle flaps. Plast Reconstr Surg. 1965;35:245–262.

[19] Serin M, Altinel D, Leblebici C, et al. Subdermal nitrous oxide delivery increases skin microcirculation and random flap survival in rats. J Plast Surg Hand Surg. 2018;53:37–44.

[20] Serin M, Altinel D, Leblebici C, et al. Preoperative subcutaneous sildenafil injection increases random flap survival in rats. Acta Cir Bras. 2018;33:216–222.

[21] Komorowska-Timek E, Timek TA, Brady S, et al. Perfusion, viability, and pedicle dependence in acute and delayed rat island skin flaps. Microsurgery. 2007;27:146–151.

[22] Dobryansky M, Galiano RD, Cetrulo CL, Jr, et al. Endostatin inhibits ischemia-induced neovascularization and increases ischemic tissue loss. Ann Plast Surg. 2004;52:512–518, discussion 8.

[23] Goukassian DA, Qin G, Dolan C, et al. Tumor necrosis factor-alpha receptor p75 is required in ischemia-induced neovascularization. Circulation. 2007;115:752–762.

[24] Huang PH, Chen JY, Chen CY, et al. Simvastatin pretreatment enhances ischemia-induced neovascularization and blood flow recovery in streptozotocin-treated mice. J Vasc Surg. 2016;64:1112–1120 e1.

[25] Pittman RN. Regulation of tissue oxygenation. San Rafael (CA); Morgan & Claypool Life Sciences. 2011.

[26] Tam JC, Ko CH, Lau KM, et al. A Chinese 2-herb formula (NF3) promotes hindlimb ischemia-induced neovascularization and wound healing of diabetic rats. J Diabetes Complications. 2014;28:436–447.