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Original Article

Electrical microneedling improves survival of pedicled perforator flap by enhancing neoangiogenesis: an experimental study

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Abstract: Aim: Pedicled perforator flap transfer is subject to risk of distal marginal necrosis due to insufficient perfusion. This study aimed to evaluate the effectiveness and safety of electrical microneedling in improving the survival of pedicled perforator flaps in an experimental rat model. Methods: Bilateral perforator flaps (20 pairs) pedicled with the iliolumbar artery were harvested from the dorsal skin of 20 adult male Sprague-Dawley rats. These flaps were orthotopically sutured onto the donor sites, with the right side as the experimental flap and the left side as the control. Experimental flaps were preconditioned and conditioned using electrical microneedling on preoperative day three, the day of operation, and postoperative days (PODs) three and five; while the control flaps receiving no microneedling. Main outcome measures included flap survival area, blood perfusion, angiogram, microvascular density (MVD) and cutaneous expression of vascular endothelial growth factor (VEGF). Results: On POD seven, the experimental flaps had a significantly larger survival area than the control flaps (88.2 ± 2.5% *vs.* 78.3 ± 4.1%, $P = 0.04$). Furthermore, the experimental flaps had significantly dilated choke vessels with more collateral anastomoses compared to the control flaps. In addition, the experimental flaps also had significantly greater blood perfusion (9.1 ± 1.7 *vs.* 3.6 ± 0.9 units, $P = 0.02$), MVD (200.2 ± 3.8 *vs.* 136.4 ± 8.1 per mm^2, $P < 0.001$) and cutaneous expression of VEGF (0.54 ± 0.09 *vs.* 0.11 ± 0.02 fold, $P = 0.001$). Conclusions: Preconditioning and conditioning with electrical microneedling could improve perforator flap survival by increasing flap perfusion and angiogenesis.

Keywords: Perforator flap, choke vessels, microneedling, survival, perfusion, angiogenesis

Introduction

Flap transfer is an effective treatment modality for repairing soft tissue defects after traumatic injury or radical resection of malignant tumors. Perforator flaps contains skin and subcutaneous adipose tissues with a blood supply, and are derived from perforators emerging through underlying muscles or the intermuscular septa [1]. As compared to a conventional random-pattern flap with an unknown vascular supply or an axial-pattern flap with a definitive blood supply, a flap pedicled with a perforator has good flexibility and minimal donor site morbidity. However, a major limitation of using a perforator flap is the risk of partial or complete necrosis, especially at the distal end, due to compromised blood supply [2, 3].

Microneedling is a newly-emerging cutaneous aesthetic surgical technique for treating atrophic acne scars, actinic keratosis, androgenetic alopecia, and skin rejuvenation [4-6]. The therapeutic effect of microneedling has been thought to be derived from the regional vascular rupture in the dermal layer [7, 8], which further results in the release of platelets and growth factors that favor neoangiogenesis and blood supply reconstruction [9]. Sezgin *et al.* [5] reported that preconditioning of the recipient site with microneedling could improve fat graft survival in experimental rats.

Figure 1. Flap outlining, operative procedure and microneedling: A. Outline of the iliolumbar artery perforator flap; B. Preoperative microneedling of the right-side flap (experimental flap) in contrast to the left-side flap with no preconditioning (control flap); C. Harvesting of the perforator flap. TDA, thoracodorsal artery; SCA, subcostal artery; ILA, iliolumbar artery.

There is a knowledge gap on whether perioperative microneedling can improve perforator flap survival by optimizing blood supply. This study primarily aims to evaluate the effectiveness and safety of electrical microneedling in an experimental pedicled perforator flap transfer rat model, with respect to flap survival, blood perfusion, choke vessel dilation and neoangiogenesis.

Materials and methods

Laboratory animals

This study protocol was approved by the Animal Research Committee of Shandong University. All animal experiments were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and the Animal Research: Reporting In Vivo Experiments (ARRIVE) Guidelines. Twenty adult male specific-pathogen-free Sprague-Dawley rats weighing 250-300 grams were bred and obtained from the Laboratory Animal Center of Shandong University (License No. SCXK [SD]2005-0019), and housed in individual rodent cages with access to drinking water and rodent chow ad libitum. All animals were housed in an environment of constant temperature (24 ± 1°C) and humidity (30 ± 5%) with a 12-hour/12-hour light/dark cycle.

Iliolumbar artery perforator flap outlining

Bilateral iliolumbar artery perforator flaps were outlined in a rectangular shape (2.5 cm × 9.0 cm), with the dorsal midline as the medial margin, extending from the subscapular angle towards the hip joint. Briefly, the dorsal skin was incised along the midline, with two parallel incisions 2.5 cm away from the midline on each side at an approximate length of 9.0 cm, extending 0.5 cm caudal to the subscapular angle and 0.5 cm towards the cephalic to the hip joint (**Figure 1A**). This flap outline contained three comparable angiosomes (thoracodorsal artery, subcostal artery, and iliolumbar artery angiosomes) and two choke vessel areas [10, 11].

Experimental grouping and microneedling

The right-side perforator flaps were used as experimental flaps (n = 20), and the left-side counterparts were used as control (n = 20). The experimental flaps were preconditioned in vertical full-thickness (approximately 1.2 mm) using a 1.5-mm commercially available electrical microneedle (JKL Beauty Equipment Co.

Figure 2. Gross flap pathology: (A) Gross appearance; and (B) bar chart of the survival area (%, mean ± SD) of the experimental flap *vs.* the control flap on postoperative day seven, expressed as the percentage of the total flap area (n = 20).

Ltd., Guangzhou, China; **Figure 1C**) at a power of 6 W and at a speed of 1,000 rpm for 15-20 minutes, until small dotted bleeding emerged (**Figure 1B**) [12]. Microneedling was performed for four sessions on preoperative day three, the day of operation, and postoperative days (PODs) three and five. The control flaps received no perioperative conditioning.

Operative technique

Animals were anesthetized by intraperitoneal injection of 5% phenobarbital (50 mg/kg; Wende Pharmaceutical Co., Ltd., Nanjing, China) and placed in the prone position. The dorsal skin was prepared, disinfected and draped as routine. The flap was incised along the dorsal midline to harvest three angiosomes beneath the deep fascia. The thoracodorsal and subcostal vessels were transected to configure an island flap pedicled with the iliolumbar artery alone, which contained the skin and superficial fascia (**Figure 1C**). Bilateral flaps were orthotopically sutured onto the donor site using 4-0 absorbable nylon sutures (Pudong Jinhuan Medical Products, Co., Ltd., Shanghai, China). Cardboard collars were placed on the animals after the operation, and the animals were given 100,000 units of intramuscular penicillin G (Wende Pharmaceutical) for three consecutive days.

Flap gross pathology

A blinded pathological technician was assigned to examine the viability of the flap including color, elasticity, texture, ulceration and necrosis at a daily interval during PODs 1-7. On POD seven, gross flap pathology was documented using a digital camera (Aigo, Beijing, China) equipped with an image processing software, Image-Pro® Plus version 6.0 (Media Cybernetics, Rockville, MD, USA), for quantitation of the surviving flap area; and was expressed as the percentage of the total flap area. All measurements were performed independently, in triplicate.

Flap perfusion laser Doppler imaging scan

On POD seven, animals were anesthetized and maintained in a 24°C temperature environment. Flap perfusion was evaluated using a blood perfusion monitor (PeriFlux System 5000; PERIMED, Järfälla, Sweden) starting at 60 minutes after the stabilization. Primary and secondary choke vessel areas were scanned for one minute, and flap blood flow was quantitated in perfusion units. All measurements were performed independently, in triplicate.

Flap contrast angiography

The animals were euthanized using intraperitoneal injection of 10% chloral hydrate (Wende) and perfused with lead oxide-gelatine (SH Ch-

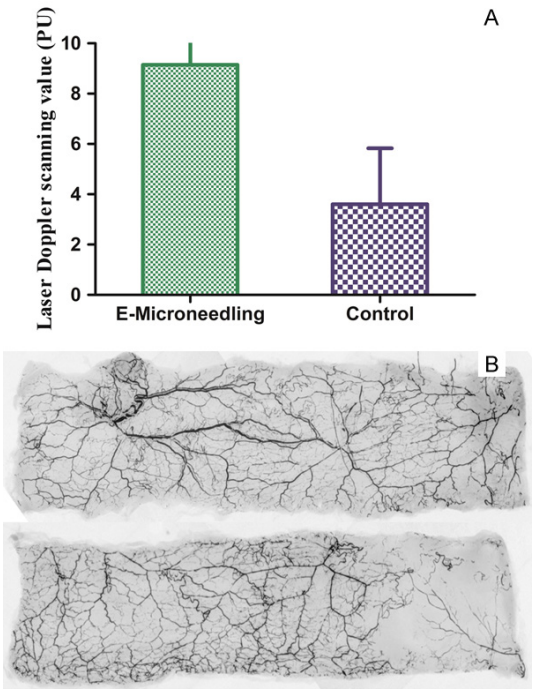

Figure 3. Flap perfusion Doppler imaging scan and angiography: A: Bar chart of the flap perfusion quantitation expressed in perfusion units (mean ± SD, *n* = 20); B: Representative angiographs of the experimental flap (upper panel) and control flap (lower panel).

emical Co., Ltd., Shanghai, China), as previously described [13]. The flap was mounted flat for computed radiography (voltage = 40 kV, amplitude = 5 mA, and capture time = 0.1 sec; Fujifilm, Tokyo, Japan), and special focus was given on choke vessels adjacently connected to the angiosomes. All measurements were performed independently, in triplicate.

Microvessel histology

Choke vessel tissues were harvested for histological examination. Specimens were fashioned into 1 × 1 cm pieces, fixed in 10% paraformaldehyde for 24 hours, embedded in paraffin, sectioned into 4-μm slices, and prepared for hematoxylin and eosin staining. The number of microvessels per unit area (mm^2) was counted using a light microscope (Leica Microsystems, Wetzlar, Germany) to represent flap microvascular density [14]. All measurements were performed independently, in triplicate.

Vascular endothelial growth factor measurement

Vascular endothelial growth factor (VEGF) was quantitated using western blotting assay to examine flap neoangiogenesis [15]. For total cellular protein extraction, dorsal midline cutaneous tissues were lysed using radioimmunoprecipitation assay lysis buffer (Santa Cruz Biotechnology, Dallas, Texas, USA) containing 50-mM Tris hydrochloride (pH = 7.4), 150-mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 1-mM of EDTA, 1-mg/mL leupeptin, 2.5-mM sodium orthovanadate and 1-mg/mL aprotinin. Specimens were homogenized and centrifuged at 4°C and at 12,000 g for 10 minutes. The supernatant protein concentration was measured using Bradford reagent (Sigma-Aldrich, St Louis, MO, USA). Proteins were electrophoresed on a sodium dodecyl sulphate-polyacrylamide gel (Sigma-Aldrich) and transferred onto a polyvinylidene fluoride membrane (Roche Applied Science, Indianapolis, IN, USA). Protein bands were sequentially incubated with polyclonal rabbit-anti-rat primary antibodies (Santa Cruz) against VEGF (working dilution, 1:500) and β-actin (internal control; 1:500), and probed with a goat-anti-rabbit secondary antibody. Antibody-binding bands were detected using an enhanced chemiluminescence detection system and enhanced chemiluminescence plus reagent (Thermo Fisher Scientific, Rockford, IL, USA). VEGF band intensity was measured as a fold change relative to that of β-actin using Quantity One® version 4.6.2 software (Bio-Rad Laboratories, Hercules, California, USA). All measurements were performed independently, in triplicate.

Statistical analysis

SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All continuous data are expressed as mean ± SD, and the means were compared using the paired Student-*t* test or one-way repeated measures analysis of variance. A two-tailed *P*-value < 0.05 was considered statistically significant.

Results

On POD one, the experimental flaps exhibited a slightly dark and swelling appearance on the margins nourished by the thoracodorsal artery; however, these areas were relatively less marked compared to that in the control flaps. On POD five, the experimental flaps exhibited a superficial epidermal loss with a preserved smooth and soft dermis; while the control flaps

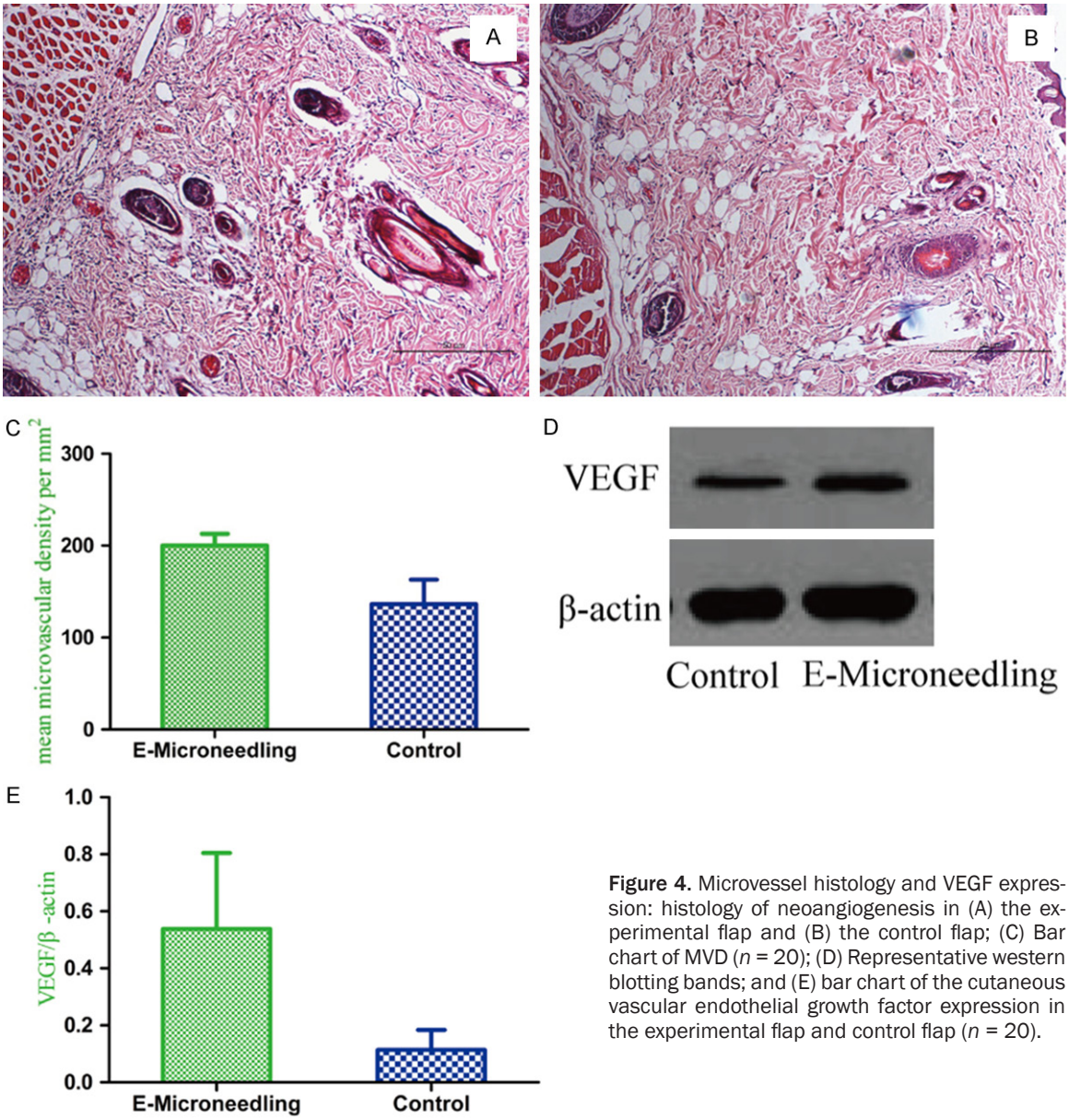

Figure 4. Microvessel histology and VEGF expression: histology of neoangiogenesis in (A) the experimental flap and (B) the control flap; (C) Bar chart of MVD (n = 20); (D) Representative western blotting bands; and (E) bar chart of the cutaneous vascular endothelial growth factor expression in the experimental flap and control flap (n = 20).

had a complete necrosis on the distal margin. Furthermore, the experimental flaps had a significantly larger survival area compared to the control flaps on POD seven (88.2 ± 2.5% *vs.* 78.3 ± 4.1%, P = 0.04; **Figure 2A** and **2B**).

On POD seven, the experimental flaps exhibited significantly greater perfusion to the secondary choke vessel area compared with the control flaps (9.1 ± 1.7 *vs.* 3.6 ± 0.9 units, P = 0.02; **Figure 3A**). On angiography, both the experimental and control flaps had vessels paralleling to the flap axis and dilated choke vessels. In the experimental flaps, choke vessels that were connected to the thoracodorsal and intercostal angiosomes became significantly more dilated with a greater number of collateral anastomoses (**Figure 3B**, upper panel), as compared to the control flaps (lower panel).

On histology, the experimental flaps had a significantly higher MVD in the choke vessel area than in the control flaps (200.2 ± 3.8 *vs.* 136.4 ± 8.1 per mm^2, P < 0.001; **Figure 4A-C**). The experimental flaps also had a significantly higher relative expression level of VEGF than in the control flaps (0.54 ± 0.09 *vs.* 0.11 ± 0.02 fold, P = 0.001; **Figure 4D** and **4E**).

Discussion

The use of a pedicled perforator flap is an effective treatment modality for reconstructing local soft tissue defects, but this technique is subject to a risk of flap devitalization due to compromised flap hemodynamics [16, 17]. Skin microneedling has been widely used in cosmetic surgery practice [18] such as acne scar removal [8], skin rejuvenation [4], hair implantation [19] and facial cosmesis [6, 20]. In conventional flap microneedling, a roller device is normally used, which may result in aggressive pain and excessive bleeding. Moreover, the penetration depth of the roller microneedle cannot be adjusted to the size of the recipient site and the flap. In our study, the use of electrical microneedling allowed a flexible penetration depth and vibration frequency. To the best of our knowledge, this study was the first to report on the experimental use of electrical microneedling for the perioperative conditioning of the perforator flap. This preconditioning technique resulted in improved flap survival and blood perfusion through a multifactorial mechanism, including dilated choke vessels and increased neoangiogenesis.

Choke vessels are reduced-caliber vessels that anastomose adjacent angiosomes of the skin. These vessels may become obviously dilated, which results in the formation of more collateral anastomoses after flap harvesting [16]. Our results revealed that choke vessels in the experimental flaps, compared to that in the control flaps, became enlarged in caliber after perforator flap transfer along with significantly greater dilation. As a result, more collateral anastomoses formed among the three angiosomes of the perforator flap after microneedling. This effect was consistent with better flap blood perfusion and the consequent improvement in flap survivability.

Increased neoangiogenesis has been thought to be the primary mechanism of microneedling for improving flap perfusion and survival. Microneedling causes extensive but mild injuries to both the epidermal and dermal layers [4-6, 8, 18-21]. These clinically unharmful injuries induce the synthesis of subcutaneous collagens and the release of angiogenic cytokines, such as VEGF, fibroblast growth factor, epidermal growth factor and angiogenin [6, 22]. Among these proangiogenic factors, VEGF, a potent endogenous stimulus of angiogenesis essential for neovascualrization [23, 24], has been widely expressed in maturing blood vessels [25]; and VEGF receptors are exclusively expressed in endothelial cells [26]. In addition, VEGF can result in vasodilation partially through the stimulation of nitric oxide synthase production in endothelial cells, and also attract cell migration and inhibit cell apoptosis [27]. Upregulated VEGF expression has been confirmed to be associated with neovascualrization and improved blood perfusion [15, 16]. Our results revealed that these experimental flaps had significantly higher MVD and relative VEGF expression levels in the choke vessel area than in the control flaps. These effects could result from the microneedling-induced release of VEGF and other proangiogenic factors, which consequently increased neoangiogenesis and blood perfusion, and improved the survivability of the pedicled perforator flap. Another potential benefit of microneedling was the improvement of venous backflows through the large number of mild skin bleeding injuries. This benefit remains to be validated in further experimental studies.

In conclusion, our experimental animal study results have demonstrated that preconditioning and conditioning with electrical microneedling could improve the survival of the pedicled perforator flap. This therapeutic benefit resulted from improved blood perfusion and increased neoangiogenesis. The upregulated cutaneous expression of VEGF might be the major molecular factor underlying the microneedling-induced therapeutic effect. This therapeutic effect needs to be further validated in large-scale, randomized controlled trials.

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Disclosure of conflict of interest

None.

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