Supercharging Strategies for Prefabricated Flaps in a Rat Model

Heng Xu, MD[1],[*]   Zvi Steinberger, MD[2],[*]   Yan Wo, MD, PhD[3]   Ke Li, MD[1]   Chenghung Kuo, MD[1]
Yun Tong, MD[4]   Yixin Zhang, MD, PhD[1]

[1] Department of Plastic Surgery, Ninth People's Hospital, Shanghai
Jiao Tong University School of Medicine, Shanghai, China
[2] Department of Orthopaedic Surgery, Sheba Medical Center,
Tel Hashomer, Israel
[3] Department of Human Anatomy, Histology and Embryology,
Shanghai Jiao Tong University School of Medicine, Shanghai, China
[4] Department of Plastic and Cosmetic surgery, Jinhua Central
Hospital, Jinhua, Zhejiang, China

Address for correspondence Yun Tong, MD, Department of Plastic
and Cosmetic surgery, Jinhua Central Hospital, Jinhua, Zhejiang, China
(e-mail: yun960@163.com).

J Reconstr Microsurg 2019;35:568–574.

Abstract

Background   Limited survival area is an intractable problem in the clinical practice of prefabricated flaps. This study compared four strategies to find an effective method and to understand the potential mechanisms for supercharging.

Methods   A prefabricated abdominal flap rodent model was prepared. Rats were randomly divided into five groups ($n = 6$/group). (A) Control group: prefabricated right side femoral vessels. Based on group A, various prefabricated vessels were added; (B) proximal venous supercharging group: right side superficial inferior epigastric vein (SIEV); (C) proximal arterial supercharging group: right side superficial inferior epigastric artery (SIEA); (D) distal venous supercharging group: left side SIEV; and (E) distal arterial supercharging group: left side SIEA. Macroscopic analysis, near-infrared fluorescence imaging, and microscopy were used to analyze the survival area, fluorescence area, and capillary density.

Results   No significant differences in survival areas were found among supercharging groups (B–E), which were larger than in the control group. Near-infrared fluorescence imaging showed the areas of control and venous supercharging groups (A, B, and D) were smaller than in arterial groups (C and E). Capillary density areas in the right part of the flap in proximal supercharging groups (B and C) and left part of the flap in distal supercharging groups (D and E) were all greater than group A, with no significant differences among the other groups.

Conclusion   Enhanced neovascularization is a useful supercharging strategy. Both arterial and venous vessel supercharging improved the survival area of prefabricated flaps.

Keywords
► prefabricated flaps
► microcirculation
► supercharging

A new technique named prefabricated flap was introduced by Yao[1] in 1980. By implanting a vascular carrier into a random skin area, the patch of skin can be converted after an extended period, when the neovascularization is complete and used as an axial flap based on the newly-introduced vascular carrier.[2]

Previously, we reported that a prefabricated flap would be a good option when faced with a specific defect, such as a cheek skin defect/scar, to achieve similar color as the surrounding tissue, as well as contour restoration which leads to high patient satisfaction.[3] However, Pribaz et al demonstrated that harvesting large prefabricated flaps could lead to partial or complete flap necrosis.[4] Although numerous strategies have been developed to enhance prefabricated flap survival, for

---

example, using angiogenic growth factors or pre-expansion, the intractable problem of limited survival area is still awaiting a more straightforward solution.

As an easy-to-approach method used in the clinic, the typical supercharging technique has been shown to be effective in improving the survival area of axial flaps by enhancing the flap blood supply—both arterial perfusion and venous drainage. Li et al demonstrated that it was also valid for prefabricated flaps.[5] Based on these, we suggested understanding the mechanisms of supercharging used in prefabricated flaps might be helpful for translating this methodology into clinical practice.

By designing and comparing the effectiveness of four different supercharging strategies (proximal vs. distal and arterial vs. venous), this study discusses the potential mechanisms, whereby a supercharging strategy was used in prefabricated flaps. We believe that the conclusion might be used as a technical reference to enable surgeons to perform supercharging prefabricated flaps with confidence.

Methods

Animal and Anesthesia

All animal care complied with the Ethics Committee regulations of Shanghai Jiao Tong University, Shanghai, China. Thirty male Sprague-Dawley (SD) rats weighing 250 to 300 g were used. Chloral hydrate (4%, 8 mL/kg, IP) was used for anesthesia. All surgeries were performed under sterile conditions.

Rat Abdominal Prefabricated Flap Model

First Stage

After prepping the surgical area and marking the flap boundaries as follows: extended from the inferior margin of the costal arch cranially to the anterior superior iliac spines caudally and to the anterior axillary lines, bilaterally, the vessels were fixed underlying the abdominal flap. Vessels derived from the left side were fixed at one-fourth of the diagonal line (from bottom left to upper right) of the rectangular flap, and vessels derived from the right side were fixed at one-fourth of the diagonal line (from bottom right to upper left) of the rectangular flaps. Incision pattern and vessel fixation position were marked with methylene blue dye (►Fig. 1A).

A longitudinal incision was used to explore and isolate the femoral vessels, the ends of which were ligated. The inferior flap border of the abdominal flap was incised. The vessels underneath the panniculus carnosus were dissected next, and the superficial inferior epigastric vessels were resected bilaterally. The distal ligated ends of the femoral vessels were
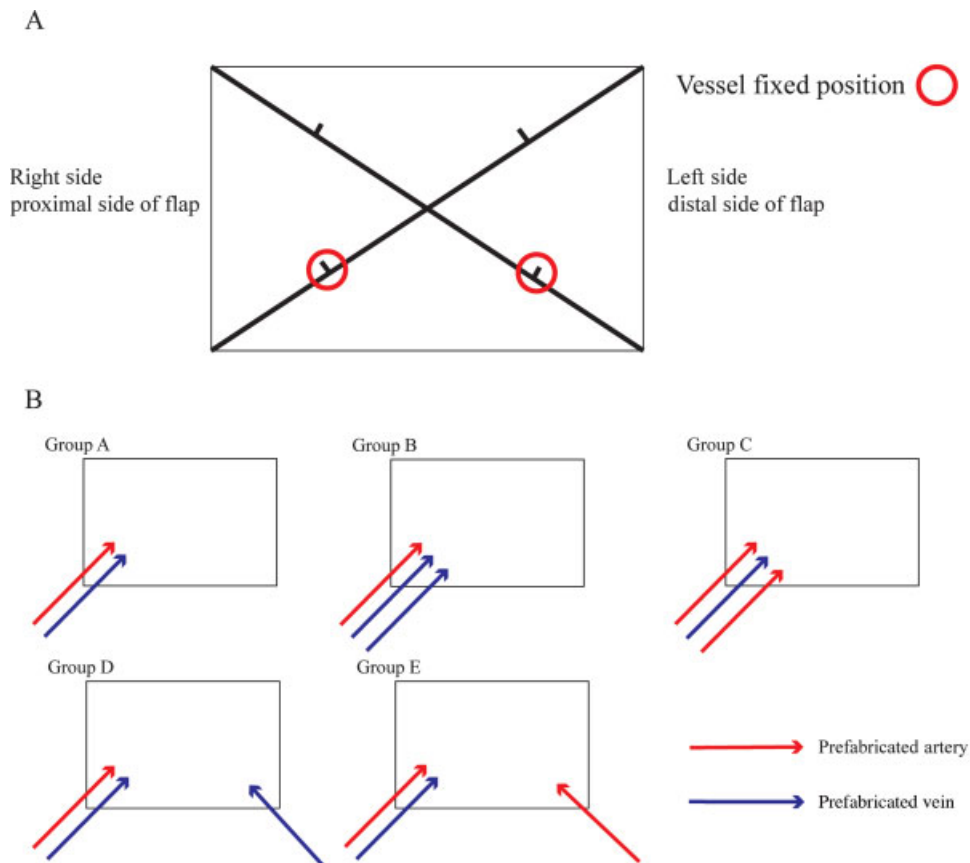

Fig. 1 Schematic drawing of model set-up and experimental flaps for groups A–E. (**A**) The right side of flap was designated as proximal and the left side as distal. Draw two diagonal lines in the rectangle flap from corner to corner. Each is divided into four equal lengths (gray circle: fixed vessel position). Right femoral and superficial inferior epigastric vessels are fixed on the right side, corresponding to the right gray circle; and left femoral and superficial inferior epigastric vessels are fixed on the left side. (**B**) Diagram of experimental designs of groups A–E. Prefabricated artery/vein refers to femoral and/or superficial inferior epigastric artery/vein.
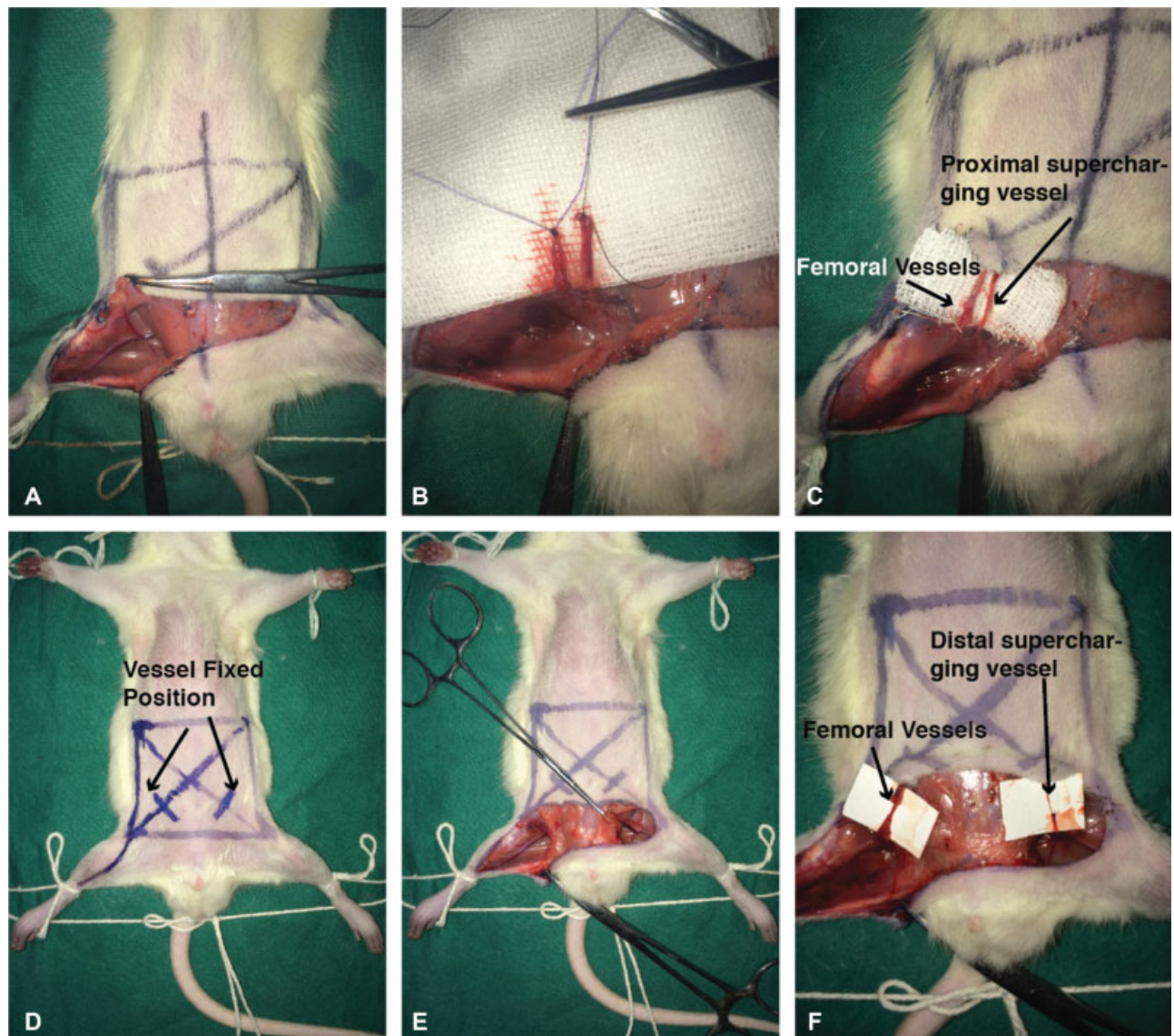
Fig. 2 Surgical set-up for the model. (**A**) Expose proximal side (right side) vessels. (**B**) Isolate femoral and supercharging vessel. (**C**) Fixed femoral vessels and proximal supercharging vessel underneath flap. (**D**) Mark the vessel fixed position. (**E**) Expose proximal and distal sides (bilateral sides) vessels. (**F**) Fixed femoral vessels and distal supercharging vessel underneath flap.

fixed to the panniculus carnosus, corresponding to the group designation and skin markings, and the incision was sutured closed (►**Fig. 2**).

Second Stage
The neovascularized vessels were allowed to grow for 2 weeks. Then, the skin flap was dissected and raised based on the implanted femoral vessels and sutured back in place.

Groups
The rats were then randomly divided into five groups (six rats per group). All vessels involved were fixed at the same position at one-fourth of the diagonal line of the rectangular flap (right or left side).

(A) Control group: prefabricated femoral artery and vein (right side; control group); (B) proximal venous supercharging group: prefabricated right side femoral artery, vein, and superficial inferior epigastric vein (right side; venous proximal [VP] group); (C) proximal arterial supercharging group: prefabricated femoral artery, vein, and superficial inferior epigastric artery (right side; arterial proximal [AP] group); (D) distal venous supercharging group: prefabricated femoral artery, vein (right side), and superficial inferior epigastric vein (left side; venous distal [VD] group); (E) distal arterial supercharging group: prefabricated femoral artery, vein (right side), and superficial inferior epigastric artery (left side; arterial distal [AD] group; ►**Fig. 1B**).

Survival Area Assessment
Seven days after the second-stage surgery, all flaps were photographed and assessed by three blinded investigators, who marked the borders of viable areas. Image Pro Plus software (6.0, Media Cybernetics, Inc., CA) was used to calculate the ratio (%) between the viable areas and the entire flap.[6]

Near-Infrared Fluorescence Imaging

During the second part of the experiment, after dissecting and raising the flap, indocyanine green (ICG; Dandong Yichuang Pharmaceutical Co., Ltd., Liaoning, China) was injected (0.08 mL/kg, dissolved in saline to a ratio of 2.5 mg/mL) through the caudal vein. After injection, fluorescent images of blood perfusion of the abdominal flap were obtained using an infrared camera system (Photodynamic Eye; Hamamatsu Photonics K. K., Hamamatsu, Japan). Fluorescent images were recorded in real time with photographs and videos.[6]

Histological Assessment of Capillary Densities

After all flaps were photographed, the rats were euthanized humanely using narcotic overdose. Prefabricated flaps were then harvested and fixed with 4% paraformaldehyde for 24 hours. For evaluation, some tissues were embedded in paraffin and sectioned. The sectioned specimens were processed by conventional hematoxylin and eosin staining, and CD31 immunohistochemical staining. Two blinded investigators assessed vessel densities by counting the number of CD31 positive vessels in three randomly chosen fields of each slide (at ×40 magnification). Hematoxylin and eosin, CD31, staining were photographed at ×10 magnification.[6]

Statistical Analysis

The data were analyzed using GraphPad Prism software (6.0, GraphPad software Inc.., MD). One-way analysis of variance (ANOVA) was used to evaluate survival percentages and vessel densities across groups A–G, Tukey's multiple comparison tests were used to compare every two groups afterward. The Kruskal–Wallis H-test was used for data that were not normally distributed, after which the Dunn's test was performed. A p-value of <0.05 was considered significant.[6]

Results

Survival Area Assessment

Based on one-way ANOVA and Tukey's multiple comparison tests, the survival areas of supercharging groups (B–E) were significantly larger than that of control group (A). However, no difference was found between every two groups among super-
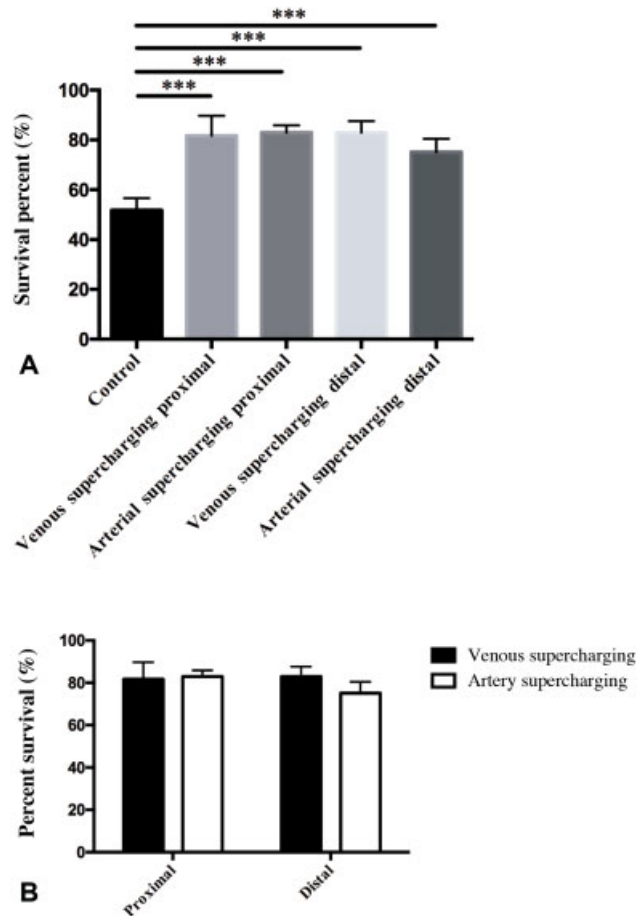

Fig. 4 (A) Bar graph comparing the percent survival among five groups (one-way ANOVA; Tukey's multiple comparison test). (B) Bar graph comparing the percent survival among groups B–E above (two-way ANOVA). A, artery; V, vein.

charging groups (►Fig. 3A–E, ►Fig. 4A, ►Table 1). Using two-way ANOVA for supercharging groups (B–E; treating the arterial and venous superchargings as row factors, and proximal and distal superchargings as column factors), venous supercharging improved the survival area by 7.52% ($p = 0.16$), and proximal supercharging improved the survival area by 7.24% ($p = 0.17$, ►Fig. 4B, ►Table 2), both of which were not significant.
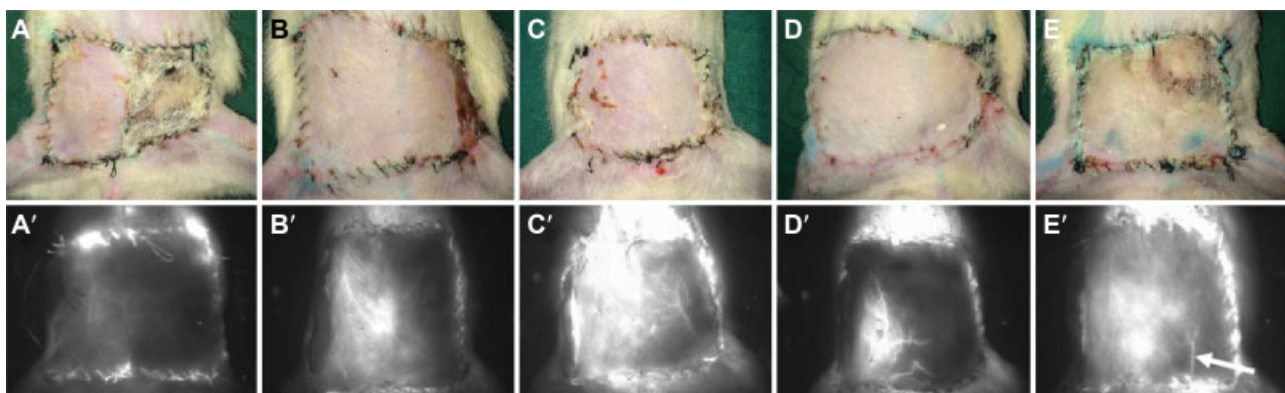

Fig. 3 The pictures present survival area (A–E). (A) Prefabricated control group; (B) venous supercharging (proximal) group; (C) arterial supercharging (proximal) group; (D) venous supercharging (distal) group; (E) arterial supercharging (distal) group. (A′–E′) Near-infrared fluorescence imaging counterparts; arrow: left arterial source.

Table 1 One-way ANOVA analysis of the five groups

Groups		Percent survival of flap (mean ± SD)	95% CI
A	Control	51.82 ± 4.80	(46.78, 56.85)
B	V proximal	81.68 ± 8.03	(73.24, 90.11)
C	A proximal	82.92 ± 2.96	(79.81, 86.03)
D	V distal	82.98 ± 4.52	(78.24, 87.72)
E	A distal	75.16 ± 5.30	(69.60, 80.72)

Abbreviations: A, arterial; ANOVA, analysis of variance; CI, confidence interval; SD, standard deviation; V, venous.
Note: Homogeneity of variance test: Bartlett's test $p = 0.4520$. Thus, both of these tests confirm the homogeneity of data to utilize one-way ANOVA analysis in proper.

Near-Infrared Fluorescence Imaging

The total success rate of near-infrared fluorescence imaging was 40.0% (12/30). No difference in appearance and survival were observed in the flaps that failed to image. The specific reason for the low rate of success is unknown but might be explained by transient vasospasm.

Using the near-infrared fluorescence imaging in control and VP, AP groups (A–C), the contrast agent flowed proximal to distal, intensity became stronger, and then gradually vanished. The outer boundaries appeared twig-like, and the fluorescence intensity was not homogenous. In VD group (D), the contrast agent flowed from the proximal to the distal side and merely appeared as a single fluorescent line. AD group (E) was similar to control and VP, AP groups, except in the distal section, the contrast agent appeared from another source to flow proximally and fuse with the proximal fluorescent region.

The area of fluorescence was not entirely consistent with the macroscopic assessment. As the fluorescence intensity observed in this study was not homogenous, we decided to compare the fluorescence area instead of fluorescence intensity. Because the number of successful near-infrared fluorescence images was insufficient for statistical evaluation, a qualitative visual analysis of fluorescence area was performed. The fluorescence areas of control, VP, VD groups (A, B, D) were smaller than those of AP, AD groups (C, E). Moreover, control and AP, AD groups (A, C, E) were consistent with the macroscopic assessment, while VP, VD groups (B, D) were assessed as smaller than the actual flap survival area (►Fig. 3A–E).

Histological Assessment of Capillary Densities

Each slice included epithelium, hair follicle, connective tissue, and vessels. No significant difference in vessel caliber was found among the five groups (►Fig. 5A–E). All skin capillary densities were calculated using CD31 staining (►Fig. 6). We calculated capillary densities under ×40 magnification and used Kruskal–Wallis H-test and Dunn's test to analyze the data because they were not normally distributed.

The samples harvested from the right flap, which corresponded to the rightside (proximal side) vessel fixed position, indicated that capillary densities in VP, AP groups (B, C) were higher than those in control group (A), while VD, AD groups (D, E) did not differ significantly from control group (►Fig. 6A–E). The samples from groups A to E harvested from the left part (distal part) flap, which corresponded to the left side vessel fixed position, showed that VD, AD groups (D, E) were larger than the other three groups (A, B, C; ►Fig. 5, ►Fig. 6A′–E′ and ►Table 3).

Discussion

Prefabricated flaps have an essential role in reconstructive surgery. However, achieving a larger and more predictable survival area is still a problem in clinical practice. As an example of ongoing research efforts, supercharging might be able to overcome these problems, which have been demonstrated in axial flaps.[7,8] In this study, we aimed to achieve a better understanding of the effects of different supercharging strategies on the survival area of prefabricated flaps, to find an effective supercharging method.

We choose to designate the right side of the flap as proximal and the left side as distal. The superficial inferior epigastric vein or artery from both right and left sides was used as the supercharging vessel to set-up four experimental groups in comparison with the control group, which was prefabricated with the right femoral vessels. The survival areas with supercharging groups (B–E) were significantly larger than that of the control group. There was no difference between arterial and venous or proximal and distal supercharging. These results indicate that supercharging is a useful strategy in promoting the survival of prefabricated flaps.

Near-infrared fluorescence imaging showed that the fluorescence area was not consistent with the macroscopic

Table 2 Two-way ANOVA analysis of the groups B–E

Column row	Venous supercharging		Arterial supercharging		
	Percent survival of flap (mean)	SD	Percent survival of flap (mean)	SD	
Proximal	81.68	8.03	82.92	2.96	
Distal	82.98	4.62	75.16	5.30	
			Effect in the total variance		
	DF	SS	MS	Simple effect (%)	F-Value	p-Value	Significance
Column factor	1.0	64.94	64.94	7.52	2.12	0.16	None
Row factor	1.0	62.60	62.60	7.24	2.04	0.1700	None

Abbreviations: ANOVA, analysis of variance; DF, degree of freedom; MS, mean square; SD, standard deviation; SS, sum of square.
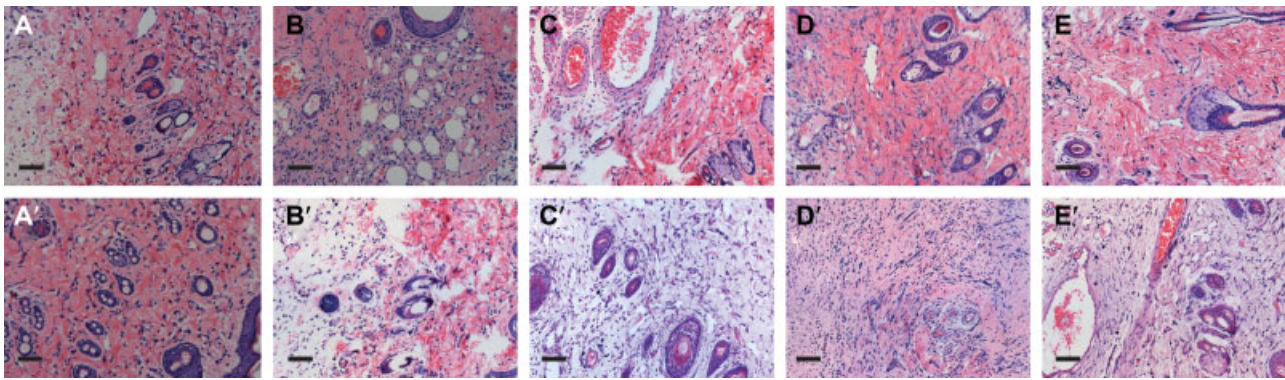
Fig. 5 Microscopic views of different regions of the prefabricated flaps (H&E staining). (**A–E**) are the photos of groups A–E (samples harvested from right side). (**A'–E'**) Photos of groups A'–E'(samples harvested from the left side); scale bar is 100 μm. H&E, hematoxylin and eosin.
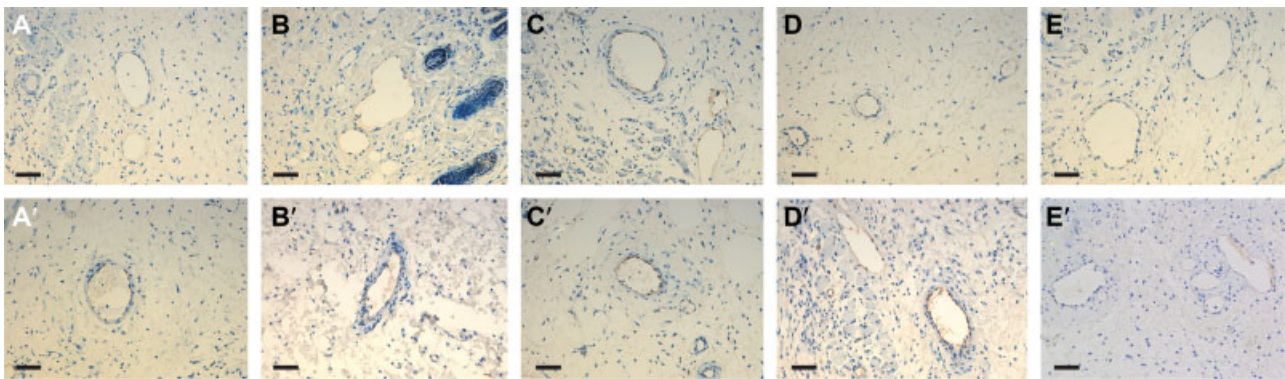

Fig. 6 Microscopic views of different areas of prefabricated flaps (IHC-CD31 staining). (**A–E** Photos of groupsA–E (samples harvested from right side). **A'–E'** are the photos of groups A'–E' (samples harvested from the left side); scale bar is 100 μm. CD31: Cluster of differentiation 31; IHC: immunohistochemistry.

Table 3 Capillary densities of the groups A–E

Group	Capillary density (mean ± SD)	Kruskal–Wallis (Dunn's test)	
		Comparison	p-Value
A	1.43 ± 1.07	Group A vs. group B	0.01
B	5.33 ± 2.73	Group A vs. group C	0.03
C	4.71 ± 2.56	Group A vs. group D	0.66
D	2.78 ± 1.48	Group A vs. group E	0.77
E	2.70 ± 1.64		
A'	1.00 ± 1.07	Group A' vs. group B'	>0.99
B'	0.90 ± 0.88	Group A' vs. group C	>0.99
C'	1.43 ± 1.27	Group A' vs. group D'	0.02
D'	3.00 ± 1.23	Group A' vs. group E'	0.03
E'	3.08 ± 1.71		

Abbreviation: SD, standard deviation.
Note: Homogeneity of variance tests has significant differences, so using the Kruskal–Wallis test is proper. Dunn's test is used for multiple comparisons. In Groups A–E, samples are harvested from the right side. Groups A'–E' are samples harvested from the left side.

assessment, although it is well-known that near-infrared fluorescence is not an objective tool for mapping the flap perfusion precisely. Venous supercharging is supposed to improve flap survival area by increasing venous drainage.[9] Venous supercharging strategies improved flap survival area but had rela-

tively smaller fluorescence area. This might indicate stronger venous drainage in venous supercharging strategy. In addition, larger areas of fluorescence were observed in arterial supercharging groups. Chang et al reported that insufficient arterial perfusion contributed to flap necrosis, and that arterial supercharging provided enough blood to nourish the flap which agree with our findings.[8]

In histological analysis, supercharging resulted in stronger vessel intensity than in the control group in both the right and left sides of the flaps. Thus, supercharging strategy is indispensable for enhancing neovascularization, which contributes to improved flap survival area. Though Guoand Pribaz showed that efficiency of the neovascularization increased with increased perfusion pressure,[10] which indicated arterial supercharging strategy, resulted in larger survival area. Limited flap size might be the reason we failed to demonstrate this point as we did previously.[11] However, this requires further investigation.

It is hypothesized that high-to-low "capacitance" flow system can force inflow to the flaps which improves to their survival.[7,12] Enhancing the perfusion and drainage capacities using arterial and venous supercharging seems reasonable. Based on previous research, we found that prefabricated vessels have relatively insufficient perfusion and drainage abilities. We also hypothesized that the supercharging strategy worked because of the suboptimal pressure gradient between the flap's artery and vein (ΔP_{A-V}).[11] Sasaki et al[13] showed that

increased flow capacity could increase flap survival area. Therefore, increasing the pressure gradient relying on arterial perfusion and/or venous drainage might also work in prefabricated flaps.

Additionally, we have to acknowledge that visual assessment is still the best tool for estimating the extent of flap survival. Thus, developing more direct tools to measure the microscopic perfusion of the flap would be worthwhile. In addition, given that single arterial or venous supercharging is used clinically much more often, we did not devise a supercharging group with both artery and vein. It is also important to note that the delay process is inherent to prefabricated flaps; therefore, its protective effects on flap survival are not negligible. These considerations could optimize the outcomes of prefabricated flaps further. Finally, the explanation for our conclusion is hypothetical and further study is needed to determine the exact mechanisms.

Conclusion

In this study, we found that using either an artery or vein as the supercharging vessel on the proximal or distal side (relative to the vascular carrier) resulted in similar improvements in the survival area of prefabricated flaps. Increased vessel intensity led to enhanced neovascularization which is the advantage of the supercharging strategy.

Conflict of Interest
The authors have declared no conflict of interests.

References

1 Yao ST. Vascular implantation into skin flap: experimental study and clinical application: a preliminary report. Plast Reconstr Surg 1981;68(03):404–410
2 Li JN, Hu Y. A review of the experimental study and clinical application of the prefabricated flap. Eur J Plast Surg 2001;24:271–274
3 Lazzeri D, Su W, Qian Y, et al. Prefabricated neck expanded skin flap with the superficial temporal vessels for facial resurfacing. J Reconstr Microsurg 2013;29(04):255–262
4 Pribaz JJ, Fine N, Orgill DP. Flap prefabrication in the head and neck: a 10-year experience. Plast Reconstr Surg 1999;103(03):808–820
5 Li GS, Zan T, Li QF, et al. Internal mammary artery perforator-supercharged prefabricated cervicothoracic flap for face and neck reconstruction. Ann Plast Surg 2015;75(01):29–33
6 Xu H, Zhang Z, Tao W, et al. Ex vivo delay: A novel approach to increase prefabricated flaps survival rate. J Reconstr Microsurg 2016;32(08):632–638
7 Chang H, Minn KW, Imanishi N, Minabe T, Nakajima H. Effect of venous superdrainage on a four-territory skin flap survival in rats. Plast Reconstr Surg 2007;119(07):2046–2051
8 Chang H, Nobuaki I, Minabe T, Nakajima H. Comparison of three different supercharging procedures in a rat skin flap model. Plast Reconstr Surg 2004;113(01):277–283
9 Chow SP, Chen DZ, Gu YD. The significance of venous drainage in free flap transfer. Plast Reconstr Surg 1993;91(04):713–715
10 Guo L, Pribaz JJ. Clinical flap prefabrication. Plast Reconstr Surg 2009;124(6, Suppl):e340–e350
11 Xu H, Feng S, Xia Y, et al. Prefabricated flaps: identification of microcirculation structure and supercharging technique improving survival area. J Reconstr Microsurg 2017;33(02):112–117
12 Hallock GG, Rice DC. Efficacy of venous supercharging of the deep inferior epigastric perforator flap in a rat model. Plast Reconstr Surg 2005;116(02):551–555, discussion 556
13 Sasaki GH, Pang CY. Hemodynamics and viability of acute neurovascular island skin flaps in rats. Plast Reconstr Surg 1980;65 (02):152–158