Supercharging Strategies for Prefabricated Flaps in a Rat Model

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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.

Keywords ► prefabricated flaps ► microcirculation ► supercharging

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

A new technique named prefabricated flap was introduced by Yao¹ 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

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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. 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

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**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.
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.
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 supercharging 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.
distal, intensity became stronger, and then gradually vanished. In VD group (D), the contrast agent appeared from another source to primarily and fuse with the proximal vessels. No difference in appearance and survival was found among the four experimental groups in proper. As 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).

### Table 1 One-way ANOVA analysis of the five groups

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

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).

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

<table>
<thead>
<tr>
<th>Column row</th>
<th>Venous supercharging</th>
<th>Arterial supercharging</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Percent survival of flap (mean ± SD)</td>
<td>SD</td>
</tr>
<tr>
<td>Proximal</td>
<td>81.68 ± 8.03 (73.24, 90.11)</td>
<td>2.96</td>
</tr>
<tr>
<td>Distal</td>
<td>82.98 ± 4.52 (78.24, 87.72)</td>
<td>7.24</td>
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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.

### 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. 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 3

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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.
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.¹ Venous supercharging strategies improved flap survival area but had relatively 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. 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 (ΔPₐᵥ).¹¹ Sasaki et al showed that

<table>
<thead>
<tr>
<th>Group</th>
<th>Capillary density (mean ± SD)</th>
<th>Kruskal–Wallis (Dunn’s test)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.43 ± 1.07</td>
<td>Group A vs. group B</td>
<td>0.01</td>
</tr>
<tr>
<td>B</td>
<td>5.33 ± 2.73</td>
<td>Group A vs. group C</td>
<td>0.03</td>
</tr>
<tr>
<td>C</td>
<td>4.71 ± 2.56</td>
<td>Group A vs. group D</td>
<td>0.66</td>
</tr>
<tr>
<td>D</td>
<td>2.78 ± 1.48</td>
<td>Group A vs. group E</td>
<td>0.77</td>
</tr>
<tr>
<td>E</td>
<td>2.70 ± 1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A’</td>
<td>1.00 ± 1.07</td>
<td>Group A’ vs. group B’</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>B’</td>
<td>0.90 ± 0.88</td>
<td>Group A’ vs. group C’</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>C’</td>
<td>1.43 ± 1.27</td>
<td>Group A’ vs. group D’</td>
<td>0.02</td>
</tr>
<tr>
<td>D’</td>
<td>3.00 ± 1.23</td>
<td>Group A’ vs. group E’</td>
<td>0.03</td>
</tr>
<tr>
<td>E’</td>
<td>3.08 ± 1.71</td>
<td></td>
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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.
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