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Submental Perforator Flap Design with a Near-Infrared Fluorescence Imaging System: The Relation between Number of Perforators, Flap Perfusion, and Venous Drainage

Aya Matsui, M.D.^{1,2,*}, Bernard T. Lee, M.D.^{3,*}, Joshua H. Winer, M.D.⁴, Rita G. Laurence, B.S.¹, and John V. Frangioni, M.D., Ph.D.^{1,5,†}

¹Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA

²Division of Cancer Diagnostics and Therapeutics, Hokkaido University Graduate School of Medicine, Sapporo, Japan

³Division of Plastic and Reconstructive Surgery, Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA

⁴Department of Surgery, Brigham & Women's Hospital, Boston, MA

⁵Department of Radiology, Beth Israel Deaconess Medical Center, Boston, MA

Abstract

Background—The submental flap is a reliable alternative to microsurgical reconstruction of facial deformities, providing an excellent cosmetic match with the contour and color of the face. In this study, we evaluated submental flap design by employing near-infrared (NIR) fluorescence angiography to identify perforator arteries (PAs). The impact of the number of preserved PAs on flap perfusion and venous drainage were quantified.

Methods—Indocyanine green was injected intravenously into n = 18 pigs. Three groups of 6 animals each had one, two, or three PAs preserved. The FLARE™ NIR fluorescence imaging system was employed for image acquisition. Images were recorded before and after flap creation, and every h, for 6 h. The time to maximum perfusion, the drainage ratio (an indicator of venous drainage), and the percentage of perfused flap area were analyzed statistically at each time point.

Results—Flaps with a single dominant PA had an initial mean perfused area of 80%, which improved to 97% at 6 h. For flaps with two and three preserved PAs, perfused area at 6 h was 99.8%

[†]**Corresponding Author:** John V. Frangioni, M.D., Ph.D., BIDMC, Room SL-B05, 330 Brookline Avenue, Boston, MA 02215, 617-667-0692 Fax: 617-667-0981, jfrangio@bidmc.harvard.edu.

*These authors contributed equally to this work.

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and 100%, respectively. A significant increase was observed in all three metrics as more vessels were preserved. Regardless of the number of PAs preserved, though, all three metrics improved over 6 h.

Conclusions—NIR fluorescence angiography can reliably identify submental PAs for flap design, and can be used to assess flap perfusion and venous drainage in real-time. Flap metrics at 6 h were equivalent when either one, or multiple PAs, were preserved.

Keywords

Submental perforator flap; near-infrared fluorescence; indocyanine green

INTRODUCTION

The submental flap has become a reliable option for the reconstruction of facial defects, providing an excellent cosmetic match with facial skin. The submental flap is also versatile, since the flap can be transferred to the upper half of the face as a pedicled flap without the need for complex microvascular procedures. Ideally, facial defects are reconstructed with flaps that are thin and pliable, while supplying sufficient mobility and coverage. The submental flap meets these criteria. Both clinical and anatomical evaluations have validated that one or two reliable perforating arteries (perforators) are present in the unilateral submental territory originating from the submental artery.¹ Questions remain, however, as to the number of perforators that must be preserved, and their impact on perfusion.

Currently, a submental flap is commonly raised as a conventional musculocutaneous flap incorporating the digastric muscle, as the pedicle is dissected off the mylohyoid muscle.² This standard technique requires meticulous dissection between the two muscles and ligation of several branching vessels, while the course of perforating vessels are safely protected surrounded by the digastric muscle. As previously reported, the location of the perforators are constant at the border of the anterior digastric belly, but not always symmetric.¹ Ishihara et al. described a small clinical experiment with submental perforator flaps where perforators were confirmed by preoperative Doppler examinations.³ The study suggested it was not necessary to include the muscle if the location of the perforator vessels was identified prior to the dissection.

We have previously described the use of an intraoperative near infrared (NIR) fluorescence imaging technique for patient-specific perforator flap design and postoperative flap monitoring.^{4,5} NIR wavelength ranging from 700 – 900 nm light take advantage of the “NIR window” where light absorption from blood and water (i.e., major light absorbers in the body) is minimal.⁶ Thus, NIR light can penetrate relatively deeply into subcutaneous tissue. We confirmed that the location of perforating vessels identified using NIR fluorescence was identical to the flap anatomy seen with both conventional X-ray angiography and surgical dissection.⁷

The technique we employ is similar to fluorescein angiography, although we use indocyanine green (ICG) as the fluorescent contrast agent. ICG has been used for medical purpose for more than 50 years with very few adverse reactions. It is rapidly eliminated from the blood by the liver with a half-life of 3 to 4 min. The limitations of fluorescein include a prolonged half-life (286 min) and dye leakage through the capillary. In contrast, more than 95% of ICG is bound to plasma proteins,⁸ minimizing leakage through the capillary walls, and providing dynamic, real-time assessment of perfusion. Because of its rapid clearance from the blood, additional ICG injections can be repeated every 10 min as needed.⁵ Fluorescence emission of ICG is at approximately 800 nm, in the middle of the NIR window, while fluorescein emission peaks at 525 nm, which is highly absorbed and scattered by blood. Fluorescein angiography requires darkness during the procedure and the use of an ultraviolet Woods light ($\lambda = 315\text{--}400\text{ nm}$),

while in NIR fluorescence imaging, white light ($\lambda = 400\text{--}650\text{ nm}$) can continue to illuminate the surgical field simultaneously with the invisible NIR light ($\lambda \approx 760\text{ nm}$) necessary for NIR fluorophore excitation.

We hypothesized that the perfusion to submental flaps could be measured prior to flap elevation using NIR fluorescence angiography, and could be followed reliably over time. In this study, we quantify flap perfusion area, venous drainage, and the time to achieve peak perfusion in submental flaps as a function of the number of perforators that are preserved.

MATERIALS AND METHODS

NIR Fluorescence Imaging System

The FLARE™ (Fluorescence-Assisted Resection and Exploration) imaging system has been described in detail previously by our laboratory.^{9–11} The most recent version (Troyan et al., manuscript in review) permits positioning of the custom optics anywhere in three-dimensional space while maintaining a working distance of 45 cm between the optics and the surgical field. White (400–650 nm) light and NIR fluorescence excitation (745–779 nm) light are generated by filtered light emitting diodes (LED) over a 15-cm field-of-view.¹⁰ Color video (i.e., surgical anatomy) and NIR fluorescence (i.e., fluorophore distribution) images are obtained simultaneously and in real-time. After computer-controlled image acquisition via custom software, color video and NIR fluorescence images can be displayed individually and/or merged. The merged image consists of a grayscale NIR fluorescence image pseudo-colored in a visible color (chosen from a palette of over 256 colors) and overlaid on top of the color video image. All images are refreshed up to 15 times per second.

Animals

Animals were studied under the supervision of an approved institutional protocol. Female Yorkshire pigs (E.M. Parsons and Sons, Hadley, MA; body weight: 31.8–40.2 kg, mean: 36.9 kg) were induced with 4.4 mg/kg intramuscular Telazol™ (Fort Dodge Labs, Fort Dodge, IA), intubated, and maintained with 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL). ECG, heart rate, oxygen saturation, and body temperature were monitored during experiments.

Perforator Flap Design using NIR Fluorescence Angiography

NIR fluorescence angiography was performed in $n = 18$ pigs by injecting 2.5 mg (0.06–0.07 mg/kg) of clinical grade indocyanine green (ICG; Akorn, Decatur, IL) diluted in 10 ml saline at the time points indicated. Each injection was via rapid bolus into a central venous line in the femoral vein. Images were acquired at a 67-msec exposure every 500 msec for the first 1 min, every 1 sec for the next 1 min, and every 1 min from 3 to 10 min post-injection. After identification of skin perforators using the initial injection, an 8×8 cm elliptical submental flap was designed, which encompassed the selected perforator(s) as shown in Figure 1 (top row). Animals were divided into 3 groups depending on the number of perforators (Table 1). Details of selected perforators were recorded in terms of the location and the dominance as detailed in Figure 2. Perforators were described as dominant if they had higher fluorescence intensity, and area perfused, than others in the same flap. Subsequently the flap was raised and another ICG injection was given to confirm flap perfusion (Figure 1, bottom row). Flaps were evaluated repeatedly for the purpose of tracking changes in perfusion and venous drainage every hour for 6 h. Multiple ICG injections were given at a minimal time interval of 10 min apart to reduce dose stacking.⁵ For histological evaluation, three samples were taken from each flap, including areas that by NIR fluorescence angiography were well-perfused, areas that were poorly perfused, and tissue outside of the flap for use as a control.

Quantitative Assessment of Flap Perfusion and Venous Drainage

All image acquisition and processing was performed via custom Lab-VIEW software (Lab-VIEW, National Instruments, Austin, TX). Quantitation of fluorescence intensity was described in detail previously.⁴ Briefly, a small circle was drawn as a region-of-interest (ROI) at a perforating artery (PA) identified in the NIR fluorescence image. Another small circle was made in the corner of the image outside of the flap to assess background (BG) fluorescence. Fluorescence intensity (FI) of the ROI and the background was quantified using 12-bit pixel intensity (range 0–4095). Contrast-to-background-ratio (CBR) was defined as (mean FI of ROI – mean FI of BG) / mean FI of BG. Of note, ROIs were defined at a set location for each flap through all subsequent injections. The CBR pattern for each injection was recorded graphically and analyzed. Maximum CBR ($I_{\max}$) and CBR at 2 min post-injection (I_{120}) were obtained in individual injections. The Drainage Ratio (DR) was evaluated to quantify venous drainage, defined as $DR = I_{120}/I_{\max} * 100$ (%). We had previously identified that a $DR > 85\%$ indicated venous congestion.⁴ Using Vision Assistant (National Instruments) software, the perfused area as a percentage of total flap area was quantified using the NIR fluorescence images. The analysis was performed for all conditions at the time point at which maximum perfusion was achieved, and maximum perfusion was set at 100% (Figure 3). The time to maximum perfusion and the DR were also quantified over the 6 h of the experiment.

Statistical Analysis

Either an unpaired Student t-test or analysis of variance (ANOVA) was employed to determine the statistical difference between two groups, or between multiple groups, respectively. Statistical significance was set at $p < 0.05$. The analyzed values were described as mean $\pm$ SEM.

RESULTS

NIR Fluorescence Angiography of Perforators

A single bolus injection of ICG provided reproducible identification of both dominant and non-dominant perforators in real-time (Figure 1 and Figure 2). Flaps could be assessed pre-operatively, throughout elevation, and post-operatively (Figure 1 and Figure 3). The ability to overlay NIR fluorescence and color video images permitted precise planning of flap boundaries (Figure 1–3). Post-operatively, ICG appeared at the preserved perforators within 20 sec after injection (Figure 1, bottom), then quickly spread into the flap (Figure 3). At 30 to 60 sec post-injection, NIR fluorescence was seen in the vein(s) and then gradually returned to its baseline, pre-injection level within 10 to 20 min.

Impact of Preserved Perforator Number Over Time

Pre-operative NIR fluorescence angiography permitted selection of the location and number of perforators to be preserved in each flap (Figure 1). We therefore studied the impact of 1, 2, or 3 preserved perforators on the three key metrics of flap function: the time to maximum perfusion, venous drainage, and percent of total flap area perfused.

The baseline characteristics of the $n = 6$ animals in each group (Table 1) were statistically matched ($p = 0.5533$). All three metrics of flap function were compromised immediately after flap creation (Figure 4). The time to maximum perfusion was markedly prolonged in flaps with a single perforator (Figure 4A). Mean values for 1, 2, and 3 perforators were 5.8 ± 0.7 , 3.3 ± 0.4 , and 2.1 ± 0.3 min, respectively. Statistically, there was advantage for multiple perforators, with the p value for 1 vs. 2, 1 vs. 3, and 2 vs. 3 perforators being 0.009, 0.0002, and 0.0268, respectively. DR also indicated that the venous drainage in flaps with multiple perforators was more favorable than in flaps with a single perforator (Figure 4B). Mean values for 1, 2, and 3

perforators were $75.3 \pm 4.7\%$, $61.5 \pm 3.7\%$, and $52.7 \pm 2.2\%$, respectively. *P* values for 1 vs. 2, 1 vs. 3, and 2 vs. 3 perforators were 0.036, 0.0005, and 0.0582, respectively. Flaps with multiple perforators were also superior in terms of perfused area as a function of total flap area, although there was no significant difference between flaps with 1 and 2 perforators ($p = 0.1830$) and between flaps with 2 and 3 perforators ($p = 0.2172$) (Figure 4C). The *p* value for 1 vs. 3 perforators was 0.0144. Immediately after flap creation ($T = 0$ h), mean perfused area for flaps with 1, 2, and 3 perforators were $80.3 \pm 3.6\%$, $84.5 \pm 2.6\%$, and $91.7 \pm 6.2\%$, respectively. After 6 h, those values improved to $97.1 \pm 1.7\%$, $99.8 \pm 0.2\%$, and 100%, respectively.

Histological Evaluation

There were no histological changes seen in tissue sampled from poorly perfused areas of flaps, normally perfused areas of flap, or nearby non-flap control areas (data not shown). In particular, there was no evidence of fat necrosis in any samples.

DISCUSSION

In this study, we demonstrated that the submental perforator flap has reliable perfusion and venous drainage as demonstrated using NIR fluorescence angiography. We followed each flap for 6 h, and although there was a statistically significant advantage in all metrics for multiple perforators, perfusion was adequate even when a single perforator was preserved. One clear advantage was that with more perforators preserved, there was a shorter time to maximum perfusion. The advantage of multiple perforators was not clearly seen in comparing the percentage of perfused area, though, since all flaps eventually reached nearly 100% perfusion.

Even with a single perforator flap, the areas most distant from the perforator eventually regained proper perfusion at 6 h. This most likely represents changes in flap physiology, as a single perforator flap can potentially redistribute blood flow by 6 h. However, areas that took a long time to recover could have unrecognized areas with poor blood supply, which may cause long-term perfusion problems in the flap such as fat necrosis. A further study is now ongoing to investigate the long-term outcome of the relationship between perfusion and tissue viability.

To assess venous drainage, we previously identified the drainage ratio (DR), and set 85% as an upper limit of normal providing the highest accuracy for detecting venous congestion.⁴ At 6 h, none of the flaps developed venous congestion, as the DR was less than 85%. Importantly, flaps with 3 perforators never showed a DR greater than 85%, even immediately after raising the flap.

Gill et al. reported a positive correlation between the number of perforators and the incidence of fat necrosis in deep inferior epigastric perforator (DIEP) flaps.¹² Fat necrosis is a chronic complication, thus, our study could not identify these areas as we only examined the acute phase after the surgery. In all three metrics we used to assess flap function, no negative correlations with perfusion or venous drainage was seen with increasing numbers of preserved perforators. The dominance of the selected perforators was not mentioned in that study, and in our study we mostly used dominant perforators (Table 1). Further studies to assess the long term impact of perforator dominance and location are underway. In the present study, histological examination revealed no findings suggestive of fat necrosis, even in samples taken from areas judged by NIR fluorescence to have poor perfusion.

In order to give a flap sufficient mobility, it is necessary to minimize the number of perforators preserved. Another reason to reduce the number of perforators is to decrease dissection and additional muscle sacrifice at the donor site. It is still uncertain whether the number of perforators, the location, the dominance, or size has the highest priority for perforator selection. Patel et al. described how the blood flow could be changed depending on the number, length

and diameter of selected perforators using a mathematical model.¹³ The study suggested that the benefits of preserving multiple non-dominant perforators must be weighed against the morbidity of additional muscle and fascial trauma. Since that model did not account for the effects of internal nitric oxide-derived vascular dilation and arteriovenous shunts, which normally occur in capillary vessels under ischemic conditions, we believe *in vivo* studies may be better suited to evaluate clinically relevant outcomes in flaps. In this study, we focused on the impact of the number of perforators preserved, a result with potential relevance to other perforator flaps such as a DIEP flap.

We found that flaps with a single perforator initially showed the least perfusion and venous drainage; however, this steadily recovered over time. The percentage of perfused area in flaps with a single perforator at 0 and 6 h after flap isolation was $80.3 \pm 3.6\%$ and $97.1 \pm 1.7\%$, respectively, and all metrics showed continuing recovery at 6 h. We conclude that a perforator flap with a single perforator may minimize morbidity of the donor site and maximize mobility while providing adequate perfusion. Further investigation is essential to elucidate the ultimate fate of areas that recover slowly in NIR fluorescence images, since these areas may potentially develop fat necrosis long term.

CONCLUSIONS

We demonstrate that NIR fluorescence angiography can identify perforating arteries in submental flaps with a high degree of reliability, assist with flap design, and quantify flap perfusion. Although flaps with a single dominant perforator initially showed signs of altered flap metrics, by 6 h the perfused area of the flap was equivalent to flaps having multiple preserved perforators.

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Figure 1. Evaluation of Perforator Flaps using NIR Fluorescence Imaging

Perforator artery (PA) location (circles) was mapped pre-operatively (top) and post-operatively (bottom). Shown are the color video image (left column), NIR fluorescence image (middle column), and a pseudo-colored (lime green) merged image of the two (right column). Flap design is indicated by black skin marker on the color video image. Four identified perforating arteries (PA1-PA4) identified pre-operatively were reduced to three after flap elevation.

Color Video

NIR-Fluorescence

Figure 2. Location and Dominance of Selected Perforator Arteries

Each submental flap was divided into the regions shown on the color video image (left): C = Central 2 cm diameter, RUQ = right upper quadrant, RLQ = right lower quadrant, LUQ = left upper quadrant, and LLQ = left lower quadrant. Perforator arteries (PAs) identified using NIR fluorescence imaging of the same flap (right) reveal dominant vessels (D; solid circles) and non-dominant vessels (ND; dashed circles). Terminology describing the numbering and location of the identified PAs is as follows: 1: LUQ (D), 2: LUQ/RUQ (ND), 3: RUQ (D), 4: C (D), 5: LLQ (D), 6: C (ND).

Figure 3. NIR Fluorescence Image at Maximum Perfusion

Shown are the NIR fluorescence images, from a submental flap having three dominant perforator arteries, taken pre-operatively (left), after flap elevation (middle), and 1 h post-operatively (right). Quantitation of the perfused area in these three examples was 100%, 86%, and 97%, respectively.

Figure 4. Quantitation of Flap Perfusion over Time using NIR Fluorescence

Each of the following parameters were measured as a function of time and number of perforator arteries (PAs) within the flap:

- A. The time in min required to achieve peak flap perfusion.
- B. The drainage ratio (DR) of the flap.
- C. The total perfused area of the flap.

Table 1

Location and Dominance of Selected Perforators in Each Animal.

Number of Perforators	1	2	3
Animals per group (n)	6	6	6
Body weight (kg)	36.0 ± 1.1	37.2 ± 1.2	37.5 ± 0.7
Location and dominance (D) of perforators from each animal	LLQ/RLQ (D)	C (D)	RUQ (D)
	LLQ/RLQ (D)	RUQ (D)	RUQ/LLQ (D)
	C (D)	C (D)	RLQ (D)
	RUQ (D)	RLQ (D)	LLQ (D)
	RUQ (D)	LLQ (D)	LLQ (ND)
	LLQ (D)	LLQ (D)	C (D)
	LLQ (D)	RUQ (D)	RUQ (D)
		RUQ (D)	RUQ/RLQ (D)
			RUQ/LLQ (D)
			LLQ (D)
			LLQ (D)
			LUQ (D)
			RUQ/LLQ (D)

D: dominant, ND: non-dominant, C: central, RUQ: right upper quadrant, RLQ: right lower quadrant, LUQ: left upper quadrant, LLQ: left lower quadrant.