Published in final edited form as:

Plast Reconstr Surg. 2009 August ; 124(2): 451–460. doi:10.1097/PRS.0b013e3181adcf7d.

Quantitative Assessment of Perfusion and Vascular Compromise in Perforator Flaps using a Near-Infrared Fluorescence Guided Imaging System

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Abstract

Background—Techniques currently used to determine flap perfusion are mainly subjective, with the majority of reconstructive surgeons still relying on clinical examination. In this study, we demonstrate the use of near-infrared (NIR) fluorescence angiography to directly quantify normal and abnormal perfusion in perforator flaps.

Methods—Indocyanine green was intravenously injected into anesthetized adult pigs (n = 38). A custom NIR fluorescence imaging system was employed for image acquisition and quantitation. Thirty-nine flaps were designed based on identified perforators, and post-operative imaging was performed for comparison. In select flaps, isolated occlusion of the arterial and venous pedicle was performed. In select flaps, vascular spasm was induced by local irrigation of the vessels with

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Presented as a poster at the World Molecular Imaging Conference in Nice, France, September 10-13, 2008.

Author Statements of Financial Interest:

Aya Matsui, M.D.: None

Bernard T. Lee, M.D.: None

Joshua H. Winer, M.D.: None

Rita G. Laurence, B.S.: None

John V. Frangioni, M.D., Ph.D.: All intellectual property for the intraoperative near-infrared fluorescence imaging system is owned by Dr. Frangioni's employer, the Beth Israel Deaconess Medical Center (BIDMC), a teaching hospital of Harvard Medical School. The patent rights have been licensed non-exclusively by the BIDMC to GE Healthcare. As inventor of the technology, Dr. Frangioni may someday receive royalties if a product is ever developed. Dr. Frangioni has no real or deferred equity interests, whatsoever, in this, or any other technology. Dr. Frangioni does not consult for any company. GE Global Research sponsors unrelated research in Dr. Frangioni's laboratory.

Devices Used: Custom-built intraoperative near-infrared fluorescence imaging system with simultaneous color video and near-infrared fluorescence capabilities.

Drugs Used: Indocyanine Green (Cardiogreen, Sigma-Aldrich, St. Louis, MO/ IC-GREEN™, Akorn, Decatur, IL), Epinephrine (Hospira, Lake Forest, IL).

epinephrine. The fluorescence intensities (FI) of select regions-of-interest (ROI) were quantified. From these data, we defined two indices for abnormal perfusion: the $T_{\max}$ ratio (TR) and the drainage ratio (DR).

Results—We identified a normal pattern of perfusion prior to flap elevation, composed of a distinct FI peak at maximal arterial inflow followed by a smooth drop representing venous drainage. Delay of this peak after flap elevation, as indicated by TR, identified vascular spasm and arterial occlusion ($p < 0.0001$). Abnormal fall of FI after this peak, as indicated by DR, identified venous occlusion ($p < 0.0001$).

Conclusions—Quantitation of FI by NIR angiography accurately characterizes arterial and venous compromise. Our technique can assess perfusion characteristics during the intra-operative and post-operative periods, and therefore complements clinically-based subjective criteria now used for flap assessment.

Keywords

Quantitative flap assessment; Near-infrared fluorescence; Microsurgery; Flap failure

INTRODUCTION

Despite the numerous methods that have been introduced to evaluate flap perfusion, the most common technique still used is clinical evaluation. In a study of reconstructive surgeons in the United Kingdom, 95.1% used only clinical findings for flap monitoring.¹ Although skin color, turgor, temperature, and capillary refill are frequently used for flap assessment, the subjective nature in interpretation is highly variable. A white flap, with little or no capillary refill, can represent either arterial occlusion or arterial spasm. On the other hand, a red or purple flap with rapid capillary refill would lead one to suspect venous congestion.

Recent animal studies, however, demonstrated that skin color was not directly related to the degree of venous congestion.² As guidelines for skin color and capillary refill are based on evaluator experience, it can be difficult to distinguish normal from abnormal. The experience with temperature monitoring has also been highly variable since studies have shown that in obviously necrotic flaps the temperature did not change.^{3,4}

Complication rates after microsurgery commonly focus on total flap loss, with reported rates from 0% to 7.3%.⁵⁻¹⁶ Re-exploration for vascular compromise in multiple large series ranged from 2% to 16%,^{5,7,9,10,13,16} and flap salvage rate was highly dependent on timely recognition. In addition, partial flap loss and fat necrosis can also represent complications related to inadequate perfusion. In head and neck reconstruction, Jones et al. demonstrated that once a complication occurred, longer hospital stays were seen and excessive resource costs were to be expected.¹³

Since flap salvage is highly dependent on early exploration, monitoring techniques must identify problems early, and, ideally, in the operating room.^{5,9,10,11,16} Late recognition of both arterial and venous problems results in thrombosis formation, which often represents an unsalvageable state. Multiple studies have demonstrated a positive correlation between the salvage rate and time interval to re-exploration.^{5,9-11,16}

We have previously described the use of intraoperative near-infrared (NIR) fluorescence angiography to identify the location and distribution of perforating vessels, permitting a patient-specific flap design regardless of anatomic variability.¹⁷ Our technique is similar to fluorescein angiography, although we use an FDA-approved NIR fluorophore, indocyanine green (ICG) as a contrast agent. ICG has favorable pharmacokinetics with more rapid clearance

when compared to fluorescein. With this rapid clearance, additional injections can be repeated every 10 min if necessary.¹⁷ Another drawback to fluorescein is the need for darkness with the use of an ultraviolet Woods light. On the contrary, in NIR fluorescence imaging, white light ($\lambda = 400\text{-}650\text{ nm}$) continues to illuminate the surgical field simultaneously with the invisible NIR light ($\lambda = 750\text{-}850\text{ nm}$) necessary for NIR fluorophore detection. Custom optics and computer control permits quantitation of fluorescence intensity (FI) and contrast-to-background-ratio (CBR) on selected regions of interest (ROI), thus eliminating operator variability.

This study included a total of 345 injections evaluating 39 perforator flaps in 38 pigs. Injections were performed prior to flap elevation, immediately after flap creation, and during the immediate post-operative course. In addition, we isolated the pedicle in 7 flaps with sequential clamping of the artery and the vein, and in 4 flaps we induced vascular spasm with local irrigation of the vessels with epinephrine. By grouping patterns of normal and abnormal circulation, in this study we were able to define two numerical indices that could reliably identify flaps with vascular compromise.

MATERIALS AND METHODS

NIR Fluorescence Imaging System

The imaging system (Figure 1) has been described in detail previously by our laboratory.^{18, 19} The newest version permits positioning of the custom optics anywhere in three-dimensional space while maintaining a working distance of 18" between the optics and the surgical field. White (400-650 nm) light and NIR fluorescence excitation (745-779 nm) light are generated by light emitting diodes (LED) over a 15-cm field-of-view. Color video (i.e., the surgical field) 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. 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: 22.1-41.7 kg, mean: 36.5 kg) were induced with 4.4 mg/kg intramuscular TelazolTM (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 with NIR Fluorescence Angiography

Animals ($n = 38$) were injected with ICG as a rapid bolus via either femoral vein or external jugular vein. Due to manufacturer problems, two different ICG formulations were administered: chemical grade (Cardiogreen, Sigma-Aldrich, St Louis, MO; $n = 31$ animals) and clinical grade (IC-GREENTM, Akorn, Decatur, IL; $n = 7$ animals). No difference in performance was noted. ICG dose ranged from 0.01 to 5.0 mg (mean: 2.5 mg) per injection, with each diluted in 10 ml saline. Images were acquired at 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 perforators using the initial injection, a flap was designed which encompassed the selected perforator(s) (Figure 2A). Subsequently the flap was raised and another ICG injection was given to confirm flap perfusion (Figure 2B). Some flaps were evaluated repeatedly for the purpose of tracking changes in perfusion over time. Multiple injections were given at a minimal time interval of 10 min to reduce the dose stacking effect of ICG.¹⁷ All injections investigated were made within 6 hours of the flap isolation. Twenty-

two deep superior epigastric artery (DSEA) flaps and 17 submental flaps were included in this study.

Vascular Occlusion and Drug-Induced Spasm Models

As physiological references, we created vascular occlusion models by clamping the artery or vein at the level of the DSEA (or DSEV; deep superior epigastric vein; $n = 7$ animals). Occlusive clamps were placed within 3 min before the injection. In 4 animals, 5 ml of 1000 mg/ml epinephrine (Hospira, Lake Forest, IL) diluted in saline to a final concentration of 100 mg/ml was directly applied as a local irrigant on the dissected pedicle to induce vascular spasm. As a control, subcutaneous injection of the same epinephrine solution was performed at a nipple outside of the flap. Images were obtained after clinical evidence of vascular spasm in the control nipple after 5 min.

Quantitation of the Fluorescence Intensity

All image acquisition and processing was performed via custom Lab-VIEW software (Lab-VIEW, National Instruments, Austin, TX). A small circle was drawn as a region-of-interest (ROI) at a perforating artery (PA) identified on the NIR fluorescence image (Figure 2). Another small circle was made in the corner of the image outside of the flap to assess background (BG) fluorescence (Figure 2). Fluorescence intensity (FI) of the ROI and the background was quantified using 12-bit pixel intensity (range 0-4095). Automatic display of maximum, minimum, and mean FI of up to 16 ROIs at a time facilitated quantitation of the ≈ 250 images generated by each study. 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 the subsequent injections.

Analysis of FI Patterns

The CBR pattern for each injection was recorded graphically and analyzed. Maximum CBR ($I_{\max}$), time to $I_{\max}$ ($T_{\max}$) and CBR at 2 min post-injection (I_{120}) was obtained in individual injections (Figure 2C). The Drainage Ratio (DR) and $T_{\max}$ Ratio (TR) were developed to quantify venous drainage and arterial inflow, respectively. $DR = FI_{120 \text{ sec}} / FI_{\max} * 100 (\%)$. $TR = T_{\max}' / T_{\max} * 100 (\%)$, where $T_{\max}$ and $T_{\max}'$ are defined as the time of maximal FI after pre-operative and post-operative injections, respectively.

Either Student t-test or analysis of variance (ANOVA) was employed to determine the statistical difference in DR and TR between two groups or between multiple groups. DR and TR were assessed pre-operatively, post-operatively, in arterial and venous occlusion, and in epinephrine-induced spasm models. Statistical significance was set at $p < 0.05$. The analyzed values were described as mean $\pm$ S.D.

RESULTS

Visual Overview of the NIR Fluorescence Perforator Angiography

Typically, ICG NIR fluorescence appeared at the preserved perforators within 20 sec after injection, then quickly spread into the flap. At 30 to 60 sec post-injection, fluorescence was seen in the vein(s) and then was gradually eliminated within 10 to 20 min down to the pre-injection level. No fluorescence was found in flaps with arterial occlusion, whereas nearby background tissue increased in fluorescence (Figure 3). In flaps with venous occlusion, ICG exhibited minimal clearance compared to background (Figure 3).

CBR Patterns within Flaps

Six different CBR patterns were found: 1) Pre-operative evaluation: CBR of the pre-operative injections consistently showed a sharp peak immediately after the injection as a result of arterial inflow, followed by an unimpeded fall with venous drainage (Figure 4A, Pre-OP). 2) Post-operative evaluation: While CBR of the post-operative injections showed a pattern comparable to that of pre-operative injections, it typically remained at a higher level after the first peak (Figure 4A, Post-OP). 3) Spasm pattern (SP pattern): In 9 post-operative injections in the setting of arterial spasm, a blunted peak was observed with a delayed time to inflow peak (Figure 4A, Arterial Spasm). These 9 results were investigated separately from other post-operative evaluations. 4) Arterial Occlusion (AO): CBR stayed at or around the baseline level without any inflow peak (Figure 4A, Total Arterial Occlusion). Neither DR nor TR was evaluated because of a lack of a definitive T_{max} and I_{max} . 5) Venous Occlusion (VO): CBR stayed continuously elevated after the inflow peak (Figure 4A, Total Venous Occlusion). 6) Epinephrine-induced spasm (EI-SP): Similar to the spasm pattern, time to the inflow peak was delayed, which resulted in a characteristic blunted peak with a slower clearance of fluorescence over time (Figure 4A, Epinephrine).

Statistical Analysis of DR and TR

DR and TR were analyzed using the CBR patterns described above (Table 1). With respect to DR, ICG clearance was generally prolonged, to varying degrees, in post-operative evaluations. Post-operative DR was significantly greater than pre-operative DR ($p < 0.0001$). We also found a statistical difference between VO and post-operative evaluations ($p < 0.0001$). The differences between SP patterns and EI-SP were not significant ($p = 0.0622$) and there was no difference between post-operative evaluations and SP patterns ($p = 0.1095$). Interestingly, SP pattern was significantly different from VO ($p = 0.0069$), while EI-SP and VO were comparable ($p = 0.1413$). ANOVA showed a significant difference between multiple groups that included pre-operative, post-operative, VO, EI-SP, and SP patterns ($p < 0.0001$; Figure 4B, Drainage Ratio).

With respect to TR, T_{max} of the initial and second pre-operative injections became shorter during latter injections ($p = 0.0054$, data not shown). When multiple pre-operative injections were made, T_{max} of the last pre-operative injection was used as the denominator for TR calculations. Statistical differences were found between post-operative evaluations and VO ($p < 0.0001$), and between multiple groups that included post-operative, VO, EI-SP, and SP patterns ($p < 0.0001$). The difference between SP patterns and EI-SP was not significant ($p = 0.6671$; Figure 4B, T_{max} Ratio).

Determination of the Normal Range

The optimal set point for the lower limit of “normal” DR and TR was investigated. For DR, sensitivity, specificity, and accuracy were calculated from flaps with VO compared to normal flaps. A lower limit of normal set at 86% provided 100% sensitivity, 98.9% specificity, and 99.4% accuracy. For TR, flaps with EI-SP were compared to normal post-operative flaps. A lower limit of normal set at 25th percentile of EI-SP (200%) resulted in a 75% sensitivity, 100% specificity, and 87.5% accuracy. Using these values, a diagnostic algorithm was created to identify cases of abnormal perfusion (Figure 5).

DISCUSSION

The ideal flap evaluation system would be non-invasive, assess large areas of skin, provide quantifiable data, and accurately distinguish between arterial and venous compromise in a timely fashion. No such system currently exists; however, our NIR fluorescence imaging system comes close to meeting these criteria. The greatest clinical benefit with our system is

the ability to evaluate flaps in the operating room, with real-time overlay of flap physiology (i.e., using invisible NIR fluorescent light) with surgical anatomy (i.e., using color video). The portability of our system also permits its use in the post-operative setting when vascular compromise is suspected.

Currently, handheld Doppler is the most frequently used technique for flap monitoring.¹ It is an easy monitoring device to use, while being inexpensive and non-invasive. A major limitation, however, is that it only evaluates a pinpoint site within the flap and may not distinguish global perfusion problems. In addition, it is difficult to quantify and to compare data. Newer methods of flap monitoring such as tissue oximetry (ViOptix, Fremont, CA) have attempted to eliminate the subjective nature of flap assessment. Tissue oximetry provides continuous monitoring that is quantifiable. This technique, however, is a poor evaluator of global perfusion as it provides data for a 1 cm area below the probe. In addition, the quantitative criteria for vascular compromise are still being established.

Our NIR imaging system can identify and quantify normal flap perfusion curves based on fluorescence intensity. More importantly, by comparing injections prior to flap elevation and after flap elevation, we were able to distinguish arterial and venous compromise with defined DR and TR values. We set 86% and 200% as the lower limit of the normal range for DR and TR, respectively, as these two values provided the highest accuracy. Using the diagnostic algorithm described in Figure 5, 27% of our 190 post-operative evaluations had an abnormal DR and venous congestion. In contrast, 4.5% had an abnormal TR, which represents arterial spasm. The ultimate fate of flaps with such abnormalities is now being investigated.

We also compared the DR before and after flap elevation ($p < 0.0001$). The higher DR in post-operative evaluations most likely represents a redistribution of venous outflow seen in normal flap physiology. While the higher DR did not result ultimately in flap congestion, it may explain how early skin color changes after flap elevation such as hyperemia or rapid capillary refill can resolve and not manifest as flap failure. We are currently designing long-term survival studies to examine improvements in flap physiology.

NIR fluorescence angiography can also identify flaps with both arterial and venous compromise. Epinephrine induces both arterial and venous spasm non-selectively with local application, and all 9 injections after epinephrine irrigation resulted in epinephrine induced-spasm patterns that exceeded DR and TR thresholds. As for DR, the difference between spasm and venous occlusion patterns was significant, while venous occlusion and epinephrine induced-spasm were comparable. The difference in TR between spasm and epinephrine induced-spasm patterns was not significant. These results are consistent as spasm induced by epinephrine and surgical dissection is similar.

Previously reported NIR fluorescence angiography studies validated the clinical relevance of ICG fluorescence to tissue perfusion.^{20,21} At the time, it was found to be “too sensitive” for clinical use.^{22,23} Krishnan et al. suggested that flaps could potentially heal uneventfully even after NIR fluorescence angiography demonstrated signs of venous congestion.²³ The study included only 9 patients, and leeches were applied to 2 patients with delayed clearance of ICG. There are many differences between the data from the Krishnan study and the data obtained during our study. Most importantly, their normal fluorescence curve lacked an obvious inflow peak, reaching a maximum level at 2 min that begins to fall between 8 and 10 min. The imaging device employed in that study may not have had the temporal responsivity and spatial sensitivity to capture peak arterial inflow. It is interesting that a similar CBR curve can be found utilizing our NIR imaging system when the region of interest is created away from the perforating artery. Presently, we have not determined the minimal CBR level for effective perfusion, and a long-term survival study is now underway.

Indocyanine green has been approved for medical use since 1956 and widely used for various diagnostic purposes. The incidence of severe adverse reactions with intravenous administration of ICG is extremely rare with reported rates between 0.00007 and 0.05%. There are no data available describing overdose in humans; however the LD₅₀ of ICG in rabbits is reported at 50 to 80 mg/kg. The mean dose and concentration we used per injection was 2.5 mg (0.07 mg/kg), and the theoretical dose per injection for use in a 70 kg adult is approximately 5 mg. The required dose per injection is several hundreds times smaller than the LD₅₀ in a rabbit, thus we believe the risk of ICG administration is minimal. None of the animals had clinical signs of an adverse reaction due to ICG administration in our study.

A limitation of our study is that we evaluated perfusion after flap creation with no microvascular transfer. This endpoint was chosen to facilitate collection of the large amounts of data necessary for flap assessment. Isolation and clamping of the artery and vein were performed to simulate vascular occlusion and flap failure; however, this may not properly approximate thrombus formation. Ideally, a microsurgical transfer would be performed to provide the most accurate clinical correlation and these studies are currently underway.

CONCLUSIONS

Quantitative assessment of flaps using NIR fluorescence angiography with a color video overlay is safe, simple, and non-invasive. Evaluation requires only ≈2 min, and ICG is relatively inexpensive and already FDA-approved for other indications. Using the two indices DR and TR, flaps suffering from vascular compromise can be identified accurately, suggesting that they may be of value as an adjunct to standard flap evaluation.

Acknowledgments

We thank Barbara L. Clough and Lorissa A. Moffitt for editing, and Eugenia Trabucchi for administrative assistance. This work was funded by National Institutes of Health grants R01-EB-005805 and R01-CA-115296 to JVF.

Sources of Funding: This study was funded by National Institutes of Health grants R01-EB-005805 and R01-CA-115296 to JVF.

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Figure 1. Real-Time Intraoperative Near-Infrared Fluorescence Imaging System
Schematic showing light paths and major system components.

A.

B.

C.

Figure 2. Quantitative Assessment of Flaps using NIR Fluorescence Angiography with Color Video Overlay

A. Pre-operative assessment of a DSEA flap. Images acquired pre-injection, and 20 sec and 60 sec post-injection of ICG. Shown are the color video (left column), NIR fluorescence (middle column), and a pseudo-colored (lime green) merged image of the two (right column). White arrow identifies perforating vessel. Dashed circle on merged image is signal ROI and solid circle is background ROI. T: time post-injection, N: nipple.

B. Post-operative assessment of a DSEA flap. Images acquired pre-injection, and 10 sec and 60 sec post-injection of ICG, as described for (A).

C. Typical CBR-time curve showing definitions of T_{120} , $T_{\max}$, I_{120} , and $I_{\max}$.

DSEA Arterial Occlusion

DSEA Venous Occlusion

Figure 3. Examples of Flap Vascular Compromise

DSEA arterial occlusion (top row) as imaged 20 sec post-injection of ICG. DSEA venous occlusion (bottom row) as imaged 5 min post-injection of ICG. Dashed circle on merged image is signal ROI and solid circle is background ROI. T: time post-injection, N: nipple.

Figure 4. The Six Flap CBR Patterns

A. Typical CBR curves for pre-operative evaluation, post-operative evaluation, arterial spasm, total arterial occlusion, total venous occlusion, and epinephrine-induced spasm.

B. Statistical analysis of DR and TR. Error bar shows the maximum and minimum values. The top and bottom of the box represents the upper and lower 25% quartile and the line inside the box indicates the median of each group, respectively. Pre: pre-operative assessment. Post: post-operative assessment. SP: spasm pattern. Epinephrine: epinephrine-induced spasm pattern. VO: venous occlusion.

Figure 5. A Diagnostic Algorithm for Vascular Compromise

Proposed algorithm for identification of problematic arterial and venous flow using the lower limit of DR and TR that provide the highest accuracy. Results are applicable to both arterial impairment and venous congestion.

Table 1
Drainage Ratio and T_{max} Ratio in Each CBR Pattern

Condition	n	Drainage Ratio (DR) Mean $\pm$ S.D. %	T _{max} Ratio (TR) Mean $\pm$ S.D. %
Pre-operative	96	57.61 $\pm$ 13.83	n/a
Post-operative	190	75.68 $\pm$ 16.74	101.0 $\pm$ 20.56
SP Pattern	9	84.74 $\pm$ 9.440	185.7 $\pm$ 33.93
EI-SP	10	92.43 $\pm$ 7.626	600.5 $\pm$ 417.5
VO	10	98.60 $\pm$ 10.13	703.0 $\pm$ 657.3

EI-SP = Epinephrine-induced spasm; n/a = Not applicable; SP = Spasm; VO = Venous occlusion.

Abbreviations**Table 2**

ICG: Indocyanine green
NIR: Near-infrared
FI: Fluorescence intensity
ROI: Region-of-interest
CBR: Contrast-to-background ratio
BG: Background
AO: Arterial occlusion
VO: Venous occlusion
EI-SP: Epinephrine induced-spasm
SP: Spasm
DR: Drainage ratio
TR: T _{max} ratio