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Near-Infrared Imaging for the Assessment of Anastomotic Patency, Thrombosis, and Reperfusion in Microsurgery: A Pilot Study in a Porcine Model

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Abstract

Background—Advances in microsurgical techniques have increased the use of free tissue transfer. Methods of intraoperative flap perfusion assessment, however, still rely primarily on subjective evaluation of traditional clinical parameters. Anastomotic thrombosis, if not expeditiously identified and revised, can result in flap loss with significant associated morbidity. This study aims to evaluate the use of near-infrared (NIR) fluorescence imaging in the assessment of microsurgical anastomotic patency, thrombosis, and vascular revision.

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Materials and Methods—A model of pedicle thrombosis was created using bilateral abdominal flaps isolated on deep superior epigastric vascular pedicles in four Yorkshire pigs. Following flap elevation, microvascular arterial and venous anastomoses were performed unilaterally, preserving an intact contralateral control flap. Thrombosis was induced at the arterial anastomosis site using ferric chloride, and both flaps imaged using NIR fluorescence angiography. The thrombosed vascular segments were subsequently excised and new anastomoses performed to restore flow. Follow-up imaging of both flaps was then obtained to confirm patency using fluorescence imaging technology.

Results—Pedicled abdominal flaps were created and successful anastomotic thrombosis was induced unilaterally in each pig. Fluorescence imaging technology identified large decreases in tissue perfusion of the thrombosed flap within 2 minutes. After successful revision anastomosis, NIR imaging demonstrated dramatic increase in flow to the reconstructed flap, but intensity did not return to pre-thrombosis levels.

Conclusions—Early identification of anastomotic thrombosis is important in successful free tissue transfer. Real-time, intraoperative evaluation of flap perfusion, anastomotic thrombosis, and successful revision can be performed using NIR fluorescence imaging.

Keywords

Microsurgery; vessel thrombosis; near-infrared imaging

Introduction

Well over 17,000 reconstructive microsurgery procedures are performed by plastic surgeons in the United States each year.¹ Despite advancements in surgical technique, flap failure continues to represent a serious adverse outcome with significant resulting morbidity. Rates of free flap failure have been reported to range from 2.5% in trunk reconstruction to 4.9% in breast flaps, 5.6% in extremity flaps, and 9.6% in head and neck reconstructions.² If unrecognized, these events lead to partial or total flap loss and fat necrosis. Each may be associated with significant morbidity, and affected patients frequently require additional reconstruction.³ Early recognition of vascular compromise and prompt surgical intervention have been recognized as important contributors to successful flap salvage when complications do occur.⁴

Currently, clinical assessment of skin warmth, color, capillary refill, and dermal bleeding comprise the standard flap monitoring protocol at most institutions. These subjective measures often rely on relatively late signs of flap failure; as such, numerous modalities have been proposed to identify vascular compromise earlier. Duplex ultrasound is highly operator-dependent and requires familiarity with both performance and interpretation of the exam.⁵ Hand-held Doppler signals are qualitative, can be confounded by signals from neighboring vessels, and require close proximity to the vasculature being interrogated.⁶ Tissue oximetry probes measure tissue oxygenation over a very small area, requiring prior knowledge of the location of a potential perfusion defect.⁷ All three methods require direct contact with the flap. Flow imaging techniques such as LASER Doppler or Speckle are limited to superficial assessment of the flap capillary flow.^{8,9} Thermographic imaging

typically requires a “cold challenge” that makes it impractical for routine clinical use.¹⁰ Because of the limitations of these techniques, none has replaced traditional clinical assessment in routine flap evaluation.

Near-infrared (NIR) fluorescence imaging has been used to assess perfusion in cutaneous flaps during reconstructive surgery, as well as in other organs.^{11,12} This technology uses NIR light (650 to 900 nm) in combination with the injection of a fluorescent angiographic tracer to evaluate tissue perfusion.¹³ Indocyanine green (ICG), an FDA approved fluorescent tracer, was used off label as the angiographic tracer in this study. NIR images were acquired using the real-time Fluorescence-Assisted Resection and Exploration (FLARE) system, developed in the Center for Molecular Imaging at the Beth Israel Deaconess Medical Center.^{14–16} Using this technology, NIR fluorescence images are displayed alongside color video, allowing the surgeon to visualize the angiographic tracer within the surgical field in real time.

Our previously published data have demonstrated that intraoperative NIR fluorescence angiography can be used to quantify tissue perfusion, aid in perforator identification, and assist in flap design in large animal models.^{17–22} These findings were validated with x-ray angiography,²³ and translated to the operating room in a human pilot study.²⁴ The FLARE system provided dynamic, real-time, intraoperative guidance in each of these applications without cannulation of, or contact with, the target vessels. In this pilot study, we aim to evaluate NIR fluorescence imaging of microsurgical anastomosis, thrombosis, and subsequent vascular revision with flap reperfusion as an important step in the translation of this technology.

Materials and Methods

Animal studies were performed under the supervision of the Beth Israel Deaconess Medical Center Institutional Animal Care and Use Committee (IACUC), in accordance with approved institutional protocol #034-2013. Female, 35 kg Yorkshire pigs (E.M. Parsons and Sons, Hadley, MA) were used in this study. Anesthesia was induced with 4.4mg/kg intramuscular Telazol (Fort Dodge Animal Health, Fort Dodge, IA); animals were subsequently intubated and ventilated using a Quantiflex ventilator (Matrix Medical, Inc.; Orchard Park, NY), and maintained using 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL) in oxygen. All animals included in this study were healthy, with no prior history of allosensitization. A 16-gauge central venous catheter was inserted in the femoral vein prior to surgery. Physiologic parameters (electrocardiography, heart rate, oxygen saturation, and body temperature) were monitored during all experiments.

NIR Fluorescence Imaging

The current FLARE imaging system includes a light source and optical apparatus attached to an articulated arm, which allows for positioning in three-dimensional space while maintaining a working distance of 18 inches between the optics and the surgical field.¹³ A light guide-coupled radiofrequency plasma light source generates >40,000 lux of white light (400 to 650 nm), and a fiber coupled laser-diode source provides approximately 10 mW/cm² of 760 nm NIR fluorescence excitation light over an area of 15×11 cm.²⁵ Color and NIR

fluorescence images of the surgical field are acquired simultaneously via custom-designed optics and software and displayed in real time with color video. For NIR angiography, 1.3 mg of indocyanine green (ICG) (Akorn Inc.; Decatur, IL) was injected as a rapid intravenous bolus in 10 cc of sterile water. The surgical field was recorded for 2 minutes after each injection, and fluorescence intensity (FI) was measured using the FLARE imaging system.

Abdominal flaps

Flaps were designed bilaterally in Yorkshire pigs to include skin, multiple nipples, subcutaneous fat tissue, rectus abdominis muscle, and pedicle vasculature, each measuring 8×8 cm (Figure 1). Flaps were raised with careful preservation of the deep superior epigastric artery and vein pedicle and its branches to the flap. After symmetric, bilateral flaps were elevated, pedicle vasculature was isolated in one of the flaps, and artery and vein were transected and microsurgically reanastomosed using 8-0 nylon suture (reconstructed flap).

Thrombosis model

A total of 8 flaps were created in 4 Yorkshire pigs. Following unilateral microvascular arterial and venous anastomosis, thrombosis was induced at the arterial anastomotic site using ferric chloride (FeCl_3), as previously described.^{26–30} Briefly, a 0.3×1 cm Whatman Grade 413 Filter Paper (GE Healthcare Bio-Sciences; Pittsburgh, PA) was saturated with a 50% ferric chloride solution (Sigma-Aldrich; St Louis, MO) and placed beneath the anastomotic site. Parafilm (Pechiney; Chicago, IL) was used to protect surrounding tissue from oxidative injury induced by the FeCl_3 solution. After a 10 min exposure to FeCl_3 , flow was evaluated using Doppler ultrasound to confirm pedicle thrombosis. Then ICG was injected and both flaps were imaged using the FLARE system. Microsurgical revision of the thrombosed artery was performed and evaluated with Doppler ultrasound to confirm reperfusion prior to final imaging. Total ischemia time was approximately 30 to 45 minutes. Heparin was given intravenously at four specified time points: immediately after flap elevation, prior to flap harvest, after pedicle anastomosis, and after the revision anastomosis.

Perfusion assessment

Flap tissue perfusion was assessed using the FLARE system by observing the spatial distribution of fluorescence intensity (FI) over the surgical field. FI was recorded continuously for 2 minutes following each intravenous injection of ICG. Two regions of interest (ROIs) were identified for comparison: the first on the control flap, and the second on the reconstructed flap (dashed circles in Figures 2, 3, and 4). Tissue perfusion was then measured using both the evolution of FI over time and the spatial distribution of fluorescence throughout the flap. Imaging was performed following bilateral flap elevation and unilateral microvascular anastomoses, following induction of thrombosis, and following excision and microvascular re-anastomosis (Figures 2–4).

Results

Bilateral abdominal flaps were created in each pig and perfusion confirmed using ICG as well as traditional clinical parameters. Following unilateral flap harvest and re-anastomosis, FLARE assessment of dynamic FI was similar between each pair of reconstructed and control flaps. FI peaked in both flaps less than 30 seconds following ICG injection, and maximal values were similar in each. Peak FI of the control flap increased to 383% of the initial value, and intensity of the control flap increased to 417%. Spatial distribution using NIR fluorescence also appeared to be similar between control and reconstructed flaps (Figure 2).

Pedicle thrombosis was successfully induced during each trial, and the accompanying perfusion defects confirmed using handheld Doppler, clinical observation, and NIR fluorescence angiography. After thrombus was induced, no notable increase in perfusion of the reconstructed flap was demonstrated by the FI measurement following ICG administration, while FI in the control flap climbed to 300% of the initial value. Concordantly, no fluorescence appeared using spatial mapping of the reconstructed flap during the 2-minute image acquisition period (Figure 3).

Each of the thrombosed pedicles in the reconstructed flaps was successfully surgically revised and patency confirmed using handheld Doppler, clinical examination, and NIR fluorescence angiography. After microvascular revision and re-anastomosis of the pedicle artery, tissue perfusion in the reconstructed flap was restored; total ischemia time was approximately 30–45 minutes. Delayed filling was noted compared with the control flap, and peak FI was lower than in the pre-thrombosis images, at 217% of the initial value. Spatial fluorescence mapping confirmed a delay in tissue perfusion relative to the contralateral control flap, and appeared to have areas of regional variation (Figure 4).

Discussion

As microsurgical techniques continue to evolve, increasingly complex free tissue transfers are being performed for a broader range of indications on patients with more comorbidities.^{2,31–33} Early recognition of flap vascular compromise is known to improve rates of flap salvage.³⁴ The ideal method for earlier recognition and revision of compromised flaps, however, has yet to be established. In this pilot study, a model of pedicle thrombosis was used to determine the capability of FLARE technology to assess flap perfusion in the setting of thrombotic anastomotic occlusion and following microvascular revision.

In this large animal model, NIR fluorescence angiography successfully demonstrated fluorescence intensity and distribution of perfusion in the flap at all three time points during the procedure. Lack of flow secondary to thrombosis was evident approximately 30 seconds following intravenous ICG injection - as was reperfusion following vascular revision. We were able to visually assess spatial flap perfusion in real time and to identify relative changes in fluorescence intensity intraoperatively in both reconstructed and control flaps.

Fluorescence angiography also provided immediate confirmation of reperfusion following pedicle revision.

Interestingly, despite successful reperfusion, peak fluorescence intensity after pedicle thrombosis, revision, and flap reperfusion did not reach the pre-thrombotic level. Areas of relative regional perfusion defects were also noted using spatial fluorescence mapping. The decrease in perfusion may reflect microthrombosis within the flap or ischemia-reperfusion injury to the tissue during the 30–45 minute period of ischemia and pedicle revision. Additional investigation will be necessary to determine the nature and extent of the observed decrease in perfusion after revascularization. In addition, future studies will evaluate the selective thrombosis of the venous pedicle as well as partial thrombosis and spasm.

Although previous studies have investigated the use of laser Doppler techniques in the assessment of microthrombosis and reperfusion injury,^{35–37} to our knowledge, NIR technology has not been evaluated for potential use in this clinical application. NIR imaging has several important advantages in this setting. Whereas laser Doppler measurements rely on velocity of blood flow within a flap, NIR angiography provides a direct indicator of tissue perfusion with a fluorescent intravascular tracer. NIR imaging provides an objective, noncontact method of assessing an entire flap simultaneously in real time, and is not affected by changes in erythrocyte concentration or motion artifact. NIR imaging is, however, affected by dermal thickness and the presence of scars or other defects that scatter light.

Our laboratory has previously demonstrated real-time intraoperative NIR fluorescence angiography as a means of determining perforator location in addition to flap perfusion.^{14,15,20,21,23,24,38,39} NIR angiography has also been evaluated for the detection of flow changes following graded experimental arterial stenosis in a rodent model with encouraging results.⁴⁰ The addition of real-time perfusion imaging in the setting of vascular thrombosis is an important aspect of the translation of this technology to the operating room. By incorporating the techniques described, NIR imaging can aid in flap design, tissue selection, microvascular anastomosis, and final perfusion assessment, as well as early detection of a thrombotic compromise with subsequent revision. As repeated injection of ICG is possible, evaluation at each of these time points is possible in clinical use. Collectively, these studies demonstrate the utility of NIR fluorescence as a comprehensive tool for guidance during tissue flap transplantation. Further study will be necessary to determine optimal use in evaluating other flaps, such as muscle; although pilot studies have examined use in vascularized bone flaps.³⁸

NIR fluorescence imaging represents an objective, non-contact method of assisting surgeons during free tissue transfer by providing real-time, intraoperative visualization of tissue perfusion. The ability to evaluate the integrity of microvascular anastomoses using this technology could provide earlier and more accurate assessment of flap perfusion, compromise, and reperfusion. Additional studies are planned using this technology to detect partial pedicle thrombosis as well as reperfusion injury. This study supports the use of NIR imaging in the rapid identification of pedicle thrombosis and associated changes in flap tissue perfusion before and after microvascular revision.

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Figure 1. Abdominal Flaps

A. Schematic drawing of an abdominal flap elevated using the superior epigastric artery and veins including rectus abdominis muscle, several nipples, skin, and subcutaneous fat. Ventral and dorsal aspects are shown.

B. Bilateral abdominal flaps, ventral surface.

Figure 2. Fluorescence Intensity and NIR Fluorescence Imaging following Unilateral Microanastomosis

1.3 mg of ICG was injected into the femoral vein followed by 2 minutes of continuous NIR image acquisition. Fluorescence intensity (FI) was measured in both the control flap (C) and the reconstructed flap (R) during ICG administration (top). Color and NIR fluorescence images are shown at four time points (dashed lines) after injection (bottom): 1. baseline; 2. the first appearance of fluorescence in the flaps, 3. maximal reconstructed flap fluorescence, and 4. 2 minutes following ICG injection. Regions of interest (ROIs) are indicated by dashed circles. A.U., arbitrary units.

Figure 3. Fluorescence Intensity and NIR Fluorescence Imaging after Thrombosis

Following induction of microthrombosis using FeCl_3 , 1.3 mg of ICG was injected via the femoral vein and 2 minutes of continuous NIR imaging acquired. FI is demonstrated for both the control flap and thrombosed reconstructed flap over the 2-minute period following ICG administration (top). Color and NIR fluorescence images are shown at four time points during the acquisition period (bottom): 1. baseline; 2. the first appearance of fluorescence in the flaps, 3. maximal reconstructed flap fluorescence, and 4. 2 minutes following ICG injection. ROIs are indicated by dashed circles.

Figure 4. Fluorescence Intensity and NIR Fluorescence Imaging following Thrombosis and subsequent Vascular Revision

Following completion of thrombosis induction and corresponding image acquisition, revision of the thrombosed anastomosis was performed to restore vessel patency. A final 1.3 mg of ICG was injected and 2 minutes of continuous NIR imaging performed. FI is graphed over the imaging period for both the control flap and the reconstructed, thrombosed, revised flap (top). Color and NIR fluorescence images are shown at four time points during image acquisition (bottom): 1. baseline; 2. the first appearance of fluorescence in the flaps, 3. maximal reconstructed flap fluorescence, and 4. 2 minutes following ICG injection. ROIs are indicated by dashed circles.