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Face Transplant Perfusion Assessment using Near-Infrared Fluorescence Imaging

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Abstract

Background—Composite tissue allografts (CTAs) including partial face transplantation have been achieved clinically. However, risks of complications including tissue ischemia, rejection, and transplant failure are significant. Safe and effective techniques to assess perfusion are needed to decrease complications in composite tissue flaps. Near-infrared (NIR) fluorescence imaging has been previously shown to provide a real-time, intraoperative evaluation of perfusion. This study investigates the use of NIR imaging in partial face CTA harvest.

Materials and Methods—Hemifacial CTAs (N = 8) were created using an established porcine model. This included ear cartilage, nerve, lymphoid tissue, muscle, and skin with perfusion by the carotid artery and external jugular vein. Animals were injected systemically with indocyanine green (ICG), and NIR fluorescence images were obtained simultaneously with color video. In addition, the elevated hemifacial flaps were assessed using standard of care, i.e. clinical examination and Doppler.

Results—Flap design was facilitated by NIR imaging with localization of perforators to the hemifacial CTA flap. In particular, an arterial and venous phase could be clearly identified. Perfusion of the flap was assessed by NIR fluorescence intensity following injection of ICG. Sequential clamping of the artery and vein confirmed correlation of perfusion deficits with NIR imaging as well as with clinical examination and Doppler.

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All FLARE™ technology is owned by Beth Israel Deaconess Medical Center, a teaching hospital of Harvard Medical School. As inventor, Dr. Frangioni may someday receive royalties if products are commercialized. Dr. Frangioni is the founder and unpaid director of The FLARE Foundation, a nonprofit organization focused on promoting the dissemination of medical imaging technology for research and clinical use.

Conclusions—Evaluation and assessment of perfusion is important in facial transplantation. The results from our pilot study indicate that NIR imaging has the capability to assess perfusion of partial facial CTAs. This emergent technology shows promise in assessing tissue perfusion in a composite flap.

Keywords

Near-infrared imaging; face transplantation; composite tissue allotransplantation; microsurgery; free flap

INTRODUCTION

Advances in reconstructive microsurgery and immunosuppressive therapy have led to a growing interest in composite tissue allotransplantation (CTA) and its varied clinical applications. CTA represents a viable alternative to conventional reconstructive methods and is considered superior on the basis of its potential for replacing defective or absent structures with anatomically identical tissues [1]. In 2006, the first successful partial face transplantation was described by Devauchelle et al. in a 38-year-old woman after injury from a dog bite. Since then, numerous facial allograft transplantations have been performed worldwide [2]. These successes speak not only to the feasibility of the current technology, but hint at CTA use as an addition to the reconstructive ladder.

In total, 13 facial transplantations have been performed worldwide in patients with defects from trauma or tumors. Transplantation is associated with many risks and complications including flap failure, flap rejection, immunosuppression toxicity, increased risk of malignancy, psychosocial issues, and excessive financial cost. The risk of acute immunologic rejection is difficult to estimate, as there are few subjects to study; however, data suggests a range between 10% and 70% [3]. Composite tissue allotransplants consist of heterogeneous tissues and are thought to elicit a stronger response than solid organs [4]. The rates of chronic rejection with composite tissues are not yet known. Newer data suggest chronic rejection rates of 10% over 5 years [5].

More importantly, facial transplantation requires complex microsurgery. Given the complex 3-dimensional nature of the harvested flap, as well as the recipient facial architecture, significant planning is necessary for optimal outcomes. In addition, there is a large body of literature documenting the risks and complications unique to microsurgical transfer, especially in the head and neck region [6]. Complications of vessel thrombosis, partial flap loss, fat necrosis, and total flap loss are often associated with poor outcomes. Early recognition of vascular compromise has been shown to be associated with flap salvage and success [7]. These issues are particularly important in facial transplantation, as many of the candidates for surgery have undergone prior reconstructive attempts that have depleted common recipient vessels for anastomosis [8]. In light of the vascular paucity in transplant recipients, early detection of vascular compromise and inadequate perfusion within the allograft is necessary to facilitate timely salvage operations. As facial CTA becomes more prevalent, it is increasingly important to have reliable and effective means of acquiring high-resolution arterial and venous images for preoperative vascular planning.

Current methods for preoperative vascular mapping are primarily based on computed tomographic (CT) angiography and magnetic resonance (MR) angiography. While CT angiography is considered the first-line modality owing to its superior ability for visualization of smaller vessels with fewer artifacts [8–9], it carries the added risks that come with ionizing radiation and administration of required contrast agents. On the other hand, MR angiography can be difficult in patients with previous hardware; in addition, it is

an expensive imaging modality. Even with these imaging studies, we still rely on clinical intraoperative assessment of vessel size and flap perfusion (skin color, turgor, temperature, and capillary refill). One of the more important aspects specific to flap surgery is localization of the underlying perforators. The intraoperative evaluation of perfusion is essential to accurate flap design. Flap design currently relies on hand-held Doppler for perforator identification and postoperative monitoring. Studies have shown hand-held Doppler has limited accuracy in the design of certain flaps [10–11], as it is highly operator dependent and can be unreliable [12].

NIR fluorescence imaging capitalizes on the deep photon penetration of near-infrared light (700–900 nm) into living tissues. When coupled with the injection of a NIR fluorophore, this technology permits rapid and quantitative assessment of local tissue perfusion at depths less than ≈ 5 mm [13]. NIR fluorescence was first introduced to the field of reconstructive surgery when it was used to evaluate adequacy of perfusion in cutaneous flaps [14]. These early imaging systems, however, failed to gain popularity because of their complexity and the lack of adequate technology for widespread dissemination. Recent technological advances in camera systems, optics, and light sources have led to the design of inexpensive, efficient, and ergonomic NIR imaging systems that can easily be integrated within the clinical workflow. Such an imaging modality offers several attractive features, which include real-time assessment of tissue perfusion, provision of the former without modification to the appearance of the surgical field, and the ability to capture images without contacting the operative field while maintaining sterility.

Our laboratory has previously reported the use of the Fluorescence-Assisted Resection and Exploration (FLARE™) system in combination with intravenous injection of indocyanine green (ICG) for NIR fluorescence angiography [13,15–18]. We have validated its role in the identification of suitable perforators for flap design, compared the location and number of perforators identified against a gold standard (X-ray angiography), assessed its potential for monitoring perfusion of various perforator flaps, and formulated indices for the quantification of venous drainage and arterial inflow [16–19]. Most recently, we successfully translated the technology to patients undergoing deep inferior epigastric perforator flap breast reconstruction by capturing perfusion within hemiabdominal flaps prior to and after elevation, and following microsurgical transfer and inset [20]. We report on the application of NIR fluorescence imaging as a novel approach for preoperative vascular planning and monitoring of tissue perfusion in porcine hemifacial composite tissue allografts. This imaging modality has far-reaching implications for the future of human facial allotransplantation, and serves as a useful addition to the armamentarium of the plastic surgeon.

MATERIALS AND METHODS

Animals

Animal studies were performed under the supervision of Beth Israel Deaconess Medical Center's Institutional Animal Care and Use Committee (IACUC) in accordance with approved institutional protocol #155-2008. Female 35-kg Yorkshire pigs (E. M. Parsons and Sons, Hadley, MA) were used during our study. The pigs were induced with 4.4-mg/kg intramuscular Telazol (Fort Dodge Animal Health, Fort Dodge, IA), intubated, and maintained with 2% isoflurane (Baxter Healthcare Corp., Deerfield, IL). Physiological parameters were monitored during all experiments. Five pigs were used in our study, and 8 hemifacial face transplant flaps were harvested. All animals included in the study were healthy with no previous history of allosensitization. The pigs adhered to a standard dietary regimen, and were provided with water *ad libitum*. Each was housed in its own cage, and

temperature, light, and airflow were controlled. To assess the wellbeing of the animals, an initial physical examination was performed in addition to baseline laboratory tests.

Anesthesia

On the day of surgery, the animals were first premedicated with xylazine (Rompun, Bayer, Shawnee Mission, KS, 2 mg/kg) and ketamine hydrochloride (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, 20 m/kg). Thiopental sodium (Pentothal, Abbott Laboratories, Chicago, IL, 50mg.) and atropine (0.5 mg) were then administered. The pigs were intubated and consequently connected to a Quantiflex ventilator (Matrix Medical, Inc., Orchard Park, NY). They were maintained on anesthesia throughout the procedure using 2% isoflurane (IsoFlo) in oxygen.

Surgical Procedure

The hemifacial flap was designed on each animal including perforators, ear cartilage, and parotid gland (Figure 1A) [21]. The skin was incised to the depth of the platysma muscle in the anterior neck and cleidooccipitalis and cleidomastoideus muscle in the posterior neck, to the depth of masseter muscles in the facial region, and above the skull in the parietal region. A periorbital incision is performed to exclude the orbit and contents from our dissection. In the facial region, the facial nerve was transected. Dissection was performed above the masseter muscle toward the ear as the ear cartilage was transected. To preserve the vascular structures, the parotid gland was included in the flap. In the posterior neck region, the flap was elevated above the trapezius, cleidooccipitalis muscle, and cleidomastoideus muscle. In the anterior neck region, once the platysma muscle and parotid salivary gland were transected, the external jugular vein (EJV) was exposed, which was used as the pedicle vein. Dissection was performed around the EJV toward the peripheral area of the flap. In the retroauricular region, the maxillary vein, a branch of the external jugular vein, was ligated and transected to elevate the flap. To expose the pedicle artery, the tendon of the sternomastoideus muscle was transected and the mandibular salivary gland was elevated. The common carotid artery was identified under the mandibular salivary gland. After osteotomy of the paracondylar process, the common carotid artery and its main branches, the external and internal carotid arteries, were exposed. The vascular distribution of the facial flap was supplied by the superficial temporal artery (STA), one of the two terminal branches of the external carotid artery. On the other hand, the maxillary artery, another branch from external carotid artery, was ligated and transected to elevate the flap completely. The external carotid artery and external jugular vein were used as the vascular pedicle of the flap (Figure 1A,B). A handheld Doppler was used during flap elevation to assist with identification and preservation of the vessels.

Imaging

NIR Fluorescence Imaging System—The FLARE™ imaging system has been previously described by our laboratory [13,15–18]. The imaging system used in this study incorporates subtle changes, as the FLARE™ system constantly undergoes improvements for better intraoperative imaging capability. White light is provided using a RF-plasma light source and carried over the surgical field using a 1/2 inch light guide, projecting above 40,000 lux of white light at the recommended color temperature and field distribution as defined in IEC#60601. NIR excitation light is provided using a multiple laser-diode light source fiber coupled to an optical fiber bundle, illuminating the surgical field with ≈ 10 mW/cm² of 760-nm light over a 15 × 11-cm area [22]. The light source and optics are housed in an articulated arm, which permits positioning anywhere over the surgical field. The working distance from the bottom of the imaging head to the patient is 18 inches, and there is no contact with the patient. The electronics, computer, and monitors are housed

within a portable cart. The surgical field is displayed in real time with color video, and captured images are refreshed at rates up to 15 times per s (15 Hz). NIR fluorescence images are obtained simultaneously via custom-designed optics and software [16]. The acquired images can then be reviewed as a snapshot or cine loop (i.e., movie), and color video and NIR fluorescence images can be displayed separately or merged. In this study, the merged images are formed by selecting an unnatural pseudo-color, green, and converting the grayscale NIR image to this pseudo-color with an overlay on top of the color video image. ICG (1.25 mg; 36 µg/kg) was injected as a rapid bolus in 10 cc of saline.

RESULTS

NIR Fluorescence-Guided Flap Design

Composite tissue flaps were created in all pigs, and ICG was initially injected systemically to identify and locate skin perforators for flap creation. Preoperative identification of perforators and flap perfusion is illustrated by ICG fluorescence (Figure 2). Dissection and creation of the flap was then performed. The flap was then assessed using traditional methods of clinical examination (skin color, turgor, and capillary refill), as well as hand-held Doppler. The current scope of our work addresses technical strategies of the application in addition to developing a normalized scale for quantitative scoring. We used signal-to-background ratio (SBR) for quantitation of fluorescence intensity (FI). SBR was calculated as follows; FI of region of interest (ROI) / FI of background (BG) (Figure 3).

Postoperative flap imaging confirmed perforator preservation and flap perfusion in all flaps (N = 8). Systemic injection of ICG performed with NIR fluorescence detected arterial flow in less than 1 min. Venous outflow and ICG clearance occurred after a period of over 10 min in all vessels and tissue of the composite flap.

Clamping trials were then performed after flap elevation in partial hemifacial flaps. Arterial clamping showed absence of ICG in the hemifacial flap with illumination of adjacent nonoperated skin. Venous clamping showed faint residual ICG with delayed clearance indicative of venous congestion (Figure 2). There was 100% correlation of arterial and venous clamping with clinical examination and Doppler evaluation. Timing between each successive clamping trial was 1 hour in order to allow for adequate clearance of ICG.

Pedicle assessment and flap perfusion could be also performed after flap elevation (Figure 4). This required direct examination and NIR imaging of the vessels rather than the skin surface. Perfusion defects (from arterial and venous clamps) correlated with NIR imaging.

DISCUSSION

The results of our pilot study suggest that NIR fluorescence has the capability to assess perfusion and viability of partial facial CTAs. Accurate prediction of global flap perfusion can often be challenging during surgery. This new modality can potentially reduce complications and improve outcomes. In this study, we performed real-time, intraoperative NIR fluorescence angiography for evaluation of perforator location, as well as flap perfusion on a porcine hemifacial model.

ICG is currently FDA approved for use in determining cardiac output, hepatic function, liver blood flow, and for ophthalmic angiography. ICG angiography with NIR fluorescence has been used clinically in reconstructive surgery for assessment of global flap perfusion [19]. Using a systemic injection of ICG, we found that identification of the underlying perforators is possible prior to surgery thereby facilitating accurate flap design.

The direct identification of the perforators prior to and during flap elevation provided correlation of the vessel location using NIR imaging. Throughout the design and elevation of our hemifacial flap, the FLARE™ imaging system was able to provide dynamic intraoperative guidance. The vascular pedicle as well as the perfused areas of a flap can be visualized. Once the flap is elevated, NIR imaging once again provides confirmation that the flap contains the appropriate perforators, and defines areas of concern for poor perfusion. The vascular pedicle can also be evaluated to determine patency of the vessels; this can be used to evaluate flow and even the presence of a clot. In addition, we were able to show that arterial and venous occlusion and clamping fully correlated with NIR imaging as well as clinical examination and hand held Doppler. In addition, we currently have the ability to quantify perfusion based on the intensity of the NIR fluorescence in the flap. This may provide critical aid in situations of impending flap loss and vessel compromise.

This emergent technology shows promise in the ability to assess tissue perfusion during the elevation and harvest of a composite flap, in addition to monitoring perfusion after microsurgical anastomosis. In this pilot study, NIR fluorescence imaging proves to be highly valuable in partial face transplant design and assessment of perfusion intraoperatively. This provides the framework for future studies, as clinical translation of NIR fluorescence imaging for facial transplantation can be both feasible and safe.

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A

**Elevated Partial
Hemi-Facial Flap**

Vascular pedicle

B

Figure 1.

A: Hemifacial composite tissue allotransplant design. Design of the flap after elevation (left), and during flap harvest with vascular pedicle and anatomic dissection (right). Scale bar = 3 cm.

1B: Diagram of the anatomy and vascular pedicle. Scale bar = 3 cm. (Delete (left) and anatomic dissection (right)).

Figure 2.

Imaging of the hemifacial flap, prior to elevation (top row), after elevation (second row), after isolated arterial clamping (middle row), and after isolated venous clamping (bottom row). Time between each image is shown (right). The imaging system provides surgical anatomy (left column), NIR fluorescence imaging (middle column), and a merged image of the two (right column). The arrow depicts the perforator location within the flap. Scale bar = 3 cm.

Quantitation of Facial Flap Perfusion

$$\text{SBR} = \text{Fluorescence Intensity (FI) of Region of Interest} / \text{FI of Background (BG)}$$

Figure 3. Quantitation of facial flap perfusion over time (seconds). Signal-to-background ratio (SBR) for quantitation of fluorescence intensity (FI). SBR was calculated as follows; FI of region of interest (ROI) / FI of background (BG). The black line represents the flap after elevation with no clamping of the vessels. Arterial and venous clamping is depicted in red and blue respectively. These are compared to a control region of normal skin in gray.

Figure 4. Imaging of the vascular pedicle prior to injection (top row), during the arterial phase (middle row), and during the venous phase (bottom row). Arrows depict carotid artery and external jugular vein. Scale bar = 3 cm.