

Redefining the Anatomic Boundaries for Safe Dissection of the Skin Paddle in a Gracilis Myofasciocutaneous Free Flap: An Indocyanine Green Cadaveric Injection Study

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Summary: The gracilis free flap remains a versatile option in the reconstructive ladder. The flap itself can be harvested with or without a skin paddle. The gracilis myocutaneous free flap, however, is known for partial skin flap necrosis, especially in the distal one-third of the skin island. The gracilis myofasciocutaneous flap has been previously described as a technique to improve perfusion to the skin by harvesting surrounding deep fascia in a pedicled flap. However, limitations to this study required injection of multiple pedicles to demonstrate its perfusion. We demonstrate a novel technique using a cadaveric model that shows perfusion through injection via a single dominant pedicle (medial circumflex) with a large cutaneous paddle (average 770 cm²) with included deep fascia, using indocyanine green and near-infrared imaging. For comparison, we are also able to confirm the lack of perfusion to the distal cutaneous paddle when the fascia is not harvested, correlating with previous findings and ink injection studies. This novel technique is versatile, relatively inexpensive, and can demonstrate perfusion patterns via perforasomes that were otherwise not possible from previous techniques. Additionally, real-time imaging is possible, helping to elucidate the sequence of flow into the flap and potentially predict areas of flap necrosis. (*Plast Reconstr Surg Glob Open* 2018;6:e1994; doi: 10.1097/GOX.0000000000001994; Published online 12 December 2018.)

INTRODUCTION

The gracilis flap remains a versatile option in reconstructive plastic surgery. Originally described as a pedicled flap by Orticochea et al¹ in 1972 for lower extremity reconstruction¹ and later McCraw² for pedicled rotational flap coverage of vaginal defects, its role has continued to evolve.^{3–11} Tamai et al.¹² first described functional restoration using a transferred muscle in a canine model in 1970.¹³ The first reported use of the gracilis as a free muscle flap was described by Harii and Ohmori¹⁴ in 1976

for coverage of head and neck and lower extremity defects. In 1978, Manktelow and McKee¹⁵ described the free gracilis muscle to replace the long flexor musculature of the finger, and later went on to describe its use in replacing forearm musculature, facial reanimation, and anterior lower leg to treat foot drop.^{16–18}

The gracilis flap can be harvested with or without a skin paddle. The traditional gracilis myocutaneous flap was known for partial skin flap necrosis, especially in the distal one-third to one-half of the skin island.² Based on the unreliable distal skin paddle, McCraw² described the musculocutaneous gracilis pedicled flap in 1976, stating “If the gracilis muscle is divided into thirds, the distal limit of the cutaneous flap lies at the junction of the middle and distal thirds of the muscle.” Our understanding of the gracilis myofasciocutaneous flap perfusion, however, was expanded in Whetzel and Lechtman’s 1997 article.¹⁹ They described harvesting a gracilis pedicled flap with adjacent

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fascia to improve vascularity of the skin paddle in vaginal wall reconstruction and used a cadaveric ink injection study to demonstrate separate vascular territories distally over the gracilis muscle.

Despite this, there is still a wide spread belief that the gracilis myocutaneous free flap does not have a reliable distal skin paddle, and that surgeons should be extremely cautious—even avoid harvesting the distal skin altogether. Giordano et al.²⁰ in 1990 described in their anatomic study that the distal 40% of the skin overlying the gracilis muscle belonged to the sartorius muscle system and should be delayed to improve safety of harvest. Wei and Mardini²¹ state in their book of microsurgical flaps that the gracilis free flap “skin island over the distal half of the thigh is not reliable.” Juricic et al.²² performed a meta-analysis and found that 13 of 192 (6.8%) gracilis musculocutaneous flaps had total skin necrosis.

Our positive clinical experience with the free gracilis myofasciocutaneous flap in both free muscle coverage and free functional muscle transfer has led us to seek to redefine the safe boundaries for harvesting a reliable and robust skin paddle.^{23,24} Until now, cadaveric injection techniques were not able to definitively confirm our hypothesis that extended harvest of deep adjacent fascia could reliably perfuse a large skin paddle based off the single dominant pedicle.

We are now able to corroborate our original hypothesis using a cadaveric dissection model. By enlisting a novel cadaveric injection technique for free flap perfusion via the dominant single pedicle using cadaveric blood containing indocyanine green (ICG), we are able to assess skin paddle perfusion with near-infrared imaging technology (SPY-PHI, Stryker, Kalamazoo, MI). We demonstrate our findings in 9 cadaveric gracilis free flaps containing large cutaneous paddles (average 770 cm²: range 507–912 cm² or 39×19 cm: range 36–41×13–24 cm) and accompanying harvested deep fascia. With this same perfusion technique, we also confirm the lack of extended skin paddle perfusion when harvesting the myocutaneous gracilis free

flap without deep fascia harvest, consistent with previous findings by prior authors and injection studies.

MATERIALS AND METHODS

Human Tissue

This cadaveric study was approved as an educational study by the Los Angeles County + University of Southern California Fresh Tissue Dissection Laboratory (LAC + USC FTDL) Steering and Ethics Committee. LAC + USC FTDL housed all of the cadavers used in this study for dissection and were obtained within 2–4 weeks of death according to the specifications set within the Standard Operating Procedures of the laboratory. Cadavers were kept refrigerated until 1 to 2 hours before use.

Cadaver Dissections

Nine fresh cadavers (11 total flaps) underwent dissection between December 2017 and June 2018. Of the 9 cadavers, 4 had bilateral gracilis flaps harvested. In 9 of the 11 flap dissections, we harvested the flaps as gracilis myofasciocutaneous flaps using principles previously described at our institution.^{23,25} In 2 of the 11 flaps, we harvested them as gracilis myocutaneous flaps without the surrounding deep fascia. These flaps were harvested as control flaps during our bilateral dissections. From those findings, we decided to not proceed with harvesting additional gracilis myocutaneous flaps without adjacent fascia.

To visualize the limits of cutaneous perfusion, a large skin paddle was designed to include the underlying fascia of the anterior, medial, and posterior thigh of each cadaver (Fig. 1). Proximally, the landmark used for the apex of the flap was the ischiopubic ramus at the level of the adductor longus tendon origin. The anterolateral boundary of the flap was carried to the mid anterior thigh overlying the vastus medialis muscle, and the posteromedial border as the semitendinosus muscle and tendon. The most dis-

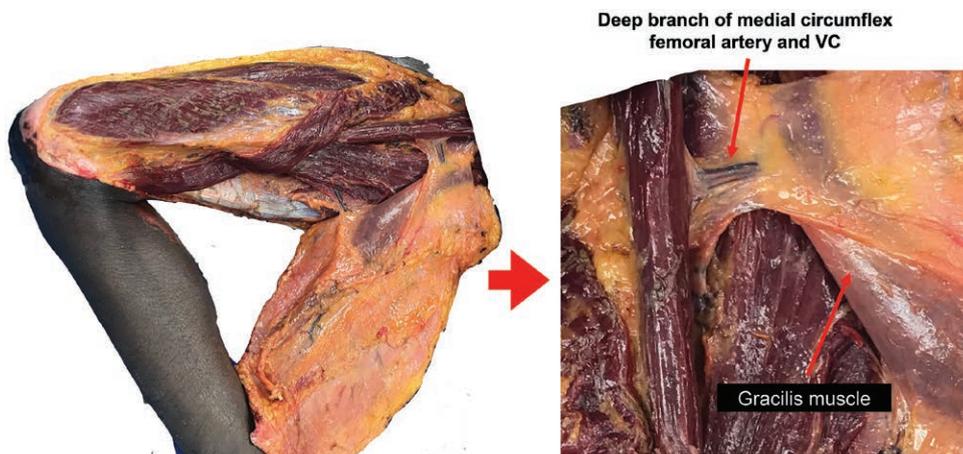


Fig. 1. Extended gracilis myofasciocutaneous flap harvest. The left image demonstrates the large flap dissection boundaries across the anterolateral thigh to the posteromedial thigh, and from the gracilis origin to insertion. The right image shows the gracilis muscle with its preserved fascia and the dominant pedicle dissection before harvest and injection.

tal aspect of the skin paddle was near the insertion of the gracilis at the medial tibial condyle.

The dissection of the gracilis myofasciocutaneous free flap began with incision through the skin and subcutaneous tissue down through the deep fascia using electrocautery, with care to keep the deep fascia attached to the skin using 3-0 interrupted polyglactin 910 suture along the edges in approximately half-inch intervals. This helped decrease the amount of shearing forces that would otherwise disrupt the small blood vessels traversing the skin and fascia. The gracilis tendon was identified distally, transected, and then secured to the skin paddle using polyglactin 910 suture. The dissection proceeded from distal to proximal, with care to harvest all of the deep fascia including the sartorius fascia and the thickened band of fascia at the junction of the adductor longus and vastus medialis. Distal perforating vessels and pedicles were cauterized carefully as the gracilis was elevated. Care was taken to not disturb the fascia on the deep surface of the gracilis muscle. The skin paddle was incised proximally and the gracilis muscle divided near its origin at the ischiopubic ramus using electrocautery. Once the distal aspect of the myofasciocutaneous flap was elevated, we identified the dominant pedicle as it traverses deep to the adductor longus and the branches were carefully clipped with surgical hemoclips. The medial circumflex artery and its venae comitantes were identified and dissected proximally until sufficient length and diameter was available for cannulation. The vessels were sharply divided, and the flap removed from the cadaver. The obturator nerve was also identified and transected proximally.

For dissection of the gracilis myocutaneous flaps as our control, the above steps were taken except the adjacent deep fascia was not harvested with the skin paddle. The fascia surrounding the gracilis muscle was preserved, as in the traditional dissection described by previous authors.^{2,22}

The flaps were harvested and weighed before injection. We recorded flap width, length, and thickness. Using a 22-gauge angiocatheter and microsurgical instruments, the pedicle was cannulated and secured in the vessel using 4-0 silk suture. Water was used to flush out the vessels and to identify any leaks that could be cauterized or clipped before injection of the ICG and cadaveric blood mixture.

Preparation of Indocyanine Green in Cadaveric Blood

We adapted a method previously described by our institution preparation of the ICG in cadaveric blood.^{26,27} The dilution was as follows: 10 mL sterile water used to reconstitute a 25 mg vial of ICG to 2.5 mg/mL. The 10 mL diluted vial of ICG was then added to 500 mL of 0.9% NaCl. This was then added to 500 mL of refrigerated cadaveric blood and then 1 L of the mixture was injected into an empty 1 L IV bag for ease of injection. A 60 mL Leur lock syringe was used with an 18-gauge needle to draw up the cadaveric blood + ICG mixture. This was then injected into the flap via the 22-gauge angiocatheter.

Injection of ICG + Cadaveric Blood into the Gracilis Free Flap and SPY-PHI (Stryker) Imaging

The flap was placed on a Mayo stand on several blue surgical towels. The 60 mL Leur lock syringe filled with



Video Graphic 1. See video, Supplemental Digital Content 1, which demonstrates injection technique in cadaveric myofasciocutaneous gracilis free flaps using cadaveric blood and indocyanine green with SPY PHI (Stryker) near-infrared technology for imaging, <http://links.lww.com/PRSGO/A928>.

ICG + cadaver blood was then secured to the angiocatheter and injected into the flap via the dominant pedicle. The SPY-PHI imaging system (Stryker, Kalamazoo, MI) was then used to record real-time flow of perfusate into the flap. A total of 60–180 mL of ICG + cadaveric blood was injected into the flap [see video, Supplemental Digital Content 1, which demonstrates injection technique in cadaveric myofasciocutaneous gracilis free flaps using cadaveric blood and indocyanine green with SPY PHI (Stryker, Kalamazoo, MI) near-infrared technology for imaging, <http://links.lww.com/PRSGO/A928>]. Once this was completed, imaging was again used to record all edges of the flap to visualize the skin paddle perfusion. We recorded and marked on the skin paddle the area of skin that was perfused. The percentage of skin paddle perfused was then calculated (dimensions of skin paddle lighting up under SPY-PHI imaging/total flap dimensions in centimeters) using pixel counting within Photoshop Creative Cloud (Adobe). The flap was then turned over to assess the muscle and fascia perfusion, and another real-time sequence was recorded. Because of the sensitivity of the near-infrared imaging, special care was taken not to contaminate the surfaces of the flap or cadaver when injecting the ICG with cadaver blood, including frequent change of gloves; keeping contaminated instruments separate; and imaging the flap before, during and after injection.

Statistics

The collected data were statistically analyzed using the 2-sided Student *t* test function in Microsoft Excel (2018) for Mac (Microsoft, Redmond, Wash.).

RESULTS

Cadaveric Flap Dissection Harvested Size

Nine extended gracilis myofasciocutaneous free flaps containing deep fascia had an average harvested size of 770 cm² (range, 507.0–912.0 cm²) or 39 × 19 cm (range, 36–41 × 13–24 cm). The average flap thickness was 2.1 cm (range, 1.1–3.0 cm). The control gracilis myocutaneous

Table 1. Myofasciocutaneous Gracilis Free Flaps

Myofasciocutaneous Gracilis Free Flaps						
Flap #	Laterality	Total Flap Length (cm)	Total Flap Width (cm)	Total Flap Surface Area (cm ²)	Perfused Flap Surface Area (cm ²)	Percentage of Flap Perfused (%)
1	Right	36	20	720	720	100
2	Right	40	17	680	680	100
3	Right	38	24	912	912	100
4	Right	39	13	507	404	80
5	Right	41	21	861	388	45
6	Right	40	18	720	361	50
7	Left	39	22	858	691	81
8	Right	36	22	792	654	83
9	Right	40	22	880	622	71
Average		38	20	770*	603	79†
Control Myocutaneous Flaps						
1	Left	40	21	840	242	29
2	Left	36	22	792	243	31
Average		38	21	816*	242	30†

The total flap dimensions were recorded and then perfused via the single dominant pedicle using ICG and cadaveric blood. The area perfused was recorded and percentage of flap perfusion calculated. For comparison, 2 control myocutaneous flaps are listed here. There was no significant difference in the sizes of the harvested flaps in each group ($P = 0.64$), but there was a statistically significant difference in percentage of flap perfused when comparing myofasciocutaneous to myocutaneous gracilis free flaps ($P = 0.005$).

* $P = 0.64$;

† $P = 0.005$.

flaps were harvested with a comparable average size at 816 cm² or 38 × 21.5 cm. There was not a significant size difference of the harvested control flaps when compared with the myofasciocutaneous flap sizes ($P = 0.64$). Average weight of the flaps was 674 g (range, 317–1,268 g) before injection of ICG + blood and 749.0 g (range, 368.8–1,392 g) after injection (Table 1 and Fig. 2).

Skin Paddle Perfusion

The average percentage of skin paddle perfusion for the gracilis myofasciocutaneous free flap with complete deep fascia harvest was 79% (range, 45.0–100%) or 603 cm² (range, 361–912 cm²) or 31.7 cm (range, 20–40 cm) × 16.6 cm (range, 12–24 cm; Figs. 3, 4). For the control gracilis myocutaneous free flaps, the percentage of skin paddle perfusion was 30% (range, 29–31%) or 242 cm² (range, 242–243 cm²) or 24.2 × 10 cm. This is a significant difference of over 263% ($P = 0.005$) in the

amount of skin paddle perfused in the gracilis harvested with extended fascia compared with the control myocutaneous flaps without deep fascia (Fig. 5 and **Supplemental Digital Content 1**).

DISCUSSION

The gracilis flap has several advantages, including ease of harvest; reliable dominant pedicle; pedicle length and size (average 1.5 mm); overall length of flap (strap muscle); minimal donor-site morbidity; well-hidden scar; ability to be used as a free or pedicled flap; has a large, long (8–10 cm) accompanying motor nerve (obturator) for free functional muscle transfer; 2 venae comitantes for excellent outflow (average diameter of 2.0 mm); a useful tendon for fixation and inset. Additionally, the obturator nerve has 2 separate fascicles that allow for separate reconstruction of individual flexors during gracilis free functional muscle transfer in the upper extremity.¹⁸

However, there are disadvantages to the myocutaneous gracilis flap that were originally described, especially its unreliable skin paddle in the distal one-third to one-half of the skin overlying the muscle. Several reports have demonstrated partial flap necrosis when basing the entire flap off the medial circumflex artery and its accompanying venae comitantes.^{20,22,28–30} Core et al.³¹ even went so far as to use a known perforator off the superficial femoral artery system as a second free flap anastomosis during surgery to ensure adequate flow to the entire skin paddle.

In light of this, Whetzel and Lechtman¹⁹ described the gracilis myofasciocutaneous pedicled flap, using cadaver studies to harvest the surrounding fascia with the skin and muscle, and serial injection of ink into defined pedicles (profunda femoris and superficial femoral arteries) of the gracilis muscle to demonstrate improved perfusion into the skin paddle, even distally for use in rotational coverage for vaginal wall defects.¹⁹ However,

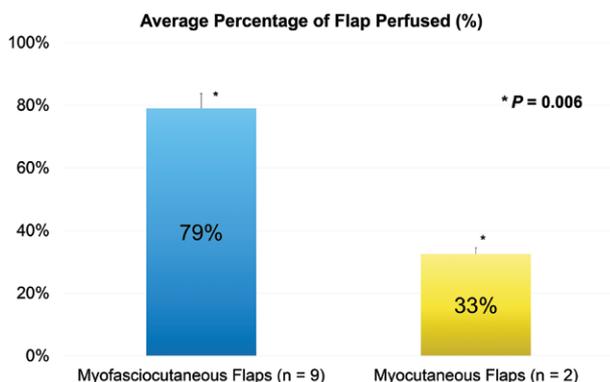


Fig. 2. Average percentage of flap perfused. This graph demonstrates the difference in average surface area perfused in the myofasciocutaneous and the myocutaneous gracilis free flaps after ICG injection with cadaveric blood. There was a significant difference in total flap perfusion, $P = 0.006$.

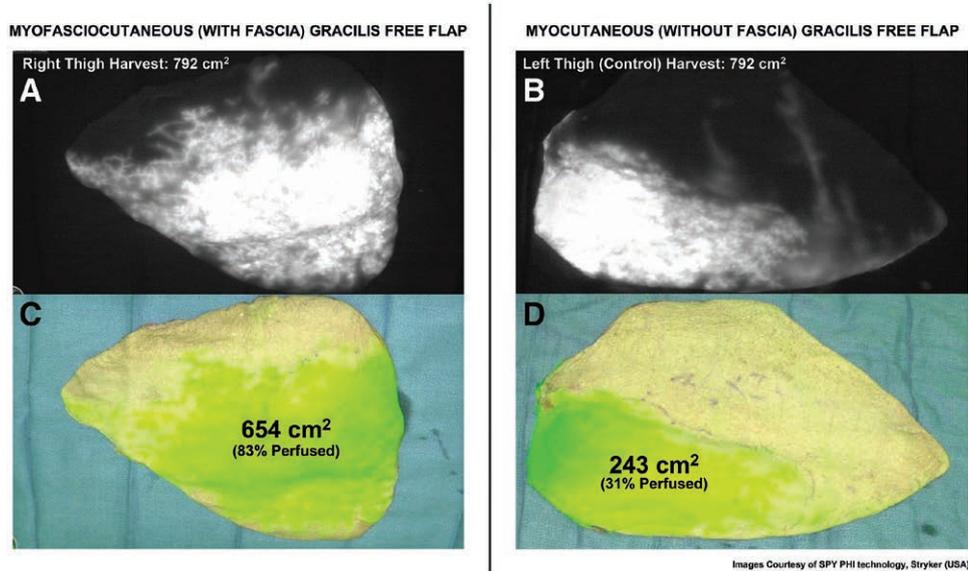


Fig. 3. Comparison of extended myofasciocutaneous gracilis flap to myocutaneous (control) gracilis flap perfusion following ICG injection with cadaveric blood into the single dominant pedicle. In these 2 flaps, they were harvested from the same cadaver with identical sizes (792 cm²). A, SPY PHI imaging (Stryker) demonstrates area of ICG and blood perfusion in white. Areas not perfused are in black. B, Area of control flap perfused. C, SPY PHI Imaging (Stryker) demonstrates area on the skin paddle surface of perfusion, for a total of 654 cm², or 83% of the total flap surface area. D, For comparison, the control flap without extended fascia harvest only demonstrated perfusion to 243 cm², or 31% of the total surface area.

MYOFASCIOCUTANEOUS GRACILIS FREE FLAP ZONE OF SKIN PADDLE PERFUSION



Fig. 4. The green highlighted zone is the superimposed area that demonstrated perfusion in the harvested extended gracilis myofasciocutaneous free flap with deep fascia on near-infrared imaging. Nearly the entire flap demonstrated perfusion through the dominant pedicle (38 × 22 cm).

this study was limited as it was not able to demonstrate perfusion of the entire cutaneous paddle through injection of a single pedicle proximally, as would be necessary in free flap reconstruction.

With the era of microsurgery and perforasomes, new techniques are becoming available to assess flap perfusion, both in vivo and in situ. ICG fluorescence angiography is being used in breast reconstruction, free flap, and abdominal surgery to assess intraoperative perfusion of

tissues. Recently, 1 feasibility study demonstrated the use of indocyanine green injected with methylene blue in a cadaver model to evaluate perforasomes (ie, arterial supply from a single pedicle to skin) of the descending genicular artery and the superficial circumflex femoral artery. They concluded that ICG fluorescence angiography is a potentially useful tool in perfusion studies for the visualization of perforasomes, especially when compared with conventional dyes.³²

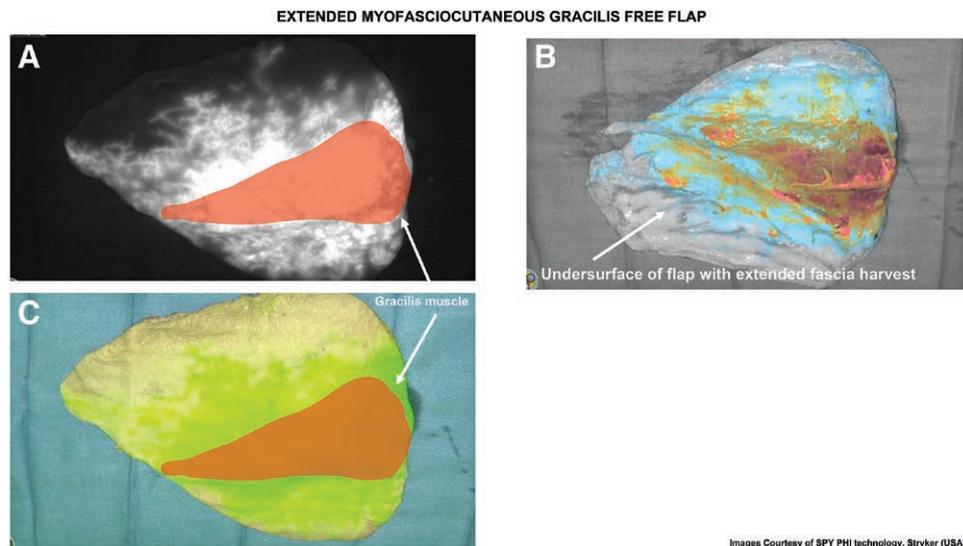


Fig. 5. Extended myofasciocutaneous gracilis free flap. A and C, White and green areas using near-infrared imaging demonstrate skin perfused with ICG and cadaveric blood (827.4 cm²). Red highlighted area shows where the gracilis muscle is in relation to the area perfused. B, SPY PHI imaging (Stryker) demonstrates intensity of ICG present in the undersurface of the flap. The gracilis muscles is also visible here, in the dark reddish brown color, with adjacent fascia perfusion in light blue.

We have previously published our technique for gracilis myofasciocutaneous flap harvest in clinical implementation.²⁵ To date, there are no published studies that look at the perfusion of a gracilis myofasciocutaneous free flap through a single dominant pedicle that demonstrates sufficient perfusion to a large skin paddle along the entire course of the muscle. Giordano's anatomic study used methylene blue to inject the dominant proximal pedicle in 10 cadavers and found a skin paddle area of 16.5 cm in length by 11 cm in width. The distal 40% of the gracilis muscle did not consistently stain with dye. It was also noted that the skin paddle proximally was typically twice as wide as the muscle.²⁰ Although we did not measure the width of our gracilis muscle specimens, the average width of skin paddle perfused was several times the width of the average gracilis muscle (proximal 4.85 ± 0.2 cm). In the study by Whetzel and Lechtman¹⁹, injection of ink into the medial femoral circumflex vessel stained a cutaneous paddle of 95.3 cm² (range, 36–144 cm²), despite having harvested the adjacent deep fascia. Injecting the other 2 pedicles separately yielded an additional 120 cm² (range, 28–336 cm²).¹⁹ Coquerel-Beghin et al.³³ performed a methylene blue and colored fluid latex injection study on the gracilia and found a mean dimension of skin paddle perfused averaging 11 cm in length and 9 cm in width (mean of 79.5 cm²). Kappler et al.³⁴ reported a study with a skin paddle size of 88.0 cm². For comparison, our study using ICG with cadaver blood perfused an average skin paddle territory of 603 cm² (range, 361–912 cm²). This reveals the limitations of both methylene blue and ink injection studies, along with their limited ability to analyze the gracilis muscle and its deep fascial vascular communications with the skin.

We understand that it is impractical to harvest an extremely large skin paddle with the gracilis muscle, as we did in this cadaveric study. This was a proof of concept for our current clinical practice of taking adjacent deep fascia during free flap harvest and demonstrates that it is technically possible to obtain a safe, viable skin paddle with this method.

Our findings could further increase the versatility of this flap's use, as it potentially provides larger skin paddles for breast reconstruction, large lower extremity defects needing durable coverage, large groin or vaginal wall defects, or even head and neck reconstruction to cover exposed hardware. We know that it is possible to safely harvest a large enough skin paddle (that can be primarily closed longitudinally) from the proximal end of the gracilis muscle to the distal portion of the muscle without any skin paddle loss. Now, we are able to demonstrate how this is possible through the deep fascia perforators using indocyanine green and near-infrared imaging.

Additionally, ICG use eliminates the toxicity of lead oxide-based studies in cadavers,³⁵ and because of its solubility in blood, it can mimic physiologic findings and provide detailed clinical correlations. Ink and latex are limited by their inability to pass through "choke vessels" and cannot stain adjacent vascular territories.⁹ Coupled with near-infrared technology and high-resolution imaging, it is possible to record real-time the course of the vessels as they fill the flap over time using ICG. Some limitations may be related to control of intravascular pressures during injection and the possibility of rupturing capillaries. Despite this, we suggest that indocyanine green with cadaveric blood should become the new gold standard in cadaver-based flap perfusion and perforator studies.

CONCLUSIONS

Wide dissection of the deep fascia beyond the margins of the skin paddle in a gracilis myofasciocutaneous flap proves that the skin paddle over the entire gracilis muscle can safely be harvested. Moreover, it can potentially increase the skin paddle harvested by over two and a half times the traditionally described surface area. Lastly, indocyanine green with cadaveric blood can be an extremely valuable tool for evaluating perforasomes in cadaveric flap dissection models.

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