

Feasibility of Bone Perfusion Evaluation in Cadavers Using Indocyanine Green Fluorescence Angiography

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Summary: Bone perfusion evaluation methods in cadaver studies have yet to be established. The aim of this report was to introduce and validate the feasibility of indocyanine green (ICG) fluorescence angiography for evaluation of bone perfusion in the femoral medial condyle in cadavers. In 4 fresh nonembalmed cadavers (2 female), the descending genicular artery was dissected and carefully cannulated bilaterally. A 10 mL solution containing 5 mL ICG solution and 5 mL methylene blue solution was injected into the descending genicular artery. After the injection, the medial femoral condyle was cut with an oscillating saw. A photograph was taken of the cut ends of the bone. The cut ends of the bones were observed using a near-infrared camera. Images corresponding to the previously taken photographs of the cut ends were captured for comparative analysis. After injection of methylene blue and ICG, the blue dye could be seen in the periosteum in all specimens, but not inside the cortex or the cancellous region of the bone. When observed with ICG fluorescence angiography, however, the cancellous region was highlighted through small perforators penetrating the periosteum. Perfusion inside the medial femoral condyle in cadavers was confirmed using ICG fluorescence angiography. Our method can be especially beneficial in confirming the bone perfusion of a new bone flap based on a particular artery, both in cadavers as well as in patients, because ICG can be injected into specific arteries. (*Plast Reconstr Surg Glob Open* 2017; 5:e1570; doi: 10.1097/GOX.0000000000001570; Published online 20 November 2017.)

INTRODUCTION

Vascularized bone flaps have been used for reconstruction of bony defects when free bone grafts do not meet requirements. The fibula, the iliac bone, and the scapula are commonly used vascularized bone flaps. The use of the medial femoral condyle has been revived lately, especially for treatment of nonunion and avascular necrosis of the bones.¹⁻⁴

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Although the use of new flaps has been reported every year since the introduction of the perforator concept, reports on new vascularized bone flaps have been limited: one of the reasons is that bone perfusion evaluation methods in cadaver studies have yet to be established.

Recently, the lead oxide–gelatin technique has become the gold standard for visualization of vessels in cadavers, yielding a high-quality staining of angiosomes.⁵ In addition to radio-opaque materials, ink injections including India ink and methylene blue have been widely used.⁶⁻⁹ However, whether due to the physical characteristics of the materials or to limitations in their sensitivity, no study has yet evaluated perfusion inside the bone in cadavers. The conventional methods are capable of visualizing the periosteum, but there are no reports on visualization of the cancellous regions. Although this may not be a problem when evaluating a bone flap with small portions of cancellous bone, as in a fibula flap, accurate identification of the perfused region becomes crucial in a bone flap rich in cancellous bones, as in an iliac bone flap.

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The aim of this report was to introduce and validate the feasibility of indocyanine green (ICG) fluorescence angiography for evaluation of bone perfusion in the femoral medial condyle in cadavers.

MATERIALS AND METHODS

In 4 fresh nonembalmed cadavers (2 male, 2 female), the descending genicular artery (DGA) was dissected and carefully cannulated bilaterally in the following approach. An incision was made from a point 1 cm medial to the patella extending proximally to the mid thigh. The vastus medialis was retracted anteriorly, and the DGA was located running atop and into the periosteum of the distal femur. After meticulous dissection of the DGA, a 10 mL solution containing 5 mL ICG solution (ICC-Pulsion, Pulsion Medical System, Germany) and 5 mL methylene blue solution [Methylenblau (C.I. 52015) reinst. Fa. LabChem Röttinger, Germany] was injected into the DGA (Fig. 1). The volume of 10 cc showed most reliable staining of the region of interest in preliminary injection studies. We therefore decided to use 10 cc as standard injection volume for this study. Further dissection followed immediately afterward to avoid unwanted intravascular dissemination of ICG to distant regions. The medial femoral condyle was cut with an oscillating saw on the sagittal plane 2 cm medial to the medial edge of the femoral condyle and on

the axial plane 2 cm superior to the distal edge of the bone. A photograph was taken of the cut ends of the bone. The cut ends of the bones were observed using a near-infrared camera (Sony HD Handycam CM05; Sony Corp., Tokyo, Japan). Images corresponding to the previously taken photographs of the cut ends were captured for comparative analysis.

All cadavers were from body donors who donated their bodies to the Institute of Anatomy for academic purposes. This study was performed according to local ethics regulations.

RESULTS

After injection of methylene blue and ICG, the blue dye could be seen in the periosteum in all specimens, but not inside the cortex or the cancellous region of the bone. However, when observed with ICG fluorescence angiography, the cancellous region was highlighted through small perforators penetrating the periosteum (Fig. 2). Images of arterioles running inside the cancellous bone were also visualized (Fig. 3; see video, **Supplemental Digital Content 1**, which displays a video demonstrating ICG fluorescence angiography. When the cut surface of the bone was observed using a near-infrared camera, the cancellous region was clearly highlighted. An enhanced arteriole was picked up with forceps, <http://links.lww.com/PRSGO/A608>).

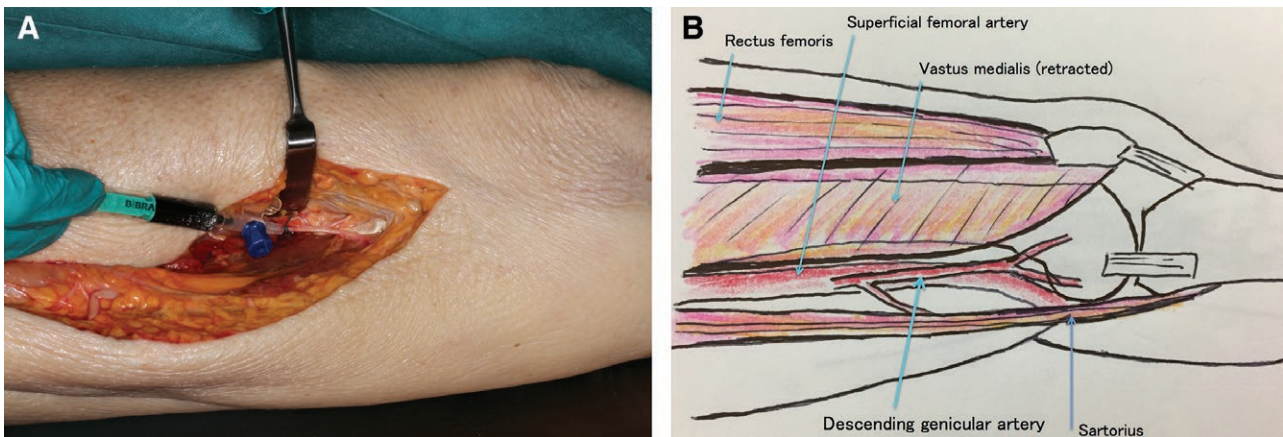


Fig. 1. Dissection and cannulation of the DGA (A) and a schematic illustration (B). A 10 mL solution containing 5 mL ICG solution and 5 mL methylene blue solution was injected into the DGA.

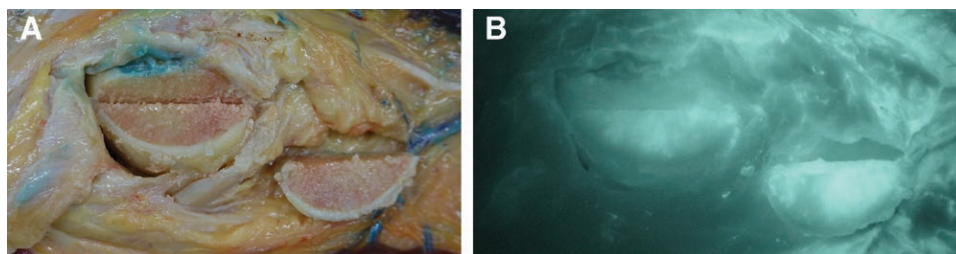


Fig. 2. A, After injection of methylene blue and ICG, the blue dye could be seen in the periosteum, but not inside the cortex or the cancellous region of the bone. B, When observed with ICG fluorescence angiography, the cancellous region was highlighted through small perforators penetrating the periosteum.

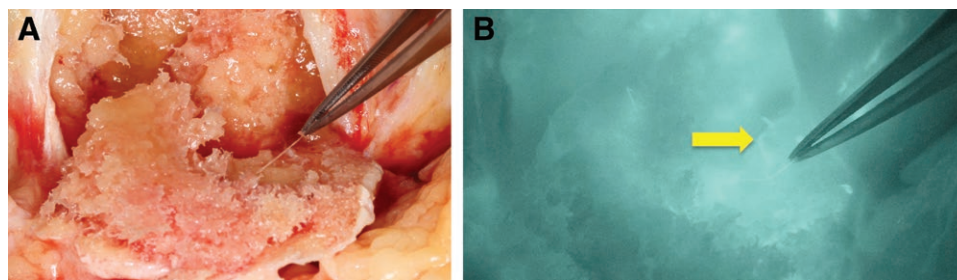
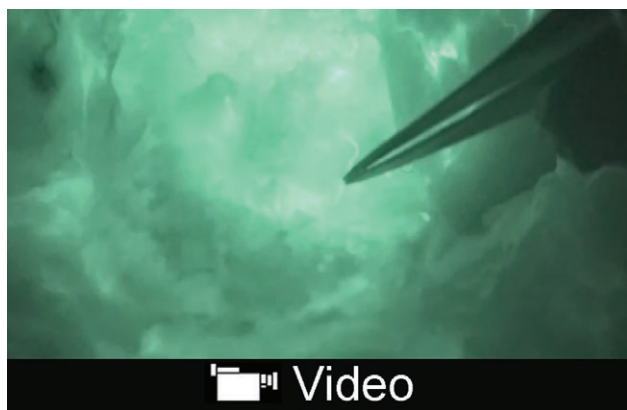


Fig. 3. A, A photograph of the cut ends of the bone after injection of ICG. B, The same region observed using a near-infrared camera. Images of arterioles running inside the cancellous bone were also visualized.



Video Graphic 1. See video, Supplemental Digital Content 1, which displays a video demonstrating ICG fluorescence angiography. When the cut surface of the bone was observed using a near-infrared camera, the cancellous region was clearly highlighted. An enhanced arteriole was picked up with forceps, <http://links.lww.com/PRSGO/A608>.

DISCUSSION

Our study confirmed that ICG fluorescence angiography could visualize bone perfusion in an established vascularized bone flap, such as a medial femoral condyle flap. On the other hand, with methylene blue, no bone perfusion inside the bone could be confirmed. We speculate that since the dye enters the bone via small capillaries, the amount of dye entering the bone marrow was too small to be observed. This can be confirmed by the fact that the green color of the ICG solution could not be visualized without the use of the near-infrared camera. In addition, this may explain why the maximum vascular territory was underestimated in previous cadaveric studies on flap perfusion.¹⁰

Limitations in cadaveric studies are inevitable. One of them could be the oversensitivity of this method, which may overestimate rather than underestimate the maximum vascular territory of vascularized bone flaps. For more accurate evaluation of bone perfusion, corresponding clinical studies using larger number of cadavers are required to address the issue.

There have been previous reports on the use of ICG fluorescence angiography for confirmation of bone perfusion: 1 in clinical settings and the other using porcine models.^{11,12} However, to the best of our knowledge, we found no report on the use of ICG fluorescence angiography for evaluation of bone perfusion in cadavers. Our method can be

especially beneficial in confirming the bone perfusion of a new bone flap based on a particular artery, both in cadavers and in patients, because ICG can be injected into specific arteries. For example, in this series, we can conclude that the fixed portion of the femoral medial condyle (2 cm medially from the medial edge and 2 cm superiorly from the distal edge) was perfused by the DGA. ICG's capability to evaluate perfusion in the cancellous bone provides another edge over other dyes in defining the perfused regions in a bone flap rich in cancellous bones. ICG fluorescence angiography can also be used to evaluate the specific perfused region, the "bone angiosome," in known bone flaps.

CONCLUSIONS

Perfusion inside the medial femoral condyle in cadavers was confirmed using ICG fluorescence angiography. ICG fluorescence angiography is potentially a powerful tool in the development of new vascularized bone flaps.

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REFERENCES

1. Iorio ML, Masden DL, Higgins JP. Cutaneous angiosome territory of the medial femoral condyle osteocutaneous flap. *J Hand Surg Am.* 2012;37:1033–1041.
2. Endara MR, Brown BJ, Shuck J, et al. Torsional stability of the femur after harvest of the medial femoral condyle corticocancellous flap. *J Reconstr Microsurg.* 2015;31:364–368.
3. Wong VW, Higgins JP. Medial femoral condyle flap. *Plast Reconstr Surg Glob Open.* 2016;4:e834.
4. Bürger HK, Windhofer C, Gaggl AJ, et al. Vascularized medial femoral trochlea osteochondral flap reconstruction of proximal pole scaphoid nonunions. *J Hand Surg Am.* 2013;38:690–700.
5. Bergeron L, Tang M, Morris SF. A review of vascular injection techniques for the study of perforator flaps. *Plast Reconstr Surg.* 2006;117:2050–2057.
6. Palmer JH, Taylor GI. The vascular territories of the anterior chest wall. *Br J Plast Surg.* 1986;39:287–299.
7. Taylor GI, Townsend P, Corlett R. Superiority of the deep circumflex iliac vessels as the supply for free groin flaps. Clinical work. *Plast Reconstr Surg.* 1979;64:745–759.
8. Stokes RB, Whetzel TP, Sommerhaug E, et al. Arterial vascular anatomy of the umbilicus. *Plast Reconstr Surg.* 1998;102:761–764.

9. Taylor GI, Palmer JH. The vascular territories (angiosomes) of the body: experimental study (angiosomes) of the body: experimental study and clinical applications. *Br J Plast Surg*. 1987;40:113.
10. Acland RD. Outlining a free flap exactly. *Plast Reconstr Surg*. 1977;59:113.
11. Nguyen JT, Ashitate Y, Buchanan IA, et al. Bone flap perfusion assessment using near-infrared fluorescence imaging. *J Surg Res*. 2012;178:e43–e50.
12. Valerio I, Green JM 3rd, Sacks JM, et al. Vascularized osseous flaps and assessing their bipartate perfusion pattern via intraoperative fluorescence angiography. *J Reconstr Microsurg*. 2015;31:45–53.