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Photoacoustic Tomography Shows the Branching Pattern of Anterolateral Thigh Perforators in Vivo

Abstract

Background

The distal branching pattern of perforators is associated with thin anterolateral thigh (ALT) flap failure. The purpose of this study is to investigate the feasibility of using photoacoustic tomography (PAT) as a diagnostic imaging modality to identify ALT perforators and their branching patterns in the subcutaneous layer.

Methods

Ten thighs in five healthy males were studied. The anterolateral aspect of the mid-thigh was examined using PAT. The correlation between PAT and ultrasound (US) findings was evaluated. To determine the detectability of PAT by depth, the depth of vessels in the stem portion was compared to the depth of the deep fascia measured by US. Branching patterns of vessels in the adipose and suprafascial layers were evaluated by three-dimensional observation.

Results

A total of 18 perforators were visualized by PAT. PAT and US had comparable diagnostic potential for the detection of perforators. PAT visualized microvessels in the subcutaneous layer, especially those in oblique or horizontal orientations. The estimated mean depth of visualized vessels was 9 mm; the maximum was 13
mm. There was a strong correlation between the depth of visualized vessels in the stem portion and the depth of the deep fascia. Three-dimensional observation of PAT images showed the branching morphology of perforators.

**Conclusions**

This study showed the applicability of PAT to identification of the branching patterns of ALT perforators in vivo although limited visualization of subfascial vessels is a technical issue. We believe PAT has the potential to be a new imaging modality for thin ALT flap surgery.

**Key words**

Anterolateral thigh, imaging, perforator, photoacoustic tomography, thin flap
Introduction

Flap thinning\(^1\) is technically challenging. In particular, a high rate of failure has been reported for the thin anterolateral thigh (ALT) flap.\(^2\) Previous experimental studies have suggested that skin perfusion is changed during thinning as a result of injuries to vessels in the suprafascial layer.\(^3\) Thus, an appreciation of the individualized vascular anatomy of the thigh is essential for thin flap planning; however, suitable imaging methods that can be used safely and easily \textit{in vivo} are lacking. Photoacoustic tomography (PAT) is an emerging vascular imaging modality that has been used in basic research and clinical practice, which allows for visualization of subcutaneous vessels on the basis of the photoacoustic mechanism. Energy from a near-infrared pulse laser is introduced into hemoglobin. As a result, red blood cells become swollen and release ultrasonic waves, which allows vessels to be visualized without contrast agents.\(^4\) The purpose of this study is to investigate the feasibility of using PAT as an imaging tool for identifying branching vessels in the subcutaneous layer associated with the thin ALT flap.

Materials and methods

After approval by our institutional ethics committee and clinical trial registration, we recruited five healthy male adults (mean age, 41 years; mean body mass index, 23.1 kg/m\(^2\)) for this study. PAT was performed bilaterally in all subjects.

The PAT we employed was the same system that was used for a previous study on breast cancer
angiogenesis. In that study, the estimated maximum depth of vessels visualized was 27 mm. The system comprised a Ti:sapphire laser transmitter and a tabletop scanning platform (Supplemental Digital Content Figure 1). The wavelength of the laser was 797 nm. The maximum laser power was set to less than the half the maximum permissible exposure recommended by the American National Standards Institute. Multiple ultrasonic transducers were placed into a hemispherical cup built into the platform. Each subject was placed on the platform in the semi-prone position with the anterolateral surface of the mid-thigh facing the cup. The space between the skin and the cup was filled with de-aired water for smooth transmission of ultrasonic waves. Each scan took approximately 2 minutes for a 14 × 14 cm² region. Acquired data were processed three-dimensionally using laboratory-made imaging software. Visualization of vessels in the adipose or suprafascial layer was performed using the following steps. First, the surface of the skin was identified automatically using signals from the dermis. Next, color gradation was applied to the images according to the depth from the skin surface. Subdermal venous networks in the most superficial layer were visualized in blue. Finally, vessels in the adipose layer or deeper were visualized independent of the venous networks by semi-automatically cropping out the superficial layer that included the networks (Fig. 1).

To study the diagnostic potential of PAT, the correlation between the locations of perforators identified on PAT versus conventional ultrasound (US) was evaluated. To determine the detectability of PAT by depth, the depth of vessels in the stem portion was measured and compared to the depth of the deep fascia measured using US. The orientation of perforators in the stem portion was also evaluated. The branching morphology
of vessels in the adipose and suprafascial layers was evaluated using three-dimensional observation.

**Results**

PAT visualized the branching vessels of perforators in the subcutaneous layer in all subjects. PAT showed 18 perforators and US showed 15 perforators, all of which were visualized by PAT. However, discrepancies in location of less than 10 mm were frequently observed. The estimated mean depth of vessels visualized by PAT was 9 mm; the maximum was 13 mm. Unlike US, PAT does not visualize the soft tissue components. Comparison of the depth of a vessel visualized by PAT with the depth of the deep fascia visualized by US at the corresponding site revealed a strong correlation (Pearson's correlation = 0.8). Among the 18 perforators, nine had an orientation of approximately 30 degrees relative to the horizontal plane and the remaining nine had an orientation of 30–60 degrees.

Three-dimensional observation of PAT images showed the branching pattern of perforators. Fourteen Type I and four Type II perforators according to a classification system proposed by Schaverien et al.⁶ were observed. Figure 2 is a representative image showing a Type II perforator with horizontal branches visualized in the deepest layer and oblique branches more superficially (see also Supplemental Digital Content Video 1). Such branching vessels in the suprafascial layer were almost always undetectable by Doppler mode of US (Supplemental Digital Content Figure 2).
Discussion

This is the first report to show the feasibility of using PAT imaging to visualize the branching morphology of perforators in the subcutaneous layer in the anterolateral thigh. The three-dimensional nature of PAT imaging contributed to identification of the branching morphology of perforators.

Compared with conventional US, PAT visualized vessels to the level of the deep fascia. By contrast, vessels deeper than the fascia were undetectable, suggesting that the deep fascia could be a factor limiting the depth performance of PAT. The lack of anatomical references of PAT imaging is a barrier to its intraoperative use. Cooperation of US with PAT would resolve these issues.

PAT has two other technical limitations. First, there are currently no established methods to distinguish arteries from veins using PAT, thus a triple bundle appearance was common, especially in vessels near the stem portion (Fig. 2). The subdermal venous networks were distinguishable owing to their superficial location and polygonal morphology. Second, the orientation of a vessel affects its visualization by PAT. This is known as the limited view problem. More vertically distributed vessels are reportedly less likely to be visualized.

Indeed, no vertical vessels were observed in this study, although vessels at an angle of approximately 60 degrees were visualized. This might be the reason why no Type III perforators were found in this study.

Although further technical refinements are needed for PAT to establish its clinical utility, we believe that PAT would be a promising new imaging modality for thin ALT flap surgery.
Authors’ role/participation in the authorship of the manuscript

SS conducted the study and IT performed the experiments. HS developed imaging software. IT, AY and SS analyzed the data. All the authors contributed to writing of the manuscript.

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References


Figure legends

Fig. 1
Anteroposterior photoacoustic tomography images in the all-layer (left) and deep-layer views (right). The color corresponds to the depth from the skin surface. Note that the blue superficial venous networks in the all-layer image are not shown in the deep-layer image; deep branching vessels in red are visualized separately. Scale bar = 20 mm.

Fig. 2.
Photoacoustic tomography projection images showing a Type II branching pattern in a perforator. Insets are lateral views for the regions indicated in the anteroposterior image. In the inset A–B, a perforator bifurcates into vessels coursing horizontally and oblique to the skin surface. In the inset C–D, a branch coursing obliquely from the bifurcation changes its direction, becoming more horizontal in the distal portion. Note the triple bundle appearance of the branches (arrowhead). Scale bars = 10 mm. See also Supplementary video 1.

Supplemental Digital Content Figure 1
Schematic of the photoacoustic tomography system.
Images show a difference in visual performance between PAT and Doppler ultrasound. Note that the oblique portion of the perforator system in the subcutaneous layer shown by PAT is not visualized by Doppler US (arrowhead).

The video highlights three-dimensional visualization of the branching vessels in Fig. 2. The color of the vessels corresponds to the depth from the skin. X, Y, and Z coordinates indicate the medial, distal, and posterior directions, respectively.
Supplemental Digital Content Figure 1

Supplemental Digital Content Figure 2