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OCT-based microangiography for reactive hyperemia assessment within residual limb skin of people with lower-limb loss

Utku Baran^{1,2,*}, Eric Swanson^{1,*}, Joan E. Sanders¹, and Ruikang K. Wang¹

¹Department of Bioengineering, University of Washington, Seattle, WA, USA

²Department of Electrical Engineering, University of Washington, Seattle, WA, USA

For individuals with lower-limb loss, the residual limb skin is subjected to continuous, repetitive normal and shear forces at its interface with the prosthetic socket. As a result, many lower-limb amputees suffer from frequent skin irritation and breakdown [1,2]. These injuries are often painful, reduce mobility, and in severe cases can lead to more proximal amputations [3]. Visual inspection remains the current gold standard for evaluating skin health in individuals at risk for skin breakdown [4]. Skin temperature measurements have also been suggested as an alternative skin evaluation technique, however this method has not been extensively adopted in clinics [5, 6]. Although these methods are non-invasive, they can be subjective and are vulnerable to many confounding factors. There is a need for more accurate skin assessment techniques.

Post-occlusive reactive hyperemia (PORH) is an important protective mechanism of skin that enables it to recover from an arterial occlusion. It has been suggested that the measurement of the skin's PORH response following an applied physical stress can serve as a biomarker for skin health by indicating the tissue's blood reperfusion capability [6]. PORH measurement has the potential to provide valuable insight into the assessment of tissue health for individuals with lower-limb loss, whose skin is subjected to repetitive, occlusive stresses due to use of a prosthesis.

Optical coherence tomography (OCT) is an emerging non-invasive imaging modality that can provide high-resolution volumetric images of *in vivo* tissue structure and microvasculature. Here, we explore the feasibility of using OCT-based microangiography (OMAG) [7, 8] as a method for non-invasive PORH monitoring within *in vivo* human skin at highly loaded areas of the residual limb of individuals with trans-tibial limb loss. A proof-of-concept for this measurement technique is also presented in able-bodied participants over multiple days. With further development this technique could potentially augment visual inspection and improve skin care for people with lower-limb loss by non-invasively identifying microvascular changes within the skin.

Correspondence: Dr. Ruikang K. Wang., Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle, WA, 98195, USA. wangrk@uw.edu.

*Equally contributed authors.

Disclosure

The authors state no conflict of interest.

A real-time swept source OCT system [9] was used in this study to acquire volumetric images of *in vivo* limb skin of able-bodied individuals and those with trans-tibial amputation. The system operated on a central wavelength of 1310 nm with an A-line rate of 100 kHz, and with an extended imaging range (12 mm in air). The field of view was set either to 5 mm $\times$ 5 mm with 512 A-lines per B-frame or to 2.5 mm $\times$ 2.5 mm with 256 A-lines per B-frame for faster data acquisition. Following a 10-minute acclimation period, PORH responses of lower-limb skin in able-bodied participants and in participants with limb loss were induced with 5 minutes of walking in place using a stiff foot-offloading brace (Fig. 1A) or prosthetic limb, respectively. After brace or prosthesis removal, OMAG images were captured at multiple time-points for 10–15 minutes at a location that was previously determined to be an area of high stress due to the presence of skin redness after doffing the device (Fig. 1B). The blood flow responses were compared by quantification of the vessel density within the maximum intensity projection (MIP) map of each image (Fig. 1C).

The study was reviewed and approved by a University of Washington Institutional Review Board and informed consent of all participants occurred prior to participating in research activities. Able-bodied participants (N = 2) were healthy males, ages 28 and 24, between 80–85 kg. Participants with limb loss (N = 2) were male, each with a trans-tibial amputation that occurred >7 years prior to the study and each having a lock and pin style suspension prosthesis. Participant 1 was 69 years of age, 93 kg, with a history of high blood pressure, and experienced his amputation as a result of traumatic injury; and Participant 2 was 60 years old, 86 kg, with a history of high blood pressure and diabetes mellitus with neuropathy and experienced his amputation secondary to diabetes.

For the first able-bodied participant, the OMAG MIP images of skin at the high-stress region exhibited a distinct PORH response. A clear increase in blood flow over the first 1–3 minutes after doffing occurred, as vessels dilated and more capillaries filled, followed by a gradual decrease and re-stabilization near baseline, where blood flow was detectable primarily in larger vessels only (Fig. 2A). To quantify this PORH response, we plotted the vessel density values for each image over the 5 trials (Fig. 2B). In each trial, the average vessel density quickly increased significantly (to 4.4–7.3 times baseline), and then gradually returned to the baseline after 10–15 minutes, which is consistent with a typical PORH response of a healthy microvascular network. Peak blood flow was reached 3.5 minutes or less post-doffing. As daily stress sessions continued, the return to baseline occurred more rapidly in each subsequent session.

It is important to note that OMAG images for the first able-bodied participant were obtained using a glass cover slip and mineral oil. While this protocol improved image quality thus providing more accurate blood flow information, it required preparation time prior to collecting the first image. This made it difficult to observe the exact timing of the PORH peak after load removal, which may be of clinical interest. For OMAG imaging of the second able-bodied participant and the participants with lower-limb loss we developed a faster protocol by eliminating the glass and oil and decreasing the imaging field to 2.5 $\times$ 2.5 mm; however, several seconds were still lost during doffing of the brace or prosthesis and the arrangement of the probe on the skin.

The results from OMAG imaging of an able-bodied participant and the highly loaded skin of a participant with a lower-limb amputation are shown in Fig. 3. Fig. 3A shows that the second able-bodied participant had a similar PORH response compared to the first able-bodied participant (Fig. 2). During each of 4 trials, this individual exhibited an increase in vessel density to 1.5–2.3 times baseline, occurring 60–90 seconds after doffing. Vessel density returned to baseline 3–5 minutes after doffing. Unlike the first participant, this participant did not demonstrate a more rapid return to baseline as subsequent stress sessions were performed.

The skin of the first participant with lower-limb loss (Fig. 3B) had images with smaller, denser vessels and a faster PORH return to baseline as compared to the able-bodied participants (Fig. 2, Fig. 3A). The quantification of the OMAG images from participants with limb loss (1 trial each) indicated an increased peak vessel density to 2.6 times baseline approximately 21 seconds after doffing (3 times faster than the fastest of the two able-bodied participants) for participant 1, and a peak increase to 1.6 times baseline occurring 78 seconds after doffing for participant 2. Moreover, the vessel density returned to baseline 2.3 times faster for participant 1 versus participant 2 and greater than 3.8 times faster than the able-bodied participants.

The results of this preliminary study demonstrated that OMAG can provide qualitative morphological vessel information as well as quantitative measurements of blood perfusion in the dermis of lower limb skin following an applied stress. Furthermore, the timescale and magnitude of the PORH response quantified by OMAG vessel density were consistent with previous PORH studies using alternative measurement techniques [10]. OMAG offers advantages over methods previously employed to measure PORH by allowing for microvascular visualization and depth information.

It was interesting to find that as the first able-bodied participant completed more daily stress sessions, distinct trends could be noted in his PORH response. The blood flow return to baseline following the PORH peak became faster with subsequent sessions. Similarly, the time-to-peak appeared to decrease with each trial; though by trial 3 this reached the time limit required by the instrument to obtain the first image. One possible explanation for these trends is that repeatedly stressing the tissue to a threshold level using the stiff brace may have induced physiological adaptation in limb skin, making it more resistant to continual loading. This follows the Physical Stress Theory described by Mueller in 2002 which states that tissue responds to physical stress through biological changes that are dependent on the magnitude and type of stress applied to the tissue [11]. Skin adaptation studies suggest that one of these biological changes may be the alteration of collagen architecture to allow skin to bear more load with less damage. In a 2001 study by Sanders and Goldstein, electron microscopy of porcine skin following repetitive compressive and shear stresses revealed that collagen fibril diameters increased in stressed versus unstressed areas, though overall collagen content remained constant [12]. This phenomenon could help explain why limb skin may become more tolerant to the stresses acting on them; however other physiological mechanisms are likely to be involved as well.

Although the increasing rate of PORH recovery was an interesting finding, it must be noted that this effect was seen in only one participant, and that these trends were not noticeable even in the second able-bodied participant within this study. This could have been due to the differences in imaging protocols used, differences in stress magnitude that may have occurred at the region (as suggested by the less-pronounced PORH in participant 2), or the absence of adaptation in the second able-bodied participant. Further studies are warranted and should include a more rapid imaging protocol that will allow for a more thorough characterization of the entire PORH response. Additionally, using more consistent imaging and stress application protocols across all participants will help to better identify and evaluate trends.

In summary, these experiments demonstrated the utility of using OMAG to study PORH responses within residual limb skin of individuals with lower-limb loss. It is important to emphasize that all observations discussed here are based on preliminary data and must be verified with a larger study group. If future studies are successful, OMAG has the potential to enhance the current understanding of skin problems caused by the use of prosthetic devices. Moreover, the OMAG technique could be similarly applied to the study of other stress-related skin complications such as diabetic foot ulcers or skin injuries affecting individuals with spinal cord injury.

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Figure 1.

OCT system and imaging protocol for able-bodied skin. (A) Able-bodied participant wearing foot off-loading brace to stress limb skin. (B) Close-up picture of imaging probe on skin. (C) Volumetric image and maximum intensity projection (MIP) of OMAG imaging results from human skin *in vivo* with 5 mm $\times$ 5 mm field of view.

Figure 2.

(A) MIP images of OMAG show PORH response of skin microvasculature over 15 min following 5 min walk-in-place stress on able-bodied participant using foot off-loading brace (5 mm × 5 mm). (B) OMAG vessel density change following stress over 5 trials on consecutive days. Each trial shows a similar rapid rise to initial peak and a progressively faster return to baseline.

Figure 3.

MIP images of OMAG (2.5 mm $\times$ 2.5 mm) using faster imaging protocol, showing PORH in highly stressed regions of skin after a 5 min walk-in-place using a stiff brace or prosthetic socket. (A) OMAG images from the second able-bodied participant, showing similar results to the first able-bodied participant of Fig. 2. (B) Skin of the first participant with lower-limb loss has a different microvascular morphology and faster PORH recovery compared to the able-bodied participant. Similar results were obtained for the second participant with limb loss except the PORH response was less pronounced.