Original Article

Dynamic Optical Coherence Tomography Is a New Technique for Imaging Skin Around Lower Extremity Wounds

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Abstract

Chronic wounds such as venous leg ulcers invariably heal slowly and recur. In the case of venous leg ulcers, poor healing of chronic wounds is variously attributed to ambulatory hypertension, impaired perfusion and diffusion, presence of chronic inflammation at wound sites, lipodermatosclerosis, and senescence. The aim of this study was to investigate whether a new technique, optical coherence tomography (OCT), which permits imaging of blood capillaries in the peri-wound skin, can provide new insights into the pathology. OCT and its recent variant, dynamic OCT, permit rapid noninvasive depth-resolved imaging of the capillaries in the superficial dermis via a handheld probe, showing the morphology and density of vessels down to 20 µm in diameter. We used dynamic OCT to investigate 15 chronic wounds and assess characteristics of the vessels at the 4 poles around the wounds, the wound bed, adjacent dermatosclerosis, and unaffected skin. The results of the study show that both vessel morphology and density in the wound edges are dramatically different from that in healthy skin, showing clusters of glomuleri-like vessels (knot-like forms or clumps) and an absence of linear branching vessels, and also greater blood perfusion. Such vessel shapes are reported to be associated with tissue growth. The OCT imaging procedure was rapid and well tolerated by patients and provided new information not available from other devices. Thus, OCT appears to have great promise as a tool for the evaluation and study of chronic ulcers.

Keywords

chronic wounds, optical coherence tomography, capillary imaging, venous leg ulcer

Active and healed venous ulcers, the most common of lower extremity chronic wounds, have a prevalence of 0.7% in Germany.[1] The economic and patient's own burden of disease is considerable.[2,3] Chronic wounds such as venous leg ulcers (VLUs) invariably heal slowly and recur. Poor healing of chronic wounds is variously attributed to etiology—ambulatory hypertension in the case of VLU, impaired perfusion and oxygen diffusion, the presence of chronic inflammation at wound sites, changes in skin and subcutaneous tissue, which in VLU is described as lipodermatosclerosis and in general, senescence.[4] Previous reports showed normal cutaneous perfusion and tissue oxygen levels assessed using skin surface sensors, which had little bearing on the outcome of treatment.[5] While this may be a reflection of unsuccessful treatment, the findings provoked the thought that monitoring oxygen on skin offered no benefits to clinical management. Since then, wider use of surface sensors to measure tissue oxygen tension transcutaneously has led to evidence-based recommendation for its use in the management of diabetic foot ulcer for determining levels of amputation.[6]

In VLU, the oxygen levels of the skin surface adjacent to the ulcer may be low: intracutaneous oxygen measurements show still lower levels compared with heathy skin, but no signs of hypoxia were observed.[7] This raises the question why: It has been argued that tissue oxygen measured on skin ($TcPO_2$) varies with perfusion as well as the resistance to oxygen diffusion offered by skin thickness and intracapillary distance, which is affected by the presence of edema.[8] Skin thickness is associated with lipodermatosclerosis that is common in VLU. Edema is another common clinical

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finding in VLU. These physical changes could diminish oxygen available for healing regardless of the levels of cutaneous perfusion. For this reason, we initiated a study of the blood vessels supplying chronic wounds.

New Technique for Visualizing Blood Vessels

Optical coherence tomography (OCT) is gaining acceptance as a useful device to aid in the diagnosis of basal cell carcinoma, as well as for research into many skin conditions and their treatments.[9-14] OCT was first described by Huang and coworkers at Massachusetts Institute of Technology (MIT) in 1991 and has been very successfully applied to the field of ophthalmology for imaging the subsurface structures of the retina and other avascular parts of the eye.[15] Welzel et al reported the use of OCT to obtain structural images of the most superficial layers skin.[16] "Dynamic" OCT (D-OCT) is a further development that allows visualization of the skin vasculature by detection of motion from speckle variance.[17,18] Early images of the blood vessels in burns and wounds are promising; the development of D-OCT permits imaging of capillaries of diameters as small as 20 μm as well as of perfusion in the mid/upper dermis that may enable an understanding of the nutritional supply to wounds.[19] This offers potential advantages on photoplethysmographic techniques that detect changes in venous volumes in deeper tissues but no interpretation of capillary function. For these reasons, the D-OCT technique was extended to examine peri-wound skin.

OCT is used to form images by projection of a tightly focused scanned laser beam into skin; back-scattered light from the tissue subsurface structures is collected by the scanning optical system and directed by optical fibers to detectors, where it interferes with an internally generated reference beam taken from the same laser, providing precise indication of scattering intensity at each depth. Light that has been multiply scattered within the tissue loses coherence and so does not produce a strong interference signal. The interference signal is used to build up an image of the subsurface tissue structures, in a manner loosely analogous to ultrasound imaging. OCT has inherently higher imaging resolution than ultrasound ($<$10 μm) but with lower depth penetration (~1 mm). It is easily capable of imaging the epidermis and upper dermis including the papillary dermis and upper reticular dermis, but is usually not able to reach the subcutaneous layers except on very thin skin. No coupling gel or special preparation of the skin is required.

Since 2015, software-based methods for detecting blood flow from the OCT images were developed, by detecting microscopic changes between OCT images captured within a very short time interval. These methods are known variously as speckle-variance OCT,[17] decorrelation-mapping OCT,[20] optical microangiography,[21] and collectively as OCT-angiography or D-OCT ("D-OCT" in the remainder of this article). D-OCT is significantly different from Doppler-OCT because it utilizes changes in intensity rather than in phase to detect motion. Although Doppler-OCT has also been shown to be capable of detecting blood flow in skin, it lacks sensitivity due to a high noise floor and because most blood flow is perpendicular to the laser beam.[22,23] D-OCT has strong similarities to laser speckle contrast imaging, but has the added capability of being able to resolve depth below the skin and has sufficient resolution to resolve individual blood capillaries.[24] D-OCT detects motion of blood, which is of the order of 0.1 to 1.0 mm/s. Therefore, it does not detect blood vessels in which blood is stationary or flowing very slowly. D-OCT is also not able to detect the much slower flow of fluid in lymph vessels.

This capability to image small blood vessels in high resolution together with depth measurement opens up new possibilities in the field of wound healing as it is well established that angiogenesis plays a key role in the healing process and persistently low oxygen supply is the main reason for disturbed wound healing. For evaluation of vessel pathologies and blood perfusion in wounds, there are only few noninvasive methods established like laser Doppler flowmetry, lacking high resolution. Therefore, D-OCT may provide new means to noninvasively image and quantify blood perfusion in wounds that may prove to be of value to the wound specialist in diagnosing wounds, differentiating venous ulcers from other types, selecting therapies, and monitoring their effects. The aim of this study was to image capillaries in chronic wounds for a fixed duration.

Materials and Methods

Imaging

The OCT device used in this study was a commercially available OCT scanner, "VivoSight" (Michelson Diagnostics Ltd, Maidstone, Kent, UK), equipped with D-OCT processing software. The OCT probe was equipped with presterilized disposable standoffs and disposable transparent sterile sheaths to fully encase the OCT probe and conduit to the scanner console. The disposable standoff is placed in contact with the tissue, setting the correct distance from the imaging lens to the tissue, and fits over the sheath (see Figure 1), thus ensuring that only disposable sterilized parts can come into contact with the wound during the scan procedure.

The image resolution of VivoSight is $<$7.5 μm over a focal depth of 1.0 mm and field of view 6 mm $\times$ 6 mm. The image penetration depth is 1 mm, but the D-OCT function works well to a penetration depth of at 0.5 mm. The handheld probe is placed onto the target skin before starting the scan; in 30 seconds, the device captures a 3-dimensional block of data comprising 120 D-OCT image frames of size 6 mm $\times$ 2 mm with pixel size 4.3 μm over the field of view. The probe is equipped with a color camera showing the exact area of skin being scanned.

Figure 2 shows vertical and en-face OCT images of healthy normal skin of a volunteer with D-OCT enabled.

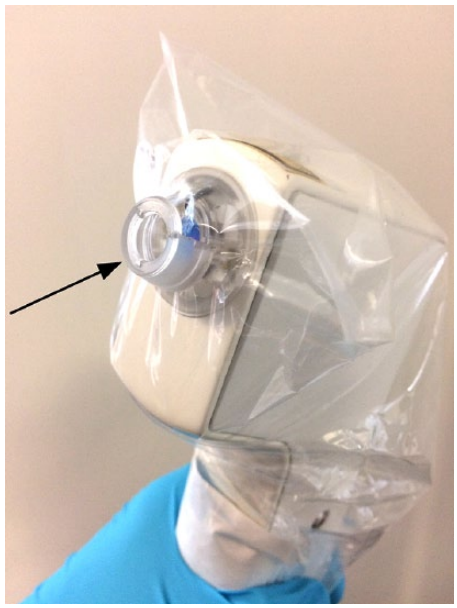


Figure 1. Optical coherence tomography probe, enclosed in sterile sheath over which is fitted a sterilized "standoff" (arrowed) to set skin at the correct focal distance.

Structural features such as epidermis, hair follicles, and blood vessels in the superficial vascular plexus are identifiable in the cross-sectional image (Figure 2a); their size and depth can be measured with an accuracy of ~10 μm. The en-face image (Figure 2b) displays the vasculature with a network of branching vessels.

Study

Eleven patients (7 females, 4 males; median age 73 years, range 50-88 years) with 15 lower extremity chronic wounds were recruited for this study. Patients aged more than 18 years, attending the hospital for treatment of difficult to heal chronic lower extremity wounds, were included in the study with informed written consent. The study was carried from May 31 to October 4, 2017, at the Department of Dermatology and Allergology of the General Hospital in Augsburg with approval from the Ethical Committee of the Ludwig-Maximilian University (Project Number 755-16). The study was conducted according to the principles of the Declaration of Helsinki and international guidelines concerning human studies.

Severe peripheral arterial occlusive disease that required revascularization was an exclusion criterion. In addition, patients under treatment for cancer, those with a history of decompensated cardiac failure, clinically significant renal failure defined as serum creatinine level elevated by a factor of 2, stroke, rheumatoid arthritis, scleroderma, Raynaud's phenomena, or pregnancy were excluded. Ulcers with a superinfection were either excluded from the study or treated before the start of the study.

Five patients suffered from type 2 diabetes. One patient had psoriasis without systemic treatment. One patient took a β-blocker (propranolol). Six patients underwent anticoagulation, 2 with phenprocoumon, 3 with acetylsalicylic acid, and 1 with acetylsalicylic acid/clopidogrel.

Patients included had Ankle Brachial Pressure Index (ABPI) $\geq$0.5 to $<$1.2 in the ulcerated leg among whom ABPI in 6 ulcerated legs was below 0.9. All patients received the local vascular workup (Duplex ultrasound) prior to the study as part of routine diagnostics. Evidence of venous incompetence (in the deep and/or superficial veins) was found in all patients, confirming the venous disease. Thus, the included cohort of wounds in the study ranged from pure venous to mixed arteriovenous disease. All wounds were treated according to the local standard protocol. The preexisting duration of ulcers ranged from 14 months to 15 years.

At the first visit, the ABPI of all patients was measured. The wounds were photographed with a Canon Powershot A1200, and the wound dimensions were measured. The calf circumference was measured at the midpoint of legs using a paper tape measure and the patients sitting with elevated legs.

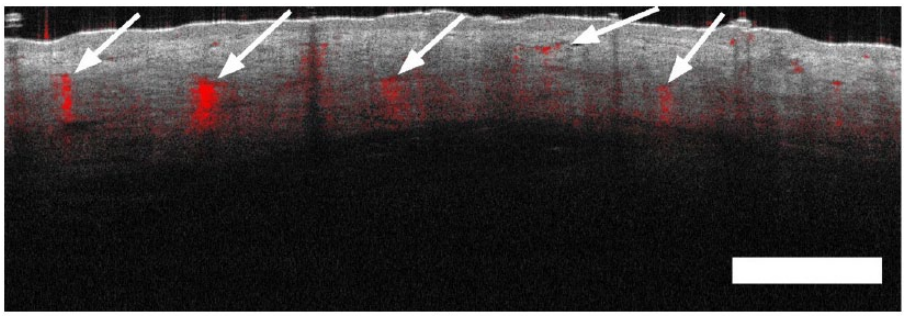
All patients received standardized treatment, which was wound care according to local protocol followed by appropriate bandaging. D-OCT imaging was performed when the patients attended the hospital for a dressing change according to their treatment plan.

All ulcers were imaged with D-OCT. The scanning process was easy to perform and well tolerated by the patients, taking approximately 30 seconds for each scan location. As the probe scanning area is much smaller than the ulcer surface area, scans were made at several places on the wound at standardized locations: at 4 sides of the wound edges measurements were done at the upper, right, lower, and left border (the "poles of the wound") so that the wound bed was partly in the scanner field of view. In addition, images were collected from the center of the wound bed, on nonulcerated but dermatosclerotic skin from at least 2 cm outside the wound and from nonulcerated skin of the contralateral leg.

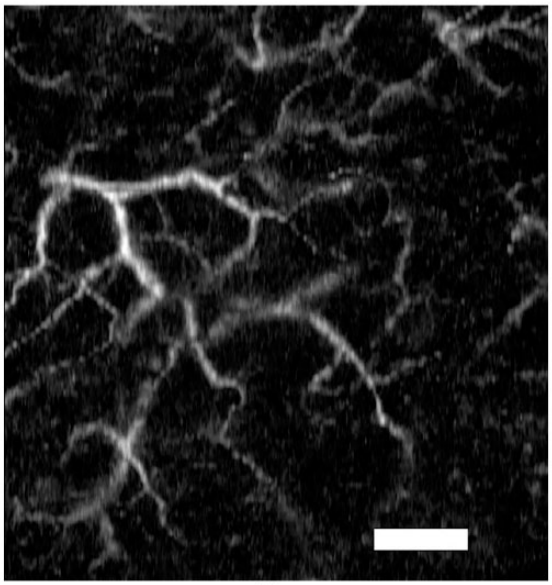
Statistical analysis for comparison between ulcer center, ulcer border, adjacent dermatosclerotic, and contralateral skin was performed using the Wilcoxon test for matched pairs; statistical significance was assumed with differences at $P < .05$.

Analysis of Images

The vessel morphology was assessed visually from the horizontal "en-face" projections of the captured D-OCT images produced by use of the VolumeViewer tool of ImageJ image



(a)



(b)

Figure 2. (a) Example vertical optical coherence tomography (OCT) scan of skin of limb of healthy 52-year-old male. Red areas (arrowed) indicate blood flow motion in dermal vessels detected by D-OCT (dynamic-OCT) processing. The vessels produce a vertical D-OCT "shadow" artefact beneath the area of motion. Scale bar 1 mm. (b) En-face projection of D-OCT scan from skin of lower limb of a healthy 52-year-old male. Branching vessels are clearly evident. Image size 6 mm × 6 mm; scale bar 1 mm.

processing software.[25] This tool provides a top view of the blood vessel shapes in captured D-OCT data volume over 6 mm × 6 mm skin area. The vessel morphology was analyzed and described using the methodology provided by Ulrich et al, with an additional morphological category we called "clump," which we used to describe the appearance of knot-like vessel shapes.[26] The prevalence of each vessel morphology was visually estimated on a scale of 0 to 3, with 0 = absent, 1 = a few, 2 = some, and 3 = many. Blood vessel density as a percentage of the scanned tissue volume was obtained by using the "Measure" tool of ImageJ, which provides the average pixel intensity in the projected D-OCT dataset. For both vessel morphology and density, the 4 "poles" of the wound were independently assessed and then the average taken to obtain the "border" value.

Results

Figures 3 and 4 show example en-face projection views of scans from 2 edges of 2 venous leg ulcers, which are typical for many of the results. Also shown are the views provided from the OCT probe camera, with an overlay showing the location of the OCT scan, and clinical photographs taken of the whole wound showing the location of the scan.

Healthy skin is characterized by vessels branched in 3 dimensions. In most cases, D-OCT scans in the peri-wound areas did not show the familiar branching vessel patterns as seen in healthy skin (see Figure 2b); instead blood flow appear to be focused in coiled, knotted vessel clumps, also termed glomeruli-like vessels. These were generally observed in clusters so that the image skin has a mottled appearance, as clearly seen in Figure 3. The size of the

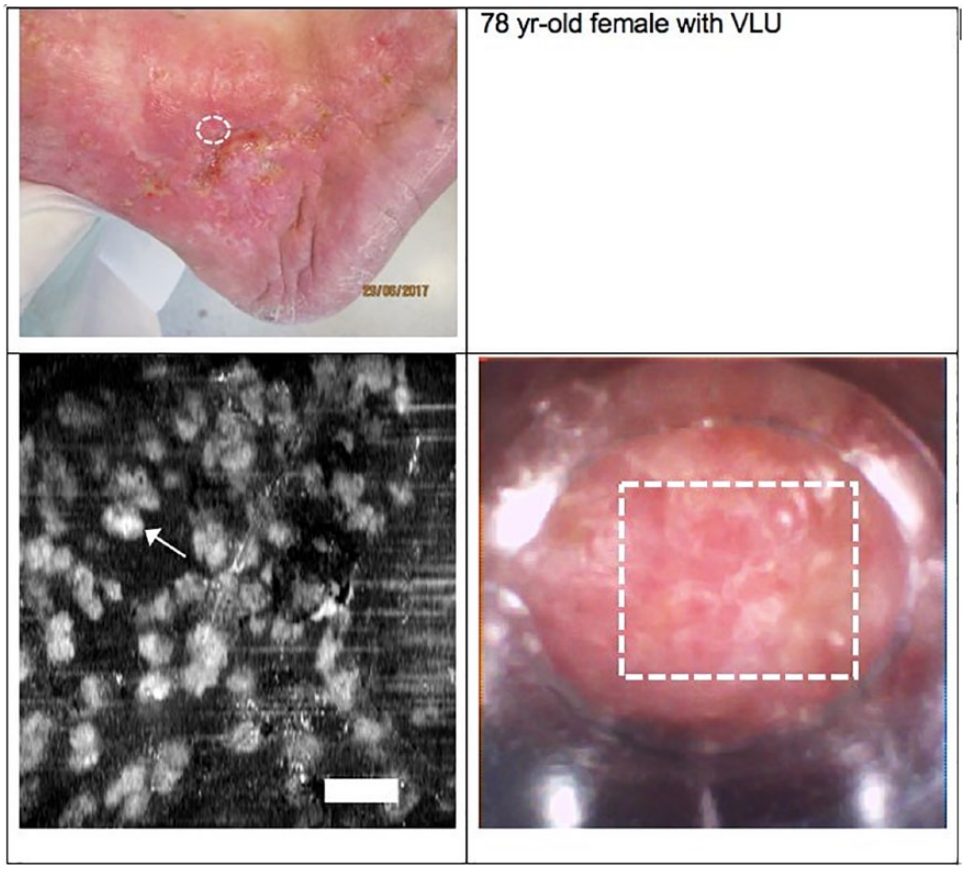


Figure 3. Example dynamic optical coherence tomography (D-OCT) scan of wound edge. Top left, clinical photograph showing location of D-OCT scan shown below at bottom left. Arrowed is a typical knot-like vessel clump. Bottom right, OCT probe camera view of scanned area. The box shows the location of the 6 mm × 6 mm D-OCT scan in the camera field of view. The wound bed is seen at the right side of the scan. Scale bar 1 mm.

clumps varied from 50 to 500 μm in diameter, but generally all of the clumps in a scanned area of skin had broadly similar size; thus, either clusters of small clumps or clusters of large clumps were seen. The vessel clumps were typically located 200 to 400 μm deep in the upper dermis.

Figure 5 shows a scan of skin 2 cm outside of the wound edge. A serpiginous vessel is clearly visible. Most D-OCT scans of the adjacent sclerotic skin exhibited presence of linear vessels, additionally in many cases serpiginous vessels with branches. The density of the vessels varied from isolated and very sparse to normal. Even in skin of the contralateral limb, serpiginous vessels were observed, which is an unusual vessel pattern in persons without venous insufficiency.

Figure 6 shows the semiquantitative prevalence of each vessel morphology on a scale of 0 to 3 assessed from OCT images at the wound border, wound center, adjacent skin, and on healthy appearing skin on the contralateral leg. There are clear differences between each location. At the wound border, coils and clumps are the dominant morphology and there are fewer linear or curved vessels. The wound center does not show a clear dominant shape but lacks curved or serpiginous vessels. By contrast, in the adjacent liposclerotic skin and in contralateral skin, linear and serpiginous vessels were predominant, with fewer coils or clumps.

Figure 7 shows that the measured density of vessels varied from 10% to 15% of the scanned tissue volume. The wound border and wound center showed clearly higher vessel densities than adjacent skin or healthy appearing skin of the contralateral leg. Statistical analysis showed no significant differences between the center and the border ($P > .05$ NS), but there were statistically significant higher densities of vessels at the ulcer border compared with adjacent ($P = .04$) and skin in contralateral limb ($P = .005$), respectively. Statistically significant higher vessel density at the center compared with contralateral skin ($P = .01$) was also measured. Coils were significantly more frequent at the center compared with adjacent skin ($P = .03$). The border showed significantly more clumps compared with adjacent and

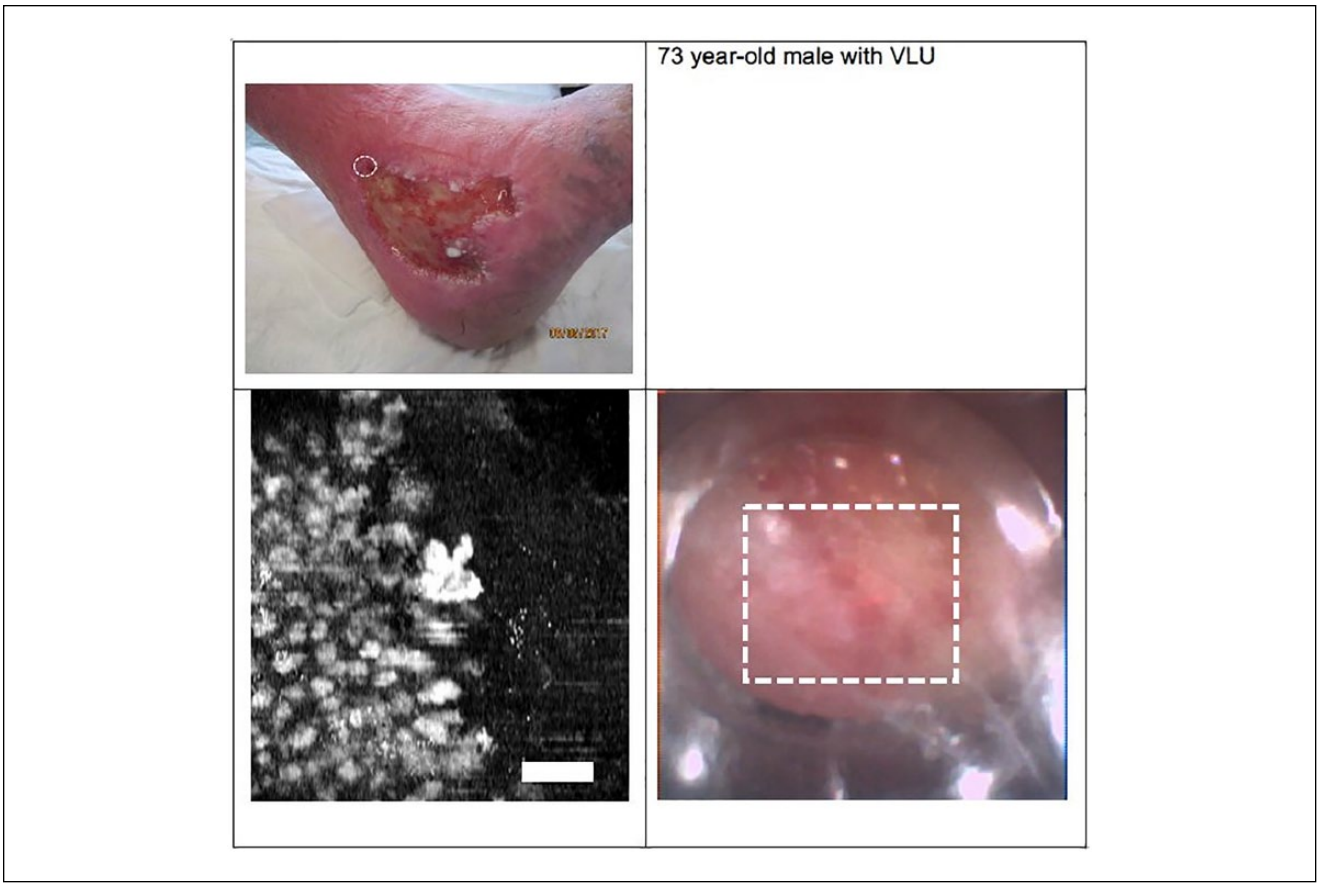


Figure 4. Example dynamic optical coherence tomography (D-OCT) scan of wound edge. Top left, clinical photograph showing location of D-OCT scan shown below at bottom left. There are many clumps seen at the left side. Bottom right, OCT probe camera view of scanned area. The box shows the location of the 6 mm × 6 mm D-OCT scan in the camera field of view. The wound bed is seen at the right side of the scan. Scale bar 1 mm.

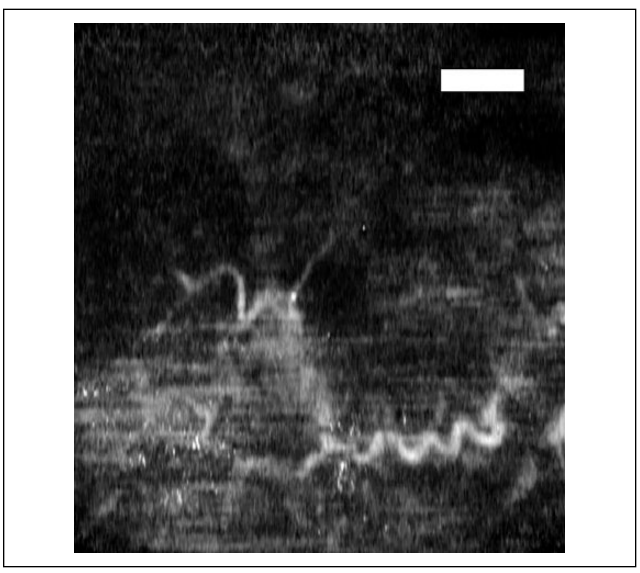


Figure 5. Example dynamic optical coherence tomography scan of dermatosclerotic skin 2 cm adjacent to wound edge. Arrowed is a serpiginous vessel. Scale bar 1 mm.

contralateral skin (both $P = .005$). Serpiginous vessels were significantly less frequent at the border compared with contralateral skin ($P = .008$).

Discussion

The purpose of this study was to use a new technique, D-OCT, to study capillaries in peri-wound skin. In 11 patients with chronic wounds (of venous or arteriovenous etiology) of long duration, we studied capillaries at the 4 poles along the wound edge, the center of the wounds, skin at a fixed distance proximal to the wounds, and at a control site. The scanner was easy to use and well tolerated by all patients in this study.

Our motivation in performing this study was to assess what new useful information about the vessel morphology in chronic lower extremity wounds such as venous and arteriovenous ulcers, OCT, and in particular its variant D-OCT, can reveal. The results show that capillaries along the wound edges and the center may be grouped in coils and clumps, whereas further away from the wound, as well as in

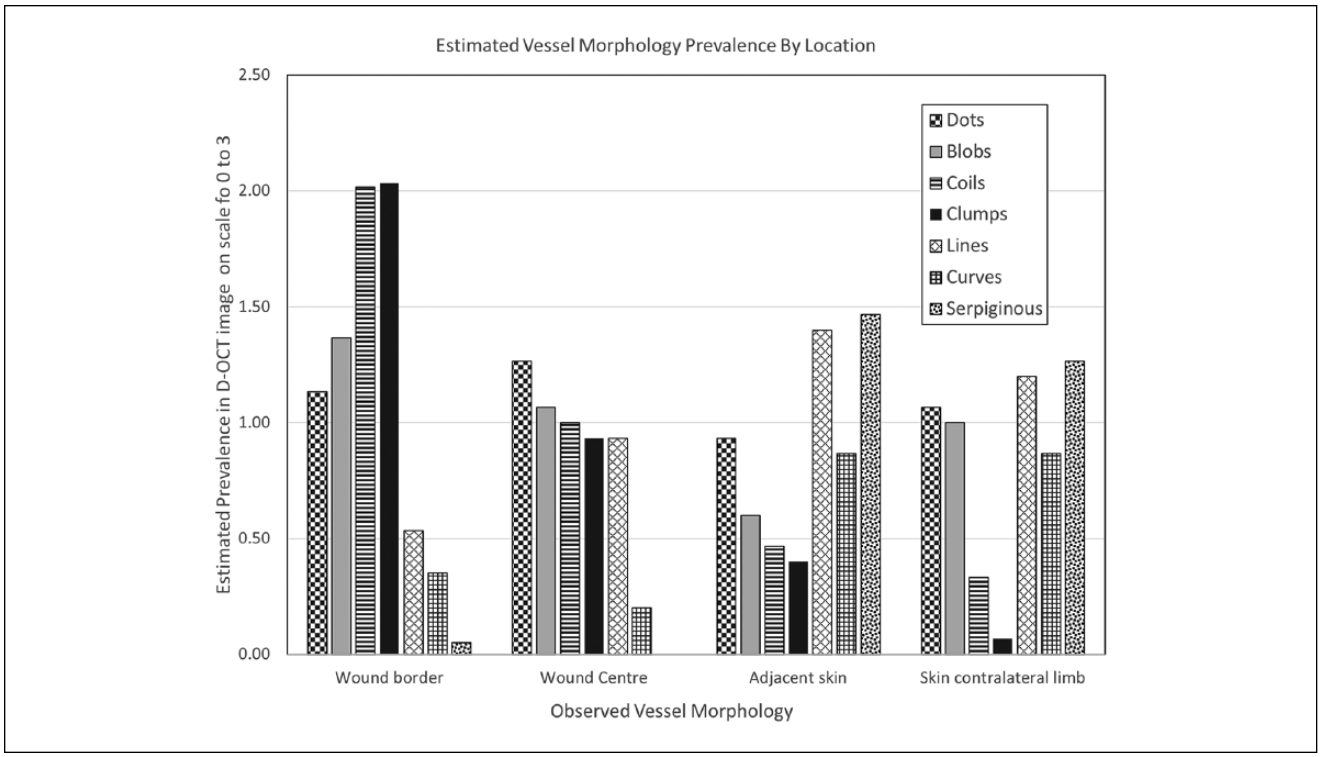


Figure 6. Vessel morphologies observed. The vessel shapes were counted in a semiquantitative way: no = 0, yes (+) = 1, yes (++) = 2, and yes (+++) = 3. The *y*-axis shows the mean value of this semiquantitative score; n = 15.

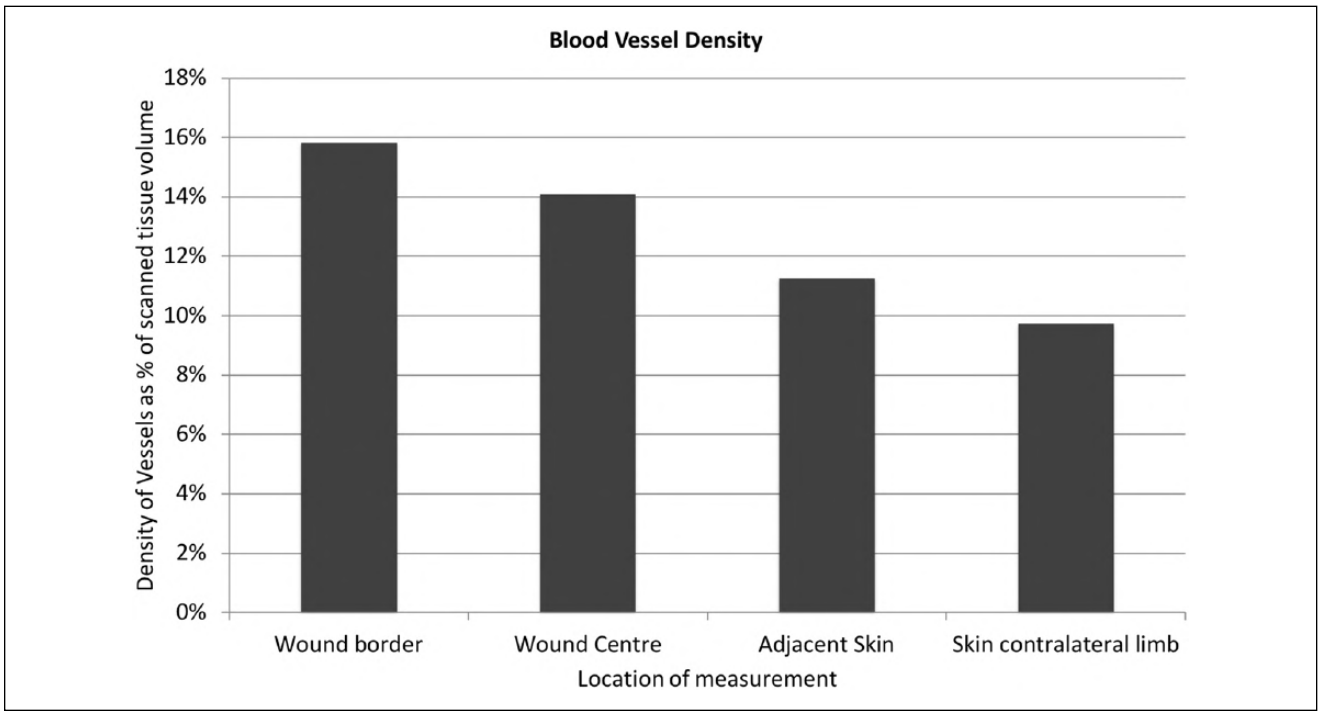


Figure 7. Vessel densities observed. The mean vessel density as percentage of vessels within the whole image of 6 mm × 6 mm is calculated; n = 15.

contralateral skin, vessels are found in lines or curves and very often in a serpiginous manner. These findings are consistent with the observations in acute wounds.[27]

To our knowledge, this is the first attempt to investigate and characterize vessel morphology of peri-wound skin and adjacent skin of venous leg ulcer in humans with D-OCT.

OCT has previously been used to study both microcirculation in skin and acute wound healing. Yuan and coworkers reported success in using OCT to monitor diabetic wound healing in mice including reepithelialization, inflammatory response, and granulation tissue formation in hydrogel-assisted wound healing.[28] Polarization-sensitive OCT has also been applied to assess burns and infected wounds, utilizing the fact that healthy collagen is birefringent.[29,30] OCT has also been used to monitor fractional laser-induced wounds and also laser treatment of scars.[31-34] More recently, in 2014, Greaves and coworkers performed studies of biopsy wounds in human subjects over 4 weeks correlated with histology at 7-day intervals and showed a correlation between OCT signal intensity and fibrosis ($P = .038$).[35,36] OCT has also been used to monitor the rate of epithelialization with validation by histology.[37,38]

Turning to vascular aspects of wound healing, Kuck and coworkers first reported that OCT could be used to detect vasoconstriction, vasodilation, and reepithelization in wounds.[39] When D-OCT/angiographic OCT techniques were developed, Yousefi and coworkers demonstrated that it could be used to visualize vessel growth during wound healing phases in a mouse model.[40] In 2016, Themstrup and coworkers showed that D-OCT measurements of the density of blood vessels in upper dermis of human volunteers treated with vasoconstriction and vasodilation agents positively correlated with measurements made by other techniques including chromameter and laser speckle contrast imaging.[18,41] They also reported that changes in the vascular morphology produced by changes in blood flow were reliably observed and accurately discriminated by blinded observers. Their research showed that D-OCT images reveal the structure of the superficial vascular plexus in the upper dermis and that changes in individual capillaries responding to vasoconstriction agents could be directly visualized. These studies show that D-OCT can both image skin capillary structure and quantify changes leading directly to the question of whether D-OCT may have a useful role in assessing chronic wound healing, for the above-mentioned motivations.

Our results show that D-OCT can be used to image vessel morphology in the peri-wound skin and adjacent skin of both VLU and mixed arteriovenous ulcers and that these D-OCT images reveal the presence of non-normal vessel morphology that is quite distinct in character from vessels in skin of healthy subjects. It is interesting that the scans from the ulcer center and border show evidence of glomeruli-like vessel structures, as known from histopathology and, furthermore, that the size and density of these structures varies from wound to wound and from location to location around the edge of a given wound.[42] It may be that the vessel morphologies observed are associated with specific wound tissue pathologies and/or tissue healing states, and our studies do not permit further elucidation. It is also possible that the capability of D-OCT to quantify the density of vessels, equivalently the blood perfusion, may prove useful in determining whether a wound is healing, or which parts of a wound are healing. These coils and clumps are very typical for chronically wounded skin and the ulcer border in VLU, whereas the vessel shape changed markedly in a greater distance to the border. In dermatosclerotic skin and even in healthy looking skin at the same or contralateral leg, serpiginous vessels were often detected. These snake-like vessels are normally not found in healthy skin of persons without venous conditions and may be interpreted as micro-varicosities although other explanations are possible. The differences between ulcer border and the adjacent skin were highly significant regarding vessel density and shape, and we consider this an important finding.

Thus, our results provide evidence that D-OCT may have a useful role in the differential diagnosis of chronic wounds, assessment of wound healing, and provide new insights into the morphology of blood vessels at the edges of chronic venous leg ulcers.

Limitations of the study include the small size of the sample that was restricted to lower extremity wounds, the majority of which were of venous etiology, and the subjectivity of human observer assessment of vessel prevalence. Our study lacks histological data to confirm the findings, or co-validation data with other devices to obtain comments on the images obtained with D-OCT. D-OCT provides direct visualization of capillaries in the upper dermis even in skin that is thickened and toughened by ambulatory venous hypertension.

In conclusion, this pilot study provided clear images of wound edges from where healing occurs, wound centers, as well as healthy appearing skin. Future work to determine more quantitative data in larger studies is under way.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Author Jon Holmes is an employee and stockholder of the company that manufactures the OCT device used in this study. The other authors have no conflicts of interest to disclose.

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Ethical Approval

The protocol was approved by the Ethical Committee of the Ludwig-Maximilian University (Project Number 755-16).

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