Imaging Blood Flow in Human Port-wine Stain In Situ and in Real Time Using Optical Doppler Tomography

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**Background:** Optical Doppler tomography (ODT) combines laser Doppler flowmetry with optical coherence tomography to obtain high-resolution images of blood flow in human skin in situ and in real time.

**Observations:** We present a case in which ODT was used on a patient with a port-wine stain (PWS) birthmark to document the change of blood flow in response to laser therapy. It might be possible to use ODT blood flow measurements in situ to assist in assessing the efficacy of laser PWS therapy. If partial restoration of flow occurs immediately or shortly after laser exposure, indicative of reperfusion due to inadequate blood vessel injury, the PWS can be retreated using higher light dosages. Retreatment is continued until the measured Doppler shift is zero due to a permanent reduction in blood flow, indicative of irreversible microthrombus formation in the PWS vessels.

**Conclusions:** We have demonstrated that ODT may be used for noninvasive imaging of blood vessels in PWS skin. Moreover, ODT will potentially allow laser therapy to be optimized on an individual patient basis by providing a fast, semiquantitative evaluation of the efficacy of PWS laser therapy in situ and in real time.

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MATERIALS AND METHODS

We developed an ODT instrument that uses a fiber-optic Michelson interferometer (Figure 1) with a broadband light source (center wavelength, 1300 nm; output power, 5 mW; and bandwidth, 65 nm). Light from the source was coupled into a fiber interferometer using a 2 × 2 coupler and then split equally into reference and target beams. In the reference beam, a rapid-scanning optical delay line was used to change the optical path length for axial scanning. In the reference beam, light backscattered from the skin was coupled back into the fiber and formed interference fringes at the photodetector. Interference fringes were observed only when the optical path length difference between the sample and reference beams was less than the coherence length of the source. The fringe signals were then processed by a computer to generate conventional OCT and ODT images. Images from OCT were obtained by calculating the amplitude of the fringe signals. Optical Doppler tomographic images were obtained by calculating the phase change of the fringe signals.

Light in the sample path was focused onto a 10-µm spot on the skin surface using a 1:1 magnification gradient index lens (NA=0.2). The probing beam was aligned with the optical axis oriented at an angle of 5° to 10° from the skin surface so that blood flow parallel to the surface could produce a Doppler frequency shift. Using our instrument, the approximate time to record conventional OCT and ODT blood flow images was 2 seconds, with a velocity sensitivity of 10 µm/s. After scanning, the time required to reconstruct the images was 7 seconds using a workstation platform.

To prevent surface movement, the area imaged was in tight contact with a glass window, and an index-matching oil was inserted between the glass and the PWS to decrease light reflection from the skin surface. The oil also helped to flatten the skin surface so that wavefront distortion of the probing beam at the skin surface was minimized.

A 62-year-old white man with a PWS on the left hand was recruited from the outpatient population of patients available at the Beckman Laser Institute and Medical Clinic, University of California, Irvine; the protocol was approved by the institutional review board. Informed consent was obtained.

To monitor the efficacy of PWS treatment in situ, we constructed a handpiece that combined the laser irradiation with the ODT probe at the same site. The conventional OCT and ODT images were obtained from the exact same site immediately before and after the laser pulse was delivered without moving the probe. The laser used for PWS irradiation was a ScleroPlus (Candela Laser Corp, Wayland, Mass) pulsed dye laser (wavelength, 595 nm; pulse width, 1.5 milliseconds; spot diameter, 7 mm; and fluence, 12 J/cm²).

After laser irradiation, a 3-mm punch biopsy specimen was obtained from the center of the 7-mm irradiated spot and fixed in 10% formalin. The tissue sample was embedded in paraffin, sectioned, and stained with hematoxylin-eosin. The remaining laser-irradiated area was followed up with a subsequent ODT measurement and evaluation of blanching 3 months after laser exposure.

RESULTS

Figure 2 shows conventional OCT and ODT images taken in situ from human skin with a PWS. The scanning range is 2 mm (lateral) by 2 mm (axial), but only the linear part (1.25 mm) of the axial scan is shown in the images. The image size is 800 (lateral) by 500 (axial) pixels, with a size of 2.5 µm/pixel. The images in Figure 2 were taken from the palm-side surface of the index finger.

In the conventional OCT image before laser exposure (Figure 2A), the boundary between the stratum corneum and the epidermis is clearly visible, as is an organized network of collagen fibers in the dermis. The conventional OCT image after laser exposure (not shown) did not reveal any notable changes compared with that taken before laser exposure.
Figure 2B-C are color-coded tomographic images of blood flow velocity. In the ODT image (Figure 2B), taken from the exact same site as the conventional OCT image, many PWS vessels are detected in the dermis up to 500 µm below the skin surface before laser exposure. In Figure 2B, static regions appear dark, and blood moving at different velocities is evident. Immediately after pulsed laser exposure using a fluence of 12 J/cm², no blood flow is noted in the ODT image, indicative of microthrombus formation in the PWS blood vessels (Figure 2C). Figure 2D is a hematoxylin-eosin–stained histologic section obtained from the punch biopsy specimen taken at the imaged site. Comparable PWS blood vessels are noted in images B and D.

In this observation, we demonstrated how ODT can measure the vascular response of patients with PWSs undergoing laser therapy. Port-wine stain is a congenital, progressive vascular malformation in the dermis; histopathological studies of PWS show an abnormal plexus of layers of dilated blood vessels 150 to 750 µm below the skin surface having diameters varying on an individual patient basis, and even from site to site on the same patient, from 10 to 150 µm.13

Use of the pulsed dye laser can induce microthrombus formation14 and selectively coagulate PWS vessels.15-17 At low light dosages, pulsed dye laser induces only temporary effects on the PWS vasculature; reperfusion occurs and blood flow returns to preirradiation levels. Higher light dosages might effectively form a totally occluding microthrombus, leading to a reduction in blood flow, which approaches zero immediately after laser exposure and does not return to preirradiation values. It might be possible to use ODT blood flow measurements in situ to assess the efficacy of laser PWS therapy. If partial restoration of flow occurs immediately or shortly after pulsed laser exposure, indicative of reperfusion due to inadequate blood vessel injury, the PWS can be retreated using higher light dosages. Retreatment is continued until the measured Doppler shift is zero because of a permanent reduction in blood flow, indicative of irreversible microthrombus formation in the PWS vessels.

Recently published studies18,19 have described another modality, confocal microscopy, which can pro-
vide in situ real-time images of trafficking red and white blood cells in human skin. Although this modality has many potential uses not possible with ODT,20 confocal microscopy does not specifically measure blood flow. Comparatively, ODT images clearly demonstrate flow changes in a distinct false-color map.

In summary, we demonstrated that ODT can be used for noninvasive imaging of blood vessels in patients with PWSs. Moreover, ODT will potentially allow laser therapy to be optimized on an individual basis by providing fast, semiquantitative evaluation of the efficacy of PWS laser therapy in situ and in real time.

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