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Assessing vessel damage with color Doppler optical coherence tomography following irradiations with cooling

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ABSTRACT

The effects of cooling, laser irradiation, and laser irradiation with cooling on blood vessels were investigated with Color Doppler Optical Coherence Tomography (CDOCT). CDOCT may contribute to an understanding of the dynamics of laser-blood vessel interactions and aid in better optimization of laser parameters to be used. In this study, hamster dorsal skin flap window vessels were irradiated with a KTP laser operating at 532 nm. Irradiation sites were imaged with CDOCT prior to, during, immediately after, and several days after irradiation. KTP laser parameters were: radiant exposures in the range of 7-14 J/cm^2, 3 mm spot size, and 10 ms pulse duration. Magnitude and color Doppler images provided information such as vessel size, depth, and changes in blood flow velocity. Vessel constriction, temporary occlusion, and changes in flow were frequent results of laser irradiation visualized with CDOCT. In addition, the effects of cooling alone were imaged with CDOCT and its effects on blood vessel flow and morphology were investigated before and after laser irradiation.

Keywords: pulsed dye laser, KTP laser, vessel coagulation, cooling

1. INTRODUCTION

A number of vascular disorders benefit from the destruction of blood vessels by laser irradiation. Examples are port wine stains, leg telangiectasias, and leg veins. The aim is to destroy the target vessels with no or minimal damage to the surrounding tissue. While lasers have been in use for this application, the exact nature of the mechanisms of vessel destruction are not fully understood, nor do standardized parameters exist for the treatment of abnormal blood vessels.

Selective destruction of blood vessels without damage to superficial structures is an issue of great importance in laser-mediated therapeutic procedures. Treatment of cutaneous vascular disorders commonly involves the use of a cooling device to minimize the damage to superficial layers [1-3]. Particularly, at 532 nm and 585 nm there is still absorption of the light by the surrounding dermis and epidermis. Cryogen spray cooling has been utilized to cool the epidermis to protect it while unaffecting the temperatures or characteristics of the targeted blood vessels. Chilled tip devices have also been used for protection of superficial layers, but the cooling is not restricted to these superficial layers. [4]

The aim of this study is to use Color Doppler Optical Coherence Tomography (CDOCT) to investigate the effects of cooling and laser irradiation on blood vessels. First, CDOCT was used to follow the healing response of blood vessels irradiated with a KTP laser in order to determine parameters for effective blood vessel coagulation. The effects of simple cooling using a cryogen spray device and a chilling tip on dermal temperature and blood flow were then investigated. Finally, CDOCT was used to follow the healing response of blood vessels pre-cooled with a chilled tip device and irradiated with a 532 nm KTP laser.

2. MATERIALS AND METHODS

2.1 Hamster Skin Flap Window Model

Syrian Golden hamsters weighing 80-120 g were used for the study. The hamsters were anesthetized with a 3:4 mixture of Xylazine and Ketamine during surgical procedures in addition to times of laser irradiation and imaging.

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A hamster skin flap model described previously[5] was used for direct observation of sub-dermal blood vessels. The window preparation involves lifting a double thickness of shaved and epilated skin from the dorsal area and suturing it to an aluminum fixture. One thickness of skin is then cut out in the shape of a circle, 1cm in diameter. A glass window is used to cover this exposed sub-dermal area. The window allows the unique opportunity to view both sides of a full thickness of skin *in vivo*. On the sub-dermal side, only a thin layer of connective tissue covers blood vessels.

The window preparation may be left on a hamster for over a week without affecting the morphology of cutaneous blood flow. However, over time growth of connective tissue over the sub-dermal side of the window occurs and makes direct observation of the blood vessels difficult.

2.2 KTP laser irradiation

Blood vessels on the sub-dermal side of the window preparation were irradiated at various radiant exposures using a frequency doubled Nd:YAG (KTP) operating at 532 nm with a 3 mm spot size and pulse duration of 10 ms. The set-up for video recording of the window preparation prior to, during, and following laser irradiation is shown in Figure 1.

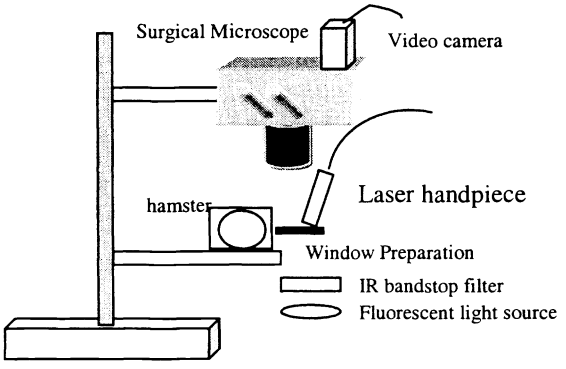

Figure 1 : Experimental setup for laser irradiations and video recording.

2.3 CDOCT

A CDOCT system, described in a number of vessel coagulation studies, was used for these experiments [6,7]. Briefly, the system is a fiber optic-based Michelson interferometer. Amplitude images are constructed by use of a heterodyne detection technique. The CDOCT system incorporates a 1280 nm center wavelength with a 30 nm full width half max (FWHM) bandwidth superluminescent diode. Scans in depth (a-scans) are acquired by translation of a reference mirror to change the length of the reference arm. Scanning mirrors are then used to scan laterally across the sample. This allows the construction of a two-dimensional cross sectional image of the tissue. The system has a 20 μm resolution in both the lateral and axial directions. In addition, it has a high dynamic range (>100 dB). A schematic of the system is shown in Figure 2.

2.4 Temperature Measurements

Temperature monitoring of the sub-dermal side of the window preparation was done using a 237 ± 10 μm diameter Omega type K thermocouple. The voltage-temperature conversion was done using a Lutron temperature adaptor (DH-802C). The signal was then amplified and lowpass filtered with a two pole Butterworth analog filter (cutoff at 40 Hz). The amplified and filtered signal was input into the A/D of a 16-bit National Instruments DAQ device (AT-MIO-16E-1) for data acquisition at 10 Hz. For temperature measurements on the hamster window preparation, the thermocouple tip was embedded under connective tissue on the sub-dermal side or between the two thicknesses of skin in cases where a small flap of skin was left attached from the cut out side.

2.5 Cooling Effects

Two types of cooling devices were investigated for effects on sub-dermal temperature and vascular blood flow or diameter changes: 1) a chilled tip cooling device (VersaSpot F, Coherent Medical Group) and 2) a cryogen based cooling device (Dyanamic Cooling Device, Candela Corporation). For the chilled tip, a layer of thermally conductive gel was placed

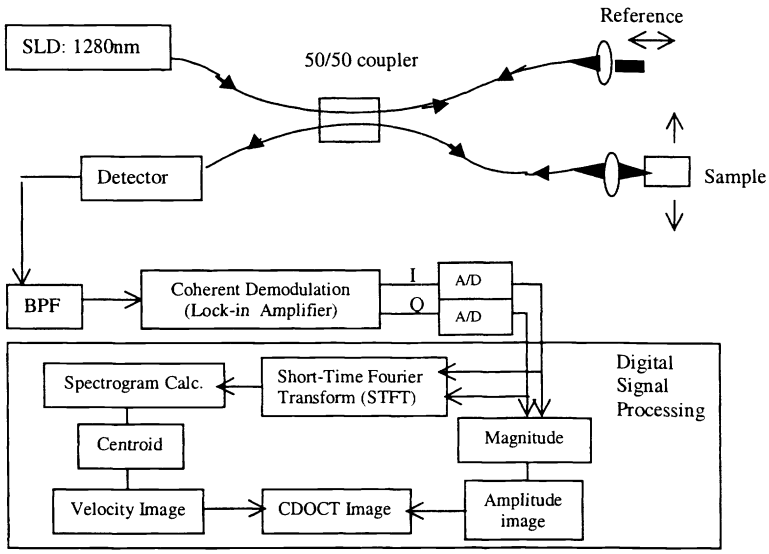

Figure 2. Diagram of CDOCT system

between the tip and the epidermis of the window preparation while temperature measurements were taken with the thermocouple on the sub-dermal side of the skin. The chilled water was preset at a temperature of 4°C, the temperature-controlled pump was turned on and temperature monitored by the thermocouple placed on the sub-dermis.

Temperature measurements were performed in the same manner for the cryogen spray cooling. Single spurts were applied in durations of 20 to 100 ms with 10 ms intervals with the handpiece held 20 mm from the skin surface. In addition, CDOCT scans were taken of blood vessels on the sub-dermal side of the window model prior to and immediately after bursts of cryogen were applied to the epidermal side. Although, not used clinically, to investigate cooling on vasculature, multiple pulses were applied while CDOCT scans taken or the changes in microcirculation were video recorded.

In order to confirm the degree of cooling measured with the thermocouple embedded in the window preparation, temperature measurements were performed without the window preparation through a single thickness of skin. To do this, a 1cm long incision was made in the dorsal skin and the thermocouple inserted 11/2 inches into the incision site. The site was then closed. Single cryogen bursts were applied in spray durations ranging from 20 to 100 ms, while temperature readings were acquired from the thermocouple. The same procedure was performed with the chilled tip by placing it against the skin for a total time of 1 second or 10 seconds. The skin was allowed to return to initial temperature after each reading, before being cooled again. Thermocouple depth was determined by CDOCT.

2.6 Vessel irradiation with pre-cooling

A number of irradiations with the KTP 532 nm laser were performed in order to assess the fluences required for permanent coagulation of blood vessels in the hamster window preparation when the dermal layers were cooled. The skin was cooled with a chilled tip cooling device held to the epidermal side of the skin. Once the temperature had reached a value of 20°C, a single laser pulse was applied to the sub-dermal side targeting an arteriole-venule pair and the cooled tip was pulled away. Each study began with a radiant exposure found in previous studies[8] to be unlikely to damage the vessels in cases without cooling. The irradiation process was videotaped and damage was confirmed visually in that way. In addition, CDOCT imaging was done immediately after the pulse and several minutes later. If no changes were seen to occur in either visual appearance of the vessel or in flow, the same pair was irradiated with a 1 J/cm^2 higher exposure and so on. If any changes were seen to occur in either morphology or flow, the pair was imaged under CDOCT an hour after irradiation, then 24 hours later. Any vessels which appeared to be fully or partially occluded, or constricted were checked at 48 hours to follow the progress of damage. If the vessels appeared to recover, a higher radiant exposure was applied. This continued until permanent vessel coagulation occurred.

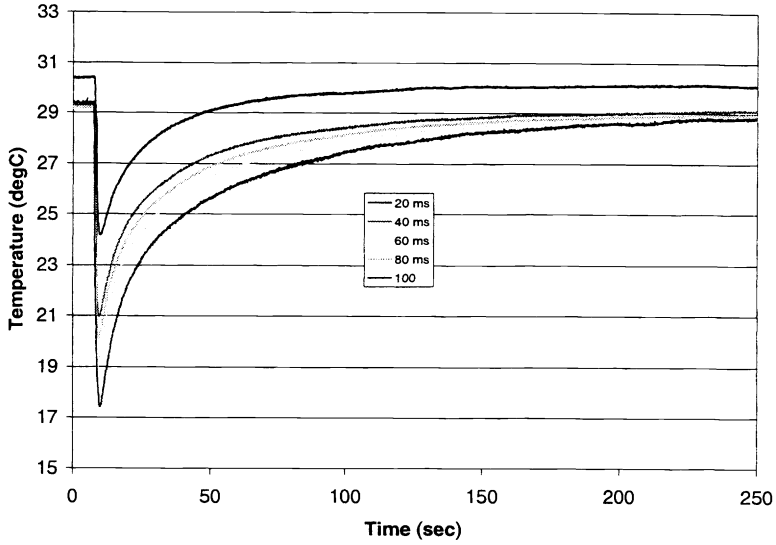

Figure 3. Temperature profiles at a depth of 625 μm for various bursts of cryogen applied to the surface of *in vivo* hamster skin.

3. RESULTS AND DISCUSSION

3.1 Cooling Effects

Temperature measurements revealed that even with a short cryogen spurt to the epidermal side of the skin, there are cooling effects as far down as the sub-dermal tissue. Shown in Figure 3 are the temperature profiles found for the single pulse bursts. The thermocouple was embedded 625 μm below the skin surface. The cooled area was approximately 8 mm. The data demonstrates there is a significant drop in temperature even for the shortest duration burst of 20 ms. This is contrary to theory which predicts minimal changes in tissue depths beyond 200 μm.[4] The total time for the temperature to drop to a minimum was measured to be approximately 1 to 1 ½ seconds. Attempts to confirm the temperature measurements with a thermal

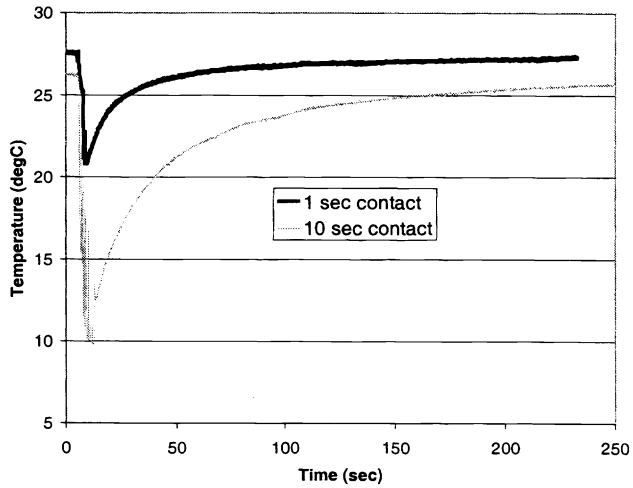

Figure 4. Temperature profiles at 655μm depth for the chilled tip device placed on the skin surface for 1 sec or 10 sec.

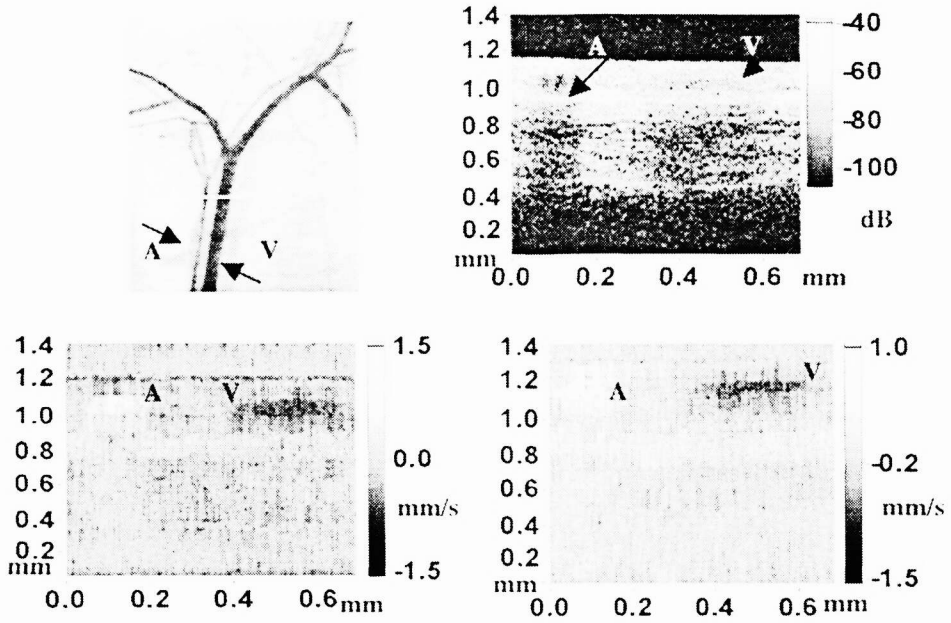

Figure 5. Effect of 100ms cryogen spurt. (a) White line shows cross-section of CDOCT images taken of this arteriole-venule pair. (b) amplitude image (c) CDOCT scan before spurt (d) CDOCT scan after cryogen spurt (2 minutes).

camera were made. Unfortunately, a blackbody source properly calibrated to room temperature and below was unavailable, making the results only qualitative. The method did confirm there was a detectable temperature drop at the sub-dermal tissue when cooling was applied to the skin surface.

Figure 4 shows the temperature profiles measured for the chilled tip in contact with the skin surface and the thermocouple at a depth of 675± 50 μm. The error in the depth measurement is due to uncertainty as to how much the chilled tip pressed on the skin, making it thinner than normal.

The average time required for minimum temperature to be reached in the case of the chilled tip was 4.1 seconds for the short contact time, and 6.6 seconds for the longer contact time. While clinically, the laser pulse is normally applied within 100msec after the area is cooled, so that the vessels may not have reached their minimum temperature at the time of irradiation, radial temperature effects could certainly contribute to pre-cooled vessels for later laser pulses in a nearby region. The core temperature of vessels could be significantly reduced. An increase in the radiant exposure could be required to permanently coagulate vessels and offset any advantages gained from cooling the superficial layers. Knowing the degree of

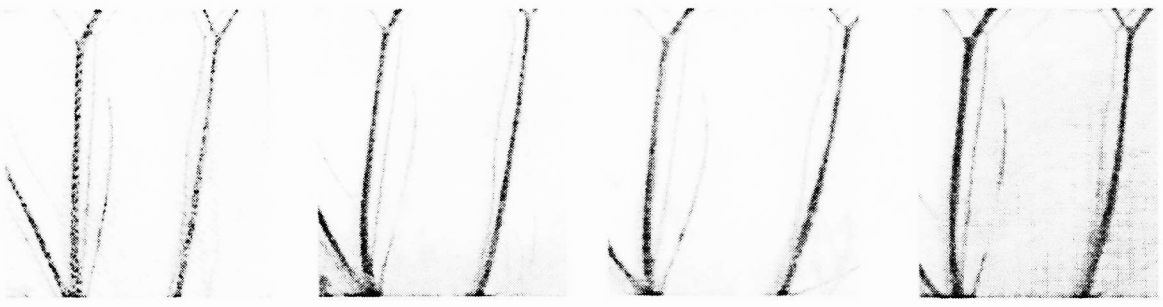

Figure 6. Visual effect of multiple (4) 70 ms cryogen spurts (100 ms apart) on an arteriole. (a) Window preparation before cooling, (b) immediately following 4th burst (c) 10 minutes later (d) immediately following 4 bursts of cryogen.

cooling within the tissue is crucial for the determination of the proper parameters to be used clinically in laser mediated therapies.

While there were considerable temperature changes deeper than expected, we saw few changes in blood flow or diameter with the single bursts of cryogen. This was true for all burst durations ranging from 20 to 100 ms. The same was true for the chilled tip within the period of time the device would be used clinically.

CDOCT was used in combination with video recording of the window preparation to determine if cooling by cryogen or chilled tip affects blood flow and/or vessel diameter. An example of CDOCT images taken before and after a 100 ms cryogen burst are shown in Figure 5. A visible change was a slight decrease in arteriole flow for all cases. For instance in the figures shown, the arteriole had an initial blood flow velocity of 1.5 mm/sec. Within a minute the flow was down to 1 mm/sec. Within 20 minutes after the burst, the flow was 0.9 mm/sec. This characteristic was typical of all vessels hit with a single 100 msec spurt. For spurts of 20 and 60 msec, no visible change was seen. The slight decrease in flow, could be within the errors seen in the images, however, the changes, although slight were consistent over time and for several trials.

Visual changes for 100 msec cryogen spurts were seen for a brief time. Figure 6a shows a window preparation before being cooled. Figure 6b shows the brief focal constriction which occurred immediately following four consecutive 70 msec bursts of the cryogen (100 msec delay) where the temperature reached a minimum of 8°C. The region was allowed to return to the baseline skin temperature (29 °C). It was again cooled in the same way. Again, one sees the focal constriction in the arteriole in Figure 6d. This effect lasted between 1 and 2 seconds and was repeatable.

Since video taping and OCT imaging could not be done simultaneously, the experiment was repeated with OCT while cooling in the same way. These results are shown in Figure 7. The scans for images (c) and (d) were taken 4 minutes after the spurts, but are indicative of the flow changes which occurred immediately following the cooling and continued for a long duration.

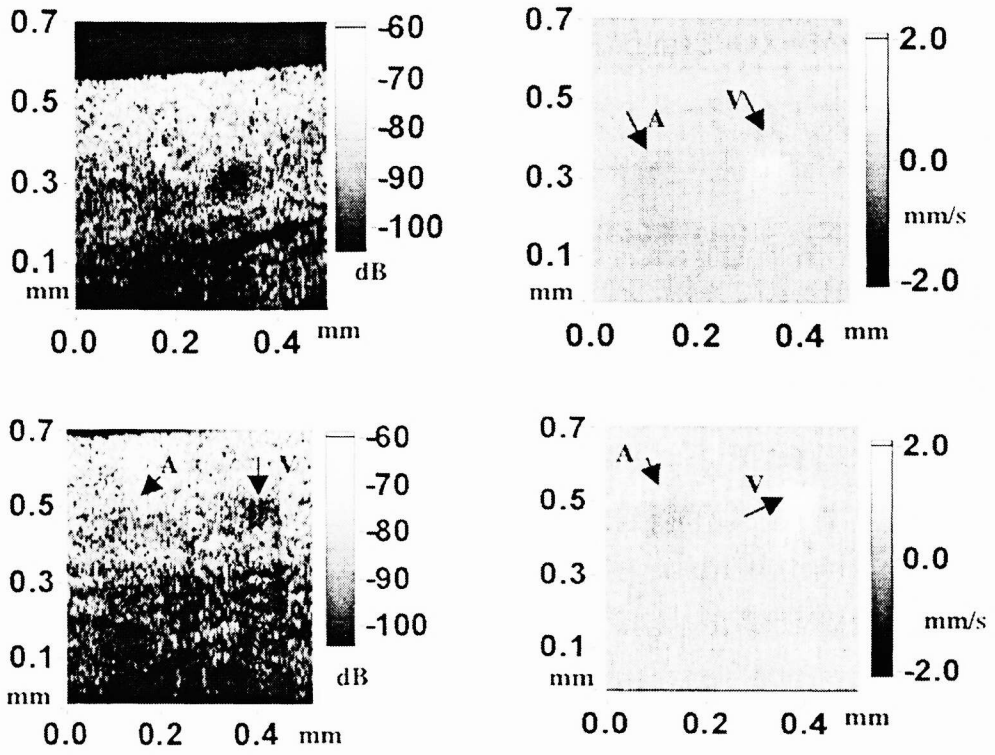

Figure 7. Effects of multiple cryogen spurts on blood vessel flow. (a) Amplitude image before cooling. (b) CDOCT image of venule-arteriole pair before cooling. (c) Amplitude image after 4 consecutive spurts of 70 msec spurts 100 msec apart (d) CDOCT image after cooling. Notice flow has increased slightly.

Although, cooling effects were also investigated using a chilled tip, CDOCT scans could not be taken during the cooling process, since vibrations from the pumping of the chilled water considerable motion artifact.

3.2 Vessel coagulation in a cooled window

We performed preliminary studies to determine how low temperatures might affect the radiant exposure required to permanently coagulate blood vessels in the window preparation. An example of events following irradiation with cooling is given in the text and figures below. Figure 8 shows the events which occurred immediately before and after a 9.5 J/cm2 irradiation. Vessels from a window preparation are shown in Figure 8a where a main venule-arteriole pair (marked 1) branch into two smaller pairs (2 and 3). An OCT amplitude image and velocity image corresponding to the pair at site 1 are shown in Figure 8b and c. The window was irradiated with a radiant exposure of 9.5 J/cm2 (532 nm , 10 ms, and 3 mm spot size) in the area marked by the circle (an initial irradiation of 8 J/cm2 yielded no changes visually or in CDOCT). Immediately following irradiation, focal constriction in the two smaller branches occurred (Figure 8d). CDOCT images of the main pair at site 1 showed little change in flow and size (not shown), however the constriction at site 2 was confirmed by CDOCT. Figures 8e is a velocity image at site 2 before the irradiation, and 8f is the image within minutes of irradiation. The location of the venule and arteriole at site 2 are shown by the arrows in Figure 8e. Neither vessel showed up in the velocity image after irradiation indicating there was no detectable flow.

One hour after focal constriction was shown to occur and CDOCT images were taken, the window was again imaged. Figure 9a shows that focal constriction is still present in both smaller branches. CDOCT imaging revealed that there was still some flow present in both the arteriole and venule and that the venule certainly was constricted. Not much change is seen in the arteriole diameter. Again, flow at site 1 remained unaffected (not shown).

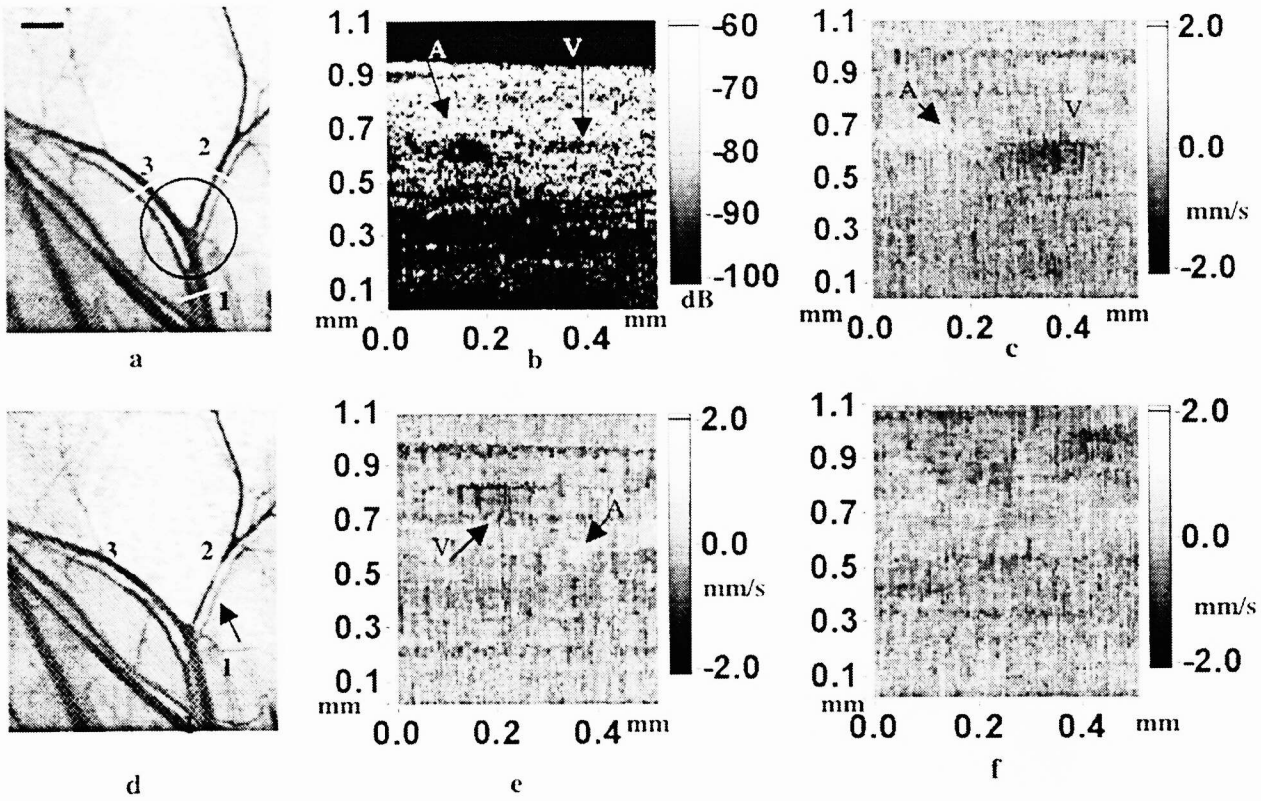

Figure 8. Example of irradiated venule-arteriole pair (before-after) (a) Image before – circle indicates the irradiation site – scale 1mm (b) Amplitude image across main branch (1) (c) CDOCT image across main branch (d) Focal constriction following 9.5 J/cm2 laser pulse (e) CDOCT across venule and arteriole at 2 before irradiation (f) CDOCT across constriction site 2 after irradiation. Flow in the main branch remained unchanged.

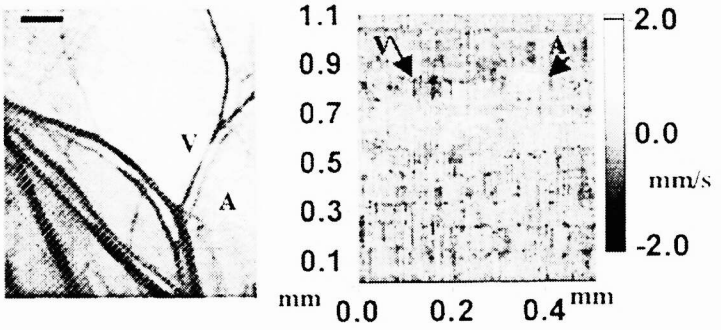

Figure 9. Focal constriction seen 1 hour post-irradiation at site 2 (9.5 J/cm2). Scale: 1mm

Observation of the window was performed after 24 hours. Although the venule and arteriole at site 2 appeared constricted (Figure 10a) and the venule initially appeared blocked by a coagulum (Figure 10b) -the coagulum flowed out of view within minutes of the start of video recording. CDOCT revealed that there was still flow present at site 2 (Figure 10d). Since none of the vessels of interest were permanently damaged, an 11 J/cm2 radiant exposure was given in the same location. The venule was damaged, while flow remained in the arteriole (not shown). It took a radiant exposure of 12 J/cm^2 to permanently damage the arteriole. This was confirmed 48 hours later.

An interesting thing to note is that after the 12 J/cm2 radiant exposure, there was still flow at site 1, even up to 2 hours after irradiation. Observation at 24 hours revealed all flow had stopped in the main branch at site 1 and in the branch at 3. This is an example where damage up or downstream can led to permanent damage of much larger vessels.

In all, 6 venules and 5 arterioles were irradiated after the window was cooled in the manner described. Since this is not enough data to make a statistical determination of the effect of cooling, we will only present the individual data shown in relation to data taken on 250 vessels in the case where cooling was not applied.

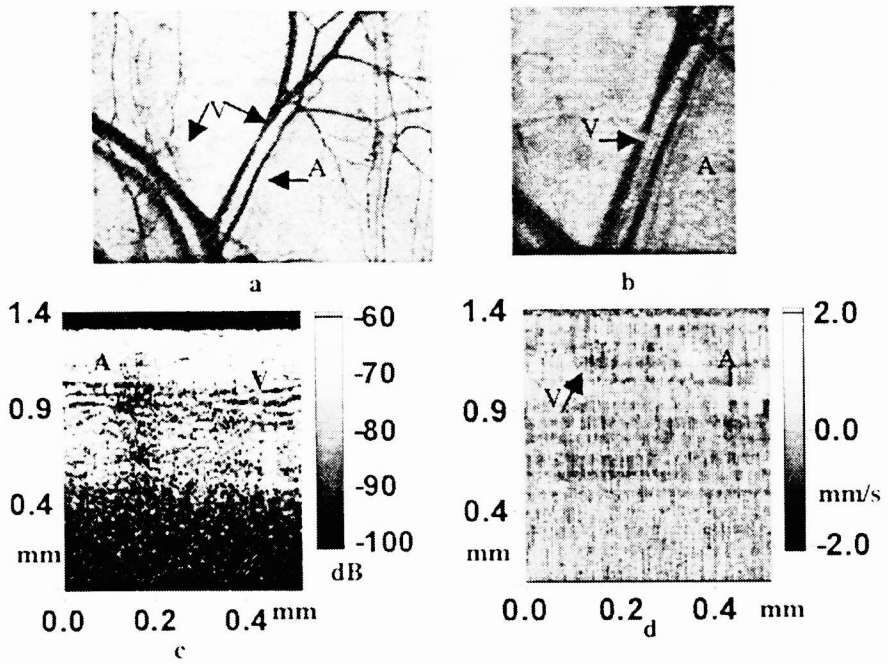

Figure 10. 24 hours post- irradiation (9.5 J/cm^2). (a) Some focal constriction at site 2 is still seen. (b) A close-up of site two shows partial occlusion of venule. (c) Amplitude image: both the venule and arteriole sites can be seen with significant shadowing under the venule. (d) velocity image reveals there may still be some flow in the venule.

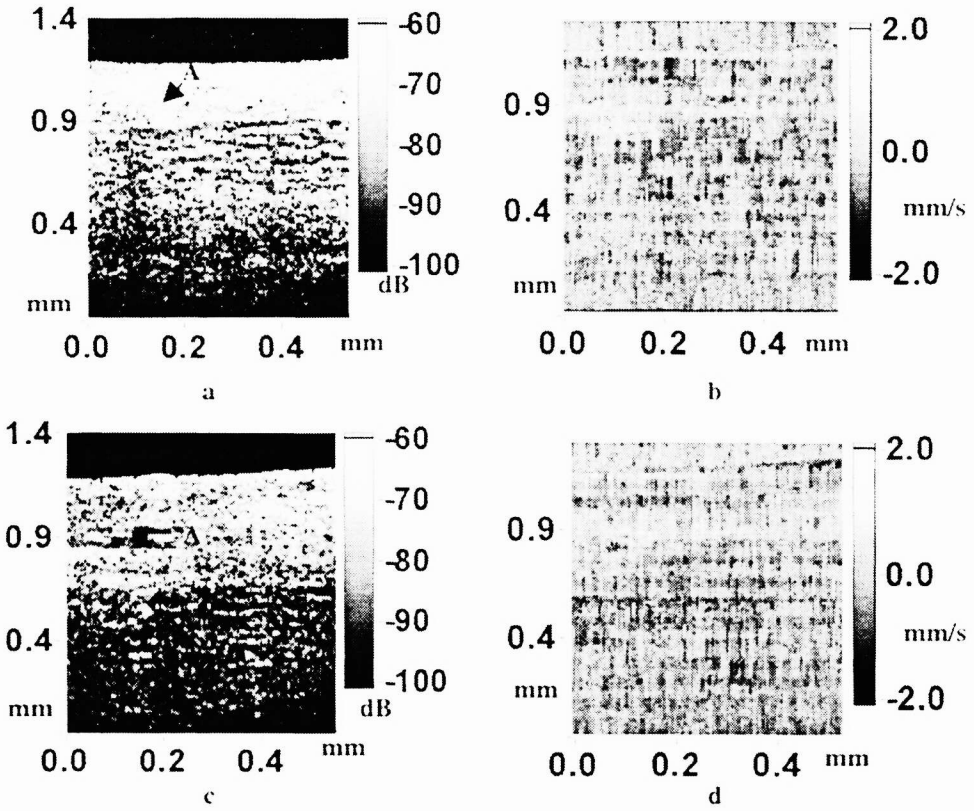

Figure 11. 24 hours after 12 J/cm^2 irradiation. (a) Amplitude image at site 2. (b) Velocity flow image at site 2 – there is no sign of flow in the arteriole or venule. (c) Amplitude image at site 1. The site indicated by the arrow may be an area of pooled blood in the damaged venule. (d) Doppler imaging indicates no flow in the main branch after 48 hours.

The details of the experiments leading to this data can be found in a previous publication [8] Shown in Figure 12 is the result of these experiments. Probit analysis was used to determine the 50 percent probability for permanent damage to blood vessels for the 532 nm, 10 ms, 3 mm spot size laser. The 50 percent probability determined as a result of those irradiations are shown by the dashed and solid lines. The radiant exposure required to permanently damage the vessels with cooling applied are shown on the same plot as individual marks. Note that these are not ED50 values, but individual trial results. From this small amount of data it is not possible to say anything conclusively, but in general the trend was that a slightly higher radiant exposure was required to damage either arterioles or venules. Further investigation is required to determine if this is indeed true. In addition, it is of interest to determine the effect on the radiant exposure required to damage vessels when both cooling and irradiations are done from the epidermal side.

4. CONCLUSIONS

CDOCT was successfully used to monitor changes in morphology of the microcirculation of the hamster window model. It provided valuable information on the changes that occurred after vessels were cooled and irradiated. One limitation is that we could not investigate capillary function due to insufficient resolution of the CDOCT and surgical microscope. Temperature measurements revealed that regardless of the cooling method used, significant temperature changes occur below superficial layers. This may have a consequence on the degree of radiant exposures required to cause permanent vessel damage, resulting in a situation where the superficial layers are damaged. In the small sample pool it appeared the trend was that a higher radiant exposure is needed for permanent vessel damage. A very large number of samples is required to reduce uncertainty.

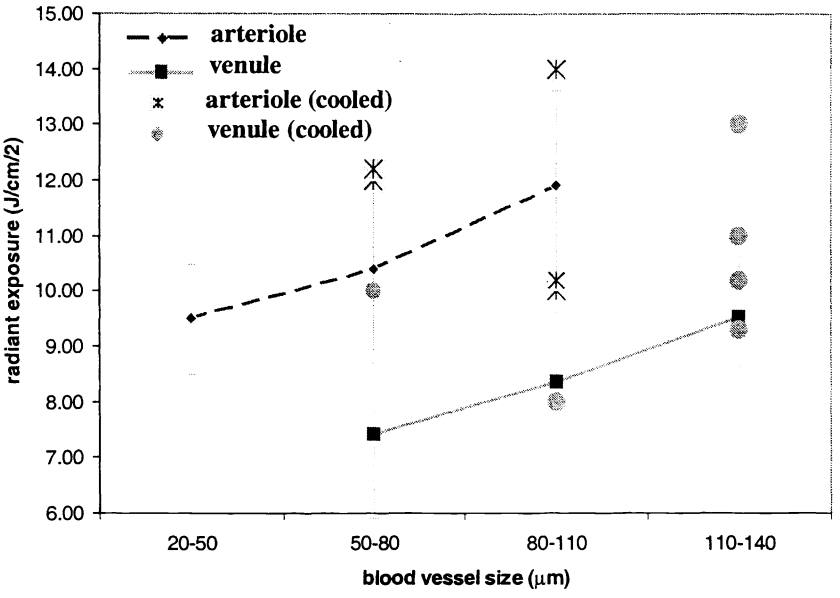

Figure 12. Radiant Exposures required to cause permanent vessel damage. ED50 values for permanent damage without cooling are show by the dashed and solid lines. Individual circles (venules) and crosses (arterioles) represent vessels which were cooled prior to irradiation (these are not ED50 values)

5. ACKNOWLEDGEMENTS

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