

Mechanisms of Microvascular Response to Laser Pulses

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“Selective photothermolysis” is widely used for treating vascular lesions. In order to understand mechanisms of response, we investigated fast events during pulsed laser treatment of microvessels. A high-speed (2000 fps) CCD camera and microscope were used to image hamster cheek pouch microvessels during and after 532 nm and 1064 nm laser pulse exposures. Pulse duration and fluence were varied systematically (1–50 ms, 0–600 J per cm²). Threshold fluences for fast events were determined. On a millisecond time-scale, a specific series of fast events occur, which are wavelength, fluence, irradiance, and pulse duration dependent. In order of increasing fluence we observed: blood coagulation, vasoconstriction, thread-like appearance of the treated vascular segment, vessel disappearance, intravascular cavitation, bubble formation, vessel wall rupture and hemorrhage, and shrinkage of perivascular tissue. With increasing pulse duration, the threshold fluences for coagulation, vessel disappearance, and cavitation increase, and cavitation becomes less violent, conforming to the vessel lumen. Intravascular cavitation did not always rupture the vessel wall, and is not the mechanism for immediate vessel disappearance, a desired endpoint for treating vascular lesions. The apparent mechanism for immediate vessel disappearance is contraction of intravascular blood and perivascular collagen after thermal denaturation. This study suggests that detecting fast events in humans, in real time, may provide useful feedback signals for “smarter” laser devices.

Key words: laser/port wine stain/cavitation/microvessel.
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Laser wavelengths absorbed by hemoglobin are used to treat cutaneous vascular lesions such as port-wine stains (PWS), hemangioma, spider angioma, cherry angioma, venous lake, and telangiectasia of the face and legs. The basic objective in treating vascular lesions is to damage irreversibly abnormal blood vessels while sparing the surrounding skin tissue. Many problems, however, are yet to be solved, including occasional unresponsiveness, recurrence, and side-effects. Argon and other continuous and quasicontinuous visible light lasers were first used to treat PWS. The risk of scarring in the pink and immature group was as high as 25% (Silver, 1986). The pulsed dye laser (PDL) was then developed based on the concept of selective photothermolysis (Anderson and Parrish, 1983). Although results are very good in many cases, PWS seldom clear completely, multiple treatments are necessary to achieve maximal lightening and purpura is a typical side-effect. About 20% of PWS are resistant to PDL, but the reason for resistance is unknown (Renfro and Geronemus, 1992). Potential side-effects include epidermal damage (crusting, erosion, and blistering), purpura, pigmentary alteration, and scarring. A variety of lasers have been developed for treating vascular lesions. These include long-pulsed PDL, 532 nm pulsed lasers with pulse duration of 1 to 100 ms, pulsed alexandrite laser at 755 nm with pulse duration up to 20 ms, pulsed 800 nm diode lasers with pulse durations from 5 to 1000 ms and pulsed 1064 nm Nd:YAG laser with pulse duration up to 100 ms.

Wavelength affects both depth of treatment and the uniformity of heating luminal blood. Generally, the longer the

laser wavelength up to 1200 nm the deeper the penetration (Anderson *et al*, 1983; van Gemert *et al*, 1986; Tan *et al*, 1989) due to lower scattering and absorption. For large leg veins, it is hard to heat uniformly the entire luminal blood content. At wavelengths weakly absorbed by hemoglobins, such as 700 to 1100 nm, high treatment fluences are needed. Pulse duration, also called pulse width, affects spatial confinement of the thermal energy within the target vessel (Anderson and Parrish, 1983; van Gemert and Welch, 1989; Dierickx *et al*, 1995). Ideally, pulse width should be about equal to the thermal relaxation time for the target vessel, which is proportional to the square of vessel diameter (Dierickx *et al*, 1995). Smaller vessels have shorter thermal relaxation time and may be best treated with pulses less than 10 ms. When the pulse width exceeds the thermal relaxation time, more heat diffuses outside the vessel during laser exposure causing thermal damage to surrounding tissue. In contrast, very short pulses generate high local peak temperature potentially leading to intravascular cavitation from explosive vaporization that may result in vessel rupture (Paul *et al*, 1983). Fluence is defined as energy delivered per unit area. Ideally, fluence may be chosen to produce permanent vessel damage, without rupturing the wall to cause hemorrhage. The threshold fluence for a given response can often be determined, and is a useful quantitative endpoint. Exposure spot size affects the distribution of light within skin. Because of multiple scattering, the intensity of a narrow beam (small spot size) tends to suffer greater loss by diffusion. Larger exposure spot sizes are therefore desirable when the target vessels are deeper in skin (Jacques, 1992).

The aim of this study was to gain an understanding of fast events occurring during laser exposure of blood vessels, and how those events correspond to responses of blood vessels exposed to different wavelengths and pulse widths.

Results

A specific series of fast events, which were fluence, irradiance, and pulse width dependent, occurred in blood vessels during laser exposures. The events occurring during laser pulses in order of increasing fluence were: blood coagulation, vasoconstriction, thread-like appearance of the treated vascular segment, vessel disappearance, intravascular cavitation, extravascular cavitation, vessel wall rupture and hemorrhage, denaturation and shrinkage of perivascular collagen. Responses of blood vessels to 532 and 1064 nm wavelengths were qualitatively similar but different quantitatively. At fluences less than threshold for blood coagulation there was either no apparent change in the blood vessel or occasional transient vasodilation. At the threshold for blood coagulation, an intravascular coagulum of blood was observed, sometimes embolized by blood flow (Fig 1), but more commonly adherent to the vessel wall.

The coagulum appeared dark in both imaging systems, consistent with an increase in optical scattering and/or absorption. At higher fluences, but lower than the threshold for cavitation, the column of intravascular coagulated blood separated and retracted from the center of the irradiated vascular segment, forming plugs at both ends of a constricted or an empty vessel segment. These responses were called thread-like appearance (Fig 2) and vessel disappearance (Figs 3, 4), respectively. The rate of coagulum separation increased with increasing fluence. Short pulse durations (1, 3, and 5 ms) did not cause vessel disappearance, but with longer pulse durations (10, 25, and 50 ms) vessel disappearance was reliably present above a threshold. Longer pulses yielded easier and more extensive vessel disappearance.

At fluences equal to or higher than the threshold for cavitation, a transparent vapor cavity arose inside the vessel lumen, expanded and collapsed rapidly after the laser pulse. The vapor cavity typically expanded along the length of vessel lumen, further displacing the plugs formed by blood coagulation. Variably, intravascular cavitation ruptured the vessel walls (Figs 5–7). At high fluence and with pulse durations equal to or longer than 10 ms, intravascular cavitation was followed by explosive bubble expansion and rupture of the vessel wall (extravascular cavitation, Figs 8,

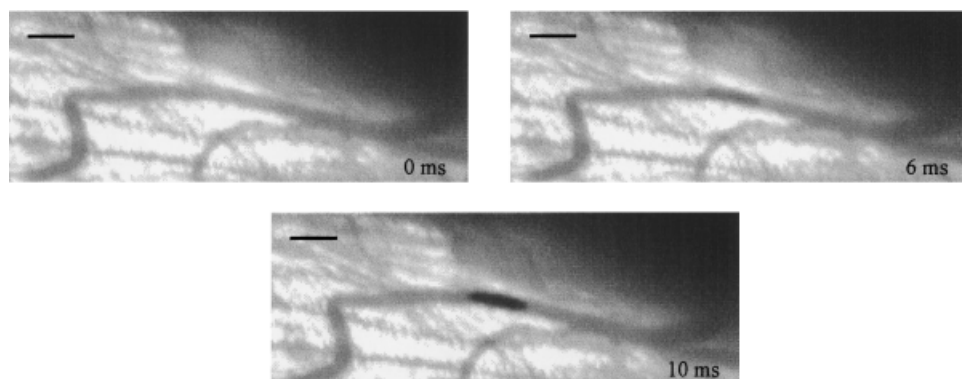


Figure 1
Near the threshold fluence for blood coagulation (60 J per cm², 1064 nm, 10 ms laser pulse), an intravascular coagulum appears at 6 ms into the laser pulse, then embolizes along the blood stream. Scale bar: 500 μM.

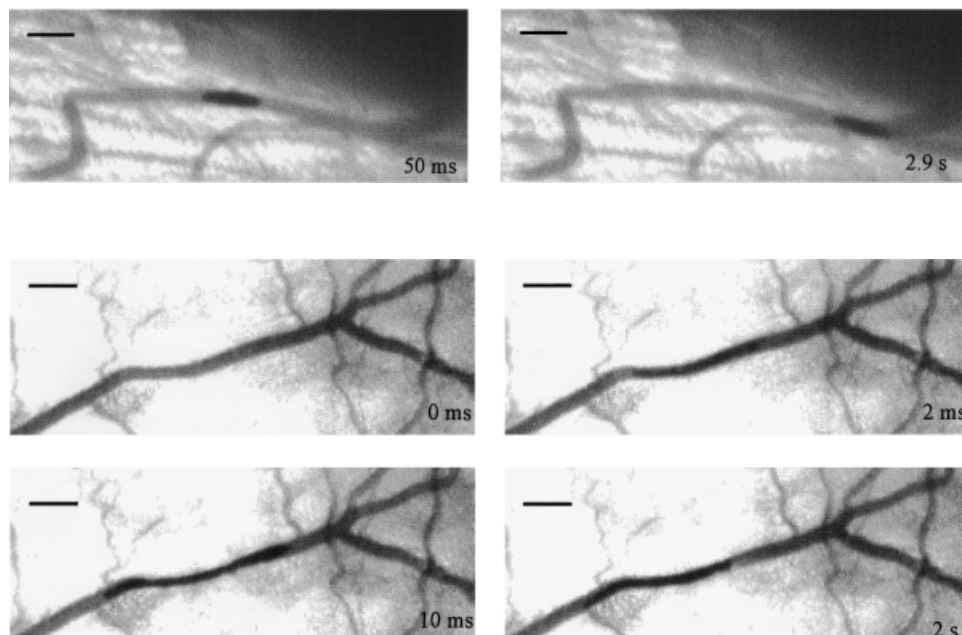


Figure 2
Above the threshold for blood coagulation (10 J per cm², 532 nm, 10 ms laser pulse) a coagulum appears at 2 ms into the laser pulse, elongates, and forms a plug in the vessel blocking blood flow. Scale bar: 500 μM.

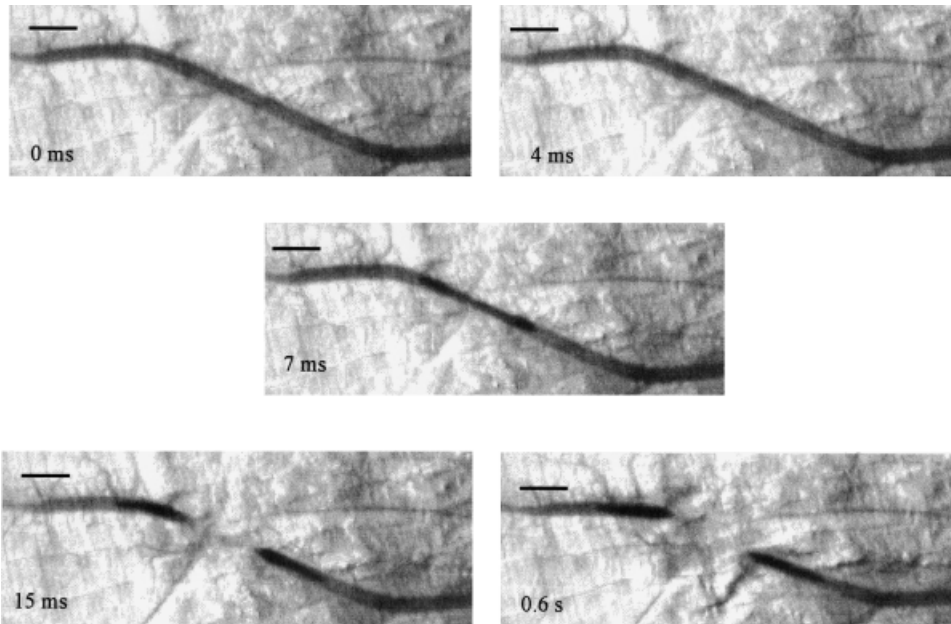


Figure 3
Immediate vessel disappearance occurs well below the threshold for cavitation (20 J per cm², 532 nm, 25 ms laser pulse). Blood coagulation begins 4 ms into the laser pulse, elongates and begins to separate from the center of irradiated area at 7 ms. The irradiated vascular segment disappears at 15 ms into the pulse. This sequence may be ideal for therapy. Scale bar: 500 μM.

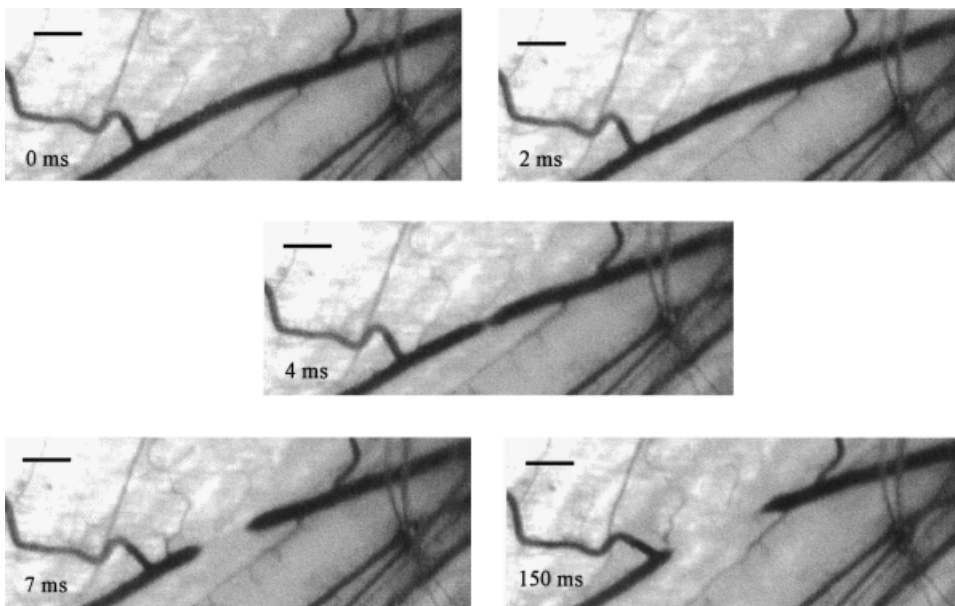


Figure 4
Vessel disappearance without any cavitation occurs below the threshold for cavitation (380 J per cm², 1064 nm, 25 ms laser pulse). Blood coagulation begins 2 ms into the laser pulse, elongates, and begins to separate from the center of irradiated area at 4 ms. The irradiated vascular segment disappears at 7 ms into the pulse. Scale bar: 500 μM.

9). Bubbles up to 20 times the blood vessel diameter were noted at very high fluences. Rarely, bubble formation occurred without apparent intravascular cavitation. The final appearance of the irradiated vessel after collapse of a large bubble was either thread-like or hemorrhagic, with perivascular tissue obviously shrunken and damaged.

Much higher fluences were needed to produce the same events at 1064 nm compared with 532 nm, and bubble formation with vessel wall rupture and hemorrhage was less likely to occur with the 1064 nm wavelength. Responses to the 532 and 1064 nm wavelengths, and the threshold fluences for coagulation, vessel disappearance, and cavitation are summarized in Table I and Table II, respectively.

Measured response threshold fluences are shown in Fig 10. With the 532 nm wavelength, the threshold fluences for coagulation, vessel disappearance, and cavitation increased with increasing pulse duration. For cavitation the

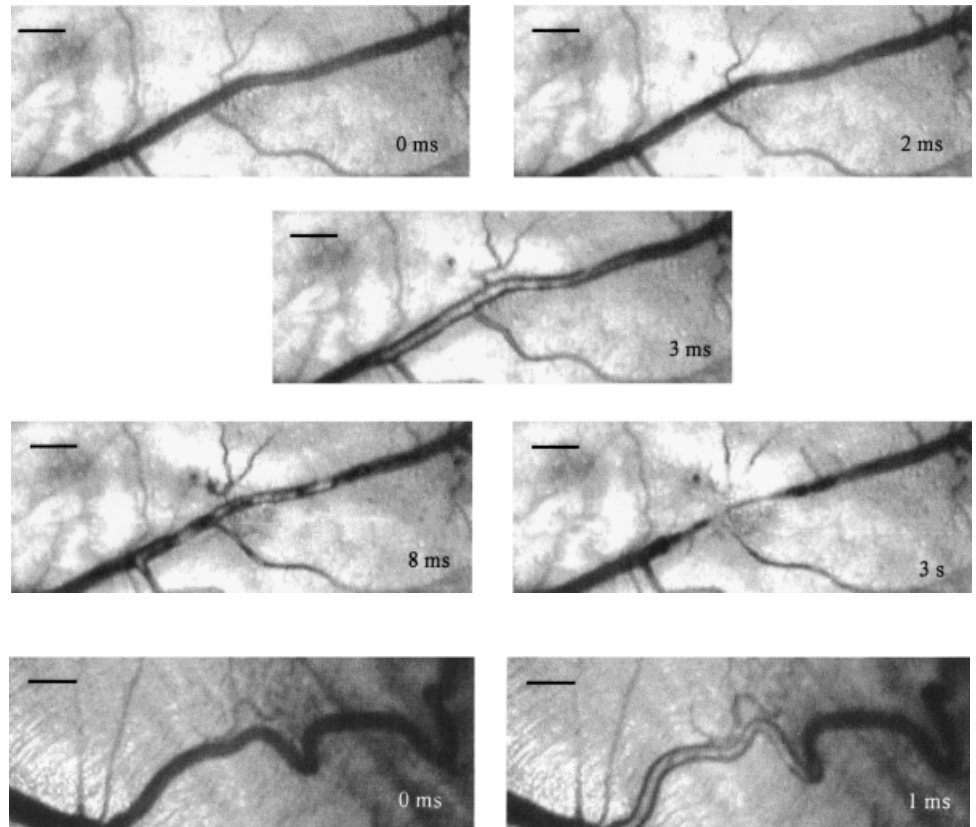
slope was much higher. Irradiance (W per cm²) expresses the incident power per unit area, and is equal to fluence divided by pulse duration. With the 532 nm wavelength, the threshold irradiances for coagulation and cavitation decreased with pulse duration up to 10 ms, but with pulse durations longer than 10 ms they stayed almost constant (Fig 11). The threshold irradiance for vessel disappearance decreased between 10 and 50 ms pulse duration (Fig 11). With the 1064 nm wavelength, threshold fluences and irradiances were qualitatively similar to 532 nm, but quantitatively much larger (Figs 10, 11).

Discussion

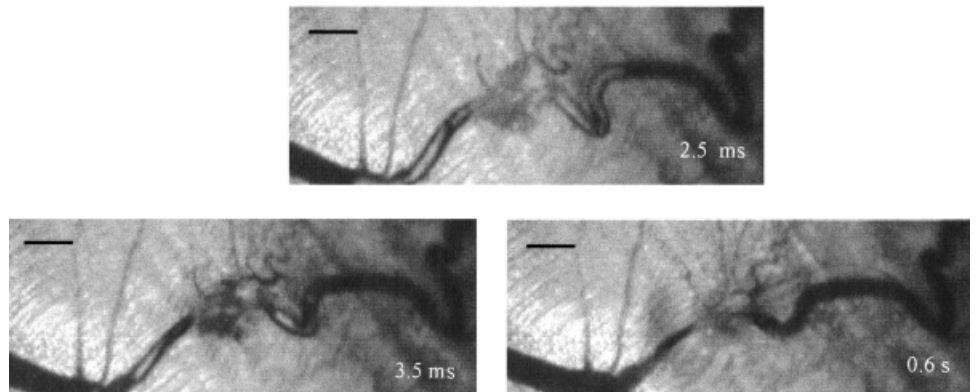
This study has practical implications for improving treatment of abnormal blood vessels by selective photothermo-

Figure 5

Intravascular cavitation without vessel wall rupture occurs above the threshold for cavitation (20 J per cm², 532 nm, 10 ms laser pulse). Blood coagulation and cavitation begin 2 ms and 3 ms into the laser pulse, respectively. The vapor bubble spreads out along the vessel. Coagulum forms plugs at both ends of the irradiated area, which finally appears irregular and thread-like. Scale bar: 500 μ M.

**Figure 6**

Intravascular cavitation with vessel wall rupture occurs well above the threshold for cavitation (20 J per cm², 532 nm, 3 ms laser pulse). Cavitation occurs at 1 ms into the laser pulse and the vapor spreads out along the vessel. The vessel wall begins to rupture at 2.5 ms into the pulse. The final appearance of the irradiated vascular segment is hemorrhagic. Scale bar: 500 μ M.



lysis. High-fluence Nd:YAG laser pulses at 1064 nm, which penetrate much deeper into skin than green or yellow light, produced the same sequence of fast events as green 532 nm pulses. This suggests that 1064 nm and similar infrared sources may ultimately have advantages for treating microvascular skin lesions. The results also suggest how to make "smart" sources, incorporating *in vivo* detection of fast events during each optical pulse, to optimize results. The findings further support previous studies (Kimel *et al*, 1994; Dierickx *et al*, 1995), which suggest that a pulse duration nearly equal to the thermal relaxation time of a target microvessel are optimal. Pulses between about 10 and 30 ms were optimal for selective photothermolysis of the 120 to 150 μ m vessels used in this study, corresponding well to the estimated thermal relaxation time.

Selective photothermolysis is driven by absorption of optical energy by hemoglobins, leading to confined heating, and thermally mediated injury. Although widely used with

good clinical results and a minimal risk of scarring, many important details remain speculative or unknown, and present treatments are far from perfect. Much of our understanding of selective photothermolysis of blood vessels has come from simplified theoretical models (Anderson and Parrish, 1981; van Gemert *et al*, 1986; Pickering *et al*, 1989; Dierickx *et al*, 1995) studies of histopathology and non-invasive imaging after laser treatment (Greenwald *et al*, 1981; Tan *et al*, 1986; Fiskerstrand *et al*, 1996b; Nelson *et al*, 2001), laser parametric studies (Paul *et al*, 1983; Garden *et al*, 1986; Tan *et al*, 1989), and clinical trials (Renfro and Geronemus, 1993; Fiskerstrand *et al*, 1996a; Hohenleutner *et al*, 2001; Chang *et al*, 2002). None of these approaches directly reveal the events occurring during laser exposure.

This study provides a qualitative and quantitative understanding of fast events occurring during selective photothermolysis of blood vessels. Specifically, we intended to discover the events leading to immediate vessel disap-

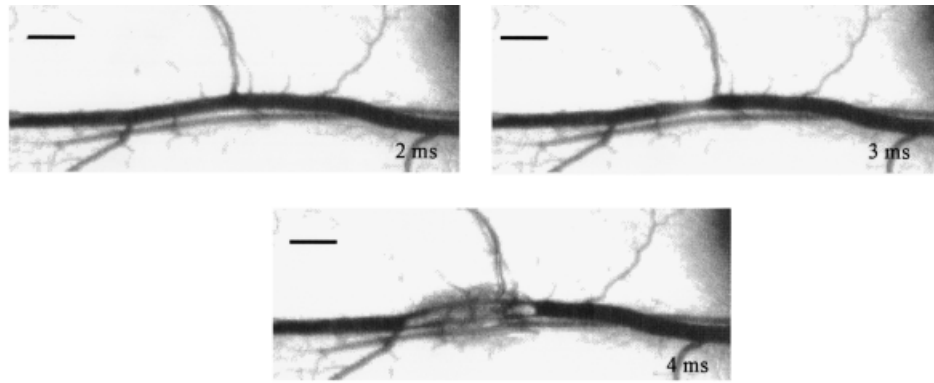


Figure 7

Intravascular cavitation with vessel wall rupture occurs at the threshold for cavitation (400 J per cm^2 , 1064 nm , 25 ms laser pulse). Blood coagulation begins 2 ms into the laser pulse, elongates, and begins to separate from the center of irradiated area at 3 ms. Cavitation and vessel wall rupture occurs at 4 ms into the laser pulse. The final appearance of the irradiated vessel segment is hemorrhagic. Scale bar: $500 \mu\text{M}$.

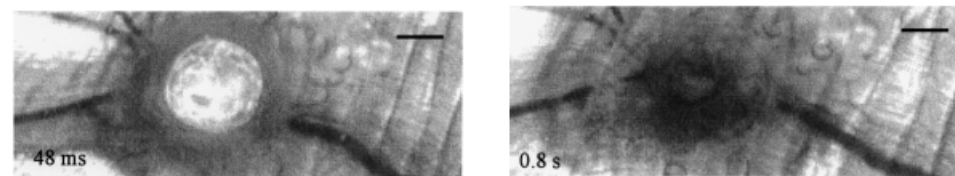
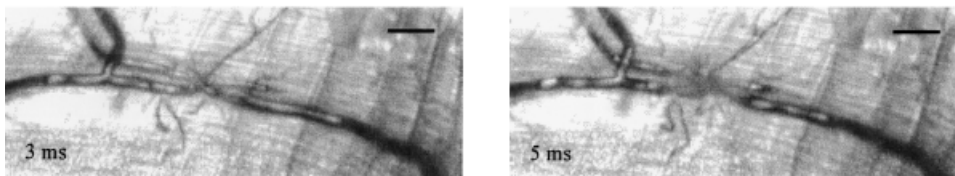
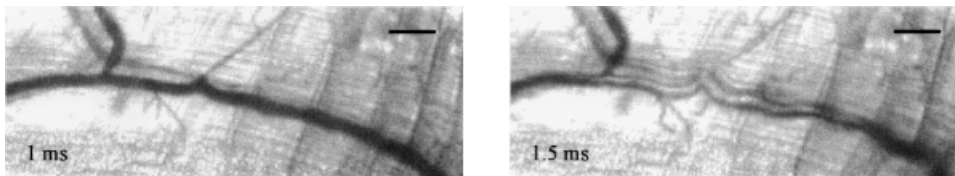
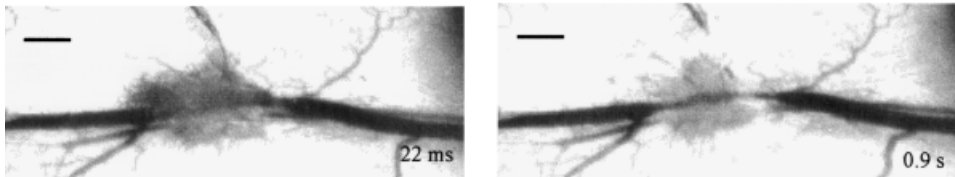


Figure 8

Intravascular cavitation with huge extravascular bubble occurs well above the threshold for cavitation (160 J per cm^2 , 532 nm , 50 ms laser pulse). Blood coagulation and intravascular cavitation begin 1 ms and 1.5 ms into the laser pulse, respectively. The vapor bubble spreads out along the vessel. Vessel wall rupture and huge bubble begins to form at 5 ms into the pulse. There is marked hemorrhagic and perivascular collagen damaged. Scale bar: $500 \mu\text{M}$.

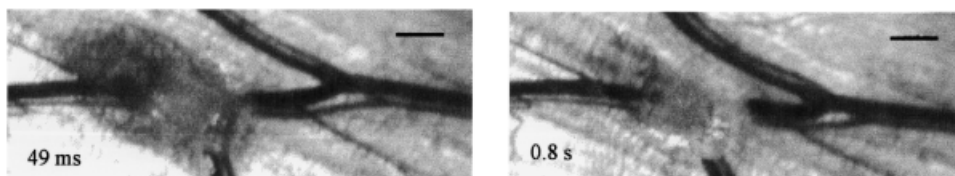
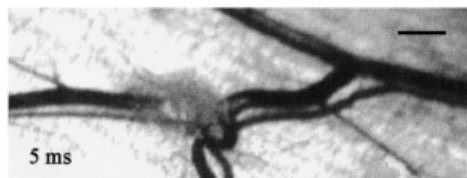
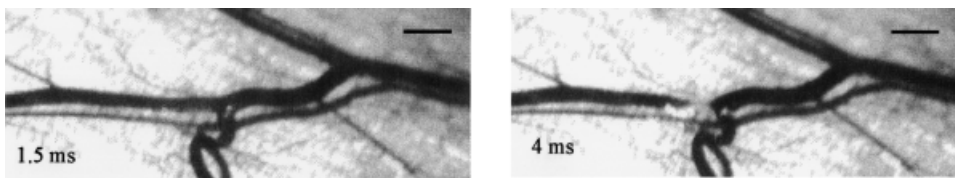


Figure 9

Intravascular cavitation with huge extravascular bubble occurs well above the threshold for cavitation (500 J per cm^2 , 1064 nm , 50 ms laser pulse). Blood coagulation begins 1.5 ms into the laser pulse, elongates, and begins to separate from the center of irradiated area at 4 ms. Vessel wall rupture and huge bubble begins to form at 5 ms into the pulse. There is marked hemorrhagic and perivascular collagen damaged. Scale bar: $500 \mu\text{M}$.

Table I. Fast events and their fluence thresholds for 532 nm wavelength at different pulse durations

Pulse duration	1 ms	3 ms	5 ms	10 ms	25 ms	50 ms
Coagulation	Y (2) ^a	Y (2) ^a	Y (3) ^a	Y (3) ^a	Y (4) ^a	Y (5) ^a
Constriction	Y	Y	Y	Y	Y	Y
Thread-like appearance	N (≤ 10) ^b	Y	Y	Y	Y	Y
Vessel disappearance	N (≤ 10) ^b	N (≤ 23) ^b	N (≤ 38) ^b	Y (10) ^a	Y (13) ^a	Y (15) ^a
Cavitation	Y (5) ^a	Y (8) ^a	Y (10) ^a	Y (18) ^a	Y (38) ^a	Y (85) ^a
Bubble formation	N (≤ 10) ^b	N (≤ 23) ^b	N (≤ 38) ^b	Y	Y	Y
Hemorrhage	N (≤ 10) ^b	Y	Y	Y	Y	Y
Collagen damage	N (≤ 10) ^b	N (≤ 23) ^b	N (≤ 38) ^b	Y	Y	Y

^aValues (in J per cm²) in parenthesis are threshold fluences for the particular event.

^bValues (in J per cm²) in parenthesis are maximum fluences available for a given pulse width.

Y indicates an observed response; N indicates not observed, up to the highest fluence (b) available.

Table II. Fast events and their fluence thresholds for 1064 nm wavelength at different pulse durations

	1 ms	3 ms	5 ms	10 ms	25 ms	50 ms
Coagulation	N (≤ 20) ^b	Y (35) ^a	Y (37) ^a	Y (40) ^a	Y (45) ^a	Y (64) ^a
Constriction	N (≤ 20) ^b	Y	Y	Y	Y	Y
Thread-like appearance	N (≤ 20) ^b	N (≤ 60) ^b	Y	Y	Y	Y
Vessel disappearance	N (≤ 20) ^b	N (≤ 60) ^b	N (≤ 100) ^b	Y (120) ^a	Y (280) ^a	Y (350) ^a
Cavitation	N (≤ 20) ^b	N (≤ 60) ^b	Y (90) ^a	Y (175) ^a	Y (400) ^a	Y (500) ^a
Bubble formation	N (≤ 20) ^b	N (≤ 60) ^b	N (≤ 100) ^b	N (≤ 200) ^b	Y	Y
Hemorrhage	N (≤ 20) ^b	N (≤ 60) ^b	Y	Y	Y	Y
Collagen damage	N (≤ 20) ^b	N (≤ 60) ^b	N (≤ 100) ^b	Y	Y	Y

^aValues (in J per cm²) in parenthesis are threshold fluences for the particular event.

^bValues (in J per cm²) in parenthesis are maximum fluences available for a given pulse width.

Y indicates an observed response; N indicates not observed, up to the highest fluence (b) available.

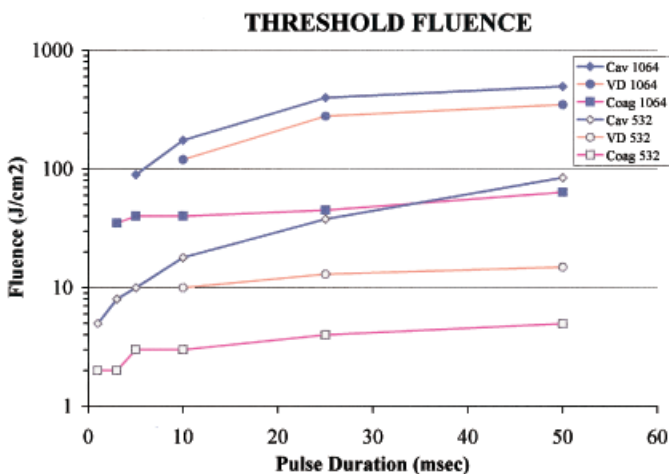


Figure 10
Response threshold fluence versus pulse duration, for 532 nm and 1064 nm laser pulses. Cav, cavitation; VD, vessel disappearance; Coag, blood coagulation.

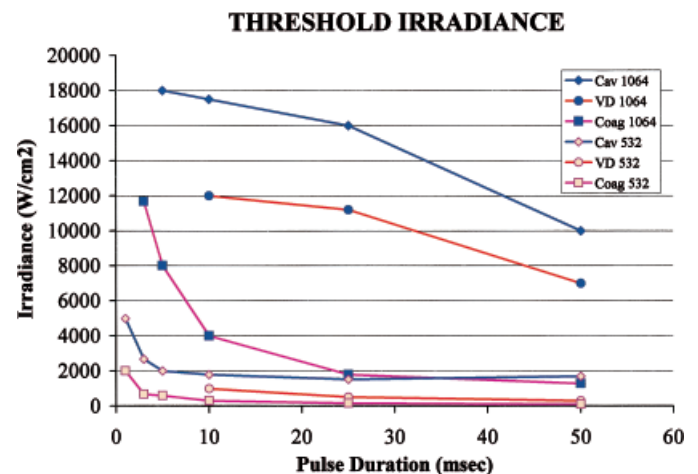


Figure 11
Response threshold irradiance versus pulse duration, for 532 nm and 1064 nm laser pulses. Cav, cavitation; VD, vessel disappearance; Coag, blood coagulation.

pearance without hemorrhage, which is a useful clinical endpoint associated with good results. Before the study, we surmised that intravascular cavitation, i.e., steam bubble formation in blood, was necessary to produce vessel disappearance. Clearly, this speculation was incorrect.

Although intravascular cavitation can occur and can drive blood along the length of vessel (Figs 5, 6), it is obvious that immediate vessel disappearance does not require cavitation (Figs 3, 4). Moreover, cavitation was frequently associated with vessel rupture and hemorrhage (Figs 7–9),

generally considered to be unwanted side-effects. This study clearly shows that immediate disappearance of a target vessel is caused by splitting and retraction of the intravascular thermal coagulum, which typically occurs before and without cavitation. The potential for recanalization (survival) of such "disappeared" target vessels merits further study.

In practice, a host of patient-specific variables are at play during selective photothermolysis. These variables include at least skin pigmentation, target vessel size, depth of target vessels, and hemoglobin concentration. It is difficult for even a seasoned therapist to optimize empirically treatment parameters. An appealing alternative, is to design pulsed light sources (lasers, intense pulsed light) that detect rapid events in the target vessels, and cease optical exposure when a desired response has been achieved. For example, detection of the onset of cavitation might be used as a "stop" signal for optical pulses during treatment. Furthermore, irradiance of the source could be adjusted such that the desired response was obtained in approximately 1 thermal relaxation time for the size of the target vessels involved.

The events observed with increasing fluence in this study were: intravascular thermal coagulum (which may or may not embolize), vessel disappearance, intravascular cavitation expanding along the vessel lumen, violent bubble-like cavitation, hemorrhage, and perivascular tissue injury. These events occurred or were initiated during the laser pulse. These events occurred in the same sequence, and with similar dependence on pulse duration at both the 532 and 1064 nm wavelengths. For a given wavelength, pulse duration, and spot size, models of selective photothermolysis predict that peak temperature of the target vessel increases with increasing fluence. Therefore, the sequence of events listed above may also be taken to represent events occurring as a function of increasing target vessel temperature.

As expected, much higher fluence was necessary to achieve a given response at 1064 nm, compared with 532 nm. The absorption coefficient of oxygenated blood at 532 nm is 260 per cm (van Gemet *et al*, 1992) and at 1064 nm is only 5.6 per cm (Yaroslasvsky *et al*, 1996), a ratio of 46. Quantitatively, fluence thresholds at 1064 nm for any given response are about 10 to 20 times those at 532 nm, less than expected based on blood's optical absorption. This discrepancy is consistent with a process that enhances absorption of 1064 nm in blood during laser exposure. These findings support recent observations of Barton *et al* (1999), who reported a large increase in 1064 nm absorption of blood due to the formation of methemoglobin (metHb) during thermal denaturation. MetHb is an oxidized form of hemoglobin, with a distinct absorption band near 630 nm. The intravascular coagulum caused by thermal denaturation of blood was easily seen as an opaque object in this study (Fig 1), by laser stroboscopic transillumination at 620 nm. Potentially, formation of metHb during a laser pulse may offer another useful signal for feedback, e.g., by monitoring changes in skin reflectance near 630 nm.

In summary, this study reveals fast events occurring during selective photothermolysis of small blood vessels. Pulse durations of 10 to 30 ms, equivalent to approximately

the thermal relaxation time of the target vessels used in this study, produced selective thermal injury without gross mechanical injury when the fluence was sufficient for vessel disappearance, but less than that causing cavitation (vaporization) and hemorrhage. "Smart" sources, which detect the presence of an intravascular coagulum and vessel disappearance, and stop exposure before or at the onset of cavitation, appear to be promising. The 1064 nm Nd:YAG laser, operating near 10 ms pulse duration and delivered with appropriate skin cooling and feedback controls, may provide a superior treatment source.

Materials and Methods

Laser and irradiation procedures A long-pulse Nd:YAG/KTP laser (Orion model; Laserscope, Santa Clara, California) was used, delivered via a 600 μm core diameter fiber-optic and hand piece that projected a uniform circular beam 1 mm in diameter, at the exposure site. This laser system emits either 532 or 1064 nm wavelengths. Pulse width is adjustable from 1 to 50 ms. Fluences could be varied from 1 to 380 J per cm^2 with the 532 nm wavelength and from 1 to 1000 J per cm^2 with the 1064 nm wavelength. The laser output consists of a train of 200 ns Q-switched pulses of frequency of 25 kHz (25 micropulses per ms). Blood vessels were exposed to either the 532 or 1064 nm wavelength as described below. Pulse durations of 1, 3, 5, 10, 25, and 50 ms were used. The fluences were varied systematically from 1 to 600 J per cm^2 . Two hundred and 25 vessels were treated in this study. Threshold fluences and threshold irradiances for blood coagulation, vessel disappearance, and intravascular cavitation at each pulse duration were determined by varying fluence in approximately 5 to 10% increments until a minimum value for reproducing a specific event was found. At least three observations were repeated independently to confirm each threshold.

Preparation of blood vessels This study was approved by the research subcommittee of Massachusetts General Hospital. Syrian hamster cheek pouch blood vessels were used as a microvascular target model in this study. The hamster was anesthetized with an intramuscular mixture of ketamine and xylazine HCl. Doses of ketamine were 250 to 750 mg per kg and of xylazine HCl were 0.4 to 1 mg per kg body weight. The animal was laid on a clear plastic platform, one end of which had a circular channel milled out to give a viewing pedestal for the pouch. The blind end of the cheek pouch was grasped and everted using small blunt forceps or cotton buds. A single layered preparation was made and loose connective tissue was removed for optimum visibility of the microcirculation. Diameters of the blood vessels were determined with a dissecting microscope (Wild, model 355110, Heerbrugg, Switzerland) before experiments. The blood vessels chosen for study were between 120 and 150 μm in diameter. This diameter is similar, for example, to that of adult PWS vessels, and fine telangiectases.

Imaging method 1: laser stroboscope A coaxial flashlamp-pumped dye laser (Candela Co., Wayland, Massachusetts) with a nominal pulse duration of 0.3 μs , and a wavelength of 620 nm was used at low fluence as a flash illumination source. The illumination light was delivered through a 1 mm diameter fiber to the experiment site. A delay generator (Stanford Research Systems, model DG535, Sunnyvale, California) was used to delay the illumination pulse relative to the start of treating laser pulse and also to trigger a computer (MacIntosh, Quadra 650, Cupertino, California) to capture digitally the image at selected time intervals. The time delay was confirmed using a dual channel 175 MHz oscilloscope (LeCroy Corporation, Model 9400, Spring Valley, New York) and two photodiodes (Thor Labs, model DET 200, Newton, New Jersey). A CCD camera (Pulnix, model TM 745 E, Sunnyvale, California) was attached to a dual head dissecting microscope with

a magnifying power of 6 to 50 × and used to capture images. A 620 nm long pass filter and an optical 1064 nm band pass filter were used to block the reflected 532 and 1064 nm treatment laser beam, respectively, so as not to interfere with the imaging. NIH image software version 1.49 was used to capture and analyze the data.

Imaging method 2: fast video A high-speed CCD camera (Redlake, model 2000-S, Morgan Hill, California) was also used. This camera captures images up to 2000 frames per second and for a duration of up to 4 s, and was used with a 5 × objective lens with a numerical aperture of 0.12. The output images were delivered to a computer (EPIQ Pentium III, 450 MHz, Merrimack, New Hampshire) equipped with PCI controller board and monitor. Images were stored with a compact disc writer. For illumination, a dual fiber illuminator (Bausch and Lomb, model Fiber Lite, Rochester, New York) was used. The same delay generator was used to control the onset time and duration of image capturing.

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References

- Anderson RR, Parrish JA: Microvasculature can be selectively damaged using dye lasers: A basic theory and experimental evidence in human skin. *Lasers Surg Med* 1:263–276, 1981
- Anderson RR, Parrish JA: Selective photothermolysis: Precise microsurgery by selective absorption of pulsed radiation. *Science* 220:524–527, 1983
- Anderson RR, Jaenicke KF, Parrish JA: Mechanisms of selective vascular changes caused by dye lasers. *Lasers Surg Med* 3:211–215, 1983
- Barton JK, Hammer DX, Pfefer TJ, Lund DJ, Stuck BE, Welch AJ: Simultaneous irradiation and imaging of blood vessels during pulsed laser delivery. *Lasers Surg Med* 24:236–243, 1999
- Chang CJ, Kelly KM, Van Gemert MJ, Nelson JS: Comparing the effectiveness of 585-nm vs 595-nm wavelength pulsed dye laser treatment of port wine stains in conjunction with cryogen spray cooling. *Lasers Surg Med* 31:352–358, 2002
- Dierickx CC, Casparian JM, Venugopalan V, Farinelli WA, Anderson RR: Thermal relaxation of port-wine stain vessels probed *in vivo*: The need for 1–10-millisecond laser pulse treatment. *J Invest Dermatol* 105:709–714, 1995
- Fiskerstrand EJ, Svaasand LO, Kopstad G, Dalaker M, Norvang LT, Volden G: Laser treatment of port wine stains: Therapeutic outcome in relation to morphological parameters. *Br J Dermatol* 134:1039–1043, 1996a
- Fiskerstrand EJ, Svaasand LO, Kopstad G, Ryggen K, Aase S: Photothermally induced vessel-wall necrosis after pulsed dye laser treatment. Lack of response in port-wine stains with small sized or deeply located vessels. *J Invest Dermatol* 107:671–675, 1996b
- Garden JM, Tan OT, Kerschmann R, Boll J, Furumoto H, Anderson RR, Parrish JA: Effect of dye laser pulse duration on selective cutaneous vascular injury. *J Invest Dermatol* 87:653–657, 1986
- van Gemert MJ, Welch AJ: Time constants in thermal laser medicine. *Lasers Surg Med* 9:405–421, 1989
- van Gemert MJ, Welch AJ, Amin AP: Is there an optimal laser treatment for port wine stains? *Lasers Surg Med* 6:76–83, 1986
- van Gemert MJ, Pickering JW, Welch AJ: Modeling laser treatment of port-wine stains. In: Tan OT (ed.) *Book Modeling Laser Treatment of Port-Wine Stains*. Philadelphia: Lea & Febiger, 1992; p 24–47
- Greenwald J, Rosen S, Anderson RR, Harrist T, MacFarland F, Noe J, Parrish JA: Comparative histological studies of the tunable dye (at 577 nm) laser and argon laser: The specific vascular effects of the dye laser. *J Invest Dermatol* 77:305–310, 1981
- Hohenleutner S, Badur-Ganter E, Landthaler M, Hohenleutner U: Long-term results in the treatment of childhood hemangioma with the flashlamp-pumped pulsed dye laser: An evaluation of 617 cases. *Lasers Surg Med* 28:273–277, 2001
- Jacques SL: Simple optical theory for light dosimetry during PDT. *Proc SPIE* 1645:155–165, 1992
- Kimel S, Svaasand LO, Hammer-Wilson M, Schell MJ, Milner TE, Nelson JS, Berns MW: Differential vascular response to laser photothermolysis. *J Invest Dermatol* 103:693–700, 1994
- Nelson JS, Kelly KM, Zhao Y, Chen Z: Imaging blood flow in human port-wine stain *in situ* and in real time using optical Doppler tomography. *Arch Dermatol* 137:741–744, 2001
- Paul BS, Anderson RR, Jarve J, Parrish JA: The effect of temperature and other factors on selective microvascular damage caused by pulsed dye laser. *J Invest Dermatol* 81:333–336, 1983
- Pickering JW, Butler PH, Ring BJ, Walker EP: Computed temperature distributions around ectatic capillaries exposed to yellow (578 nm) laser light. *Phys Med Biol* 34:1247–1258, 1989
- Renfro L, Geronemus RG: Anatomical differences in response to treatment of port wine stain by the pulsed dye laser. *Proc SPIE* 1643:310–316, 1992
- Renfro L, Geronemus RG: Anatomical differences of port-wine stains in response to treatment with the pulsed dye laser. *Arch Dermatol* 129:182–188, 1993
- Silver L: Argon laser photocoagulation of port wine stain hemangiomas. *Lasers Surg Med* 6:24–28, 1986
- Tan OT, Carney JM, Margolis R, Seki Y, Boll J, Anderson RR, Parrish JA: Histologic responses of port-wine stains treated by argon, carbon dioxide, and tunable dye lasers. A preliminary report. *Arch Dermatol* 122:1016–1022, 1986
- Tan OT, Murray S, Kurban AK: Action spectrum of vascular specific injury using pulsed irradiation. *J Invest Dermatol* 92:868–871, 1989
- Yaroslavsky AN, Yaroslavsky IV, Goldbach T, Schwarzmaier HJ: The optical properties of blood in the near infrared spectral range. *Proc SPIE* 2678:314–324, 1996