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Differential Vascular Response to Laser Photothermolysis

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Individual blood vessels in the chick chorioallantoic membrane were selectively coagulated through photothermolysis, using pulsed laser irradiation at 585 nm. Pulse durations were chosen to be 0.45 ms and 10 ms, which correspond to the thermal relaxation times in blood vessels of 30 μm and 150 μm diameter, respectively. The short pulses, at a light fluence $F = 3 \text{ Jcm}^{-2}$, caused permanent occlusion of vessels of 40 μm diameter or less, whereas larger caliber vessels (60–120 μm) required $F = 4–5 \text{ Jcm}^{-2}$. The long-duration pulses, at $F = 7 \text{ Jcm}^{-2}$, caused coagulation of the larger diameter vessels; the small-caliber vessels and capillaries showed resistance to photothermolysis and required multiple exposures to achieve coagulation. The fluence versus diameter (F versus d) relationship for coagulation was calculated for the two pulse durations. The energy de-

posited in a cylindrical absorber of diameter d by an optical field, incident perpendicular to the vessel, was expressed analytically and compared with the energy required to coagulate a blood vessel of the same lumen diameter. When thermal diffusion is incorporated into the model, our findings can be accounted for quantitatively. This information will be of use for improving the laser treatment of port wine stains and other vasculopathies. A surprising observation was that arterioles were damaged at lower incident energy densities than venules having the same lumen diameter, despite the fact that absorbance in oxygen-rich and oxygen-poor blood is the same at 585 nm. **Key words:** arteries and veins/CAM/hemostasis/pulsed dye laser. *J Invest Dermatol* 103:693–700, 1994

The objective of this study is to gain an understanding of the biophysical principles underlying permanent coagulation of blood vessels, through conversion of selectively absorbed radiant energy into thermal energy. Such understanding is of importance in dermatologic laser treatments, such as port wine stains (PWS) [1–4], telangiectasias [5,6], or hemangiomas [7] and may also be of benefit in the treatment of choroidal neovascularization [8,9]. This information is expected to assist physicians in improving laser treatments of vascular lesions, which at the present time are not totally effective.

The chick chorioallantoic membrane (CAM) is an established *in vivo* model for studying microvascular effects [10]. The CAM vasculature is located in a transparent matrix [11] that allows direct visualization of blood flow as well as real-time observation of photothermal effects on blood vessels, such as vessel dilation, constriction, hemostasis, and rupture. The CAM matrix does not significantly absorb or scatter radiation. Thus, the influence of the pertinent laser parameters (wavelength, pulse duration, and fluence) that affect the CAM vasculature can be studied conveniently. Moreover, the CAM is a self-contained system that lends itself to mathematical modeling of optical and thermal effects [12]. Preliminary

considerations are presented here for the choice of laser parameters, which are fully described in *Materials and Methods*.

Wavelength (λ) Light anywhere within the visible spectrum may be used to irradiate the transparent CAM blood vessels without encountering absorption in overlying epidermis that normally accompanies irradiation of dermal tissues [13,14]. In accordance with general considerations associated with increased light penetration in tissue at longer wavelengths [1,14–16] the absorption of blood in the yellow-red spectral region around 585 nm was chosen for irradiation. A singular feature of the CAM is the possibility to study coagulation patterns in individual arterioles and venules. The primary targets for radiative heating are the red blood cells (RBCs), which undergo morphologic changes following pulsed laser irradiation [17]. We seek, therefore, to equalize photothermal effects due to light absorption by the two endogenous chromophores, oxyhemoglobin (HbO_2) and hemoglobin (Hb), present in the RBCs. Equal absorption is achieved at the isosbestic point ($\lambda = 585 \text{ nm}$) in the absorption spectra of these two target chromophores. Because of deeper tissue penetration, this wavelength is of clinical interest, even though the absorption coefficient at 585 nm is 50% lower than the absorption peak of HbO_2 at $\lambda = 577 \text{ nm}$ [15,16].

Pulse Duration (t_p) The pulse duration governs the spatial confinement of the thermal energy within the targeted vessel [1,18]. Ideally, the pulse duration (t_p) should be compatible with the diameter (d) of the vessel and be about equal to the thermal relaxation time (τ_d) for that dimension ($\tau_d = d^2/16\chi$, where χ is the thermal diffusivity). This is defined as the time required for the instantaneous temperature, generated inside the target after exposure to the

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Abbreviations: CAM, chorioallantoic membrane; Hb, hemoglobin; HbO_2 , oxyhemoglobin, PWS, port wine stains; RBC, red blood cell.

laser pulse, to decrease by 50% (see *Discussion*). Taking $\chi = 1.4 \times 10^{-7} \text{ m}^2\text{seconds}^{-1}$, corresponding to water, typical values for τ_d are 0.2 mseconds for $d = 20 \mu\text{m}$ and 4.5 mseconds for $d = 100 \mu\text{m}$. If $t_p \gg \tau_d$, heat diffuses outside the vessel during the laser exposure, reducing the target specificity, and can cause extra thermal damage to surrounding tissue. A very short pulse, $t_p \ll \tau_d$, will generate a high-peak intravascular temperature rise, leading to localized explosive vaporization of tissue water, or to photoacoustic transients, which will result in vessel rupture [19]. In such cases, repair mechanisms may revascularize the tissue [1].

Radiant Energy of Pulse per Unit Area, or Fluence (F) For PWS therapy, it is important to know the damage-threshold fluence in hypervascular skin, $F_{th}(t_p)$, sufficient to effect selective, irreversible thermal injury to vessel wall structures, without causing rupture of the targeted vessel with subsequent hemorrhage. The magnitude of $F_{th}(t_p)$ is difficult to establish by theoretical modeling, because of epidermal melanin absorption, multiple scattering events within the skin, and the fact that blood vessels are located at different dermal depths [16]. In the CAM, scattering and nonspecific absorption are small and measurements are made on identifiable targets. The depth of the targeted vessel in the CAM is of little consequence, provided no other superficial vessel is seen to shield part of the incident beam. Thus, measurements in the CAM will provide single-vessel values of the damage-threshold fluence, $F_0(d, t_p)$, which can be used as input in the derivation of the damage-threshold fluence for the composite vasculature, $F_{th}(t_p)$.

MATERIALS AND METHODS

Laser Parameters A continuous wave (CW) Argon-ion pumped dye laser (model 920, Coherent, Palo Alto, CA) was used for single-vessel irradiation. The laser delivered a maximum power of 1.4 W at 585 nm, as measured with a power meter (model 210, Coherent). The laser beam was transmitted through an 80- μm core-diameter multimode fiber terminated with an adjustable focusing microlens positioned in a hand piece. The diameter of the beam at the focus was 500 μm , giving, at maximum, a fluence $F = 700 t_p = 7 \text{ Jcm}^{-2}/\text{pulse}$. The CW laser was pulsed with a foot-pedal-controlled mechanical shutter; the pulse duration was preselected at the shortest attainable setting of $t_p = 10 \text{ ms}$.

A flashlamp-pumped dye laser (model SPTL-1, Candela, Weyland, MA) was used for multiple-vessel irradiation. The laser was tuned to $\lambda = 585 \text{ nm}$ and delivered pulses of duration $t_p = 0.45 \text{ ms}$. The beam was coupled into a 1-mm core-diameter multimode fiber terminated with a microlens that focused the laser output to a 5-mm diameter circular spot of uniform light intensity. The optical energy exiting the fiber was varied between 0.7 and 1.3 J, as measured with a calibrated energy meter (Ophir, model 10A-P), giving fluences on the CAM membrane ranging between $F = 3$ and $6 \text{ Jcm}^{-2}/\text{pulse}$.

Preparation of Intact CAMs The protocol for CAM preparation was a modification of a previously described technique [10]. Fertilized eggs (Hy-line W36 white leghorn) were washed with 70% alcohol, incubated at 37°C in 60% humidity, and rolled over hourly. On day 3–4 of embryonic development, a hole was drilled in the apex and 2–3 ml albumin was aspirated from each egg to create a false air sac. On the following day, part of the CAM was exposed by opening a round window of 20-mm diameter in the shell that was covered with a Petri dish. The eggs were placed in a stationary incubator until the CAM was fully developed and ready for experimentation. On day 10–12, sterile teflon O-rings (6.2 mm inner diameter, 9 mm outer diameter, and 1.4 mm annular width) were placed on the surface of the CAM, each demarcating a location where individual blood vessels and capillaries were clearly visible and to which the laser beam was directed. A drop of normal saline was added within the ring area to reduce spurious light reflection and to prevent desiccation of the CAM during the experiment [12]. Outside of the incubator, eggs were kept at 35°C in a heating block filled with glass beads. At the time of irradiation, the CAM was illuminated with a cold white-light fiber-optic source (Volpi, Intralux, model 100 HL) and placed under a stereomicroscope (Olympus, model SZH), equipped with a video camera (Panasonic, model AC-2510), giving a total magnification of 70 $\times$ on a color monitor (Sony, model KV-1393R).

Vessel Selection It was convenient to subdivide the extensive microvascular network of the CAM according to the following branching pattern [20]. The capillaries served as a reference and were designated vessels of "order-0." The smallest precapillary vessels (arterioles, a) as well as the smallest postcapillary vessels (venules, v) were assigned "order 1." The

Table I. The Mean Number n (per cm^2), the Mean Length l (μm), and the Mean Diameter d (μm) of Blood Vessels in the CAM at Day 10 (after [20])

Vessel Type	Vessel Order	n	l (μm)	d (μm)
Precapillary (artery)	3	16	1570	112
	2	57	740	71
	1	167	320	45
Capillary	0			
	1	98	410	54
Postcapillary (vein)	2	33	900	76
	3	15	1980	113

convergence of two order 1 vessels was assigned as an "order 2" vessel and similarly two order 2 vessels formed an "order 3" vessel. **Table I** presents, for each order, the mean number n of blood vessels per square centimeter, the mean vessel length l (μm) and diameter d (μm) in a mature CAM at day 10 [20]. The CAM area, viewed at magnification 70 $\times$ on the monitor during laser irradiation (see **Fig 1A**), had a diameter of 3 mm and typically comprised 1–2 vessels of order 3, six of order 2, and approximately 15 of order 1; these were about equally divided between precapillaries (a) and postcapillaries (v). The mean number of vessels in the capillary bed (order 0) was difficult to determine at the magnification used, but based on the mean intercapillary distance of 15 μm , as reported by DeFouw [20], about 100 capillaries were estimated to be in the field of view.

Irradiation Procedures The long-pulse laser was used for precise micro-spot irradiation of individual target vessels of a given type (a or v) and order (1, 2, or 3). Focusing adjustment and diameters of specific vessels to be irradiated were ascertained *in situ* by videotaping the field of view with the aiming beam in place and comparing it with the 1.4-mm annular width of the teflon ring (see **Fig 1**). Laser exposures were performed under standard conditions: $t_p = 10 \text{ mseconds}$, spot size 200 μm , and $F = 7 \text{ Jcm}^{-2}$. Each vessel was exposed three times at the same site, keeping the time interval between sequential exposures at 30 seconds, so that the subsequent irradiation interacted with a vessel that had cooled down to ambient temperature and in which the exposed blood had been replaced. Repeated exposures caused cumulative thermal damage to the vessel wall, eventually leading to occlusion or to hemorrhage (when the exposures were stopped).

The short-pulse laser was used to irradiate a field of vessels located inside a teflon ring on the CAM (see **Fig 2**). The laser pulse irradiance was increased, from a subthreshold fluence $F = 3 \text{ Jcm}^{-2}$ to $F = 6 \text{ Jcm}^{-2}$, in increments of 0.5 Jcm^{-2} . Each field was exposed to three laser pulses, at 30-second intervals, unless hemorrhage occurred in an order 1 or higher-order vessel following the first or second exposure—at which point irradiation was stopped. When irradiating additional fields in the same CAM, care was taken to assure that the arterial and venous trees were not compromised by the previous exposures in an adjacent field.

After laser irradiation, the eggs were covered and returned to a stationary incubator. Selected specimens (among those that had not undergone massive hemorrhage) were inspected 24 h later for re-perfusion of the vessels.

Damage Assessment and Statistical Analysis The laser-induced vascular damage, recorded on videotape, was evaluated in a double-blind fashion and graded as follows: 0, no observable damage; 1, slight damage, vasodilation/constriction; temporary occlusion; 2, moderate damage, permanent occlusion; 3, severe damage, capillary extravasation; hemorrhage.

Chi-square tests using stepwise logistic regression analysis [21] was used to assess the statistical significance of vessel type (arteriole versus venule), vessel order (1 versus 2 and 3; 1 and 2 versus 3), and energy level (for the short-pulse laser only). The two dependent variables analyzed were the number of exposures to any vessel damage (grade > 0) and the number of exposures to moderate or severe damage (grade > 1). When occlusion or hemorrhage occurred after 1 or 2 pulses, irradiation was stopped. For the short-pulse irradiation, this resulted in some vessels in the same exposure field not being graded at all three exposures.

RESULTS

Long-Pulse Laser The fluence $F = 7 \text{ Jcm}^{-2}$ was marginally sufficient to cause injury and vessel damage (mostly grade 1) was observed to occur only in the beam center (spot size $\approx 200 \mu\text{m}$). Order 1 vessels had significantly less damage compared to higher-order vessels ($p = 0.0001$). Forty-three percent of order 1 vessels were damaged after a single pulse compared to 75% for vessels of order 2 or 3; this differential was preserved after additional exposures (**Fig**

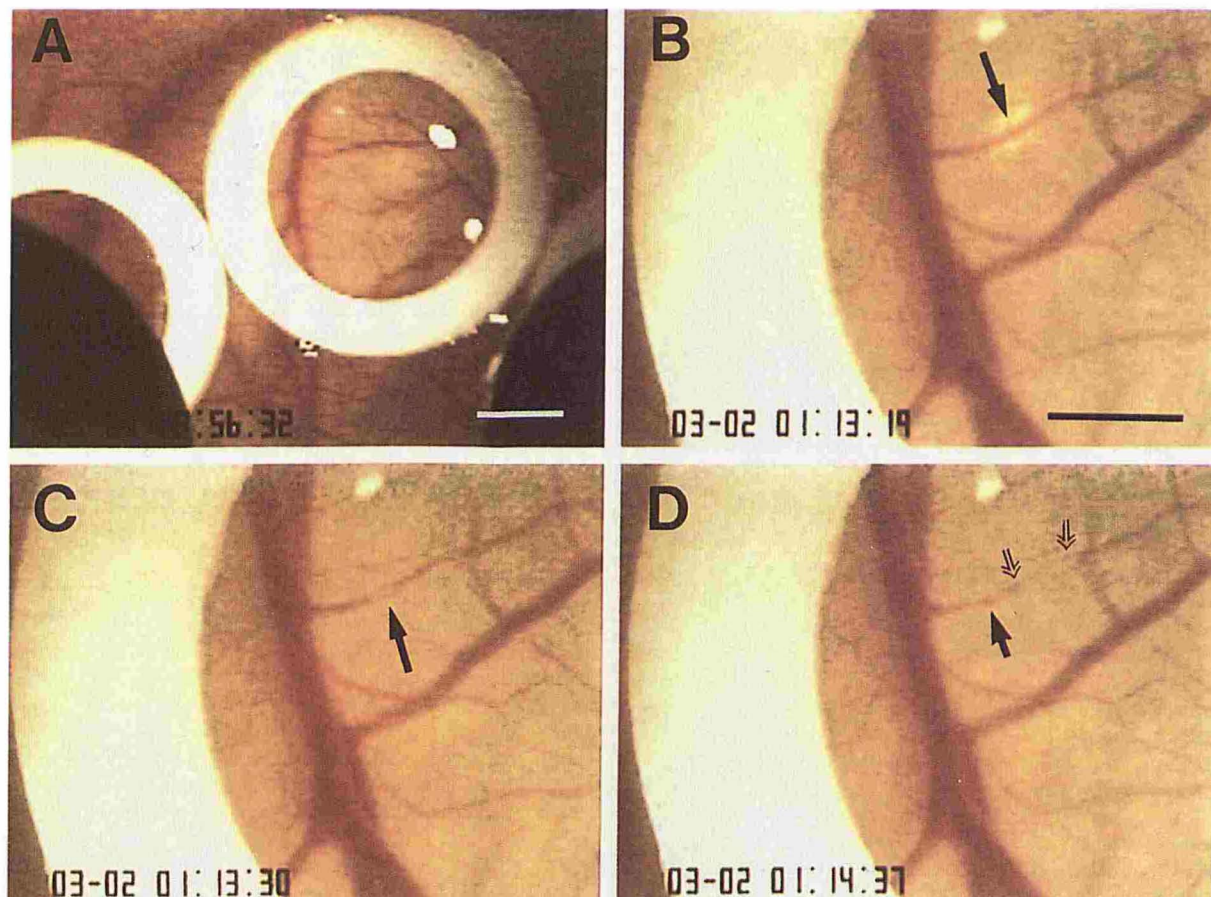


Figure 1. Video micrographs of a typical irradiation procedure with the long-pulse laser ($t_p = 10$ msecond). The last portion of the digital display in the lower left corner of each panel gives the running time of the video in h : min : seconds. *A*: Low-power magnification view of ring placement on the area of the CAM selected for treatment. Bar, 2 mm. *B*: High-power magnification view of an order 2 arteriole selected for irradiation. Arrow, aiming beam over the target area. Bar, 1 mm. *C*: Target area of the arteriole in *B* immediately following laser exposure with a single pulse of laser light (7 Jcm^{-2}). Arrow, area irradiated that shows "blanching" of the RBCs within the vessel. *D*: Target area of the arteriole in *B* 7 seconds after the third exposure to laser light. Solid arrow, irradiation point at which a thrombus has formed, blocking the vessel and causing the vessel to begin to "empty" between the open arrows.

3). Arterioles were significantly more vulnerable to moderate or severe damage than venules ($p = 0.001$). Fifteen of 89 (17%) arterioles sustained moderate or severe damage after three exposures. By contrast, only three of 102 (3%) of the venules sustained comparable damage.

Short-Pulse Laser Order 1 vessels were most vulnerable to damage; vessels of order 3 were most resistant. Arterioles were more vulnerable than venules and higher energy exposures resulted in more damage. Upon multiple exposures, injury first occurred to the capillary system, next to arterioles a1, and then to vessels a2 and v1, as was observed on the video screen (Fig 2). All p values were less than 0.0001.

Figure 4a,b shows the percentage of arterioles and venules with any (grade > 0) damage and with moderate or severe (grade > 1) damage, respectively, after one, two, and three exposures. Notably, nearly all arterioles of order 1 are damaged after one single exposure, whereas arterioles of order 3 have a similar damage profile as venules of order 2. A general comparison of thermal damage may be made between arterioles of "order n " and venules of "order $n - 1$." Figure 4a,b presents cumulative results obtained for a total of 688 vessels, irradiated with fluences distributed among three fluence ranges: $F = 3 - 4 \text{ Jcm}^{-2}$ (247 vessels), $F = 4 - 5 \text{ Jcm}^{-2}$ (257 vessels), and $F = 5 - 6 \text{ Jcm}^{-2}$ (184 vessels). Figure 4c,d shows the effect of fluence on vessel (arterioles and venules) damage.

All damage to capillaries (less than order 1) was graded as either moderate or severe. Of 159 capillaries, 49 (31%) were damaged

after one pulse, six additional capillaries (4%) were damaged on the second exposure, and two (1%) were damaged on the third exposure; 102 (64%) were not damaged. Laser fluence was not statistically correlated with capillary damage.

DISCUSSION

Absorption by a Cylindrical Vessel in a Uniform Optical Field Consider a cylindrically shaped blood vessel, with outer diameter d and inner (lumen) diameter d_i , lying along the y -direction and exposed over length l to a pulsed, collimated light beam propagating in the z -direction as illustrated in Fig 5. It is assumed that the diameter of the beam (and thus l) is larger than d . Thermal injury of the vessel wall occurs through heat conducted from the RBCs that have absorbed incoming light. To a first approximation, the energy q required to induce thermal coagulation of blood in a vessel of unit length is given as

$$q/l = \rho c \pi (d/2)^2 (T_f - T_i) \quad (1)$$

Here ρ is the mass density (gcm^{-3}) and c is the specific heat of blood ($4.2 \text{ Jg}^{-1}\text{K}^{-1}$) taken to be equal to the corresponding values for water; T_i and T_f denote, respectively, the temperature before (35°C) and immediately after the laser pulse.

The critical temperature T_f at the vessel wall required for thermal denaturation of the tissue proteins, causing permanent occlusion of blood vessels, is expected to be higher than the T_f required for thermal denaturation of RBCs or circulating proteins, which may cause transient microvascular hemostasis ($T_f = 70^\circ\text{C}$) due to coagu-

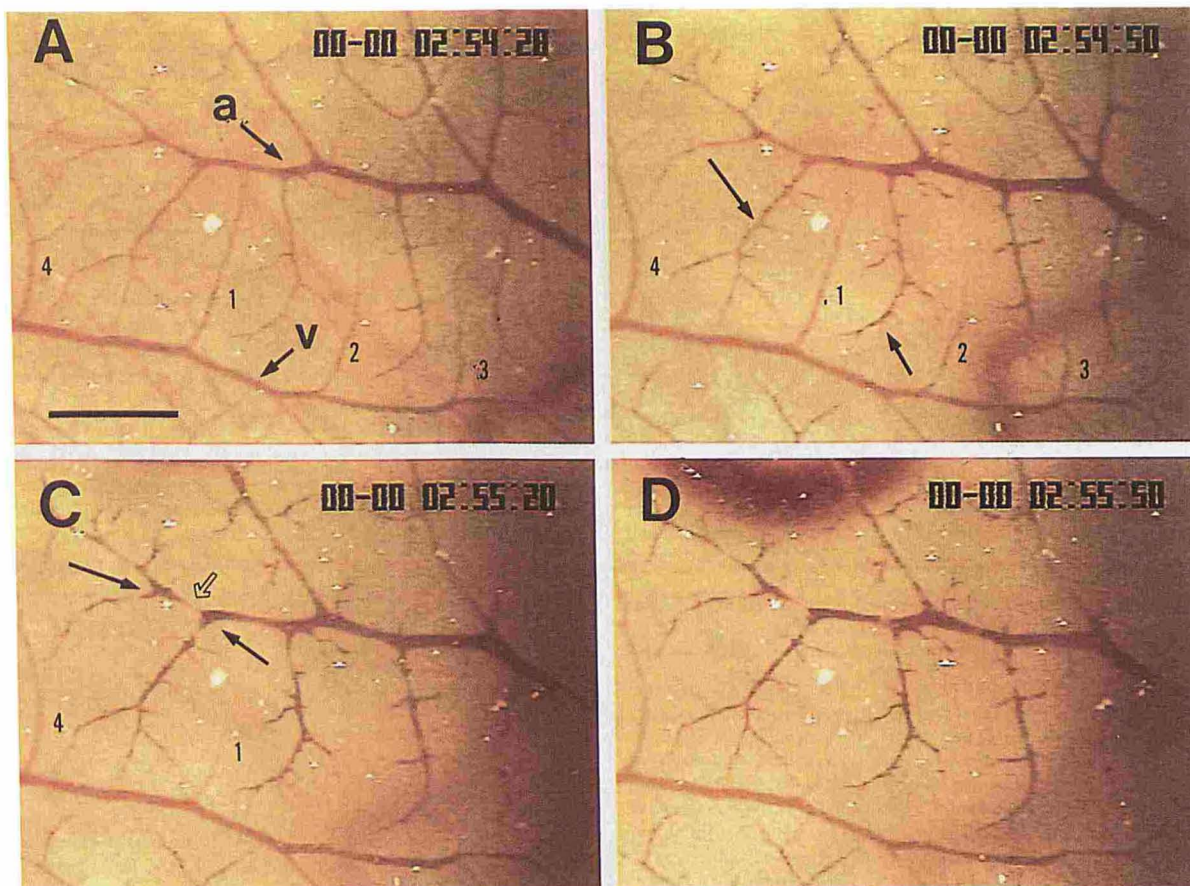


Figure 2. Video micrographs of a typical irradiation procedure with the short-pulse laser ($t_p = 0.45$ msecond). The digital display in the upper right hand corner of each panel gives the running time of the video in h : min : seconds. In B–D the entire visible field was irradiated. All panels are at the same magnification. A: The target field pre-irradiation shows an arterial “tree” (a) and a venous “tree” (v), each having a main vessel of order 2 with numerous order 1 branches interdigitating with one another. Vessels 1–3 connect the venous system with the arterial system through the common capillary bed. Vessel 4 connects the venous system to an arterial “tree” located outside the irradiation field. Bar, 1 mm. B: Target field 5 seconds after the first irradiation pulse (5 Jcm^{-2}). The order 1 arterioles are becoming blocked by small thrombi (arrows). The order 2 arterioles as well as the order 1 and 2 venules remain open. C: Target field 5 seconds after the second irradiation pulse (5 Jcm^{-2}). The order 1 arterioles remain blocked and thrombi begin to form at the bifurcation point between orders 1 and 2 (solid arrows). The vessel between the clots becomes empty (open arrow). Venules 1, 2, and 3 have emptied due to lack of flow through the capillary bed fed by the arterioles. The wall of the empty arteriole 1 remains visible. Vessel 4 (fed from outside the field) and the order 2 venule (also fed from outside) remain open. D: Target field 5 seconds after the third irradiation (5 Jcm^{-2}). The arterial “tree” is completely thrombosed. The order 1 venules fed from the blocked arterioles are empty. The order 1 and 2 venules fed from outside remain open.

lation of vessel content [13,22]. In the sample calculations of q presented in Table II we have assumed $T_f = 90^\circ\text{C}$. When considering long-pulse microspot irradiation, values of q in Table II should be corrected for the cooling effect of blood flow in the axial direction during the time t_p [23]. Measured flow velocities ($\mathbf{v}$) in the CAM vessels ranged from $\mathbf{v} = 0.7 \text{ mm} \cdot \text{seconds}^{-1}$ when $d = 40 \mu\text{m}$ to $\mathbf{v} = 1.4 \text{ mmseconds}^{-1}$ when $d = 100 \mu\text{m}$ [12]. For example, during $t_p = 10$ mseconds, the blood flow in a vessel of $l = 200 \mu\text{m}$ and $d = 100 \mu\text{m}$ replaces about $t_p \mathbf{v} / l \approx 7\%$ of the total volume in the exposed part of the vessel and q is correspondingly larger (neglected in Table II). Convective heat loss by blood perfusion is even less important with short-pulse exposure since t_p is 20 times shorter and l is generally much larger than in the long-pulse microspot exposure. Spots effects following pulsed irradiation, as observed in highly scattering skin tissue [24], are probably not important in the non-scattering CAM matrix. This is expected to be true even for the widely different spotsizes employed in this study. A definitive answer must await pathologic analysis of damaged vessels. Such a study is envisaged by us, in conjunction with measurement of the temporal evolution of temperatures in individual vessels during and following irradiation.

When we neglect light scattering and also reflections at the air/CAM and the CAM/vessel interfaces, the energy per pulse, Q_A ,

deposited in a blood vessel due to absorption from a uniform optical field, can be expressed analytically. In a cylindrical vessel of lumen diameter d_i and length l the net absorbed energy is

$$Q_A = \int_0^1 dy \int_{-d_i/2}^{d_i/2} dx \int_0^{2\sqrt{(d_i/2)^2 - x^2}} F \mu_a e^{-\mu_a z} dz \quad (2)$$

$$= (\pi/2) F l d_i [I_1(\mu_a d_i) - L_1(\mu_a d_i)].$$

Here F (Jcm^{-2}) is the incident energy fluence; $l d_i$ is the target area intercepting the light beam; $\mu_a = \mu_a(\lambda)$ is the absorption coefficient of blood; I_1 and L_1 are, respectively, the first-order modified Bessel and Struve functions [25], which have been tabulated [26]. To visualize the role of $\mu_a d_i$ on Q_A we reformulate Eq. 2 in terms of the dimensionless variable $u = (1 - x^2/r^2)^{1/2}$,

$$Q_A = F l d_i \cdot \mu_a d_i \cdot \int_0^1 e^{-\mu_a d_i u} \sqrt{1 - u^2} du, \quad (3)$$

and plot $f = Q_A / F l d_i$ versus $\mu_a d_i$ (see Fig 6). This result is completely general. It represents the fraction of incident optical energy absorbed by a cylinder, situated perpendicular to the direction of light propagation. The cylinder volume contains a homogeneous absorber, e.g., blood characterized by an absorption coefficient μ_a (585 nm) = 170 cm^{-1} or μ_a (577 nm) = 430 cm^{-1} [16], or blood with

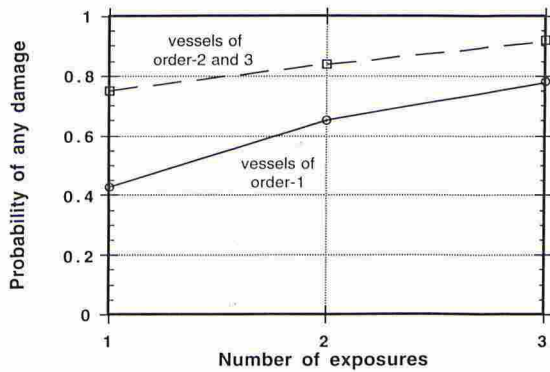


Figure 3. Probability of any vessel (arteriole or venule) damage (grade 1,2, or 3) versus number of long-pulse ($t_p = 10$ msec) exposures.

exogenous chromophores such as fluorescein or indocyanine green. Blood has a rather large absorbance at $\lambda = 585$ nm and for $d_i > 100$ μm ($\mu_a d_i > 1.7$) it can be seen (Fig 6) that more than 70% of the light incident on the upper surface of the vessel is absorbed. In Table II, Q denotes the energy absorbed by a blood vessel when irradiated with unit fluence ($F = 1$ Jcm^{-2}) at $\lambda = 585$ nm. The values show that for a blood vessel with a lumen diameter $d_i = 20$ μm , the coagulation energy q is equal to the energy intercepted by that vessel from an optical field having $F \geq 1.4$ Jcm^{-2} . When $d_i = 120$ μm , an incident energy density $F \geq 2.9$ Jcm^{-2} is predicted to affect coagulation.

Effects of Thermal Diffusion The effect of radial thermal diffusion out of the heated vessel into the surrounding tissue will now be considered. It is particularly relevant for small diameter vessels and/or long irradiation times, i.e., $t_p \gg \tau_d = d^2/16\chi$. We make the ansatz that thermal energy diffuses out of a vessel in an exponential fashion, so that for $t > t'$,

$$dQ(t, t') = dQ_A(t')e^{-(t-t')/\tau_d} \quad (4)$$

Here $dQ_A(t')$ denotes the incremental amount of optical energy absorbed in the exposed lumen during dt' at a time t' ; $dQ(t, t')$ denotes the corresponding thermal energy after the time interval $(t - t')$. The thermal energy remaining in the vessel at time t is found by integrating Eq. (4) over the duration of the laser pulse, $0 < t' < t_p$. The result is

$$Q_t = Q_A(\tau_d/t_p)[1 - e^{-t_p/\tau_d}], \quad t \leq t_p \quad (5a)$$

$$= \Delta T \rho c \pi (d/2)^2. \quad (5b)$$

Because we are interested in the coagulation temperature of the entire vessel (i.e., lumen and vessel wall) d in Eq. (5b) is taken to be the outer diameter of the vessel. After inspecting histologic sections of our CAM preparations, we established that $\langle d_i \rangle = 0.93 \langle d \rangle$ for venules and $\langle d_i \rangle = 0.88 \langle d \rangle$ for arterioles, with smaller vessels (capillaries or order 1) having relatively thicker walls. Taking as an average $d_i = 0.9d$, we use in Eq. (5b) for the specific heat of the vessel

$$\rho c = 4.18(d_i/d)^2 + 3.50[1 - (d_i/d)^2] = 4.05 \text{ Jcm}^{-3}\text{K}^{-1},$$

where 4.18 and 3.50 $\text{Jcm}^{-3}\text{K}^{-1}$ are, respectively, the specific heat of the lumen and vessel wall cellular materials and the mass density is taken to be $\rho = 1$ gcm^{-3} .

In Fig 7 we have plotted the temperature rise ΔT given by Eq. (5b) for long-pulse irradiation ($t_p = 10$ msec) and $F = 7$ Jcm^{-2} and for short-pulse irradiation ($t_p = 0.45$ msec) and $F = 3$ Jcm^{-2} . For the two curves in Fig 7, a striking feature is the different dependence of ΔT on the vessel diameter d . The long-pulse exposure causes a monotonic temperature rise with d over the given range $d < 130$ μm . At larger d , the temperature rise will reach a maximum and, eventually, decrease as $1/d$. In contrast, the temper-

ature rise due to the short-pulse exposure reaches its maximum at a smaller diameter. Consequently, for a critical temperature $T_f = 90^\circ\text{C}$ ($\Delta T = 55^\circ\text{C}$), Fig 7 indicates that the short-pulse exposure at $F = 3$ Jcm^{-2} affects predominantly small diameter vessels (of order 1 and 2), whereas the long-pulse exposure will damage larger diameter vessels. Figure 7 may also be used to interpret vessel damage reported in the hamster cheek pouch microvasculature at two temperatures $T_i = 34^\circ\text{C}$ and $T_i = 8^\circ\text{C}$ (Fig 2 in [27]). A fluence increase of $\Delta F = 1.4$ Jcm^{-2} was required to obtain an identical (72%) "percentage of vessels showing any visible change in response to a single laser pulse" delivered from a short-pulse Candela laser similar to the one used in the present study. The ordinate in Fig 7 scales linearly with fluence [cf. Eqs. (3) and (5)]. Thus, if for short-pulse irradiation $\Delta T = 60^\circ\text{C}$ is reached for $F = 3$ Jcm^{-2} , an additional temperature rise $\Delta T = 34^\circ - 8^\circ\text{C} = 26^\circ\text{C}$ requires correspondingly a fluence increment $\Delta F = 1.3$ Jcm^{-2} , in excellent agreement with the experimental value 1.4 Jcm^{-2} .

Instantaneous Temperature Increase Inside an Absorbing Cylinder For adiabatic laser heating ($t_p \ll \tau_d$), $Q_i \approx Q_A$ [in Eq. (5a)]. The absorbed optical energy gives rise to a non-uniform temperature distribution in the lumen

$$\Delta T(x, z, t_p) = (1/\rho c) \frac{dQ_A}{dV}(x, z) = (F\mu_a/\rho c)e^{-\mu_a z(x)}, \quad (6)$$

where dQ_A/dV is the absorbed energy density (Jcm^{-3}) and the other parameters are as defined in Eqs. (1) and (2). Upon substituting $z(x)$, as defined in Eq. (7) (see Fig 5),

$$z(x) = z - d/2 + \sqrt{(d/2)^2 - x^2}, \quad (7)$$

into Eq. (6) the spatial distribution of the temperature rise in the lumen is obtained. In Fig 8, the cross sections of vessels of order 1, 2, and 3 with (see Table I) $d = 50, 80$, and 110 μm , respectively, are diagrammed. Inside each diagram are plotted the isotherm curves ($\Delta T = 55^\circ\text{C}$) for $F = 3$ and 5 Jcm^{-2} . The curves define the transition zones between damaged ($T_f > 90^\circ\text{C}$) and non-damaged ($T_f < 90^\circ\text{C}$) lumen volume. It is evident that smaller caliber vessels are more easily coagulated than larger ones. According to the hemodynamic criterion, total occlusion occurs when at least 61% of the lumen has coagulated, i.e., the critical temperature $T_f = T_i + 55$ $\text{K} = 90^\circ\text{C}$ reaches halfway through the vessel diameter [16].

Also, we note that at the lowest fluence ($F = 3$ Jcm^{-2}) only the small diameter vessels show thrombosis ($>61\%$ of lumen has coagulated), whereas at $F = 5$ Jcm^{-2} the small and medium caliber vessels will have coagulated completely and the larger vessels of order 3 will show partial occlusion. This behavior is borne out by the results for the short-pulse laser presented in Fig 4.

Long-Pulse Photothermolysis For the long-pulse exposure the results given above must be modified to include the full effects of thermal diffusion. The cooling effect due to radial thermal diffusion at the vessel periphery will cause a nonuniform spatial distribution of temperature within the vessel, with a peak temperature in the center of the vessel (displaced somewhat toward the upper part facing the light beam) and lower temperatures at the periphery of the vessel [28]. This situation has been modeled analytically [13] and numerically [16,28–30] but is beyond the scope of this discussion. Our main aim has been to present analytical results that provide direct answers for single vessel exposure.

Arterial and Venous Response One of the more salient phenomena observed in the present study was the higher vulnerability for thermal injury of arterioles as compared to venules. This occurred for the three vessel calibers considered and for both short-pulse and long-pulse exposures. In the CAM, arterial (oxygen poor) and venous (oxygen rich) blood possess equal light absorbance at 585 nm; thus, vessels of the same lumen diameter are expected to undergo similar thermal stress. In a study of prolonged steady-state

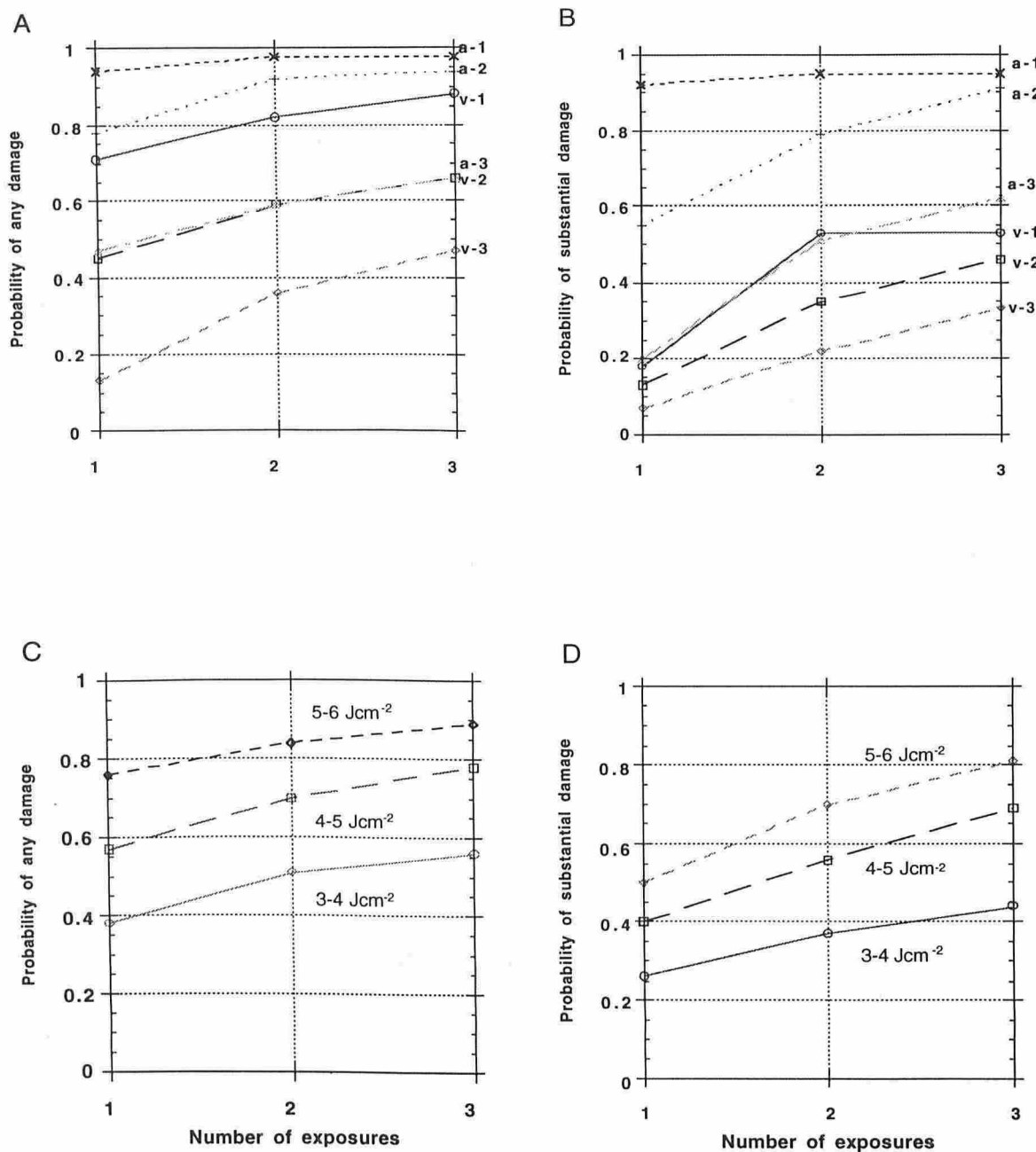


Figure 4. Probability of laser damage versus number of short-pulse ($t_p = 0.45$ mseconds) exposures. *A*: Probability of any damage (grade 1, 2, or 3) to arterioles and venules of orders 1, 2, and 3. *B*: Probability of moderate or severe damage (grade 2 or 3) to arterioles and venules of order 1, 2, and 3. *C*: Probability of damage to all vessel types (arterioles or venules of any order) versus incident laser fluence. *D*: As *C* for moderate or severe vessel damage.

(16 seconds) heating of blood vessels immersed in a waterbath it was reported that arteries showed less vasoconstriction (and faster post-heating recovery) than veins [31]. This variance with our observations is probably due to the nature of waterbath heating, when heat slowly ($\Delta T \approx 10^\circ \text{Csecond}^{-1}$) and nonspecifically diffuses into vessel walls. In another study it was reported [32] that CW laser irradiation at 514 nm and 595 nm caused vasodilation in precontracted venules but not in arterioles. The irradiance ranged from 1.4 to 14 Wcm^{-2} and was applied for 20 seconds or more; at 12 Wcm^{-2} the steady-state temperature rise was 17°C ($\Delta T < 1^\circ \text{Csecond}^{-1}$). A possible explanation of these findings [31,32] might be based on considerations of vascular anatomy. The arteriolar walls consist of three concentric layers: an endothelial tube, an intermediate layer of smooth muscle cells, and an outer coat of fibrous elements. The

thickness of the arteriolar wall varies with vessel caliber and function; the walls of venules are always thinner than those of arterioles of equal caliber. In the case of steady-state heating one might anticipate less damage to the thicker, more resilient arteriolar wall. In contrast, pulsed radiation at 585 nm generates heat rapidly ($\Delta T \approx 10^5^\circ \text{Csecond}^{-1}$) and intraluminally. In this case, the venous-arterial lumen difference may assume significance. However, in the CAM the difference in vascular cross section is generally small. If we take as typical wall thicknesses $0.06d$ and $0.035d$ for arterioles and venules, respectively, the ratio for the lumen volume of arterioles and venules for two vessels with the same outer diameter will be $(0.88/0.93)^2 = 0.9$. This ratio is too close to unity to explain our observations. Moreover, contrary to the observations, the long-pulse laser would then be expected (see Fig 7) to damage preferentially the

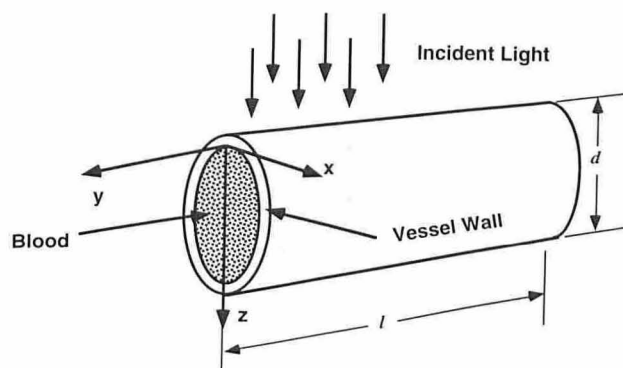


Figure 5. Coordinate geometry for calculation of light absorption in a vessel.

venules, whereas the short-pulse laser would preferentially damage the arterioles.

Another point of difference is the platelet aggregation initiated by the chain of biochemical reactions triggered by thermal trauma, which is different in arterioles and venules. This seems to be consistent with reports of PDT-induced vasoconstriction, where it was shown that 90% of the arterioles were affected by photochemical injury versus 70% of the venules [33]. However, on the time scale of photothermolysis ($t \approx 10$ mseconds) no platelet aggregation is expected to occur in real time.

Finally, it should be noted that coagulated blood emboli, consisting of agglutinated damaged RBCs [17], can be transported downstream in venules, but not in arterioles because they get blocked in the capillaries. This difference in permanent clotting is a component in our interpretation for the lower threshold for arteriolar photothermolysis.

In conclusion, this study described the first controlled experiment of the relationship between laser fluence and vessel diameter in the photothermolysis of single blood vessels *in vivo*. Small-caliber vessels are relatively spared when using long-duration exposures. Arterioles were found to be significantly more sensitive to thermal damage than venules of comparable size. The observations of damage-threshold fluence versus vessel diameter were quantified for two widely different pulse durations. They were interpreted using a theoretical model that has applicability to a number of vasculopathies. On the basis of our results it would appear that a pulse duration between 1 and 5 mseconds might be better suited to treat PWS than the two pulse durations used in this study. However, individual variations in the diameter of the ectatic venules in PWS (depending on age of patients) and their depths in the dermis [34] prevent specifying any generally applicable optimal pulselength.

Table II. Thermal Relaxation Times τ_p in Blood Vessels with Lumen Diameter d_i ^a

Vessel Order	d_i (μm)	τ_p (msecond)	q (mJ)	Q (mJ)
0	20	0.18	0.7	0.5
1	40	0.71	2.9	1.6
1	60	1.61	6.5	3.3
2	80	2.86	11.6	5.1
2	100	4.46	18.1	7.2
3	120	6.43	26.0	9.1
3	140	8.75	35.4	11.6

^a The coagulation energy q [Eq. (1)] for a vessel of unit length (1 cm) is compared with the optical energy Q [Eq. (3)] absorbed when that vessel is exposed to pulsed light (thermal diffusion neglected, $t_p \ll \tau_d$) at $\lambda = 585$ nm at a fluence $F = 1$ Jcm⁻².

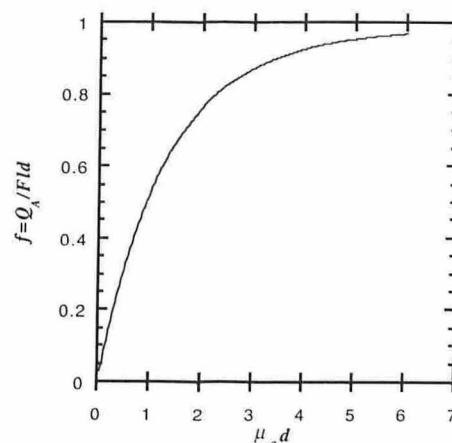


Figure 6. Fraction (f) of incoming light absorbed by a homogeneous cylindrical volume with diameter d and absorption coefficient μ_a .

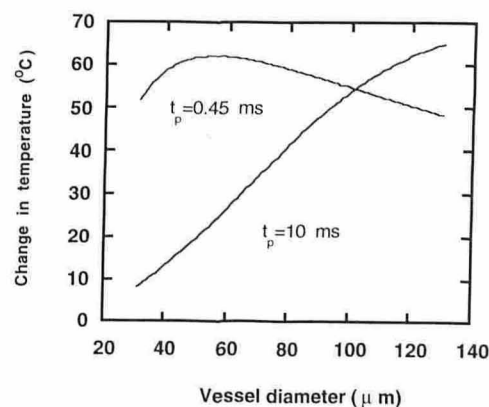


Figure 7. Change in vessel temperature for short- and long-pulse laser irradiation.

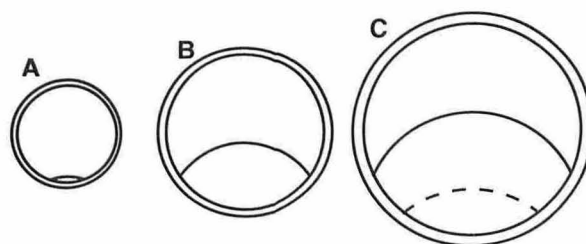


Figure 8. Isotherm curves ($\Delta T = 55^\circ\text{C}$) in vessels of various diameters (d) for a given fluence (F). A: $d = 50$ μm and $F = 3$ Jcm⁻². B: $d = 80$ μm and $F = 3$ Jcm⁻². C: $d = 110$ μm and $F = 3$ (—) or 5 (---) Jcm⁻².

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