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A Useful Algorithm for Determining Fluence and Pulse Width for Vascular Targets Using 1,064 nm Nd:YAG Laser in an Animal Model

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Background and Objectives: Many current parameters to ablate vascular beds using 1,064 nm lasers are based on high-energy settings and often fail to consider vessel diameter and/or pulse width. This study attempts to define the minimal effective dosage (MED) of energy and pulse width for specific vessel diameters in an animal model.

Study Design/Materials and Methods: 1,064 nm Nd:YAG was used in 15 Sprague–Dawley rats. Bilateral extended dorsolateral skin flaps were elevated and vessel diameters from 0.1 to 1 mm were identified. Pulse widths (PW) in a range of 15–60 milliseconds and fluences between 70–110 J/cm^2 with contact cooling at 5°C (Celsius) were utilized. Results were determined clinically and histologically.

Results: Ideal pulse width and MED for each vessel diameter were determined using a 6 mm spot size. Histology showed early hemostasis and subsequent thrombosis, which are consistent with clinical findings.

Conclusions: This model allows in vivo monitoring of vessel ablation.Optimal pulse width and MED levels versus vessel diameter determined in this animal model provide a useful algorithm that may allow for more effective treatment of vascular targets utilizing the 1,064 nm Nd:YAG laser. Lasers Surg. Med. 34:420–425, 2004.

Key words: 1,064 nm Nd:YAG laser; optimal pulse width; vessel diameter; extended dorsolateral flap

INTRODUCTION

A wide variety of lasers and light sources have been studied for the treatment of vessels over the last 25 years [1–5]. Early treatments used a variety of wavelengths and fluences resulting in lack of vascular selectivity and thermal confinement leading in many cases to unacceptable results and scarring. However, shorter wavelength and pulse widths (PW) of some laser systems enabled effective treatment for smaller diameter vascular targets. Recent efforts have focused on developing lasers that maintain vascular selectivity while providing sufficient depth of penetration, fluence, and PW to effectively photocoagulate larger leg telengiectasias and feeding reticular veins while decreasing the likelihood of untoward effects. Pulsed alexandrite, diode, and Nd:YAG lasers have been used to treat larger vascular targets [6–11].

Anderson and Parrish [12] first described selective photothermolysis (SP), the selective absorption of light pulses by pigmented targets to achieve thermally-mediated injury. They described the thermal relaxation time (TRT) as the unit of time, a specific target dissipates at least 50% of its absorbed energy in the form of heat to surrounding tissue. The thermal containment time (TCT) is the optimal time to delivery energy to a selective target, which is less than TRT. This is the foundation of SP. Furthermore, the TRT in seconds is about equal to the square of the target dimension in millimeters. Anderson [13] also suggests that the TCT should be 1/4 to 3/4 of TRT. Clarck described an algorithm considering the ideal pulse width and MED by using a frequency doubled Nd:YAG laser system at 532 nm wavelength [14].

This study demonstrates MED, ideal pulse width, and fluence for vascular targets with a 1,064 nm system using a novel animal model.

MATERIALS AND METHODS

Each animal received care in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals as monitored by IACUC committee at The University of Texas Southwestern Medical Center. Fifteen [15] Sprague–Dawley rats weighing 350–500 g were used. All animals were anesthetized with an intraperitoneal injection of a combination of xylazaine hydrochloride (5 mg/kg body weight) and ketamine hydrochloride (75 mg/kg body weight). Five of them were not treated and used as the control group.

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The 1,064 nm Nd:YAG laser (Sciton Profile, Inc., Palo Alto, CA, USA) was used in this study design. Targets were treated with both 3 and 6 mm spot sizes. Laser treatment was initiated from the periphery of the vasculature targeting the smaller diameter of the vessel. Treatment progressed proximally allowing the treatment of progressively enlarging diameters. If treatment was performed initially at proximal sites, blood flow to the vessels would have been interrupted and subsequent treatments of the peripheral, smaller vessels would be invalid.

Initial settings of 15 milliseconds, 60 J/cm^2 were evaluated. Ideal PW were determined by vessel diameter. Fluences were increased in 5 J/cm^2 increments until vessel obliteration was achieved. PW were extrapolated from Anderson and Parrish's study, "SP" [12]. In this article, they described TRTs of vascular targets varying with vessel size. Ideal pulse width values for various diameter vessels were evaluated by use of the equation in their work. Once the pulse width was extrapolated, higher and lower values were used to verify the ideal pulse width value for each certain diameter vessel. This principle was then used as a guideline for various vessel diameters. Furthermore, each evaluation began at a fluence of 110 J/cm^2. Once the ideal pulse width was determined, the fluence was decreased by increments at 5 J/cm^2 in an effort to find the best amount of energy required for the ideal pulse width.

5°C (Celsius) contact cooling was used in all cases to prevent superficial thermal injury. Vessel diameters were determined by use of a vein guide (Cynosure, Inc., MA, USA) in 0.1 mm increments.

Macroscopic observation, video recordings, and histology were used for evaluation. The dorsal hair of the rat was removed by use of an electric shaver and a commercial depilatory cream 1-day before the procedure. All surgical procedures were performed under sterile conditions. Two extended dorsolateral skin flaps including panniculus carnosus were elevated through a dorsal midline incision 10 cm in length, bilaterally. The lateral thoracic, deep circumflex, and the posterior intercostal vasculature were included in the flaps with their branches. The skin flap was positioned as perpendicular to rat dorsum with two skin hooks at the edges. Loose areolar tissue underneath the panniculus carnosus was trimmed. Both flaps were used for treatment, respectively. A digital camcorder (Sony Handycam DCR-PC 120, Japan) was mounted on a tripod and set at a 35 cm distance from the flap in order to record the response of the vascular targets to treatment from the undersurface of the flap (Fig. 1). The LCD monitor was turned back to face to the laser physician and the camcorder was zoomed in to visualize the subdermal vasculature clearly. For each specific diameter vessel, 10 treatments were performed with a selected PW and fluence, and real time recordings were obtained. Three physicians analyzed the video to evaluate vessel ablation.

Full thickness skin biopsies were obtained from each treatment area either just after laser treatment of the vessels (No:5 rats) or at post-treatment day 7 (No:5 rats). Specimens were transferred into formalin, fixed in paraffin, and sections (3 mm) were prepared and stained with both Hematoxylene Eosin (H&E) and Trichrome. Skin thickness, vessel diameter, and vessel depth were recorded for all animals. Average of skin thickness measurements and vessel parameters were determined by descriptive statistics for this animal model.

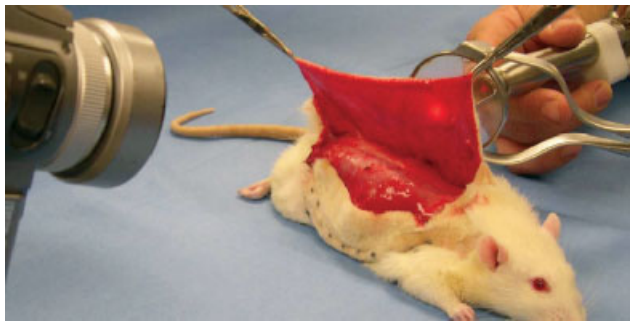

Fig. 1. Experimental set-up including the digital video recorder, the elevated flap, and the laser device is seen.

RESULTS

Data from 15 rats were presented in Table 1. Skin thickness values were obtained from histological assessments. From each slide, five independent values were determined for each animal. The average skin thickness was 1,343 ± 71 μm (range: 1.08–1.6 mm). In this model system, the posterior intercostal, the lateral thoracic, and the deep circumflex vasculatures were available for examining the effects of the laser energy on vessels with 0.6, 0.8, and 1 mm diameters, respectively (Fig. 2). The effects on veins with the accompanying arteries were also examined. Histologically, the vessels ranging from 20 to 150 μm in diameter were located in the deep dermis. In the immediate subdermal area (depth range: 1,150–1,660 μm from the skin surface), vessel diameters were 150–400 μm. In the subcutaneous area (depth range: 1,380–3,840 μm from the skin surface), vessel diameters ranged from 400 μm to 1 mm. Although larger vessels up to 1.3 mm diameter can be found proximally, the variability of their depth prohibited objective evaluation.

Consistent closure of vessels with diameters 0.2–1 mm was not achieved by using a 3 mm spot size. Using a 6 mm spot size, the vessels were ablated by varying PWs (Table 2). By macroscopic observations of vessel closure, ideal PW and MED values were determined for each vessel diameter. An

TABLE 1. Vessel Parameters for This Model Are Seen

	Diameter (μm)	Depth in tissue (μm)
Vessels	20–150	800–1,200
	150–400	1,150–1,660
	400–1,000	1,380–3,840
Posterior intercostal vessels	≤600	1,800
Lateral thoracic vessels	≤800	3,200
Deep circumflex vessels	≤1000	3,840

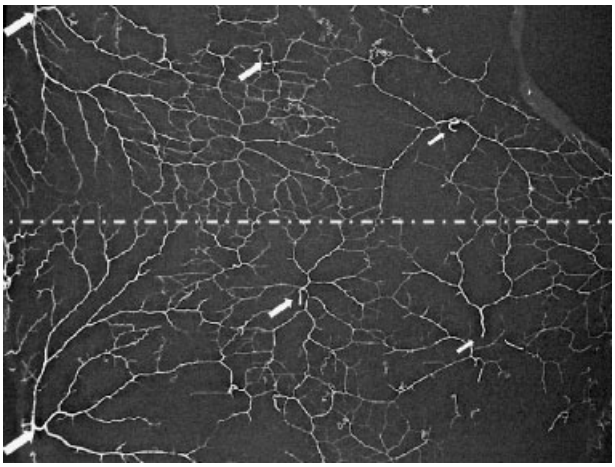

Fig. 2. Microangiography of the dorsolateral skin shows the three major vascular supplies including the lateral thoracic (big arrow), posterior intercostals (medium arrow), and the deep circumflex vessels (small arrow).

exponential correlation was observed between minimal PW levels that closed vessels and the respective vessel diameters ($R^2 = 0.9385$). A narrow range of PW was determined for specific vessel diameters. In no case, did fluence exceed 110 J/cm^2.

Histological analyses showed that all target vessels were located within 1,330–3,600 μm depths. No change in pathology was observed in the control group (Fig. 3a). Coagulation of the vessels with thrombostasis and moderate wall injury without any rupture was seen in the early post-treatment period (Fig. 3b). No significant surrounding tissue damage was observed. At 7 days, persistent thrombosis at the target vessel was observed with no collateral tissue injury. Longer PW closed the selectively targeted deeper large diameter vessels while sparing the more superficial smaller vascular targets (Fig. 3c).

DISCUSSION

This study describes optimal PW settings with defined minimal energy levels for vascular targets using 1,064 nm Nd:YAG laser in an animal model. This is the first report, to our knowledge, that describes a scientifically based treatment algorithm for vascular targets using the 1,064 nm Nd:YAG laser.

TABLE 2. Ideal PW and MED Values for Certain Diameter Vessels Are Seen

Vessel diameter (mm)	Pulse width (millisecond)	Fluence (J/cm^2)
0.2	15	80
0.3	20	90
0.4	20	90
0.5	25–30	100
0.6	30	100
0.7	35	100
0.8	35–40	100
0.9	45–50	110
1	60	110

Taylor and Minabe [15] first described the vascular anatomy of an extended dorsal flap with three vascular territories in a rat model. This study provided a consistent vascular model to evaluate laser technology on blood vessels. Furthermore, the diameters of the vessels both in superficial and deep dermis are comparable to the human skin microvasculature [16,17]. The vessels as small as 0.1 mm in diameter at the periphery of these vascular systems can be easily observed and recorded during treatment. This model was not able to evaluate vascular targets larger than 1 mm.

The thickness of the skin varies over surface of the human body from less than 1 mm in the eyelids to more than 5 mm in the sole of the foot [18]. The average skin thickness in this model was comparable to the thickness values of the face and leg skin seen in humans. The diameter of postcapillary venules ranges from 12 to 35 μm, while collecting venules range in size from 40 to 60 μm in the upper and middermis and enlarge to 100–400 μm in diameter in the deeper tissues of humans [16]. Superficial leg telangiectases measure 0.03–0.3 mm in diameter and connect with postcapillary venulectases (0.4–2.0 mm in diameter), which connect to reticular veins (2.0–4.0 mm in diameter) and larger varicosities [10,16]. The vessel sizes treated in this study are comparable with the superficial telengiectasias and some postcapillary venulectasias. The deeper location of large vessels resembles the anatomic localization of leg venulectasias of human. Since, complete closure of the vessels were confirmed by histology and were consistent with live observation, we found this model reliable and useful for in vivo investigation. This model allows for extrapolation of the data to humans. A potential limitation of this model system is that laser settings in human skin may produce purpura and hyperpigmentation. Although not observed in the animal model, future studies in human skin will be required to address possible side effects.

The use of Nd: YAG 1,064 nm laser for vascular targets has been reported previously [3,8–10,19] (Table 3). Weiss and Weiss [3] published the first report of a long-pulsed Nd:YAG laser for treatment of leg veins. For small vessels (0.3–0.6 mm), a triple synchronized pulse of 3–4 milliseconds at a fluence of 80–110 J/cm^2, and for medium-sized vessels (0.6–1.0 mm), a double synchronized pulse of 7 milliseconds separated by 20–30 milliseconds at a fluence of 90–120 J/cm^2, or a single pulse of 10 milliseconds at a fluence of 100–110 J/cm^2 were used. These PW values seem to be low for effective complete closure of the vessels compared to our results. Our study would suggest usage of 25–60 milliseconds PW to treat the vessel diameters 0.6–1.0 mm at a fluence of 100–110 J/cm^2.

Sadick [8] recently treated 25 female patients with 1–3 treatment sessions at 6 week intervals using a long-pulsed Nd:YAG laser with a spot size of 6 mm. Vessels 0.2–2.0 mm in diameter were treated with a 6 mm spot size using a double pulse of 7 milliseconds at 120 J/cm^2, and vessels 2.0–4.0 mm in diameter were treated with a single pulse of

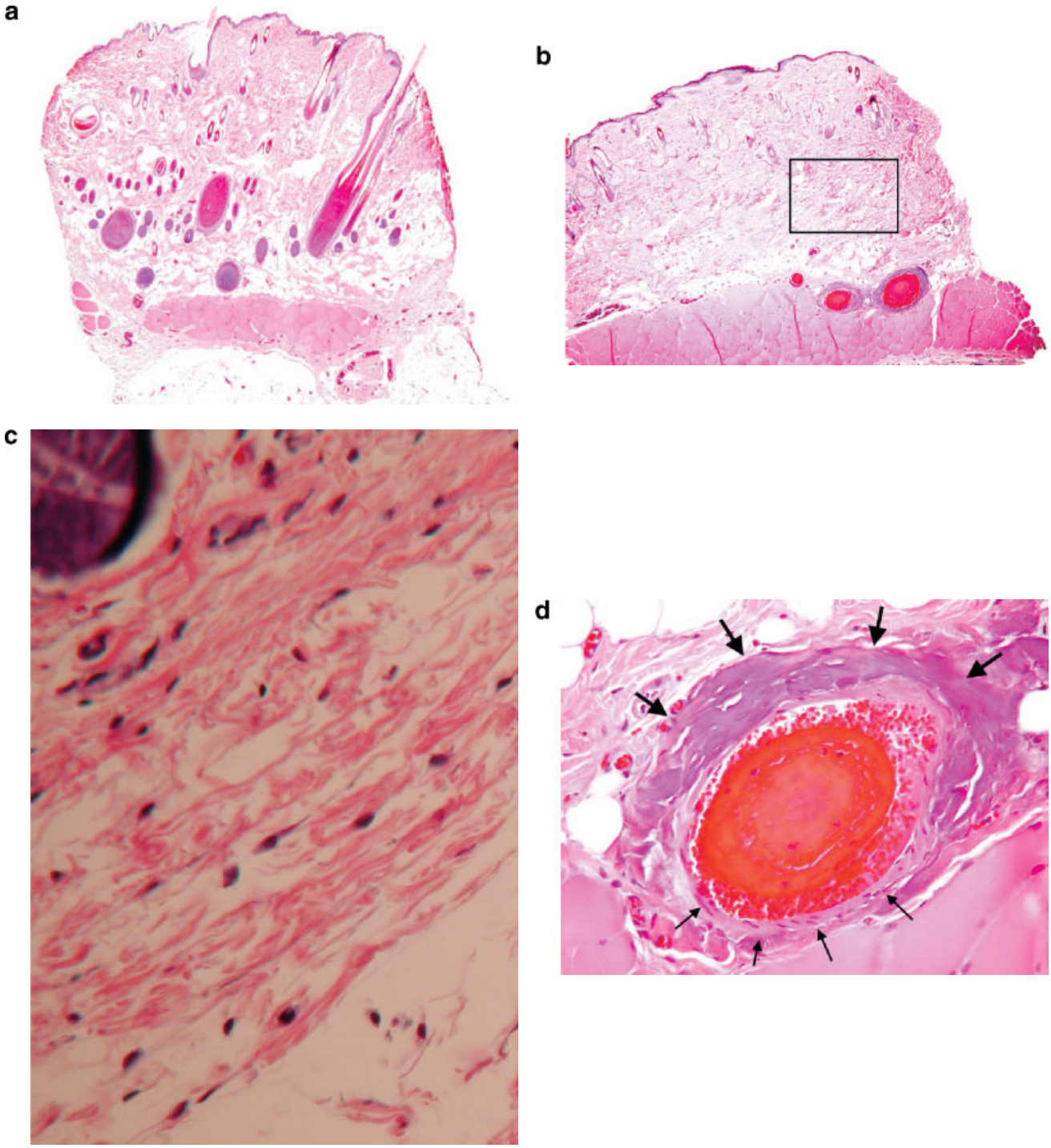

Fig. 3. **a**: Normal rat skin histology shows the deep larger vessel up to 1 mm diameter (arrow) and the smaller superficial vessels. (H&E, ×20). **b**: Panoramic view of the skin and subcutaneous tissue. The larger vessels (800–1,000 μm) show congestion and coagulative necrosis of the wall. (H&E, ×20). **c**: The high power view of the diagrammed area of the b showing intact small vessels (150–400 μm in diameter and 1,150–1,660 μm in depth) without damage to the wall or thrombosis. (H&E, ×200). **d**: The high power view of the large vessel (900 μm in diameter and 3,600 μm in depth) shows coagulative necrosis of the wall to the direction of laser application (thick arrows). No significant necrosis is seen in the wall of the opposite site (thin arrows). The vessel shows marked congestion and early thrombosis. (H&E, ×200).

TABLE 3. Combined Clinical Data From Recent Publications and Our Experience With 1,064 nm Nd:YAG Laser Are Shown

Vessel diameter (mm)	Weiss and Weiss [3]			Sadick [8,9]			Omura et al. [10]			UTSW Medical Center		
	PW	Fluency	No. of treatments	PW	Fluency	No. of treatments	PW	Fluency	No. of treatments	PW	Fluency	No. of treatments
0.1–0.3	—	—	—	7	120	Double	—	—	—	25	90–120	Single
0.3–0.6	3–4	80–110	Triple	7	120	Double	—	—	—	25–35	90–110	Single
0.6–1.0	7 milliseconds	90–120	Double	7	120	Double	—	—	—	45–60	100–110	Double
	10 milliseconds	100–110	Single									
1.0–2.0	14–16	110–130	Single	7	120	Double	50	100	Single	—	—	—
2.0–3.0	14–16	110–130	Single	14	130	Single	50	100	Single	—	—	—
3.0–4.0	—	—	—	14	130	Single	—	—	—	—	—	—

14 milliseconds, at a fluence of 130 J/cm^2. Using these settings, greater than 75% clearance was reported in 64% of the patients after a maximum of three treatments. Our study suggests that longer PW may allow for improved ablation and possibly decreased treatment sessions.

In a recent study by Omura et al. [10], high clearance of leg reticular veins was reported with 1,064 nm Nd:YAG laser by using 50 millisecond PW and a fluence of 100 J/cm^2 with a 4°C (Celsius) contact cooling. By using this pulse width and fluence, it is reasonable to assume that vessels of diameter around 0.9 mm would be closed. With a similar setting, Omura et al. [10] confirmed our results in that a single treatment may be enough for successful vessel ablation. Furthermore, selecting the right PW decreases the fluency required for closure of the vessels.

This study showed that effective treatment of vessels is dependent on PW. A narrow pulse width range was determined for each specific vessel diameter. The optimal relationship between PW and vessel diameter closure was exponential as the TRT is proportional to the square of the size [12,20]. Therefore, selectivity of larger vessel damage may be possible by choosing laser exposures that exceed thermal relaxation times of capillaries. In no cases did fluence exceed 110 J/cm^2. Increasing fluence did not compensate for incorrect PW. Clinically, arterial flow typically requires higher fluences and shorter PW. In this model we were not able to appreciate a significant difference between arteries and veins with regard to laser settings.

Longer PW were required to close the selectively targeted larger, deeper diameter vessels while sparing the more superficial smaller vascular targets. Larger vessels have longer TRT and this require longer PW for effective obliteration. Because the more superficial, smaller vessels have shorter TRT, they are spared during treatment with longer PW.

An interesting finding was coagulative necrosis of the superficial side of a large diameter vessel wall (Fig. 3d). Shorter PW will ineffectively treat larger vessels unless a high fluence is utilized. Longer PW will allow for a more uniform treatment of the vessel.

Ablation of vessels with diameters 0.2–1 mm was not achieved by using a 3 mm spot size in this model. This was confirmed grossly and histologically in both early and late periods. When the beam radius is less than or approximately equal to the penetration depth of the laser, intensity within the skin decreases much more rapidly with depth, because of beam broadening from optical scattering to the sides [20]. Larger spot sizes help minimize the effects of beam broadening providing more consistent results for deeper targets. The increased efficiency with larger spot diameters is the result of multiple scattering in the superficial dermis. This scattering allows more photons to remain within the diameter of the incident beam than with a smaller spot. This model is different from the clinical setting as the flap is elevated off of the tissue. Loss of the deeper tissues in this model eliminates a source of heat dissipation and scatter. This possibly could result in loss of overall heat generated at the treatment site. Conversely, the lack of deeper tissues will allow for a more uniform

treatment of the target. These two effects while important, may offset each other in this model. This may explain why the 3 mm spot size at the settings presented did not successfully obliterate the vasculature. While a 3 mm spot size may result in less patient discomfort, it typically requires higher fluences which may result in an increased risk for tissue necrosis, particularly when pulse stacking is incorporated. We have utilized the 6 mm spot clinically, despite its associated pain, for the following reasons: (1) less energy required for obliteration; (2) decreased morbidity associated with higher fluences including hyperpigmentation,ecchymoses; and (3) risk for tissue necrosis with pulse stacking. We seldom treat patients over 120 J/cm^2.

In conclusion, the combination of longer PW, longer wavelengths (1,064 nm Nd:YAG laser) with consistent laser fluences provide more optimal laser parameters for treating deep dermal vascular targets with diminished untoward effects. The animal model described in this study serves as a useful tool for evaluating technology focused on vascular obliteration. This study presents a useful algorithm that may guide the clinician when treating vascular targets within the dermis at diminished fluences which may lead to less overall morbidity.

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