The Effects of Pulse Dye Laser Double-Pass Treatment Intervals on Depth of Vessel Coagulation

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Background and Objective: Multi-pass treatments with pulse dye lasers (PDLs) are avoided due to perceived side effects. Proper multi-pass techniques allow for deeper vascular injury. New extended PDLs allow use of multi-pass procedures. This study evaluates how the time between pulses, inter-pulse interval [IPI] affect extent of vascular treatment.
Study Design/Materials and Methods: Sixteen subjects were exposed to a series of exposures on normal skin to determine depth of injury for various IPI. Subjects were exposed to single pass, and 4 double-pass intervals. Tests included exposures at 0.5 milliseconds, 2–7 j/cm². Exposures included one and two passes, IPI of 1, 10, 30, and 60 seconds; 5 and 30 minutes. Treatments were done with PhotoGenica V-Star (595-nm), SmartCool air cooling. Biopsies were taken: single pass and double pass purpuric thresholds; and at 7 j/cm² to determine depth of vascular coagulation.
Results: Histology revealed increased vascular coagulation depth at purpura threshold for intervals of 1, 10, 30, and 60 seconds between passes compared to single pass treatment, and a significant monotonic increase in depth of vascular injury at 7 j/cm² with increasing IPI.
Conclusions: The use of multiple passes increases depth of vascular injury, which may increase the efficacy of treatment without significant increase in purpura or risk of scarring for treatments at purpura threshold. At purpura threshold, the depth of vascular injury increases with increasing IPI up to 60 seconds. Above purpura threshold, there is a monotonic increase in depth of vascular injury for IPI up to 30 minutes. These observations suggest multi-pass treatment methods may be beneficial when employed with PDLs. Lasers Surg. Med. 38:16–21, 2006.
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Key words: vascular lesion; selective photothermolysis; purpura; met-hemoglobin

INTRODUCTION

Since its inception, the pulse dye laser (PDL) has become the standard by which all vascular lasers are evaluated. The PDL was the first laser designed using the concept of Selective Photothermolysis for the treatment of vascular lesions [1,2]. The dye laser has an exceptional safety record [3]. The use of multiple pulses, passes, or "pulse stacking," was originally employed in an attempt to improve lesion clearance, and to reduce the total number of treatments required for acceptable outcomes. With the advent of longer pulse durations, pulse stacking has been employed to provide more effective treatment while avoiding or limiting purpura [4].

Multi-pass treatments encompass complex phenomena. Using 0.45 millisecond pulse duration, 577-nm wavelength and a 3 second IPI, Tan et al. [5] found that while the degree of vascular injury could be increased, the depth of effective treatment was unaffected by up to five overlapping pulses. In contrast, Koster et al. [6] published that using similar parameters at 585-nm wavelength resulted in a 30% increase in the depth of vascular injury. Using a much shorter IPI of 10 millisecond, Dierickx et al. [7] found that two stacked pulses below the purpuric threshold provided increased purpuric thresholds, consistent with thermal diffusion models.

A consequence of PDL treatment is purpura, which typically occurs at therapeutic fluences, often providing a guide for proper treatment. This unpleasant bruising, due to the non-selective coagulation of capillary-sized blood vessels [8] can last well over a week in some cases, leading some clinicians and patients to choose less effective treatments. Several technical advances and treatment methods, including longer pulse durations, laser emissions consisting of multiple sub-pulses [9], and multiple passes [10] have been used in an attempt to limit or eliminate purpura, while providing effective vessel clearance in one or two treatments.

There are several distinct phenomena, which are often misinterpreted in the discussion of purpura and non-purpuric treatments. "True" purpura (Fig. 1) is distinguished by producing a bruise, which mimics the laser spot in size and shape. Purpura is an acute (or delayed) response caused by overheating and damage to normal sized capillaries with diameters of 40 μm or less, in non-port-wine-stain lesions [8]. Purpura in the case of PDL at

Fig. 1. Purpura resulting from treatment with a 595-nm pulse dye laser (PDL).

Fig. 2. Typical test are as 24 hours following laser exposure showing purpura thresholds for 0.5-millisecond pulse duration at IPI from 1P = 1 pass to 2P60, two passes 60 seconds apart, and associated biopsy locations (circled) at observer determined thresholds.

0.5 millisecond pulse duration is typically associated with the delivery of laser energy sufficient to destroy the target blood vessel, resulting in the collateral coagulation of capillary-sized vessels. Iterative studies comparing clinical results of extended pulse durations with analytical models provide the basis for this definition [11].

Until recently, treatment of vascular lesions with multiple pulses on the same site has typically been avoided due to a perceived increased risk of side effects. However, proper understanding and use of multi-pass techniques should allow for additive damage and deeper vascular injury, without an increased occurrence of side effects.

MATERIALS AND METHODS

Over a period of 3 years, a total of 16 subjects (6 M, 10 F), ages ranging from 27 to 60 years, were recruited for this study. Enrollment was limited due to the invasive nature of the study. All had normal appearing skin in the test areas. Subjects were exposed to a series of test spots on normal buttocks skin to determine purpuric threshold and depth of injury for a variety of pulsewidths, inter-pulse intervals, and number of passes.

Each subject was exposed to single pulse, and 4 double-pass intervals. The test spots consisted of exposures at 0.5 milliseconds from 2 to 7 j/cm^2 (Fig. 2). Exposures consisted of one, and two passes, at intervals of 1, 5, 10, 30, and 60 seconds between consecutive pulses. All subjects were tested for single-pulse threshold and double pass with a 1 second IPI, along with three other IPI. Timing of inter-pulse intervals for intervals of 1–10 seconds were based on firing a series of pulses matching the desired IPI laser pulse timing at 1 Hz pulse frequency (e.g., a 5 second IPI would be timed by firing a series of five pulses then returning to the first pulse to commence the second pass), intervals over 10 seconds were manually timed using digital stopwatches. All treatments were done using the PhotoGenica V-Star (Cynosure, Inc., Chelmsford, MA)

operated at 595-nm, 7-mm handpiece; and were done in conjunction with forced air cooling. The laser was in good working order and maintained calibration throughout the period of the study. Areas were evaluated and photographed immediately and 24 hours after laser exposure. Observed purpura threshold was defined as the fluence at which the pattern of bruise or erythema fills the spot size delivered. This was determined for each test condition by direct observation by the investigators. At the 24-hour follow-up visit, biopsies were taken at the fluence of single pass, and double pass purpuric thresholds (Fig. 3a and at 0.5 millisecond, 7 j/cm^2 (Fig. 3b)) exposures, to determine the depth of vascular coagulation associated with multiple passes. All biopsies were preserved and submitted for histological evaluation to a qualified pathologist. Hematoxylin and Eosin staining was used to determine the depth of vascular injury associated with laser treatment.

Depths of vascular injury were pooled based on test condition. Data was then compared to single pass treatment using Wilcoxon Signed Rank statistical test.

RESULTS

All tests were well tolerated by subjects. There were no side effects associated with treatment or biopsy, other than transient purpura. No scarring was produced by any test parameter on treated normal buttock skin.

At purpuric threshold, defined as minimal visible purpura covering the diameter of the laser spot on tissue; 0.5 milliseconds, single pass depth of vascular injury averaged 0.71 ± 0.3-mm (Table 1). Double pass depth of vascular injury ranged from 0.88 ± 0.3-mm (5 and 30 minutes inter-pulse interval) to 1.34 ± 0.5-mm (60 second inter-pulse interval) and exhibited a trend of greater
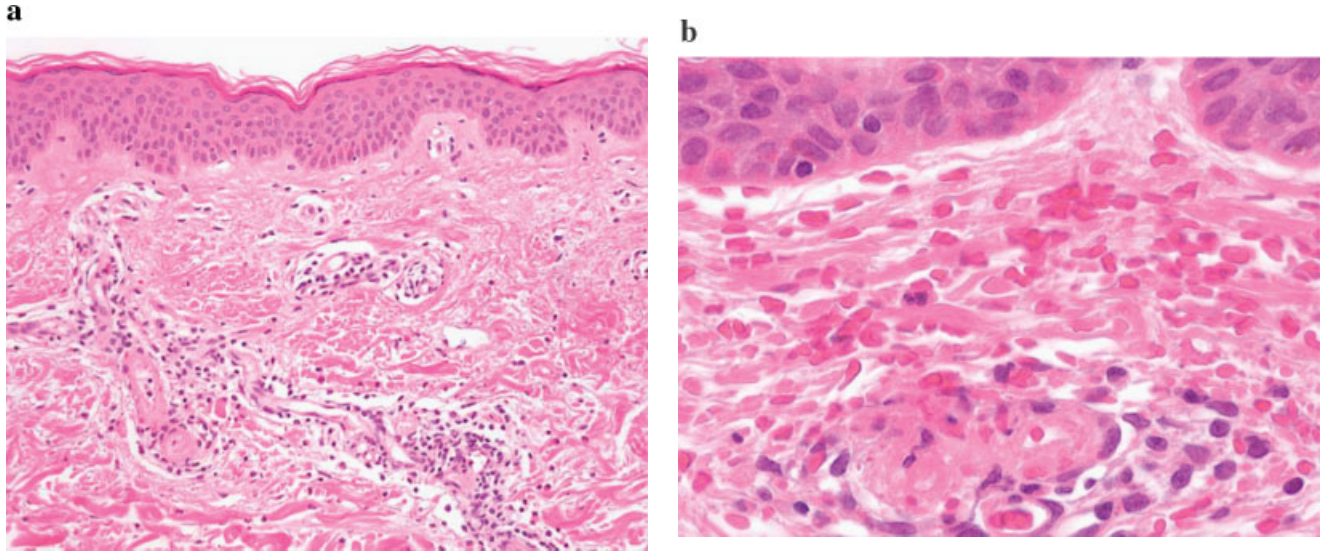
Fig. 3. **a**: Histology of 0.5 millisecond, double pass test site treated at purpura threshold with inter-pulse interval of 1 second (**b**) of severe purpura for IPI of 10 seconds; 400× magnification.

depth of vascular injury for intervals between 1 and 60 seconds (Fig. 4). The average depth for inter-pulse interval of 1 second was significantly greater than single pass. Depth of injury at 10, 30, and 60 second intervals did not reach the level of significance required, although they exhibit a trend of greater depth of injury. At intervals of 5 and 30 minutes, the depth of injury measured similar to single pulse exposures.

For 0.5 millisecond pulse format at 7 j/cm^2 (treatment resulting in purpura, uniform bruising covering the entire treatment spot, 24 hours following treatment) (Table 2), single pulse depth of vascular injury averaged 0.9 ± 0.2-mm. This compared to depths ranging from 1.2 ± 0.7-mm to 2.78 ± 0.7-mm for inter-pulse intervals of 1 second−30 minutes, respectively. The depth of vascular injury was monotonically increasing with increasing inter-pulse

TABLE 1. Depth of Vasculitis [mm] Measured on Histology for Laser Exposure With 0.5 millisecond Pulse Duration, at Purpura Threshold for the Labeled Conditions

Subject	1p	2p1	2p10	2p30	2p60	2p5m	2p30m
A1	0.3-mm	0.7-mm	0.9-mm	1.5-mm	1.1-mm	1.0-mm	0.9-mm
B1	1.0-mm	1.1-mm	1.9-mm	1.6-mm	1.9-mm	0.6-mm	0.8-mm
C1	0.8-mm	0.8-mm	1.7-mm	1.0-mm	1.1-mm	0.9-mm	0.4-mm
D1	1.5-mm	1.2-mm	1.2-mm	0.8-mm	0.8-mm	0.7-mm	1.0-mm
E1	1.0-mm	1.5-mm	0.8-mm	2.0-mm	1.8-mm	0.5-mm	0.6-mm
A2	0.4-mm	0.6-mm	1.3-mm	1.5-mm		0.9-mm	0.9-mm
B2	0.7-mm	1.2-mm	1.1-mm	0.9-mm			
C2	0.4-mm	1.2-mm	1.6-mm	0.5-mm			
D2	0.7-mm	0.6-mm	0.9-mm	0.9-mm			
E2	1.0-mm	1.3-mm	0.6-mm	0.5-mm			
A3	0.5-mm	0.7-mm		1.3-mm			
B3	0.5-mm	0.6-mm					
C3	0.7-mm	0.7-mm					
D3	0.6-mm	0.5-mm					
E3	0.6-mm	0.5-mm					
F3	0.6-mm	0.8-mm					
Average	0.71	0.88*	1.20	1.14	1.34	0.77	0.77
Standard deviation	0.30	0.32	0.42	0.48	0.48	0.20	0.23

Key 1p = 1 pulse, 2p1* = 2 pulses 1 second apart, 2p10 = 2 pulses 10 seconds apart, 2p30 = 2 pulses 30 seconds apart, 2p60 = 2 pulses 60 seconds apart, 2p5m = 2 pulses 5 minutes apart, 2p30m = 2 pulses 30 minutes apart.
*Statistically significant $P \leq 0.05$.

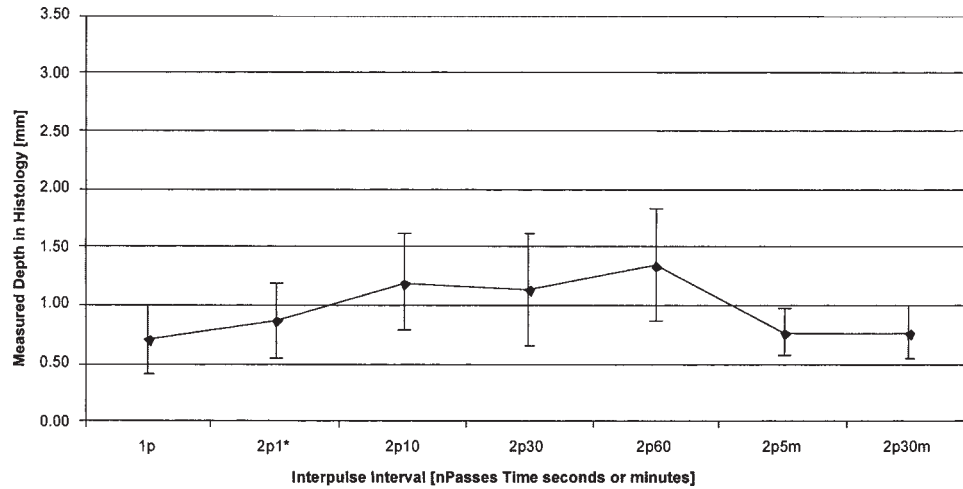**0.5-msec 595-nm w/ cooling multipass depth of vasculitis at threshold**

Fig. 4. Average depth of vascular injury versus inter-pulse interval for 0.5 millisecond pulse duration PDL exposures, at purpura threshold, for single pulse and double pulse exposures between 1 second and 30 minutes. (* statistically significant $P \leq 0.05$).

interval (Fig. 5). The depth of vascular injury was significantly greater than single pulse ($P \leq 0.05$) for intervals of 5 and 30 minutes.

DISCUSSION AND CONCLUSIONS

In an attempt to develop a clearer theory of multi-pass tissue effects, this study focused on the depth of vascular injury associated with the 0.5-millisecond pulse duration. Two cases were evaluated for the 0.5 millisecond situation, at purpura threshold (subject dependent), and at a constant fluence significantly above purpura threshold (fixed at 7 j/cm^2). While the sample size is small, the study provides significant results for several study conditions, warranting further investigation with larger samples and more precise tools.

Successive pulses of PDL, when delivered at purpuric threshold fluences exhibited increasing depth in vascular coagulation with increasing IPI from 1 to 60 seconds, returning to single-pulse depth for IPI of 5 minutes or more. At fluences above purpura threshold, depth of injury increased monotonically with increasing IPI up to 30 minutes, the limit investigated in this study. Based on tissue thermodynamics, the prevailing view has been that shorter intervals between pulses result in greater tissue injury. This study contradicts this assumption, with vascular coagulation depth exhibiting a complex temporal dependence.

At purpura threshold, there was a statistically significant ($P \leq 0.05$) difference in depth of vascular injury between single pass and the double pass interval of 1 second. The 1 second IPI was used throughout all subjects as a reference point, thus the sample size was larger than other IPI timing. Figure 4, exhibits a trend of increasing depth of injury with increasing inter-pulse intervals, with a

TABLE 2. Depth of Vasculitis [mm] Measured on Histology for Laser Exposure With 0.5 millisecond Pulse Duration, at 7 j/cm^2 (Resulting in Frank Purpura) for the Labeled Conditions

Subject	7j1p	7j2p1	7j2p30	7j2p5m	7j2p30m
A3	0.8-mm	0.6-mm	0.6-mm	0.9-mm	1.6-mm
B3	1.0-mm	1.5-mm	1.0-mm	2.0-mm	3.2-mm
C3	0.7-mm	0.6-mm	0.7-mm	2.2-mm	3.1-mm
D3	0.9-mm	2.2-mm	1.5-mm	2.5-mm	2.8-mm
G3	1.3-mm	0.9-mm	2.4-mm	2.1-mm	3.2-mm
Average	0.94	1.16	1.24	1.94	2.78
Standard deviation	0.23	0.69	0.74	0.61	0.68

Key 7j1p = 7 j/cm^2 1 pulse, 7j2p1 = 2 pulses 1 second apart, 7j2p30 = 7 j/cm^2 2 pulses 30 seconds apart, 7j2p5m = 7 j/cm^2 2 pulses 5 minutes apart, 7j 2p30m = 7 j/cm^2 2 pulses 30 minutes apart.
*Statistically significant $P \leq 0.05$.

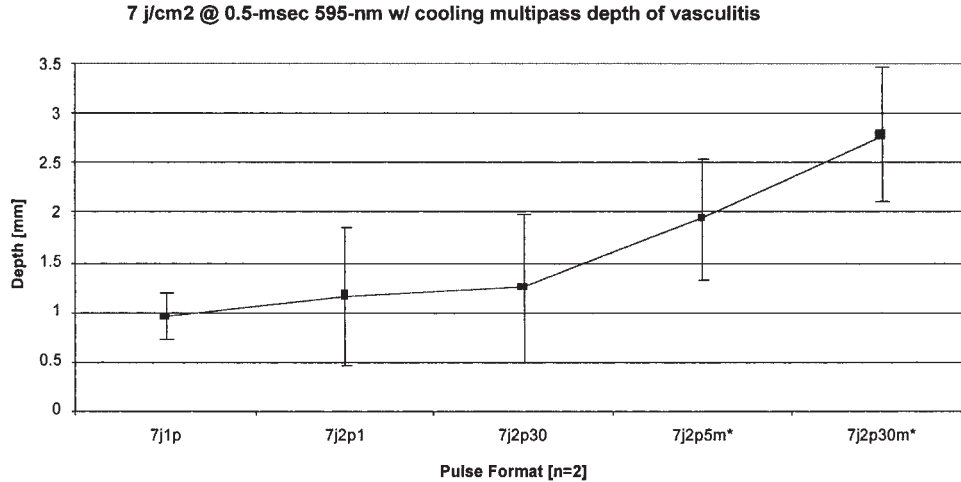**7 j/cm2 @ 0.5-msec 595-nm w/ cooling multipass depth of vasculitis**

Fig. 5. Average depth of vascular injury versus inter-pulse interval for 0.5 millisecond pulse duration PDL exposures, for exposures purpuric treatments at 7 j/cm^2, for single pulse and double pulse exposures between 1 second and 30 minutes. (*statistically significant $P \leq 0.05$).

plateau between 10 and 60 seconds. At 5 minutes, the depth of injury returns to single pulse levels. This trend suggests a transient phenomena occurring on a time scale that begins to occur within 1 second, reaches its maximum between 10 and 60 seconds, and has resolved within a few minutes. This phenomenon increases the depth of injury caused by subsequent pulses, without an attendant change in purpuric threshold. The time scale suggests biological, rather than thermodynamic cause, as effects persist well in excess of associated thermal relaxation times. The lack of persistence of the phenomena, at purpura threshold, beyond a few minutes suggests either the tissue recovers; or that the cause diffuses or is carried by circulation out of the treatment area.

Above purpura threshold, a very different effect is seen. As shown in Figure 5, depth of vascular injury increases monotonically with increasing interval, resulting in depth of injury on the order of three times single pass treatment, when multiple passes are set 30 minutes apart. This suggests a progressive and irreversible phenomenon that increases the depth of effect over the course of 30 minutes.

We postulate that the two effects seen are the result of heating of blood creating, at temperatures of approximately 65–72°C, Methemoglobin [Met-Hb] and other denaturation products, and above approximately 80°C, the development of clot, with attendant changes in wavelength absorption [12].

Met-Hb is a compound formed by the alteration of blood by heating and a variety of processes [13,14]. At 595-nm wavelength, Met-Hb exhibits approximately 1.8 times greater molar (absorption/Mole) absorption, 2.5 times lower absorption at 585-nm, and 3–5 times greater absorption at 1,064-nm, than oxyhemoglobin [15,16]. This is a molar conversion process, in which each molecule of hemoglobin is converted one-for-one to Met-Hb. Under conditions, such as at purpura threshold, of sufficient pulsed laser exposure; conversion occurs on a time scale of

10–30-milliseconds [13]. Thus absorption becomes a dynamic process for extended pulse durations in excess of 10 milliseconds. Randeberg et al. [17] have observed both an increase in tissue blood fraction and production of Met-Hb due to 585-nm PDL laser exposure resulting in increased optical absorption.

Previous work has suggested that multiple pulses and a combination of wavelengths during treatment can lead to improved outcomes in the treatment of recalcitrant Port Wine Stains [18], and other lesions [19]. Data for cooperative wavelength treatment, combining 595 and 1,064 wavelengths at IPI of 30–60 seconds appear to exhibit a cooperative effect, with increased depth of vascular injury [20].

Since, it is derived from hemoglobin in the blood, this reaction results in a molecule-for-molecule substitution of this preferentially absorbing compound. This results in increased absorption of later laser exposures, leading to enhanced depth of injury. The formation of Met-Hb does not, by itself, result in clot formation. Red blood cells containing Met-Hb remain viable, and continue to circulate [21], allowing propagation of Met-Hb through local tissues and eventually to be carried out of the local tissue by normal perfusion. Met-Hb is also converted back to hemoglobin by methemoglobin reductase over the course of several minutes [22], preventing it from building up in the system. Although the kinetics of both transport and conversion are not well understood, this and similar compounds are likely candidates for explanation of the transient nature of increased depth of vascular injury when treating at or near purpura threshold.

When applying multiple passes of PDL energy above purpura threshold, explanation of observed effects is straight forward. Work by Barton et al. [13] has shown that clot formed by laser exposure exhibits increased wavelength absorption at 595 nm. Clot formation and propagation occurs on a time scale similar to the observed

effects [15]. This suggests that the monotonic increase in depth of vascular injury for PDL treatment above purpura threshold is directly related to the development and propagation of intravascular clot.

The plurality of data suggests mechanisms to safely increase treatment depth when using PDLs. The use of multiple passes of PDL energy in the treatment of vascular lesions can increase the depth of treatment (vascular injury), when treating at fluences both near the purpura threshold and well above purpura threshold, and may improve clinical outcomes. When used at fluences, at or moderately above purpura threshold, the treatment has not been associated with an increased occurrence of side effects. This contrasts with the use of high fluences in single passes to increase the depth of vascular injury, in which significant permanent collateral damage can occur to the epidermis. It should be noted that the combination of vascular specific absorption and typically high peak powers associated with the PDL are specific to treatment with PDL and thus the results of this study should not be generalized to other wavelengths and/or other lasers. These results do, however, provide tantalizing clues to take advantage of multi-wavelength vascular photocoagulation.

When treating at purpura threshold, increased depth of treatment is correlated with inter-pulse interval, developing by 1 second and resolving on the order of 1 minute. When treating above purpura threshold, depth of treatment is directly correlated with inter-pulse interval for intervals up to at least 30 minutes. This study suggests that when using double pulse treatments, certain time frames may improve outcomes. The utility of this method in the treatment of specific classes of lesions should be evaluated.

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