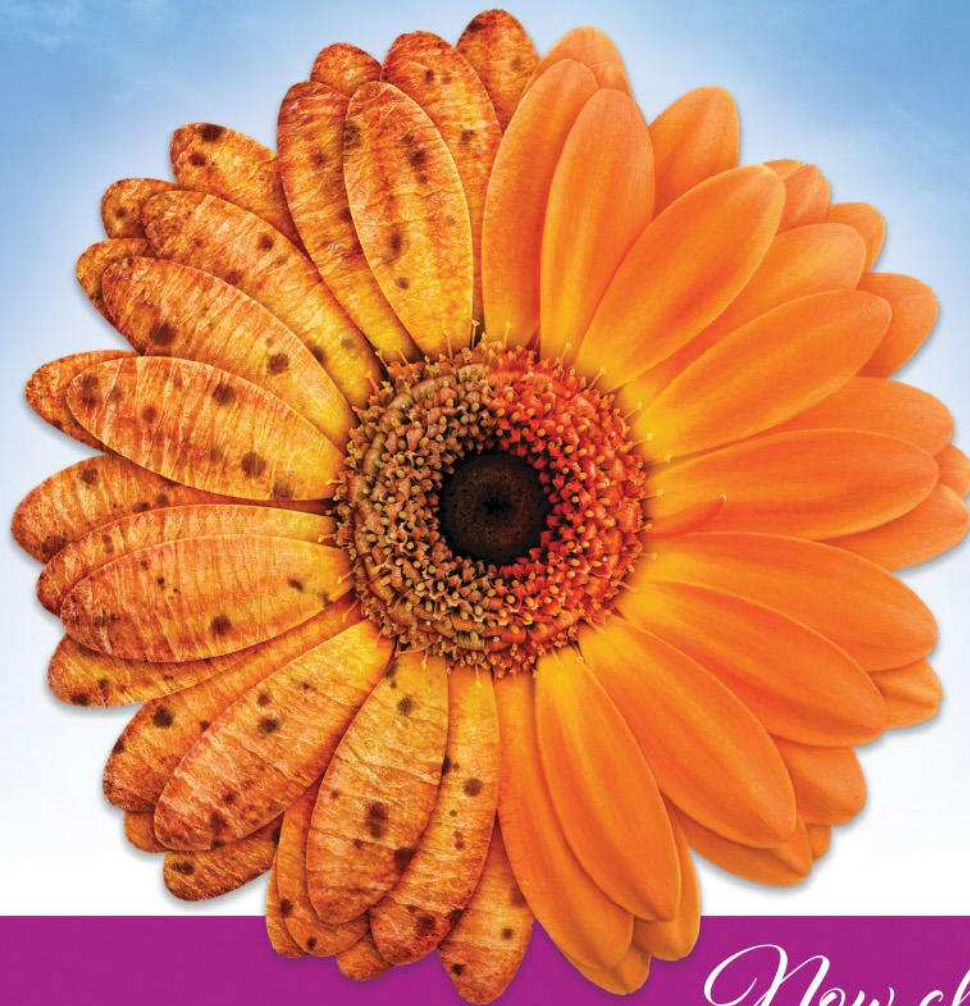


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A Comparative Study With a 755 nm Picosecond Alexandrite Laser With a Diffractive Lens Array and a 532 nm/1,064 nm Nd:YAG With a Holographic Optic

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Background and Objectives: This study was performed to better understand the cutaneous effects of using a fractional picosecond laser at 755 nm with a diffractive lens array and a picosecond Nd:YAG laser at 532 nm and 1,064 nm with a holographic optic. We characterized the injuries created by these devices on skin clinically and histologically over 24 hours. With this information we modeled the effects of these devices on a cutaneous target.

Methods: Eight patients, representing Fitzpatrick skin types I–VI, were treated on their backs with a picosecond Alexandrite laser with a diffractive lens array, as well as a picosecond Nd:YAG laser at 532 nm and 1,064 nm with a holographic optic. Photographs were taken 15 minutes and 24 hours after treatments. Punch biopsies were obtained at 24 hours and examined histologically.

Results: Treatment with the picosecond Nd:YAG laser at both 532 nm and 1,064 nm with the holographic optic revealed erythema and small scattered areas of petechial hemorrhage areas immediately and in many cases at 24 hours after treatment. The 755 nm picosecond Alexandrite laser with diffractive lens array produced erythema immediately after treatment, which largely dissipated 24 hours later. Histologies revealed intra-epidermal vacuoles with all three wavelengths. Fractional picosecond Nd:YAG laser at 532 nm and 1064 nm with the holographic optic showed focal areas of dermal and intra-epidermal hemorrhage with areas of vascular damage in some patients.

Conclusions: This study demonstrates that both fractional picosecond devices produce vacuoles in the skin, which are most likely due to areas of laser induced optical breakdown (LIOB). In the patients (skin type II–IV) we observed scatter areas of hemorrhage in the skin, due to vascular damage with the 532 nm and 1,064 nm, but not with 755 nm wavelengths. *Lasers Surg. Med.* © 2017 Wiley Periodicals, Inc.

Key words: laser induced optical breakdown (LIOB); superficial hemorrhage; fractional picosecond laser; holographic optic; diffractive lens array

INTRODUCTION

The use of picosecond lasers with fractional optics have provided an opportunity to deliver high energy short

pulses of light to the skin. The picosecond Alexandrite 755 nm laser with a diffractive lens array has been used successfully to treat acne scars [1], photo-damaged skin [2], and melasma [3]. We have described the creation of intra-epidermal vacuoles, which appear to be the result of areas of LIOBs from the absorption of high energy 755 nm laser light by melanin in the granular layer of the epidermis [4]. This localized damage has been associated with the production of new collagen and elastic tissue. It is possible that the production of epidermally generated growth factors, cytokines, and chemokines could be responsible for these changes [1,5]. The immediate clinical effects of transient erythema, heat, and swelling with this device lasting less than 24 hours has been well characterized [5]. In individuals with skin types I and II with melanin index (MI) of 12 or less we have described small transient areas of hemorrhage which suggests that hemoglobin in superficial dermal vessels can be a target for this laser in the absence of a sufficient amount of melanin in the epidermis. In individuals with a modest amount of melanin (MI greater than 12) and especially in darker skin types, only short lived erythema and slight swelling is observed.

We have also studied picosecond 532 nm, 1,064 nm, and 755 nm light with a diffractive lens array in order to better understand the comparative effects of these wavelengths on the skin [6]. Clinically, we observed more erythema and areas of hemorrhage across all skin types with the 532 nm and 1,064 nm wavelengths immediately after treatment and at 24 hours on trunk skin. In contrast, treatment with 755 nm with the diffractive lens array showed short lived erythema and mild induration lasting only a few hours. Skin biopsies done 24 hours after treatments with the 532 nm and 1,064 nm wavelengths with the diffractive

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optic showed focal areas of epidermal necrosis and epidermal vacuoles with scattered and focal areas of dermal vascular damage with extravasation of red blood cells across all MI's and skin types. In contrast, those individuals with MI's greater than 12 treated with the 755 nm picosecond laser with the diffractive optic showed epidermal vacuoles without hemorrhage or damage to the dermal vasculature. Our findings suggested that the delivery of high energy 755 nm picosecond light to the skin was more selectively absorbed by melanin in the epidermis creating LIOBs only in that region. The high energy 1,064 nm and 532 nm picosecond light was absorbed by melanin in the epidermis and also by hemoglobin in the superficial cutaneous vasculature resulting in LIOBs in the epidermis and hemorrhage seen both in the epidermis and dermis.

These clinical and histological findings do have practical implications. Cutaneous hemorrhage often remains visible for days and can be difficult to camouflage. The picosecond 532 nm/1,064 nm device with the diffractive lens array, which we used in that study, was a prototype laser. There is a commercially available picosecond 532 nm/1,064 nm Nd:YAG laser with a holographic optic which was developed to deliver high energy fractional pulses to the skin for the treatment of acne scars, photo damaged skin, and abnormalities of pigmentation. Our goal in this investigation was to compare the commercially available 755 nm picosecond Alexandrite laser with the diffractive lens array to this commercially available Nd:YAG laser with the holographic optic specifically studying the clinical and histological findings over 24 hours on the skin. We will present a model to better understand our observations.

MATERIALS AND METHODS

This was a single center IRB approved study. Eight female patients were treated on their backs with a picosecond 532 nm and 1,064 nm laser with a holographic optic (Syneron-Candela) and a picosecond 755 nm Alexandrite laser with a diffractive lens array (Cynosure). Energies were 0.2 mJ/dot, 0.24 mJ/dot, 0.28 mJ/dot at 532 nm, 1.3 mJ/dot, 1.7 mJ/dot, 2.1 mJ/dot for 1,064 nm and 0.25 J/cm², 0.4 J/cm², and 0.71 J/cm² for 755 nm. These treatment parameters were marked in a grids on the backs of the patients labeled A, B, and C with the corresponding energies and wavelengths (Table 1). The settings for these devices were in the ranges suggested by the manufactures when the study was performed. MI's from 11, 12, 15, 18, 19, 24, 30, and 95 (skin types I–VI) were studied with histologies obtained 24 hours after treatment of three

passes with each wavelength. 3.5 mm punch biopsies were taken from each side, formalin fixed and examined with hematoxylin and eosin staining. Clinical photographs were obtained 15 minutes and 24 hours after treatments.

RESULTS

Erythema was seen immediately after treatment with all wavelengths. One thousand and sixty-four nanometer was more prominent than 532 nm, which was more noticeable than 755 nm. At 24 hours erythema with small areas of petechial hemorrhage was seen with 1,064 nm and to a lesser extent with 532 nm. Petechiae were not seen in the treatment sites with 755 nm. Only faint erythema was seen with this wave length at 24 hours (Fig. 1).

With the picosecond 532 nm Nd:YAG laser and the holographic optic, we observed focal areas of epidermal necrosis on histological examination. We also observed scattered areas of superficial dermal hemorrhage associated with focal areas of vascular damage with the lighter skin types MI 12–15 and the lower energies (Fig. 2). As the energy and the MI increases, focal epidermal vacuoles are observed with areas of dermal hemorrhage. In the more pigmented individual MI 24, 30, and 95 more vacuoles were observed, which became larger and more numerous as the MI and energy increased. Fewer areas of dermal hemorrhage were seen in the darker skin types.

In patients treated with picosecond 1,064 nm Nd:YAG and the holographic optic, intra-epidermal vacuoles accompanied by focal areas of dermal hemorrhage associated with scattered areas of vascular damage were routinely observed across the fluences and skin types studied (Fig. 3). In the darker skin types the vacuoles and areas of damage became larger and more superficially situated.

In the regions treated with the picosecond Alexandrite laser epidermal vacuoles were generally observed with the higher fluences (Fig. 4). Rare areas of hemorrhage were seen only with the patient with an MI of 11. Intra-epidermal vacuoles were routinely observed in with the higher energies in patients with MI 18–24. In darker skin types, MI 30 and above, these vacuoles were seen with all the energies studied. No hemorrhage was observed in patients with MIs over 12.

DISCUSSION

The advent of high energy picosecond lasers with fractional methods of delivery has provided an opportunity to explore a new way to wound the skin creating very localized areas of injury with very little adjacent tissue damage. This process appears to be initiated in the skin by the absorption of the light by melanin in the epidermis, which results in the production of a seed electron. As the energy is delivered during the laser pulse this process continues with the creation of a very localized area of heating by the creation of a small steam bubble. This results in small 40–60 micron vacuoles in the epidermis with very little adjacent tissue damage.

TABLE 1. Listed are the Energies Used Which Each of the Three Wavelengths. Three Passes Were Performed with Each Setting

	532 nm	1,064 nm	755 nm
A	0.2 mJ/dot	1.3 mJ/dot	0.25 J/cm ²
B	0.24 mJ/dot	1.7 mJ/dot	0.4 J/cm ²
C	0.28 mJ/dot	2.1 mJ/dot	0.71 J/cm ²

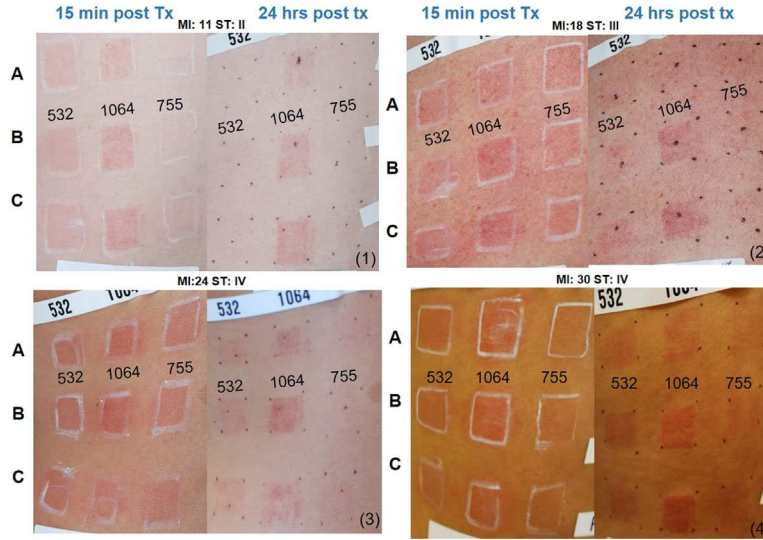


Fig. 1. Refer to Table 1 for specific parameters. Erythema was seen immediately after treatment with all wave lengths. One thousand and sixty-four nanometer was more prominent than 532 nm, which was more noticeable than 755 nm. At 24 hours erythema with small areas of petechial hemorrhage was seen with 1,064 nm and to a lesser extent with 532 nm. Petechiae were not seen in the treatment sites with 755 nm. Only faint erythema was seen with this wave length at 24 hours.

When delivering 755 nm light with the diffractive lens array in the absence of a critical amount of melanin, such as a patient with vitiligo or a low MI of 11 or less, we have observed areas of epidermal and superficial dermal hemorrhage. This appears to be due to the absorption of this energy by hemoglobin in the superficial capillary loops which are located in the lower layers of the epidermis and the superficial dermis. Fortunately most patients have MIs

greater than 12 with superficial hemorrhage not being a problem. It has been well documented that the darker skin types do well with this type of wounding without the dyspigmentation as seen with other non-ablative devices [7].

When considering the epidermal absorption of 532 nm, 1,064 nm, and 755 nm radiation we can see not only the effect of absorption of the first chromophore and melanin,

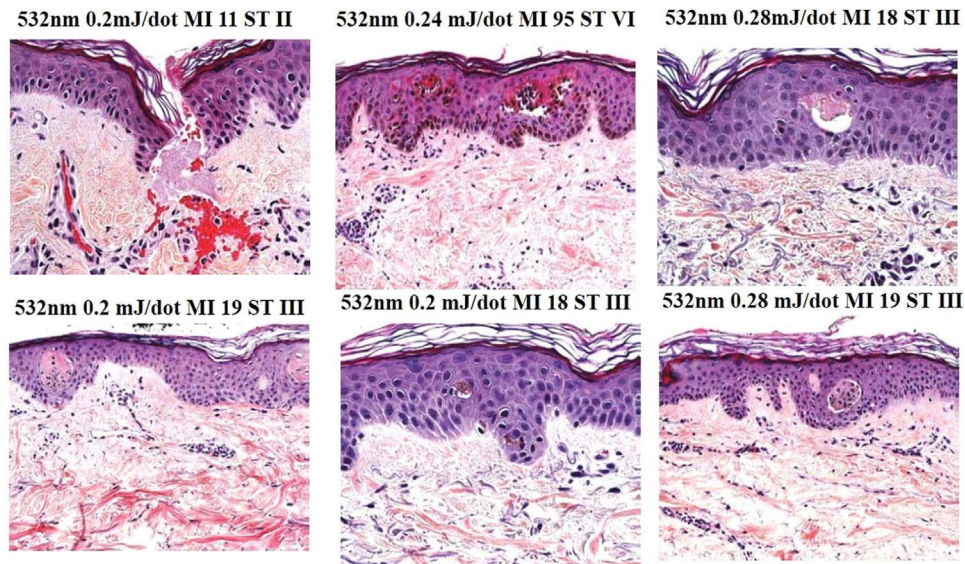


Fig. 2. Five hundred and thirty-two nanometer- focal areas of epidermal necrosis and areas of superficial dermal hemorrhage is seen in the lighter skin types MI 12–15 with the lower energies. As the energy increases and the MI increases focal epidermal vacuoles are observed with areas of dermal hemorrhage. In the more pigmented individual MI 24, 30, and 95, more vacuoles were observed which became larger and more numerous as the MI and energy increased. Fewer areas of dermal hemorrhage were seen in the darker skin types.

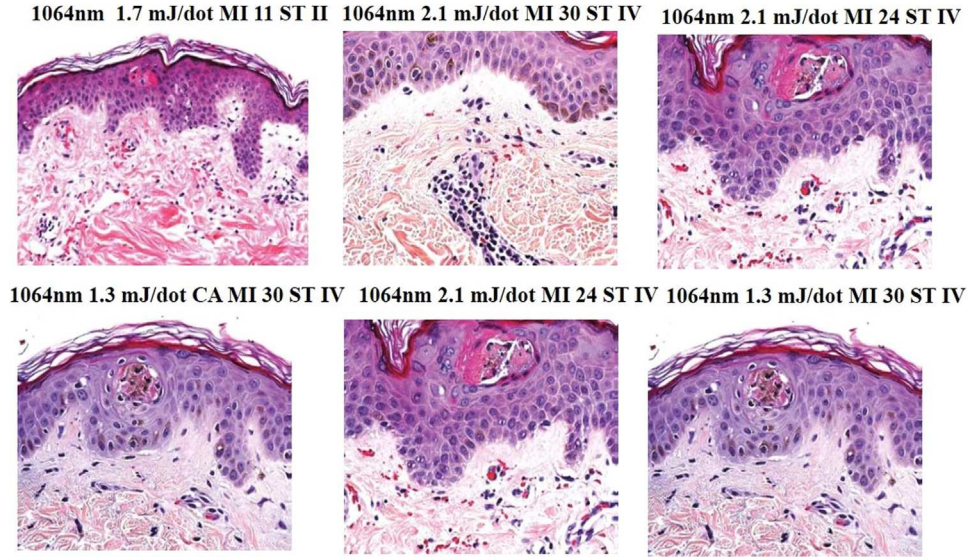


Fig. 3. One thousand and sixty-four nanometer- intra-epidermal vacuoles accompanied by focal areas of dermal hemorrhage were observed were routinely observed across the fluences and skin types studied. In the darker skin types the vacuoles and areas of damage became larger and more superficially situated. Focal areas of hemorrhage were appreciated.

in the path of the energy emitted from the optic, but also the effect of the lower absorption coefficient of the secondary chromophores oxy or deoxy hemoglobin in the blood vessels (Fig. 5). Despite the higher melanin absorption versus hemoglobin we have observed evidence of blood vessel damage and disruption most likely due to the hemoglobin absorption. We have observed occasional vacuoles at the dermal-epidermal junction in patients treated with 532 nm and 1,064 nm light, which were associated with vascular damage. We have not seen dermal vacuoles that were not associated with vascular damage.

When examined we can see that the absorption of melanin relative to blood is the greatest for 755 nm with a 54 to 1 ratio, followed by 1,064 nm with a 16 to 1 ratio, and with 532 nm at a 2.4 to 1 ratio (Table 2). In Table 2, we have summarized calculations based on the formalism described in the Appendix. The most important parameter in an

LIOB based treatment is the fluence threshold for thermionic emission of a seed electron. We present the LIOB threshold in joules/cm² in the epidermis calculated from equation (A4) for the three laser wavelength under consideration. For each of the three laser wavelengths the temperature rise in a blood vessel is calculated from equation (A5) when the corresponding laser delivers the calculated LIOB threshold fluence. For the considered three wavelengths melanin and blood absorption we can see that the greatest blood temperature rise occurs with 532 nm light followed by 1,064 nm with the least occurring at 755 nm. The calculated values for LIOB threshold fluence and the corresponding temperature rises in blood vessels depend only on material properties and epidermal melanin content. The calculated values in Table 2 are not dependent on the type of optical delivery system or the spatial intensity distributions created by the optical delivery system. A consistent treatment based on

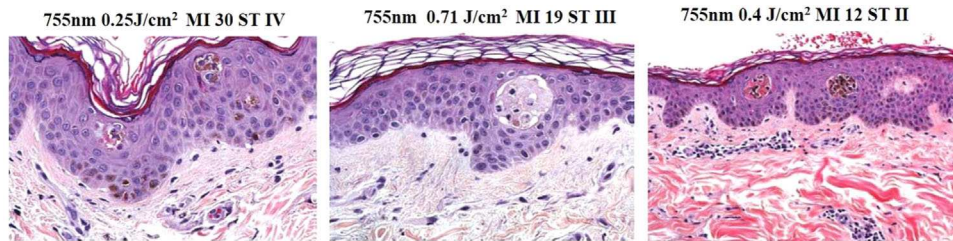


Fig. 4. Seven hundred and fifty-five nanometer- in patients with MI 11–15 intra-epidermal vacuoles were generally observed with the higher fluences. Rare areas of hemorrhage only with the patient with an MI of 11. Intra-epidermal vacuoles were routinely observed in with the higher energies with in patients with MI18–24 in. In darker skin types MI 30 and above these vacuoles were seen with all the energies studied. No hemorrhage was observed.

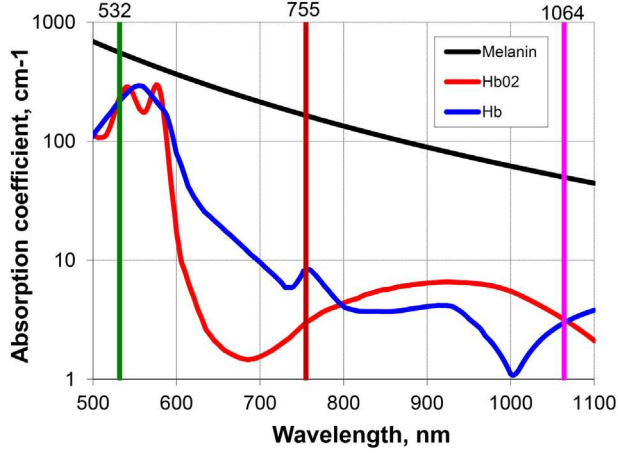


Fig. 5. The absorption by melanin versus hgb species at 755 nm displays preference for 755 nm over 532 nm and 1064 nm.

intra-epidermal LIOB injuries requires fluence in the high intensity regions that is equal or higher than the LIOB threshold for the treatment laser wavelength.

Correlating this data with our observations, the 1,064 nm picosecond Nd:YAG laser with the holographic optic gave us the most hemorrhage followed by 532 nm picosecond Nd:YAG laser with none seen with 755 nm picosecond Alexandrite laser in individuals with MI's greater than 12. We can conclude that there is an advantage in using a fractional mode of delivery of the 755 nm relative to 1,064 nm and 532 nm if our goal is to selectively create LIOB's in the epidermis via melanin absorption without superficial vascular damage.

While our modeling indicated that the fractional delivery of 532 nm light should produce more damage to the superficial vessels, our clinical observations, and histologies showed more vascular damage with fractional 1,064 nm energy. This finding suggests that the fluence in the high intensity regions used at 1,064 nm is larger than the 1,064 nm LIOB threshold fluence. The relatively lower vascular damage at 532 nm suggests that the fluence in the high intensity regions employed at 532 nm is equal to or lower than the 532 nm LIOB threshold fluence. Our data and modeling also indicate that as the epidermal melanin

concentration rises with darker skin types, the heating of the superficial blood vessels is diminished due to the larger amount of melanin available to absorb the energy delivered to the epidermis. If hemorrhage avoidance is a goal, darker skin types have a distinct advantage with the use of a fractional picosecond 532 nm, 1,064 nm, and 755 nm.

Insights on the histological changes caused by a high energy picosecond 1,064 nm pulse focused into the superficial dermis have been reported by Habbema [9]. In one part of the investigation in *ex-vivo* skin they described the production of LIOBs with a morphology that was similar to the histological changes that we described in the epidermis. However, when the treatments were performed in *vivo* petechiae were noted on the skin clinically lasting a number of days. On pathological examination, the well formed LIOBs seen in *ex vivo* skin were not observed. Instead, irregular spaces and areas that appeared to have damaged blood vessels surrounded by extravasated blood was seen. These two sets of observations would suggest that the target with both experiments is blood and that the differences observed could be due to the dynamic changes that occur with a moving target both during and after the laser pulse in *in vivo* skin. There is also a substantially different absorption of the clotted blood observed in *ex vivo* skin compared to the largely oxyhemoglobin and deoxyhemoglobin that occurs in a vessel of a living subject. In addition, this group focused the beam to 190 microns in the dermis and was largely able to avoid significant melanin heating in the epidermis. If we compare these observations with our own, the degree of the changes that are seen are less dramatic with areas of vascular damage and hemorrhage, but we have to acknowledge that the amount of energy reaching the target is less in this series of experiments. In a previous study we were able to visualize the superficial vasculature using a confocal microscope. We noted pulsating capillary loops in the lower portion of the epidermis and the superficial papillary dermis which could likely be a target for the devices used in our investigation [4].

There is a recent report in this journal of the use of a multiphoton microscope to study the effects on the skin at a number of time points after treatment with a fractional picosecond Nd:YAG with a holographic optic at both 532 nm and 1,064 nm [8]. They used the maximum energy

TABLE 2. Absorption Coefficients for Melanin and Blood for the Three Laser Wavelengths Used for Calculation of the LIOB Threshold Fluence and the Corresponding Temperature Rise in Blood Filled Capillaries at the Dermal/Epidermal Junction

Wavelength, nm	532			1,064			755		
Melanin abs, cm ⁻¹	555			50			163		
Blood abs, cm ⁻¹	235			3.2			3.0		
Absorption ratio	2.4:1			16:1			54:1		
Epid. Melanin %	2%	15%	43%	2%	15%	43%	2%	15%	43%
LIQB Thresh, J/cm ²	2.8	2.7	2.6	31	30	29	9.5	9.1	8.8
Blood Temp rise, °C	172	105	40	28	25	22	7.8	6.5	4.8

Values colored green are the most advantageous for avoidance of side effects in a clinical treatment, values colored red are the least advantageous and yellow is in intermediate.

for each wavelength but treated only with a single pass. They were able to visualize the LIOB injury zones in the epidermis. They did not see or document any evidence of dermal damage or hemorrhage. Unfortunately, this investigation was only done on one patient with one pass in a skin type II patient. We treated our patients with a variety of skin types with three passes at the fluences that were recommended for treatments by the manufacturer. The hemorrhage was mild but visible lasting only for a few days. The red blood cell seen in the dermis appeared to be associated with damage to blood vessel or an LIOB at the dermal-epidermal junction. The histological method that we employed in our study allowed us to see the entire specimen and to view the entire epidermis and dermis in fine detail. The treatment of only one patient of a single skin type, the use of one pass, and the limitation of the multiphoton microscope to see deeper or finer details might explain the difference seen in tier investigation. It is of interest to note that in our darkest skin type no hemorrhage was observed with any device. The large amount of melanin in this patient appeared to absorb enough of the laser energy to prevent significant blood absorption.

CONCLUSION

This study supports the earlier observations that the fractional delivery of high energy picosecond light at 532 nm and 1,064 nm with an Nd:YAG laser, whether it is by a diffractive lens array or with a holographic optic, does produce LIOBs in the epidermis, but also heats and damages the superficial blood vessels resulting in cutaneous hemorrhage. It also confirms that fractional high energy picosecond Alexandrite laser at 755 nm creates LIOBs in the epidermis with the lowest heating of the superficial vasculature relative to these other wavelengths. The modeling framework indicates that the superior vasculature safety of the 755 nm picosecond laser is due to preferential absorption in melanin versus blood at 755 nm and it is not related to the optical delivery system.

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APPENDIX

Seed electron generation for laser induced optical breakdown in the epidermis and associated thermal effects in vasculature at the dermal/epidermal junction

The distinct feature of the treatment with a picosecond laser creating multiple high intensity exposures in the epidermis are the multiple epidermal voids observed in histology. The native absorption of epidermal melanin is insufficient to account for the observed void diameters. A chromophore with approximately 100 times higher absorption than melanin is needed to account for the observed voids [10,11]. The process of laser induced optical breakdown (LIOB) creates the needed transient high absorption chromophore consisting of high density electron plasma. The key first step in the LIOB process is the generation of a "lucky" seed electron. The seed electrons in the epidermis can be generated either through a multiphoton absorption and ionization or through a thermal ionization also called thermionic emission. The multiphoton

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absorption and ionization is relatively inefficient for the typical 500–750 ps pulse durations and fluence of a few Joules per cm². The thermionic emission is the more efficient of the two processes and depends on the native absorption of the epidermal melanin and its ionization potential.

The generation of a single seed electron or a small number of seed electrons from heated epidermal melanin can be described as a Poisson process. The first step is to derive a relationship between the epidermal fluence in the high intensity regions irradiated on the skin and the average expected value of the Poisson process. In the following considerations the threshold epidermal fluence H_T is interpreted as the lowest value leading to the generation of one seed electron, on average, in the large number of high intensity regions irradiated on the skin. The probability of generation of one or more seed electrons by epidermal fluence H_T approximately follows a Poisson distribution. A laser delivery system set to deliver exactly fluence H_T will generate one or more seed electrons in a fraction f_I of the high intensity regions, $f_I = 1 - e^{-1} = 0.63$. In a clinical setting the laser is set to deliver in the high intensity regions fluence larger than H_T in order to have consistent generation of LIOBs and corresponding clinical effect.

The average expected value of seed electron generation in the epidermis will be calculated using the mathematical formalism for thermal ionization describing the density of electrons and ions of various level of ionization developed originally by Saha [12,13] and later expanded for microscopic solid particles [14]. The Saha equation for the number density of free electrons n_e , singly ionized melanin n_i , and non-ionized melanin n can be written as

$$\frac{n_i n_e}{n} = 2 \frac{(2\pi m_e k T)^{\frac{3}{2}}}{h^3} \exp\left(-\frac{\varphi}{k T}\right) \quad (\text{A1})$$

where m_e is the mass of the electron, k is the Boltzmann's constant, h is the Plank's constant, φ is the melanin ionization potential and T is the melanin temperature. The Saha equation implicitly assumes a Boltzman distribution for energy of the melanin particles in the targeted region. That assumption is mostly satisfied during the initial heating of the melanin particles when the thermally generated electrons are very few or non-existent.

The typical 500–750 ps laser pulse durations generated by the lasers used in the experiments are much shorter than the 0.5 μ s thermal relaxation time of the typical $\sim 1 \mu$ m melanosome. That means the laser heating of a melanosome is thermally confined. Then for melanin with absorption coefficient μ_m the melanosome temperature can be written as

$$T = T_0 + \frac{\mu_m H_0}{\rho_m C_m} \quad (\text{A2})$$

where T_0 is the normal skin temperature, H_0 is the fluence in the high intensity region, ρ_m is the melanin density and C_m is the melanin heat capacity.

The thermionic emission is the only source of free electrons in the epidermis and multiple ionization of melanin molecules is ignored because of the higher ionization potential. That means for every free electron generated by thermionic emission there will be a singly ionized melanin ion. Then the number density of free electrons n_e is equal to the number density of singly ionized melanin n_i . The number density of melanin n in the epidermis is dependent on skin pigmentation. The skin pigmentation can be taken into account by introducing a volume fraction of melanin in the epidermis f_m that takes values between 1 and 43% [15]. Then using that $n_e = n_i$, Equation (A1) can be written for the number density of free electrons in the epidermis as

$$n_e = \sqrt{2 f_m \frac{N_A}{M_m} \rho_m \frac{(2\pi m_e k T)^{\frac{3}{2}}}{h^3} \exp\left(-\frac{\varphi}{k T}\right)} \quad (\text{A3})$$

where the number density of melanin n was written as $n = \frac{N_A}{M_m} \rho_m$, N_A is the Avogadro's number, M_m is the melanin molecular weight and ρ_m is the melanin mass density. The last equation can be rewritten for the number density of the free electrons n_e corresponding to one seed electron in the targeted region in the epidermis.

Substitution of Equation (A2) and (A3) leads to a relationship between the number density corresponding to one seed electron in the targeted region and the threshold fluence in the high intensity regions of the epidermis H_T .

$$n_1 = \sqrt{2 f_m \frac{N_A}{M_m} \rho_m \frac{\left[2\pi m_e \left(k T_0 + \frac{k \mu_m H_T}{\rho_m C_m}\right)\right]^{\frac{3}{2}}}{h^3} \exp\left(-\frac{\varphi}{k T_0 + \frac{k \mu_m H_T}{\rho_m C_m}}\right)} \quad (\text{A4})$$

Equation (A4) can be considered as an implicit equation for the threshold epidermal fluence H_T that leads to average expected value of one seed electron for the Poisson process in the targeted region. Equation (A4) is solved numerically for

the threshold epidermal fluence H_T . It is remarkable that the calculated threshold fluence H_T depends only on fundamental constants, material properties of melanin and epidermal melanin content. It does not depend on the type of optical delivery system or the spatial intensity distributions created by the optical delivery system.

The energy delivered in the high intensity regions does not always get fully absorbed in the epidermis. In the regions where there was no generation of a seed electron, or where the seed electron was generated late during the pulse, a fraction of the delivered energy, up to 100%, reaches the dermal/epidermal junction and propagates deeper in the dermis. In such high intensity regions capillaries near the dermal/epidermal junction are exposed to fluence equal to or larger than $H_T \exp(-f_m \mu_m d)$, where μ_m is the absorption coefficient of melanin, f_m is the volume fraction of melanin in the epidermis and d is the epidermal thickness. The capillary heating is considered thermally confined because the 500–750 ps laser pulse durations are much shorter than the thermal relaxation time of 47–190 μs for a typical 10 to 20 μm capillary. Then the temperature rise in a blood filled capillary at the dermal/epidermal junction can be expressed as

$$\Delta T_B = \frac{\mu_B H_T \exp(-f_m \mu_m d)}{\rho_B C_B} \quad (\text{A5})$$

where μ_B is the blood absorption coefficient, ρ_B is the blood density and C_B is the blood heat capacity.