Lasers Med Sci 2002, 17:154–164

In Vivo Study of Intradermal Focusing for Tattoo Removal

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Abstract. Delivery of intradermally focused nanosecond laser pulses with small energy as an alternate technique applicable to clinical procedures in dermatological and plastic surgery is an area of relatively new interest with multiple potential applications. We assessed this approach on common tattoo pigments in dermis in an in vivo study using a wavelength of 1064 nm. Paired micropigs were tattooed with standard blue, black, green and red pigments. The tattoos were allowed to mature and then treated by 12 ns pulses in a focused beam of 11.4° cone angle. Visual observation and histological analysis of biopsies were performed to evaluate results. Significant reduction in pulse energy and collateral damage was achieved with pulse energy ranging between 38 to 63 mJ. Blue and black tattoos were found to respond well from a clinical standpoint. The depth dependence of tissue response and pigment redistributions at 1 hour, 1 week and 1 month after laser treatment was quantitatively analysed through biopsies and a strong relationship was demonstrated between tattoo response and laser-induced dermal vacuolation. The optical absorption coefficients of the four tattoo pigments were measured to be approximately the same and the laser-induced plasma is suggested to be responsible for the pigment redistribution. As we hypothesised, intradermal focusing of nanosecond pulses significantly reduced required pulse energy for tattoo ablation to about 60 mJ or less. These results stimulate a number of additional questions relevant not only to clinical applications but also to the understanding of the fundamental process of laser–pigment interaction in the dermis as it relates to tattoo removal.

Keywords: Intradermal focusing; Laser surgery; Tattoo removal

INTRODUCTION

There is a tremendous volume of research in both basic and clinical sciences investigating the medical applications of laser technology. The development of laser systems capable of scanning a beam of small energy and intradermally focused nanosecond (ns) pulses pose the question of how these systems could augment or improve clinical procedures in dermatological and plastic surgery. The potential areas of application could include not only tattoo removal and pigmented lesions but extend also to vascular malformations and other lesions. The process of tattooing is one that lends itself readily to in vivo clinical investigations and the potential for developing new laser systems for more effective tattoo removal could be very beneficial considering the current worldwide trend of increased popularity of tattooing and the subsequent desire for removal. The study of tattoo removal also allows for the academic pursuit of basic biophysics in understanding the complexity and fundamental problems of light distribution in the skin and the pigment ablation process.

Q-switched lasers are widely used in tattoo removal as the sources of nanosecond pulses due to their relatively simple system configurations and high reliability in comparison to other short-pulsed lasers. Although a selective photothermolysis model [1] has been extensively applied to interpret the data obtained with the ns pulses [2–6], very few quantitative in vivo investigations have been conducted to provide critical insight into the mechanism of the ablation process within the skin. As a result, fundamental problems remain unanswered. For example, what is the role of the tissue or pigment absorption in the ablation process in relation to the strong electromagnetic field created by the pulse during

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the short time scales of nanoseconds? Additionally, what is the effect of cavitation and transient acoustics induced by the ns laser pulses on the pigments and pigment mobilisation? Finally, can one reduce further the collateral tissue damage by decreasing the energy of a ns pulse delivered into the skin? This lack of a clear understanding of the mechanism underlying skin and pigment ablation by ns pulses has impeded the further improvement of the way we deliver the pulses for treating skin lesions ever since the Q-switched lasers were introduced into dermatological clinics in the 1980s [8], with a telling manifestation of this situation demonstrated by the fact that multiple Q-switched laser systems with pulse energy of 1 J or larger are needed in most clinics practising laser tattoo removal procedures for treating different coloured pigments [6]. Recently, we have studied the mechanism of skin ablation by ns laser pulses [9] and skin optics [10] in vitro in our efforts to investigate a new approach to delivering ns pulses in treating multicolour pigmented lesions with reduced collateral tissue damage [11]. As a prelude to our efforts in identifying an efficient clinical method of treating skin lesions with short laser pulses, we studied tattoo removal in vivo in a micropig animal model using a focused beam. In this paper we report initial results from two micropigs using 12 ns pulses at 1064 nm wavelength from a Q-switched Nd:YAG laser and pulse energies between 38 and 63 mJ.

MATERIALS AND METHODS

Two female Sinclair Yucatan micropigs (Charles River Laboratories, Inc.) were used in our study because the histology and healing rate of porcine skin are similar to that of human skin [4]. All manipulations involving the micropigs were accomplished in strict accordance with requirements of the Animal Welfare Act and the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals' and followed an Animal Use Protocol approved by the Animal Care and Use Committee of East Carolina University. The 3-month-old pigs, weighing approximately 12 kg, underwent tattooing procedures at the onset of the research project. Under general anaesthesia, each pig received four tattoo areas each consisting of four tattoo strips, 70 mm long (x-axis) by 10 mm wide (y-axis), spaced about 35 mm apart to facilitate later laser treatment and biopsy procedures. In addition, a circular spot of about 10 mm diameter and of the same colour was tattooed near one end of each tattoo strip. These circular spots were not treated by laser and were used as the baseline for evaluation of removal efficacy on the tattooed strips. The tattoo areas were placed just behind the shoulder region and just anterior to the hip roughly midway between the spinal column and sternum. A clinical nurse specialist experienced in the process performed all tattooing procedures using a medical tattoo unit. Four different pigments (Lasting Impression I, Inc.) were used in each tattoo area and all sites were monitored for 10 days postprocedure with no observed infection.

The absorption coefficients of the tattoo pigments were determined by measuring the collimated transmittance with a cw laser beam of power of 0.3 mW from a Nd:YAG laser at a wavelength of 1064 nm. The laser beam was modulated at 1 kHz and transmittance was measured by a photodiode and a lock-in amplifier. All pigments were dissolved in 50% alcohol at a concentration of 1% by weight and contained in a glass cuvette of path length 12.4 mm. A weak scattering component in the transmitted light was filtered out by two 2 mm apertures, separated by a distance of 100 mm, before the photodiode. Since the tattoo pigment particles are mostly metallic and thus have a mass density larger than that of the alcohol, care was taken to ensure the homogeneity of the solutions during the measurements. The results, shown in Table 1, were confirmed with absorbance measurements of the pigments in further diluted solutions by a factor of 8 using a UV-VIS spectrophotometer (8453E, Agilent Technologies). In comparison, the absorption coefficient of 50% alcohol at 1064 nm was measured to be only $7.38 \times 10^{-3}\,\mathrm{cm}^{-1}$. We found that variation in the absorption coefficient of the pigment solutions at 1064 nm was less than $\pm 6\%$, with the blue and red absorbing less than black and green. These findings were in marked contrast to the large difference in the visible region.

Approximately 40 days after tattooing and under general anaesthesia, the tattoo strips on each micropig were treated with 12 ns pulses at 1064 nm from a Q-switched Nd:YAG laser (Surelite I, Continuum). The pulse repetition rate was set at 10 Hz and the M^2 factor for the beam quality was measured as 1.30. The laser

Table 1. Properties of tattoo pigments used in the study

Pigment	Blue (navy liner)	Black	Red (red lip liner 3)	Green (green 2)
Chemical composition[a]	Iron oxide, ultramarine blue and violet, glycerin, isopropyl alcohol, and titanium dioxide	Iron oxide, glycerin, isopropyl alcohol, titanium dioxide	(Organic) iron oxide, D & C red no. 30, D & C red no. 7, glycerin, and isopropyl alcohol	Chromium hydroxide green, glycerin, isopropyl alcohol, and titanium dioxide
Absorption coefficient (cm^{-1})[b]	4.97	5.63	5.08	5.52

[a]Obtained from the pigment bottle labels.
[b]The absorption coefficients of pigments (0.1 g) dissolved in 50% alcohol (10 g) were determined through measurement of collimated transmission at 1064 nm.

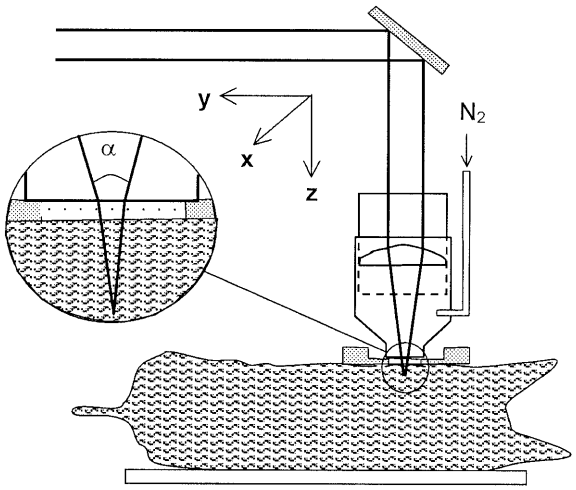

Fig. 1. Schematic side view of the experimental set-up with an intradermally focused laser beam and the micropig translated by a motorised table. The insert shows the focused beam penetrating through a saline film on the skin with a cone angle α exaggerated for better viewing.

beam was collimated and expanded to a diameter of 15 mm using a pair of spherical lenses before entering a specially designed focusing cup (Fig. 1). The focusing cup consisted of two parts with the outer part firmly adjacent to a template on the skin and the inner part containing a planoconvex lens of 75 mm focal length and 25 mm diameter to produce a converging beam with a full cone angle of $\alpha = 11.4^{\circ}$ in the air. The inner part carrying the focusing lens was adjustable along the laser beam axis (z-axis) with a 0.1 mm precision to vary the depth of the focal point below the pig skin surface. To reduce the beam defocusing due to the refractive index mismatch at the rough skin surface [12], we employed a 1 mm thick film of 0.9% saline solution. This film was formed within the template that was pressed against the skin with a rectangular opening of $60 \times 7\ mm^2$ area centred over a tattoo strip. Dry nitrogen gas was circulated continuously through the cup chamber during the treatment to reduce moisture condensation on the focusing lens and the lens was cleaned between each treatment procedure. The pulse energy cited in Tables 2–5 was determined after the focusing lens from the average power measured before and after each treatment using a power meter (30-15-A-P-HE, Ophire) with the fluctuation in average power less than 3%. A schematic side view of the set-up is shown in Fig. 1.

A clinical version with a beam-scanning device is yet to be designed for this system. Therefore, in the experimental system the animal was used as a moving target on a motor-driven translation table as opposed to scanning the laser beam. The pig was translated along the longitudinal direction of the tattoo strip (x-axis) at a constant speed to ensure that only one pulse was delivered per spot with a spot-to-spot distance of 100 μm over the 60 mm length of the treated tattoo strip. After a complete pass, the table was translated manually by 190 μm in the y-axis followed by a reversed x-axis translation. Treatment of one tattoo strip was completed with about 30 passes. Digital photos and punch biopsies of the tattooed skin for each test strip were obtained immediately prior to lasering and at 1 hour, 1 week, 2 weeks and 1 month after laser treatment for clinical and histological evaluation. The biopsies were fixed in 10% buffered formalin and processed by routine histological

Table 2. Response of blue tattoos

Tattoo ID	0C	0F	0K	0N	9C	9F	9K	9N
Pulse energy (mJ)	63	63	63	49	56	55	55	55
Focal depth (mm)	5.9	5.9	4.7	4.7	4.7	7.1	2.4	2.4
Petechial haemorrhage[a]	Mod.	Mod.	Mod.	Mod.	Min.	Min.	Mod.	Min.
Tattoo removal[a]	Good	Good	Exc.	Good	Good	Exc.	Good	Good
Hypopigmentation[a]	Min.	Min.	Min.	Min.	Mod.	Mod.	Mod.	Mod.
Index of epidermis necrosis – 1 hour[b]	0	0	0	0	0	0	0	0
Sum index of vacuolation – 1 hour[b]	11	0	4	18	13	9	20	10
Sum index of aggregated pigment – before[b]	34	12	17	12	34	12	17	12
Sum index of aggregated pigment – 1 hour[b]	15	0	5	16	20	9	23	12
Sum index of aggregated pigment – 1 week[b]	0	0	0	0	4	0	0	0
Sum index of aggregated pigment – 1 month[b]	0	4	20	20	15	21	0	15

[a]Descriptions of the scales are given in the Results section.
[b]Measured from biopsy slides immediately before, 1 h after, 1 week after and 1 month after laser treatment.

Table 3. Response of black tattoos

Tattoo ID	0A	0H	0I	0P	9A	9H	9I	9P
Pulse energy (mJ)	62	63	63	49	55	55	55	38
Focal depth (mm)	5.9	5.9	5.9	4.7	3.6	5.9	2.4	1.3
Petechial haemorrhage[a]	Min.	Mod.	Min.	Mod.	Mod.	Mod.	Min.	Mod.
Tattoo removal[a]	Fair	Good	Mod.	Mod.	Good	Good	Mod.	Mod.
Hypopigmentation[a]	Min.	Min.	Min.	Min.	Min.	Mild	Min.	Min.
Index of epidermis necrosis – 1 hour[b]	4	0	3	1	2	0	0	1
Sum index of vacuolation – 1 hour[b]	34	26	41	20	0	30	18	16
Sum index of aggregated pigment – before[b]	42	21	15	16	42	21	15	16
Sum index of aggregated pigment – 1 hour[b]	23	10	14	11	0	21	12	16
Sum index of aggregated pigment – 1 week[b]	36	22	2	9	0	0	16	15
Sum index of aggregated pigment – 1 month[b]	20	3	15	0	18	16	29	21

[a]Descriptions of the scales are given in the Results section.
[b]Measured from biopsy slides immediately before, 1 h after, 1 week after and 1 month after laser treatment.

procedures to obtain stained slides. Additional gross examinations were made and photos taken three months after the laser treatment immediately before the animals were euthanised to evaluate the late phase condition of the treated skin areas. The system of evaluation consisted of skin appearance assessment and histological analysis. Visual appearance assessment was carried out in a blinded fashion by a plastic surgeon experienced in the clinical treatment of tattoos using photographs of tattoo test areas and was subsequently confirmed by another evaluator. Histological analysis was conducted in two ways: the first in analysing the actual tissue damage and the second in quantifying the pigment mobilisation through depth dependence.

RESULTS

Because of the relatively small cone angle of 11.4° used in our study, laser-induced breakdown in the saline on the skin surface in which shockwaves were clearly audible occurred frequently for treatments with focal depths less than 3 mm. Consequently, the actual pulse energies delivered inside the skin were expected to be smaller than those measured before the saline film and cited in Tables 2–5 because of the shielding effect of laser-induced plasma in the saline. Previous results [12] indicated that for a 6ns pulse at 1064 nm with a cone angle of 22° the pulse transmission through a plasma induced in water decreased to about 50% at the breakdown threshold from nearly 100% below the threshold. The

Table 4. Response of green tattoos

Tattoo ID	0D	0E	0L	0M[c]	9D	9E	9L	9M
Pulse energy (mJ)	63	64	64	49	55	55	55	55
Focal depth (mm)	5.9	5.9	4.7	4.7	4.7	5.9	2.4	2.4
Petechial haemorrhage[a]	Mod.	Mod.	Mod.	Mod.	None	Min.	Mod.	Mod.
Tattoo removal[a]	Fair	Poor	Poor	Fair	Fair	Fair	Fair	Mod.
Hypopigmentation[a]	Min.	Min.	Min.	Min.	Mild	Min.	Min.	Mild
Index of epidermis necrosis – 1 hour[b]	0	0	0	0	0	0	0	1
Sum index of vacuolation – 1 hour[b]	0	0	0	0	0	0	0	0
Sum index of aggregated pigment – before[b]	31	18	14	11	31	18	14	11
Sum index of aggregated pigment – 1 hour[b]	13	0	15	5	15	0	24	17
Sum index of aggregated pigment – 1 week[b]	27	21	24	3	5	16	10	17
Sum index of aggregated pigment – 1 month[b]	8	27	10	7	22	15	36	15

[a]Descriptions of the scales are given in the Results section.
[b]Measured from biopsy slides immediately before, 1 hour after, 1 week after and 1 month after laser treatment.
[c]Laser ablation without the saline film on skin surface.

Table 5. Response of red tattoos

Tattoo ID	0B	0G	0J	0O	9B	9G	9J	9O
Pulse energy (mJ)	62	63	62	49	55	55	55	52
Focal depth (mm)	5.9	5.9	5.9	4.7	3.6	7.1	2.4	2.4
Petechial haemorrhage[a]	None	Min.	Min.	Min.	None	None	Min.	None
Tattoo removal[a]	Poor	Fair	Poor	Fair	Good	Fair	Mod.	Fair
Hypopigmentation[a]	Min.	Min.	Min.	Min.	Mild	Mod.	Mild	Mild
Index of epidermis necrosis – 1 hour[b]	0	0	0	0	0	0	0	0
Sum index of vacuolation – 1 hour[b]	0	0	0	0	0	0	0	0
Sum index of aggregated pigment – before[b]	0	0	0	0	0	0	0	0
Sum index of aggregated pigment – 1 hour[b]	0	0	0	0	0	0	0	0
Sum index of aggregated pigment – 1 week[b]	0	0	0	0	0	0	0	0
Sum index of aggregated pigment – 1 month[b]	0	0	0	0	0	0	0	0

[a]The descriptions of the scales are given in the Results section.
[b]Measured from biopsy slides immediately before, 1 hour after, 1 week after and 1 month after laser treatment.

increased loss of pulse energy due to the saline breakdown prompted us to use focal depths larger than 3 mm and we estimated that the actual pulse energy could be less than half of the values cited in Tables 2–5 for treatments with focal depth of 2.4 mm and 1.3 mm.

The saline film covering the skin during ablation procedures served the dual purpose of reducing the index mismatch at the skin surface and tissue cooling. The laser treatment lasted about 30 min for each tattoo strip. Regardless of tattoo colour, treated areas of the skin appeared white after removal of the template because of the laser-induced vacuolation under the surface. Although no significant immediate haemorrhage was observed in any of the test areas, varying degrees of delayed foci of petechial haemorrhage with little visible epidermal damage were observed in most tattoo strips about 30 min post-treatment. Based on gross examination and review of photographs of the skin surface, we graded the degree of petechiation per treated area of 3.4 cm^2 in each tattoo strip within 1 hour of laser treatment using a standardised scale based on the number of foci: (1) none, (2) minimal, <10, (3) moderate, 11–25, (4) significant, >26 foci. Grading was performed within one hour of laser treatment based on the combined evaluations of two investigators (WAW and XHH) and results, grouped by the tattoo colours, are listed in Tables 2–5. Comparing the appearance of the four tattoo groups at similar ranges of pulse energy and focal depth, the blue and black pigments showed the most intense petechiation or bruising, green less

and red the least. These observations agree well with the acute histological responses of the skin tissues showing epidermal necrosis and laser-induced vacuolation in biopsies taken 1 hour after the laser treatment and are consistent both with previous results [3,5] and our own clinical experience in tattoo removal using commercial Q-switched laser systems. Surprisingly, the close correlation of the acute responses with the pigment colour is in strong contrast with the fact that the absorption coefficients of the tattoo pigments dispersed in solutions are nearly the same at 1064 nm (see Table 1), suggesting that effects of presently undefined factors other than pigment absorption alone are involved in ns laser pulse removal of tattoos.

The clinical appearance of each tattoo strip for tattoo ablation was evaluated by comparing the photos of untreated baseline pigment spots to each treated tattoo strip 1 month post-treatment using a subjective scale of: (1) poor, <20%, (2) fair, 20–40%, (3) moderate, 40–60%, (4) good, 60–80%, (5) excellent, >80% clearance of pigmentation compared to baseline immediate post-laser control. Grading scores were assigned based on review and consensus of two investigators (WAW and XHH) and results, grouped by the tattoo colours, are listed in Tables 2–5. Visual evaluation of clinical tattoo ablation efficacy as assessed through these comparisons demonstrated a removal grading for blue pigment tattoos ranging from good to excellent. The same grading for black test sites ranged from fair to good whereas the degree of removal for red and green pigments was judged less than for either blue or black, with green grading slightly better than red.

A final index used in evaluating the gross visual appearance of test sites was the degree of observable hypopigmentation. An exit clinical examination of the micropigs was conducted 3 months post-treatment and late phase photos were taken to complete the study. Detailed evaluation of these photographs revealed no evidence of hypertrophic scars in any of the treated fields at this late time point regardless of the acute response to treatment. Similarly, we found no incidences of hyperpigmention in any treated area although the potential for this in the pig model used in this study has not been defined. Hypopigmentation was noted at this time point and was graded as: (1) minimal, >30%, (2) mild, 30–60%, (3) moderate, >60% compared to control. Results of hypopigmentation scoring were again achieved by consensus of two of the investigators, and are also presented in Tables 2–5. Treated fields in one pig with freckling were observed to develop the common freckling seen with maturation of the pigs 3 months after the laser treatment. The pigmentation seemed to be in a natural and organised form and crossed over into the zones of treatment in a grossly normal appearance, indicating the continued presence of some normal, physiologically functioning melanocytes within the basal layer of the epidermis. From these observations we concluded that the treatment of pigmented lesions with a focused beam of 12 ns pulse with energy in the region of 50 mJ may cause a moderate amount of petechiation and/or bruising in the acute response of the skin but is within the relative safe limits for protecting the basal layer near the epidermis–dermis junction as indicated by 66% of reviewed samples being rated as minimal, i.e. less than 30% hypopigmentation, for this index.

Biopsies taken immediately before laser treatment, 1 hour, 1 week and 1 month after treatment have been quantitatively analysed for histological parameters by two investigators (MJC and GWK) in a double-blind study. To evaluate the tissue response and tattoo pigment redistribution according to the depth in the dermis or the distance from the epidermis–dermis junction, an ocular grid with 20×20 squares was used in an optical microscope to divide each biopsy slide into an epidermal layer and seven dermal layers from the epidermis–dermis junction at $40\times$ magnification. The squares of the ocular grid in each layer correspond to 0.12 mm squares on the biopsy. Highly localised laser-induced necrosis in the epidermis and vacuolation in the dermis were observed in biopsies taken at 1 hour after laser treatment. A necrosis index was defined to measure the acute damage in the four sublayers of epidermis (stratum corneum, stratum granulosum, stratum spinosum and stratum basale), which is equal to the number of damaged sublayers ranging from 0 to 4. Using the ocular grid, we further defined an index of vacuolation based on the number of squares containing vacuolations for each dermis layer consisting of 20 squares. Both epidermal necrosis and dermal vacuolation were found to disappear in biopsies taken 1 week or later after the laser treatment. Granules of aggregated tattoo pigments of sizes on the order of 10 μm were observed to exist in the upper dermis of

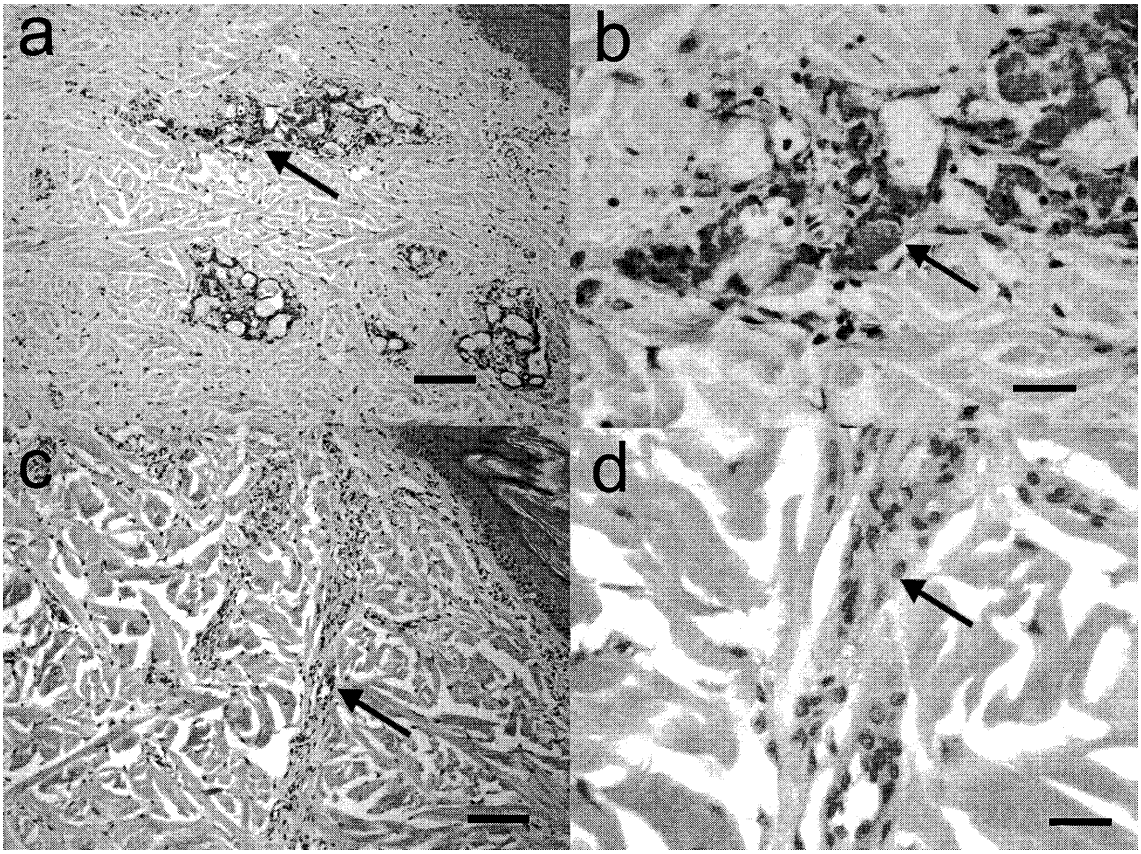

Fig. 2. Histological micrograph images of biopsies taken 1 hour after laser treatment with the arrow indicating the tattoo pigments. (a) The blue tattoo (ID: 0C) (100×), bar=100 μm; (b) the same slide as (a) (400×), bar=25 μm; (c) the red tattoo (ID: 9B) (100×); bar=100 μm; (d) the same slide as (c) (400×), bar=25 μm.

biopsies with blue, black and green tattoos. To quantify the distribution of the aggregated pigments in the dermis, we defined a pigment index as the number of squares containing aggregated pigments for each layer covering the dermis at the 40 × magnification. The vacuolation and pigment indices of the seven grid layers within the dermis were then added to yield a sum index for each biopsy specimen. We list the necrosis index for the epidermis and sum indices of vacuolation and aggregated pigments for the dermis from biopsies obtained at different times relative to the laser treatment in Tables 2–5. It should be noted that a pigment index of 0 does not exclude the existence of tattoo pigments. For biopsies with red tattoo, the pigment was widely dispersed with sizes less than 5 μm and could only be identified under higher magnification (400 ×), resulting in these specimens receiving a value of 0 for pigment index at the 40 × parameter. Figure 2 presents two micrographs of blue and red tattoo biopsies taken 1 hour after laser treatment to show the different forms of tattoo pigment aggregation and vacuolations at magnifications of 100 × and 400 × . Under the 400 × magnification, we have observed the coexistence of macrophages showing evidence of actively phagocytosing the laser-dispersed tattoo pigment. There is no indication that these macrophages migrate any appreciable distance from the site of laser activity.

Significant correlation was observed from the depth dependence of the pigment distribution and the vacuolation in the dermis in all biopsies. Two representative groups of blue, black and green tattoo biopsies are shown in Fig. 3 with pulse energy of 63 mJ and focal depth of 5.9 mm and in Fig. 4 with 55 mJ and 2.4 mm as examples. In biopsies of red and green tattooed tissues no laser-induced vacuolation was seen, indicating that the laser-induced breakdown either did not occur or was much weaker than that in blue and black. This is consistent with the different degrees of the postprocedural petechial haemorrhage, caused by either breakdown of or thermal damage to the blood vessels, noted in the independent clinical examination of the skin surface. Comparing the pigment index of

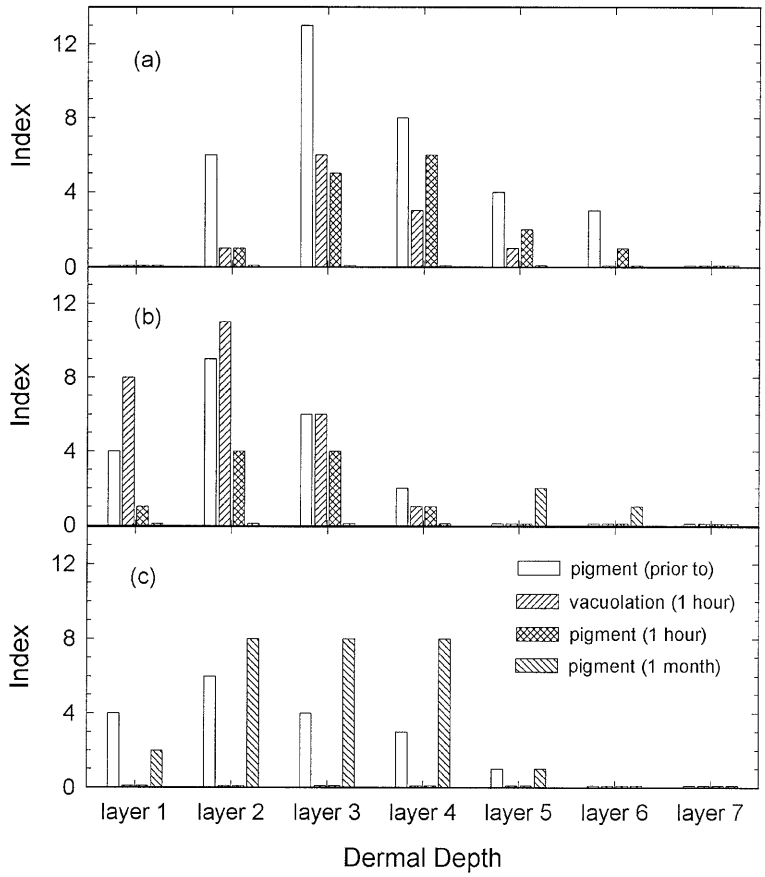

Fig. 3. Depth dependence of the aggregated pigment index from four biopsies immediately before, 1 hour and 1 month after the laser treatment and the vacuolation index 1 hour after the laser treatment with laser pulse energy of 63 mJ and focal depth of 5.9 mm: (a) blue tattoo (ID: 0C); (b) black tattoo (ID: 0H); (c) green tattoo (ID: 0E).

biopsies taken before, 1 hour and 1 month after the laser treatment, we found two changes that may be responsible for the tattoo removal, i.e. the overall decrease in pigment density in each layer and the pigment mobilisation into deeper layers. It was noted that for blue tattoos the pigment reduction at 1 month after treatment was evident in nearly all layers whereas for black, the corresponding pigment reduction occurred most prevalently in those layers having relatively large vacuolation indices. We further noted that black tattoos exhibited a trend of tattoo reduction in the shallow layers and an increase in the deeper layers as shown in Fig. 5.

DISCUSSION

In the current approach of treating pigmented lesions by ns pulses, large pulse energies up to and even exceeding 1 J must be used which frequently results in considerable collateral tissue damage [6]. We proved in this initial in vivo study that the pulse energy can be significantly reduced to between 40 and 60 mJ at 1064 nm wavelength through intradermal focusing. The team hopes to pursue a series of subsequent experiments with the ultimate aim being the development of a compact laser system possessing beam scanning capability for treating different coloured pigments using a single wavelength and shortened treatment times as an alternative to the existing methods. We would like to point out that the question as to how this type of Q-switched intradermally focused laser system could be applied in an efficient clinical environment is yet to be answered. Likewise, a number of questions arising from the optical properties of the dermis and laser interactions with pigments within the dermis will also need to be examined further. Also to be refined, is the potential of developing a clinically applicable scanning system, which would allow the comparison in clinical

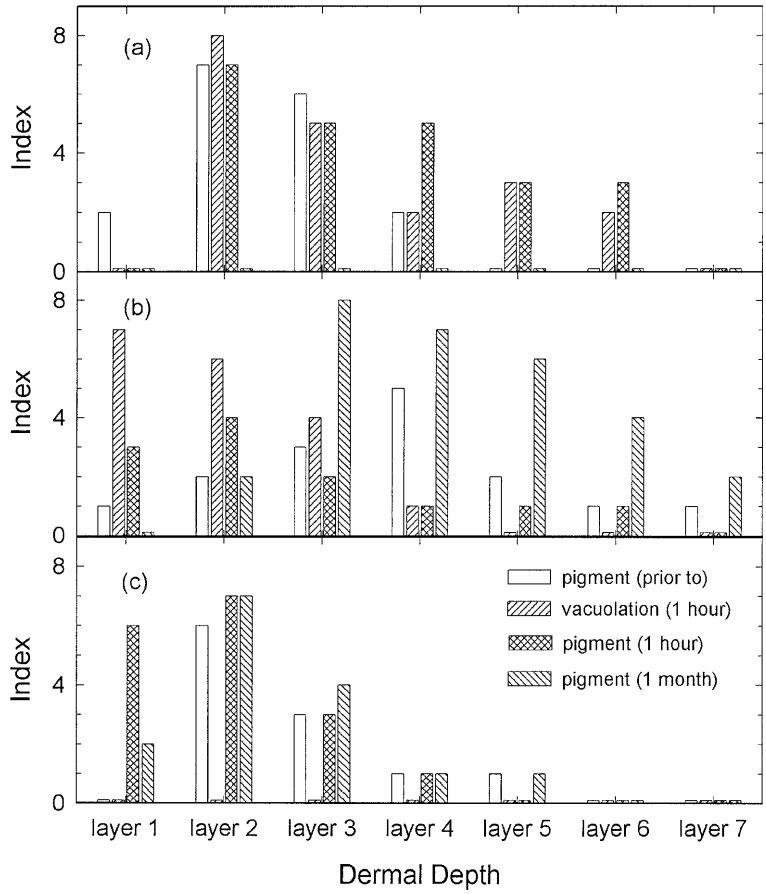

Fig. 4. Depth dependence of the aggregated pigment index from biopsies immediately before, 1 hour and 1 month after the laser treatment and the vacuolation index 1 hour after the laser treatment with laser pulse energy of 55 mJ and focal depth of 2.4 mm: (a) blue tattoo (ID: 9K); (b) black tattoo (ID: 9I); (c) green tattoo (biopsy ID: 9M).

applications to standard Q-switched laser systems commercially available in dermatological clinics.

Because of the strong light scattering described in pig skin [10], we expected that the distribution of pulse energy inside porcine skin would become much wider than that of the direct beam in the cases of no scattering [13]. Consequently, pulses with energies in the region of 50 mJ were used in this study to achieve a visible acute response that was much larger than the threshold estimated at a few millijoules from our previous study on the surface ablation in isolated pig skin with similar beam configurations [9]. Histological analyses of biopsies taken 1 hour after laser treatment have shown that the epidermis in all biopsies remained essentially intact, except for highly localised damage in one strip with black tattoos. This can be attributed to the combination of small pulse energy and the saline film used for index matching and tissue cooling. Due to the high density of ablated spots per tattoo strip, large laser fluences, between 200 J/cm^2 and 340 J/cm^2 are accumulated during the half-hour treatment. These results show that collateral tissue damage is primarily determined by the pulse energy if the thermal relaxation of absorbed energy is allowed. This is consistent with previous results on ablating ocular tissues by nanosecond pulses [14].

Comparing the results of acute responses to laser treatment from gross observation and biopsy histology on a total of 32 treated tattoos (see Tables 2–5), we observed a strong relation between the degree of tattoo removal and the extent of long-lasting vacuolation observable in biopsies at 1 hour after laser treatment for tattoo removal. Previous studies have shown that the long-lasting vacuolation in soft biological tissues is a result of shock waves and acoustic transients by the laser-induced plasma [14,15]. The observations thus suggest that pigment breakdown and tattoo removal can be efficiently achieved through intradermal focusing with reduced pulse energy. From the layer dependence of the aggregated

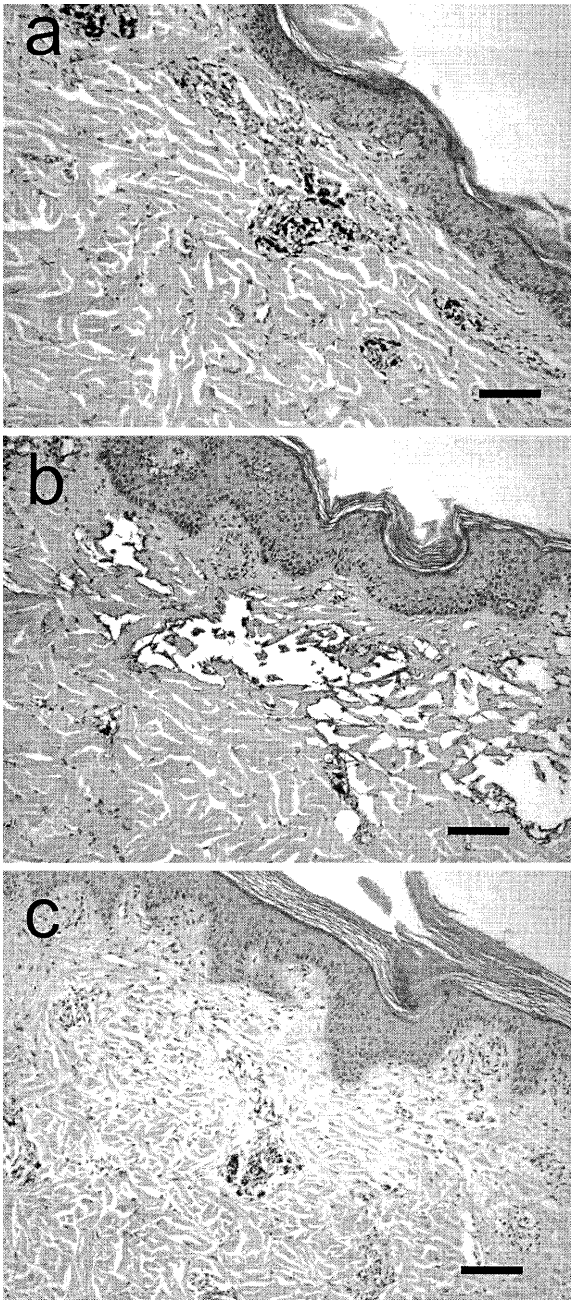

Fig. 5. Distribution of aggregated black pigments (ID: 9H) in biopsies taken (a) before, (b) 1 hour after, and (c) 1 month after the laser treatment (100×), bar=100 μm.

pigment indices from biopsies immediately prior to and 1 month after laser treatment (Figs 3–5) we also noticed that in the blue and black tattooed skin, treatment had either reduced the amount of pigment granules in all grid layers and/or redistributed the pigment granules deeper into the dermis. This provides direct microscopic evidences that tattoo removal by short laser pulses is, to a certain degree, due to pigment mobilisation. Since the pigment redistribution could also be accomplished through the thermal damage and corresponding recovery process in skin, it may be responsible for tattoo removal in some cases of green and red tattoos.

Two models have been proposed to explain the soft tissue ablation by ns laser pulses in visible and near-infrared spectral regions: (1) the selective-photothermolysis model for skin and pigment ablation [1]: and (2) the plasma ablation model for ocular tissue ablation in which tissue absorption is negligible [16,17]. The fundamental difference between the two models lies in whether the ablation process is caused by a localised plasma induced by the strong electromagnetic field of ns pulses. Although the tissue absorption dominates the laser–tissue interaction described by the photothermolysis model, its role in the existing plasma ablation model remains unknown [16,17]. In a previous study we reported experimental results on the surface ablation of porcine skin using ns pulses at 1064, 532, 266 and 213 nm, in which the optical absorption coefficient of the skin increases by two orders of magnitude as the laser wavelength decreases, and we found that the ablation data can only be satisfactorily explained by a plasma-mediated ablation model [9]. The theoretical modelling of the above experimental results, to be published elsewhere, suggested to us that the initial seed electrons are generated in tissue chromophores through a thermal ionisation pathway and energised by the strong electric field of the ns pulse to initiate subsequent avalanche ionisation that leads to the formation of plasma. Therefore, the ablation thresholds in these cases of substantial light absorption are determined mainly by the generation of initial seed electrons and thus the local temperature reached in tissue chromophores. Since the local temperature depends on both the chromophore absorption cross-section and the packing configuration of the chromophores, we expect that the ablation thresholds of different tattoo pigments depend on the form of pigment aggregates in addition to the pigment absorption. Based on the above discussion it is plausible to attribute the least response of the red tattoo pigments to the ns laser pulses to the very dispersed distribution of the red pigments which facilitate the diffusion of the localised thermal energy in these pigments and consequently increase the corresponding ablation threshold. It seems clear that better comprehension of these complex processes will require further quantitative

studies to achieve a clear understanding of the fundamental mechanism underlying the treatment of pigmented lesions by ns pulses.

In summary, we have carried out an initial in vivo study of tattoo removal in micropigs by 12 ns pulses in a converging beam of 11.4° cone angle at 1064 nm wavelength. We achieved significant tattoo pigment mobilisation for the blue and black pigments with a nominal range of pulse energy from 38 to 63 mJ and found a strong relation between the degree of tattoo removal and laser-induced vacuolation in the dermis. Furthermore, we established that the variation between optical absorption of tattoo pigment was too small to be responsible for the large difference in the responses between the blue/black and green/red tattoos. These results are very encouraging for possible development of a new generation of compact Q-switched lasers for dermatological and plastic surgery with high efficiency diode-laser pumping and automated scanning delivery system. We are currently preparing to study the use of a converging beam with a larger cone angle up to 30° which is expected to further reduce the pulse energy, collateral tissue damage and, more importantly, to achieve better responses from the green and red tattoos by exceeding their respective breakdown thresholds without causing breakdown in the saline film at smaller focal depths.

ACKNOWLEDGEMENTS

The authors thank Kenneth Jacobs for constructing the translation table and the focusing cup for this project and Pat Vore, RN, CPRSN for providing tattooing expertise.

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Paper received 1 November 2001;
accepted after revision 14 December 2001