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Clinical, Histologic, and Ultrastructural Evaluation of Tattoos Treated With Three Laser Systems

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We examined the response of tattoo pigments treated with three commercially available lasers: Q-switched ruby, Q-Switched neodynium:yttrium,aluminum,garnet (Nd:YAG), and the alexandrite. Tattoos applied to hairless guinea pigs and treated with the aforementioned lasers were evaluated clinically, histologically, and ultrastructurally. Clinical evaluation showed red brown, dark brown, and orange pigment responded best to the Nd:YAG laser (1064 nm). The alexandrite laser was most effective for removing blue and green pigment, the Q-switched ruby laser was most effective for removing purple and violet pigment, and the Nd:YAG laser (532 nm) removed red pigment the best. Black pigment was lightened equally with the Nd:YAG laser (1064 nm) and (532 nm) and the alexandrite laser (755 nm). No clinical scarring was observed; however, some colors turned black after treatment. Histologic and ultrastructural examination showed epidermal and dermal damage to be most evident after treatment with the Nd:YAG laser. Our study shows that certain tattoo pigments respond better to different laser systems. © 1994 Wiley-Liss, Inc.

Key words: Alexandrite, electron microscopy, Q-switiched ND:YAG, Q-switched ruby

INTRODUCTION

Lasers are capable of interacting with specific structures within the skin. "Selective photothermolysis" is a term used to describe this targeted thermally mediated injury [1]. By altering the wavelength and pulse duration, laser energy can be engineered to interact with a variety of unique structures. Current lasers are capable of destroying small cutaneous blood vessels and pigment with little to no damage to the surrounding tissue.

Several lasers are now being used successfully to treat exogenous pigment within the skin, such as tattoos [2–6]. Currently, three lasers are used selectively to treat tattoos. These are the Q-switched ruby (QSR), the Q-switched neodynium:yttrium,aluminum,garnet (Nd:YAG), and the alexandrite lasers. Clinical experience and previous studies have shown that amateur tattoos respond better than professional tattoos to laser treatment, and different lasers are better at removing different colored pigments [4].

The reason for these findings, as well as the mechanism responsible for lightening the tattoos, are not known. In this study we evaluated the treatment response of different colored pigments to three commercially available laser systems. To

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date, this is the first report comparing the results of all three laser systems in the treatment of different tattoo pigments.

MATERIALS AND METHODS

Hairless guinea pigs were anesthetized with intramuscular injection of ketamine, xylazine, and atropine. The back of each animal was tattooed with seven 1.0 cm × 4.5 cm bands. Pigments were obtained from Spaulding and Rogers (Voorheesville, NY). We selected all of our pigments from this supplier based upon a telephone survey of tattooists located in Minnesota. Of the nine tattooists questioned, all stated that they buy most of their inks from Spaulding and Rogers. Many also mentioned that they add their own "secret" ingredients to the pigments.

Six weeks after tattooing, the animals were anesthetized and treated with the lasers listed in Table 1. Fluence and spot size are major factors in treatment response. Instead of trying to determine the optimum parameters for laser tattoo removal, our attempt was to replicate our current clinical setting as closely as possible. The spot sizes and fluences chosen were those currently being used in our clinical practice. Fluence was determined by the clinical appearance of the treated skin immediately after treatment. Due to the differences in thickness and pigmentation of guinea pig and human skin, the fluences were determined by treating the tattoos at starting at 2 J/cm^2 and increasing at ¼–½ J/cm^2 increments until the desired clinical response was obtained. After treatment with the QSR laser, the treated area had a gray-white discoloration with rare scattered pinpoint bleeding. The treatment site after Nd:YAG (1,064 nm) laser treatment showed uniform pinpoint bleeding, whereas after treatment with the Nd:YAG (532 nm) there was gray-white discoloration with scattered sites of purpura. After treatment with the alexandrite laser, the skin had a gray-white discoloration with minimal to no bleeding or purpura.

TABLE 1. Laser Parameters*

Lasers used:	*QSR*	*Nd:YAG (1064)*	*Nd:YAG (532)*	*A*
Wavelength (nm):	694.3	1,064	532	755
Pulse duration:	20–40 ns	10–12 ns	10–12 ns	100 ns
Spot size (mm):	6.5	2	2	3
Fluence (J/cm2):	4.5	7	4	4

*QSR = Q-switched ruby; Nd:YAG = Q-switched neodynium: yttrium, aluminum, garnet; A = alexandrite.

Clinical Evaluation

Six weeks after treatment, the tattoos were photographed using the same film and lighting as the pretreatment photographs. Projections of 35 mm slides taken of the tattoos prior to and 6 weeks after treatment were scored by three independent blinded observers (see Acknowledgments). Each observer was unaware of the study protocol and evaluated the tattoos on a clinical scale of 0–4, where 0 = no change, 1 = 1–25% lightening, 2 = 26–50% lightening, 3 = 51–75% lightening, and 4 = 76–99% lightening. The sites were also evaluated with a Minolta (Osaka, Japan) chroma meter with readings taken prior to and 6 weeks after treatment. A detailed description of this instrument is published elsewhere [7]. A color is expressed using the CIE 1976 L a b color scale in a three-dimensional coordinate system with an a-axis (green to red, where red is positive), a b-axis (yellow to blue, where yellow is positive), and an L-axis, (brightness, where the brighter colors are numerically larger). For the purpose of this study we examined color change for red and green tattoo pigment using readings from the a-axis, for yellow and blue tattoo pigment using readings from the b-axis, and for white and black tattoo pigment using readings from the L-axis. The total color difference for all colors was also determined by calculating delta E using the following equation: $\Delta E_{ab} = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$.

Histologic and Ultrastructural Evaluation

Immediately prior to and after laser treatment, 4 mm punch biopsies were taken and again 6 weeks after treatment. The biopsies were bisected and half was fixed in 10% buffered formalin and embedded in paraffin. These samples were the cut and stained with hematoxalin and eosin and evaluated by a blinded observer. The other half of the pigments listed in Table 3 were fixed in 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and postfixed with 2% osmium tetroxide in the same buffer. The tissue was dehydrated in graded ethanol solutions and embedded in Spurr. Ultrathin sections were then cut with an LKB microtome, picked up on copper grids, and stained with uranyl acetate and lead citrate. The tissue was then examined in a blind fashion with a RCA EMU 4 electron microscope.

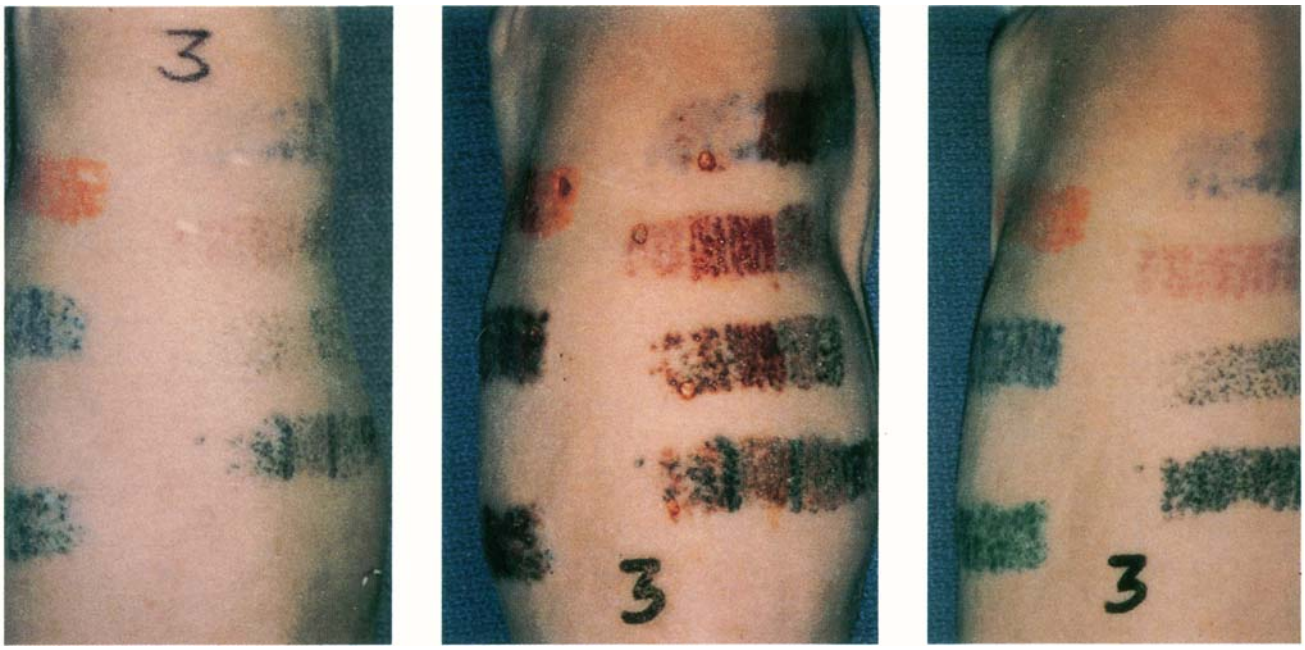

Fig. 1. Hairless guinea pigs tattooed with seven different pigments. Far left photo shows tattoos just prior to laser treatment. Middle photo shows results just after laser treatment. The lasers used on each pigment stripe are (from left to right) the alexandrite, the Q-Switched Nd:YAG (532 nm), the Q-Switched Nd:YAG (1064 nm), and the Q-switched ruby. Far right photo shows the tattoos 6 weeks after treatment.

RESULTS

Clinical Evaluation

The clinical appearance of the guinea pigs prior to, just after, and 6 weeks after laser treatment can be seen in Figure 1. Clinical evaluation 6 weeks after treatment are seen in Figure 2 a–c. A clinical score of 3 or 4 after treatment was seen in eight pigments with the Nd:YAG (1,064 nm), seven pigments with the QSR laser, and five pigments with both the Nd:YAG (532 nm) and alexandrite lasers. Clinical scoring showed red brown, dark brown, and orange pigment responded best to the Nd:YAG laser (1,064 nm). The alexandrite laser was most effective for removing blue and green pigment, the Q-switched ruby laser was most effective for removing purple and violet pigment, and the Nd:YAG laser (532 nm) removed red pigment the best. Black pigment was lightened equally with the Nd:YAG laser (1,064 nm) and (532 nm) and the alexandrite laser. Table 2 lists pigments that turned black after laser treatment. Iron oxide is known to be an ingredient in most of these pigments.

The treatment response was also evaluated with a Minolta chroma meter. The total color change from pre- to posttreatment as determined by the delta E readings is seen in Figure 3 a–c. The specific color change for white, black, red, green, yellow, and blue are seen in Figure 4 a–c. Quantitation using the L scale showed white to be lightened the most by the QSR and Nd:YAG (532 nm) lasers and then by the Nd:YAG laser (1,064 nm) and alexandrite lasers. Readings from the *a* scale show that red pigment is removed to a greater extent by the Nd:YAG (532 nm) laser and green by the alexandrite laser. The *b* scale readings showed yellow to be removed best with the Nd:YAG laser (1,064 nm) and blue with the alexandrite laser.

Histology

All specimens were evaluated with particular attention to the tattoo pigment location and tissue alteration immediately posttreatment and 6 weeks later. The histologic evaluation of pretreatment tattoo biopsies showed that the majority of tattoo granules were found in the papillary and superficial dermis. Almost all colors evaluated were found within macrophages and fibroblasts. Only a minority of tattoo granules were located interstitially as small grains or clusters.

All immediate posttreatment biopsies

TABLE 2. Posttreatment Discoloration*

	BLACK DISCOLORATION			
Color	QSR	Nd:YAG (1064nm)	Nd:YAG (532 nm)	A
White	0	+	0	+
Flesh	+	+	0	+
Red	0	0	0	0
Orange	0	0	0	0
Yellow	0	+	0	+
Green	+	+	0	+
Blue	0	0	0	0
Purple	0	0	+	0
Violet	0	0	+	0
Red brown	+	+	+	+
Brown	+	+	+	+
Dark brown	+	+	+	+
Gray	0	0	0	0

*QSR = Q-switched ruby; Nd:YAG = Q-switched neodynium: yttrium, aluminum, garnet; A = alexandrite; + = Black discoloration noted clinically 6 weeks after laser treatment; 0 = no black discoloration noted clinically 6 weeks after laser treatment.

showed some loss of integrity of the pigment laden cells with tattoo granules finely dispersed in the interstitium of the superficial and middermis. The immediate-posttreatment tattoo granules were finer and less clumped with minimal loss in pigment volume compared to the pretreatment biopsy for each color evaluated. All lasers produced a similar amount of free dermal pigment. The amount of cellular damage did vary from color to color. Red tattoo pigment in particular was completely disrupted from dermal laden cells with all lasers.

The total histologic pigment loss 6 weeks after treatment, compared to original biopsy, did not correlate with clinical or chroma meter readings. There was some variation between colors and the different lasers used. Black pigment responded best to the alexandrite laser, whereas blue and orange were cleared better by the Nd: YAG (1,064).

Evaluation of the epidermal changes (spongiosis, necrosis, sloughing) immediately posttreatment showed the Nd:YAG (1,064) and Nd: YAG (532) lasers to produce more epidermal response than QSR and alexandrite lasers. The amount of dermal damage (homogenization, hemorrhage, and blood vessel changes) was greater with the Nd:YAG (532), followed by the Nd:YAG (1,064), then alexandrite and QSR lasers. The biopsies after 6 weeks showed some loss of rete ridges and mild fibrosis of the papillary and superficial reticular dermis. However, these findings were more prominent with the Nd:YAG (1,064) and Nd:YAG (532) lasers and did not seem to vary with the color of tattoo pigment.

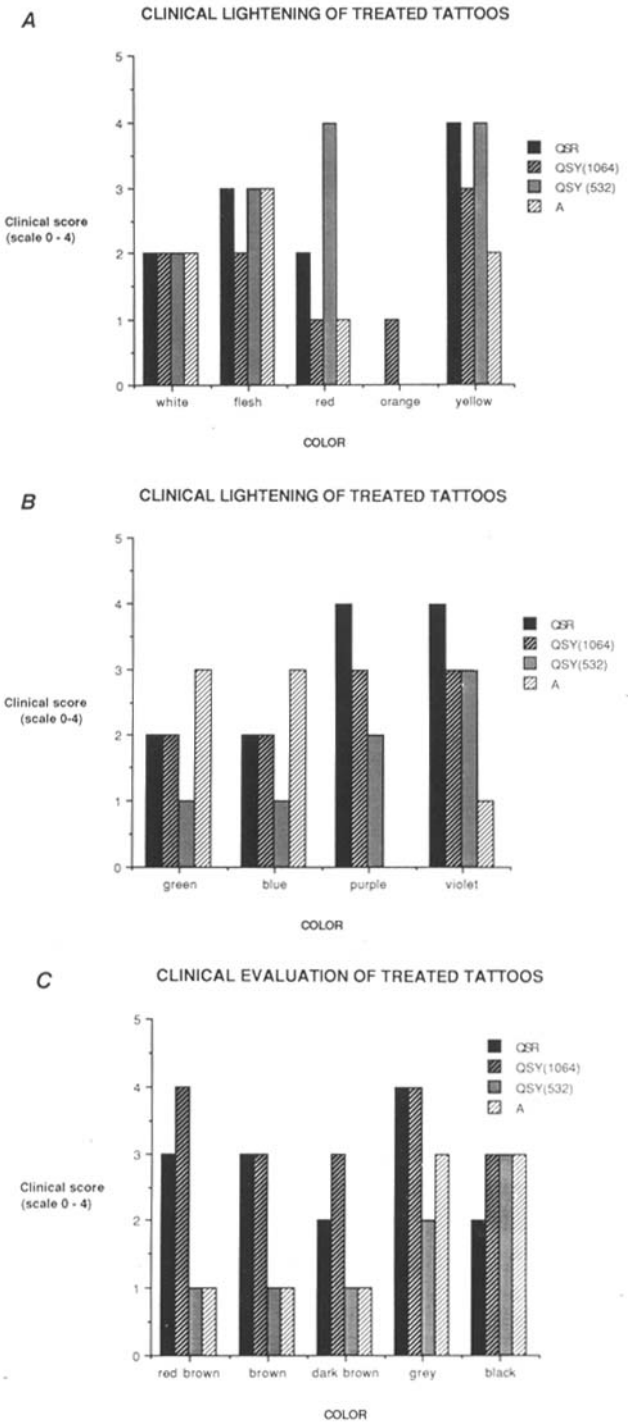

Fig. 2. Clinical evaluation of tattoo lightening 6 weeks after treatment. Lightening was assessed on a clinical scale of 1–4, where 1 = 0–25% lightening, 2 = 26–50% lightening, 3 = 51–75% lightening, and 4 = 76–99% lighting.

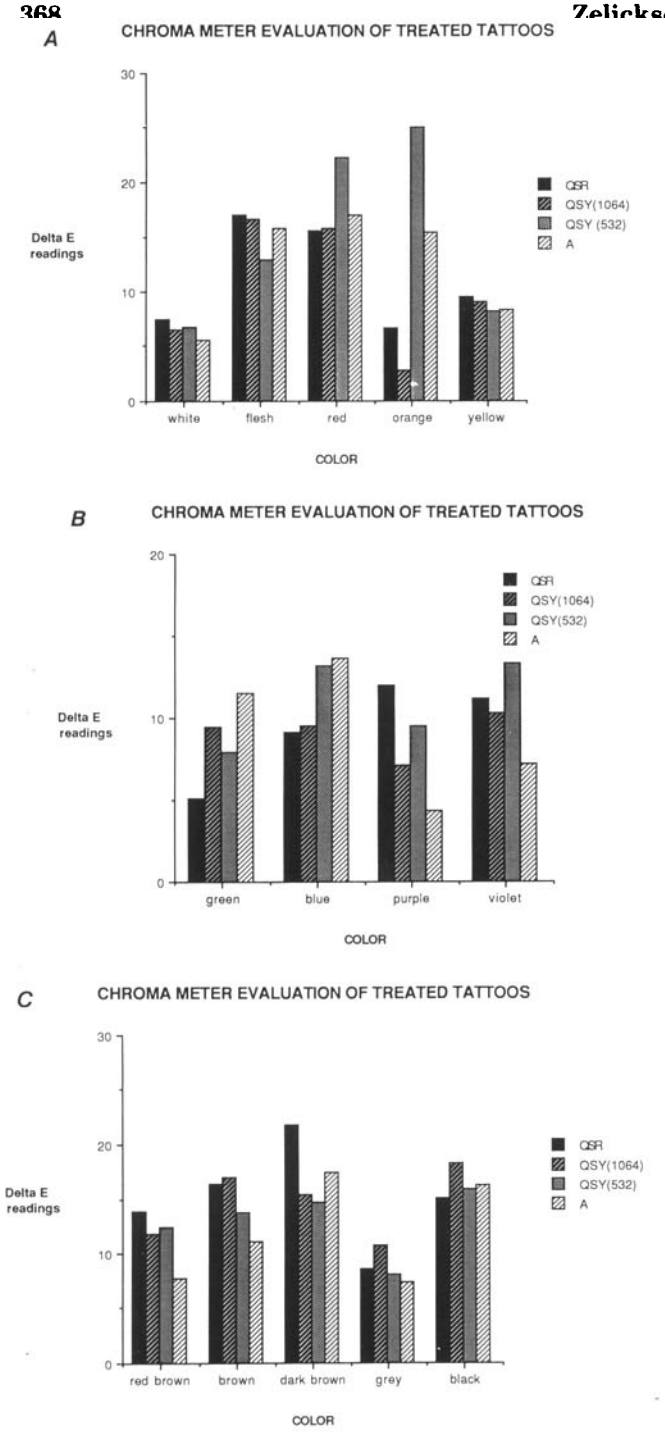

Fig. 3. Chroma meter evaluation of tattoo lightening 6 weeks after treatment. Lightening was assessed with a Minolta color meter with readings taken prior to and 6 weeks after treatment. The L a b color scale was used and lightening was determined by calculating the total color change (delta E) after treatment.

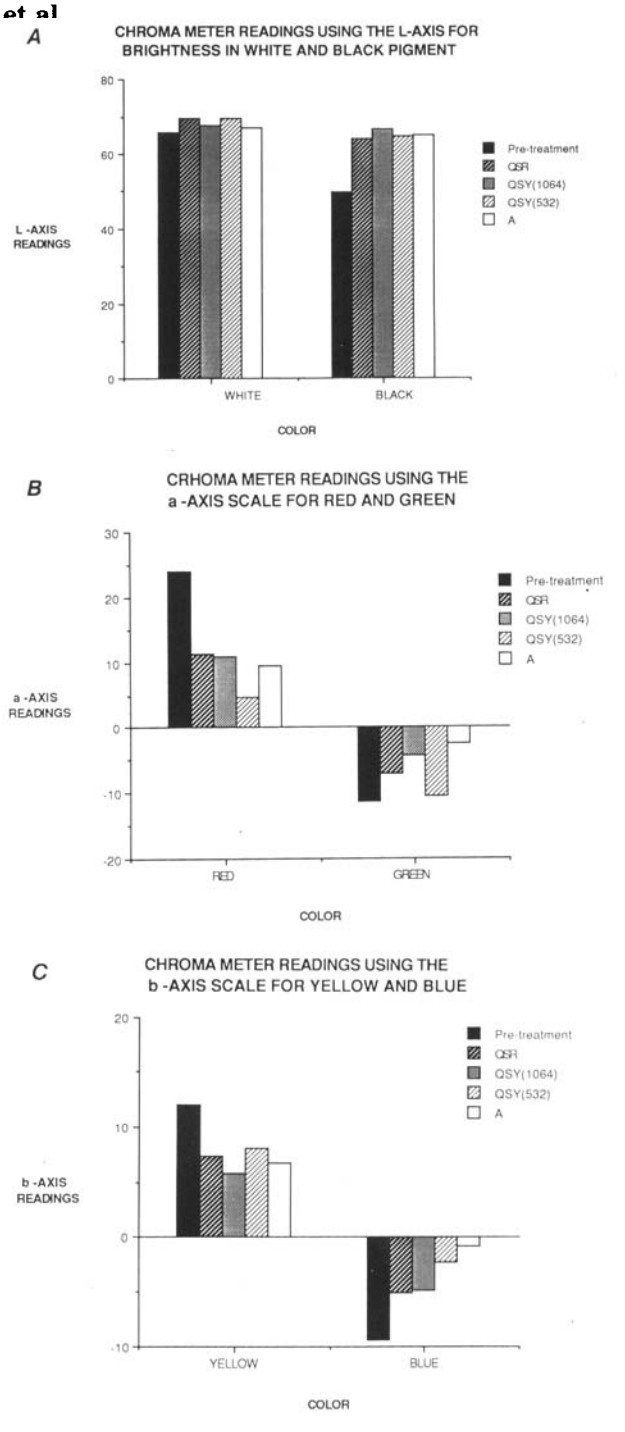

Fig. 4. Chroma meter evaluation of tattoo lightening 6 weeks after treatment. Lightening was assessed with a Minolta color meter with readings taken prior to and 6 weeks after treatment. The L a b color scale was used and lightening was determined by calculating ΔL for white–black (A), Δ a for red–green (B), and Δ b for yellow–blue (C).

Electron Microscopy

Prior to treatment, the majority of pigment was within intracellular membrane bound organelles. The pigment was detected within fibroblasts, macrophages, and occasionally within mast cells. Fibroblasts had abundant endoplasmic

TABLE 3. Electron Microscopic Evaluation of Tattoos*

Pigment Color	Prior to treatment pigment shape/size	Six weeks after treatment overall disruption
Red	polymorphous fine particles, curvilinear, linear, and circular bodies amorphous	Nd:YAG (1,064) > Nd:YAG (532) = A = QSR
Yellow	dense round globules	Nd:YAG (1,064) > QSR > A = Nd:YAG (532)
Green	dense round globules and round to polygonal particles	Nd:YAG (1,064) > Nd:YAG (532) > A > QSR
Blue	dense round globules and ill-defined round and rod-shape bodies	QSR = Nd:YAG (532) > A > Nd:YAG (1,064)
Black	well-defined round to polygonal particles.	Nd:YAG (532) > A > Nd:YAG (1,064) = QSR

*QSR = Q-switched ruby; Nd:YAG = Q-switched neodynium: yttrium,aluminum,garnet; A = alexandrite.

reticulum and numerous ribosomes. The majority of the pigment laden cells were in the superficial dermis and in perivascular locations. There was no pigment detected within the epidermis.

Table 3 shows the size and type of pigment granules seen with five pigments. Electron micrographs of pretreatment biopsies are shown in Figure 5. Immediately after treatment, pigment-laden cells showed changes ranging from complete ablation and disruption of the cytoplasm leaving a naked nucleus to cells with large cavitations within their cytoplasm (Fig. 6). The Nd:YAG (532) laser tended to cause the most disruption to the pigment laden cells. The amount of damage did not appear to correspond with the final clinical response.

Six weeks after treatment, pigment that originally contained round to polygonal particles appeared as amorphous granular material. The morphology of the other types of pigment granules were not altered after treatment.

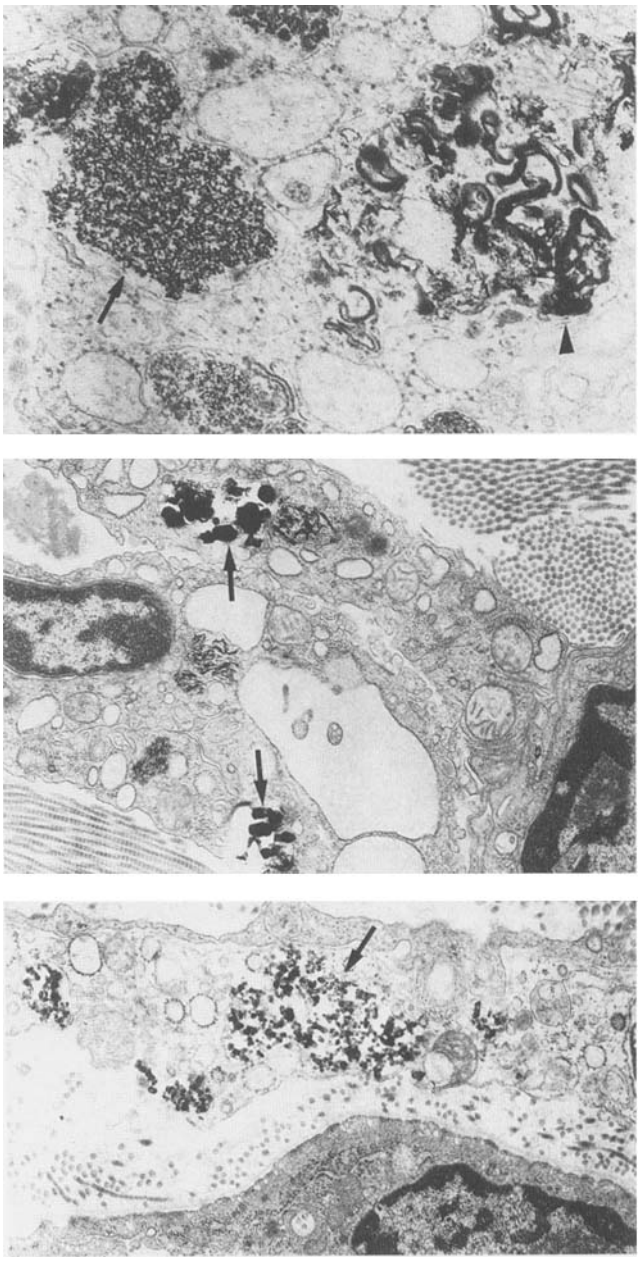

Fig. 5. Electron micrographs showing tattoo pigment within the cytoplasm of dermal scavanger cells 6 weeks after application. Top micrograph shows the polymorphous fine particles (see arrow) and curvilinear bodies (see arrowhead) seen in red pigment (magnification, ~39,000×). Middle micrograph shows the dense round globules and polygonal particles (see arrow) seen in green pigment (magnification, ~12,000×). Bottom micrograph shows collections of round to polygonal particles (see arrow) seen in black pigment (magnification, ~12,000×).

DISCUSSION

There are currently three types of commercial laser systems available for tattoo removal. These are the Q-switched ruby (QSR), the Q-switched neodynium:yttrium,aluminum,garnet (Nd:YAG), and the alexandrite lasers. Several published studies have revealed the effectiveness

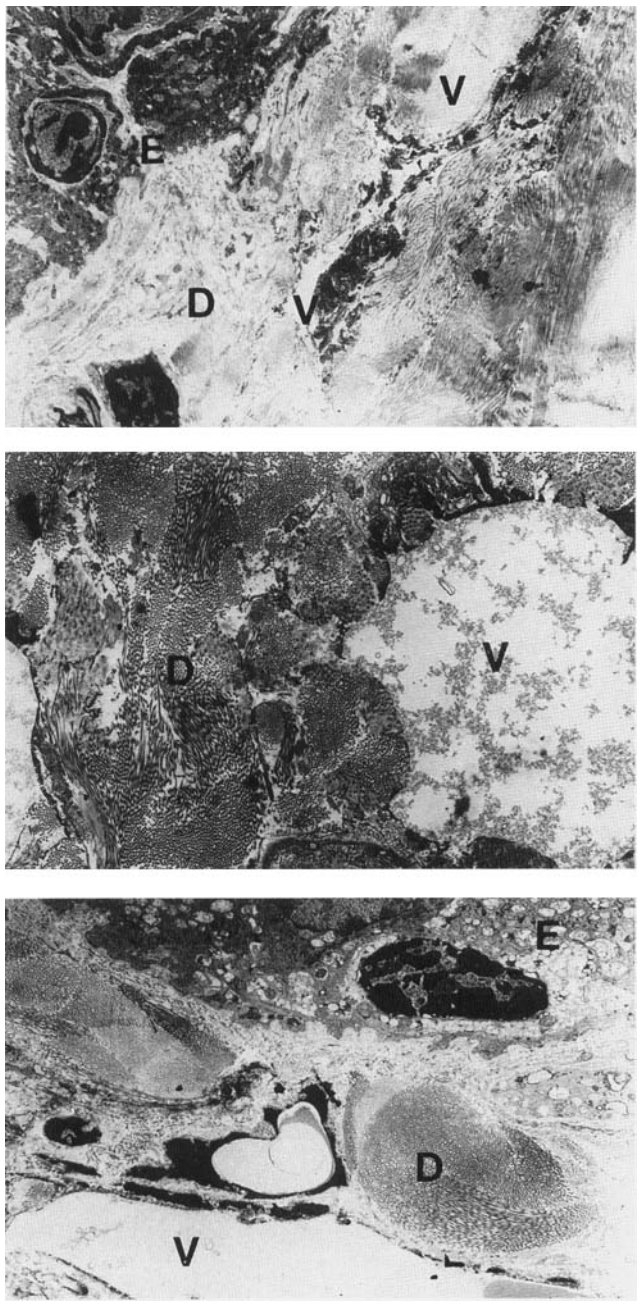

Fig. 6. Electron micrograph of tattoos immediately after treatment. Notice the large vacuoles (v) within the cytoplasm of dermal fibroblasts. The surrounding dermis (D) and epidermis (E) is still intact. Magnification from top to bottom: ~3,700×, ~2,700×, ~3,700×.

of these lasers in the lightening of tattoo pigment [2–6,8–12]. Each laser system appears able to remove certain pigments better than others. Tattoo pigments of the same color may have very different chemical compositions [13]. In clinical practice, tattoos of the same color do not always respond in the same manner after laser treatment. This may be due in part to the variation of tattoo pigment ingredients or the variation of human response to laser treatment. There are several different manufactures of tattoo pigment and tattooists use many different ingredients to mix their own unique pigments. With no uniform regulations, the comparison of laser tattoo removal based upon color is very difficult. A telephone survey of nine tattooists located in Minnesota revealed that despite mixing many of their own pigments, they all order most of their inks from Spaulding and Rogers (Voorheesville, New York). To help standardize this study, we selected all of our pigments from this supplier. Therefore, although impossible to test every variation of each pigment used for tattooing, this study focuses upon one set of pigments.

Other important variables in comparing the response to laser tattoo removal are spot size, fluence, pulse duration, and wavelength. Although much work needs to be done to define the optimum parameters for each specific tattoo pigment, this study examines current commercially available FDA approved tattoo lasers. Understanding that there are no universal treatment parameters written for laser tattoo removal, the fluences chosen for this study were based upon our current clinical treatment practice. The spot sizes used are those currently used in our clinical practice. The fluence is determined by the immediate post-treatment clinical response. This clinical response is obtained in guinea pig skin at lower fluences than that seen while treating human skin. This difference is most likely due to the differences between the thickness and pigmentation of hairless guinea pig and human skin. Further studies are needed to assess variation in spot size, fluence, pulse duration, and wavelength for removing each specific tattoo pigment.

All of the lasers were effective in lightening tattoo pigment without causing clinical scarring. However, our findings suggest that certain pigments respond better to different laser systems. Based upon a clinical score of 3 or 4, the Nd:YAG (1,064 nm) laser was able to lighten eight of the pigments tested. This was followed by the QSR laser, which was best able to lighten seven pigments, and then the Nd:YAG (532 nm) and alexandrite lasers, each of which lightened five pigments. Our results support previous reports that the Nd:YAG (532 nm) laser was the best treatment of red pigment due to the selective absorption at this wavelength. We also found that black

pigment was lightened > 50% with each laser system except the QSR, which lightened black 26–50% after one treatment. We found that the QSR laser was not as effective as the alexandrite laser in lightening green or blue pigment. The appearance of black coloration after laser treatment was noted with all lasers. Of the pigments in which we were able to know the ingredients, those containing iron oxides tended to become blackened. Ferric oxide (Fe_2O_3), which has a reddish-brown color, may be reduced by the laser impact to ferrous oxide (FeO), which is black. This finding has previously been reported by Anderson et al. [14].

Chroma meter readings for specific colors using the L a b scale for white–black, red–green, and yellow–blue gave results similar to our clinical scores. The difference seen in the L readings for white pigment were not clinically apparent; however, the L readings for black pigment showing that the QSR laser did not brighten black as well as the other lasers was seen clinically. The differences detected between the other three lasers was not clinically evident. Readings from the a scale show a definite decrease in red coloration after treatment with the Nd:YAG (532 nm) laser and in green pigment after treatment with the alexandrite laser. These findings are the same as seen by clinical score. The b scale readings for response of blue pigment clearly show that the alexandrite laser lightened this color to a greater degree than the other lasers. This finding was also seen in clinical evaluation; however, the b scale was not able to detect the remaining clinical responses seen in yellow and blue.

Delta E readings show us a global change in color after treatment. Using these readings, both the QSR and Nd:YAG lasers were best at clearing six pigments. As with the clinical scoring, the Nd:YAG (532) was the best laser to treat red pigment. Both blue and green responded best with the alexandrite laser. Most of the darker pigments had a greater delta E reading, or color change, after treatment with the Nd:YAG (1,064) laser. Despite these similarities there are discrepancies between observed clinical scoring and delta E readings. This may be due to sample error in obtaining delta E readings, inability of of the naked eye to detect subtle changes in hue, or the fact that changes in hue do not respond to observed clinical lightening.

Our histologic and ultrastructural studies did not necessarily correlate with the clinical results. Previous studies have had similar findings [11]. This is most likely due to sample error. On histologic examination we did note that the depth of cellular damage did not appear to correspond with wavelength. The ability of these pulsed lasers to generate photacoustic damage probably gives rise to this finding. By histologic examination the red pigment was disrupted to a greater extent than other colors with all lasers. This may be a result of the difficulty in seeing the small red granules with hematoxylin and eosin stain. Although not clinically evident, mild superficial scarring was seen histologically with all lasers.

The limitations of this study are several. First, although we are able to control the application and type of pigments used, this is not the case in clinical practice. We and others have attempted to discover the ingredients most commonly used in decorative tattoo pigments with little success. Without this standardization, all color-based findings must be viewed with some degree of caution. Second, the treatment parameters used were based upon our current clinical practice and the clinical tissue response immediately after treatment. Since the wavelength and pulsewidth are not easily adjustable in the current commercially available lasers, we did not attempt to alter these parameters. However, response may vary with changes in fluence and spot size. Further investigations are needed to determine how important these variables are. Third, in clinical practice we treat each tattoo 4–12 times for maximum lightening, in this study we evaluated the response after only one treatment. The effect of fluence on tattoo lightening may not be apparent until after several treatments (6).

In conclusion, these results show the need for access to several laser systems in order to obtain the best treatment for multicolored tattoos. Using this in vivo method, we are able to control the type and application of pigment. We can now further concentrate on controlling wavelength, spot size, pulsewidth, and fluence to optimize tattoo laser systems.

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