In Vitro and In Vivo Laser Treatments of Tattoos

High Efficiency and Low Fluences

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Objective: To analyze the absorption of tattoo inks related to their in vivo and in vitro behavior under laser irradiation to improve laser-assisted tattoo removal.

Design: The absorption of 21 tattoo inks in a wavelength range from 300 to 800 nm was characterized by reflection spectroscopy from samples consisting of inks mixed in gelatin. Tattoo inks were removed in vitro using pulsed laser radiation with different variables, and morphologic analysis of the irradiated areas was performed.

Setting: An interdisciplinary laser laboratory with a common industrial project with the Spanish company Milesman S.A.

Participant: One person was voluntarily tattooed with 2 of the studied inks.

Main Outcome Measures: (1) First approach to the optimum dose for pigment removal in in vitro models. (2) Correlation between the in vitro and in vivo situations at the optimum dose.

Results: Reflection spectroscopy facilitated selection of the most adequate laser wavelengths for tattoo removal. Red, orange, and rose inks were successfully lightened at 532 nm with 0.6 J/cm²; brown at 1064 nm with 0.3 J/cm²; yellow and green at 448 nm with 2.6 J/cm²; and blue at 600 nm with 0.9 J/cm². Similar colors in in vitro and in vivo tattoos responded with the same efficiency to the laser variables.

Conclusions: High efficiency is reached in the removal of in vivo tattoos by using an irradiation wavelength at which the percentage of reflection from the pigment is minimal. Under this condition, laser pulses can be used with a low fluence, minimizing adverse effects and clinical time.


THROUGH THE YEARS, MANY different methods of tattoo removal have been explored. Older techniques involve removal of the outer skin layers using mechanical (dermabrasion and salabrasion), chemical, or thermal (cryosurgery and cauterization) methods.¹⁻³ Progress in laser technology offers alternative treatments to patients with cutaneous discolorations, including senile lentigos and tattoos. According to the principle of selective photothermolysis,⁴ laser devices are chosen that provide wavelengths adapted to the size, color, and location of the lesion. Because the wavelength used for tattoo removal should match the absorption spectra of the tattoo pigment,⁵ important information for the selection of the most appropriate laser variables can be obtained from reflection, absorption, or Fourier transform infrared spectroscopy of each pigment.⁶ For each tattoo ink, the laser wavelength selected for its removal should be selectively absorbed by it, minimizing absorption by primary endogenous chromophores, hemoglobin, and melanin. Because tattoo pigments come in a variety of colors, multiple wavelengths of laser light may be needed to remove a tattoo. Because the laser pulse duration should be shorter than the thermal relaxation time of the target, short pulses in the nanosecond time scale are typically used, causing disruption of the pigment.⁷ Fragmentation of the tattoo particles leads to small pigment particles, unknown decomposition products, and newly generated chemical compounds⁸ that may then be removed from the skin by means of the lymphatic system, and, as a consequence, a noticeable lightening of a colored tattoo results.

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Until now, the laser systems used for tattoo removal have provided fixed wavelengths. The most commonly used are 510 nm (pulsed-dye laser), for removing yellow tattoos; 1064 and 532 nm (Nd:YAG laser, fundamental and second harmonic wavelengths), for removing black and red tattoos, respectively; 694 nm (ruby laser), for removing blue, black, and green tattoos; and 755 nm (alexandrite laser), for removing blue and black tattoos. The extent to which the different tattoo pigments absorb these wavelengths has not been fully assessed, and the removal of tattoos has been commonly ensured by using high fluences (approximately 9-12 J/cm²). The lightening process of tattoos performed under these laser conditions involves a high risk of losing the stratum basalis from the epidermis and of bleeding and spreading of heat to surrounding tissue, which cause unacceptable damage. During the past few years, there has been a tendency to continuously increase the laser fluence to remove tattoos by using a unique, nonoptimized wavelength with all kinds of inks and pigments. This use of higher fluences, increasing the amount of light delivered to superficial layers of the skin, increases adverse effects with little or no improvement in efficacy. Adverse effects could be lessened and the efficacy of tattoo removal improved by delivering lower fluences in picosecond laser pulses, although clinical application of picosecond lasers is still a challenge due to their technological complexity, high cost, and difficulty to operate. Another approach to help avoid damage when using nonoptimized laser pulses could be using dermal clearing agents after partial removal of the tattoo with low-fluence laser pulses.

The duration of treatment depends mainly on the immune response of the patient, the tone of the skin (more success in clear skin), the color of the pigment (yellow and light green are poorly eliminated), the source of the ink (not regularized, amateur tattoos respond better than professional tattoos), and the depth of the tattoo in the dermis. Generally, an average estimate for complete lightening is 6 to 12 exhibitions (1 session every 6 or 8 weeks). Tattoos exhibit different colors obtained from a variety of pigments, and neither the tattoo artist nor the patient have any information on the substances punctured into skin. In fact, although knowledge of the nature and characteristics of the pigments used in tattooing is essential to develop strategies to remove them, only a few studies have been performed regarding the in vitro analysis of tattoo pigments.

The aim of the present work is to improve the understanding of the optimal laser variables for the treatment of tattoos. To this end, we first characterized a variety of marketed tattoo inks by means of reflection measurements. Because modern tattoo needles place granules of ink in the mid dermis, which makes deep penetration of laser light into the skin necessary to achieve tattoo removal, we incorporated selected and well-characterized pigments into in vitro experimental models of skin made of gelatin and proceeded to irradiate the samples under controlled laser variables. Considering that the engulfment of the tattoo particles by macrophages or their incorporation into fibroblasts may change these optical properties, a volunteer was tattooed using 2 of the well-characterized inks, and the evolution of the in vivo tattoos and the skin reaction to laser irradiation was carefully controlled. In the present work, inks from different worldwide suppliers, including pigments of similar but not identical color, were first characterized and then used. This study’s conclusions might provide general guidance for optimizing lightening therapy.

## Methods

### Tattoo Inks

A variety of tattoo inks were selected from those used in Spanish (Sani Color; Madrid, Spain) and American (VooDoo Brand; Spaulding Color Corp, Voorheesville, New York) markets. Twenty-one different inks (9 Spanish and 12 American) were representatively selected for this study: yellow (amarillo canario and sun yellow), orange (naranja and light orange), pink (rosa, Dusty Rose, monterey, and poppy), red (rojo fuego, rojo ciruela, Lava Red, blood red, and Dyn-o-Mite red), blue (azul marino and blue jay), green (verde prado and Irish green), brown (marrón and russet brown), and black (negro tribal and midnite black).

### Reflection Spectra

Diffuse reflection spectra (wavelength coverage from 220-900 nm) of the tattoo inks embedded in gelatin were recorded using a fiberoptic spectrophotometer (model USB2000) and a reflection probe (model R400-7-VIS-NIR) placed in a holder (model RPH-1) (all from Ocean Optics, Dunedin, Florida) that keeps it at 45° from the surface.

### In Vitro Tattoo Removal Study

Considering the in vitro methods reported in the literature, and trying to simulate the conditions of tattooed skin, where the ink particles are located in the dermis, an experimental model of skin was developed according to the following protocol: 0.35 mL of a suspension of ink in water (100 mg/mL) is added to 2.0 mL of a solution of gelatin (Royal [A type, 240° Bloom, transparent] and the laser emission spectral regions); Kraft Foods, Madrid, Spain) in water (120 mg/mL). The solution was poured into a Petri dish to reach a thickness of approximately 2 mm once solidified, which is the average thickness of human dermis. The concentration of ink was chosen to be near saturation so that the most unfavorable conditions for its elimination were achieved. The upper surface of each gelatin slab was coated with a cellulose-hydrated membrane (Celle-Sep; Barcellona, Spain) measuring 0.1 mm thick, simulating the 2 layers (dermis and epidermis) existing in skin. Scattering of pump radiation from this membrane would take into account, at least partially, scattering from the epidermis in vivo systems. The model skin was tattooed with the different tattoo inks from the Spanish and American markets described in the “Tattoo Inks” subsection. The behavior of this model under laser irradiation was studied as a function of different laser variables, such as wavelength (λ), fluence, spot size, and repetition rate.

The model skin does not incorporate hemoglobin, melanin, and other physiologic chromophores present in human skin. Although these skin components can interact with the laser radiation, because we are pursuing selective photothermolysis using laser wavelengths corresponding to the absorption maximum of the pigments, the incoming radiation should be absorbed mainly by ink particles. Thus, although incomplete, the...
model skin should allow us to establish some initial laser variables to safely begin the in vivo treatment. This initial selection of laser variables cannot be made on the basis of existing bibliographic data owing not only to the wide differences in the described experimental conditions but also to the differences between these conditions and those encountered in clinical practice.

LASER TREATMENT

Two light sources were selected for laser treatment: (1) Q-switched Nd:YAG laser (Lotis TII SL-2132; Lotis TII, Minsk, Belarus), with pulses at 1064 nm (energy up to 137 mJ/pulse, an approximately 11-nanosecond pulse duration [full-width at half maximum]), 332 nm (up to 90 mJ/pulse, approximately 11 nanoseconds), and 355 nm (up to 20 mJ/pulse, approximately 18 nanoseconds); repetition rates from 1 to 15 Hz; and spot size of 2.6 × 3.4 mm; and (2) laser radiation at 448 and 600 nm generated from an Nd:YAG laser (Spectron SL 803G; Spectron Laser Systems Ltd, Rugby, England) pumped pulsed-dye laser (Spectron SL 4000; Spectron Laser Systems Ltd) using a methanolic solution of coumarin 440 (9 × 10⁻⁴ M) (to generate radiation at 448 nm) and sulfanilamide B (5 × 10⁻⁴ M) (to generate radiation at 600 nm). The dyes (laser grade) were purchased from Exciton (Dayton, Ohio). The laser characteristics were as follows: output energy, up to 1.5 mJ; repetition rate, up to 10 Hz; pulse duration, 4 nanoseconds; and diameter of spot, 0.22 mm.

The samples were mounted on an XY translation stage (New Focus, San Jose, California) and were placed normal to the incident focused laser beam. The laser beam was focused on the sample using a BK7 spherical lens with a 250-mm focal length. Energy measurements of the laser pulse incident on the sample were performed using a pyroelectric detector (Gentec-DE 500; Gentec Electro-Optics Inc, Quebec, Quebec, Canada) combined with a Gentec-EO SOLO console. The fluence (energy/surface) of the radiation incident on the sample surface was controlled by either varying the lens to sample distance or reducing the pump energy. Spot size was estimated from the area of the incident laser pulse focused into the sample, as registered on photographic paper.

MORPHOLOGIC ANALYSIS

The in vitro tattoo lightening process was evaluated by performing a morphologic analysis of the irradiated zones using a stereomicroscope (model DM-143; Motic, Richmond, British Columbia, Canada) coupled to a digital camera (Moticam 2300, 3.0 megapixels; Motic). The photographs of the progression of the bleaching process were taken by means of reflection, illuminating the samples with visible light.

IN VIVO TATTOO REMOVAL STUDY

We selected 2 inks, Lava Red and Dusty Rose (VooDoo Brand), which in the study in model skin had been shown to be selectively removed under irradiation at 532 nm. One volunteer (with Fitzpatrick skin type I [defined as pale white skin, blue or hazel eyes, and blond or red hair]) to minimize absorption by melanin) was tattooed with 2 stars, each with a different ink. Six months after the tattooing process, we proceeded to laser irradiation of both tattooed stars in several sessions, and the progress in the removal of the tattoos was carefully monitored. The Q-switched Nd:YAG laser system was used (see the “Laser Treatment” subsection), operating at the second harmonic (532 nm) and delivering the radiation through an optical fiber coupled to the head of the laser. Output energy ranged from 2.6 to 6.0 mJ/pulse, with a spot diameter of 0.7 mm, fluence of 0.7 to 1.6 J/cm², and a repetition rate of 1 to 5 Hz. Written consent was secured from the participant. This study was approved by the ethics committee of Hospital Clínicó San Carlos, Madrid.

At the different tattoo removal sessions, photographs were taken to document the progress of the lightening process. At each session, a selected area of each tattooed star was irradiated. Note that correct selection of the appropriate laser variables resulted in the participant requiring neither anesthesia during the process of tattoo elimination nor treatment with anti-inflammatory drugs after treatment. Local anesthesia was applied to the irradiated area only under the highest laser fluence selected herein (1.6 mJ/cm²), with no requirement for any other pretreatment or posttreatment.

RESULTS

The reflection spectra of the different pigments are shown in Figure 1. It can be appreciated that tattoo inks with similar colors may contain completely different pigments and, therefore, exhibit clear differences in their reflection behaviors.

The in vitro lightening process of the different inks was carefully analyzed as a function of the different laser variables in a morphologic study with optical microscopy of the irradiated areas. Figure 2 shows microphotographs of irradiated in vitro model skin tattooed with a red ink as a function of laser fluence and repetition rate at 532 nm. In trying to simulate what would be a session of tattoo removal, the laser beam was scanned at a constant speed over an expanded area of the model skin in gelatin. Because the pigment selectively absorbs the wavelength of radiation, lightening of the ink is observed at low fluences even with a single pulse.

The laser pulse repetition rate was found to be a relevant variable in the efficiency of the process and in the extension of collateral damage. For a fluence of 0.9 J/cm², the increase in the repetition rate up to 5 Hz originates a darkening in the central area of the irradiated spot with clear areas at the lateral edges due to the gaussian geometry of the laser beam. At a given fluence and repetition rate, exposure of the irradiated area to more than 3 pulses induces darkening of the tattoo ink. This behavior could be related to oxidation of 1 or more pigments in the ink (Figure 2B, D, and F). Excessive fluence or repetition rate could cause water vaporization and damage to the surrounding tissue (Figure 2D–F).

Laser irradiation at 532 nm, with fluences ranging from 0.6 to 0.9 J/cm² at 1 Hz, efficiently lightens the 5 red inks selected herein independent of market supplier and elemental composition. Laser variables demonstrated to be appropriate for removal of the red inks were successfully applied to lighten other pigments with similar reflection spectra, such as orange (Figure 3A) and the 4 studied pink inks (Figure 3B). However, and contrary to what might be expected according to their reflection spectrum, brown inks were eliminated under irradiation at 1064 nm only (Figure 3C), with the best results being obtained at a high repetition rate (5 Hz), with no induction in this case of any significant morphologic alterations. Model skin samples tattooed with yellow (Figure 3D) and green (Figure 3E) inks
were lightened under laser irradiation at 448 nm, 2.6 J/cm², and 1 Hz. Blue inks were efficiently removed at 600 nm, 0.9 J/cm², and 1 Hz (Figure 3F).

One important difference between in vivo and in vitro tattoos is that in vivo, the tattoo particles are engulfed in macrophages or are incorporated into fibroblasts, which affects the optical properties of the medium. Nevertheless, the results obtained in the in vitro studies were a good guide to optimizing the removal of tattoos in vivo. Thus, in the first phase of the study, the 2 in vivo tattoos were irradiated using the laser variables that had produced the best results in the in vitro study (532 nm, 0.7 J/cm², and 1 Hz). The use of laser fluences much lower than those used up to now in standard clinical methods resulted in a drastic decrease in collateral effects, such as bleeding, swelling, and scarring. This lack of adverse effects allowed irradiation of the tattooed areas at 2-week intervals instead of the usual 6- to 8-week intervals required in standard clinical practice. Multiple pulses were delivered in each treatment to continuously irradiate the selected tattooed areas.

Figure 4 shows the evolution of the irradiated in vivo tattoos after 1, 2, and 3 laser treatments at 2-week intervals. The rose star tattoo had an excellent response. In contrast, the red star tattoo, which according to Figure 1 should exhibit better absorption at 532 nm than the tattoo with rose ink, did not respond to the laser therapy under the laser variables selected in this first phase of the study because of the higher concentration of pigments present in the red ink. Consequently, in the second phase of the study, higher exposure doses of laser radiation were used in the red tattoo. By increasing the fluence to 1.6 J/cm², delivered in a single pulse, an excellent response was achieved after just 2 treatments (Figure 4B). Personal circumstances of the patient did not allow further tattoo removal treatment. Inspection of the tattoos 1 month after the last session showed that the lightening process of both tattoos had been performed without any laser-induced postinflammatory hypopigmentation or hyperpigmentation and with no allergic reactions or scarring.

As tattooing becomes increasingly popular, the need for tattoo removal will also increase. Despite the improvement in tattoo-clearing laser therapy, more efforts are required to optimize the efficiency of the treatments and the simplicity of procedures to decrease the number of sessions and reduce the adverse effects. The present study correlates reflection spectra, in vitro and in vivo responses to laser irradiation, leading to several novel findings with interesting implications.

One of the basic principles of selective photothermolysis implies that the wavelength used for tattoo removal should match the absorption spectra of the tattoo pigment. The characteristic absorption of pigments from different market suppliers can be successfully analyzed using reflection spectroscopy and, consequently, may help optimize the choice of the best-available wavelength. This fact, together with the easy implementation of the detection system, allows performance of direct analysis of in vivo tattoos, something not possible on the basis of absorption spectroscopy.
Because the laser wavelength should match the absorption range of the ink, selectivity of the lightening process could be achieved by using wavelengths of approximately 440 nm for treating yellow pigments; approximately 535 nm for orange, pink, red, and brown pigments; approximately 600 nm for blue pigments; and approximately 650 or 450 nm for green pigments. Microscopic examination after irradiation of the in vitro model skin samples provided useful information about the most appropriate laser variables for lightening of the different tattoo inks, avoiding darkening and excessive heating of the gelatin.

At irradiation wavelengths where a minimal percentage of reflection from the pigment was observed, just 1 treatment session with the appropriate nanosecond-pulsed laser system provided excellent results in the removal of all the tested inks using a low fluence, a low repetition rate, and a small number of pulses. Adverse effects and morphologic and pigmentation effects were minimized. The effectiveness of the clearing process did not improve by delivering more fluence, increasing the number of pulses, or using a higher repetition rate. On the contrary, treatment of tattoos at the optimal irradiation wavelengths with laser fluences significantly higher than those reported previously herein resulted in excessive scarring while leaving much of the tattoo pigment behind.

Some researchers have argued that the optimal wavelength for the treatment of green, blue, and blue-green pigments is not necessarily that corresponding to the highest absorption peak of the pigment. In this regard, we note the good response we observed in the treatment of green tattoos under irradiation at 448 nm in contrast to the low efficiency obtained under irradiation with red light, which is absorbed less efficiently than is the 448-nm radiation by the green inks used. Inks with other colors, such as yellows, which are difficult to treat with a commonly used Q-switched laser, were also successfully cleared under dye-laser irradiation tuned at 448 nm. Nevertheless, these results should be taken with some caution because they were obtained in the in vitro experiments with the model skin. The high absorption of the 448-nm radiation by epithelial melanin decreases the penetration depth in skin of laser light with this wavelength, which could complicate treatment in darkly pigmented individuals.

The studies herein demonstrate that the selection of adequate laser variables guarantees the selective elimination of tattoo pigments in vivo, even using a density of energy significantly lower than those usually reported in the literature. We found that under the present conditions, fluences of 0.7 to 1.6 J/cm² were effective in the removal of tattoos compared with fluences of 9.0 to 12.0 J/cm² commonly reported in the literature. In addition, the tattoo removal process in the present case required shorter therapy sessions and spacing between sessions of 1 to 2 weeks compared with the 4 to 8 weeks needed in other laser treatments.

The present study should be considered initial, and there are some venues that should be further explored. We limited this study to tattoo pigments from just 2 different representative manufacturers. Subsequent studies should be extended to cover inks supplied from most of the worldwide market suppliers. Pigment spectra were measured only up to 900 nm and did not include measurements at 1064 nm, which is one of the commonly selected wavelengths for tattoo removal. Pulsed-dye laser wavelengths other than 448 or 600 nm were not tested in the in vitro experiments, and, thus, no extended correlation with reflection spectra could be made. In vivo experiments were performed by analyzing just 2 tattoos.
in a unique patient. Despite these limitations, we succeeded in demonstrating a high-efficiency laser protocol to clear 2 particular pigments in vivo, opening the way to eliminate effectively and with no secondary effects similar colors, even from different suppliers, despite the complex composition of the ink tattoos.

The Q-switched laser systems already being used for tattoo removal operate at the fixed wavelengths of 510, 532, 694, 755, and 1064 nm. The present case study illustrates that the improvement in clinical outcome for lightening multicolored and single-colored tattoos requires multiple-wavelength laser systems. Thus, it is clear that tattoo removal presents new challenges for laser technology. An attractive alternative to the existing laser systems would be a solid-state dye laser. Such a laser could be built to be a compact, versatile, hazardless, and easy-to-handle system, allowing broad, rapid wavelength tuning over the entire visible spectral region. With this objective in mind, we have dedicated our recent systematic efforts to design and synthesize laser-efficient and photostable dye-doped optical materials to build an industrial prototype of a solid-state dye laser. As demonstrated herein, such a laser system will have immediate applications in tattoo removal processes. On the other hand, developments leading to new biodegradable tattoo inks, feedback systems to detect the absorbance characteristic of tattoo inks, dermal clearing agents, and perhaps even lasers with shorter pulse durations might also improve therapy results in the future.
sis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

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REFERENCES


Announcement

Volunteering With Health Volunteers Overseas

The American Academy of Dermatology partnered with Health Volunteers Overseas (HVO) in 2004 to match interested dermatologists with overseas volunteer opportunities. Through HVO programs, volunteer dermatologists train local health care providers, giving them the knowledge and skills to make a difference in their own communities.

The major goal of the HVO dermatology programs is to build capacity through training local health care providers (ranging from dermatology residents to primary care health workers) in clinical dermatology.

Sites with volunteer opportunities for dermatologists include Costa Rica, Palau, India, Peru, Uganda, Cambodia, and Saint Lucia. Volunteers generally serve for 2 to 4 weeks, although shorter and longer assignments are possible.

A private, nonprofit membership organization, HVO was founded in 1986 to improve global health through education. HVO designs and implements clinical education programs across the spectrum of health specialties. To learn more about volunteering with HVO, visit the Web site (www.hvousa.org) or contact the HVO Program Department at (202) 296-0928.