Tattoo Darkening and Nonresponse After Laser Treatment

A Possible Role for Titanium Dioxide

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Objective: To examine relationships between chemical composition, biopsy findings, and clinical outcome in laser-treated tattoos.

Design: Observational nonblinded retrospective study.

Settings: University-based dermatology clinic and private practice.

Participants: Twenty patients who underwent biopsy of laser-treated tattoos.

Main Outcome Measures: Biopsy specimens were analyzed after laser treatment, and the depths of changed particles were recorded. Ultrastructure of the changed particles was examined by electron microscopy. Presence of inorganic chemicals was determined by x-ray diffraction. Correlation between x-ray diffraction, microscopy, and clinical response was attempted.

Results: Of the 20 tattoos, 7 lightened, 9 failed to change, and 4 darkened after laser treatment. There was a significant association between presence of titanium dioxide and poor response to laser therapy. Microscopic studies showed variable changes in the ink particles, but there was a trend toward residual deep green pigment in the resistant tattoos. Also, round dark stippling was observed superficially in the darkened specimens.

Conclusions: Titanium is overrepresented in tattoos that respond poorly to laser treatment. Further studies are necessary to show whether this metal is the primary cause of this poor response.

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MANY TATTOOS are resistant to laser treatment, particularly those containing blue, purple, yellow, green, and flesh-toned dyes. For example, while some green tattoos clear completely after only 2 to 3 treatment sessions, others do not lighten or may even become darker.

The mechanism by which tattoos darken after laser treatment is not completely understood. One factor may be the laser-induced reduction of metallic compounds used in certain dyes. A potential offender is titanium dioxide (TiO2), which is an increasingly popular white ink used to enhance the brilliance of tattoos. Titanium is most commonly found in green, white, and flesh-colored tattoos; however, it has been identified in tattoos of almost any color. To explore a possible association between TiO2 and tattoo response to laser treatment, we examined the metallic composition of a series of treated tattoos in this pilot study.

Tattoo colors and metal compositions are summarized in Table 1. Of the 20 tattoos studied, 7 lightened significantly throughout the whole tattoo, 9 were at least partly resistant, and 4 became darker. Of the 13 tattoos that responded poorly, all except 2 contained titanium, whereas in the 7 tattoos that lightened considerably after laser treatment, only 2 contained titanium. With the use of a Fisher exact test, there was a significant association between the presence of titanium and a poor response to laser therapy (P = .02).

In general, tattoos that failed to lighten showed higher ink density than those that responded favorably (Table 2). All samples of colored (nonblack) pigment appeared to be heterogeneous by light microscopy, showing mixtures of colored and indeterminate brown-black granules. Within the group of resistant green tattoos, 2 types of pigment granules were seen: (1) a large and rounded globular granule (2-5 µm in diameter; Figure 1A)
SUBJECTS AND METHODS

Biopsy specimens were obtained in this retrospective analysis from consenting patients participating in tattoo treatment protocols with the use of Q-switched ruby (Spectrum Medical Technologies, Natick, Mass) or Nd:YAG (ConBio, Livermore, Calif) lasers. Each patient was treated by an experienced dermatologist with a protocol expected to achieve an adequate clinical response. Green tattoos were treated with the ruby laser with fluences of 4 to 7 J/cm² and spot sizes of 5 to 6 mm. Red tattoos were treated with a frequency-doubled Nd:YAG laser with fluences of 2 to 4 J/cm² and spot sizes of 2 to 3 mm. Black tattoos were treated with an Nd:YAG laser at 1064 nm with fluences of 4 to 8 J/cm² and a spot size of 2 to 3 mm. Patients received at least 6 treatments with 1 or a combination of lasers before biopsy. Tattoo colors are summarized in Table 1. A total of 20 patients were included in the study.

For most of the tattoos, only posttreatment biopsy specimens were available. Biopsy samples were handled in 1 of 2 ways. Some tissue was processed for paraffin embedding and stained with hematoxylin-eosin. Representative sections were left unstained and were used to assess the density of ink particles and depth of microscopic lightening induced by laser. The rest of the tissue was processed for electron microscopy. Specimens were fixed in 4% glutaraldehyde in 0.1-mol/L cacodylate buffer, dehydrated, and embedded in epoxy resin. Postfixation in osmium tetroxide was omitted to enhance the x-ray analysis.

To observe the direct effects of laser irradiation on titanium, a 5% TiO₂ cream (Ti-Screen Natural; Pedinol, Farmingdale, NY) was irradiated with a Q-switched Nd:YAG laser (Schwartz Electro-optics, Orlando, Fla) with a fluence of 7 J/cm². The rest of the tissue was processed for electron microscopy (Figure 1A). Scattered among the deeper granules were crystalline particles evenly distributed in the cytoplasm (Figure 1B). (Note: In this study, we defined granule as the smallest identifiable structure on electron microscopy. This is distinguished from crystalline, which we defined as the smallest structure observed on routine light microscopy.)

Table 1. Summary of Tattoo Features

<table>
<thead>
<tr>
<th>Tattoo Color and Constituents (No. of Tattoos)</th>
<th>Resistant (n = 9)</th>
<th>Darkening (n = 4)</th>
<th>Good Response (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green, titanium (5)</td>
<td>White/flesh</td>
<td>Black, no peak</td>
<td>Black, titanium</td>
</tr>
<tr>
<td>and chromium (1)</td>
<td>(2)</td>
<td>(5)</td>
<td>(1)</td>
</tr>
<tr>
<td>Green, chromium (1)</td>
<td>Red, mercury</td>
<td>Green, titanium</td>
<td></td>
</tr>
<tr>
<td>Black, titanium (1)</td>
<td>Purple, titanium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red, titanium (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Electron Microscopic Findings of Particle, Granule, and Density of Tattoos

<table>
<thead>
<tr>
<th>Granule depth, µm</th>
<th>Resistant</th>
<th>Darkening</th>
<th>Good Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>200-1300</td>
<td>200-1000</td>
<td>200-1000</td>
</tr>
<tr>
<td>Mean</td>
<td>700</td>
<td>600</td>
<td>500</td>
</tr>
<tr>
<td>Particle size, nm</td>
<td>200-1000</td>
<td>60-300</td>
<td>40</td>
</tr>
<tr>
<td>Superficial</td>
<td>15-250</td>
<td>20-300</td>
<td>60</td>
</tr>
<tr>
<td>Granule size, µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td>1-5</td>
<td>2-5</td>
<td>1-3</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>1-2</td>
<td>2-5</td>
<td>1-3</td>
</tr>
<tr>
<td>Density of ink (light microscopy)</td>
<td>4+</td>
<td>3+</td>
<td>2+</td>
</tr>
</tbody>
</table>

*Globular/crystalline.

that tended to break down into smaller round particles of various sizes when treated (Figure 1B) and (2) crystalline granules (5-10 µm in diameter; Figure 1C) that showed a tendency to “splinter” with therapy (Figure 1D). Changes in granule shape were less conspicuous with increasing depth of the samples. In both types of specimens after treatment, there was a transition from translucent brownish granules (0.5-1 µm) with small black inclusions (like a stippling) located superficially, to larger, more opaque bright-green granules (1-3 µm) appearing unchanged by the laser starting about 500 µm deep in the dermis (Figure 1A). Scattered among the deeper granules were stippled aggregates of smaller 1-µm granules similar to those noted in the superficial biopsy regions. The number of these smaller dark dots appeared constant from 500 to 1500 µm deep in the skin. However, more superficially, there was a progressive decrease in their number such that they were rarely observed in the uppermost sections of the specimen (200 µm deep).

By transmission electron microscopy, resistant green tattoos showed some cells with lysosomes containing pale material with a few dark particles in treated areas (Figure 1B). (Note: In this study, we defined particle as the smallest identifiable structure on electron microscopy. This is distinguished from granule, which we defined as the smallest structure observed on routine light microscopy, usually 0.5 to 10 µm in diameter, ie, the particle-containing lysosome.) Other specimens showed more crystalline particles evenly distributed in the cytoplasm (Figure 1D). In both cases (globular and crystalline), Sections 1 µm thick were examined either unstained or stained with toluidine blue O. Thin sections were stained with saturated uranyl acetate and Sato lead stain or left unstained and examined in an electron microscope (CM10; Phillips, Hillsboro, Ore).

All specimens underwent x-ray diffraction studies for detection of metallic elements. Paraffin-embedded specimens were cut at 10 µm and mounted directly on an aluminum stub for use in scanning electron microscopy. Paraffin was removed from the sample with xylene, and a layer of carbon was applied to the surface of the section. These samples underwent x-ray microanalysis on a scanning electron microscope (Amray 1400; Amray, Bedford, Mass) with an x-ray detector (Kevex, Valencia, Calif). Samples processed in epoxy resin were cut at 90 nm and placed on a carbon and polyvinyl formal–coated grid for examination with a transmission electron microscope (Philips Bio-twin; Phillips) equipped with an x-ray detector. Both methods produced spectral signatures for elements present within the tissue.

A Fisher test was used to determine if there was a significant correlation between presence of titanium and tattoo resistance and/or darkening. We excluded tattoos found to contain iron oxides from the data analysis, as they are known to darken with treatment.

To observe the direct effects of laser irradiation on titanium, a 5% TiO₂ cream (Ti-Screen Natural; Pedinol, Farmingdale, NY) was irradiated with a Q-switched Nd:YAG laser (Schwartz Electro-optics, Orlando, Fla) with a fluence of 7 J/cm².
areas from the deeper dermis, the presumed untreated regions, showed larger, more homogeneous particles.

In the 4 tattoos that underwent darkening, light microscopy showed large granules (1-3 µm) deeper in the dermis that appeared unaltered by laser treatment. Exclusively in a thin band (about 100 µm thick; Figure 2A) in the superficial parts of the specimens (about 200-300 µm in depth), smaller (1 µm), darker round bodies appeared as stippling within these granules. In the more superficial ("treated") areas of the specimens, these small bodies were more loosely distributed, as compared with a more aggregated, denser, clumped distribution in the untreated deeper regions of the sections. By transmission electron microscopy, the appearance was similar, with more heterogeneity seen superficially, where specimens showed a mixture of apparently unchanged particles (approximately 500 nm in diameter on electron microscopy) and smaller (50-100 nm) and "changed" darkened particles (Figure 2B and C).

Mercury was found in the one red specimen that darkened. The histologic findings were similar to those of the other 3 darkened tattoos, with the exception that in the superficial (ie, treated) dermis there were both larger reddish granules and smaller, presumably changed, granules.

Figure 1. A, Light micrograph of an unstained 6-µm paraffin section showing the presence of large globular green pigment (original magnification ×100). B, Electron micrograph of a treated region of the same tattoo shown in A. There are large lysosomal structures that contain round pigment particles (arrowheads) of varying sizes. Most of these particles are much smaller than the pigment particles found in the untreated areas (bar indicates 0.2 µm). All particles retain a round shape. C, Light micrograph of an unstained 6-µm paraffin section showing large green crystalline structures (original magnification ×100). D, Electron micrograph of a treated region of the tattoo shown in C. Note the large electron-dense structures as well as the smaller similarly dense structures around them (arrowheads). C indicates crystal (bar indicates 0.2 µm).
Microscopically, in treated tattoos that lightened considerably (most of which were black), there was smudging and a light-brown color superficially, with little or no peppering. Deeper in the dermis, homogeneous black granules were noted. By transmission electron microscopy, treated parts of the specimens showed a mixture of electron-dense and electron-lucent particles, with many of the electron-lucent (changed) particles showing slightly increased size (60-100 vs 40 nm) compared with their unchanged electron-dense (native) counterparts.

Laser irradiation of the 5% TiO2 cream resulted in a dramatic immediate color transformation from bright white to bluish-black.

**COMMENT**

Mechanisms for tattoo removal after Q-switched laser irradiation are not clearly understood. It is presumed that the absorption of short-pulse energy produces high temperatures in the ink particles, resulting in death of ink-laden phagocytic cells. Tattoo clearing may occur via particle fragmentation, phagocyte cell death and subsequent egress via lymphatics, transepidermal elimination, or intrinsic optical property changes in the pigment granules. Resistance to laser treatment may be related to early repopulation of particles by fibroblasts, excessive amounts of ink, depth of particles, failure of temperature and pressure stresses to alter particles, and electrochemical changes in the valence state of inorganic dyes subjected to high-power laser irradiation, as described below.

Tattoos containing ferric oxide, a brown-red ingredient widely used in red, pink, and flesh-colored tattoos, have been reported to result in a black discoloration when treated with the Q-switched ruby laser. The mechanism is thought to involve the reduction of ferric oxide, which is rust colored, to ferrous oxide, which is jet black. A similar phenomenon may be involved in white and other iron-free inks that contain titanium. In the untreated tattoo, titanium is in the TiO2 form, which is bright white. High-intensity laser irradiation has been shown to result in the reduction of Ti4+ to Ti3+, which is responsible for the blue color. We demonstrated this color change by irradiating a titanium-enriched sunscreen.

Tope et al studied tattoo ink gels containing TiO2 and iron oxide and found that changes were both wavelength and pulse duration dependent. Most important, they were unable to induce tattoo ink darkening in tattoos with pulse durations greater than 1 millisecond, suggesting that a threshold power density is required for tattoo ink darkening.

Interestingly, there is evidence to support spontaneous bleaching of ink darkening in tattoos containing titanium. This observation is supported in vitro, where, on exposure to air, there is “bleaching” of the blue discoloration after 3 to 4 months.

White ink, composed of about 95% TiO2, is commonly used to brighten green, blue, yellow, and purple tattoos. The resulting mixtures are made such that green inks, for example, are typically composed of about 30% to 40% TiO2. These data were obtained in a recently completed pilot study with a newer energy dispersive x-ray microanalysis device. Therefore, it is possible that tattoos with a large titanium fraction turn deeply black with Q-switched laser treatment, whereas other tattoos with smaller titanium “burdens” darken so little with laser irradiation that they appear grossly not to lighten. With repeated treatments of green tattoos, one possible scenario is that the green “organic” portion, at least superficially, is being eliminated, whereas the titanium portion is darkening.

One of the difficulties in this study is correlating the electron microscopy and light microscopy results with the clinical findings. For example, we have noted that, in resistant green biopsy specimens, there is considerable remaining green ink at the base of the gross specimen, with less gross green color superficially. This was confirmed his-
is evidence that red light (alexandrite or ruby laser) is well responded. Thus, color is a confounding variable; unfortunately, most green tattoo inks contain TiO$_2$. It is interesting that clinicians generally note that green tattoos are the most difficult to treat. Often a green tattoo lightens about 30% after 2 to 4 treatments, only to fail to lighten considerably more with even up to 20 additional treatments. The lack of response in green tattoos is interesting, as there is evidence that red light (alexandrite or ruby laser) is well absorbed by green inks. It is unknown, however, whether TiO$_2$ is playing a role in resistance or, alternatively, whether there is simply a higher damage threshold for the organic azo-dyes commonly used as green tattoo inks.

Green tattoos are not singularly resistant to treatment; other ink colors are also characteristically resistant. For example, we have found that purple and yellow inks are difficult to treat, even though they, too, show at least fair absorption of one or another commonly used Q-switched laser; these ink colors also usually contain TiO$_2$. Probably, green has become such a nuisance color largely because of its prevalence. It is our experience that the 3 most common tattoo colors are black, green, and red. Of these, red and black respond well in most instances, and these inks typically contain no titanium.

The ultimate test of TiO$_2$ as an independent risk factor for resistance would be a comparison of green inks with and without TiO$_2$. However, although our study incorporated 6 colors, we believe that the overrepresentation of TiO$_2$ in poor responders and darkening tattoos is due at least partly to elemental composition and not solely tattoo color. Moreover, at least for the darkening tattoos, the microscopic correlation (increased stippling and heterogeneity of granules after treatment) and presence of TiO$_2$ make a compelling argument for titanium’s role in the gross observations. The results of this study should encourage other investigations of tattoo ink composition and its relation to laser treatment success. A larger, more systematic study is necessary to individually assess the roles of titanium, ink densities, ink depth, and ink color. Experiments in animal models with the placement of controlled tattoos with known concentrations of inks and metal components would be invaluable. If additional compounds are identified that darken with laser irradiation, it may be prudent for manufacturers to search for alternative dyes so that “permanent” but “laser-friendly” tattoos might be available in the future.

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REFERENCES