The Q-switched neodymium:YAG laser and tattoos: a microscopic analysis of laser–tattoo interactions

J.E.FERGUSON, S.M.ANDREW,* C.J.P.JONES* AND P.J.AUGUST
The Dermatology Centre, Hope Hospital, Stott Lane, Salford M6 8HD and *Department of Pathological Sciences, University of Manchester, Oxford Rd, Manchester, U.K.
Accepted for publication 10 April 1997

Summary
The Nd:YAG laser effectively removes or lightens amateur and professional tattoos. The biomechanics of the removal of tattoo particles at the cellular level are incompletely understood. We examined biopsies obtained from 35 amateur and professional tattoos (including coloured tattoos), treated on three or more occasions with the Nd:YAG laser. Biopsies taken immediately after laser treatment showed vacuolation with complete clearance of tattoo particles in the most superficial layers of the dermis, as assessed by light and electron microscopy. We propose that the ‘disappearance’ of the tattoo particle arises from the formation of atomic species and gaseous products, which are rapidly dissolved in the extracellular fluid. Residual fragmented particles that are commonly found in the mid- and lower dermis are rephagocytosed. The interaction between the Nd:YAG laser and black tattoo particles at 1064 nm, and red tattoo particles at 532 nm, appears to be specific, as there was little evidence of thermal damage to adjacent cells or stromal collagen.

Recent clinical studies show that the Q-switched neodymium:YAG (Nd:YAG) laser is an effective treatment for amateur and professional tattoos.1–3 The Nd:YAG laser emits radiation at 1064 nm and 532 nm; the infra-red emission (1064 nm) interacts with black and blue-black tattoo inks and most tattoos of this type can be removed without trace. The visible green emission (532 nm) interacts with some but not all red tattoo pigments. The risk of scarring at both wavelengths is low.

The action of the lasers principally used for removing tattoos, i.e. the Q-switched ruby (QSRL), the alexandrite and the Nd:YAG lasers, depends upon selective photothermolysis: brief laser pulses are preferentially absorbed by cells containing pigment, resulting in their selective destruction by heat. Thermal injury to adjacent non-pigmented cells and tissues is minimized.4

The fate of tattoo particles and the mechanism which renders tattoos invisible is poorly understood. Several mechanisms, based on histological studies of tattoos treated with the QSRL, have been proposed: tattoo pigment may be reduced by fragmentation and subsequent redistribution by inflammatory cells, or eliminated transepidermally by desquamation of the epidermis.5 It is unknown which, if any, of these factors contribute to tattoo lightening after treatment with the Nd:YAG laser.

Materials and methods
Tattoos were treated on at least three occasions at monthly intervals using a Q-switched Nd:YAG laser (Lynton Lasers Ltd, Manchester, U.K.). The operating characteristics of the laser were: wavelengths of 1064 nm and 532 nm with a spot size of 2 mm and 1.5 mm, respectively, a frequency of 1–10 Hz, pulse width 10 ns and the energy flux fixed at 10 J/cm².

All tattoos were initially irradiated at 1064 nm and those colours (red, green, yellow, orange, blue) failing to lighten were subsequently irradiated at 532 nm. Skin biopsies were obtained from 22 amateur and 13 professional tattoos, including coloured areas. Punch biopsies (3 mm) were taken from the tattooed site after injecting subcutaneously with 1% lignocaine and adrenaline. Two biopsies were obtained from each tattoo before treatment and two at one time (immediately, 2, 7, 30, 60 or 90 days) after treatment. The study was approved by the ethics committee of Salford Royal Hospitals and all subjects gave written informed consent.

One of each pair of biopsies was fixed in 10% formalin, processed for routine paraffin sections and stained with haematoxylin and eosin (H&E). Several sections were stained with Picrosirius red to enhance visualization of collagen fibres and with Gomori aldehyde-fuchsin to highlight elastin. The other biopsy

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was fixed in 4% glutaraldehyde in 0-1 mol/L cacodylate buffer (pH 7.3) and processed for electron microscopy. The sections were contrasted with uranyl acetate and lead citrate, or left unstained, and examined with a Philips EM 301 transmission electron microscope.

Results

Black tattoos before treatment

Light microscopy. There was no tattoo pigment in the epidermis; pigment particles were found predominantly within mononuclear cells in the dermis (Fig. 1a). Pigment-laden cells were clustered around small vessels in the papillary dermis and around vessels, hair follicles and sebaceous glands in the reticular dermis (Fig. 1b). Occasional cells containing pigment were found in the subcutaneous fat and around eccrine glands. Tattoo pigment was concentrated in the reticular dermis and was sparse in the papillary dermis. The depth of pigment varied within and between different tattoos. The mean depth measured from the granular layer was 2 mm and ranged from 1.1 to 2.9 mm. The dermal collagen appearance was not altered by the presence of the tattoo. Professional tattoos contained greater quantities of pigment than amateur tattoos. Dermal scarring was observed in one professional black tattoo before treatment.

Electron microscopy. At the ultrastructural level, tattoo pigment was exclusively intracellular; individual pigment particles and clumps of particles were sequestered within membrane-bound spherical structures, identified as secondary lysosomes. Lysosomes containing pigment were distributed throughout the cytoplasm of mononuclear cells, some of which could be identified as fibroblasts by the presence of abundant rough endoplasmic reticulum. Occasional pigment-laden secondary lysosomes were seen within mast cells and macrophages.

Black tattoo particles were electron dense, irregularly shaped with smooth edges, and varied enormously in size. Tiny particles (<0.1 μm) were found singly or in groups along with larger particles (up to 5 μm) and particle clumps (up to 100 μm) within the same lysosome. In addition, lysosomes contained some less electron-dense particles with indistinct outlines and amorphous material. Apart from the lysosomal contents, the ultrastructural features were normal.

Black tattoos immediately after laser treatment

Light microscopy. Laser treatment affected dermal cells containing tattoo pigment; cells with no pigment were unaffected. Vacuoles of various sizes (0.01–0.1 mm) were visible throughout the dermis (Fig. 1c). Vacuoles in the papillary dermis were well defined and contained tattoo pigment; cells with no pigment were unaffected. Vacuoles of various sizes (0.01–0.1 mm) were visible throughout the dermis (Fig. 1c). Vacuoles in the papillary dermis were well defined and contained

![Figure 1.](image-url)
no visible tattoo particles within the lumen or vacuole wall. The appearance of the vacuoles in the reticular dermis varied according to depth; the most superficial vacuoles had a rim of brownish material composed of fragmented pigment (Fig. 1d). Other vacuoles were within or adjacent to large clumps of residual tattoo. Vacuoles were formed as deep as 2 mm from the skin surface. Below this, tattoo pigment was intact, reflecting the limits of penetration of the laser at 1064 nm. In places, the epidermis was perforated by multiple bleeding points corresponding to the centre portion of the laser beam. These appeared to arise from small vessels which ruptured upwards through the epidermis. This was presumed to be a mechanical effect as there was little evidence of coagulation or vessel wall necrosis. Melanocytes were not affected by the laser at 1064 nm.

Electron microscopy. At higher magnification, the vacuole contents appeared homogeneous. Vacuoles were lined by remnants of pigment-containing cells and flattened cell nuclei (Fig. 2a). Some vacuolar walls contained fragmented extracellular pigment particles. Lysosomes containing pigment were disrupted in some fibroblasts, which were immediately adjacent to vacuoles. These cells contained altered pigment lying free in the cytoplasm and showed signs of necrosis as a result of thermal damage. Cells without pigment, which were adjacent to vacuoles, were not affected. Some of the dermal collagen fibres in a rim about 10 μm deep around vacuoles had lost their typical banding, providing evidence of thermal damage; outside this narrow rim the collagen, elastin and cells were normal (Fig. 2b).

Black tattoos 48 h to 90 days after treatment

Light microscopy. Dermal vacuoles were no longer visible; instead, there was a moderate mixed inflammatory cell infiltrate of neutrophils and lymphocytes around vessels and adnexal structures. Residual pigment, seen predominantly in professional tattoos, consisted of altered small brown fragments that were now extracellular. There were areas of scale-crust over some parts of the epidermis.

One week after treatment, epidermal surface scale was minimal or absent. There was a moderate chronic inflammatory cell infiltrate in the dermis. All tattoo particles had been phagocytosed and were now intracellular within fibroblasts and macrophages. After 1 month there was a mild residual inflammatory infiltrate around dermal vessels and adnexae. All residual pigment particles were within lysosomes. Many lysosomes contained visibly fewer particles than before treatment.

After 3 months, the inflammatory changes had settled and the skin appeared normal (Fig. 1e). Histological scarring, consisting of parallel bundles of collagen in the reticular dermis, with loss of adnexal structures, was seen in one red and one black tattoo. Light amateur tattoos were histologically clear after two or three treatments. Clearing in professional or dense amateur tattoos was gradual; the most superficial parts of the dermis were clear, middle parts were partially clear with residual altered pigment, and deep parts contained unaltered pigment (Fig. 1f).

Late biopsies (>6 months) were taken from patients with persistent faint outlines of tattoo that were unresponsive to a series of treatments. At tissue depths of >1.5 mm, tattoo particles were mostly

Figure 2. (a) Electron microscopy immediately after laser treatment. A large intracellular vacuole (V) is lined with residual pigment and compressed cellular components (arrow). Other cytoplasmic organelles (O) appear intact. (Uranyl acetate and lead citrate, x11,000). (b) Residual pigment (arrows) along the edge of a vacuole (V). Chromatin within the nucleus appears undamaged. Collagen fibres (C) adjacent to the vacuole show normal banding and structure. (Uranyl acetate and lead citrate, x38,000).
unchanged, indicating poor penetration of the laser into the mid- and lower dermis. This occurred even when treatment was continued after the upper dermis was relatively clear of tattoo.

**Coloured tattoos—before and after laser treatment**

*Light microscopy.* Only professional tattoos contained coloured pigments; green tattoos contained bright green pigment particles admixed with black pigment (Fig. 3a). After irradiation at 1064 nm, vacuoles were found within deposits of black pigment, but there was no evidence of laser interaction with the green component (Fig. 3b). With successive treatments, black pigment was completely removed. Clinically, this had the effect of brightening the appearance of the green tattoo. Yellow tattoos contained small and large brown particles. Orange, blue and turquiose tattoos contained particles of the corresponding single colour.

Red tattoos usually consisted of red particles, orange particles or red and black particles mixed together (Fig. 3c). Immediately after irradiation at 532 nm, vacuoles were visible to a maximum depth of 0·6 mm. Vacuoles in the papillary dermis were clear of pigment. As depth increased, vacuoles contained altered pigment or intact pigment at the edges. In the epidermis there was ballooning of individual melanocytes in the basal and suprabasal layers (Fig. 3d). Subepidermal bullae were seen occasionally but there was no evidence of epidermal necrosis.

*Electron microscopy.* Ultrastructurally, red tattoos comprised particles of medium to high electron density, up to about 0·3 μm in their longest dimension, enclosed within vacuoles of up to 3·5 μm in diameter that also contained amorphous material (Fig. 4a). There were visibly fewer electron-dense particles after laser treatment (Fig. 4b).

Blue and yellow tattoos were composed of two distinct types of pigment: an extremely electron-dense component with rounded edges and a less electron-dense component. The former measured up to about 0·6 μm in their longest dimension and were often contained within lysosomes up to 1 μm in diameter, although huge, apparently cohesive masses were seen. The latter, less electron-dense particles were enclosed in large vacuoles and were composed of tightly packed, overlapping, elongated structures up to 0·2 μm long. Yellow, orange and blue tattoos were not altered by laser treatment at 1064 or 532 nm.

**Discussion**

The Nd:YAG laser interacts specifically with tattoo pigment, with minimal damage to surrounding structures. Initial vacuolation of the dermis is followed by an

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**Figure 3.** Professional tattoos (all stained with H&E). (a) Green tattoo comprising green and black pigment particles within dermal mononuclear cells (×240); (b) vacuolation of a black but not green tattoo at 1064 nm (×240); (c) red tattoo containing red particles (×240); (d) vacuolation (arrows) of epidermal melanocytes (m) and upper dermis (d) immediately after laser treatment at 532 nm (×240).
acute and chronic inflammatory infiltrate, then rapid healing with complete reconstitution of the dermal architecture. Providing the bulk of the pigment lies within the upper and mid-dermis, black and red tattoos can be removed without trace. When substantial pigment remains in the deeper parts of the dermis, beyond the effective depth of penetration of the laser, this manifests clinically as a residual ‘ghost’ of the original tattoo.

The earliest visible manifestation of interactions of the Nd:YAG laser and tattooed skin is the appearance of an ash-white maculopapule, corresponding to the laser spot. The ash-white lesion is transient and fades to normal skin colour within minutes. This phenomenon of transient opaqueness of the skin also occurs after treatment of tattoos with the QSRL and the alexandrite laser, but its origin and resolution are poorly understood.

Biopsies of ash-white lesions show empty vacuoles throughout the dermis. Attempts have been made to correlate the appearance immediately after treating the tattoos with the histological observations; an early belief was that the initial opacity was due to coagulation of proteins in the surrounding tissues. Taylor et al. proposed that steam or gas was formed around pigment in the dermis. However, even brief exposure to steam would thermocoagulate tissues and, although minor damage to collagen was observed within a few micrometres of the vacuolar edges, the present study found no evidence of the gross thermocoagulation required to render the skin opaque. Furthermore, once thermocoagulation of collagen occurs, it is irreversible and thus cannot account for the return to normal skin colour only minutes after laser exposure.

We speculate that the vacuoles are occupied by gaseous products resulting from the fragmentation of both cells and pigment. The gas-filled spaces in previously continuous tissues cause an increase in the back-scattering of visible light from the skin, and hence whitening. The spaces then rapidly fill with extracellular fluid, which are closer in refractive index to the surrounding tissue than gas. The previously enhanced scattering of light resolves and the whitening fades.

The mechanism by which the pigment completely disappears is unknown. We speculate that the energy (10 J/cm²) absorbed by a pigment particle 25 µm in diameter would be enough to heat it to a temperature of >1000°C (J. Colles, personal communication). This would be sufficient to reduce pigment and immediately adjacent cell components to atomic species, resulting in a ‘mini-explosion’. The process of atomization and explosion, including the generation of sound (there is an audible detonation and a palpable shock-wave when the laser beam contacts the tattoo) would rapidly dissipate the heat, so that there is only minimal damage to adjacent collagen. The most superficial pigment is exposed to the highest laser energy and is totally converted into gas, which is rapidly dissipated in the extracellular fluid; if there are remnants of these particles, they are so small and infrequent that they were undetectable by electron microscopy. Deeper pigment exposed to a lower incident energy fragments into small particles that are distributed around the surface of the vacuole and then either rephagocytosed or ingested by tissue macrophages and fibroblasts. Below a certain level, energy transfer is insufficient to alter the tattoo pigment.

Dermal scarring occurred very rarely after laser...
treatment. This is remarkable considering the extent of vacuolation of the dermis immediately after laser treatment. At the ultrastructural level, visible changes in collagen were limited to within a few micrometres of the vacuole edge. The absence of disruption or necrosis of collagen suggests that vacuolation may cause reversible deformation of collagen without significantly altering the dermal architecture. Without significant tissue destruction, the stimulus to healing with fibrosis is minimized.

Red tattoos responded efficiently to the 532 nm output at a low energy density (2.5 J/cm²). The uniformity of the response to laser treatment is surprising given that red tattoos are heterogeneous. Some contain organic dyes and others contain elements such as Hg, Al and Cl in salt form. We would expect heating and laser-ablation characteristics to differ according to the composition of the red tattoo. The energy and parameters used were clearly above threshold for all our red tattoos but the extent of over-treatment is unknown.

Most other colours failed to improve after multiple treatments at 1064 and 532 nm, suggesting that these wavelengths were poorly absorbed. Unlike black tattoos which are carbon-based, coloured professional tattoos contain a wide variety of metals, including mercury (red), copper (blue), chromium (blue-green), iron (yellow and brown) and titanium (green), and organic dyes. The components of a particular colour are often specific to the tattooist. By examining the absorption spectra of individual pigments it is possible to ascertain which wavelengths are likely to be absorbed by the coloured pigment in situ. Unfortunately, the lasers currently available (QSRL, alexandrite and Nd:YAG) are effective only for black, green and red tattoos, and additional lasers would be required for other colours.

References