

INVESTIGATIVE REPORT

Titanium, a Major Constituent of Blue Ink, Causes Resistance to Nd-YAG (1064 nm) Laser: Results of Animal Experiments

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A blue tattoo is more resistant to laser therapy than black or brown tattoos. This study aimed to confirm titanium as a key response-disturbing constituent in a blue tattoo ink after Nd-YAG (1064 nm) laser treatment by animal experiments. Rabbits' backs were tattooed with four ink colours, and the Nd-YAG (1064 nm) laser was used to remove the tattoos. The response to the laser treatment in the rabbits was evaluated and electron microscopic studies were also performed. Excellent to fair responses were observed for the black, brown and dark brown inks, but the blue ink responded poorly to the laser. Histological examination indicated that the blue pigments were unchanged even after the laser treatment. Quantitative energy dispersive spectrometry revealed that blue ink contained high amounts of titanium. Our animal experiments confirm that a blue tattoo ink containing titanium, is a key element in poor response to the Nd-YAG laser. Key words: blue tattoo; Nd-YAG; titanium.

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Laser systems have become a major therapeutic tool in dermatology, and several lasers have been used effectively to remove tattoos. Currently, the Q-switched ruby laser, Q-switched neodymium:yttrium, aluminum, garnet (Nd:YAG) laser and Alexandrite laser have been widely used (1). The Q-switched Nd-YAG laser is available with two wavelengths, 1064 nm and 532 nm, which can treat black and red tattoo inks, respectively (2, 3).

In Korea, professional tattoos using various coloured tattoo inks are not so popular, but amateur tattooing along eyebrows is widely practised for cosmetic reasons (4). For this purpose, black inks are commonly used. However, brown inks are preferred nowadays due to their natural colouring, and even blue inks are increasingly being used. Amateur tattooing by poorly trained people or with impure tattoo inks results in many medical complications including tattoo granuloma, which need to be treated by the laser removal of tattoo inks. Despite the

use of laser treatment as a standard method to remove tattoos, some tattoo inks are difficult to remove, due partly to oxidative-reductive changes in the metal components of the tattoo inks with laser excitation (5). For example, ferric oxide, which is included in pink, red and flesh-coloured inks, can result in a black discoloration after Q-switched ruby laser treatment. Titanium, which is present as a form of TiO₂ in green, blue and yellow inks, was reported to be another response-disturbing material in laser treatment (6, 7).

In this study, the major constituents of different coloured tattoo inks were analysed by quantitative energy dispersive spectrometry (EDS), and the response to the Nd-YAG laser was compared in four different coloured tattoo inks in animal experiments. From this study, we could confirm that titanium is a major component of blue tattoo ink which induces resistance to treatment with Nd-YAG laser.

MATERIALS AND METHODS

Animals and tattoo inks

White rabbits (New Zealand White strain) weighing approximately 3 kg were used for the tattoo experiments. Rabbits were fed commercial rabbit chow and tap water available ad libitum, and kept at room temperature and in normal light conditions. Four tattoo ink colours (black, dark brown, brown and blue) were purchased from a commercial ink supplier via an internet shopping mall (www.body-art.co.kr, www.capricornslair.com and tattoosuperstore.com). The Q-switched Nd-YAG laser (Medlite, Continuum Biomedical Inc, Livermore, CA, USA) was used for experiments. Animal experiments were performed as requested by rules of the Committee for Animal Experimental Study of the Chonnam National University Medical School. Artificial tattooing with the four different coloured inks was repeatedly performed on the backs of five rabbits.

Methods

Both qualitative and quantitative analyses of ink constituents were performed using a scanning electron microscope (S-4700, Hitachi, Japan) equipped with EDS (Sigma, Kevex, CA). After anaesthetizing the rabbits with ketamine and xylazine, the rabbits' backs were shaved with an electric razor. Tattooing with the four different coloured inks was repeatedly performed on the backs of five rabbits. Tattooing was performed with the inks using a conventional 26-gauge needle, in a band-like manner along the back. Each band was approximately 8 cm in length and 0.5 cm in width. After 4, 8 and 12 weeks, the left-hand halves

of the bands were treated with the Nd-YAG laser with 5.5 J/cm² fluence at a wavelength of 1064 nm, with pulse duration of 5 nsec and an exposure spot size of 3 mm. Both the spot size and the output energy remained constant throughout the study. The degree of tattoo lightening was arbitrarily graded by the naked eye as follows: clear (<95%), excellent (76–95%), good (51–75%), fair (25–50%) and poor (<20%). For histological study, 4-mm punch biopsy specimens were taken from the treated and non-treated sites at the ninth week after tattooing. Each biopsy specimen was divided into two pieces. One piece was fixed in 10% buffered formalin, embedded in paraffin and then stained with haematoxylin and eosin. Histologically, the ink particles were identified as black dots of irregular shapes by optical microscopy, and the efficacy of the laser treatment was quantitatively compared with the non-treated area. The other piece was fixed in 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer (pH 7.4) and post-fixed with 2% osmium tetroxide. The tissue was dehydrated and embedded in epoxy resin. An ultra-thin section was then cut using a microtome, placed on a copper grid and stained with uranyl acetate and lead citrate. The tissue was then examined with a transmission electron microscope (100CXII, JEOL, Japan).

RESULTS

The results of the quantitative EDS analysis are listed in Table I, in which the concentration of each element in the tattoo ink compounds is shown as an atomic percentage. As previously reported (5), the blue ink contained a significant amount of titanium, and the brown and dark brown inks contained a considerable amount of iron.

The lightening response of tattoo inks to the Nd:YAG laser differed according to their colours (Table II). As described previously (1), black tattoo ink responded well to the 1064 nm Nd-YAG laser with clear to excellent degrees of response. However, brown and dark brown tattoo inks responded moderately to the laser, demonstrating good to fair degrees of response. In particular, the tattoo colour produced by the blue ink was hardly removed by the Nd:YAG laser. In histological studies, black pigments became sparse and faint in the dermis after laser treatment, but blue pigments were unchanged after laser treatment (Fig. 1). Electron microscopy

Table I. Relative amounts of elemental compositions of four different coloured tattoo inks as determined by quantitative energy dispersive spectrometry analysis

Colour*	Element (%)									
	Ti	O	C	Fe	Al	Cl	Si	Cu	K	Na
Blue	36.8	31.9	20.7		4.8	0.5	4.5	0.9		
Brown		13.1	14.2	72.7						
Dark brown		23.3	7.9	65.4	3.4					
Black		43.6	32.8	1.0		2.0	10.1		1.4	9.0

Ti, titanium; O, oxygen; C, carbon; Fe, iron; Al, aluminium; Cl, chloride; Si, silica; Cu, copper; K, potassium; Na, sodium.

*Tattoo ink colour from www.body-art.co.kr, www.capricornslair.com and tattoosuperstore.com. See Materials and methods section.

Table II. Response of four different coloured tattoo inks to the Nd-YAG laser (1064 nm)*

Animal	Colour of pigments			
	Black	Brown	Dark brown	Blue
Rabbit 1	Excellent	Good	Good	Poor
Rabbit 2	Cleared	Good	Fair	Poor
Rabbit 3	Excellent	Good	Fair	Poor
Rabbit 4	Excellent	Fair	Fair	Fair
Rabbit 5	Cleared	Good	Fair	Poor

*The degree of tattoo lightening was arbitrarily graded as follows: clear (>95%), excellent (76–95%), good (51–75%), fair (25–50%) and poor (<25%).

revealed that the black particles were phagocytosed by macrophages, producing many phagosomes in the cytoplasm (Fig. 2).

DISCUSSION

The selective photothermolysis of pigmented structures was first achieved by treating tattoos with the Q-switched ruby laser (8). Currently, three types of laser systems are commonly used to treat tattoos; the Q-switched ruby laser, Q-switched Nd-YAG laser and Alexandrite laser (1, 9, 10). Because of the selective absorption characteristics of tattoo pigments and the wavelength of each laser, no single laser can remove all the tattoo colour effectively (1). The absorption of laser energy is the highest in the carbon particles contained in black inks (8).

There is a significant difference in the degree of lightening after laser treatment in terms of the colours of tattoo inks. The Q-switched Nd-YAG laser (1064 nm) is known

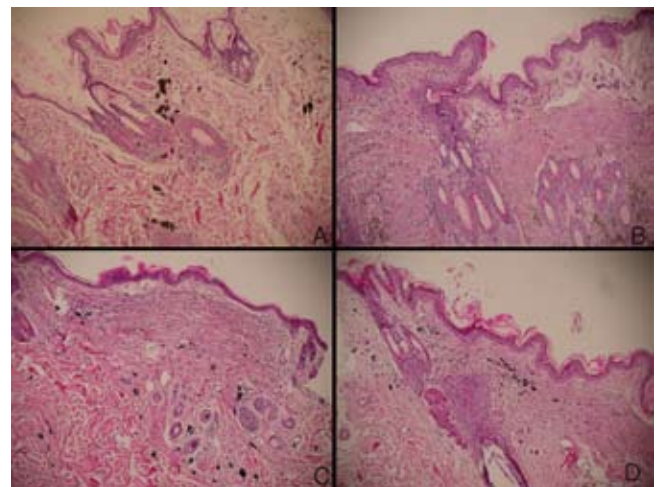


Fig. 1. Histological findings of the black and blue tattoos in the rabbit skin before and after laser treatment (9 weeks after tattooing). Black tattoos in the dermis before laser treatment (A) and absent after treatment (B). Blue tattoos in the dermis before laser treatment (C) and remaining after treatment (D) (original magnification ×100).

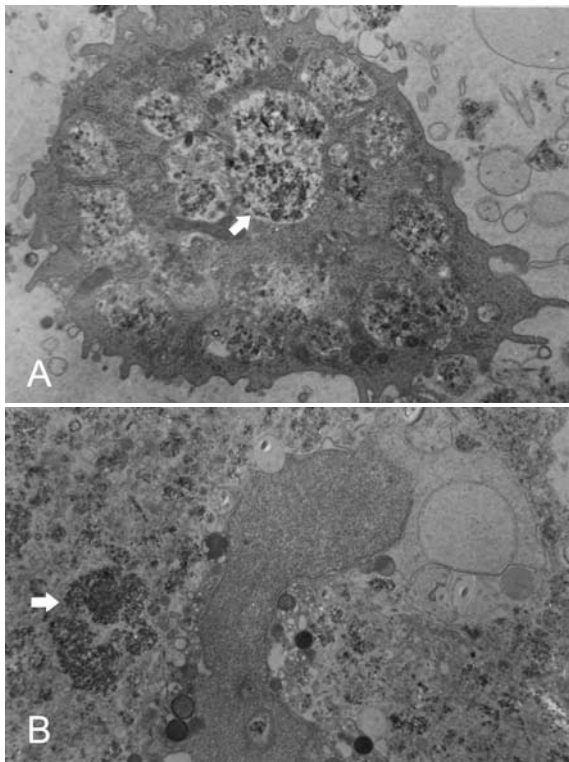


Fig. 2. (A) Electron microscopic findings of the Nd-YAG treated black tattoo. Black particles were contained in phagosomes of the cytoplasm (arrow) (original magnification $\times 8000$). (B) Higher power view of the same sample showing the presence of particles in a phagosome (arrow) (original magnification $\times 15\,000$).

to effectively treat almost all colours except for red and green inks (8). In our animal experiments, blue tattoo ink was confirmed to be the least responder to the Nd:YAG laser compared with other tattoo inks. Quantitative X-ray microanalysis confirmed that the blue ink contained titanium, which was not found in other coloured inks.

TiO₂ is added to various paints and tattoo pigments as a brightening agent, and is also a common ingredient in sunscreens (5). In its particulate form, TiO₂ is a good reflector to lighten the colour due to its reflecting and light scattering effects. Therefore, many light-coloured tattoos have a high probability of containing TiO₂. Unfortunately, TiO₂ is known to have a darkening reaction after absorbing laser light, which is based on the reduction of TiO₂ (5, 11). Recently, Ross et al. (7) – using X-ray diffraction and paraffin-embedded specimens – suggested that TiO₂ may play a role in the non-response of tattoos to therapy. In a previous study on the composition of cosmetic tattoos that have been sold in the USA, TiO₂ was found in almost all blue tattoo inks (5). In our study, a commercially available blue ink was revealed to contain a high concentration of titanium (36.82%) by quantitative electron X-ray microanalysis. It is still uncertain as to whether the poor lightening of titanium-containing tattoo inks originates from the darkening of TiO₂ alone or reflects

another mechanism involving the photocatalytic activity of titanium-containing inks (11).

The other component known to cause the laser darkening of tattoo inks is ferric oxide (12). Ferric oxide, which is present in pink, red and flesh-coloured inks, was reported to produce a black discoloration after treatment with the Q-switched ruby laser. The discoloration was suggested to be caused by the reduction of rust-coloured ferric oxide to jet-black-coloured ferrous oxide (12). Consistently, in our experiments, X-ray analysis indicated a large amount of iron (65.4–72.7%) in the brown and dark brown inks, causing moderate response to the Nd:YAG laser.

The mechanism of tattoo lightening or disappearance after laser treatment is not clearly understood. It has been suggested that photopyrolysis (irreversible high-temperature chemistry) of the phagocytosed ink particles and/or the photoacoustic disruption of particles are the principal events, which then enable the particles to be transported away either through the lymphatic vessels, transcutaneous elimination via the epidermal damage, or by the macrophage-mediated rephagocytosis (3, 13, 14). Our data showed that phagocytosed black ink particles were contained in the macrophages (Fig. 2), which supports the rephagocytosis of the tattoo particles by macrophages.

In conclusion, different responses to the Nd:YAG laser were found among coloured tattoos, probably due to differences in constituents of the inks. In particular, titanium-containing blue tattoo inks should be used cautiously in eyebrow tattooing, because some populations have allergic or foreign body reactions to the tattoo, which need to be removed by the Nd:YAG laser.

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