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Quantitative evaluation of enhanced laser tattoo removal by skin optical clearing

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Lasers have been widely used for tattoo removal, but the limited light penetration depth caused by high skin scattering property restricts the therapeutic outcome of deep tattoo. Skin optical clearing method, by introducing optical clearing agent (OCA) into skin, has shown some improvement in the effect of laser tattoo removal. In this study, the enhanced laser tattoo removal has been quantitatively assessed. OCA was applied to the skin of tattoo animal model and Q-switched Nd:YAG laser (1064 nm) irradiation was used to remove the tattoo. The skin evaluation instrument (Mexameter probe, MPA580) was applied to measure the content of tattoo pigment before and after laser treatment, and then the clearance rate of pigment was calculated. Further, Monte Carlo (MC) method was utilized to simulate the effect of skin optical clearing on light transmission in tattoo skin model. By comparing the pigment change of tattoo areas respectively treated with OCA plus laser and single laser, it was found that pigment clearance of the former tattoo area was increased by 1.5-fold. Further, the MC simulation verified that the reduced light scattering in skin could increase the effective dose of luminous flux reaching to the deep tattoo regions. It can be concluded from both experiment and theoretical

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simulations that skin optical clearing technique could improve the outcome of laser tattoo removal, which should be beneficial for clinical laser tattoo removal and other laser pigment elimination.

Keywords: Skin optical clearing; laser tattoo removal; Q-switched Nd:YAG laser; Monte Carlo simulation.

1. Introduction

The issue of tattoo removal has evolved in concert with the evolution of tattooing.[1] Dermabrasion,[2] salabrasion,[3] liquid nitrogen[4] and thermal methods[5] were firstly used to remove tattoo. But they all belong to destructive methods. Lasers have been used to treat tattoo since 1970s, including argon laser (488 and 514 nm), CO_2 laser (10,600 nm)[6–8] and Q-switched lasers such as Q-switched ruby laser (694 nm),[9] alexandrite laser (755 nm) and Nd:YAG laser (532 and 1064 nm).[10] As the size of tattoo particles ranges between 40 and 300 nm,[11,12] the Q-switched lasers would restrict the thermal injury to the targeted tattoo pigment, allowing for selective photothermolysis.[1,8,13,14]

However, single laser treatment of tattoo should not work very well actually because of the high scattering property of skin as well as the hemoglobin absorption in the tattoo regions. Generally, multiple therapeutic sessions are typically required.[1,14,15] For large, deep and complex tattoos, much more sessions and increased laser intensity are recommended to compensate the energy loss caused by tissue scattering and absorption,[16] yet which can cause many side effects such as purpura, blistering and damage to the surrounding tissues.[15,16]

The skin optical clearing method, by introducing optical clearing agent (OCA) which is of high refractive index, hyperosmosis and biocompatibility into skin, could reduce skin scattering,[17–19] and has been reported to be effective to improve laser tattoo removal. Prior application of the OCA (PPG:PEG) has been shown to be effective in improving the outcome of tattoo removal by Q-switched 532 and 694 nm laser treatment.[20] But the report was just from one case. Besides, the optical clearing method was effective to increase the tattoo image contrast. Monte Carlo (MC) simulation predicated that 60–70% radiation power would remove the tattoo if the optical clearing method was used.[16] However, the opinion needs to be further demonstrated by experiments.

In this study, the skin optical clearing method was used to improve the effect of tattoo removal with Q-switched Nd:YAG laser (1064 nm) irradiation. The skin evaluation instrument (Mexameter probe, MPA580) was applied to quantitatively assess the improvement of laser tattoo removal by skin optical clearing method. Further, the MC method was utilized to simulate the effect of skin optical clearing on light transmission, and the radial distribution and luminous flux at tattoo layer were calculated.

2. Materials and Methods

2.1. *Tattoo animal model*

This animal study was approved by Institutional Review Board for Animal Study of Huazhong University of Science and Technology. Sprague-Dawley (SD) rats, 100–120 g body weight, purchased from Hubei Health and Epidemic Prevention Station (Wuhan, China), were fed under specific pathogen-free (SPF) conditions. After three days of environment adaptation, animals were intraperitoneally anesthetized using a mixture of chloral hydrate (0.02 g/mL) mixed with ethylurethanm (0.1 g/mL). The dorsal hair of rats was depilated by a depilatory cream (Veet hair removal cream, Reckitt Benckiser, produced in China) before experiments. Then the dorsal skin was divided into 9–12 areas, for each area about a total of 0.2 mL Indian ink was intradermally injected with a micro-syringe. About 7 to 10 days later, regrown hair was depilated again and the animals were used for experiments.

2.2. *Experimental protocols*

In this study, Q-switched Nd:YAG laser (1064 nm, made by Eraser-K, MEDITECH, Korea) was used for tattoo removal, with laser pulse energy of 20, 40, 60, 80 and 120 mJ, spot diameter of 4–5 mm and frequency of 1 Hz. The first aim was to screen optimal treated parameter which could eliminate tattoo pigment effectively and cause little side effects.

Next, the effect of OCA on laser tattoo removal was investigated. Tattoo skin of animal models were cleaned with alcohol and stripped for three times with adhesive tape. The tattoo regions were divided into three groups, OCA plus laser treated group, single laser treated group and untreated group. Then high-effect OCA, composed of PEG400 and Thiazone (19:1, v:v) was applied to experimental tattoo regions.[21] After massaging for 15 min, laser irradiation was applied. At the end of treatment, saline was used to wipe off the residual OCA.

2.3. *Evaluation of pigmentation clearance*

The method of scale score was adopted to evaluate the effect of laser tattoo removal with different pulse energies. Score of 0, 1, 2, 3 and 4 mean pigment clearance degree of no change, slightly lessening, lessening, obvious lessening and significant lessening, respectively. Similarly, the same scale method was also used for evaluation of side effects.

Further, the pigment detecting probe Mexameter 18 (MX18) of MPA580 (Courage + Khazaka (CK) electronic GmbH, Germany) was utilized to measure the pigment before and after laser treatment. During the measurement, the MX18 was contacted vertically and tightly to tattoo skin surface until the result was recorded. Then the probe was relaxed and slightly moved to the nearby sub-area. The melanin value of each area was the average of six sub-area measurements. And a total of six tattoo areas for each group (PT + laser, laser and intact) were measured. The measurement principle is mainly based on the absorption principle. Light with three defined wavelengths (green light: 568 ± 3 nm; red light: 660 ± 3 nm; infrared light: 870 ± 10 nm) is emitted by MX18 to the skin surface, and the reflected light is measured by a receiver. The pigmentation (melanin) was calculated based on different absorption rates at 660 and 880 nm, shown as Eq. (1).[22,23]

$$M_v = \frac{500}{\log 5} \times \left(\log \frac{R_{880}}{R_{660}} + \log 5\right). \quad (1)$$

Here M_v means the melanin value, and R_{660} and R_{880} mean the skin reflectance at wavelength of 660 and 880 nm, respectively.

And the clearance rate (C_r) of tattoo pigment was calculated as Eq. (2).

$$C_r = \frac{M_{v0} - M_{v1}}{M_{v0}}. \quad (2)$$

The M_{v0} and M_{v1} mean the content of tattoo pigment before and after the treatment, respectively. The treatment included OCA plus laser and single laser irradiation.

2.4. *MC simulation*

The effect of skin optical clearing on photon delivery in tattoo skin was simulated by MC method under various skin optical properties.[24,25] Four layers skin model, including epidermis, upper dermis, tattoo layer and lower dermis, was used in the simulation. The parameters of refractive index, absorption coefficient, scattering coefficient and anisotropic factor (n, μ_a, μ_s, g) are shown in Table 1.[16,26,27] As the principle of skin optical clearing is replacement of tissue matrix by OCA, inducing increase of refractive index of the tissue background,[17,28] the scattering property of tissue was thus changed as Eq. (3) to characterize the skin optical effect.

$$n = n_0 + (n_{\mathrm{OCA}} - n_0) \times k \quad (3)$$

Here, n means the total refractive index of skin, n_0 and n_{OCA} means the initial refractive index of different skin layers and OCA, respectively. The k means the percentage of matrix water replaced by OCA, from 0% to 80%, with 20% interval.

Further, the change of radial distribution, represented as the change of radius (r) corresponding to $1/e$ light flux, and the improvement of luminous flux at tattoo layer were calculated.

Table 1. Optical parameters of different layers of skin model.

Skin layers	n	μ_a (cm^{-1})	μ_s (cm^{-1})	g	Thickness (mm)
Epidermis	1.45	0.34	182.21	0.89	0.1
Dermis 1	1.35	2.96	80.43	0.9	0.9
India ink	1.46	33.3	3.33	0.9	0.05
Dermis 2	1.35	1.96	80.43	0.9	0.75

3. Results

3.1. *Pigment change and side effects of laser tattoo removal with different radiated doses*

Q-switched Nd:YAG laser with different radiated doses were firstly used to remove the tattoo. The change of tattoo pigment and side effects are shown in Table 2. In general, the higher the laser energy, the more score the laser tattoo removal, revealing the better tattoo clearance effect. Nevertheless, higher laser energy leads to high risk of side effects such as blister and bleeding, shown as higher score of side effects. It can be seen that laser irradiation with 60 mJ could clear tattoo pigment obviously with only slight side effects.

3.2. *Enhancement of laser tattoo removal by skin optical clearing effect*

Typical macroscopical result of the effect of OCA on laser tattoo removal is shown in Fig. 1. The tattoo pigment has been obviously cleared in region 1 which has been pretreated with OCA and then laser irradiation, with only pigment residual in the injected points is left. The tattoo pigment in region 2 has also been cleared to some extent, but there is still pigment in skin in and around the injected sites. For the untreated region 3, the tattoo region shows no detectable change.

Table 2. Score scale of tattoo pigment change and side effects of laser tattoo removal with different energies.

Wavelength (nm)	Energy (mJ)	Laser beam (mm)	Frequency (Hz)	Pigment change (score)	Side effect (score)
1064	20	4	1	1 ± 0.4	0.2 ± 0.32
	40	4	1	2 ± 0.4	0.4 ± 0.42
	60	4	1	3 ± 0.33	0.83 ± 0.27
	80	4	1	3.6 ± 0.48	1.8 ± 0.32
	120	4	1	3.8 ± 0.32	2

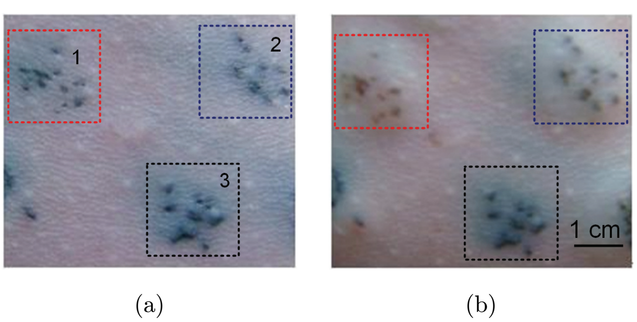

Fig. 1. Improvement of the effect of laser tattoo removal by skin OCA. (a) and (b) reveal the macroscopic pigmentation before and after treatment, respectively. Area 1: PT + laser irradiation; area 2: laser irradiation; area 3: without treatment. Scale bar: 1 cm.

3.3. *Quantitative evaluation of the effect of OCA enhanced laser tattoo removal*

The pigment value of tattoo regions before and after treatment was measured by Mexameter 18, and the clearance rate of pigment was calculated, as shown in Fig. 2. As for the tattoo region pretreated with OCA and then with laser irradiation, the clearance rate of pigment reaches up to 82%, while the single laser treated area shows only 55% of pigment clearance rate. It can be said that the OCA pre-treatment of skin could enhance the effect of laser tattoo removal by about 1.5-fold. What is more interesting, the intact control area without treatment also shows some degree of pigment decrease.

3.4. *MC simulation of the improvement of light distribution in tattoo area by skin optical clearing*

Improvement of light delivery into tattoo region by skin optical clearing was shown in Fig. 3. At the initial state, the light distribution in four layers is

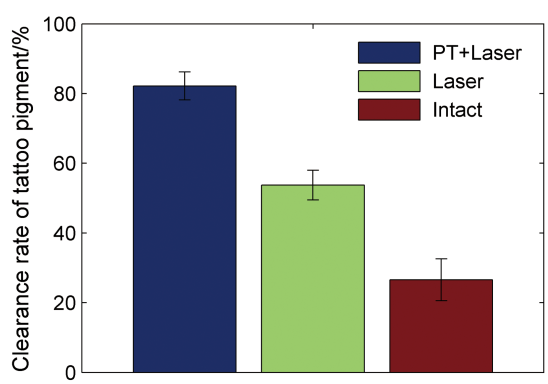

Fig. 2. Quantification of pigment clearance in tattoo areas with different treatments. PT + laser: the area treated with OCA of PT then irradiated with Q-switched laser (1064 nm, 60 mJ); Laser: tattoo area treated with single Q-switched laser (1064 nm, 60 mJ); Intact: tattoo area without treatment. Data shown as mean ± SD ($n = 6$).

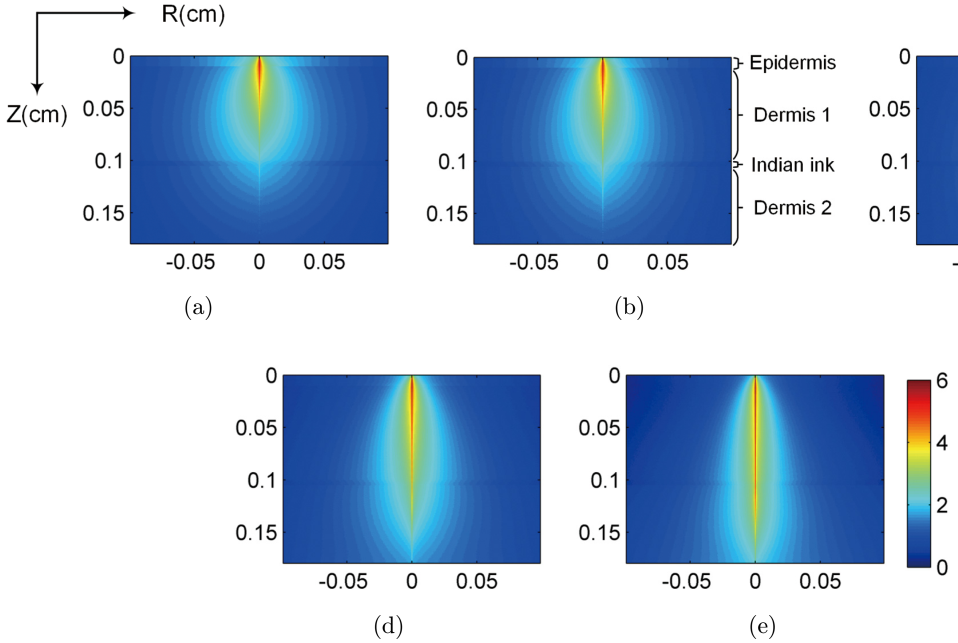

Fig. 3. Distribution of light flux in the tattoo model with different degree of skin optical clearing simulated by MC method. (a) initial state; (b)–(e) skin with optical clearing efficacy of 20%, 40%, 60% and 80%, respectively. The tattoo model is of four layers: epidermis, dermis 1, Indian ink layer and dermis 2. All figures have the same color bars.

quite different. Most photons are distributed in the epidermis and upper dermis [Fig. 3(a)]. Then more and more photons get to the deeper tattoo regions and lower dermis along with the increase in skin optical clearing efficacy from 20–80% [Figs. 3(b)–3(e)], leaving less photons distribute in the epidermis and upper dermis, which means that the distribution of luminous flux in different layers of skin are more homogeneous.

Besides, from the change of radiation radius (r) corresponding to $1/e$ light flux with the increase of skin optical clearing, it can be found that the r is decreased from 650 to 175 μm, revealing the radial distribution of light is narrowed [Fig. 4(a)], which means the light is more targeted. The further quantification of light flux in tattoo layer shows that the total luminous flux is increased by about 14.9%, 31.2%, 46.2% and 58.7% with the increase of

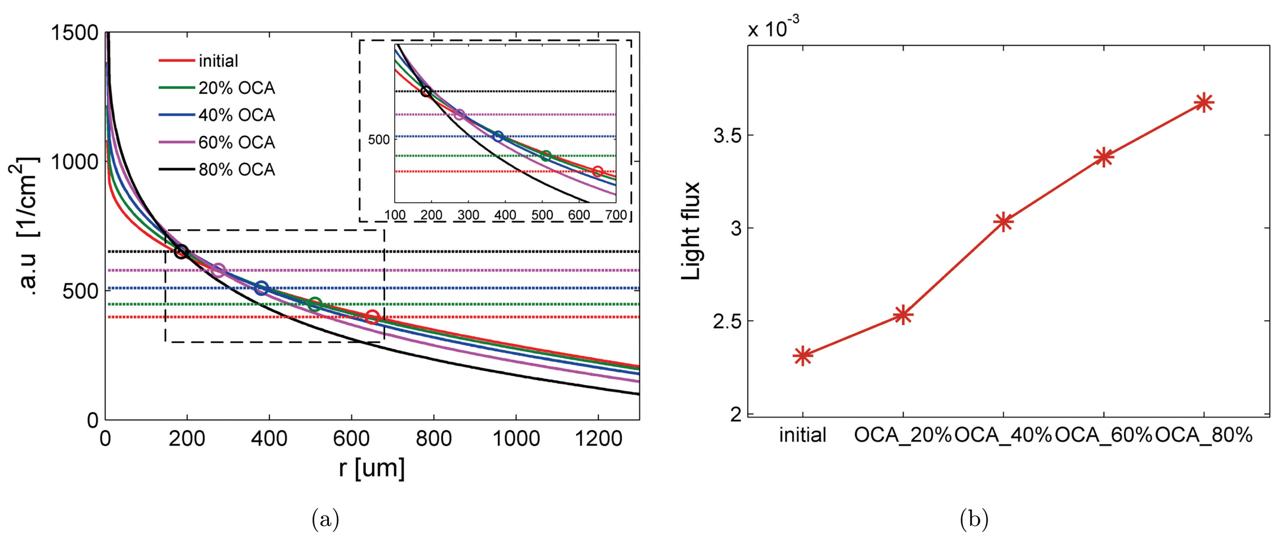

Fig. 4. Improvement of light distribution in skin by optical clearing effect. (a) Change of radial distribution and (b) improvement of light flux at tattoo layer by skin optical clearing effect from 20% to 80%.

optical clearing efficacy to 20%, 40%, 60% and 80%, respectively [Fig. 4(b)].

4. Discussion

Lasers have been widely used to remove tattoo pigments. However, the high scattering property of skin to light limits the curative effect, particularly for large and deep tattoos. In this study, the skin optical clearing method has been attempted to enhance the effect of laser tattoo removal.

As we know, tattoo pigment granules are mainly placed in the mid-dermis, a perivascular region, so there are great demands for lasers. On one hand, the laser radiation can penetrate deep enough to achieve the tattoo region, on the another hand lasers can be absorbed strongly by the pigment granules.[1] In these circumstances, the Q-switched lasers with red and near-infrared emitting wavelengths have become the best choice in recent years for tattoo removal.

In this study, the Q-switched Nd:YAG laser (1064 nm) with different irradiated doses were performed to remove tattoo pigment. Similar to the one reported previously, the 1064 nm Q-switched Nd: YAG laser irradiation could clear tattoo pigment to some extent, and the higher the laser energy, the better the tattoo clearance effect.[29] However, it was found that there was still some pigment residual after the laser treatment (Fig. 1, region 2), which should account for the required multiple therapeutic sessions.[1,14,15] Besides, it was also found that higher laser energy could lead to higher risk of side effects such as blisters and bleeding (Table 1).

Instead, the pre-treatment of tattoo region with OCA could enhance the tattoo clearance by subsequent laser irradiation with the same parameters. In this study, the pigment probe MX18 of MPA580 was used to quantitatively evaluate the degree of tattoo removal. The clearance rate of tattoo pigment is more than 1.5 times (Fig. 3) for the combination method of OCA pretreatment with laser irradiation than that for single laser irradiation. The improvement of tattoo removal is similar to that shown in a case report.[20] However, their result was merely shown in photographs and was intuitive though more commonly used. The quantitative measurement of tattoo pigment in this study could be helpful for the correct evaluation of the degree of tattoo removal.

As we know, OCAs have hyper refractive index. The infiltration of OCA into skin dermis could replace the water in dermal matrix,[28,30] and make collagen to dissociate,[31] which would match the refractive indices of different components and thereby decrease the light scattering within skin, making the skin to be light transparent. The decreased tissue scattering, shown as results of the MC simulation, allowed more effective photons to get deep into the targeted tattoo layer and increased the luminous flux in tattoo layer, facilitating the pigment clearance and disintegration [Fig. 1, region 2; Figs. 4(b)–4(e)]. In other words, the optical clearing method could decrease the required power for tattoo removal.[16] Besides, the much deeper penetration of photons to tattoo layer will lead to less damage to the upper skin layers.[20]

There was another interesting finding from the quantification of laser tattoo removal that skin pigmentation was also decreased by about 22% in the untreated area. This should due to the native defensive function, which is the essential phagocytosis of external pigment by macrophages.[32] Besides, the self-renewal of epidermis by upward moving of basal layer proliferation and differentiation as well attenuation of some of the pigment granules. What is more, the fast re-growing hair in the depilated dorsal skin was also supposed to play a role in pigmentation decrease.

5. Conclusion

In this work, both experiment and theoretical simulations were taken to quantitatively study the effect of skin optical clearing on laser tattoo removal. It was found that the pretreatment of skin by OCA application improved the effect of laser tattoo removal, increasing the pigment clearance by 1.5 times. Further, the MC simulation showed that the reduced light scattering in tissue could increase the effect of luminous flux reaching to the deep tattoo regions, and increase the tattoo targeting. It can be concluded that the skin optical clearing method could improve the effect of laser tattoo removal, and should be beneficial for clinical laser therapy.

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