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Efficient Picosecond Laser for Tattoo Removal in Rat Models

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Background: Tattoos are popular in modern times. Due to the occurrence of adverse effects such as poor aesthetic value, scar hyperplasia, and abnormal pigments, there is a high demand for uniform operation standards as well as standards for tattoo technologies. In the present study we used Sprague-Dawley rats to assess the tattoo removal efficacy of use of a picosecond laser at various energy values.





Material/Methods: Tattoos were made on the backs of rats, then we used a picosecond laser set at various energy parameters to remove the tattoos. After performing the removal procedure in multiple groups, we selected the most suitable energy levels with corresponding parameters for the tattoo removal. We recruited human volunteers who wanted their tattoos removed and used the energy level found to perform best during tattoo removal experiments. The tattoo removal effects were evaluated and verified. Four tattoo volunteers were treated by using the optimal energy parameters for picosecond laser technology.

Results: Through characterization observation and pathological staining results, it was demonstrated that the 1.9 mJ/μbeam energy laser had the best hollowing effect and the most complete pigment particle crushing effect in the rat skin, and had the best tattoo removal effect.

Conclusions: We leveraged the evaluation standard to choose the most suitable energy value of the picosecond laser, which had a good tattoo removal effect and could be employed as a reference for clinical removal of tattoos. This process provides criteria for tattoo removal evaluations as well as alternatives for tattoo removal in clinical practice.

MeSH Keywords: **Laser Therapy • Lasers, Dye • Skin Abnormalities • Tattooing**

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Background

Tattoos are an ancient custom that remains particularly popular in many local regions nowadays. However, more and more people with tattoos desire to have them removed for various reasons, including dissatisfaction with the color or adverse reactions. At present, there is a high demand of uniform operation standards in clinics as well as standards for tattoo removal technology. There is also a lack of quality standard supervision over the production of tattoo dyes, which has led to more and more problems such as poor aesthetic value, abnormal pigments, scar hyperplasia, and poor healing. Bad dye implantation can even cause cancers such as skin cancer. A growing number of patients desire to have tattoos removed safely, quickly, and effectively. To remove their own tattoos, dermatologists have used electrocution, grinding, freezing, and skin grafting to remove them, but the results have not been satisfactory. In the 1960s, with the advent of laser technology, Laub et al. introduced use of ruby lasers to remove human tattoos [1], and the applications of various laser technologies on tattoo treatments emerged gradually from then on. The Q-switch laser treatment has recently become the method of choice for tattoo removal.

Although the selective heat effect of the Q-switch laser has the advantage of breaking the pigment as well as reducing skin damage, there are problems such as long treatment intervals, long treatment times, and nodus in the treatment of the body pigment, as well as outline residue. Scarring and texture changes are potential irreversible complications of tattoo removal [2]. The organic action of short-pulse oscillating (nanosecond) ruby/alexandrite/YAG lasers employed in benign pigmentation disorder treatments is photomechanical and photothermal. Tattoo therapy needs to destroy the tattoo particles without too much photothermal effect. The thermal relaxation time of tattoo ink is 10–1000 picoseconds. With nanosecond lasers, treatment effectiveness is low and the residue rate is relatively high [3]. Moreover, since the Q-switch laser is based on the theory of selective photothermal therapy, only melanin is the target base, but it is still not suitable for the removal of professional tattoo dyes such as red and green. Therefore, the search for more extensive, efficient, and low-damage tattoo removal has become important in clinical practice.

Picosecond lasers are widely used by clinical practitioners to remove tattoos. Short picosecond pulse width in picosecond laser treatment has more photomechanical or light breakdown effects, which crush the pigment particles more thoroughly with low energy density. In addition, due to short pulse width and low energy density, the tissue damage around the target base is minimized using a picosecond laser, which improves the curative effect and reduces the adverse reactions [4]. The picosecond laser has distinct advantages in eliminating

tattoos, especially stubborn tattoos and intractable yellow tattoos [5–7]. Also, the picosecond laser has good performance in light aging treatment [8–11]. However, the application of picosecond lasers in the field of dermatology is still at its initial stage, and further research is needed to achieve better efficacy. Therefore, it is critical to conduct proactive comparative studies on the standardization of treatment settings and treatment strategies for use of picosecond oscillating lasers.

In the present study, the effects of various energy values for picosecond laser in tattoo removal were investigated in an animal tattoo model. To provide a theoretical and data basis for the clinical application of picosecond lasers in tattoo removal, we investigated the causes and mechanisms of the effects and assessed the effects of various energy levels.

Material and Methods

Sprague-Dawley rats

We purchased 100 Sprague-Dawley rats (50 male and 50 female) weighing 160–190 g from Jinan Pengyue Experimental Animal Breeding Co. [SCXK(LU) 20140007]. Breeding and experiments are carried out in the barrier environmental facilities of Yantai Langdi Biotechnology Co. [SYXK(LU) 20170028]. Chloral hydrate and tattoo dye were obtained from Suzhou Naton Bio-nanotechnology Co.

Preparation of tattoo model

After 1 week of adaptive feeding, we depilated a 3×3 cm area on the backs of rats with a depilating agent. After alcohol wiping and disinfection, we utilized a 0.5-mm tattoo needle to tattoo black dye into the exposed rat skin. The tattoo area was 1×1 cm with a depth 0.5 mm. All tattooed rats were fed normally. After 15 days, the inflammatory response disappeared and the pigment stabilized. The scab on the tattoo site fell off after 15 days and there was no obvious exudation. The pigment color on the tattoo surface was stable and no longer continued to deepen. Then, the picosecond laser was used to remove the tattoo.

Picosecond laser irradiation

The interval between the 2 picosecond laser treatments was 15–20 days. According to the energy of picosecond laser, rats were divided into a high-energy group (2.5–2.9 mJ/μbeam), a medium-energy group (1.7–2.1 mJ/μbeam), and a low-energy group (0.7–1.1 mJ/μbeam). After choosing the energy group with the best performance, rats were divided into subgroups further assessment. The treatment was delivered with the 1064-nm neodymium-doped yttrium aluminum garnet (Nd: YAG)

picosecond laser (PicoWay, Syneron Candela) with a 1064-nm resolve handpiece, spot size 6x6 mm, pulse width 450 ps, 3~5 passes, and frequency 2~3 Hz. Protective eyewear was utilized during all treatments.

Characteristic observation

During the experiments, the inflammatory reaction, scab formation, and pigment stability were recorded every day after the tattoo. After the picosecond laser treatment, the foaming, swelling and color fading of the tattoo site were monitored, and the effect of the picosecond laser on pigment removal was evaluated from the surface. We evaluated the effects based on the diminished color and the reduced area of skin lesions. There were 3 categories of tattoo pigmentation fading. In Grade 1 there was mild pigmentation fading with dark brown color. In Grade 2 there was moderate pigmentation fading with grayish brown color. In Grade 3 there was severe pigmentation fading with light brown color. Moderate and severe regressions are considered as effective. Photoshop software was utilized to calculate the area of tattoo pigment retreat, and the area of retreat above 60% was regarded as effective.

Histological observation

When the tattoo pigment was stable after the picosecond laser processing, and the skin was stable, 3 rats in each group were randomly selected at each time point and were killed. The tattooed skin samples were fixed in 10% formaldehyde solution and underwent conventional dehydration, paraffin-embedding, slicing, and HE staining. Afterwards, we observed the organization structure and the changes of pigment dyes using an optical microscope.

Statistical analysis

SPSS 26.0 statistical software SPSS version 26.0 for Windows (SPSS, Inc., Chicago, IL, USA) was utilized to analyze the data. The measurement data are expressed as mean±standard deviation (SD), and the count data are expressed in percentage. The area of dye and pigment fading after irradiation with different energy level lasers were tested through R by C chi-square test. After adjusting *P* values, a pairwise comparison was made. *P*<0.05 was considered as statistically significant.

Ethical approval

This study was approved by the Ethics Committee of Shanghai Skin Disease Hospital, Tongji University School of Medicine.



Figure 1. The skin appearance of tattoo model in rats.

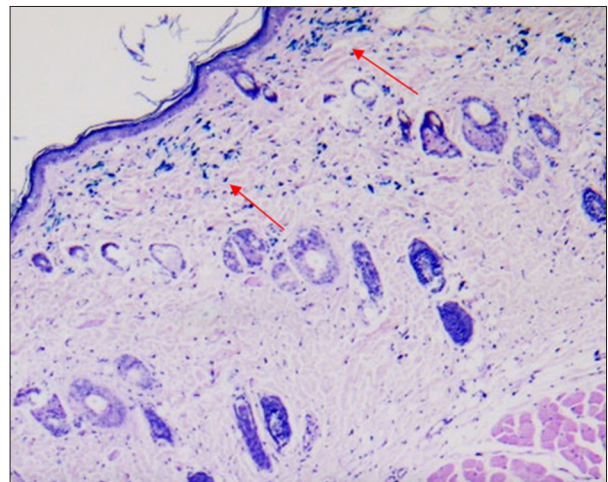


Figure 2. HE results of tattoo model. The animal model of the tattoo was successfully established, and the epidermis basically returned to normal. The arrows indicate that the tattoo dye is fixed in the dermis layer.

Results

The tattoo model

During the first 3 days after the tattoo, the redness and swelling in the tattoo area were obvious. On the 5th to 7th days, the engraving position gradually developed. On the 10th day, the scab fell off and the tattoo dye stabilized. On the 15th day, the color depth of the dye stopped changing (Figure 1). The pathological section of the skin tissue showed that there was a large amount of dye particle aggregation near the dermis and there were no changes from the 11th to 15th days (Figure 2), indicating that the dye was stable at that position, which was combined with the observation of characteristics. We determined that the tattoo dye was stabilized in the tattoo area and the model was established successfully.

Rat tattoo removal results

After laser treatments, the phenomenon of redness and swelling in the low-energy group quickly disappeared, and the

phenomenon of redness and swelling in the high-energy group was lighter compared with the medium-energy group, which disappeared in a short time (10–20 min). The phenomenon of redness and swelling in the medium-energy group was the most serious, which lasted the longest time (2–3 h) among all groups. After the first treatment, it could be observed from the spot dye color that the low-energy group tattoos were not eliminated, and the color depth of dye in the high-energy group was reduced. The color depth of the tattoo dye in the medium-energy group was significantly reduced, and the dye became scattered. These results show that the tattoo removal effect in the medium-energy group was the most obvious (Figure 3).

For this reason, when the second picosecond laser procedure was performed, the energy parameters were concentrated near the medium-energy group, which were randomly divided into multiple energy groups. We found that the tattoos in the 1.9 mJ/ μ beam energy group faded fastest and had the largest area of disappearance (Figure 3). After all rats had undergone 2 laser treatments with this energy value, the tattoos were considered to be effectively removed. We thus demonstrated that this energy value can maximize the removal of tattoo dyes.

We compared the results of the pathological sections with Masson staining of the 1.1 mJ/ μ beam, 1.9 mJ/ μ beam, 2.5 mJ/ μ beam, and 2.9 mJ/ μ beam energy arrays in the skin tissue samples (Figure 4). Only a very small amount of hollow structures existed in the skin tissue after the 1.1 mJ/ μ beam energy group was treated with a picosecond laser, the hollow structure was relatively small and located on the superficial dermis, and the dye particles are slightly dispersed after treatment but they are not obvious. After the 2.5 mJ/ μ beam and 2.9 mJ/ μ beam energy groups were treated by laser, a large number of small and dense hollow structures appeared on the epidermis and the junction of dermis or under the epidermis. There was no similar structure in the deep layer, and the shallow layer was non-clustered dye particles, and deep dye particles still existed. With 1.9 mJ/ μ beam energy laser treatment, there were many large areas of hollow structure in the middle and deep dermis, which were covered by a depth of dye etching that could be effectively absorbed and disintegrated by dye particles on the laser penetration path (Figure 4B). The alternations in the skin surface tattoos and the changes in the pathological sections further confirmed that the picosecond laser effect of 1.9 mJ/ μ beam energy can get into the deep layer of the skin, which effectively destroys the tattoo pigment in the dermis. These results provide a useful reference for the subsequent clinical use of picosecond lasers to remove tattoo dyes.

The results of statistical analysis showed that 1.9 mJ/ μ beam energy laser irradiation had the best effect on tattoo treatment (Figure 5). Figure 5 shows Group A: low-energy (0.7–1.1 mJ/ μ beam) group; Group B: medium-energy (1.7–2.1 mJ/ μ beam)

group; Group C: High-energy (2.5–2.9 mJ/ μ beam) group; and Group D: 1.9 mJ/ μ beam group. The degree of tattoo fading (Figure 5A) and the reduction area (Figure 5B) in the 1.9 mJ/ μ beam group are significantly better than those in the high-energy and low-energy groups ($P < 0.05$). In the first observation, there was a significant difference in the degree of color fading between group C and group D ($P < 0.05$). In the second observation, there were differences in the degree of color fading between Groups A and B, A and D, B and C, and C and D ($P < 0.05$). In terms of reduction area, there were differences between A and D, C and D in the first observation ($P < 0.05$), and in the second observation there were differences between A and B, A and D, and C and D ($P < 0.05$).

Discussion

Compared with the traditional short-pulse nanosecond laser, the super-pulse picosecond laser has better absorption of pigments, greatly reduces the damage to the surrounding tissues caused by non-specific photothermal therapy, and has a light inflammatory response and a lower incidence of color sinking [12,13]. Because the size of the tattoo particle is 40–300 nm in the body, most of the tattoo pigment TRTs are in the picosecond range. When the pulse width is compressed, the photomechanical effect of the picosecond laser makes it dependent on selective spectral absorption. For example, the pulse width of the 532 nm picosecond matches the particle size of the yellow tattoo pigment, so the clearance rate for the refractory yellow tattoo is also ideal [6]. A single-blind separation randomized study showed that the clearance rate of the 1064/532 nm picosecond laser to the tattoo color material was more than 75%, and the effectiveness was stronger than that of the 1064/532 nm short-pulse Q-switch laser [14].

This study found that 3 different levels of energy parameters have different penetration depths to skin. When the laser energy is high enough to approximate a certain threshold value, it will be absorbed by the specific target chromophore in the skin tissue. The free electron “seed” will be released from laser-induced thermo-ionization, and the plasma will be produced by cascade avalanche process, which creates a laser-induced optical breakdown effect in the skin cavity (LIOB). The excess energy will be absorbed by the plasma and destroys local tissue with little or no collateral damage [15,16]. When the laser intensity decreases to near or below the optical breakdown threshold, it is insufficient to cause the LIOB (plasma). The laser electrons can propagate through the epidermis to the dermis, but its intensity can cause mechanical stress and laser-induced cavitation effects (LIC) in the dermis [17]. Interestingly, with the low-energy picosecond laser irradiation, we observed the vacuolation with dye pigment as the target, but the penetration depth remained in the superficial dermis due to the energy.

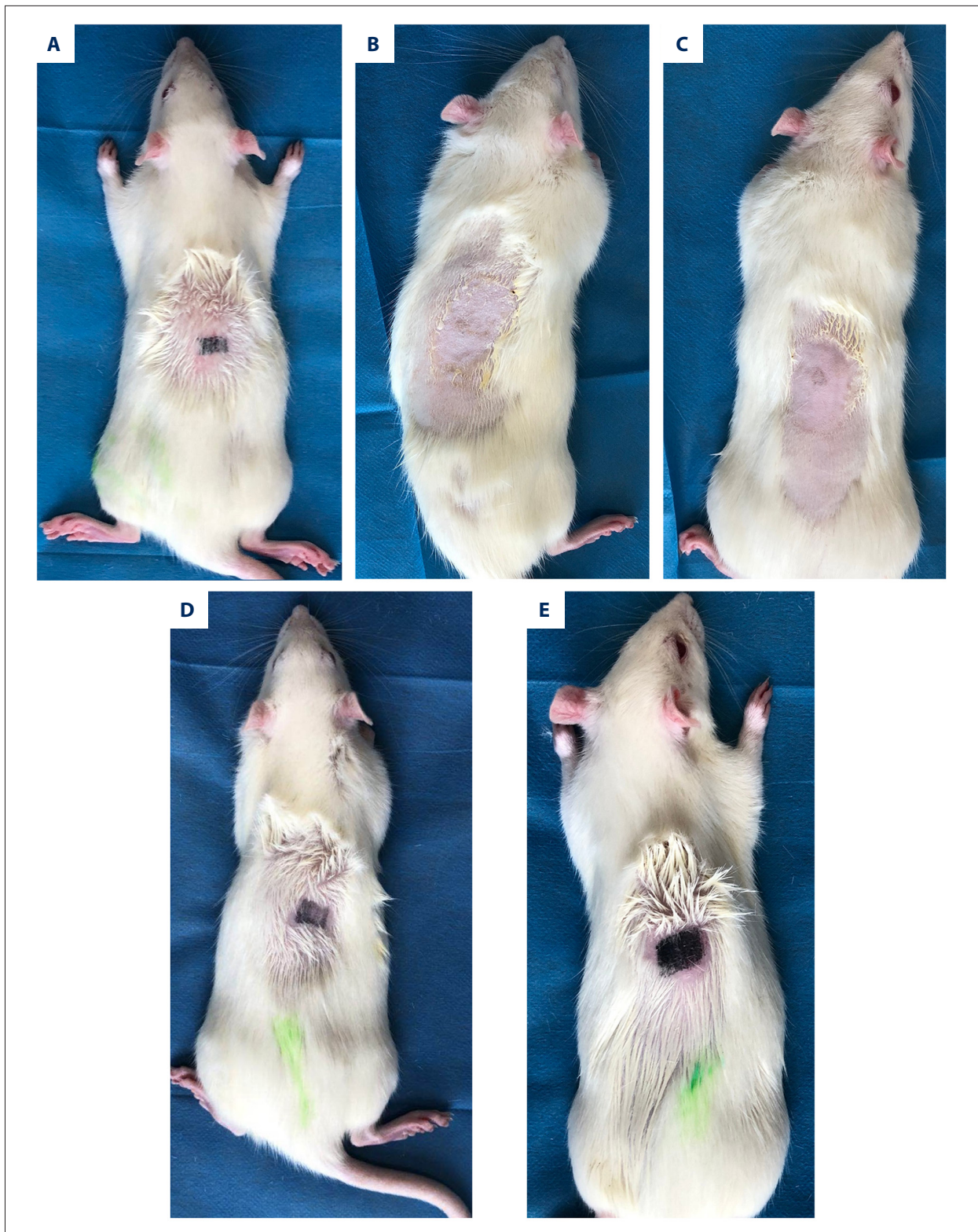


Figure 3. The tattoo removal effects of groups with different energy levels: (A) 1.1 mJ/ μ beam, (B) 1.9 mJ/ μ beam, (C) 2.5 mJ/ μ beam, (D) 2.9 mJ/ μ beam, and (E) control group. The treatment effect of picosecond laser with different energy parameters on tattoo removal illustrates that the laser removal effect of the 1.9 mJ/ μ beam (B) energy value is the best. Magnification of each figure is 5 \times .

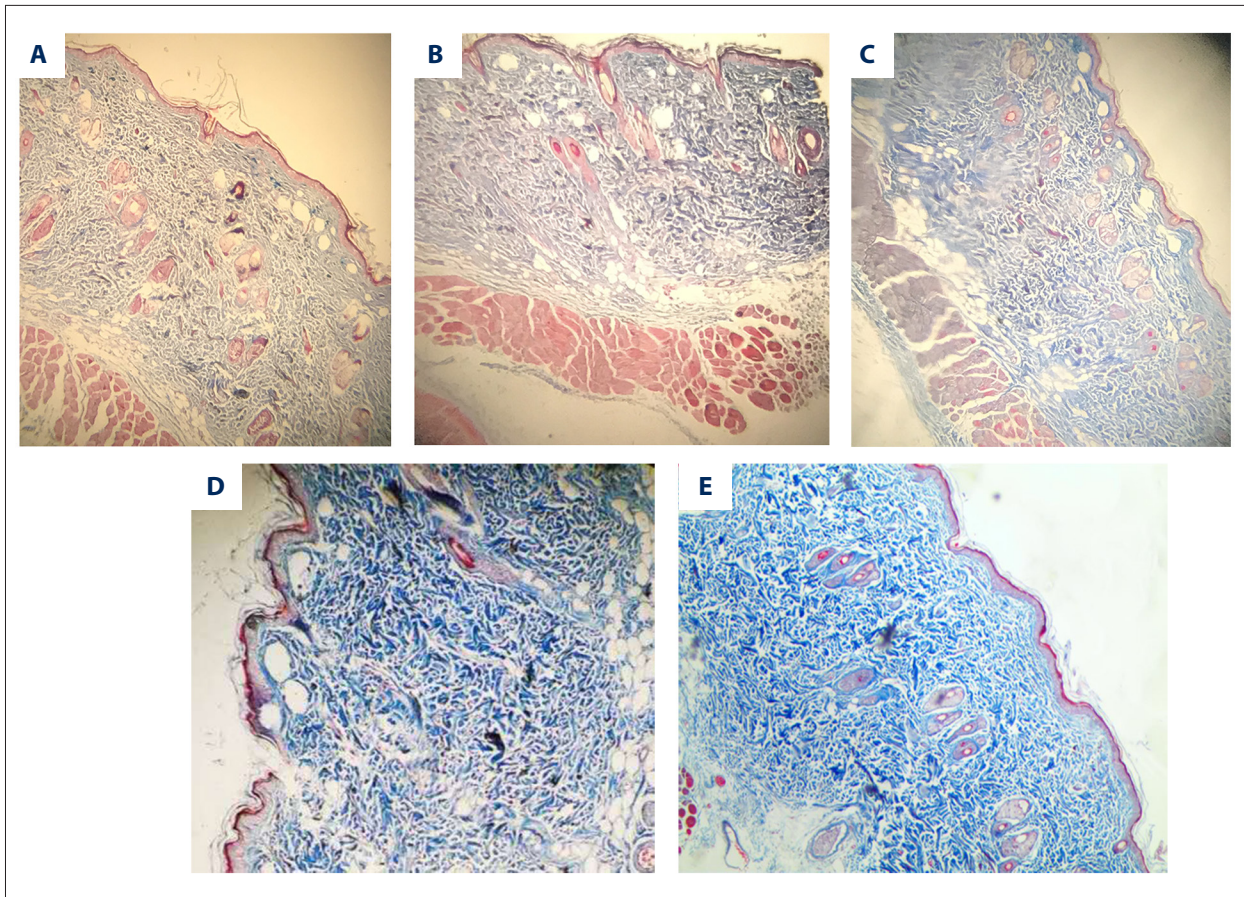


Figure 4. The Masson results in groups with different picosecond laser energy levels: (A) 1.1 mJ/μbeam, (B) 1.9 mJ/μbeam, (C) 2.5 mJ/μbeam, (D) 2.9 mJ/μbeam, and (E) control group. The penetration depth of the picosecond laser is different due to different energy parameters. The figure shows that the light penetration depth of 1.9 mJ/μbeam energy value (B) is the deepest, so its tattoo removal effect is better than others.

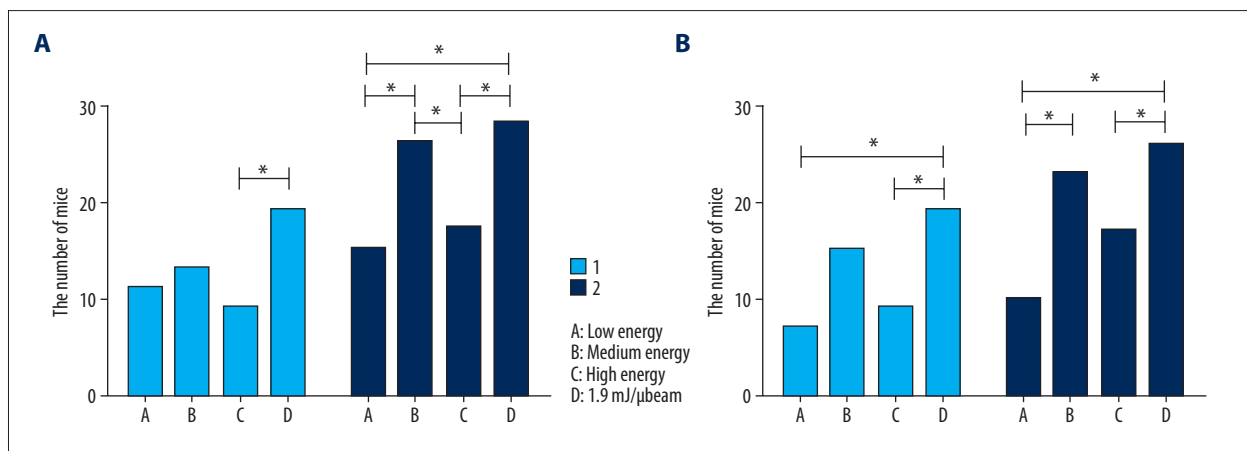


Figure 5. The efficacy of a picosecond laser with different energy in the degree of (A) color fading and (B) area reduction of tattoos. High-energy group: 2.5~2.9 mJ/μbeam; medium-energy group: 1.7~2.1 mJ/μbeam; and low-energy group: 0.7~1.1 mJ/μbeam. For each group, we conducted the picosecond laser procedure twice (labeled as '1' and '2') and collected data for analysis. (A) Demonstrates whether there is a significant difference in color fading between 2 groups. (B) Illustrates if there is a significant difference in area reduction between the 2 groups. The y axis represents the number of rats with ideal tattoo removal effects according to the 3 categories in tattoo pigmentation fading.

Since most tattoo dyes are stable in the middle layer of the dermis, theoretically, the medium energy value close to or lower than the threshold of producing LIOB is more effective in removal of tattoo dyes. Some tattoo patients have scars after tattooing, but the scars are not obvious because they are covered by pigment dye. The 1064-nm picosecond laser treatment with medium energy value can concentrate the energy on the dye layer of the tattoo, which contributes to the recovery of skin texture and the dermis remodeling effect.

In this study, the picosecond laser was used to treat the tattoo model of rats to explore the effect of different energy parameters on the removal of tattoo dyes and to observe the tattoo status at various points in time from the aspects of characterization observation and histopathology. The results showed that the 1.9 mJ/ μ beam energy laser had the most obvious hollowing effect in the skin tissue, and the pigment particle removal was the most thorough and had the best tattoo removal effect. According to animal experimental data, the study also conducted a series of human clinical trials. The tattoo removal effect of the 1.9 mJ/ μ beam energy value picosecond laser was verified. It was found that the picosecond laser with this energy value had a good removal effect. The size of the light spot is also an influencing factor that determines the penetration depth. The larger the light spot, the deeper

the penetration depth. Compared with the smaller light spots commonly used in traditional treatment, the larger light spot penetrates deeper, and the proportion of scattered photons is greater. Therefore, the therapeutic energy can be reduced to only destroy the pigments and dyes in the dermis, without obvious damage to the epidermis. In this study, medium-energy treatment of tattoos with larger light spots improved the comfort and safety of the treatment process, especially for darker-skinned Asians. Although this study explored the effects of different energy parameters on the removal of tattoos, we did not perform an in-depth study on the removal mechanism and removal path. Subsequent work will be carried out to define the underlying mechanism involved and to provide support for clinical applications.

Conclusions

Animal experiments and observations show that a picosecond laser with energy value of 1.9 mJ/ μ beam can reach deep into the skin, create a large number of holes in the pathway, and effectively crush pigment particles, showing a good therapeutic effect on tattoo removal. It can be used as a reference in clinical medicine.

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