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Research Article

The Effect of an Er:YAG Laser Combined with a 755 nm Picosecond Laser on Tattoo Removal in a Rat Model

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Laser tattoo removal is an effective technique, but multiple rounds of treatment are required to eliminate tattoos, which can be a challenge for patients who do not have the time. Additionally, there is relatively limited research on the mechanisms of laser tattoo removal, necessitating further in-depth studies. This study aimed to investigate the efficacy of an Er:YAG laser combined with a 755-picosecond laser for tattoo removal and the changes in skin diffuse reflection, pigment particle size, macrophage recruitment, and inflammatory factor levels during combined laser treatment. Our experiments used three sets of lasers—an Er:YAG laser, a 755 nm picosecond laser, and an Er:YAG laser combined with a 755 nm picosecond laser—for tattoo removal. The effects of tattoo removal at different time points were evaluated. Moreover, the mechanisms were explored using HE staining, immunohistochemistry, diffuse reflectance measurements, in vitro gel tests, mass spectrometry, and ELISA experiments. The combined treatment was more effective for tattoo removal in rats. The combined laser treatment effectively reduced diffuse reflection from the skin, thereby increasing the effective laser power, reducing the size of the pigment particles, allowing for easier removal of the pigment, and increasing the recruitment of dermal macrophages and the release of inflammatory factors, thus increasing the rate of tattoo removal in vivo.

1. Introduction

In recent years [1], the proportion of individuals with tattoos has been increasing. According to a survey on tattoos, the global tattoo rate is approximately 10–29%, with an increasing number of people showing interest in tattoos [2], and a number of people are seeking safe and effective methods for tattoo removal. Early tattoo removal methods such as skin scrubbing, cryosurgery, and chemical dissection were ineffective and had long recovery periods. In 1966, Goldman et al. [3] introduced laser tattoo removal as a safer and more effective method than previous techniques. Since then, it has become a popular method. However, how to increase the effectiveness of tattoo removal remains a challenge. Selective photothermolysis and photoacoustic principles can explain the mechanisms underlying the laser removal of tattoos [4].

Previous studies [5–7] successfully treated tattoos using a combination of Er:YAG and QS Nd:YAG lasers or a combination of CO_2 and QS Nd:YAG lasers. However, these studies did not provide detailed explanations of the mechanism behind this combined laser therapy.

Several studies have demonstrated the variability of laser diffuse reflectance in different states of the skin, and the effective power of a laser is influenced by the skin's reflection [8, 9]. Baranska et al. [10] studies demonstrated that injection site pigment particles were localized in dermal macrophages and thus proposed the pigment capture-release-recapture model. Under physiological conditions, neighbouring macrophages recapture tattoo-pigment-laden macrophages when they die. This process ensures the stability and long-term durability of the tattoo ink. According to recent studies [4, 11], following laser treatment, the

destruction of cells containing pigment particles leads to the release of pigment fragments within the tissue. Subsequently, macrophages engulf these fragments, transferring a portion of them and expelling them through the lymphatic vessels. During the laser tattoo removal process, the skin is damaged, and an inflammatory response occurs. During this process, the release of inflammatory factors such as IL-6, MCP-1/CCL-2, and INF-γ enhances the recruitment of macrophages. As the number of macrophages in the skin tissue increases, these cells play a role in phagocytizing tattoo pigments, which is beneficial for their removal.

To investigate laser tattoo removal, we conducted a preclinical study on animal tattoo models. Little research has been carried out on combined laser treatments, specifically using Er:YAG laser and 755 nm picosecond laser together. The mechanisms of tattoo removal and the effects on inflammatory factors have not been well-studied.

A 755 nm picosecond laser is a precise and efficient technology used for tattoo removal. It accurately targets the area and reduces heat damage to surrounding tissues compared to traditional QS Nd:YAG laser [4]. The Er:YAG laser with a wavelength of 2940 nm is a more precise ablation tool than CO_2, making it better for far-finer superficial ablation [6, 11–14].

Therefore, we investigated the efficacy of the Er:YAG laser combined with the 755 nm picosecond laser for tattoo removal and explored the possible underlying mechanisms to provide new ideas for the clinical treatment of tattoos.

2. Materials and Methods

2.1. Preparation of the Tattoo Model. 15 male Sprague–Dawley rats (220–250 g) were obtained from Jinan Peng Yue Experimental Animal Breeding Co. (Shandong, China) and used in this study. The Animal Care and Use Ethics Committee of Shandong Provincial Hospital approved all the animal experiments (No: 2023-59). After one week of adaptive feeding, the rats back were depilated with depilatory agents. Subsequently, the area was tattooed with a professional tattoo needle that inserts black dye (supplied by Hefei Duomo Electronic Technology Co., Ltd.) into the bare skin through continuous vibration. Multiple 1×1.5 cm deep 0.5 mm tattoos were created. After the tattooing, the rats resumed normal eating. Fifteen days later, HE staining showed that the tattoo colours had stabilized. All rats were anaesthetized by intraperitoneal injection of Avertin (0.015 ml of a 2.5% solution/g body weight). At the end of the experiment, the rats were euthanized by CO_2 asphyxiation.

2.2. Laser Treatments. After four weeks, the rats were anaesthetized and removed their back hair. The Er:YAG laser (Sciton, US, 2940 nm, 15 Hz, 8–10 J/cm^2) combined with the 755 nm picosecond laser (Cynosure, US, 755 nm, 5.0 Hz, 3.5 mm spot, 2–2.5 J/cm^2), and the combined laser treatment group first use Er:YAG laser and then use 755 nm picosecond laser 10 minutes later; the Er:YAG laser (2940 nm, 15 Hz, 8–10 J/cm^2) alone and the 755 nm picosecond laser (755 nm, 5.0 Hz, 3.5 mm spot, 2–2.5 J/cm^2)

alone were randomly applied to four distinct tattooed areas on the back. The control group received no laser treatment. The resulting groups were referred to as the combined laser treatment group (EP), the Er:YAG laser treatment group(E), the 755 nm picosecond laser treatment group(P), and the control group(Ctrl). The determination of the minimum parameter for the picosecond laser was based on the immediate appearance of a whitening reaction on the skin. The mice were treated every 15 days. When a tattoo is considered finished by a certain laser group, the treatment stops.

2.3. Effect Evaluation. Photos were taken before, immediately following, and 15 days after laser intervention. The tattoo clearance rate was assessed in a blinded manner by three dermatologists based on the conclusive outcomes of the investigation. The tattoo clearance rate after the final treatment was determined using a visual rating scale, which categorizes the results as follows: 1: poor (<25%), 2: average (25–50%), 3: good (51–75%), 4: excellent (76–95%), and 5: cured (>96%). Furthermore, statistical analysis was conducted to further analyse the data.

2.4. Histopathological Evaluation. The rats were anaesthetized, shaved, and had skin biopsies taken from tattooed areas at specific times. The biopsies were fixed with 4% paraformaldehyde and embedded in paraffin. Biopsies collected at 15 and 40 days after laser treatment was stained with H&E and observed under a microscope.

After laser treatment, specimens were obtained immediately and 1, 3, 7, and 15 days later for immunohistochemical (IHC) staining. We used a mouse antirabbit CD68 primary antibody (GB113109; Servicebio Hubei, China; 1 : 200 dilution) and a goat antirabbit IgG secondary antibody (GB23303; Servicebio, Hubei, China; 1 : 200 dilution). A standard protocol was used to perform IHC staining. The IHC sections were observed under an optical microscope, and five fields within the dermis were randomly selected at 400x magnification. The number of CD68+ cells was counted, and the mean value of five random fields was used for statistical analysis.

2.5. Tattoo Ink Spectrum Analysis and In Vitro Laser Treatment. The ink composition was analyzed using a mass spectrometer (Agilent 7000D, Germany). The ink was diluted 1 : 100 in water and then placed in a 24-well culture dish filled with an agarose gel mixture. After the ink coagulated, an Er:YAG laser (2940 nm, 15 Hz, 8–10 J/cm^2) combined with a 755 nm picosecond laser (755 nm, 5.0 Hz, 3.5 mm spot, 2–2.5 J/cm^2) or the individual lasers alone were used for irradiation. Each treatment was repeated three times, and a control group and a blank group were included. The agar of each sample was heated and diluted to 50 ml (Thermo Nanodrop2000/2000C) with an ultramicro ultraviolet spectrophotometer. The sample was scanned at full wavelength (190–800 nm). The blank gel showed very low absorption, which was reasonably ignored in the ink analysis.

2.6. Diffuse Reflectance Measurements. The diffuse reflectance of the samples was measured using a diffuse reflectometer (Hitachi 4150, Japan) at various excitation wavelengths ranging from 300 nm to 2200 nm. The samples consisted of normal rat skin (normal skin group), tattooed rat skin treated with an Er:YAG laser (Er:YAG group), and untreated tattooed rat skin (control group). To ensure the accuracy of the experiment, the experiment is repeated three times.

2.7. Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) for Ultrastructural Evaluation. The tattoo ink's 3D structure was observed using SEM (Hitachi SU8010, Japan). After 15 days of laser treatment, rats were executed to obtain skin tissue. The specimens were fixed in a 2.5% glutaraldehyde solution and embedded. Ultrathin sections were obtained using an ultramicrotome (EM UC7, Leica, Wetzlar, Germany) and examined with a transmission electron microscope (H-7650, Hitachi Company, Japan). ImageJ software (National Institutes of Health, USA) was used for quantitative analysis of pigment particles.

2.8. Enzyme-Linked Immunosorbent Assay (ELISA). The concentrations of the inflammatory factors Mcp-1/ccl-2, INF-γ, and IL-6 were assessed in the skin tissue samples of the rats at various time points (before treatment and on the 1st, 3rd, 7th, 11th, and 15th days) after laser treatment. This analysis was conducted using ELISA reagent kits (EK387-96, EK306HS, EK380/3-96; Lianke Biology, China) according to the provided instructions. The tissue concentrations were determined by converting their OD values into pg/L values.

2.9. Statistical Analyses. Statistical comparisons were made by one-way ANOVA or Student's *t*-test. At least three independent measurements were taken, and the data are presented as the mean ± SD. $P < 0.05$ was considered to indicate statistical significance. GraphPad Prism (GraphPad Software, Inc., USA) was used for statistical analyses.

3. Results

3.1. Effect of Combined Laser Treatment on Tattoo Removal. The tattooed skin became red and swollen within 3 days and then scabbed on days 5 and 7. After about 10 days, the scabs fell off and the tattoo dye became stable. By approximately 15 days, the dye remained deep in the skin tissue. Pathology analysis showed that the dye particles had gathered in the middle layer of the dermis, with no changes observed between days 15 and 40. Erythema and capillary dilation were observed after treatment with the combined lasers skin vaporization occurred immediately after Er:YAG laser treatment. A whitening reaction immediately occurred after 755 picosecond laser treatment. Within the first 3 days, all three groups showed edema around the treatment area. The group that received treatment with both the Er:YAG laser and the 755 picosecond laser experienced mild oozing.

Scabbing was observed in all groups, and the scab typically fell off between days 5 and 8.

Skin biopsies were conducted immediately before and after laser treatment on days 15 and 40. The tissue response to Er:YAG laser treatment and combined laser therapy was marked by complete ablation of the epidermis and dispersion of pigments. It is important to note that tattoo pigments are expelled through the damaged outer layer of the skin. However, pigment particles and cells in the deeper layers of the skin were unaffected in the Er:YAG laser group, possibly due to the limited depth that the Er:YAG laser can reach.

After 15 days of treatment, the skin showed thickening of the epidermis, increased fibroblasts, and returned to its original state. In comparison, all treated groups had less and more dispersed pigments compared to the control group, with the combined treatment group showing the most significant decrease and dispersion of pigments (Figure 1). After four treatments, we found that the combined laser treatment group had better results in treating tattoos. After the experiment, the skin of the rats was checked and there were no obvious abnormalities (Figure 2).

3.2. The Diffuse Reflectance of the Skin Is Significantly Reduced after Er:YAG Laser Treatment. In the wavelength range of 755 nm, the diffuse reflectance of healthy skin was 53.4%, while tattooed skin exhibited a diffuse reflectance of 14.3%. Following Er:YAG laser treatment, the diffuse reflectance of the tattooed skin decreased to 8.2%, equivalent to a 43% diffuse reflectance reduction after Er:YAG laser irradiation compared with tattooed skin and 85% compared to healthy skin. Our above results indicated that after using the Er:YAG laser, the diffuse skin reflectance was significantly reduced. However, we found that there was no significant difference in diffuse reflectance among the groups after the laser wavelength exceeded 1500 nm (Figure 3(b)).

3.3. Combined Laser Treatment Significantly Destroys Pigment Particles in Skin Tissue. We used mass spectrometry to analyse the ink used in this study, and as shown in the figure (Figure 4(b)), the major element in the ink was carbon (68%). The morphology of the ink observed by TEM and SEM showed a round or oval shape (Figure 3(c)). The in vitro ink spectrum analysis results before and after laser irradiation are shown in the figure (Figures 3(a), 3(c), and 3(e)). Electron microscopy of the agarose plate irradiated by a laser revealed that the pigment particles were fragmented, with smaller particles and a larger popcorn morphology following combination irradiation with the Er:YAG laser and 755 nm picosecond laser (Figures 3(d) and 3(e)).

A reflectance system analyzed the absorption spectra of the samples. The experimental ink showed a maximum absorption peak at 260–270 nm (Figure 3(a)). The absorbance of the Er:YAG laser combined with 755 nm picosecond laser radiation was much lower than the control group.

Next, we conducted an electron microscopy (TEM) analysis of the tattoo pigment particles in the skin. The control group had particles that were approximately
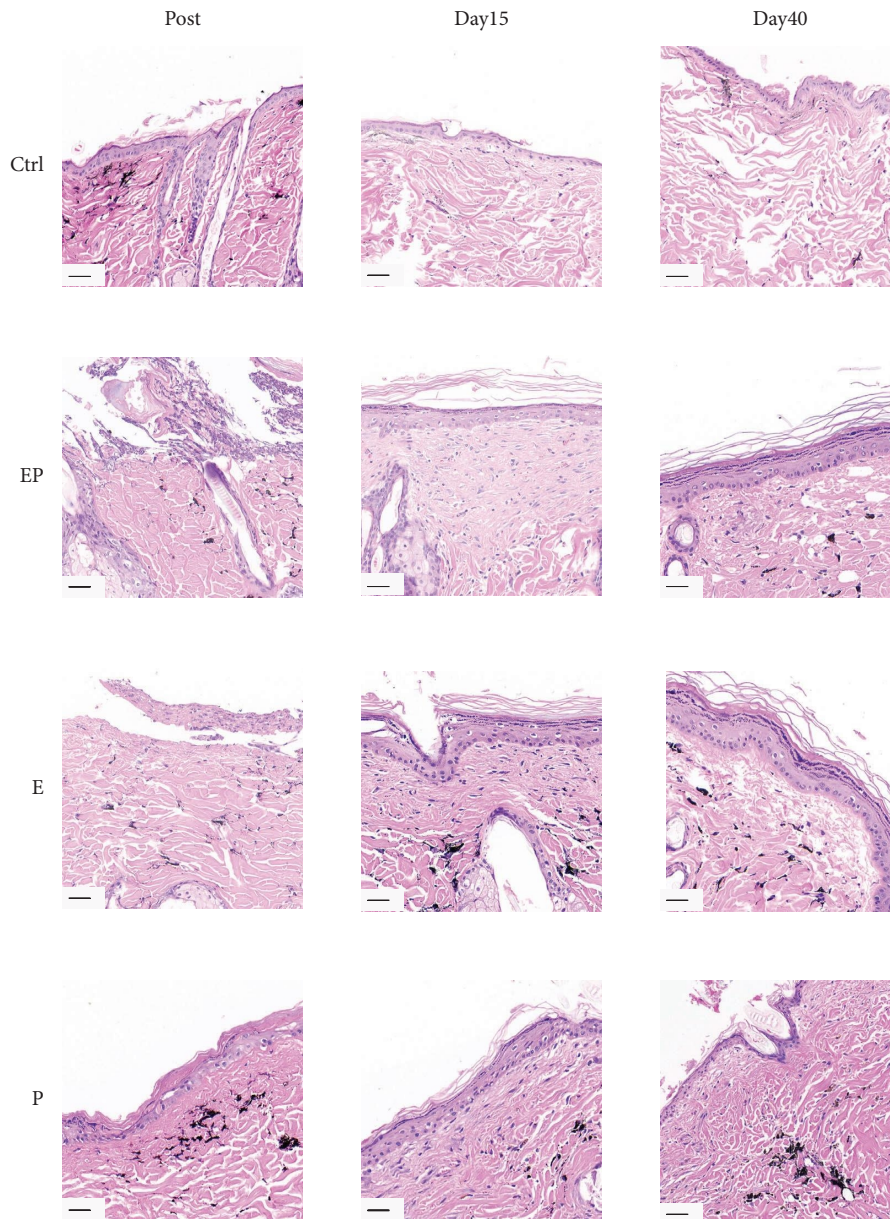
FIGURE 1: Skin biopsies were obtained from each treatment group immediately post-laser therapy 15 and 40 days after treatment. H&E staining (×400 magnification) bar = 50 μm.

78.27 ± 14.11 nm in size. After laser irradiation, the epidermis was destroyed, and cell debris was visible. Pigment particle deposition was observed in the interstitial spaces of dermal tissue. After 15 days, the pigment granules were phagocytosed by macrophages, and secondary lysosomes were present.

The combined irradiation of YAG and 755 nm picosecond laser resulted in pigment particles with a diameter of approximately 32.60 ± 5.90 nm. The 755 nm picosecond laser group had particles with a diameter of approximately 51.45 ± 10.39 nm, while the Er:YAG laser group had particles with a diameter of approximately 55.05 ± 8.44 nm (Figures 4(a) and 4(c)). The Er:YAG laser combined with the

755 nm laser group had significantly smaller particle size compared to the other groups ($P < 0.01$).

3.4. Combined Laser Therapy Can Significantly Increase the Aggregation of Macrophages in Tissues. To explore the effect of combination laser treatment on the recruitment of macrophages, we followed the methods of Xiaojie Du et al. [15] and used immunohistochemistry to identify CD68-positive cells at each specific time point (Figure 5(a)). The number of CD68-positive cells initially increased, peaked at 1~3 days, and then declined gradually. As shown in the figure (Figure 5(b)), we found that macrophage recruitment
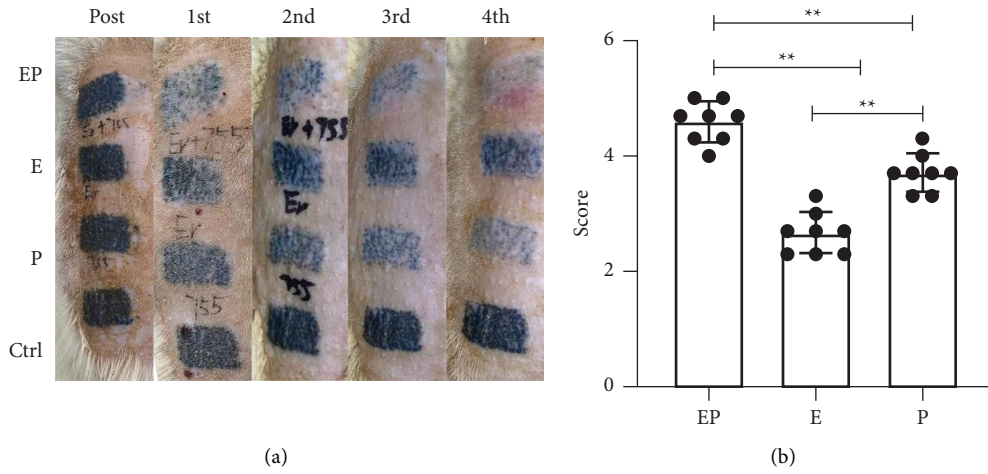
(a)

(b)

Figure 2: (a) Effects of laser treatment on tattoos. (b) After laser treatments, statistical analysis of the average scores recorded by three independent observers based on the visual rating scale. Mean ± SD of $n = 8$ separate experiments. $^{**}P < 0.01$.
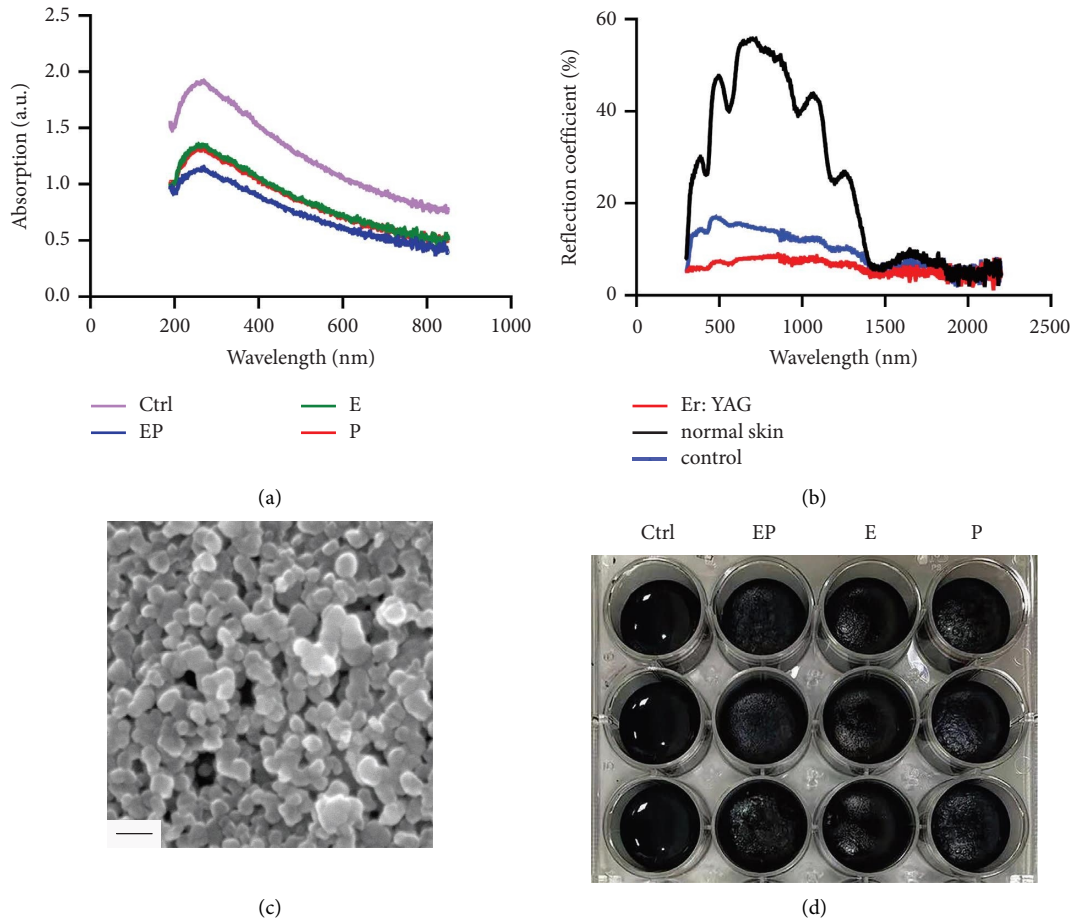

(a)

(b)

(c)

(d)

Figure 3: Continued.

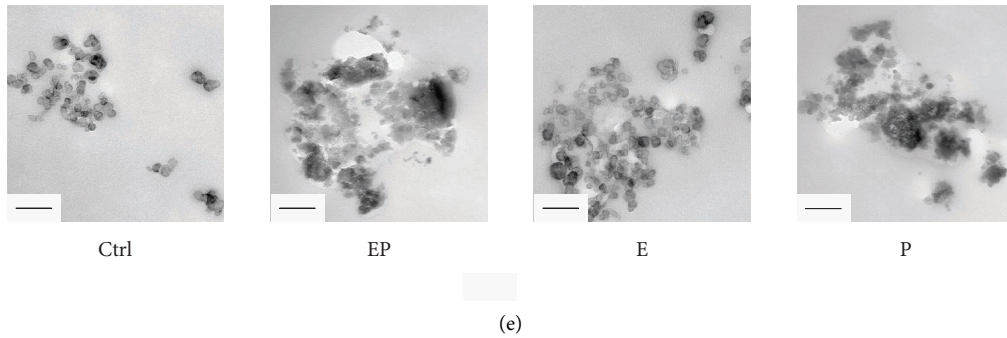Ctrl   EP   E   P

(e)

FIGURE 3: (a) Absorption spectra were analyzed by a reflectance measurement system. (b) Diffuse reflection spectroscopy. (c) Scanning electron microscopy of tattoo ink, ×100,000 magnification. Bar = 100 nm. (d) Tattoo ink agarose plates treated with Er:YAG laser, 755 nm picosecond laser, and combined lasers, 1 : 100. (e) In vitro electron microscopy images of Er:YAG laser, 755 nm picosecond laser, and combined lasers. ×50,000 magnification. Bar = 200 nm.
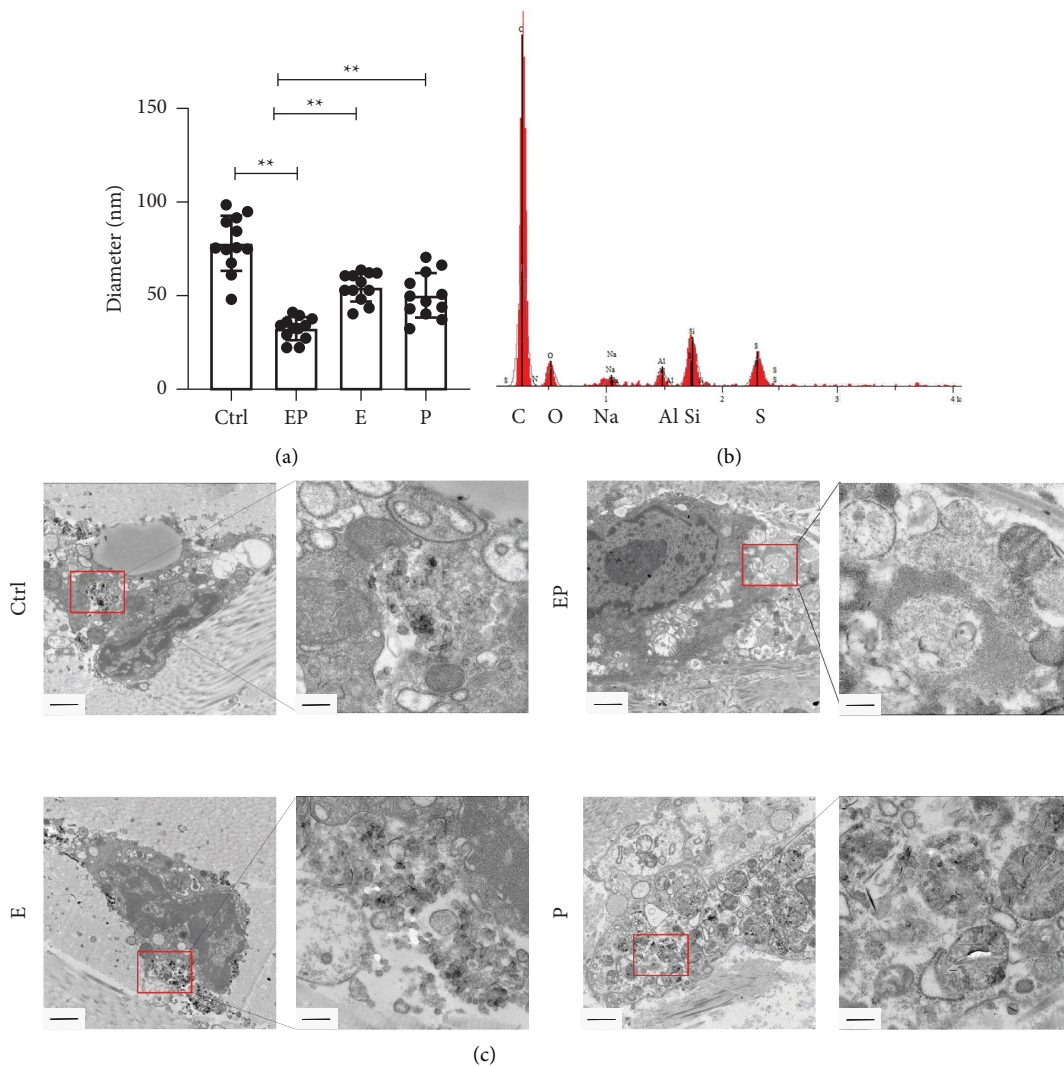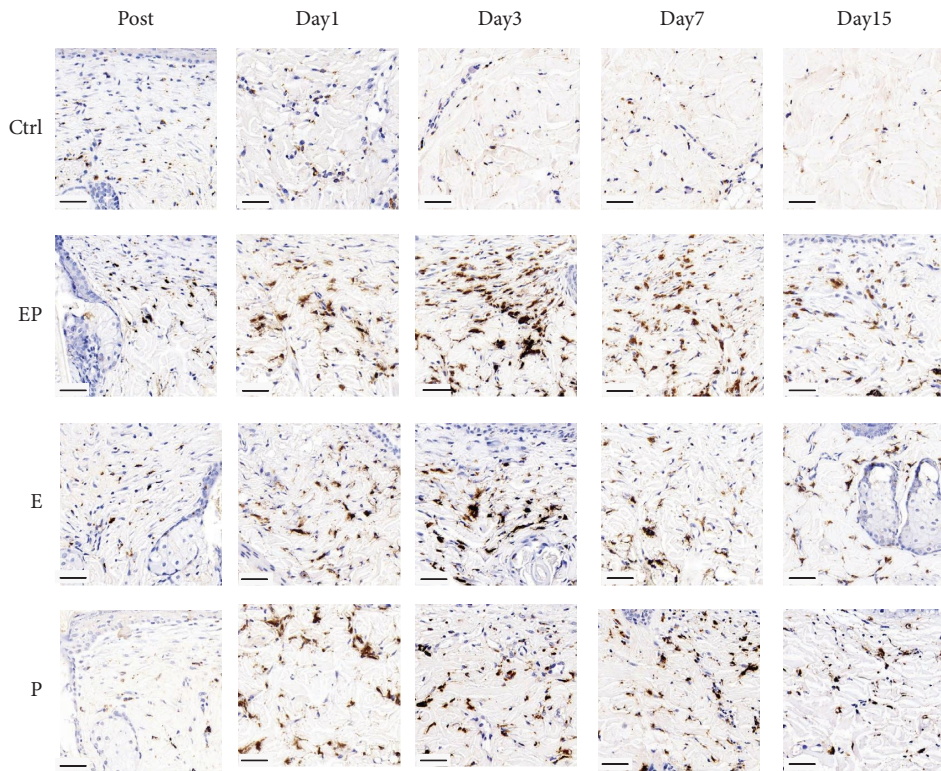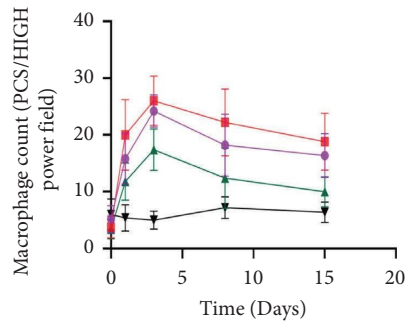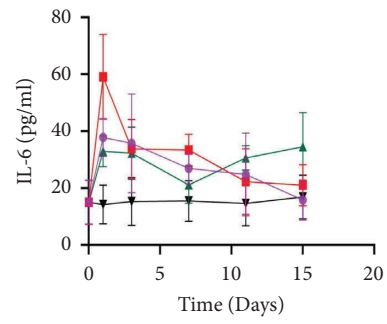

(a)

(b)

(c)

FIGURE 4: (a) Diameter of the pigment particles by respective laser treatments, mean ± SD of $n = 12$ separate experiments. $^{**}P < 0.01$. (b) Mass spectrometry analysis of tattoo ink. (c) Electron microscopy images, ×10,000 and ×200,000 magnification. Bar = 1 μm and bar = 300 nm.
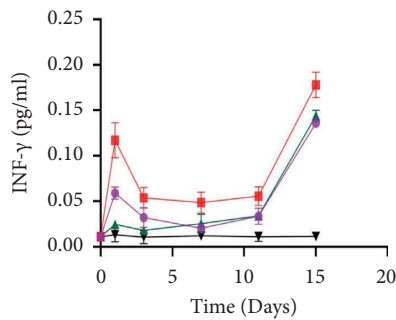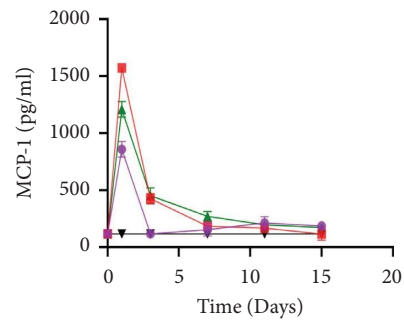
FIGURE 5: (a) CD68 staining (×400 magnification) of the sections of respective groups and time points. Bar = 20 μm. (b) Counts of CD68+ macrophages. Mean ± SD of $n = 5$ separate experiments. (c) Concentration of IL-6 of the sample on respective groups and time points. (d) Concentration of INF-γ of the sample on respective groups and time points. (e) Concentration of MCP-1 of the sample on respective groups and time points.

in the laser combination group was the fastest and that this group had the largest number of macrophages.

3.5. Combined Laser Treatment Can Promote the Release of Inflammatory Factors in Skin Tissue.

We evaluated changes in inflammatory factor levels via ELISA. The results are shown in the figures (Figures 5(c)–5(e)). The IL-6 protein levels in all the laser groups were significantly greater on day 1 (Figures 5(c)), and those in the Er:YAG laser combined with the 755 nm picosecond laser group were significantly greater than those in the other two groups. Within 1–15 days, the IL-6 levels in the Er:YAG laser combined with the 755 nm picosecond laser group and the Er:YAG laser group gradually returned to the initial level before treatment. As soon as 1 day after laser irradiation, the INF-γ protein levels were significantly greater than those in the control group (Figures 5(d)). The protein levels in all the laser groups gradually decreased from days 1 to 11 after laser irradiation, but the protein level was still significantly greater in the laser group than in the control group. However, after 11 days, INF-γ protein levels increased again and exceeded the previous highest levels. The INF-γ protein levels in the Er:YAG laser combined with the 755 nm picosecond laser group were consistently greater than those in the other groups. The MCP-1 protein levels in all the laser groups significantly increased on day 1 (Figure 5(e)) and gradually returned to the level observed before treatment within 1–15 days.

4. Discussion

This study investigated the combination of an Er:YAG laser and a 755 nm picosecond laser for tattoo removal, and the combination therapy achieved a better therapeutic effect. We subsequently explored the underlying mechanisms involved. We hope this method can provide new ideas for the clinical treatment of tattoos.

4.1. The Effect of Combined Laser Therapy on Tattoo Removal Was Superior.

We constructed an in vitro gel model and carried out TEM. The agarose gel was irradiated by the laser, which represents the state of the pigment particles of the laser-treated tattoo. We observed that the pigment particles had a popcorn-like structure. Qu and Leu et al. [16, 17] proposed that laser irradiation leads to extremely high temperatures that result in severe degradation of the entire particle, and the appearance of popcorn-like structures after laser irradiation may be due to the release of pyrolysis gas products or incomplete segregation of fragments due to microexplosions induced by superheated vapours. In this study, we observed that the Er:YAG laser combined with the 755 nm picosecond laser produced bigger popcorn-like structures with significantly smaller particles than the other groups. The absorbance decreased after laser irradiation in all the laser groups, but the optical spectrum shape did not change significantly. The results showed that the substance did not change after laser irradiation, but the change in absorbance was related to the particle size. The combination of Er:YAG laser and 755 nm picosecond laser treatment group resulted in smaller pigment particles, leading to a more significant decrease in absorbance. This means that smaller particles absorb less energy and therefore require a higher fluence rate during subsequent treatments.

4.2. Er:YAG Laser Treatment Significantly Reduces Skin Reflectance.

Calin et al. [18] reported the variability of diffuse reflectance across different states of skin. Michel and Chen et al. [8, 9] demonstrated that irradiation of the laser beam on human skin results in considerable diffuse reflection, which reduces the effective power of the laser head. Consequently, this study aimed to acquire diffuse reflectance measurements in various tissue states when subjected to laser irradiation. These findings unequivocally indicated a notable disparity in the diffuse reflectance of rat skin treated with the Er:YAG laser in comparison to the diffuse reflectance of both the control rat skin and healthy rat skin.

In addition, diffuse reflectance did not significantly differ between the treated and untreated groups when the wavelength was near 1500 nm. This may be due to the longer wavelength of the laser and the subsequent increase in the depth of penetration into the skin, which reduces diffuse reflections from the skin surface. This finding implies that opting for a laser with a longer wavelength can effectively diminish reflectance and enhance the efficacy of tattoo removal. Er:YAG laser treatment reduces the diffuse reflectance of the skin, thereby improving the therapeutic effect of 755 nm picosecond laser.

4.3. Combined Er:YAG Laser Treatment Led to the Recruitment of More Macrophages.

By ingesting pathogens, dead cells, and other particles, dermal macrophages serve as protectors of the skin, preventing the spread of potentially harmful substances. Macrophages play a crucial role in tattoo stabilization [19]. Baranska et al. [10] proposed a model for macrophage phagocytosis of pigments: The segment capture release recapture model. Our TEM results showed that 15 days after irradiation, the pigment was again phagocytosed by macrophages. Our results verified this pigment capture-release-recapture model. Senescent macrophages undergo apoptosis, release pigments, and are rephagocytized by new surrounding macrophages, which may be the cause of haloing in older tattoos.

Sepehri et al. [20] reported that some macrophages enter the lymph nodes after the recapture of pigment fragments, resulting in pigment deposition in the lymph nodes. Kurniadi et al. [4] reported that laser irradiation destroys pigment particles containing cells, which are released as fragments and phagocytosed. Pigment fragments are transferred and expelled into lymphatic ducts by dermal macrophages. Previous studies have indicated that tattoo pigment particles can disseminate via the lymphatic or blood system, and fluorescence has been detected in multiple organs, including the liver, kidneys, and intestines [16, 20, 21]. These findings are consistent with our light microscopy observation that pigment particles aggregate around blood vessels and lymphatic vessels.

In the results, it was concluded that the combination of Er:YAG laser and 755 nm picosecond laser has the strongest effect on macrophage recruitment. Based on the evidence above, combination laser treatment leads to greater efficacy because more macrophages are recruited and more pigment is transferred.

4.4. Combined Laser Treatment Can Promote the Release of Inflammatory Factors in Skin Tissue. Cytokines and chemokines are low-molecular-weight signalling molecules that mediate intercellular communication, and these molecules are produced by a variety of cell types in the immune system.

Cytokines are primarily responsible for regulating inflammation [19]. Based on previous reports, after laser irradiation, pigment and tissue fragmentation, damage, and inflammation may stimulate fibroblasts, B cells, and dendritic cells to produce chemokines that recruit macrophages [10]. To assess the release of inflammatory factors associated with macrophage recruitment in the different groups in this study, we measured the levels of tissue cytokines (IL-6, MCP-1/CCL-2, and INF-γ) in the different groups of rats using ELISA. There were nearly no relevant reports on this topic before this study.

IL-6, a versatile cytokine, exerts both proinflammatory and anti-inflammatory effects, exerting influence over a range of physiological processes encompassing immunity, tissue repair, and metabolism. IL-6 signalling can trigger the recruitment of monocytes to inflammatory sites [19, 22, 23]. The present study showed that, after laser treatment, the IL-6 protein concentration in the Er:YAG laser group significantly increased, surpassing that in the other groups. The subsequent test exhibited no clear tendency.

IFN-γ is one of the most potent macrophage activators. Interferon-gamma (IFN-γ) is a hallmark cytokine of the Th1 immune response. IFN-γ produced by Th1 cells is important for supporting and maintaining macrophages during chronic infection [23–25]. The presence of IFN-γ significantly amplifies the production of inflammatory mediators by macrophages, thereby intensifying all of these inflammatory responses. [19, 24, 26]. In our study, the IFN-γ level sharply increased after day 11, exceeding the level on day 1, and the IFN-γ level in the Er:YAG laser combined with the 755 nm picosecond laser group was always significantly greater than that in the other groups. It is likely that INF-γ enhances Th1 cell activity by stimulating antigen presentation and cytokine production in macrophages. The exchange of cytokines between macrophages and T cells creates a strong positive feedback loop between these two populations and predisposes the immune response to the Th1 mode [25]. Therefore, IFN-γ levels increased again after 11 days of laser treatment.

According to recent studies, monocyte chemoattractant protein-1 (MCP-1/CCL2) is a key chemokine involved in the migration and infiltration of monocytes/macrophages following skin injury and inflammation. MCP-1/CCL-2 can recruit macrophages, which are known for their ability to drive myeloid and lymphoid lineage cell chemotaxis. MCP-1/CCL-2 plays a crucial role in orchestrating the migration

of these cell types in both physiological immune defence and pathological contexts [27, 28]. According to the results of our study, MCP-1/CCL2 levels in the Er:YAG laser combined with the 755 nm picosecond laser group were greater than that in the other groups in the early posttreatment period.

According to the above discussion, the Er:YAG laser combined with the 755 nm laser significantly promoted the release of inflammatory factors associated with macrophage recruitment.

5. Conclusion

Er:YAG and 755 nm picosecond laser combination therapy achieved suitable therapeutic results in tattooed rats. We revealed the intrinsic mechanism of laser tattoo treatment and discussed the related factors that influence the treatment efficacy. The results showed that the effectiveness of laser treatment is closely related to the size of the pigment particles. In addition, diffuse reflection of the skin to the laser can affect the effectiveness of the laser, while related inflammatory factors can promote the recruitment of macrophages, thereby enhancing the removal effect of tattoos. These findings provide theoretical support for further optimizing laser tattoo treatment. This study was somewhat limited by the use of a single ink brand and the inherent physiological differences between human and rat skin. In addition, further studies are needed to investigate the effects of inflammatory factors on macrophages during laser treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Ethical Approval

This study was evaluated and approved by the Ethics Committee of Shandong Provincial Hospital Affiliated with Shandong First Medical University.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yongqian Cao proposed the methodology, reviewed and edited the manuscript, performed project administration, supervised the study, and was responsible for funding acquisition and overall research goal designation. Tianyu E drafted the manuscript, wrote the original draft (including substantial translation), conceptualized the study, proposed the methodology, visualized the study, curated the data, provided formal analysis, conducted research and investigation, and analyzed the data. Chen Bi investigated the study, provided formal analysis, performed project administration, organized the data, and assisted with the experimental process, making contributions to the writing of

the article. Xiaopeng Liu collected resources, curated the data, organized the data, and assisted with the experimental process, making contributions to the writing of the article. Li Lin supervised the study, reviewed and edited the manuscript, proposed the methodology, and was responsible for funding acquisition and overall research goal designation.

Acknowledgments

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