

Comparative Morphometric and Histometric Evaluation of Power-Dependent Tissue Ablation Using Fractional Carbon Dioxide Laser

Hye Guk Ryu^{1,*}, Jinyoung Park^{1,*}, Hyemin Kim¹, Wanil Kim², Hwa-Rim Lee³, Jeongah Ko⁴, Sung Bin Cho⁵

¹Clinical Development Team, Cynosure Lutronic Corporation, Goyang, Korea; ²Department of Biochemistry and Institute of Medical Science, School of Medicine, Gyeongsang National University, Jinju, Korea; ³Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Korea; ⁴Ko Jung A Clinic, Seoul, Korea; ⁵Yonsei Seran Dermatology and Laser Clinic, Seoul, Korea

*These authors contributed equally to this work

Correspondence: Sung Bin Cho, Yonsei Seran Dermatology and Laser Clinic, 224 Siheung-daero, Seoul, 08628, Korea, Tel +82 2-2135-1375, Fax +82 70-8250-1375, Email drsbcho@gmail.com

Background: Fractional carbon dioxide (CO₂) lasers are commonly used in dermatological resurfacing, offering precise ablation and controlled dermal remodeling. Although clinical outcomes vary with the energy and pulse settings, comparative morphometric and histometric data on power-dependent tissue interactions are limited.

Objective: To evaluate and compare fractional CO₂ laser-induced microscopic thermal zones (MTZs) ablation characteristics at peak powers of 30 W and 40 W using an ex vivo porcine skin model.

Methods: Ex vivo porcine skin was treated with a fractional CO₂ laser at energy levels ranging from 40 to 240 mJ under 30 W and 40 W settings. Frozen tissue was used for dermoscopic assessment of ablation diameters, while unfrozen tissue at physiological temperature (30–32°C) was analyzed histologically for ablation depth. Morphometric and histometric measurements were performed and statistically analyzed.

Results: The 30 W and 40 W settings demonstrated energy-dependent increases in ablation depth and diameter. The 40 W laser consistently generated deeper ablation columns (average 11.8% deeper) with narrower surface diameters (average 7.3% narrower) and reduced peripheral carbonization. Linear regression showed strong correlations between energy and ablation depth ($r > 0.91$) and diameter ($r > 0.91$), with higher slope coefficients in the 40 W group, indicating greater ablation efficiency.

Conclusion: The 40 W fractional CO₂ laser system offers enhanced precision, deeper dermal penetration, and reduced thermal diffusion compared with the 30 W, despite delivering equivalent total energy. These findings suggest that higher peak power settings may improve fractional resurfacing procedures' safety and clinical outcomes, particularly in populations with darker skin phototypes.

Keywords: carbon dioxide, fractional laser, ablation, ex vivo pig skin, histometric evaluation, morphometric evaluation

Introduction

Carbon dioxide (CO₂) lasers have been widely used as the gold standard for ablative skin resurfacing owing to their high absorption coefficient by water and capacity to induce controlled thermal injury to the skin. The fractional delivery of CO₂ laser energy facilitates the formation of microscopic thermal zones (MTZs) that promote dermal remodeling while minimizing downtime and adverse effects.^{1–4} The patterns of skin ablation and clinical efficacies of fractional CO₂ laser treatment vary depending on the energy settings, pulse duration, and density of the MTZs. A previous clinical comparative study demonstrated that a high-energy setting with low-density MTZs resulted in more satisfactory therapeutic outcomes for treating enlarged facial pores than a low-energy setting with high-density MTZs.⁵ In the context of chronic eczema, recent findings have also shown that a CO₂ fractional laser setting of 30 mJ with a 0.6 mm dot

spacing is the most effective among tested parameters.⁶ However, there remains a lack of studies investigating how varying peak power, while maintaining constant energy, influences tissue responses.

Biophysically, the CO₂ laser treatment at a higher peak power delivers the same amount of laser energy with a shorter pulse duration. This theoretically results in faster tissue ablation and less heat diffusion to the adjacent area.⁷ Delivering the CO₂ laser energy to the deeper dermis and reducing the extent of collateral tissue damage, accelerated wound repair with a lower risk of adverse events can be expected in Asian skin. However, morphometric and histometric data of CO₂ fractional laser-induced tissue reactions are lacking.

In this study, we compared the fractional CO₂ laser-induced tissue reactions of each MTZ, particularly skin ablation and collateral desiccation, at peak power settings of 30 W and 40 W using an ex vivo porcine skin model. To do so, the diameter of fractional CO₂ laser-induced tissue ablation was measured after delivering laser energy to frozen porcine skin to minimize immediate tissue shrinkage and was dermoscopically analyzed. The fractional CO₂ laser-induced tissue ablation depth was microscopically measured after laser treatment on porcine skin at a temperature between 30–32 °C. Moreover, our data was statistically analyzed, and the equations were developed to predict the diameter and depth of ablation after fractional CO₂ laser at different peak power settings.

Materials and Methods

Ex vivo Porcine Skin Model and Laser Treatment

Fresh ex vivo female porcine skin was purchased at 4 months of age (XP BIO Inc., Anseong, Korea). For the dermoscopic study, one part of the porcine tissue was frozen at –80 °C over 24 h. The other part was maintained at 30–32 °C in a tissue floating bath for histopathologic study. Frozen tissue was used to minimize immediate tissue contraction after laser treatment to compare the diameter of the MTZs more precisely according to the treatment parameter settings. Additionally, unfrozen tissue between 30–32 °C was prepared to compare the ablative depth of the MTZs in each treatment setting. This study used a fractional CO₂ laser (eCO2 3D™; Cynosure Lutronic Corp., Goyang, Korea), and the peak power settings of 30 W and 40 W were selected for comparative experimental purposes. The laser system was configured with a microbeam spot size of 120 µm, reflecting one of the most commonly used clinical parameters. A density of 50 MTZs/cm² was chosen to allow manual measurement of all ablation diameters and to avoid interactive thermal reactions among the MTZs. At each peak power setting, single-pass fractional CO₂ laser treatment was delivered to frozen and unfrozen porcine skin at the energy settings of 40, 60, 80, 100, 140, 160, and 240 mJ for each peak power setting, respectively.

Dermoscopic and Histometric Assessments

In this study, morphometric evaluation was performed by analyzing dermoscope images. Immediately after fractional CO₂ laser treatment, the images were captured on the surface of frozen porcine skin using dermoscope (IDS-1100; ILLUCO Corp., Gunpo, Korea) at original magnification ×10. Then, the diameters of each MTZ were measured using Image J software, version 1.48 (National Institutes of Health, Bethesda, MD, USA). Additionally, full-thickness tissue specimens, including the epidermis, dermis, and subcutaneous fat, were obtained from fractional CO₂ laser-treated unfrozen porcine skin for histometric evaluation. Each sample was fixed in 10% buffered formalin and embedded in paraffin. Next, serial tissue sections of 5-µm thickness for each treatment setting were prepared and stained with hematoxylin and eosin. Then, the depths of each MTZ were evaluated using Image J software by measuring the distance from the surface of the epidermis to the deepest part of tissue ablation in the dermis.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism, version 9 (GraphPad Software, San Diego, CA, USA). All data are presented as mean ± standard error of the mean (SEM). Group comparisons were evaluated using two-way ANOVA and Tukey's post hoc test for multiple comparisons. Pearson's correlation and linear regression analyses were conducted to evaluate the association between energy settings and tissue responses. The analyses were applied to morphometric and histometric data, including MTZ diameter and depth of tissue ablation. Pearson's correlation

coefficient (r), corresponding 95% confidence intervals, and two-tailed p -values were calculated to assess the strength and significance of the correlations. Linear regression analysis was performed using the least squares method, and the resulting regression equation was expressed as $Y = mX + b$, where m is the slope and b is the Y-intercept. The coefficient of determination (R^2) was used to assess the goodness of fit, and the statistical significance of the slope was tested using the F-test. A p -value < 0.05 was considered statistically significant for all analyses.

Results

Microscopic Comparison of Thermal Tissue Reactions at the Power of 30 and 40 W

Histological analysis using hematoxylin and eosin staining revealed distinct microscopic differences in the thermal tissue reactions between the 30 W and 40 W treatment groups (Figure 1). In both peak-power settings, the MTZs presented as conical columns of tissue ablation, extending from the stratum corneum to the dermis, with surrounding coagulation zones. As experimental energy settings increased, the MTZs of the porcine skin specimens in both groups demonstrated a gradual increase in the ablation depth and tissue coagulation area. When comparing two different peak-power settings, fractional CO₂ laser treatments at high power generated histopathological findings of dermal tissue ablation deeper into the dermis compared with low-power treatments. Moreover, skin specimens subjected to high-power treatments exhibited well-demarcated but more homogeneous coagulation zones with more constant widths surrounding the narrower ablation columns.

Prediction of Energy-Dependent Ablation Depth at the Power of 30 and 40 W

Histometric analysis of the porcine skin specimens revealed that the ablation depth increased proportionally with the delivered energy in the 30 and 40 W groups (Figure 2). Notably, the 40 W system consistently produced deeper ablation than the 30 W system at several matched energy levels, with statistically significant differences observed at 30, 50, 140, and 240 mJ ($p < 0.05$). Furthermore, Pearson correlation analysis demonstrated a strong positive correlation between energy and ablation depth in both the 30 W ($r = 0.9172$, $p < 0.0001$) and 40 W groups ($r = 0.9424$, $p < 0.0001$). The linear regression model ($Y = 4.408X + 349.2$ at 30 W and $Y = 5.005X + 377.9$ at 40 W) confirms that the energy level is a reliable predictor of penetration depth, particularly under high-power conditions (Supplementary Table 1).

Dermoscopic Comparison of Thermal Tissue Reactions at the Power of 30 and 40 W

Dermoscopic images revealed differences in the macroscopic ablation patterns between 30 and 40 W settings at matched energy levels (Figures 3–5). The skin specimens in both the 30 W and 40 W groups demonstrated round to oval, well-demarcated, and uniformly ablated MTZs, the size of which seemed to increase with increasing energy settings. Nonetheless, the 40 W CO₂ treatments generated more sharply ablated margins of each MTZ, with remarkably reduced carbonized tissue reactions, compared to the 30 W CO₂ treatments. The different ablation patterns and marginal tissue

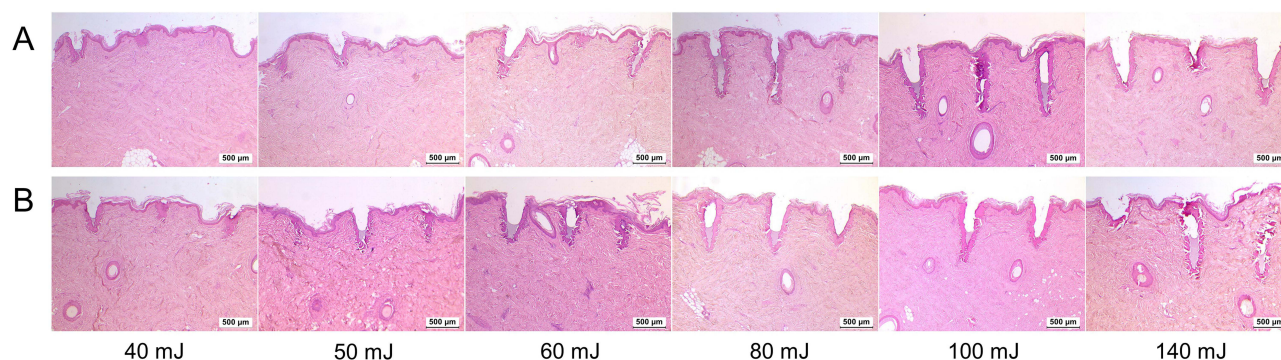


Figure 1 Histological features of microscopic thermal zones (MTZs) after fractional carbon dioxide (CO₂) laser treatments on ex vivo porcine skin. Ex vivo porcine skin was treated with a fractional CO₂ laser at (A) 30 W and (B) 40 W. The skin specimens were obtained immediately after delivering fractional CO₂ laser at the energy of 40, 50, 60, 80, 100, and 140 mJ. The 40 W-treated specimens demonstrate the narrow and deeper penetration of MTZs compared with 30 W. Hematoxylin and eosin stain, original magnification $\times 40$.

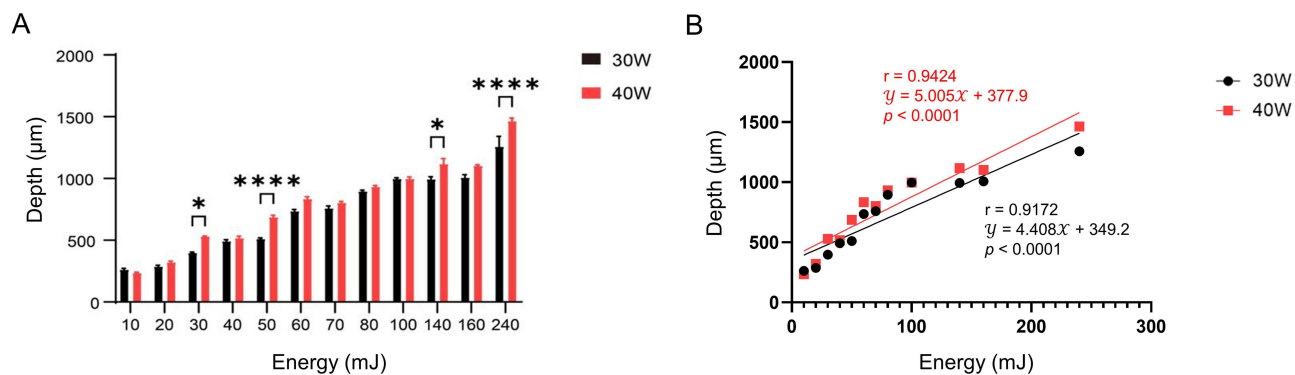


Figure 2 Comparison of energy-dependent ablation depth at the power of 30 and 40 W. **(A)** A bar graph showing the relationship between delivered energy and the depth of ablation in porcine skin (black bars, 30 W; red bars, 40 W). Asterisks denote statistically significant differences between the two groups at specific energy levels (* $p < 0.05$, *** $p < 0.0001$). **(B)** Linear correlation between energy and ablation depth in 30 and 40 W treatment groups. The 30 W group (black regression line) presented a strong correlation ($r = 0.9172$, $p < 0.0001$, $R^2 = 0.8413$, 95% CI = 0.7249 to 0.9769), and the 40 W group (red regression line) also exhibited a strong positive correlation ($r = 0.9424$, $p < 0.0001$, $R^2 = 0.8881$, 95% CI = 0.8025 to 0.9841).

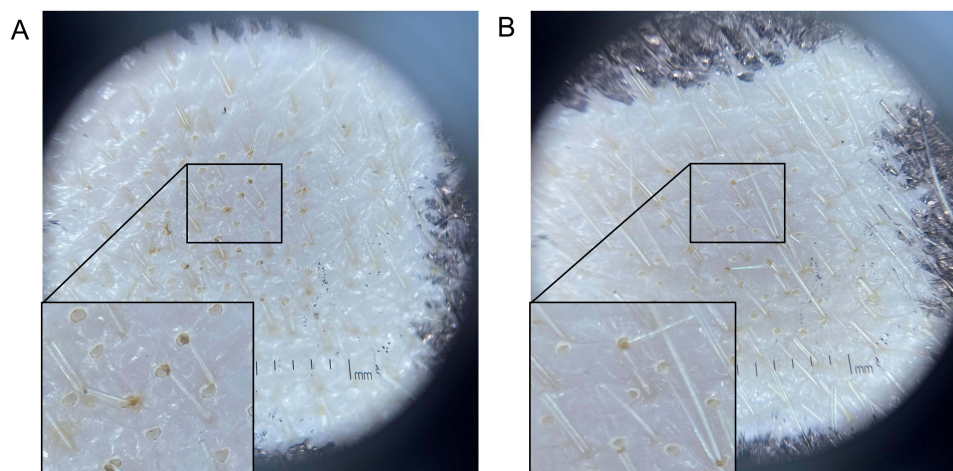


Figure 3 Dermoscopic images immediately after fractional CO₂ laser treatment at the energy of 70 mJ on the frozen porcine skin. Dermoscopic images presenting 50 MTZs after fractional CO₂ laser treatment at the energy of 70 mJ at the power setting of **(A)** 30 W and **(B)** 40 W (Insets, high power views showing ablation zones).

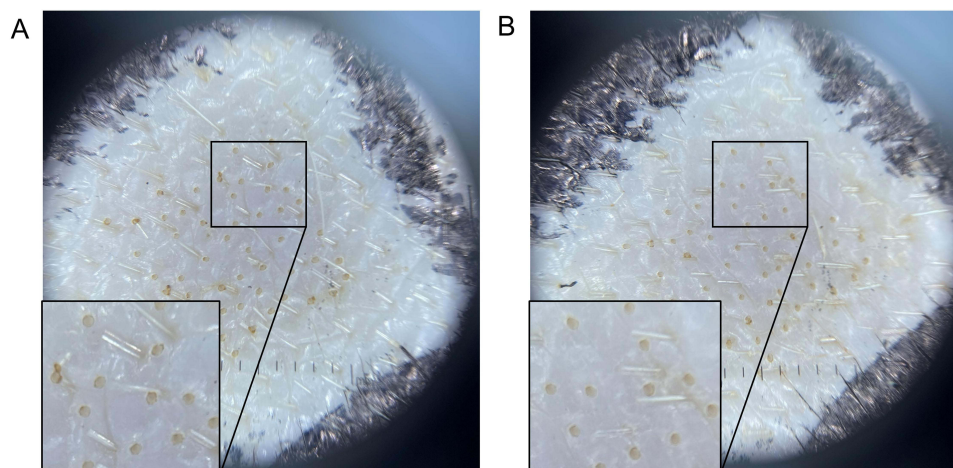


Figure 4 Dermoscopic images immediately after fractional CO₂ laser treatment at the energy of 140 mJ on the frozen porcine skin. Dermoscopic images presenting 50 MTZs after fractional CO₂ laser treatment at the energy of 140 mJ at the power setting of **(A)** 30 W and **(B)** 40 W (Insets, high power views showing ablation zones). Skin specimens, which were treated at 30 W, exhibited more irregularly carbonized surface margins of MTZs compared with 40 W.

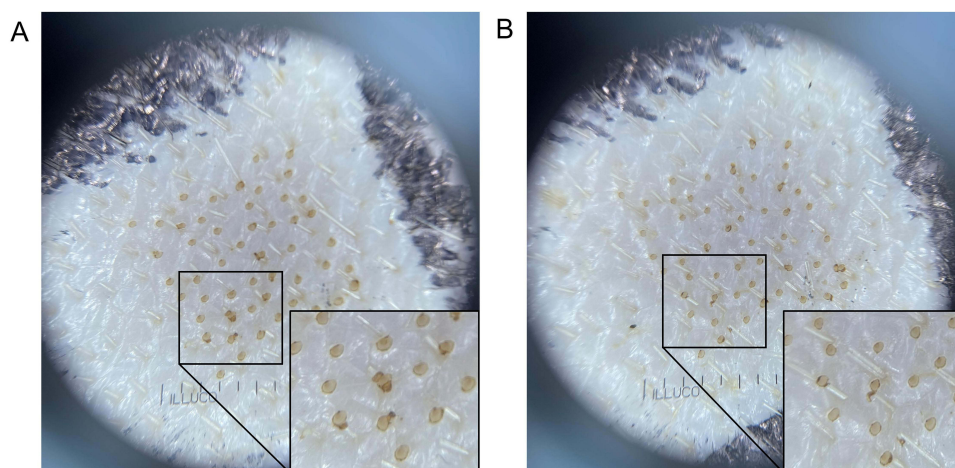


Figure 5 Dermoscopic images immediately after fractional CO₂ laser treatment at the energy of 240 mJ on the frozen porcine skin. Dermoscopic images presenting 50 MTZs after fractional CO₂ laser treatment at the energy of 240 mJ at the power setting of (A) 30 W and (B) 40 W (Insets, high power views showing ablation zones). Skin specimens, which were treated at 30 W, exhibited remarkably irregular carbonization at the surface margins of MTZs compared with 40 W.

carbonization between 30 and 40 W experimental settings when delivering higher-energy CO₂ were more pronounced to the porcine skin, particularly at 70, 140, and 240 mJ.

Prediction of Energy-Dependent Ablation Diameter at the Power of 30 and 40 W

Dermoscopy-assisted measurements of the surface diameter of fractional CO₂ treatment-induced MTZs revealed a notable increase in the ablation diameter with increasing energy delivery in both the 30 and 40 W treatments (Figure 6). Moreover, the 40 W CO₂ treatment generated smaller MTZ diameters than the 30 W CO₂ treatment at all equivalent energy settings, particularly at 50, 60, and 100 mJ. In both groups, linear regression analysis demonstrated strong positive correlations between delivered energy and ablation diameter. The correlation coefficient in the 40 W group, was $r = 0.9678$ ($p = 0.0004$), with a regression equation of $Y = 0.0008134X + 0.3224$. The 30 W group also showed a significant correlation ($r = 0.911$, $p = 0.0043$), with a regression equation of $Y = 0.0007181X + 0.3619$ (Supplementary Table 2).

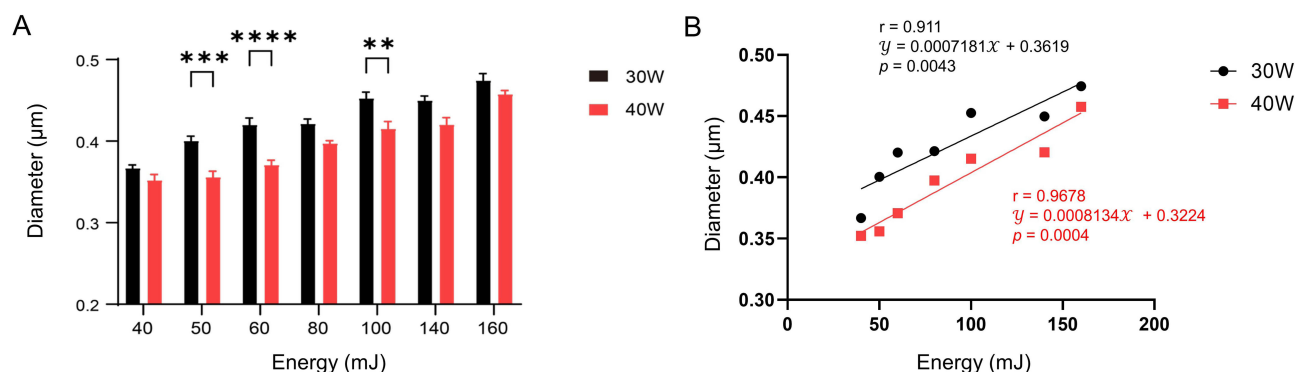


Figure 6 Comparison of energy-dependent ablation diameter at the power of 30 and 40 W. (A) A bar graph showing the relationship between delivered energy and the surface diameter of ablation in porcine skin (black bars, 30 W; red bars, 40 W). At all energy settings, MTZs after 40 W treatment generated significantly smaller ablation diameters on the skin's surface compared with 30 W treatment. Asterisks denote statistically significant differences between the two groups at specific energy levels (** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). (B) Linear correlation between energy and ablation diameter in 30 and 40 W treatment groups. The 30 W group (black regression line) presented a strong correlation ($r = 0.911$, $p = 0.0043$, $R^2 = 0.8299$, 95% CI = 0.5029 to 0.9870; $Y = 0.0007181X + 0.3619$), and the 40 W group (red regression line) also exhibited a strong positive correlation ($r = 0.9678$, $p = 0.0004$, $R^2 = 0.9366$, 95% CI = 0.7918 to 0.9954; $Y = 0.0008134X + 0.3224$).

Discussion

This study comprehensively compares fractional CO₂ laser-induced tissue responses at two different peak power settings (30 W vs 40 W) under equivalent energy delivery conditions, highlighting the impact of peak power variation on ablation and thermal effects. Based on our findings, increasing the peak power resulted in more favorable tissue interactions, characterized by significantly deeper ablation columns, sharply defined coagulation margins with consistent widths, and reduced carbonization along the ablation borders, ultimately leading to improved precision in the ablation morphology under higher-energy settings. Although achieved with the same total energy input, these differences reflect the biophysical and clinical advantages of higher peak power in fractional laser resurfacing. In contrast to the study by Jung et al,⁵ which focused on how increasing fluence (70 mJ vs 30 mJ) changed ablation depth, our work shows that even at equivalent total fluence a higher peak-power (40 W vs 30 W) pulse further deepens the ablation column while sharpening coagulation margins. This distinction underlines peak power as an independent parameter—separate from energy dose—that can be leveraged to fine-tune clinical outcomes.

The histological evaluation confirmed that the 40 W system consistently generated greater ablation depths than the 30 W system across multiple energy levels, particularly in mid-to-high settings. This suggests that increased peak power facilitates more efficient vertical energy penetration, likely owing to shorter pulse durations that reduce the thermal relaxation time and limit lateral heat diffusion.⁸ The sharply defined and smoother coagulation zones observed in the 40 W group further supported this interpretation, indicating better thermal confinement and reduced collateral tissue damage.

The dermoscopy-based assessment of the ablation morphology reinforces these observations. The 40 W system produced smaller and more uniform ablation diameters at all the tested energy levels, with less peripheral carbonization. This is particularly significant from a clinical perspective, as excessive thermal spread and surface charring are associated with delayed wound healing and a higher risk of post-inflammatory hyperpigmentation, particularly in individuals with darker Fitzpatrick skin types.^{9,10} Therefore, the more focused ablation achieved with the 40 W system may offer improved safety and cosmetic outcomes in diverse patient populations. This hypothesis aligns with Rajput et al,¹⁰ who reported that minimizing lateral thermal diffusion is critical for reducing post inflammatory hyperpigmentation (PIH) in Fitzpatrick III–V skin types. By demonstrating tighter thermal confinement at higher peak power under the same energy dose, our findings provide a mechanistic rationale for further lowering PIH risk—an enduring limitation of CO₂ fractional resurfacing. These findings suggest that a higher peak power facilitates more concentrated thermal delivery, enhancing ablation precision and limiting lateral heat diffusion. While the 30 W and 40 W systems demonstrated consistent energy-dependent modulation of the ablation morphology, the 40 W system showed greater precision, likely due to a narrower lateral energy distribution.

Quantitative correlation analyses further validated these observations. Strong linear relationships between the energy level and both the ablation depth and diameter were observed in both systems. Notably, the regression slope and intercept for the 40 W device indicated a more efficient and predictable energy-tissue interaction, supporting its potential for consistent and customizable treatment delivery. Our findings have several important implications for future research. Delivering deeper and cleaner ablation with minimal lateral thermal damage is highly desirable for procedures requiring high precision, such as periorbital rejuvenation, acne scar revision, and focal lesion targeting.^{11–13} Moreover, the improved thermal profile associated with the 40 W system may help mitigate complications such as dyspigmentation and prolonged erythema,¹⁴ which are common concerns in fractional resurfacing.

This study is limited by its *ex vivo* design, which does not fully capture dynamic biological responses, such as re-epithelialization, immune activation, or neocollagenesis. Future studies should include *in vivo* models and controlled clinical trials to evaluate real-time wound healing dynamics, collagen remodeling, and long-term efficacy. In particular, validating these findings in human skin across different Fitzpatrick types will be essential to establish optimized treatment protocols and confirm safety profiles for diverse populations.

In conclusion, the 40 W fractional CO₂ laser system showed better tissue interaction characteristics compared to the widely utilized 30 W model, particularly regarding ablation depth, uniformity, and lateral thermal confinement. These advantages highlight the clinical potential of high-peak-power fractional lasers for delivering precise and safe treatments. Nonetheless, the 30 W system remains a robust and effective platform, underscoring the importance of parameter selection tailored to specific procedural needs and patient profiles.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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