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A Comparative Study of Mucosal Wound Healing after Excision with a Scalpel, Diode Laser, or CO_2 Laser

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Background: We aimed to compare the clinical and histological secondary healing effectiveness of various types of high-level laser versus scalpel excision in mucosa frenectomy.
Methods: Forty-five Sprague Dawley rats were used in this study. These rats were divided into two laser intervention groups (CO_2, n = 15; diode, n = 15) and one control group with scalpel excision (n = 15). The effectiveness of therapy has been assessed based on the comparison of intraoperative, postoperative, and histological parameters on days 7, 21, and 35, and postoperative weight changes as pain indicator.
Results: Both laser groups demonstrated significantly ($P < 0.05$) less bleeding than did the control group during the intraoperative stage, whereas the CO_2 laser showed more precise cutting compared with the diode laser ($P < 0.05$). The highest healing score was reported in the CO_2 and scalpel groups on the first week of healing than in the diode group ($P < 0.05$). However, no significant difference was observed between the groups on days 21 and 35. Weight loss was significantly ($P < 0.05$) demonstrated in the diode group compared to the scalpel and CO_2 groups till day 7. Both laser groups demonstrated delayed healing process compared with the scalpel. Nevertheless, the CO_2 group followed the scalpel trends after day 7.
Conclusion: Scalpel and CO_2 laser yielded a superior clinical outcome compared with the diode excision of oral mucosa, whereby the CO_2 has been proposed as the most effective laser type at the end of the first postoperative month. *(Plast Reconstr Surg Glob Open 2023; 11:e5150; doi: 10.1097/GOX.0000000000005150; Published online 4 August 2023.)*

INTRODUCTION

The frenulum was historically thought of as a mucosal fold,[3] but, in recent years after a thorough revision, is now known to be a more complex structure that may contain connective tissue, fascia layers, and even muscle fibers.[1,2] As described by Priyanka et al,[4] the most notable frenal attachments in the oral cavity are the maxillary labial fraenum, mandibular labial fraenum, and lingual fraenum. Frenectomy is the excision of the entire frenulum and release of its attachment (ie, the whole frenulum). There are several techniques to perform the procedure. The conventional technique involves excision of the fraenum using a scalpel,[5] whereas the diode laser technique[6] and CO_2 laser technique are more recent alternatives.[7] Laser technology has been reported in the literature as an alternative to conventional techniques. Laser surgery is increasingly used in many fields of medicine. In particular, high-intensity lasers have proved to be an effective tool in the treatment of various lesions in medical specialties such as gynecology, plastic surgery, infertility surgery, urology, ophthalmology, otolaryngology, and oral surgery.[8] CO_2, erbium, neodymium, and diode lasers have been commonly used for soft-tissue excisions.[9] Several authors have reported that the laser cutting surgery includes visibility, hemostasis, precision, infection control and elimination of bacteremia, lack of mechanical tissue trauma, less postoperative pain, less edema, less scarring and tissue shrinkage, microsurgical capabilities, and fewer instruments at the site of operation.[10–13] However, there are some

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controversies about part of those reports. In contrast, laser surgery showed a higher level of postoperative pain and a more extended healing period, which could be explained by the effects of laser on the blood vessels.[14–16] The effectiveness of laser use in frenectomy is well documented in the literature,[10,17] but there are a very few studies that compared this laser technique with the conventional method for frenectomy in the intraoperative, clinical postoperative, and histological modalities. Knowledge of the clinical and histological healing process in frenectomy after laser cutting is critical for the procedure's short and long-term success. The aims of this study were to evaluate the secondary wound healing, macroscopic and microscopic morphological changes, and wound healing effects in Sprague Dawley rats' frenulum excised with scalpel, CO_2, and diode. The study also aimed to identify which of these tools (scapel, CO_2, and diode) causes the least amount of tissue damage and, as a result, offers a theoretical foundation for the selection and deployment of clinical laser devices.

Takeaways

Question: What is the impact of high-level laser excision compared with scalpel excision on the secondary healing process, clinically and histologically, in oral mucosa frenectomy?

Findings: The study compared high-level laser excision with scalpel excision for oral mucosa frenectomy. The results showed that both laser groups had significantly less bleeding during the surgery compared with the control group. The CO_2 laser was found to show more precise cutting than the diode laser. In terms of healing, the CO_2 and scalpel groups had the highest healing scores during the first week compared with the diode group. Laser groups showed a delayed healing process compared with the scalpel group. Nevertheless, the CO_2 group followed a trend similar to that of the scalpel group after day 7.

Meaning: The study findings suggest that both scalpel excision and CO_2 laser excision lead to better clinical outcomes compared with diode excision in oral mucosal surgery.

METHODS AND MATERIALS

Experimental Model

Forty-five male Sprague Dawley rats [Sprague Dawley outbred rats, Envigo, Rehovot, Israel; mean weight 350 g (± 10%), 12 weeks old] were involved in this study. All experiments were conducted in accordance with the guidelines on the Animal Ethics and Welfare Committee of the Technion—Israel Institute of Technology (IL0540618).

Study Group

Mucosal excision was performed at the lingual frenulum, considering its anatomy, with three different tools. The animals were randomly divided into the following groups: S: scalpel, C: CO_2 laser (10,600 nm), and D: diode laser (1470 nm). In each group, samples were taken at three time points: day 7, 25, or 35.

Surgery

General anesthesia was administered to rats using an intramuscular dose of 90 mg per kg ketamin (100 mg/mL) plus 10 mg per kg xylazine (20 mg/mL). Systemic analgesia was achieved by a subcutaneous injection of 0.01–0.05 mg per kg buprenorphine (0.3 mg/mL). After sterilization of all surgical equipment, the rats were fixed to a surgical platform. The Sprague Dawley rat mouths were stretched using rubber bands fixed on their front teeth, for a maximum oral cavity opening. The tongues were then stretched with surgical mosquito forceps to help reach the frenulum's opposite side, in order to retract the frenulum. First, an incision was made longitudinally in the midline of the tongue, and the second cutting line was made horizontally to disconnect the frenulum out of the floor of the mouth. The frenulum was then excised, leaving a diamond-shaped wound.

Postoperative Care

After surgery, the rats were housed in separate cages, and their weights were monitored by weighing them two times per week. The animals were given buprenorphine for analgesia 3 days postoperatively. Moistened food pellets and sweet jelly cubes were given to them, allowing for easier chewing and digestion.

Macroscopic Evaluation

The intraoperative parameters were evaluated as nominal parameters (yes/no) and were recorded if there was presentation of bleeding (during the procedure), carbonization (conversion of an organic substance into carbon or a carbon-containing residue through the thermal collateral damage that the lasers produced), margin integrity (continuity and integrity of the cutting site), and tissue destruction (damage to the target and adjacent tissues). The postoperative parameters were evaluated as nominal parameters and the sum of the scores was calculated to evaluate the total index score for healing at each killing point of time on day 7, 21, and 35 postoperatively. We modified the Landry Healing Index[18] according to our clinical model limitations and evaluated eight parameters.

Weight Trends

The weights of the rats were recorded in a numeric manner, and the delta differences of the day (on days 3, 7, 21, and 35) were compared. All the rats were followed up twice daily in the first 72 hours, and twice weekly after the first 72 hours until they were killed.

Tissue Harvesting

The rats were killed by CO_2 asphyxiation at one of the planned points of time: day 7, 21, or 35. The entire mandibles of the rats were harvested, and fragments were immediately fixed in 4% buffered formaldehyde for 6 hours. After 6 hours, the frenula were harvested and then placed in 4% buffered formaldehyde solution for 24 hours.

Microscopic Evaluation

The specimens were dehydrated through an ascending series of alcohol, cleared in xylene, embedded in paraffin, and subsequently cut longitudinally into 4-μm-thick sections using a microtome (Leica, Nussloch, Germany). The sections were transferred onto glass slides. For histological evaluation, tissues were deparaffinized by xylene, rehydrated through a descending alcohol series, and subsequently stained by Harris hematoxylin, Masson trichrome, and picrosirius red. The specimens were dehydrated in ascending alcohol solutions, cleared in xylene, covered in xylene-based mounting medium, and the cover slipped.

Scoring System

To evaluate the morphological wound healing events, the hematoxylin & eosin and Masson trichrome stain was used, and a modified histologic scoring system was developed specifically for this study based on the scoring system suggested by Abramov et al[19] for assessment of each parameter with scores of 0–3, as shown in Table 1. In our research, another modification was performed as described below. The qualitative histology studies were performed by analyzing the stained tissues, using a specific score for each parameter, as described below.

For each parameter, the data analysis was performed using the mean of the scores from each slice analyzed. All histological slides of the rats' frenectomies were blindly evaluated on two independent occasions, by each of the two pathologists.

Polarized Microscope

Two slides from each sample were examined under an Olympus BX51 microscope (Olympus Corp., Tokyo, Japan). Images were captured using a Retiga 2000R (QImaging, Vancouver, Canada) camera. Images were analyzed using ImageJ/Fiji 1.46 software (LOCI, University of Wisconsin, Wis.). ImageJ software was used for "splining channels" for converting RGB format of the images to red and green channel images. The threshold was adjusted to identified particles and set a point to amplify particles in the image for recognition in a set area. Particles were analyzed under automatic "particle analysis," measuring the particles in the selected area.

Picrosirius Red–stained C collagen I:III Ratio

Picrosirius red–stained collagen fibers were examined under a polarizing microscope to identify and evaluate polarization colors of thin (0.8 μm or less) and thick collagen fibers (1.6–2.4 μm). The collagen I:III ratio was calculated for each slide by dividing the red count of particle analysis by the green count of particle analysis ($\mu m^2/\mu m^2$).

Table 1. Histological Assessment Parameters and Score

Parameters	0	1	2	3
Inflammation	None	Discrete	Moderate	Abundant
Vascularization	None	Discrete	Moderate	Abundant
Epithelialization	None	Partial	Immature or thin	Complete
ECM	None	Discrete	Moderate	Abundant

Statistical Analysis

Statistical analysis was performed using the Rstudio statistical software package, version 1.3.1093 (PBC, Boston, Mass.). Frequencies (N, %) were calculated for categorical parameters. Data are presented as mean ± standard deviation (SD) in continuous parameters. Data were evaluated for normality, using the Shapiro-Wilk Test. One-way ANOVA was used for parametric test, and Kruskal Wallis test for non-parametric values, using Bonferroni adjustment method for correction. To compare between two lasers groups, pairwise Student *t* tests were performed. A P value of less than 0.05 was considered as statistically significant.

Results

Forty-five animals underwent the surgical procedure; no general-anesthesia–related death or postoperative death accrued. All animals were killed according to the veterinarian's instructions, in accordance with the accepted protocol.

Macroscopic Evaluation

Forty-five rats were evaluated intraoperatively for estimation of the surgical site, in the three groups: S, C, and D. In the S group, intraoperatively 87% (n = 13) rats had bleeding during the procedure compared with 13% (n = 2) in both laser groups; a statistically significant difference was found between those groups ($P < 0.001$). Carbonization in the C group was 86% (n = 13) when compared with 53% (n = 8) in the D group, and showed a statistically significant difference ($P < 0.05$). Margined integrity was 93% (n = 14) in both the S and C groups, whereas in the D group, it was 53% (n = 8) ($P < 0.05$). Tissue distraction was the lowest in the S group with 13% (n = 2), 20% (n = 3) in the C group, and 53% (n = 8) in the D group. No statistical difference was found in that category.

Clinical postoperative wound area was evaluated by the modified Landry Healing Index, with mean score and SD as shown in Table 2. The total score on day 7 was highest in the S group followed by the C and D groups, respectively. A statistically significant difference was found between the S and D groups ($P < 0.01$) and C and D groups ($P < 0.01$), but no statistical significance was found between the S and C groups ($P > 0.05$). Moreover, no statistical significance was found at the other points of time on days 21 and 35 ($P > 0.05$).

Weight Changes

To determine if the Sprague Dawley rats from three different vendors showed a difference in weight change after the frenectomy procedure, weights were recorded for the first 3 days and then twice weekly until the killing date, on days 3, 7, 21 and on day 35 after the procedure. Among the

Table 2. Mean and SD of the Total Score of the Modified Landry Healing Index

	Scalpel		CO_2		Diode		
	Mean	SD	Mean	SD	Mean	SD	
Day 7	6.6*	0.548	6.0†	1.000	3.0	0.707	$P > 0.001$
Day 21	7.4	0.894	7.0	0.707	6.8	0.837	
Day 35	8.0	0.000	7.8	0.447	7.6	0.548	

*Scalpel–diode laser P less than 0.05.
†CO_2 laser–diode P less than 0.05.

groups, rats lost weight at different rates; S and C group rats showed a moderate weight loss, whereas group D rats showed a more aggressive loss of weight. A critical weighing point was on the third day the after surgery; the S group showed the lowest loss of weight followed by the C group with a moderate loss of weight, and the most severe loss of weight was recorded in the D group. At that point in time, a statistically significant difference was found in weight changes between the S and D groups ($P = 0.0000$) and between the C and D groups ($P = 0.0003$). On day 7 postoperative, a statistically significant difference was found in weight changes between the S and D groups ($P = 0.0003$) and between the C and D groups ($P = 0.0004$). No statistically significant difference was found in the weight changes among the three groups, on days 21 and 35 (Fig. 1).

HISTOLOGY

Morphological Evaluation of Wound Healing

After the healing period (days 7, 21, and 35), the frenectomy sites in the scalpel and lasers groups were evaluated by using hematoxylin & eosin staining (Fig. 2) and Masson trichrome staining. The mean and SD for each parameter at each point of time are shown in Table 3. Score index of inflammation is shown in Fig. 3A. On day 7, the inflammation scores indicated significant statistical differences between S and D groups ($P = 0.0062$) and between S and C groups ($P = 0.0053$). On day 21, the trend changed, and groups S and D and C and D had statistically significant differences ($P = 0.0071$ and $P = 0.0139$, respectively). No statistically significant difference was found between S and C groups on day 21. On day 35, no statistically significant difference was found between the S and D groups and between C and D groups ($P = 0.0085$ and $P = 0.0126$, respectively). No statistically significant difference was found between S and C groups on day 35. For vascularization scores (Fig. 3B), on day 7, a statistically significant difference was found between both laser groups (C and D), which demonstrated at that point of time that they had the same score with the same SD when compared with the S group ($P = 0.0109$). On day 21, a statistically significant difference was found between the S and D groups and C and D groups ($P = 0.0053$ and $P = 0.0038$, respectively). On day 35, a statistically significant difference was found between the S and D groups and C and D groups ($P = 0.0139$ and $P = 0.0153$, respectively). No statistically significant differences were found on days 21 and 35 between the S and C groups ($P > 0.05$). For epithelization scores (Fig. 3C), on day 7, statistically significant differences were found between the S and D groups ($P = 0.0109$) and a tendency for differences between the C

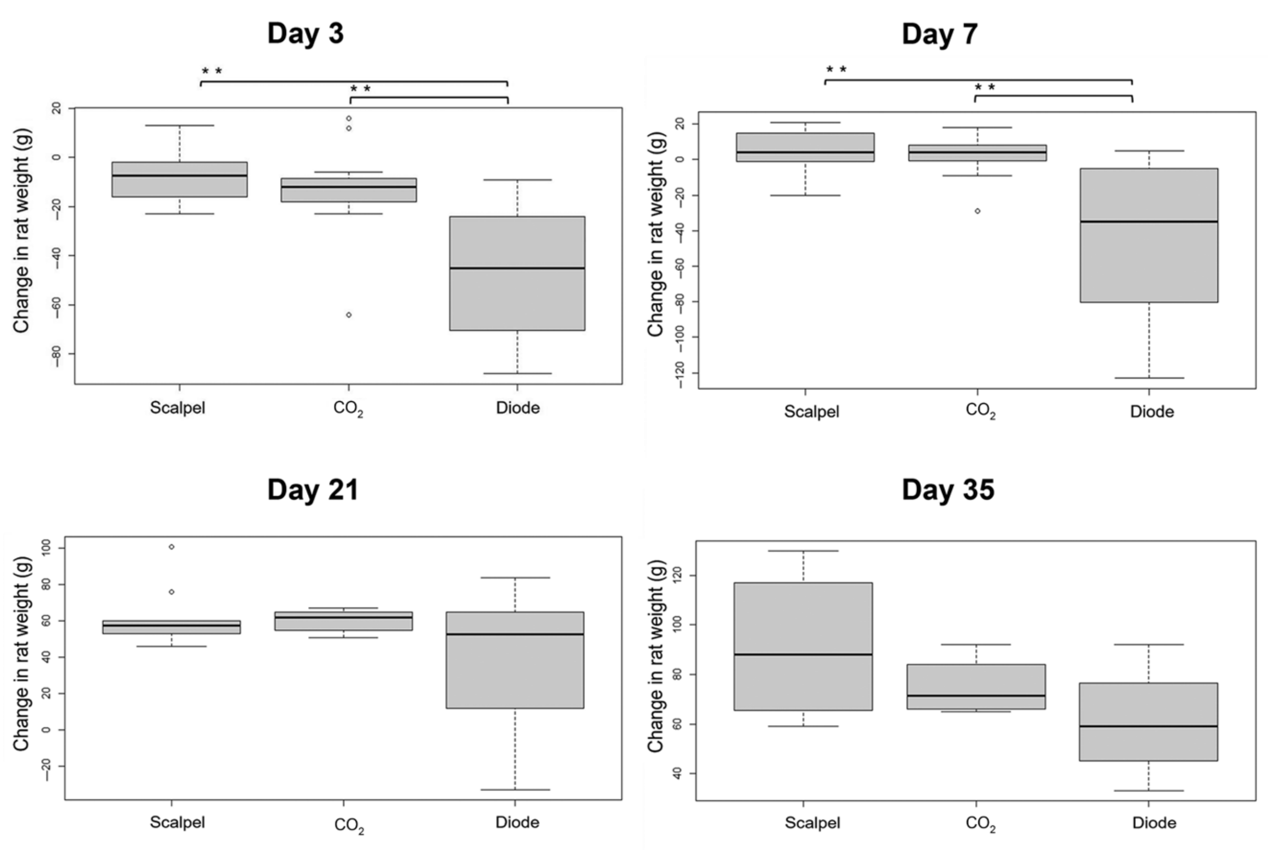

Fig. 1. Weight change in Sprague Dawley rats. The delta differences of Sprague Dawley rat body weights changed 3 days postoperative when compared with those at the starting point, with correlation to days after the frenectomy procedure, depending on the tool with which they were operated on. **P value less than 0.01.

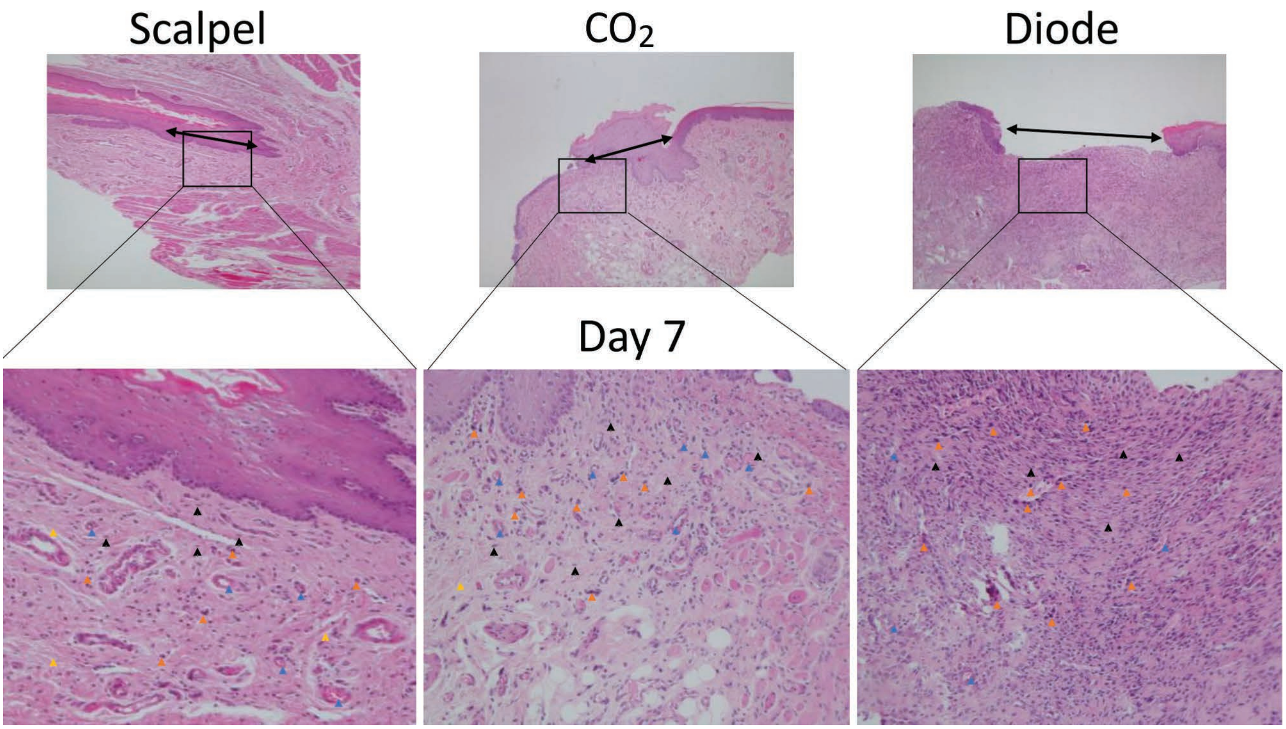

Fig. 2. Hematoxylin & eosin sections of the lingual frenulum after frenectomy day 7. Histological photomicrographs of the scalpel, CO_2, and diode groups at day 7 of the healing period. The first row demonstrates a 40× magnification of the incision site; the second row is a 100× magnification of the site. Two-sided black arrows indicate epithelization. Blue arrow heads represent vascularization; orange arrow heads, inflammatory cells; black arrow heads, fibroblast; and yellow arrow heads, collagen bundles.

Table 3. Mean and SD of the Scores of Evaluations of Histological Wound Healing

		Scalpel		CO_2		Diode		
Parameter	Day	Mean	SD	Mean	SD	Mean	SD	
Inflammation	7	0.8*†	0.447	2.2	0.447	2.6	0.548	$P < 0.01$
	21	0.6†	0.548	1.0‡	0.707	2.4	0.548	$P < 0.05$
	35	0.2†	0.447	0.4‡	0.548	2.0	0.707	$P < 0.01$
Vascularization	7	1.25§‖	0.447	2.0	0.548	2.6	0.548	$P < 0.05$
	21	0.8†	0.447	1.0¶	0.000	2.2	0.447	$P < 0.01$
	35	0.6‖	0.548	0.4‡	0.447	1.8	0.447	$P < 0.05$
Epithelization	7	2.8‖	0.447	2.4‡	0.548	1.6	0.548	$P < 0.05$
	21	3.0†	0.000	2.8‡	0.447	1.8	0.447	$P < 0.01$
	35	3.0	0.000	3.0	0.000	3.0	0.000	—
ECM	7	2.0	0.707	1.6	0.548	1.0	0.707	—
	21	2.6‖	0.548	2.6‡	0.548	1.2	0.447	$P < 0.05$
	35	1.6†	0.548	2.2‡	0.447	3.0	0.000	$P < 0.05$

*Scalpel–CO_2 laser: *P* less than 0.01.
†Scalpel–diode laser: *P* less than 0.01.
‡CO_2 laser–diode: *P* less than 0.05.
§Scalpel–CO_2 laser: *P* less than 0.05.
‖Scalpel–diode laser: *P* less than 0.05.
¶CO_2 laser–diode: *P* less than 0.01.

and D groups ($P = 0.0312$) with no statistically significant difference between the S and C groups ($P > 0.05$). On day 21, a statistically significant difference was found between the S and C groups when compared with the D group ($P = 0.0039$ and $P = 0.0153$, respectively). All the groups completed full re-epithelization at the time point on day 35, with no statistical differences found among them. For extracellular matrix (ECM) scores among the groups (Fig. 3D), on day 7, no statistically significant differences were found among the three groups ($P > 0.05$). On day 21, statistically significant differences were found between the S and C groups (which demonstrate the same score with the same SD) when compared with the D group ($P = 0.0109$). On day 35, a statistically significant difference was found between the S and C groups when compared with the D group ($P = 0.0046$ and $P = 0.0143$, respectively).

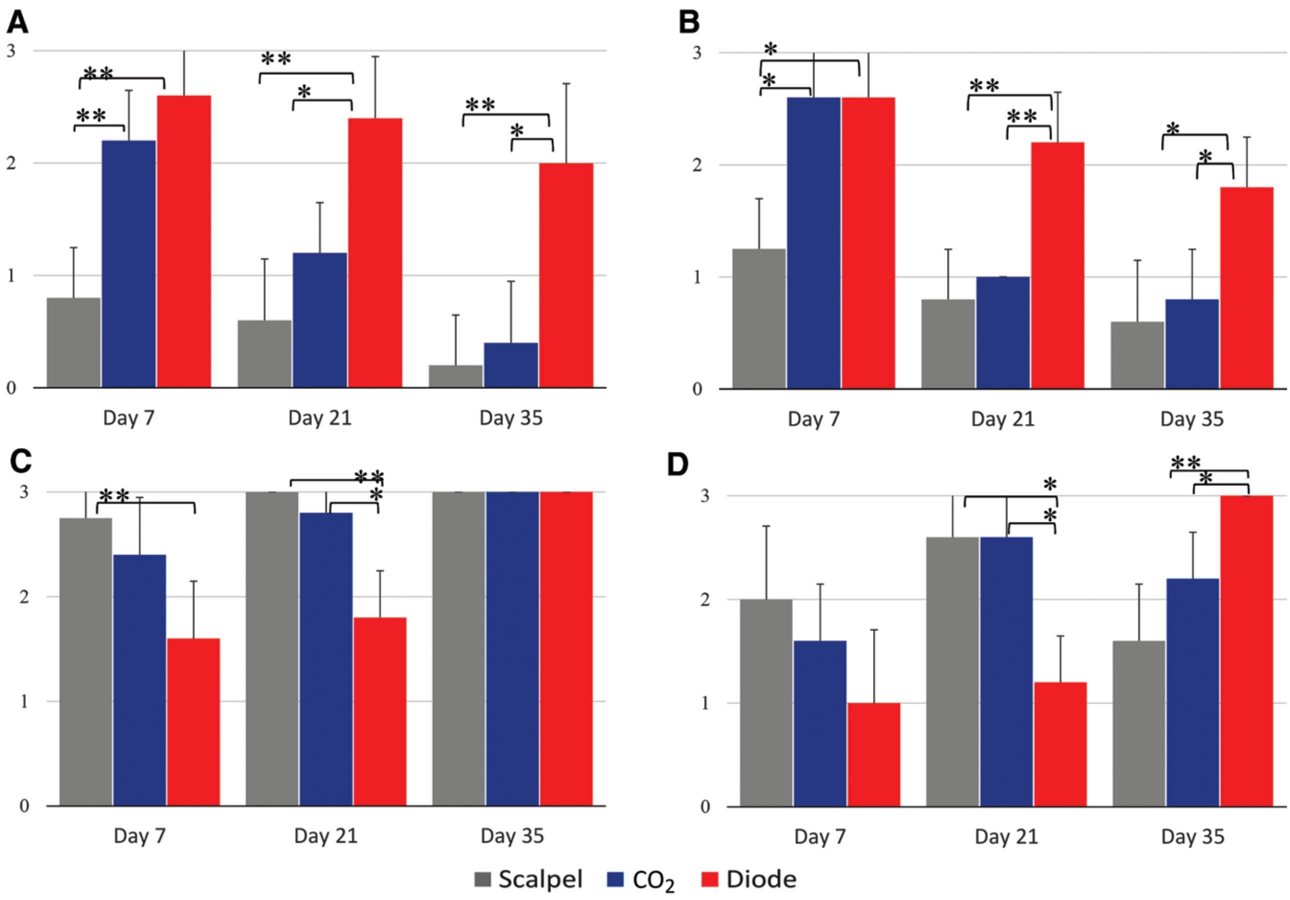

Fig. 3. Microscopic evaluation scores: evaluations of histological wound healing. A, Score of inflammation. B, Score of vascularization. C, Score of epithelization. ECM evaluation index (D) of hematoxylin and eosin and Masson trichoma staining depends on the tool used at each point of time. Gray indicates scalpel; blue, CO_2; and red, diode. $*P < 0.05$, $**P < 0.01$.

Table 4. Mean and SD of Ratio Collagen I/III

	Scalpel		CO_2		Diode		
	Mean	SD	Mean	SD	Mean	SD	
Day 7	0.71†	0.218	0.51‡	0.170	0.19	0.107	$P < 0.01$
Day 21	0.67*	0.150	0.48	0.159	0.29	0.151	$P < 0.05$
Day 35	0.94	0.181	0.70	0.292	0.74	0.147	—

*Scalpel–diode laser: *P* less than 0.05.
†Scalpel–diode laser: *P* less than 0.01.
‡CO_2 laser–diode: *P* less than 0.01.

Collagen Type I/III Ratio

Under polarized light microscopy, sirius red sections were visualized to compare the local tissue ratio of collagen I and collagen III; mean and SD were documented as shown in Table 4. Collagen I is called red, and collagen III is considered green, as presented in Figure 4.

Collagen ratio at day 7 was statistically significantly different between the S and D groups ($P = 0.009$) and between the C and D groups (0.016). On day 21, statistically significant difference was found between the S and D groups ($P = 0.01$) and not between the S and C groups or C and D groups. On day 31, no statistically significant difference was found among the groups. The red line represents the ratio of collagen in the control group that did not undergo the surgical frenectomy procedure (Fig. 5).

DISCUSSION

Although many attempts have been made to understand the laser–tissue interaction in mucosal tissue, no efficient histological evaluation has been reported in frenectomy tissue. In this study, the effects of scalpel, CO_2, and diode lasers on frenectomy were studied within an animal model for clinical and histological secondary healing process. The results showed that both CO_2 and

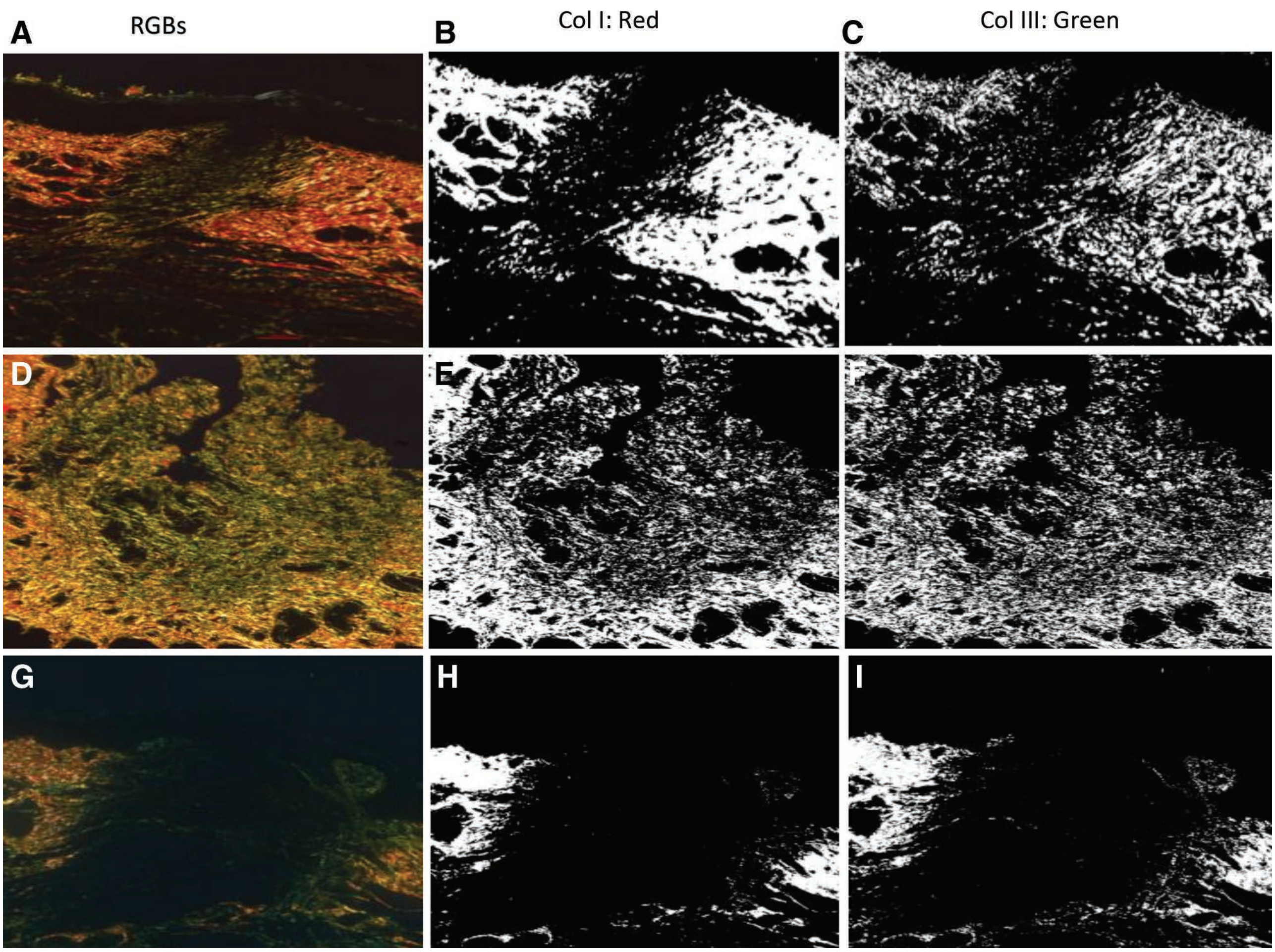

Fig. 4. Picrosirius red staining under polarized light. Collagen organization under polarized light microscopy in full color presentation, RGBs (A, D, G). Red spectrum of light represents collagen I (B, E, H), and green spectrum of color represents collagen III (C, F, I). Groups: scalpel (A–C), CO_2 (D–F), and diode (G–I). Original magnification: 100×.

diode lasers were effective in performing frenectomy in the oral cavity. Nevertheless, the scalpel presented clinically and histologically the fastest healing trends. The CO_2 group presented healing trends exceeding the diode group healing procedure among the laser groups. During the intraoperative clinical appearance, lasers have demonstrated advantages as mentioned in the literature by Pick and Colvard,[20] and less bleeding was observed in the laser groups when compared with that in the scalpel group. In the current study, the CO_2 group laser produced more carbonization than did the diode group. However, as we know from the literature, the healing process will not be delayed if the carbonization is wiped out from the surgical site.[15] The carbonization of the tissue may be controlled by the choice of selected parameters. The integrity of the incision margins was the most accurate in the scalpel and the CO_2 groups. Tissue distraction was worst in the diode group. We could assume the reason for this is due to diode laser workflow, that demands contact with the tissue during movements of the optical fiber from side to side adjacent to the target tissue, which may damage close anatomical structures. The operator should take this into consideration. The sum of the score at the postoperative surgical site at each killing point of time has demonstrated that CO_2 and scalpel groups had faster healing than did the diode group. Based on our results, the healing process from the clinical aspect was delayed at the diode group and did not demonstrate rapid healing, as mentioned by Fornaini et al.[21] Nevertheless, on days 21 and 35, clinical evaluation of the surgical site revealed no significant differences among the groups.

In the current study, where frenectomy was done on rat frenulum, weight data monitoring showed that the tool used for the frenectomy procedure had an impact on the ability of the rat to gain weight and thrive, whereas absence of weight gain could reflect suffering and pain.[22] From our results, we found that the rats in the diode group had the most significant loss of weight and for a more extended period of time. However, after 35 days, the trend ended, and the three groups reached the same level of weight. Given those results, it is essential to remember the collateral effects. Even though after 35 days, the three groups reached similar weight

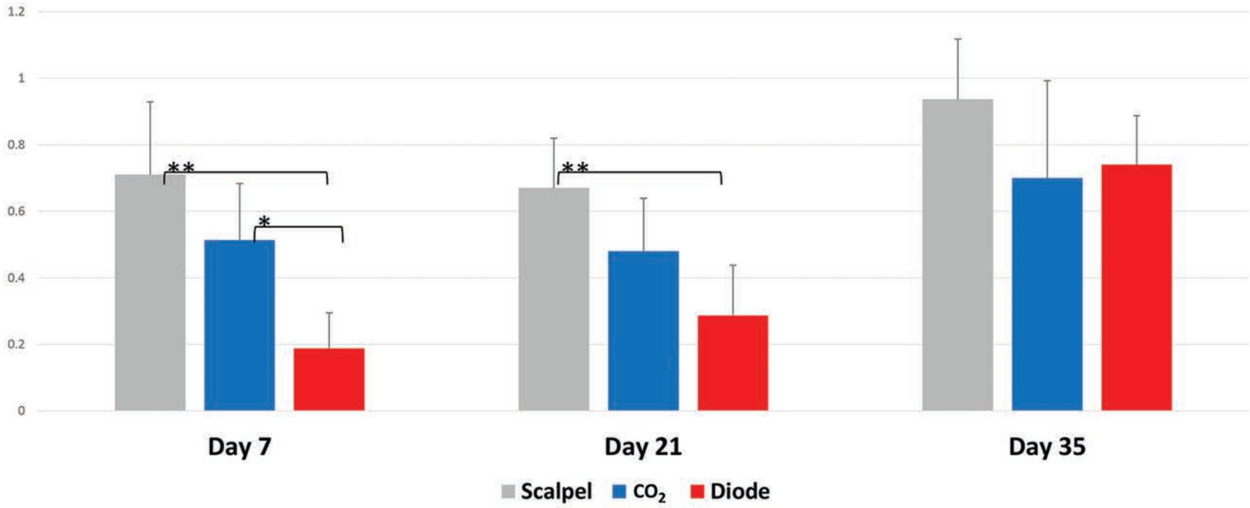

Fig. 5. Collagen ratio type I/III count at 1 week, 3 weeks, and 5 weeks postoperative. The score of evaluation index for hematoxylin and eosin staining depends on the tool used. Gray indicates scalpel; blue, CO_2; red, diode. *$P < 0.05$, **$P < 0.01$.

trends, in the diode laser group, the impact on weight loss was severe compared with in the CO_2 laser group, especially during the first 21 days postoperative. The CO_2 laser group showed more predictable results compared with the diode group. Considering our limitations, if we extrapolate our results from the rat model to the clinical aspect, we should consider the short-term and long-term impact. Gaining weight in the developmental stages of a newborn is critical, and drastic loss of weight can be traumatic.[23] In contrast, the CO_2 laser and the scalpel group's differences were insignificant even during the first days. It makes the CO_2 laser as much a predictable and reliable tool as the scalpel for frenectomy, even in newborns. As we know that the tongue plays a crucial role in eating, we can assume that in the CO_2 and scalpel groups, the rats had free movement and better functionality than those in the diode group. Moreover, reports in the literature found that laser produces less postoperative pain.[20,24] Contrary to these declarations, in this work we have assumed that the laser groups had more pain than the scalpel group, especially the diode group with the most drastic weight loss.

Researchers have debated on the period of healing after scalpel versus different laser excisions in frenectomy. Carreira and Azevedo[25] reported on the shorter time of healing and less uncomfortable period for the patient. On the other hand, other authors witnessed a more extended healing period and explained it by the effects of laser on the blood vessels.[14–16] Our findings correlated with the second opinion; we found that laser produces collateral damage to the tissue by the thermal effect and causes "delay" in the healing process. We discovered that CO_2 exhibited a significant difference than the scalpel in the histological parameters of vascular damage and inflammation. These differences presented only in the first 7 days. However, after 21 days, the CO_2 group demonstrated significantly less inflammation and ECM, and better re-epithelization than did the diode group. The CO_2 group resembled the scalpel group. It can be assumed that CO_2 laser shows a slower healing period compared with the scalpel, but it was significantly better than that of the diode laser. From our findings, we can point out that the histological data indicate that the secondary healing after frenectomy performed with scalpel suggests a faster and better healing procedure compared with the laser groups. The data in the literature indicate that there is a delay of 2–4 days in the healing procedure after cutting with laser versus scalpel. Our work found that scalpel groups exhibit an adequate healing process clinically and histologically at day 7. On the other hand, even though the surgical site's clinical evaluation reveals a proper healing process in the diode group, the histological picture indicated a poor healing process in the diode laser even on day 35, which may indicate a delay in clinical healing and an even longer delay in histological healing.

In the present study, collagen I:III ratio was higher in the scalpel and CO_2 groups compared with the diode group on day 7, and that significant difference remains between the scalpel and the diode on day 21. However, on day 35, no differences were found among the three groups. At the three points of time, the collagen ratio in the control group was still relatively far from the mean ratio when compared with that in the experimental groups; the scalpel group alone on day 35 has reached the control group collagen ratio levels, and yet the mean of the ratio was lower than that of the control. Our results demonstrated a high correlation between collagen remodeling and laser–tissue effects. We observed that both laser irradiation modalities impact the anatomical structure of frenulum collagen. Liu et al[26] explained that the photomechanical effect promotes more effectively the synthesis of collagen type III. In contrast, the photothermal effect favored the formation of collagen type I. Contrary to that explanation, our diode group produced a more extensive thermal effect than the other groups, and yet we did not find a higher collagen ratio in that group, as would be expected by that explanation. Nevertheless, it is crucial to take into consideration the fact that, based on the literature and our experiment, the laser groups have a delay in the healing process, especially the diode group, where we can assume that the remodeling phase lags after the CO_2 and scalpel groups. As mentioned by Larson et al,[27] more type III collagen fibers are found to contribute to scarless healing in oral cavity wounds, which help maintain a more flexible wound and allow for better cell migration and regeneration. The presence of type I collagen in wounds offers increased strength and stiffness to healing tissue, but may obstruct cellular migration and regeneration.[28] In accordance with our results, we assume that the balance between collagen I and collagen III is crucial for maintaining the balance between relapse on the one hand and scar formation on the other.

The ideal healing process after frenectomy would be the one that less clinically affects daily functional activities (especially eating), but histologically gives more elasticity than the base state or a condition of no elasticity when a scar has formed.

CONCLUSIONS

The scalpel exhibits its supremacy over lasers in the clinical and histological parameters of secondary wound healing in frenectomy. The scalpel demonstrated a shorter healing period, with the least impact on the epithelial and connective tissue when compared with the lasers group. Thus, it might cause more relapse in the long term. In the literature, lasers have been considered as useful, well-studied tools for mucosal surgical procedures. In our study, the application of lasers demonstrated delayed healing in the frenectomy procedure in rat models. Nevertheless, starting with day 21, using CO_2 laser and scalpel for performing lingual frenectomy in the rat model yielded significantly better outcomes than those achieved using a diode laser. These findings correlate with the histological results, which showed increased inflammation and vascularization even after 5 weeks in the diode group when compared with the scalpel and CO_2 groups. The present study demonstrates that laser application at frenectomy is a feasible treatment that has advantages and disadvantages in its clinical and histological aspects. The advantages of both lasers, as mentioned in the literature and from our practice, are the hemostasis that the laser promotes and visualization in the surgical field. The CO_2 laser demonstrated its superiority over the diode laser in the weight changes after the excision procedure, a ratio of collagen I/III, less inflammation, and less vascular damage. A key issue for future research will be finding the optimal laser and its parameters in the clinical setting to improve its effectiveness in different mucosal tissue procedures, considering the clinical and the histological aspects. Because it has been documented previously that clinical improvement is not always directly consistent with observed histological changes, similar comparative studies in human clinical settings are needed to confirm the results achieved in our experiments.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

REFERENCES

1. Walsh J, McKenna Benoit M. Ankyloglossia and other oral ties. *Otolaryngol Clin North Am.* 2019;52:795–811.
2. Mills N, Keough N, Geddes DT, et al. Defining the anatomy of the neonatal lingual frenulum. *Clin Anat.* 2019;32:824–835.
3. Mills N, Geddes DT, Amirapu S, et al. Understanding the lingual frenulum: histological structure, tissue composition, and implications for tongue tie surgery. *Int J Otolaryngol.* 2020;1:2020.
4. Priyanka M, Sruthi R, Ramakrishnan T, et al. An overview of frenal attachments. *J Indian Soc Periodontol.* 2013;17:12–15.
5. Devishree, Gujjari SK, Shubhashini PV. Frenectomy: a review with the reports of surgical techniques. *J Clin Diagn Res.* 2012;6:1587.
6. Pirnat S. Versatility of an 810 nm diode laser in dentistry: an overview. *J Laser Health Acad.* 2006;4.
7. Cunha RF, Silva JZ, Faria MD. Clinical approach of ankyloglossia in babies: report of two cases. *J Clin Pediatr Dent.* 2008;32:277–281.
8. Azadgoli B, Baker RY. Laser applications in surgery. *Ann Transl Med.* 2016;4:452–452.
9. Azma E, Safavi N. Diode laser application in soft tissue oral surgery. *J Lasers Med Sci.* 2013;4:206–211.
10. Pié-Sánchez J, España-Tost AJ, Arnabat-Domínguez J, et al. Comparative study of upper lip frenectomy with the CO_2 laser versus the Er, Cr:YSGG laser. *Med Oral Patol Oral Cir Bucal.* 2012;17:228.
11. Parker S, Cronshaw M, Anagnostaki E, et al. Current concepts of laser–oral tissue interaction. *Dent J (Basel).* 2020;8:61.
12. Kara C. Evaluation of patient perceptions of frenectomy: a comparison of Nd:YAG laser and conventional techniques. *Photomed Laser Surg.* 2008;26:147–152.
13. Convissar RA. *Principles and Practice of Laser Dentistry.* Philadelphia: Elsevier Health Sciences; 2010.
14. Schünke M, Krüss C, Mecke H, et al. Characteristic features of wound healing in laser-induced incisions. *Adv Otorhinolaryngol.* 1995;49:8–14.
15. Tichý M, Hubácek J, Jansa P, et al. Výsledný obraz reparace rány způsobené CO2-laserovým skalpelem [The final picture of wound healing in CO2 laser scalpel surgery]. *Cesk Patol.* 1986;22:217–221. [Czech.]
16. Filmar S, Jetha N, McComb P, et al. A comparative histologic study on the healing process after tissue transection. II. Carbon dioxide laser and surgical microscissors. *Am J Obstet Gynecol.* 1989;160:1068.
17. Yadav RK, Verma UP, Sajjanhar I, et al. Frenectomy with conventional scalpel and Nd:YAG laser technique: a comparative evaluation. *J Indian Soc Periodontol.* 2019;23:48.
18. Landry RG. *Effectiveness of Benzydamine HC1 in the Treatment of Periodontal Post-Surgical Patients.* Toronto: University of Toronto; 1985.
19. Abramov Y, Golden B, Sullivan M, et al. Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model. *Wound Repair Regen.* 2007;15:80.
20. Pick RM, Colvard MD. Current status of lasers in soft tissue dental surgery. *J Periodontol.* 1993;64:589–602.

21. Fornaini C, Rocca JP, Bertrand MF, et al. Nd:YAG and diode laser in the surgical management of soft tissues related to orthodontic treatment. *Photomed Laser Surg.* 2007;25:381–392.
22. Leiphart JW, Vasudevan PP, Rajjoub SR, et al. The effect of acute postoperative pain and chronic neuropathic pain on subsequent weight gain in the rat. *J Neurosurg.* 2010;112:780–783.
23. Miyoshi Y, Suenaga H, Aoki M, et al. Determinants of excessive weight loss in breastfed full-term newborns at a baby-friendly hospital: a retrospective cohort study. *Int Breastfeed J.* 2020;15:19.
24. Gáspár L, Szabó G. Manifestation of the advantages and disadvantages of using the CO_2 laser in oral surgery. *J Clin Laser Med Surg.* 1990;8:39–43.
25. Carreira L, Azevedo P. Comparison of the influence of CO_2-laser and scalpel skin incisions on the surgical wound healing process. *J Anesth.* 2016;1:1.
26. Liu H, Dang Y, Wang Z, et al. Laser induced collagen remodeling: a comparative study in vivo on mouse model. *Lasers Surg Med.* 2008;40:13–19.
27. Larson BJ, Longaker MT, Lorenz HP. Scarless fetal wound healing: a basic science review. *Plast Reconstr Surg.* 2010;126:1172–1180.
28. Merkel JR, DiPaolo BR, Hallock GG, et al. Type I and type III collagen content of healing wounds in fetal and adult rats. *Proc Soc Exp Biol Med (New York, NY).* 1988;187:493.