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Histological evaluation of the surgical margins of oral soft tissue incisions using a dual-wavelength diode laser and an Er, Cr:YSGG laser; an *ex vivo* study

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Abstract

Oral soft tissue lesions require a precise diagnosis by oral biopsy with the ability to recognize these lesions within histopathological levels, so the instrument used for the incisions should be safe and cause little to no harm to the surrounding tissue. Objective: This study compared a dual-wavelength diode laser and an Er, Cr:YSGG laser in oral soft tissue incisions to determine the most effective and safest laser system at the histopathological level. Methodology: The (810 and 980 nm) dual-wavelength diode laser was used at 1.5 W and 2.5 W (CW) power settings, and the (2780 nm) Er, Cr:YSGG laser was used at 2.5 W and 3.5 W (PW) power settings. Both laser systems were used to incise the tissues of freshly dissected sheep tongue pieces to obtain the following histopathological criteria: epithelial tissue changes, connective tissue changes, and lateral thermal damage extent by optical microscopy. Results: The epithelial and connective tissue damage scores were significantly higher in the dual-wavelength diode laser groups than in the Er, Cr:YSGG laser groups ($P<0.001$), and there was a significant difference between some groups. The extent of lateral thermal damage was also significantly higher in the diode laser groups than in the Er, Cr: YSGG laser groups ($P<0.001$), and there was a significant difference between groups. Group 2 (2.5 W) of the diode laser was the highest for all three criteria, while group 3 (2.5 W) of the Er, Cr:YSGG laser was the lowest. Conclusion: The Er, Cr:YSGG laser with an output power of 2.5 W is, histologically, the most effective and safest laser for oral soft tissue incision. The dual-wavelength diode laser causes more damage than the Er, Cr:YSGG laser, but it can be used with a low output power and 1 mm safety distance in excisional biopsy.

Keywords: Er, Cr:YSGG laser. Diode laser. Soft tissue. Histological labeling.

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Introduction

Multiple pathologic disorders of varying etiology and severity can affect the soft tissues of the oral cavity, and early identification is essential for effective treatment.[1] Oral soft tissues are commonly affected by reactive hyperplastic lesions as a result of frequent injuries.[2,3] Oral soft tissue lesions are divided into the following categories: ulcerative, vesiculobullous, white and red, pigmented, and papillary or hyperplastic. These lesions are further subclassified according to their etiology and/or pathogenesis, which refer to systemic diseases, genetic factors, environmental factors, habits, trauma, dental or prosthetic causes, or unknown causes, as in neoplastic lesions.[4,5] Major challenges in the diagnosis of these different lesions are caused by the enormous overlap of their signs and symptoms, which can only be eliminated by a thorough knowledge of the clinicopathologic features of each condition and a histological approach to diagnosis.[6]

A variety of techniques can be applied in the removal of oral soft tissue lesions, including the use of a traditional scalpel, an electric scalpel, or various types of lasers.[7,8] Any technique used should result in minimum to no damage to the surrounding tissue and the best postoperative response. Over the past 30 years, various types of lasers have been widely used as incision instruments in dentistry, including Co2, the Erbium family, neodymium:yttrium–aluminum–garnet (Nd:YAG), and Diode lasers.[9,10] When compared to the use of cold blades, the use of lasers has been shown to enhance and improve clinical and surgical procedures, resulting in a high degree of surgical site decontamination, minimal postoperative bleeding (sealing blood vessels and potentially reducing the seeding of malignant cells at the time of surgery), and a significant decrease in inflammation (sealing lymphatic vessels at the time of surgery) and postoperative pain (the ability to seal nerve endings is helpful in lessening postoperative discomfort).[11-13]

Diode lasers produce light that ranges from the visible red portion of the spectrum to the region near infrared. The efficiency, simplicity, and compactness of semiconductor lasers are their primary benefits. They do not require much additional technology and connect easily to optical fibers, which makes diode laser suitable for medical application.[11,14-16]

The erbium, chromium:yttrium scandium-gallium-garnet (Er, Cr:YSGG) 2780nm wavelength lasers belong to the Er-L family. In general practice, they are used on soft tissue lesions, tooth structure, and bone, as Er, Cr:YSGG is well-absorbed by hydroxyapatite, water, and collagen.[11,17]

The laser's photothermal interaction mechanism, which occurs when it interacts with certain tissue chromophores to heat and vaporize the targeted tissues before causing them to separate, is the basis for laser soft tissue incision. At the specimen edges, this mode of action causes thermal effects ranging from coagulation to carbonization. These can lead to inaccurate histological findings, including the presence of pseudo-dysplastic alterations, which can delay healing or at least complicate the histopathological identification of oral soft tissue disease.[9,18]

Since the heat spreading on tissues depends on the type of laser or the power, this study concerns the use of the dual-wavelength diode laser, which combines the wavelengths most commonly used (810 and 980 nm) nowadays in surgical incisions, and the Er, Cr:YSGG laser with different power outputs. Previous studies in this field have histologically compared the thermal effects of different types of lasers, but none of them has investigated the effect of these two lasers in oral soft tissue surgery.

This *ex vivo* study aims to histologically compare the dual-wavelength diode and Er, Cr:YSGG laser systems with specified different powers in the peri-incisional region to achieve an effective oral soft tissue incision with minimal thermal damage clinically.

Methodology

Ethical approval

This article does not include any human or animal studies conducted by any of the authors. This is an *ex vivo* study carried out in accordance with the ethical standards of the Institute of Laser for Postgraduate Studies, University of Baghdad in Iraq (number 1550, project no.43 in 25/01/2023).

Study design

The five tongues used in this study were taken from sheep aged 8-17 months immediately after slaughter and irradiated within five hours. Each tongue was cut into two halves and then into small pieces 10 mm thick, 20 mm wide, and 15mm long.

Laser incisions were made on the lateral side of the tongue on each piece. The sample number was 28 blocks, 14 for each type of laser and seven for each laser type parameter.

The sample size was determined using the simplest formula for randomized controlled trials comparing two groups of equal size according to information from previous literature.[19]

Laser systems

Two types of laser were used for the soft tissue incisions: the first was a dual-wavelength "810+980nm" diode laser (QUICKLASE 12W dual 4 (810+980), England, UK), used with initiated optical fiber (FC 400μm single file multimode), and the second was a 2780 nm wavelength Er, Cr:YSGG laser (WATERLASE IPLUS BIOLASE, California, USA), used with the MZ6 tip(diameter 600 μm, length 6 mm).

Group 1 (G1): The samples were cut with a diode laser at 1.5 W powers in continuous wave (CW) mode and at a power density of 1153.8 W/cm^2. Group 2 (G2): The samples were cut with a diode laser at 2.5 W powers in CW mode and a power density of 1923 W/cm^2. The optical fiber was checked with a power meter (PINTUDY, Guangzhou CN) before the incisions in each group.

Group 3 (G3): The samples were cut with an Er, Cr:YSGG laser at 2.5 W power, 50 mJ pulse energy, 892.8 W/cm^2 power density, and 71.43 W peak power. Group 4 (G4): The samples were cut with an Er, Cr:YSGG laser at 3.5 W power, 70 mJ pulse energy, 1250 W/cm^2 power density, and 100 W peak power. Both G3 and G4 were cut in pulse wave (PW) mode at a pulse duration of 700 μsec, a frequency of 50 Hz, and 10% water, 10% air.

Laser surgical procedure

The diode laser optical fiber was initiated at each power output prior to incision. The specimens from all four groups were irradiated perpendicularly in contact mode at the same room temperature (27 ºC) and by the same experienced clinician at a speed of 0.75 mm/sec, an incision length of 1.5 cm, and an adjusted exposure time of 20 seconds.

Histological evaluation

After completion of the surgical incisions, the specimens were fixed in 10% buffered formalin solution and sent to the histological laboratory for histopathological examination on the same day. The conventional processing methods in the laboratory included fixation with formalin and dehydration by graded ethanol solution then xylene in two steps to impede the tissue in paraffin wax. Each block was sectioned at 5 micrometers; the first sections were neglected, and three slides from each block were stained by Hematoxylin-Eosin (H&E) (42 slides per laser type; 21 slides per group).

A light microscope (GOWE Lab Instrument Laboratory Binocular Head Biological microscope, Japan), camera (5MP USB CMOS Camera Microscope Digital Electronic Eyepiece w/ 0.5X C Mount Lens, mainland China), and software computer program (S-EYE 2.0, China) were used in the examination of all slides to discover the following criteria established by Vescovi, et al.[20] (2010):

1) Epithelial tissue (ET) changes, scored from zero to three based on the presence (1) or absence (0) of each of the following factors: nuclear changes, cytoplasmic changes, and loss of epithelial and sub-epithelial attachment; 2) Connective tissue (CT) changes: scored from zero to three based on the presence (1) or absence (0) of each of the following factors: carbonization, desiccation (vacuolization), and vascular changes; 3) Lateral thermal damage extent: measured (in μm) from the edge of the incision to the entire healthy tissue, in all four groups.

Statistical analysis

Statistical comparisons were made using SPSS software. The Mann-Whitney test and the two-sample t-test were used to compare the samples of the two systems, while the Kruskal-Wallis test, Chi-square test, one-way ANOVA test, and post hoc Tukey test were used to compare the four groups. A 0.05 significance level was used.

Result

The results of all histological evaluation data were first compared between the two laser systems and then between the four groups. Epithelial and connective tissue changes were significantly higher in the median values of the diode laser than in those of the Er, Cr:YSGG laser according to the Mann-Whitney test, applied to all samples of the two devices ($P<0.001$) (Table 1) (Figure 1A). There was a significant difference in medians and ranges among the

Table 1- Comparison of histological criteria between all samples of the diode and Er, Cr:YSGG laser systems

Parameter	Diode N=42 Median (Range) Mean± SD	Er, Cr:YSGG N=42 Median (Range) Mean± SD	P value
Epithelial damage (Mann-Whitney test)	3 (2-3)	2 (1-3)	<0.001
Connective damage (Mann-Whitney test)	3 (2-3)	2 (1-3)	<0.001
Lateral thermal damage extent (µm) (two-sample t test)	221.24±85.44	110.6±36.14	<0.001

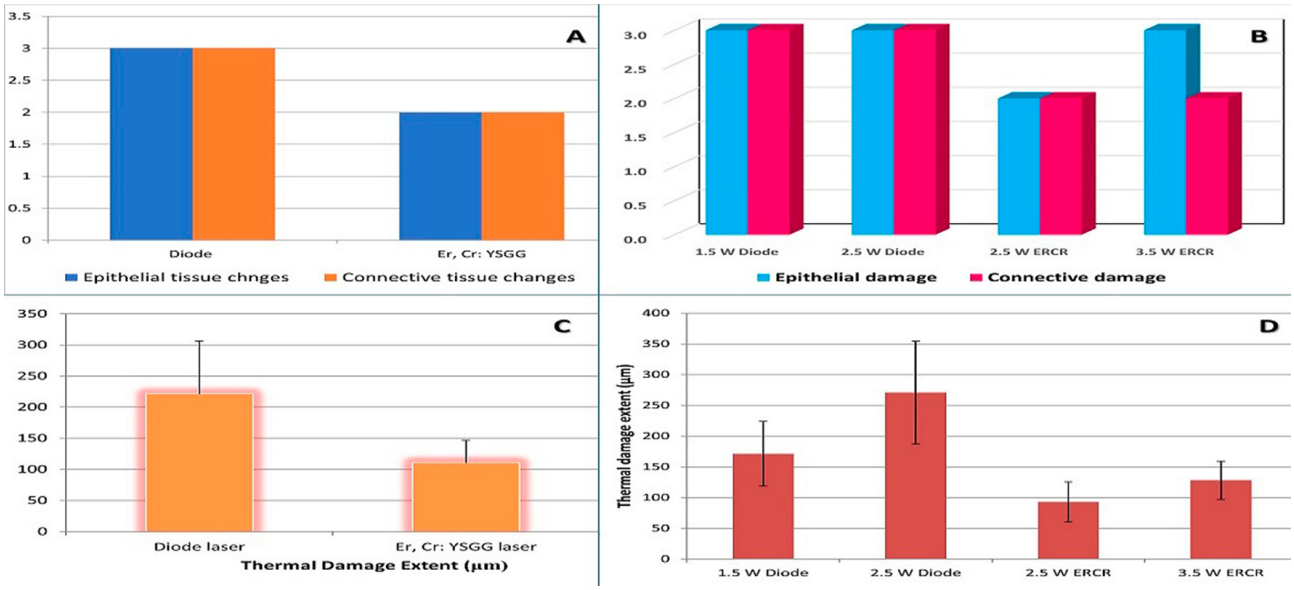

Figure 1- A) Epithelial and connective tissue changes in the Er, Cr:YSGG and diode laser samples. B) Epithelial and connective tissue changes in the four study groups of the diode and Er, Cr:YSGG lasers. C) Lateral thermal damage extent of the diode and Er, Cr:YSGG laser samples. D) Lateral thermal damage extent in the four study groups of the diode and Er, Cr:YSGG laser samples

four study groups according to the Kruskal-Wallis test (P<0.001) (Table 2) (Figure 1B). The Mann-Whitney test (Table 3) showed significantly higher epithelial and connective tissue changes in the medians of the diode laser groups than in those of the Er, Cr:YSGG laser groups, but G1 was not significantly different from G2 in terms of epithelial tissue changes, and G3 was not significantly different from G4 (3.5 W Er, Cr:YSGG laser) in terms of epithelial and connective tissue changes (P>0.05).

According to the Chi-square test, the subdivisions of epithelial tissue changes showed no significant difference between the four groups of the two laser systems in terms of nuclear changes (P>0.05). There was a significant difference between them in terms of cytoplasmic changes and loss of epithelial and sub-epithelial attachment (P<0.001), with the two changes being higher in G2 and lower in G3 (Table 2).

According to the Chi-square test, there was a significant difference between the subdivisions of connective tissue changes in the presence of carbonization (P<0.001), being higher in G1 and G2 and lower in G3. Desiccation showed no significant difference among the four groups (P>0.05). Vascular changes were significantly different between the four groups (P=0.002), being higher in G1 and G2 and lower in G3 (Table 2) (Figures 2 and 3).

The lateral thermal damage extent (LTDE) was significantly higher in the means and standard deviations (SD) of the diode laser samples than in those of the Er, Cr:YSGG laser samples (p<0.001) (Figure 1C), as shown by the two-sample t-test (Table 1). Among the four groups, the means showed a significant difference (p<0.001) according to the one-way ANOVA test (Table 2) (Figure 1D). According to a post-hoc Tukey test, between the four groups (Table 3), the mean of G1 was higher for thermal damage extent but not significantly higher than that of G4, and the mean of G4 was higher for thermal damage extent but not significantly higher than that of G3 (P>0.05).

Table 2- Comparison of histological criteria between the four groups of the diode and Er, Cr:YSGG laser systems

Parameter	G1	G2	G3	G4	P value
Nuclear Changes Yes (1) No (0) (Chi-square test)	21(100%) 0(0)	21(100%) 0(0)	21(100%) 0(0)	21(100%) 0(0)	1
Cytoplasm changes Yes (1) No (0) (Chi-square test)	21(100%) 0(0)	21(100%) 0(0)	10(47.62%) 11(52.38%)	13(61.9%) 8(38.1%)	<0.001
Loss of attachment Yes (1) No (0) (Chi-square test)	19(90.5%) 2(9.5%)	21(100%) 0(0)	9(42.9%) 12(57.1%)	16(76.2%) 5(23.8%)	<0.001
Epithelial tissue damage score (0-3) Median (Range) (Kruskal-Wallis Test)	3(2-3)	3(3-3)	2(1-3)	3(1-3)	<0.001
Carbonization Yes (1) No (0) (Chi-square test)	21(100%) 0(0)	21(100%) 0(0)	6(28.57%) 15(71.43%)	7(33.33%) 14(66.67%)	<0.001
Desiccation Yes (1) No (0) (Chi-square test)	13(61.9%) 8(38.1%)	21(100%) 0(0)	16(76.2%) 5(23.8%)	16(76.2%) 5(23.8%)	0.07
Vascular changes Yes (1) No (0) (Chi-square test)	21(100%) 0(0)	21(100%) 0(0)	13(61.9%) 8(38.1%)	19(90.5%) 2(9.5%)	0.002
Connective tissue damage score (0-3) Median (Range) (Kruskal-Wallis Test)	3 (2-3)	3(3-3)	2(1-3)	2(1-3)	<0.001
Lateral thermal damage extent mean± SD (one-way ANOVA test)	171.43±52.66	271.05±83.56	93±32.43	128.19±31.25	<0.001

Table 3- Comparison of histological criteria between each pair of power and/or diode and Er, Cr:YSGG laser systems

Parameter	1st group	2nd group	P value
Epithelial damage (Mann-Whitney test)	1.5 W Diode	2.5 W Diode	0.152
		2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	0.011
	2.5 W Diode	2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	0.001
	2.5 W Er, Cr: YSGG	3.5 W Er, Cr:YSGG	0.064
Connective damage (Mann-Whitney test)	1.5 W Diode	2.5 W Diode	0.002
		2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	0.007
	2.5 W Diode	2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	<0.001
	2.5 W Er, Cr: YSGG	3.5 W Er, Cr:YSGG	0.148
Lateral thermal damage extent (μm) (post-hoc Tukey test)	1.5 W Diode	2.5 W Diode	<0.001
		2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	0.056
	2.5 W Diode	2.5 W Er, Cr:YSGG	<0.001
		3.5 W Er, Cr:YSGG	<0.001
	2.5 W Er, Cr: YSGG	3.5 W Er, Cr:YSGG	0.162

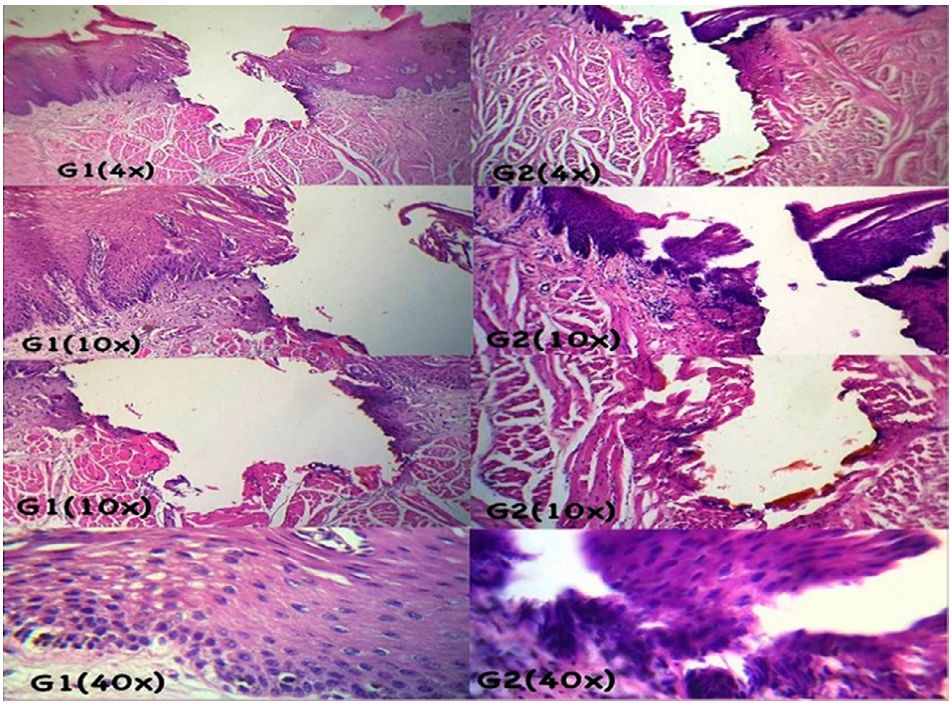

Figure 2- Cross-sectional histology of soft tissue incisions with G1 (1.5 W) and G2 (2.5 W) of the diode laser systems 4x, 10x upper part, 10x lower part, and 40x magnification

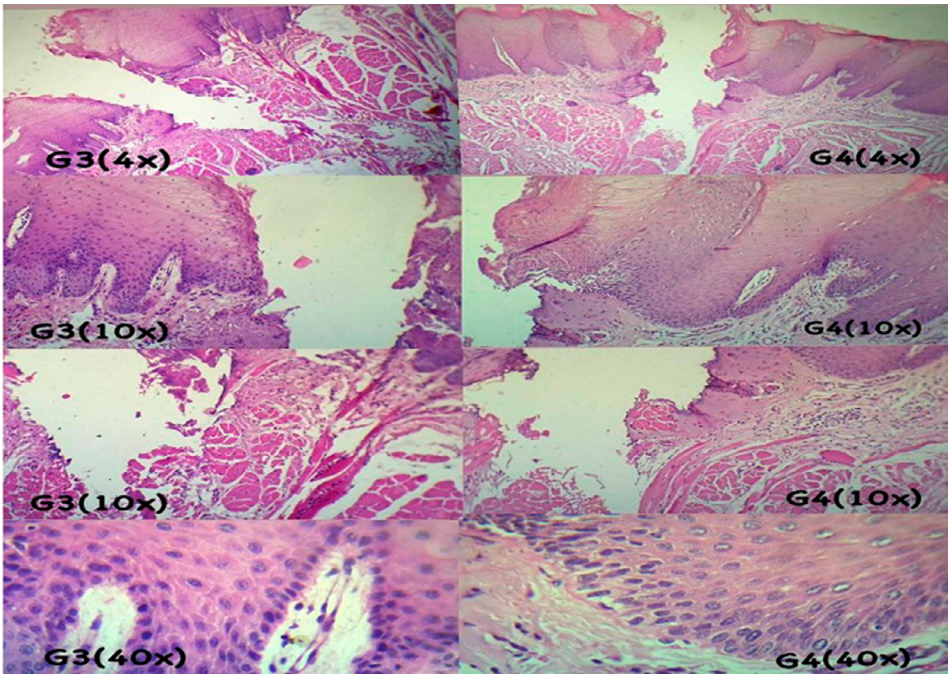

Figure 3- Cross-sectional histology of soft tissue incisions with G3 (2.5 W) and G4 (3.5 W) of the Er, Cr:YSGG laser system at 4x, 10x upper part, 10x lower part, and 40x magnification

Discussion

There are many studies discussing the use of lasers in soft tissue incisions, but few of them focus on heat conduction and tissue damage in the peri-incisional margins, and very few studies compare two types of lasers histologically, especially the studies that discuss Er, Cr:YSGG lasers in soft tissue incisions. Power, wavelength, pulse duration, frequency, emission mode, irradiated area, exposure time, tip material, incision speed, optical and chemical properties of the irradiated tissue, and the use of water or air spray are some of the internal and external factors that affect how the laser interacts with the tissue and in how much peri-incision thermal damage it results.[18,21-22]

In this study, epithelial tissue changes were higher in the diode laser groups than in the Er, Cr:YSGG laser

groups. Nuclei alterations (picnotic, spindle-shaped, and hyperchromic nuclei) were found in each group of the laser systems, but cytoplasmic alterations (hyperchromic cytoplasm, cell fusion, and loss of normal cell adhesion) and the loss of intra- and sub-epithelial attachment were higher in the diode laser groups than in the Er, Cr:YSGG laser groups, which had fewer cases of these alterations. Connective tissue changes, including carbonization (thermal necrosis) and vascular alterations (thrombosed, collapsed blood and lymphatic vessels), were higher in the diode laser groups than in the Er, Cr:YSGG laser groups, but desiccation was observed in all groups (Table 2). This is related to the photothermal interaction mechanism with different wavelengths, laser radiation power, emission mode, and tissue area irradiated, as seen in the previous studies.

In previous studies, Monteiro, et al. [23] (2019) evaluated clinically increased histological tissue changes in diode laser samples at 980 nm with a PW power of 3.5 W. They found that the score for ET changes was 2.95/(0-3) and the score for CT changes was 2.76/(0-3). Romeo, et al.[24] (2007) evaluated 980 nm in CW at 2 W in animals and showed collagen homogenization and derma-epithelial detachment, and 1.5 W generated >1000 μm ET damage and >1500 μm in CT. Azevedo, et al.[25] (2016) evaluated 980 nm at 3.5 W and Boost at 3.5 W in PW mode. They found that the mean cytoplasmic score was 1.70 and the mean CT score was 2.00. Palaia, et al.[26] (2021) showed 976 nm in CW at 4.5 and 6 W; the mean epithelial damage in the peri-incisional area was 0.2 mm in ET and 0.3 in CT.

Rizoiu, et al.[27] (2014) observed minimal edge coagulation and carbonization when using the Er, Cr:YSGG laser and found no vacuolization changes in ET and CT, in contrast to this recent study.

In the present study, the LTDE was lower in the Er, Cr:YSGG laser samples than in the diode laser samples. The biggest area of damage was present in G2 and the smallest one was present in G3, according to the different delivery systems of the two lasers, the different main tissue target chromophores for the laser wavelength used, the power density, energy density, and the different emission modes.

Previous studies have investigated the thermal impact of the 808 nm laser on soft tissues. The tested powers ranged from 1 to 3 W, and the fluency measurements varied from 284 to 2400 J/cm^2. The spot size was 300 or 320 μm in PW and CW modes. The reported thermal impact ranged from 17.92 to 473 μm.[21,24,28-31] The investigations indicated the magnitude of the thermal impact with a range of 100 to 1198.54 μm among the 940-980 nm eight diode laser studies.[23,25,26,31,32]

Previous studies of the Er, Cr:YSGG laser showed a spot size of 600 or 680 μm. One study recorded the irradiance at 707-1000 W/cm^2, and two studies reported the fluence at 35- 53 J/cm^2, and when an air/water spray was used, there was little thermal impact. The range of the total thermal impact in two trials with comparable parameters was 9.26 to 33.1 μm, with settings of 1 W in PW (20 Hz), air/water spray, and a spot diameter of 600 μm.[21,24,28,33]

Melanin, hemoglobin, and protein are the main chromophores in the 808-1064 nm wavelength range of diode lasers. Because water is not a chromophore, it interacts relatively little at diode wavelengths, even though it makes up over 70% of most tissues. Since laser energy is dispersed across a significant area within tissues, this illustrates why the diode laser is a poor option for cutting soft tissue. Although there is a significant chance of serious collateral damage, the strong blood absorption of this energy results in good coagulation and hemostasis.[34]

The study suggests that 810 nm wavelength energy provides better coagulation for highly vascularized tissue after large-scale procedures (due to a high effect on blood factors), while 980 nm wavelength energy provides better ablation (due to high absorption by water) and limited tissue involvement in regions with less vascularized tissue and narrower tissue involvement. Combining these wavelengths improves coagulation and ablation with minimal thermal damage.[35]

Comparing the dual-wavelength diode laser of the current study with other single-wavelength diode lasers from previous studies, the power setting of the latter reduced the lateral thermal damage caused.[31,35] This explanation clarifies the results of the three variables in the diode laser groups.

Water is the most abundant element in biological tissue. Erbium lasers are ideal for soft tissue surgery because their main chromophore is water (the water absorption spectrum of the Er, Cr:YSGG laser is 0.4 *10^2 cm^{-1}). A few microns of tissue will effectively absorb the Er:YAG and Er, Cr:YSGG lasers. Less collateral tissue damage results from the reduced

energy transfer to the surrounding area.[34] The use of air/water spray reduces surface temperature and removes debris, but the obstacle is that part of the laser wavelength is absorbed by the quartz tip, which generates heat. These factors affect the extent of lateral thermal damage or tissue deformation.[21] This explanation clarifies the results of the three variables of the Er, Cr: YSGG laser groups.

Conclusion

The results of this study showed that the Er, Cr:YSGG laser (2780 nm) can be used in oral soft tissue incisions more safely and effectively than the dual-wavelength diode laser (810 nm and 980 nm) in terms of changes in epithelial and connective tissues and the extent of lateral thermal damage in deep surrounding tissues. The 2.5 W output power of the Er, Cr:YSGG laser groups had the best performance for all criteria, while the 2.5 W output power of the diode laser groups had the worst results. The Er, Cr:YSGG laser caused less to no changes in epithelial and connective tissues and very little lateral thermal damage. It is worth noting that none of these lasers caused surgical margin damage greater than 0.3 mm. Surgeons should take this into account when preparing for surgery and provide additional millimeters (up to 0.5 to 1 mm, based on the laser wavelength) to normal tissue in order to minimize any potential damage.

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Conflict of interest

The authors declare no conflict of interest or financial support.

Data availability statement

The data generated and analyzed during this study are not publicly available, but are available from the corresponding author upon reasonable request.

Authors' contributions

Al-Ani, Alaa Jamal: Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Funding acquisition (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Resources (Equal); Software (Equal); Validation (Equal); Visualization (Equal); Writing – original draft (Equal); Writing – review & editing (Equal). **Taher, Hanan Jafer:** Conceptualization (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal). **Alalawi, Ammar Saleh:** Conceptualization (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Supervision (Equal); Validation (Equal); Visualization (Equal); Writing – review & editing (Equal)

References

1- Neville BW, Damm DD, Allen CM, Chi AC. Oral and maxillofacial pathology. 5th ed. Saint Louis: Elsevier; 2023.

2- Sangle V, Pooja V, Holani A, Shah N, Chaudhary M, Khanapure S. Reactive hyperplastic lesions of the oral cavity: a retrospective survey study and literature review. Indian J Dent Res. 2018;29(1):61-6. doi: 10.4103/ijdr.ijdr_599_16

3- Bede SY. Gingival and alveolar ridge tumor-like overgrowth lesions. J Bagh Coll Dent. 2014;25(3):110-4.

4- Regezi JA, Sciubba JJ, Jordan RC. Oral pathology: clinical pathologic correlations. 7th ed. Saint. Louis: Elsevier, 2017.

5- Radhi M, Abbas A, Salih N. Treatment of pyogenic granuloma and peripheral giant cell granuloma by excisional biopsy and buccal sliding flap. J Bagh College Dentistry. 2011;23(1):67-8.

6- Kahn MA, Hall JM. The ADA practical guide to soft tissue oral disease. Hoboken, Nj: Wiley; 2018. doi: 10.1002/9781119437277

7. Tiwana P, Deepak Kademani. Atlas of Oral and Maxillofacial Surgery. 2nd ed. Saint Louis: Elsevier; 2023.

8- Al-Jumaily HA. The effect of 2 different techniques in second stage implant surgery. J Bagh College Dentistry. 2019;31(1):25-30. doi: 10.26477/jbcd.v31i1.2574

9- Tenore G, Mohsen A, Nuvoli A, Palaia G, Rocchetti F, Di Gioia, et al. The impact of laser thermal effect on histological evaluation of oral soft tissue biopsy: systematic review. Dent J (Basel). 2023;11(2):28. doi: 10.3390/dj11020028

10- Mazin Akram H, Husham Ali O, Kadhum Omran N, Omran Ali A. Diode laser *versus* scalpel gingivectomy. Biomed Pharmacol J. 2017;10(4):1799-804. doi: 10.13005/bpj/1295

11- Convissar RA. Principles and practice of laser dentistry: principles and practice of laser dentistry. 3rd ed. Saint Louis: Elsevier; 2022.

12- Luke AM, Mathew S, Altawash MM, Madan BM. Lasers: a review with their applications in oral medicine. J Lasers Med Sci. 2019;10(4):324-9. doi: 10.15171/jlms.2019.52

13- Jawad M, Alam M, AbdulQader S, Al-Azzawi L, Husein A, Mahmood A. Histological healing response of soft tissue incisions made by diode laser on rabbits skin. Int Med J. 2013;20(4):502-4.

14- Rajan JS, Muhammad UN. Evolution and advancement of lasers in dentistry: a literature review. Int J Oral Hlth Sci. 2021;11(1):6-14. doi: 10.4103/ijohs.ijohs_2_21

15- Al-Khassaki A, Al-Jubori J, Al-Alawi A, Al-Maliky M, Al-Bedran G. Evaluation of anti-nociceptive activity of 940 nanometer low level laser therapy on temporomandibular joint in rats. Indian J Forensic Med Toxicol. 2019;13(1):259. doi:10.5958/0973-9130.2019.00052.5

16- Ali N, Dhahir M, Aldelaimi T. Evaluation of 940 nm diode laser in esthetic crown lengthening. Iraqi J Laser. 2023;22(1):1-8. doi: 862a34d7866714c8

17- Nazar J, Nibin TN. Laser in oral and maxillofacial surgery: review of literature. Int J Sci Res Sci &Tech. 2023;10(6):87-112. doi: 10.32628/IJSRST52310612

18- Al-Ani A, Al-Alawi A, Taher H. Analysis of the temperature elevation of the dual-wavelength diode laser and the Er, Cr: YSGG laser in oral soft tissue incisions. J Lasers Med Sci. 2023;14(37):1-6. doi: 10.34172/jlms.2023.37

19- Chan YH. Randomised controlled trials (RCTs) – sample size: the magic number? Singapore Med J. 2003;44(4):172-4.

20- Vescovi P, Corcione L, Meleti M, Merigo E, Fornaini C, Manfredi M, et al. Nd:YAG laser *versus* traditional scalpel. A preliminary histological analysis of specimens from the human oral mucosa. Lasers in Med. Sci. 2010;25(5):685-91. doi: 10.1007/s10103-010-0770-4

21- Kawamura R, Mizutani K, Lin T, Kakizaki S, Mimata A, Watanabe K, et al. *Ex vivo* evaluation of gingival ablation with various laser systems and electroscalpel. Photobiomodul Photomed Laser Surg. 2020;38(6):364-73. doi: 10.1089/ photob.2019.4713

22- Grzech-Leśniak K, Matys J, Jurczyszyn K, Ziółkowski P, Dominiak M, Brugnera A Jr, et al. Histological and thermometric examination of soft tissue de-epithelialization using digitally controlled Er:YAG laser handpiece: an *ex vivo* study. Photomed Laser Surg. 2018;36(6):313-9. doi: 10.1089/pho.2017.4413

23- Monteiro L, Delgado M, Garcês F, Machado M, Ferreira F, Martins M, et al. A histological evaluation of the surgical margins from human oral fibrous-epithelial lesions excised with CO2 laser, Diode laser, Er:YAG laser, Nd:YAG laser, electrosurgical scalpel and cold scalpel. Med Oral Patol. Oral Cir Bucal. 2019;24:271-80. doi: 10.4317/medoral.22819

24- Romeo U, Del Vecchio A, Ripari F, Palaia G, Chiappafreddo C, Tenore G, et al. Effects of different laser devices on oral soft tissues: *in vitro* experience. J Oral Laser Appl. 2007;7(3):155-9.

25- Azevedo A, Monteiro L, Ferreira F, Delgado M, Garces F, Carreira S, et al. *In vitro* histological evaluation of the surgical margins made by different laser wavelengths in tongue tissues. J Clin Exp Dent. 2016;8(4):388-96. doi:10.4317/jced.52830

26- Palaia G, Renzi F, Pergolini D, Del Vecchio A, Visca P, Tenore G, et al. Histological *ex vivo* evaluation of the suitability of a 976 nm diode laser in oral soft tissue biopsies. Int J Dent. 2021;2021: 6658268. doi: 10.1155/2021/6658268

27- Rizoiu IM, Eversole LR, Kimmel AI. Effects of an erbium, chromium: yttrium, scandium, gallium, garnet laser on mucocutanous soft tissues. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1996;82(4):386-95. doi: 10.1016/s1079-2104(96)80302-7

28- Fornaini C, Merigo E, Rocca JP, Lagori G, Raybaud H, Selleri S, et al. 450 nm blue laser and oral surgery: preliminary *ex vivo* study. J Contemp Dent Pract. 2016;17(10):795-800. doi: 10.5005/jp-journals-10024-1933

29- Angiero F, Parma L, Crippa R, Benedicenti S. Diode laser (808 nm) applied to oral soft tissue lesions: a retrospective study to assess histopathological diagnosis and evaluate physical damage. Lasers Med Sci. 2011;27(2):6. doi: 10.1007/s10103-011-0900-7

30- Romeo U, Russo C, Palaia G, Lo Giudice R, Del Vecchio A, Visca P, et al. Biopsy of different oral soft tissues lesions by KTP and diode laser: histological evaluation. Sci World J. 2014;2014:1-6. doi: 10.1155/2014/761704

31- Gill K, Sandhu SV, Sethi N, Bhandari R. Biopsy of oral soft tissue lesions by 808 nm and 980 nm diode laser: a morphological and histochemical evaluation. Lasers Dent Sci. 2021;5(4):207-22. doi: 10.1007/s41547-021-00132-z

32- Gobbo M, Bussani R, Perinetti G, Rupel K, Bevilaqua L, Ottaviani G, et al. Blue diode laser *versus* traditional infrared diode laser and quantic molecular resonance scalpel: clinical and histological findings after excisional biopsy of benign oral lesions. J. Biomed. Opt. 2017;22(12):121602. doi: 10.1117/1.jbo.22.12.121602

33- Cercadillo-Ibarguren I, Espana-Tost A, Arnabat-Dominguez J, Valmaseda-Castellon E, Berini-Aytes L, Gay-Escoda C. Histologic evaluation of thermal damage produced on soft tissues by CO2, Er,Cr:YSGG and diode lasers. Med Oral Patol Oral Cir Bucal. 2010;15:912-8. doi: 10.4317/medoral.15.e912

34- Coluzzi D; Parker SP. Lasers in Dentistry - current concepts. Cham: Springer International Publishing; 2017.

35- Ostler CD, Kengike LM, Kengike M. Histological evaluation 810nm vs 980nm wavelength laser radiation on pig liver tissue [internet]. Canterbury: QuickLase; 2018 [cited March 05, 2024] Available from: https://quicklase.com/wp-content/uploads/2013/08/DrDruain_Article.pdf