IJL, Issue 2, Vol. 24, 2025 **Research Article**
https://doi.org/10.31900/ijl.v24i2.522

IRAQI JOURNAL OF LASER

Optimization of Er, Cr: YSGG Laser Parameters for Safe and Precise Bone Ablation: An Ex Vivo Histological Study

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Article history: Received 25 Feb. 2025; Revised 24 Aug. 2025; Accepted 30 Sept. 2025; Published online 15 Dec. 2025

Abstract: The Er, Cr: YSGG laser has emerged as a promising tool for safe and precise bone ablation in dental and maxillofacial procedures. This ex vivo study aimed to optimize the laser parameters to achieve efficient bone cutting with minimal thermal side effects.
Method: This ex vivo study aimed to evaluate the histological effects of varying ER, Cr: YSGG laser parameters on bone incision quality. Mandibular bone samples were collected from three freshly slaughtered sheep and sectioned into six blocks. Osteotomies were performed using an ER, Cr: YSGG laser (2780 nm, Waterlase iPlus) at six different power-frequency combinations (2.5W/20Hz, 4W/20Hz, 6W/20Hz, 4W/30Hz, 6W/30Hz, and 8W/30Hz). Each specimen was irradiated in contact mode for 25 seconds using a MGG6-6 tip, and incisions were evaluated histologically after fixation, decalcification, and H&E staining. Measured variables included incision length, width, length-to-width ratio, carbonization, and precision.
Results: ER, CR: YSGG lasers can be considered practical, effective, and easy for bon incisions. There is no thermal damage. The quality of laser incision at 4W, 20Hz is the best among the parameters($p=0.013$).

Keywords: Bone, histology, incision, IR laser, thermal damage

1. Introduction

One of the oldest forms of orthopaedical treatment is osteotomy. It has been around for nearly a thousand years and is still used today. An osteotomy's fundamental idea is to cut or incision bone, usually to alter the shape or alignment of the bone [1]. Orthopedic surgery has employed various osteotomy techniques to treat deformities and lengthen limbs. Osteotomies are performed with drill bits, electrical saws, and Gigli saws; each has benefits and disadvantages. However, a laser is another method for osteotomy. An erbium laser is one type of laser that was initially employed in maxillofacial surgery [2]. Although the handpiece and bur for osteotomy are effective, they are in direct contact with bone during the cut, causing vibrations and making patients uncomfortable. Thus, it has been suggested that lasers be used for osteotomy treatments. Surgical lasers, however, might facilitate the removal of bone tissue without vibration noises [3]. Bone, known as specialized, living, complex connective tissue, supports and shields the body's critical organs [4]. All bones are composed of a dense outer layer of compact bone and a core of cancellous bone. The cancellous bone is the main component of a bone. It surrounds marrow voids that may contain hematological tissue and form a trabecular network [5]. The accurate, contactless laser ablation method can remove the body's hard tissue. Laser osteotomy speeds up the healing of the residual bone compared to traditional bone cutting with piezo osteotomes. Recovery is accelerated because the spongy structure is preserved[6]. Bone defect repair is still a significant clinical orthopedic issue. Bone, a highly vascularized tissue, depends on the intimate spatial and temporal relationship between blood vessels and bone cells to preserve skeletal integrity[7]. Laser energy interacts precisely with water and tissues to cut both soft and hard tissue (hydrophotonics). Emission wavelengths, tissue optical characteristics, exposure duration, laser energy, and tissue absorption of the laser energy all affect the outcome of tissue interaction with laser light [8].

Therefore, while cutting bone with a laser, there is less trauma and heat damage. It encourages healing and causes less scar tissue to form, as well as less bleeding and infection[9]. A surgeon can work on tissues more accurately and ablate tissues in hard-to-reach places by using a laser as a surgical tool. A laser can be used during bone cutting to create specific forms and reduce mechanical damage to the surrounding tissue. The properties of the tissue, including its optical characteristics, color, consistency, thermal properties, heat capacity, temperature conductivity, as well as those associated with the laser system, wavelengths, and emission parameters, such as applied power, focal spot size, and emission mode exposure time, determine how the laser acts on the tissue[10-12].

Because water is the chromophore target of the erbium laser, its usage in dentistry goes beyond soft tissues such as mucosa and gingiva to the hard ones, including enamel, dentine, bone, and carious tissue. The thermal effects of erbium lasers on the target tissues can be detected through vaporization. A photomechanical effect is created when water molecules explode, which aids in the ablative and cleaning processes[13].

The Er. Cr: YSGG laser is effective for various dental operations involving both hard and soft tissues because it shows peak absorption by hydroxyapatite (HA) and water at 2780 nm in the infrared spectrum[14]. Since water and hydroxyapatite crystals absorb a large portion of the Erbium, Chromium YSGG laser at 2780 nm wavelength, this free-running pulsed laser has shown promise in treating a variety of hard tissue procedures and soft tissue operations[15].

According to preliminary research, this kind of infrared laser may offer precise, clean, and straight ablation with little thermal harm to nearby tissue because of its water and air sprays. As a result, the Er, Cr: YSGG laser appears to be a substitute tool for oral surgery [16].The biostimulatory effects of laser light irradiation on wound healing, collagen synthesis, and fibroblast proliferation have been used in medical treatments. Additionally, laser light seems to boost adenosine triphosphate (ATP) synthesis and mitochondrial respiration[17]. In dentistry, the Er: YAG (2940 nm) and Er, Cr: YSGG (2790 nm) erbium laser wavelengths are frequently utilized. Among all infrared lasers, they have the highest absorption in water and hydroxyapatite, making them perfect for "optical drilling" in composite fillings, bone, enamel, and dentin [18].

The aim of the present study was to optimize Er, Cr: YSGG laser parameters (2780 nm) for safe and precise bone ablation by assessing histological outcomes in an ex vivo sheep mandible model.

2. Materials and methods

2.1 Sample collection

In this ex vivo study, within four to six hours following slaughter, three Iraqi sheep's mandibles (aged one to one and a half) were used. The bone was preserved in an ice box and kept humidified at the same temperature. The sheep's mandible has been cut into blocks (specimens), with measurements of 1cm in length,2cm in width, and original compact thickness, by electrical saw (Figure 1). The total number was six blocks.

2.2 Laser system

The following instruments were used in this study: Er, Cr: YSGG laser (WATERLASE I plus, BIOLASE)) with a gold handpiece and MGG6-6 laser tip in pulse emission mode (PW).

The laser device is set at six parameters. Each bone specimen was irradiated with Er, CR: YCGG laser 2790nm for 25s for each parameter to make a 2cmm incision. Different laser parameters were used in each irradiation episode. The CNC (computed numerical control) device was used in this experiment to provide a more precise irradiation procedure and verify experimental conditions. This device fixed the bone specimens and held the laser handpiece perpendicular to bone specimens in contact mode; the incision was made with a 2 cm length adjusted in 25 seconds, and the horizontal speed of the CNC device was 0.8 mm/sec.
A CNC device is composed of two parts :(Figure 2)
1-movable part: handle that holds the laser or conventional handpiece and moves in the x-direction through an electrical motor connected to the computer.
2-fix part: mounted table with two arms that hold the bone specimen firmly during laser osteotomy. Figure .3.

Figure. 1: A sheep's mandible, bone specimens.

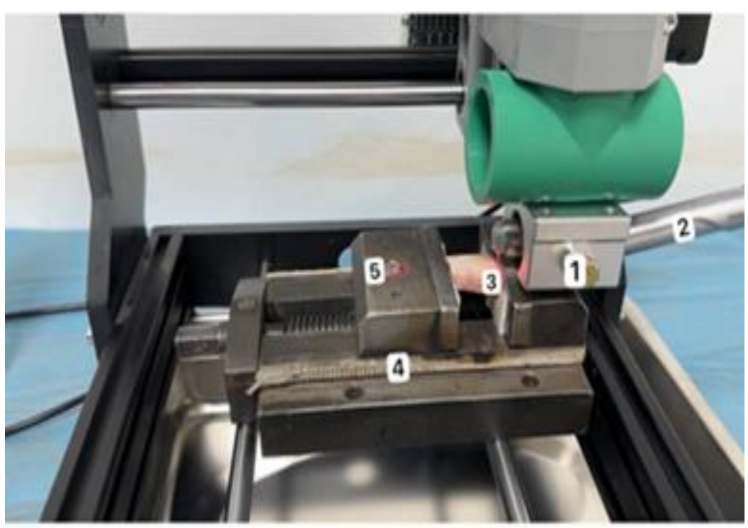

Figure 2: A CNC device:
1- handle,2-laser handpiece,3-bone specimen,4-mounted table,5-the arms that hold the specimen

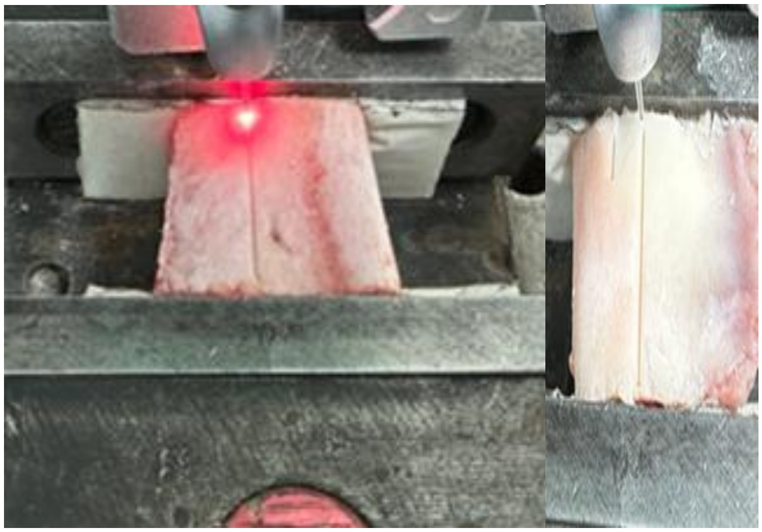

Figure 3: Laser osteotomy.

2.3 Laser Parameters

The laser parameters employed in this ex vivo investigation were selected based on prior research, physician use, and the hard tissue surgical setting of the devices. In order to accomplish the goal of the study, minor differences were made between them. According to the laser parameters setting, the groups were divided as follows: (Table 1)

Table 1. Laser parameters

Laser Group	Power (W)	Energy Per Pulse (mJ)	Water/Air (%)	Frequency (Hz)	Pulse Duration (µs)	Peak Power (W)	Power Density (W/cm²)	Fiber Tip Diameter (µm)
G1	8.0	266.7	50/50	30	60	4.45	2830.3	600
G2	6.0	200.0	50/50	30	60	3.33	2122.7	600
G3	4.0	133.3	50/50	30	60	2.22	1415.1	600
G4	4.0	200.0	50/50	20	60	3.33	1415.1	600
G5	4.0	200.0	50/50	20	60	3.33	1415.1	600
G6	2.5	125.0	50/50	20	60	2.08	884.5	600

2.4 Histological evaluation

After the laser irradiation, a 10% buffered formalin solution was used to fix the specimens. On the same day, they were then sent to the histology lab for a histological analysis. At the laboratory, the bone will be preserved in 10% nitric acid for decalcification for days or weeks according to bone fragment thickness and sequential sample observation. Then, it was put in formalin for 24 hours, dehydrated by graded alcohol, placed in xylene, processed, and embedded in paraffin to prepare a cassette. From these cassettes, sliced s of 5um thickness stained by H and E were examined under scaled lines of the microscope×10 magnifications.

- *Slide number*

Ten stained slides were obtained from each block, and 60 slides were taken.

2.5 Histological criteria

For histological assessment, each slide was examined under a light microscope by a histologist to evaluate the following for laser osteotomy incision:
1-length of incision
2-width of incision
3-length to width ratio: scored from 1-3 according to the following:
-<0.7 1
-0.7-1.5 2
-> 1.5 3
4. Carbonization: scored from 1-3 according to the following:
Sever 1
Moderate 2
Mild 3
5. Precision: scored from 1-3 according to the following:
Bad 1
Fair 2
Good 3

2.6 Statistical analysis

Continuous variables were expressed as means and standard deviations. Categorical variables were expressed as frequency and percentages. The one-way ANOVA and Tukey's post-hoc test with holm adjustment was performed to find the differences in means between the study groups. The difference between categorical variables was investigated using Fisher's exact test. A P-value less than 0.05 was considered statistically significant. R software packages (dplyr, gt_summery and ggplot) were used for data processing, visualization, and statistical analysis ("R version 4.2.2, R Foundation for Statistical Computing, Vienna, Austria").

3. Result and discussion

3.1 The length, width, and length-to-width ratio (L/W ratio)

Table 2 provides a comparative analysis of the length, width, and length-to-width ratio (L/W ratio) of bone incisions across different study groups subjected to varying laser parameters. The length of bone incision was highest in the 4 W/20 Hz group (0.91 ± 0.08 mm), Figure 7, and lowest in the 2.5 W/20 Hz fig (10), group (0.56 ± 0.04 mm), with a statistically significant difference among the groups ($P < 0.001$). Incision width was greatest in the 2.5 W/20 Hz group (0.79 ± 0.11 mm) and smallest in the 8 W/30 Hz group (0.43 ± 0.05 mm), also with a significant variation ($P < 0.001$). The L/W ratio varied significantly, with the highest ratio observed in the 8 W/30 Hz group (1.66 ± 0.44) fig (9), and the lowest in the 2.5 W/20 Hz group (0.72 ± 0.13). Grading of the L/W ratio revealed that most bone incisions in the 2.5 W/20 Hz group fell into grade 1 (<0.7), whereas incisions in the 8 W/30 Hz group predominantly belonged to grade 3 (>1.5). Statistical tests confirmed significant differences in all evaluated characteristics across groups ($P < 0.001$). Figure 4 illustrates Tukey's post-hoc test for the differences in length-to-width ratio between study groups.

3.2 The precision

Table 3 presents the precision of osteotomy observed in bone incisions subjected to different laser settings. Precision was highest in the 2.5 W/20 Hz group (2.9 ± 0.3)Fig(9), and the 4 W/20 Hz group (2.8 ± 0.4) Fig(7), while the lowest precision was noted in the 8 W/30 Hz group (1.6 ± 0.5) Fig (8).

The grading of precision showed that the majority of cuts in the 2.5 W/20 Hz and 4 W/20 Hz groups were rated as grade 3 (highest precision). In contrast, the 8 W/30 Hz group had a significant proportion of grade 1 (lowest precision). The differences in precision across groups were statistically significant (P < 0.001). Figure 5 illustrates ATukey's post-hoc test for the differences in precision scores between study groups.

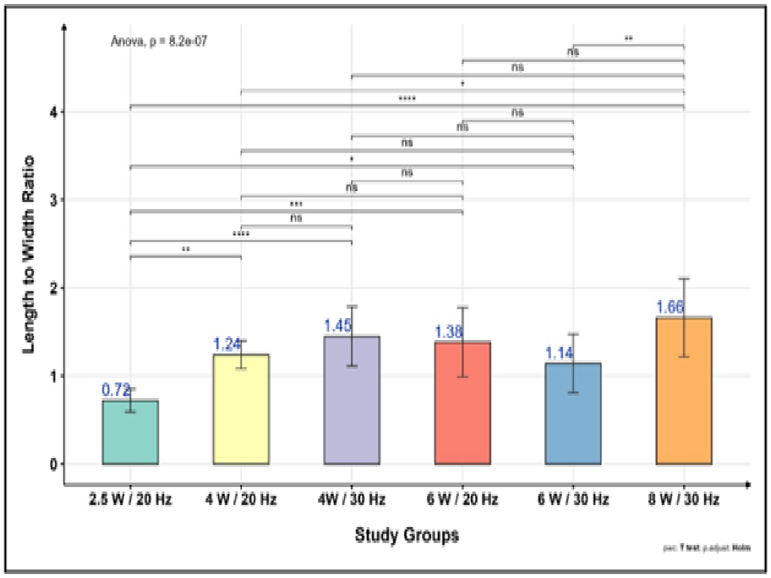

Figure 4: Tukey's post-hoc test for the differences in length-to-width ratio between study groups.

Table 2. Description of length, width, and length-to-width ratio of bone incision in different study groups.

Laser parameter	2.5 W / 20 Hz, N = 10[1]	4 W / 20 Hz, N = 10[1]	4W / 30 Hz, N = 10[1]	6 W / 20 Hz, N = 10[1]	6 W / 30 Hz, N = 10[1]	8 W / 30 Hz, N = 10[1]	P-value[2]
Length (mm)	0.56 ± 0.04[c]	0.91 ± 0.08[a]	0.83 ± 0.09[ab]	0.59 ± 0.05[c]	0.65 ± 0.11[cd]	0.73 ± 0.17[bd]	<0.001
Width (mm)	0.79 ± 0.11[b]	0.73 ± 0.05[ab]	0.60 ± 0.14[ac]	0.47 ± 0.18[c]	0.59 ± 0.16[ac]	0.43 ± 0.05[c]	<0.001
Length-to-width ratio	0.72 ± 0.13[c]	1.24 ± 0.16[ab]	1.45 ± 0.34[ab]	1.38 ± 0.39[ab]	1.14 ± 0.33[ac]	1.66 ± 0.44[b]	<0.001
Grading of L/W ratio							<0.001
1 (<0.7)	6 (60.0%)	0 (0.0%)	0 (0.0%)	3 (30.0%)	1 (10.0%)	0 (0.0%)	
2 (0.7-1.5)	4 (40.0%)	10 (100.0%)	7 (70.0%)	3 (30.0%)	8 (80.0%)	4 (40.0%)	
3 (> 1.5)	0 (0.0%)	0 (0.0%)	3 (30.0%)	4 (40.0%)	1 (10.0%)	6 (60.0%)	

[1]Mean ± SD; n (%)
[2]One-way ANOVA; Fisher's exact test

3.3 The carbonization

Table 3 presents the carbonization of osteotomy observed in bone incisions subjected to different laser settings. The mean scores ranged from 1.0 ± 0.0 in the 2.5 W/20 Hz and 6 W/30 Hz groups to 2.0 ± 0.0 in the 8 W/30 Hz group, indicating the highest degree of carbonization in the latter. Most incisions in the lower power groups (e.g., 2.5 W/20 Hz) exhibited mild carbonization, while all 8 W/30 Hz group incisions showed moderate carbonization. Severe carbonization was observed only in isolated cases within the 4 W/20 HZ group. Statistically significant differences in carbonization were noted among the study groups (P < 0.001).

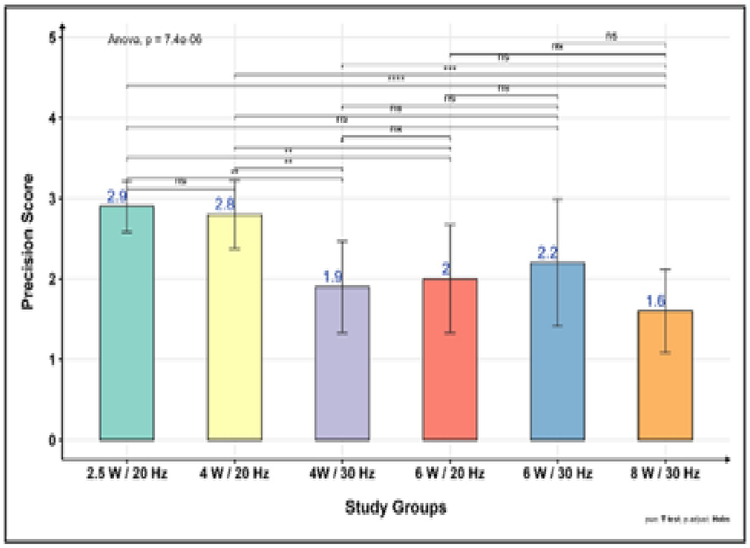

Figure 5: ATukey's post-hoc test for the differences in precision score between study groups

Table 3. Description of precision and carbonization of bone incision using different laser settings.

Laser parameter	**2.5 W / 20 Hz, N = 10**[1]	**4 W / 20 Hz, N = 10**[1]	**4W / 30 Hz, N = 10**[1]	**6 W / 20 Hz, N = 10**[1]	**6 W / 30 Hz, N = 10**[1]	**8 W / 30 Hz, N = 10**[1]	**P-value**[2]
Precision	2.9 ± 0.3[a]	2.8 ± 0.4[a]	1.9 ± 0.6[b]	2.0 ± 0.7[b]	2.2 ± 0.8[ab]	1.6 ± 0.5[a]	<0.001
1 (bad)	0 (0.0%)	0 (0.0%)	2 (20.0%)	2 (20.0%)	2 (20.0%)	4 (40.0%)	
2 (moderate)	1 (10.0%)	2 (20.0%)	7 (70.0%)	6 (60.0%)	4 (40.0%)	6 (60.0%)	
3 (good)	9 (90.0%)	8 (80.0%)	1 (10.0%)	2 (20.0%)	4 (40.0%)	0 (0.0%)	
Carbonization	1.0 ± 0.0[a]	1.3 ± 0.7[a]	1.2 ± 0.4 [a]	1.3 ± 0.5 [a]	1.0 ± 0.0 [a]	2.0 ± 0.0[b]	<0.001
1 (mild)	10 (100.0%)	8 (80.0%)	8 (80.0%)	7 (70.0%)	10 (100.0%)	0 (0.0%)	
2 (moderate)	0 (0.0%)	1 (10.0%)	2 (20.0%)	3 (30.0%)	0 (0.0%)	10 (100.0%)	
3 (severe)	0 (0.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

[1]n (%)
[2]Fisher's exact test

3.4 The total scores

Table 4 describes the total scores utilized in the study and the statistical differences observed between the study groups. The total scores, presented as mean ± standard deviation (SD), were reported for six different settings, categorized by wattage (2.5 W, 4 W, 6 W, 8 W) and frequency (20 Hz, 30 Hz). The P-value from the one-way ANOVA was 0.013, indicating significant statistical differences between the groups. The highest mean total score was observed in the 4 W / 20 Hz group (7.8 ± 0.8), while the lowest score was found in the 8 W / 30 Hz group (6.2 ± 0.6). Groups with similar total scores were marked with the same superscript letter (e.g., "a" or "b"), and significant differences were noted between groups with different superscripts. Figure 8 illustrates Tukey's post-hoc test for the differences in total scores between study groups.

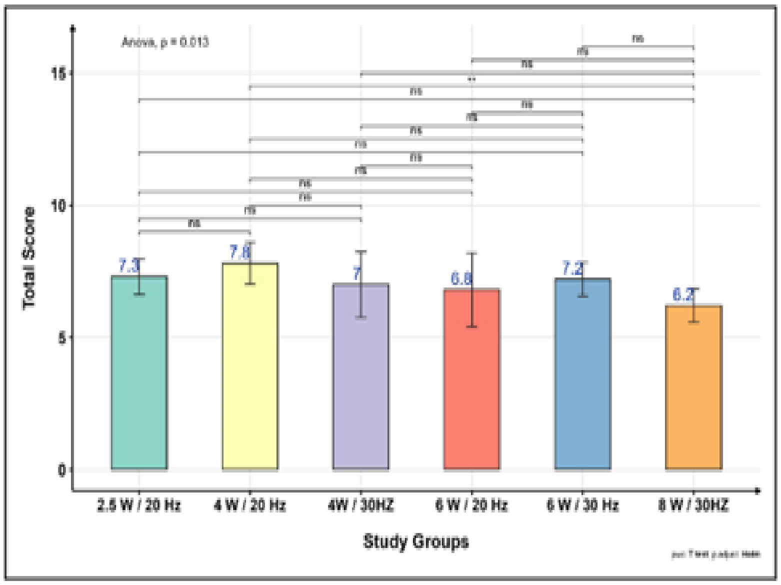

Figure 6: Tukey's post-hoc test for the differences in total scores between study groups.

Table 4: Description of the total score utilized in this study and the statistical differences between the study.

Laser parameter	2.5 W / 20 Hz, N = 10[1]	4 W / 20 Hz, N = 10[1]	4W / 30 Hz, N = 10[1]	6 W / 20 Hz, N = 10[1]	6 W / 30 Hz, N = 10[1]	8 W / 30 Hz, N = 10[1]	P-value[2]
Total score	7.3 ± 0.7[ab]	7.8 ± 0.8[a]	7.0 ± 1.2[ab]	6.8 ± 1.4 [ab]	7.2 ± 0.6 [ab]	6.2 ± 0.6[b]	0.013

[1]Mean ± SD
[2]One-way ANOVA

In laser type, the Er,Cr: YSGG laser, is ideal for surgical procedures, including osteotomy, because it can precisely coagulate arteries and ablate both soft and hard tissue while minimizing heat damage [19].
Because of its high stiffness, high mineral and water content, and quick heat dispersion from the initial absorption layer, hard bone tissue presents difficulties for laser osteotomy [20]. Unlike other laser systems, cutting hard tissue is made possible by an interaction between the laser energy and the water spray, known as the "hydrokinetic effect [21]. A good bone ablation and all forms of treatment of biological hard tissues depend on adequate cooling with an aerosol, ideally made of air and water [22]. This study shows the effect of power and frequency on the quality of laser bone incisions at the histological level.

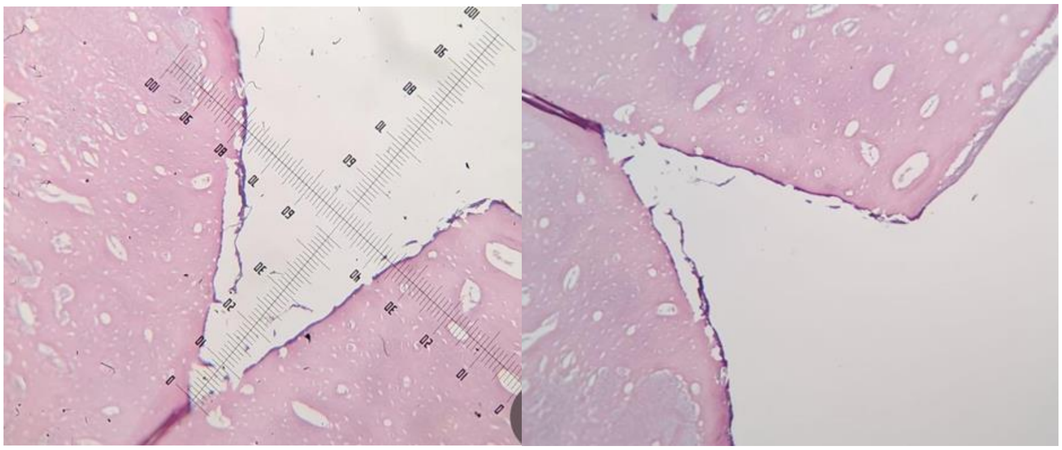

Figure 7: Histological section of Laser osteotomy of 4W 20Hz illustrate deep, precise, less carbonization incision.

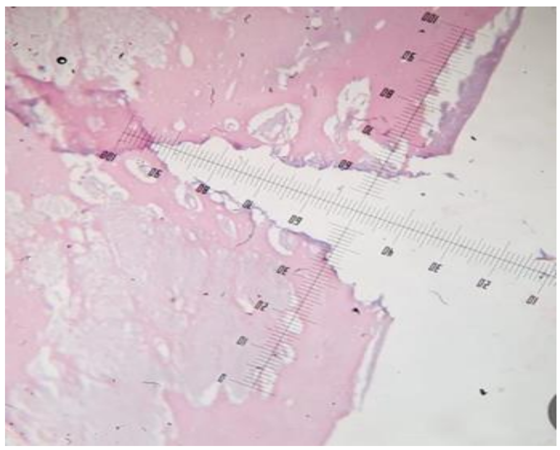

Figure 8: Histological section of laser osteotomy of 8W 30Hz shows the deep, irregular, less precise incision.

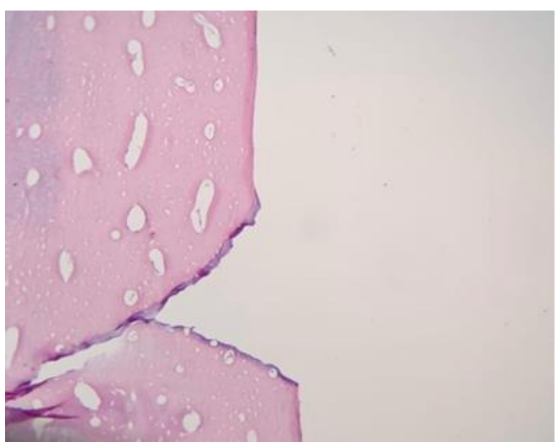

Figure 9:Histological section of laser osteotomy of 2.5W 20Hz shows a precise but shortest depth incision.

Similar to earlier studies, specimens with a high frequency and low radiation power output showed better incision quality, particularly at 20 Hz [23,24]. The incision length was highest in the 4 W/20 HZ group and lowest in the 2.5 W/20 HZ group. One of the important criteria that determines wound efficiency is the wound depth. Regarding it, an efficiency parameter depends on the clinician's need and how they want to reach their target, which is influenced by other parameters, like the thermal effect when using a laser [25].The length-to-width ratio of laser bone incisions plays a critical role in biopsy procedures, as it directly influences the precision of tissue removal, the extent of thermal damage, and the quality of the histological specimen obtained. In biopsy procedures, a minimal initial incision with a deeper or extended advancement path is preferred to reduce surface trauma and ensure representative tissue sampling [26].In excisional biopsies, specimens collected through laser bone incisions usually have a length-to-width ratio of about 3:1, a shape that improves the accuracy of tissue removal and reduces histological artifacts [27].

Carbonization, which is undesirable in medical laser applications because it increases thermal unfavorable effects on adjacent tissues and slows recovery, is reached as the temperature rises with continued laser exposure. The size, exposure time, and location of the heat deposited inside the tissue are the primary determinants of the ablation's geographic extent[28].

Heat damage and carbonizations are major concerns in the present study, but all examined samples of the laser osteotomy show no thermal damage. Because erbium lasers use water as their primary chromophore, they are a great option for surgical hard tissue procedures. The thermal relaxation time is facilitated by the erbium laser's pulse emission mode and sufficient cooling system [15]. The results of the present study match with evidence from prior ex vivo experiments that demonstrated that the Erbium laser did not cause any heat damage to bone tissue when osteotomy was carried out with cooling air/ water irrigation [29].

Finally, the histological results showed high incision quality with the Er, Cr: YSGG laser system . The interpretation related to laser-tissue interaction depends on multiple laser intrinsic and extrinsic parameters (wavelength, power, mode of emission, beam profile, pulse duration, frequency, and spot size) and the chemical and optical properties of the targeted tissue (tissue chromophores, water, and pigments)[30,31].

4. Conclusion

In ex vivo studies, the Er, Cr: YSGG laser allows for accurate surgical ablation with little thermal damage to nearby tissues. The optimal result is obtained with a 4watt, 20Hz.

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تحسين معايير ليزر Er, Cr: YSGG لتحقيق إزالة آمنة ودقيقة للعظم: دراسة نسيجية خارج الجسم على فك الأسفل للغنم

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الخلاصة : برز ليزر (Er, Cr: YSGG) كأداة واعدة للإزالة الآمنة والدقيقة للعظم في الإجراءات السنية والوجهية الفكية. هدفت هذه الدراسة خارج الجسم إلى تحسين معايير الليزر لتحقيق قطع فعال للعظم مع تقليل الآثار الجانبية الحرارية إلى الحد الأدنى. الطريقة: تم الحصول على عينات من عظام الفك السفلي لثلاثة خراف تم ذبحها حديثاً، وقُسمت إلى ست كتل. جرت عمليات قطع العظم باستخدام ليزرEr:Cr: YSGG بطول موجي قدره ٢٧٨٠ نانومتر، وذلك وفق ست مجموعات مختلفة من التوليفات بين القدرة الكهربائية والتردد، وهي: (٢.٥ واط / ٢٠ هرتز)، (٤ واط / ٢٠ هرتز)، (٦ واط / ٢٠ هرتز)، (٤ واط / ٣٠ هرتز)، (٦ واط / ٣٠ هرتز)، و(٨ واط / ٣٠ هرتز) .تم استخدام رأس ليزري خاصMGG6-6، وتم توجيه الليزر على كل عينة بوضع التلامس لمدة ٢٥ ثانية .بعد ذلك، خضعت العينات للتثبيت الكيميائي، وإزالة التكلس، ثم التلوين بصبغة الهيماتوكسيلين والإيوزين لغرض التقييم النسيجي .شملت المتغيرات التي تم قياسها: طول الشق، عرضه، النسبة بين الطول والعرض، درجة التفحم، ودقة القطع. النتائج: يمكن اعتبار ليزر (Er, Cr: YSGG) وسيلة عملية وفعالة وسهلة الاستخدام في إجراء شقوق العظام. لم تُلاحظ أي أضرار حرارية . وُجد أن جودة الشق باستخدام الليزر عند قدرة ٤ واط وتردد ٢٠ هرتز هي الأفضل مقارنة ببقية الإعدادات.

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