



Surface Topography of Primary Teeth Enamel After Sub-ablative Er;Cr:YSGG Laser Irradiation: An In Vitro Study

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Article history: Received 24 Apr. 2024; Revised 25 Aug. 2024; Accepted 27 Aug. 2024; Published online 15 Dec. 2024

Abstract

Objective: To evaluate the effect of different sub-ablative irradiation parameters of Er;Cr: YSGG laser on the surface topography of primary teeth enamel with white spot lesions.

Materials and Methods: A total of 30 primary posterior teeth with sound enamel were immersed in demineralization solution at (pH 4.4) to artificially induce enamel white spot lesions. They were randomly divided into three groups: L1, L2, and L3 groups were irradiated with Er;Cr: YSGG laser irradiation at the power of 0.75W, 0.5W, and 0.25W respectively, 20Hz frequency, and 40% air/60% water irrigation. Surface topography was evaluated with a profilometer and scanning electron microscope.

Results: Surface roughness evaluation with scanning electron microscope images revealed a non-significant increase in surface roughness after the demineralization process. Laser irradiation with different powers leads to a non-significant increased surface roughness with altered topography and a more pronounced effect with the laser group L1 and to a lower extent groups L2 and L3.

Conclusion: Increased surface roughness of the primary teeth enamel after sub-ablative power irradiation with Er;Cr: YSGG laser, with a rough and irregular surface devoid of smear layer, the roughness increase was proportional with the increased irradiation power.

Keywords: Er;Cr:YSGG. White spot lesion. Primary teeth. Roughness. SEM.

1. Introduction

The prevention and treatment of dental caries remains a pressing concern within the field of dentistry, given the implications for public health and human well-being. The development of dental caries is marked by changes in the apatite crystals of the enamel, which can result in the formation of white spot lesions, initial enamel caries, dentin involvement, and cavitation. Despite modern advances in dental care, the prevention and treatment of dental caries continue to be an active area of research worldwide. In the early stages of enamel caries, the appearance of chalky irregularities indicates a disruption in biological mineralization activity, resulting from a loss of mineral content on both the enamel surface and subsurface [1].



Contemporary approaches to caries management prioritize non-cavitated carious lesions, with an emphasis on halting or reversing progression and promoting remineralization, rather than resorting immediately to drilling and filling procedures [2].

Fluoride topical treatment is a widely used method for remineralization and stabilization of early carious lesions. Sodium fluoride, acidulated phosphate fluoride, and MI paste plus are commonly employed for this purpose. When fluoride is topically applied to white spot lesions (WSLs) *in vivo*, it can be eliminated through various mechanisms such as back diffusion, back exchange, and migration from the mineral to the surrounding tissue fluid, saliva, or plaque fluid [2]. However, the amount of fluoride released from the reservoir over time decreases. This approach of topical application of fluoride has drawbacks including the possibility of dental fluorosis, particularly in children [3]. Furthermore, the remineralization process may lead to external staining. Besides, this method requires the patient's consent and takes more time to complete. If superficial remineralization is performed while the lesion is still porous, it may cause unpredictable and persistent white discoloration [4]. Early preventive methods are insufficient to prevent new caries lesion development in high-risk individuals [2]. In recent years, however, this approach has been challenged by a need for minimally invasive and less traumatic dental caries management and prevention methods for young children. Laser irradiation is one such solution that can increase fluoride uptake [5]. Lasers are increasingly being employed in the prevention of dental cavities due to their notable impact on dental hard tissue [6]. The wavelength of Er:Cr:YSGG laser at 2780nm has many dental applications, including cavity preparation, caries removal, endodontic treatment, and surgical procedures that made the OH groups in hydroxyapatite crystals effectively absorb this wavelength which in turn made the temperature of the tooth surface rises to 800°C at the ablation threshold. This leads to crystallographic changes in the enamel, without causing the carbonated hydroxyapatite to evaporate or melt [7]. The absorbance coefficient of enamel is around 50 mm⁻¹. It was mentioned that the laser beam can pass through the enamel's outer layers at about 21 μm. The oral hard tissues are exposed to Er:Cr:YSGG laser radiation through explosive thermo-mechanical ablation. Under this mechanism, the incident radiation is absorbed by the water molecules within the hydroxyapatite crystals. The water vaporization causes an increase in internal pressure and micro explosions, which in turn cause substrate ejection in the form of inorganic particles and accurate removal of irradiated tissue [8]. This laser should have a lower energy density than ablation to prevent cavities since its absorption by hydroxyapatite hydroxyl groups and water is high at 2780nm. While an energy density of 8.5 J/cm² improves enamel acid resistance, lower fluences can be used as fluoride dentifrice substitutes. An enhanced enamel surface temperature would only produce chemical changes. Best-case laser irradiation should increase enamel hardness without affecting surface roughness [3].

Several explanations have been offered for the remineralization potential of lasers. One possibility is that laser irradiation reduces enamel permeability by melting, fusing, and recrystallization of enamel crystals [9]. Another theory proposes that the photochemical effect of laser leads to protein decomposition and reduction of carbonate content at temperatures ranging between 650°C and 1100°C rendering the enamel more acid-resistant. [10]. According to some other research, laser irradiation may encourage the development of microspaces in enamel, which would improve fluoride incorporation or diffusion across its structure and enable the creation of a fluoride reservoir important for preventing dentin erosion [11].

Surface roughness is the term used to describe irregularities or tiny indentations and projections that affect the surface's properties, including brightness, quality of adhesion, and wetting capacity. Devices like roughness meters can measure a surface's imperfections, or tiny saliencies and re-entries [12].

Roughness assessments after remineralization affect aesthetics and indicate bacterial adherence, plaque development, and exogenous staining. Compared to flat surfaces, rough and irregular surfaces create more dental biofilm that matures faster. Studies have shown that demineralized enamel, which is rougher than sound, can hold more biofilm than sound enamel [13]. Qualitative enamel surface roughness study with SEM evaluation is subjective and may favor the initial hypotheses. Since scanning electron microscopy can't provide enough quantitative data, enamel deterioration can't be continuously measured. Profilometers provide more descriptive data to quantify enamel damage severity [14].



Profilometry was used to quantify surface roughness in this study because it can accurately and precisely measure it without further measurements. Many researchers found profilometric quantitative evaluation beneficial. [14, 15, 16]

In the present study, to mimic the situations in the oral cavity adjacent to the teeth, samples with white spot lesions were used. [7]. This study aims to evaluate the surface topography of primary teeth enamel with artificially induced white spot lesions subjected to Er:Cr:YSGG sub ablative irradiation parameters as a remineralization method, evaluated with a profilometer and scanning electron microscopy (SEM).

2. Materials and Methods

2.1 Sample Collection and Preparation

For this study, 30 primary posterior teeth were used that had either been extracted for orthodontic reasons or had been naturally exfoliated. Samples were collected from private dental clinics in Baghdad city over a period of three months, From May to August of 2023. A 40X optical microscope (OLYMPUS/BX51, Korea) was used to examine the crowns and choose teeth that did not have any flaws, cracks, cavities, wear, fluorosis, or spots [15]. First, an ultrasonic scaler was used to get rid of residual tissue and other waste. The teeth were then polished with fluoride-free pumice (Perfection Plus, Hants, UK) and protective rubber caps. A water-cooled cutting disc was used to separate the roots 2 mm below the CEJ. The buccal surface of each sample was polished with Sof-Lex™ discs (3M™ ESPE, USA) on a slow-speed handpiece, disinfected for up to one week in 0.1% chloramine T solution (BDH, England), and then stored in distilled water for weekly refreshment until use [17, 18]. Samples were placed inside cold-cure acrylic cylinder blocks (Duracryl® Plus, Spofa Dental, Kerr business, Czech Republic), and only the smooth surface came out. After putting 3x3 mm adhesive tape on each enamel-polished surface, the samples were painted twice with Flormar's acid-resistant nail polish and then taken off to make a window [19]. Teeth blocks were kept in distilled water at room temperature to protect them from drying out. Teeth were put in a solution for demineralization that had 2.20 mmol/L of calcium chloride, 2.20 mmol/L of monosodium phosphate, 1 mol/L of potassium hydroxide, and 0.05 mol/L of acetic acid to make white spot enamel lesions. The solution has a pH of 4.4 and was kept in a light-resistant container in a 37° water bath (BS-11/LAB COMPANOIN, Korea) for 4 days, or until a change in the enamel surface that could be seen in both wet and dry conditions was seen [20] and confirmed by reading from a DIAGNOdent™ pen 2190 (KaVo, Biberach, Germany). The final fluorescent value for each sample was found by taking the average of three readings in a row for each tooth [21].

2.2 Experimental groups

After the WSLs were created, each sample was washed with 10 ml of deionized water and dried with a stream of compressed air. The samples were then randomly split into four groups of 10 (n=10):

- Group (L1): Samples were treated with an Er:Cr:YSGG laser at 0.75 W/20 Hz and 60% water with 40% air.
- Group (L2): Samples that were cut with an Er:Cr:YSGG laser set to 0.5 W, 20 Hz, and 60% water and 40% air.
- Group (L3): Samples were treated with an Er:Cr:YSGG laser at a power level of 0.25 w/20 Hz and 60% water and 40% air.

2.3 Laser irradiation

Irradiating the sample enamel with an Er:Cr:YSGG laser (2780nm, Waterlase iPlus; Biolase, Irvine, CA, USA) was done. The laser was used for 10 seconds at 0.25 W, 0.5 W, and 0.75 W total power, with 20 Hz repetitions. 40% air and 60% water spray were used for watering. A fiber optic system with a 600µm beam



width MZ6 sapphire gold tip and a distance of 1-2 mm in a non-contact (H) mode was used to send the energy [22]. A computerized numerical control machine (CNC) was used to standardize lasing [23]. The Laser irradiation setup is shown in Figure 2.

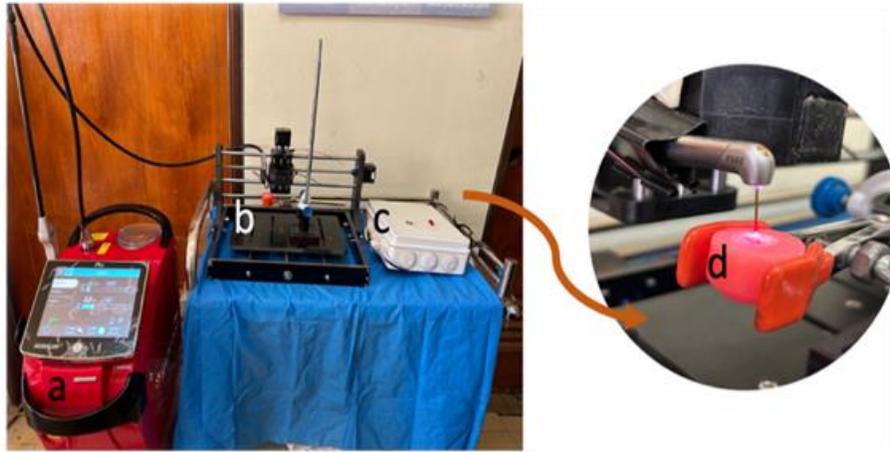


Fig.1. Left: Laser irradiation setup, a. Er:Cr:YSGG Laser, b. CNC machine, c. Power supply. Right: d. sample Irradiation.

2.4 Enamel Surface Roughness Evaluation

Samples Ra “the average distance from the profile to the mean line over the length of assessment” was found with a profilometer (SRT-6210, China). The surface roughness tester has a diamond tool with a 5 μm radius that is straight across from the sample's surface. The speed was set to 0.1 mm/sec and the cut-off distance was set to 0.25 mm. To find the surface roughness value, three measurements were taken of each sample, and the mean of those three measurements was used [24-27]. There were measurements taken before and after WSLs induction, and after laser treatment.

2.5 Scanning Electron Microscopy

A scanning electron microscope (SEM, TESCAN, VEGA II/Republic of Czech) was used to look at the features of one sample from each group. The magnification was set to 2000x. The samples were coated with gold using a sputter coating method (Emitech-K500X, Quorum Technologies, Ashford, UK) before they were analyzed by SEM.

2.6 Statistical Analysis

Different types of statistics were used to look at the data, such as descriptive statistics, a one-way analysis of variance (ANOVA), Tukey's HSD test, and the Paired t-test. The significance level that was picked was set at $p \leq 0.05$. The IBM SPSS 29 program was used to analyze the data.

4. Results

4.1 DIAGNOdent™ pen readings

According to the manufacturer's instructions, specimens with scores ranging from 14 to 20 were classified as having WSLs [28].



4.2 Enamel Surface Roughness Evaluation

The mean and standard deviation of the samples' roughness values of the study groups in addition to a one-way ANOVA test before and after WSL induction and after laser irradiation are shown in Table (1). Pairwise comparisons of roughness between groups using paired t-test after WSL induction in Table (2) and between measurements after WSL induction and after laser irradiation in Table (3).

Table 1. Roughness mean, standard deviation, and one-way ANOVA test of the study groups at baseline, after WSL induction, and after laser irradiation.

Roughness	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
Group	Baseline	After WSL induction	After Laser irradiation
0.75W (L1)	3.3 \pm 0.27	3.40 \pm 0.24	3.45 \pm 0.25
0.5W (L2)	3.19 \pm 0.21	3.31 \pm 0.24	3.43 \pm 0.27
0.25W (L3)	3.2 \pm 0.32	3.45 \pm 0.4	3.5 \pm 0.25
F value*	1.169	0.642	0.298
P-value*	0.33	0.72	0.93

*One-way ANOVA

Table 2. Pairwise comparisons of roughness between groups using paired t-test after WSL induction.

Roughness	Baseline	After WSL induction	Mean difference	t-statistics	P-value
0.75W (L1)	3.3 \pm 0.27	3.40 \pm 0.24	-0.10	-1.02	0.30
0.5W (L2)	3.19 \pm 0.21	3.31 \pm 0.24	-0.12	-1.1	0.30
0.25W (L3)	3.2 \pm 0.32	3.35 \pm 0.4	-0.15	-1.11	0.29

Table 3. Pairwise comparisons of roughness between groups using paired t-test after WSL induction and after laser irradiation.

Roughness	After WSL induction	After laser irradiation	Mean difference	t-statistics	P-value
0.75W (L1)	3.40 \pm 0.24	3.45 \pm 0.25	-0.16	-1.45	0.19
0.5W (L2)	3.31 \pm 0.24	3.43 \pm 0.27	-0.14	-1.33	0.29
0.25W (L3)	3.35 \pm 0.4	3.5 \pm 0.25	-0.11	-0.73	0.48



4.3 SEM Evaluation

Figure 2 displays the morphological properties of the samples following demineralization and after laser irradiation. The SEM photos display structures at a magnification of 2.00 k. The degraded enamel surfaces in the control sample (N) subjected to demineralization solution only, had rough and etched structures accompanied by a smear layer. The L1 sample displayed imperfections characterized by rough and uneven surfaces with sharp edges, deep craters, empty interprismatic areas, pointed enamel projections, and many porosities. Additionally, no smear layer was seen. The enamel rods displayed melting and fusing when subjected to irradiation with a laser power of 0.5W and 0.25W in L2 and L3 respectively.

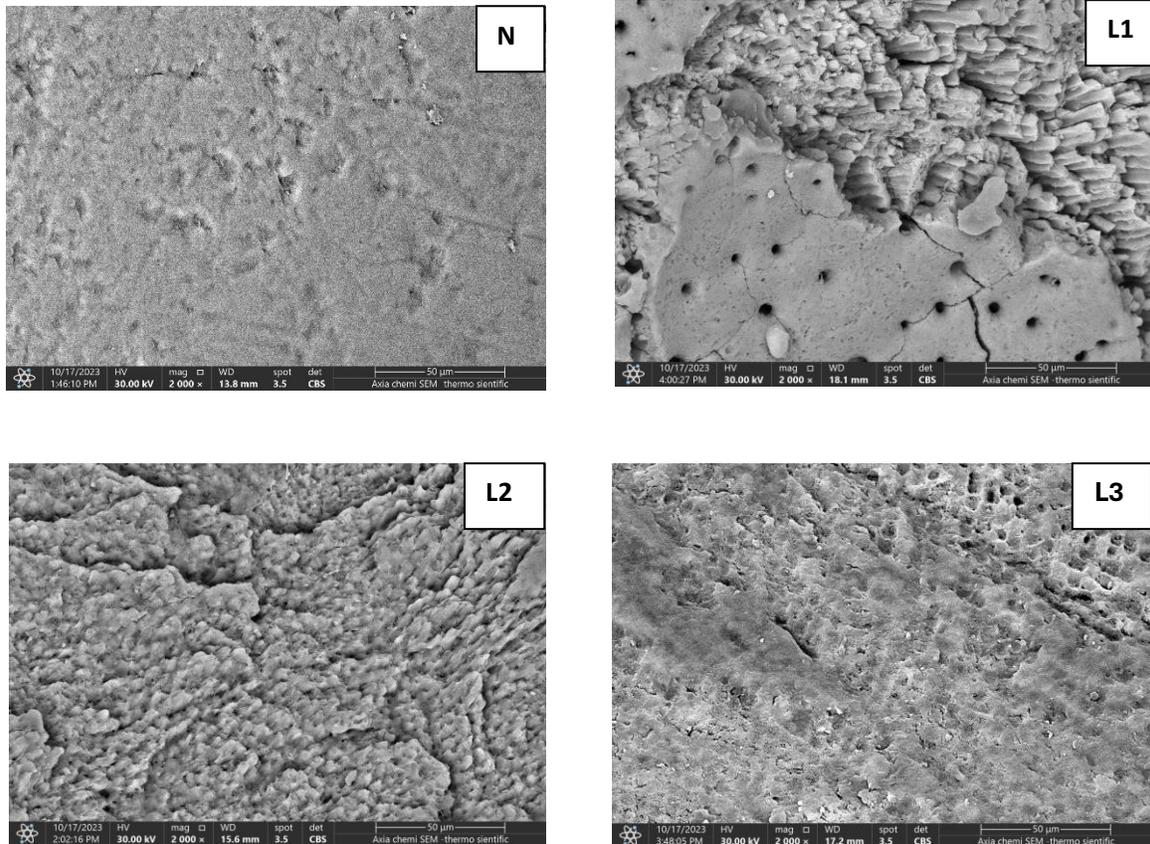


Fig.2. SEM images at 2.00k magnification of the study groups after treatment.

5. Discussion

Treatment of deciduous teeth with laser can have an impressive effect on the three heads of a triangle that involves more comfort for dentists as it decreases working time, and decreases fear feeling while child under dental work by reducing noise and vibration associated with conventional methods of drilling and more acceptable for parents. This approach enhances the patient's comfort and willingness to cooperate [29].

To simulate the demineralization process occurring in the vicinity of the teeth in vivo, the researchers utilized acetate buffer. This choice was made because acetate buffer can stimulate the development of white spot lesions (WSLs) at a deeper and more rapid rate when compared to lactate buffer to simulate carious lesion development. Concurrently, the addition of calcium and phosphate to the acidic buffer system

resulted in the solution being partially saturated, thereby creating a surface topography similar to that of natural WSLs. The surface topography of this area is characterized by an undamaged outer surface and the removal of minerals from the underlying subsurface [18]. In addition, the pH of the demineralization solution was modified to 4.4 to promote the breakdown of hydroxyapatite and fluorapatite crystals in a cumulative process within a specific time. These crystals normally need a pH of 5.5 and 4.5 respectively to be dissolved creating initial caries, which is also proposed by Gouda et al. [30].

According to the findings of Apel et al., the ablation threshold for the Er,Cr:YSGG laser ranged from 10 to 14 J/cm² [31]. The current investigation involved the application of an Er,Cr:YSGG laser beam to the experimental groups at varying sub-ablative powers of 0.25, 0.50, and 0.75 W, and a frequency of 20Hz, air/ water ratio of 40%/ 60% and H mode. When these parameters were used, in addition to pulp vitality preservation from possible thermal damage. Demineralization of enamel was significantly reduced when these parameters were used, in addition to pulp vitality preservation from possible thermal damage resulting from laser application. MZ6 laser tip with a diameter of 600µm diameter was used to achieve high energy density with low laser power in order to focus the laser irradiation within the focal length according to the manufacturer's instruction; this was also adopted by several other researchers [32-34].

The reason of choosing frequency at 20Hz is to be clinically effective and allow ample cooling between pulses achieving the desired impact and avoiding thermal pulp injury in accordance with earlier studies as Erkmén Almaz et al. and Zzell et al. [32, 34]. In addition to water irrigation to cool oral tissues and avoids the formation of acid-soluble chemical phases. It acts as a cooler during dental laser treatment, protecting the tooth and surrounding tissues. In this study, the irrigation was set to a 40% air, and 60% water ratio, which allowed for proper cleaning, and it was proven that such a ratio do not elevate pulpal temperature over the critical value preventing necrosis [7].

The finding of scanning electron microscopy (SEM) image of the control sample (N), the sample surface displayed a pattern of degraded enamel surface that was not clearly distinguishable, with a smear layer covering. The application of Er,Cr:YSGG laser, specifically at irradiation levels of 0.75W (Group L1), results in a modified morphology characterized by heightened surface irregularities, exposure of enamel rods, and non-significant increased roughness measurements with possible signs of ablation. These surface alterations and roughness were reduced as the laser power decreased. The SEM images of group L2 and L3 exhibited a decreased amount of structural alteration and fusion with partial sealing of the enamel prisms more harmonious with the 0.25W laser-irradiated group L3. Statistically, the mean difference of roughness measurements after WSL induction and after laser irradiation (L1, L2, and L3) was non-significant but increased with increased laser power. Roughness may have an impact on the preventive measures because it may enhance plaque accumulation and requires more precise oral hygiene protocols. In a study conducted by Ghabuk and Al-Shamma, treatment options with Opalustre and Sylc were investigated, and both demonstrated the ability to reduce the surface roughness of the artificial WSL. [35]

The observed augmentation of surface roughness, although it was non-significant, after laser irradiation is consistent with the conclusions made by Gouda et al., Malik et al., and Sun et al. [30, 36, 37]. Gouda et al. set that the utilization of laser irradiation on an enamel surface resulted in an increased level of roughness that subsequently creates a retentive niche for fluoride, which demonstrated improved resistance to dissolution [30]. Additionally, in a study conducted by Adel et al., they concluded that Er,Cr:YSGG laser using sub-ablative parameters in histological examination, a significantly less lesion depth compared with the control group.[38]. The Er,Cr:YSGG laser penetration depth of up to 5µm, and the WSL have a minimum depth of 300-500 µm [39, 40].

The increased roughness observed after irradiation on the enamel surface is due to the sub-ablation process. The induced structural modifications by the sub-ablation process are promoted by phase transformation or melting of inorganic compounds, in addition to the expansion of the organic matrix, as reported by Ersahan and Sabuncouglu. [12]. Among the different effects of absorbed laser energy results in thermal energy conversion, which leads to the boiling of the water inside the tooth. This will lead to steam creation at high pressure, and later on will be rapidly vaporized, eventually, the previously smooth surface tooth will change to a rough surface with sharp edges. The altered surface is usually devoid of carbonization and smear layer [41]. In addition, the inclusion of water during the process of irradiation



greatly amplifies the ablation effect, as Erbium lasers exhibit a pronounced affinity for water. Colucci et al. (2015) found that there is an inverse relationship between the quantity of water and the laser's intensity on the enamel surface [42].

The parameters used in this study could be recommended as they did not significantly increase the surface roughness of the primary teeth enamel. The determination of appropriate laser parameters is of significant relevance, as the presence of damage and alterations in the enamel surface can lead to the development of biofilms and the invasion of germs [32].

6. Conclusions

Within the limitation of this study, a non-significant increase in surface roughness of the primary teeth enamel with WSL after sub-ablative power irradiation with Er:Cr:YSGG laser, with a rough and irregular surface devoid of smear layer, the roughness increase was proportional with the increased irradiation power.

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تضاريس سطح الاسنان اللبنية بعد التشعيع بالليزر : دراسة مختبرية

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الخلاصة

الهدف: لتقييم تأثير معلمات التشعيع تحت الاستئصالي لليزر Er,Cr:YSGG على التضاريس السطحية لمينا الاسنان اللبنية مع آفات البقع البيضاء.

الطرق: تم إخضاع ما مجموعه 40 سناً خلفياً لبنياً بمينا سليمة في البداية إلى محلول نزع المعادن عند (PH 4.4) لتحفيز آفات البقع البيضاء بشكل مصطنع. ثم تم تقسيمها عشوائياً إلى أربع مجموعات (العدد=10): مجموعة التحكم (N) ، المجموعة L1 المشععة بليزر 0.75 واط، المجموعة L2 المشععة بليزر 0.5 واط، والمجموعة L3 المشععة بليزر 0.25 واط. تم تقييم التضاريس السطحية باستخدام مقياس خشونة السطح والمجهر الإلكتروني الماسح.

النتائج: كشف تقييم خشونة السطح مع صور المجهر الإلكتروني الماسح عن زيادة غير ملحوظة في خشونة السطح بعد عملية إزالة المعادن. يؤدي تشعيع الليزر بقوى مختلفة إلى زيادة غير ملحوظة في خشونة السطح مع تغير التضاريس وتأثير أكثر وضوحاً مع مجموعة الليزر L1 وبدرجة أقل المجموعتين L2 وL3.

الاستنتاجات: زيادة خشونة سطح مينا الأسنان اللبنية بعد تشعيع الطاقة شبه الاستئصالية باستخدام ليزر Er,Cr:YSGG ، مع سطح خشن وغير منظم خالٍ من طبقة اللطاخة، وكانت زيادة الخشونة متناسبة مع زيادة قوة التشعيع.

