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Original Article

Topographical Analysis of Human Enamel after Phosphoric Acid Etching and Er,Cr:YSGG Laser Irradiation

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Abstract

Introduction: Dental hard and soft tissues have been successfully removed by using the Er,Cr:YSGG laser, but there is a controversy about using lasers over conventional tooth surface preparation for bonding aesthetic restoration. Surface roughness and wettability in response to Er,Cr:YSGG laser irradiation are essential properties for restoration longevity.

Methods: Fifty-one intact human premolars removed in orthodontic treatment were included in this study and divided into three groups (n=17). The first group (G1) was the control without surface treatment, (G2) was treated with 37% phosphoric acid for 15 seconds, and (G3) was treated with the Er,Cr:YSGG laser using the following parameters: 2 W or 3 W at 20 Hz, 10% air and water ratio using the MZ6 laser tip. The standardization of laser irradiation was accomplished by a computerized numerical control unit. The surface of the samples was evaluated by using a light microscope, profilometer, atomic force microscopy (AFM), SEM, and wettability tests.

Results: The SEM examination revealed that the lased enamel surface was clean, irregular, and devoid of a smear layer, while the acid etch surface was relatively smooth and covered with a smear layer. The surface roughness of the lased enamel surface was significantly higher than that of other groups, according to the results of the profilometer as well as the AFM tests. The wettability test showed that the lased enamel surface recorded a significant reduction in the contact angle in comparison to the other groups.

Conclusion: It can be concluded that the Er,Cr:YSGG laser can be used as an alternative and safe method to the acid-etching technique for surface treatment.

Keywords: Er,Cr:YSGG; Phosphoric acid; Enamel; Scanning electron microscope; Wettability.

Introduction

The etching of dental enamel is an essential step for tooth preparation during resin bonding and orthodontic bracket attachment. The phosphoric acid etching has been a routine pretreatment method since its introduction by Buonocore in 1955.[1] Basically, phosphoric acid application on enamel surfaces will increase the surface energy and bonding area as well as the wettability of the adherent surface.[2] The possibility of decalcification of enamel surfaces is considered a disadvantage for acid etching; furthermore, precautions during acid application and those difficulties recorded in acid application during the treatment of children or old age may inspire researchers to investigate other methods that can be applied for acid etching.[3,4] Some of these methods are mechanical, like using burs or sandblasting, or optical, like using a laser.[5] Lasers are well adopted in dentistry, even being introduced in most recent treatment modalities such as dental implants.[6,7] Studies have been evaluating the effect of lasers such as CO2 and Nd:YAG on tooth structures such as enamel and dentin.[8,9] However, some laser types were unable to cut dental hard tissues effectively depending on laser and target tissue characteristics, which in turn gave different laser material interactions such as absorption, scattering, transmission, and reflection. It was proposed that some lasers induced surface modifications in enamel, such as fissuring, recrystallization, and cracking; in addition, laser energy was associated with temperature elevation that could affect dental soft tissues such as pulp and periodontal tissues.[10] After the introduction of the Er,Cr:YSGG laser, some of these shortcomings have been reevaluated. Er,Cr:YSGG laser light at 2780 nm is strongly absorbed by dental tissues, which are composed of water and hydroxyapatite crystals. Dental hard tissues are ablated and removed as a result of the thermal effect of the laser, which causes the creation of water steam, which expands to cause microexplosion.[11] The microexplosions of Er,Cr:YSGG lasers lead to micro and macro irregularities on the irradiated surfaces.[12] The strongest infrared absorption bands have been located in the 2-3 μm and 9–11 μm regions, which are easily accessible by erbium, holmium, and CO_2 laser systems.[13]

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A powerful instrument for effective hard tissue ablation and temperature control is the sapphire tip of the Er, Cr: YSGG laser, accompanied by a water and air mixture.[14] Laser application may indicate some parameters more than others, as in the etching procedure, where water or air is not essential to perform the objective; this may save time and prevent procedural errors.[15] Another advantage of lasers is that they increase enamel resistance to acid demineralization by changing or reducing the calcium-phosphate ratio, which leads to the formation of more stable and less acid-soluble compounds. This phenomenon is greatly helpful in laser caries control trials.[16] Hydroxyapatite crystal modifications with laser irradiation cannot occur with enamel temperatures lower than 100 °C. Enamel dehydration and protein denaturation, which reduce enamel permeability, occur between 60 and 200 °C, while protein breakdown, which increases enamel permeability, occurs between 350 and 400 °C. At 420 °C, carbonate breakdown leads to decreased solubility in acid.[17] Additionally, erbium lasers had different application in dentistry such as elimination of smear layer and cavity disinfection,[18] desensitization of dentin and caries prevention.[11,19] Also enhancing the bond strength between tooth and zirconia restoration and reducing nanoleakage in the composite resin.[20,21] Therefore, this trial attempts to investigate and benefit from the Er,Cr:YSGG laser in etching as an alternative to conventional acid etching method considering the effect of some laser parameters and to correlate between different surface guiding factors as surface roughness and wettability, to estimate the role of different modification methods.

Materials and Methods

Sample Collection and Preparation

Fifty-one intact, extracted human premolars for orthodontic purposes were used in this in vitro study. Teeth were cleaned from blood, debris, and periodontal ligament remnants with dental scalar and prophylactic non-fluoridated dental pumice and then stored in a 1% thymol solution until used. The preparation of the samples was done by using a dental surveyor (Dentaurum Paratherm, German), which involved sectioning the teeth into two halves mesiodistally with a diamond disk and vigorous water cooling. The enamel surface of the specimens was flattened with a polishing disk (TOR VM, 1.071, Russia) and cleaned ultrasonically. Each specimen was embedded in the self-cured acrylic block using a silicon mold, as seen in Figure 1. The distribution of the samples was as follows:

- G1: Control group without surface treatment
- G2: Samples were etched with 37% phosphoric acid gel
- G3: Samples were irradiated with the Er, Cr: YSGG laser (Biolase, iPlus, USA).

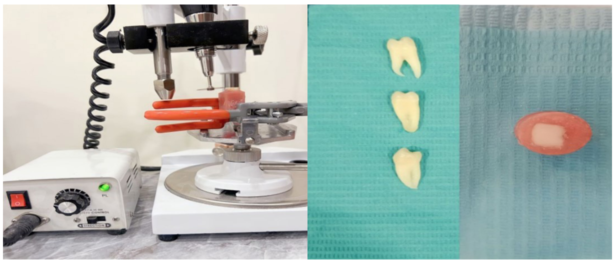

Figure 1. Sample Collection and Preparation

Phosphoric Acid Application

Enamel samples of G2 were etched with 37% orthophosphoric acid gel (3M, ESPS, USA) for 15 seconds and rinsed vigorously by using distilled water for 20 seconds according to the manufacturer's instructions.

Laser Application

An Er, Cr: YSGG dental laser (WaterLase iPlus, USA) was used to irradiate specimens of G3 with the MZ6 laser tip, (H) mode with 60 µs, 0.12 mJ, 2 W or 3 W at 20 Hz, 10% air and water ratio. The laser beam was directed upright to the target surface with a 2mm standoff distance between the laser tip and the specimen's surface. For the standardization of laser irradiation, a CNC (computerized numerical control unit) was used, as shown in Figure 2.

Light Microscopic Examination

All samples were tested under a light microscope (Euromex microscopic reflection/transmission, the Netherlands) with different magnification powers to examine the morphology of the enamel surface before and after laser irradiation or acid etching.

Surface Roughness Examination

Ten samples from each group were examined for the control group before and after laser irradiation or acid etching to determine the changes in surface roughness (Ra) by using a profilometer (SRT-6210, China). The cut-off distance of the device was 0.25 mm. Finally, the mean of the three readings was set as the roughness value for each specimen.

SEM Examination

The SEM (Inspect F-50, FEI Electron Optics International B.V, the Netherlands) examination was performed on one sample from each group to evaluate enamel surface morphology. In a vacuum chamber, the samples were exposed to a gold sputter to coat them and they were viewed in an SEM device at different magnification powers.

Wettability Test

Five samples from each group were subjected to this test. Prior to contact angle measurements, the samples were rinsed with isopropyl alcohol and then dried thoroughly to avoid any contamination during testing.[22] The contact angle measuring device (CAM 110, Taiwan) consists of a microsyringe that drops a distilled water drop (2 µL) on the surface of the specimen (as seen in Figure 3). A computerized digital camera was connected to the device, and it captured an image after 30 seconds of water dropping. Evaluation of the wettability was performed by measuring the contact angle formed between the surface of the specimen and the tangent of a water drop at room temperature.

Atomic Force Microscopy Examination
Using atomic force microscopy (AFM) (Nano Surf, Liestal, Switzerland), we tested one sample from each group. The root mean square of surface roughness was displayed in contact mode by the scanning probe of the atomic force microscope.

Results
Light Microscopic Examination
The result of a light microscope examination of enamel samples for G1 showed scratches on the enamel surface during preparation without surface modification, as seen in Figure 4A. For G2, a superficial effect of phosphoric acid appeared on the enamel surface, as seen in Figure 4B, while for G3, a uniform pattern of surface modification and roughness followed laser irradiation, as seen in Figure 4C, with no sign of adverse effects such as carbonization or cracks. However, for G3 (3W), the enamel surface appeared to have undesirable effects such as smoothing and reducing surface irregularity due to melting, as seen in Figure 4D.

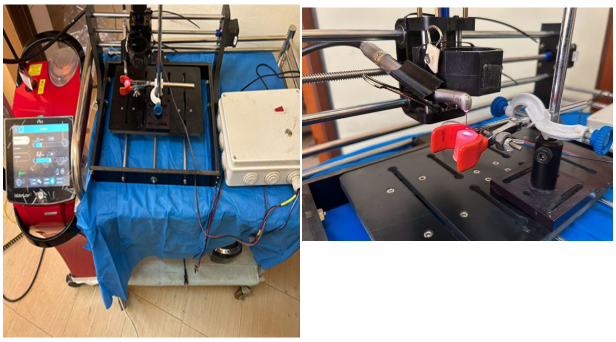

Figure 2. (A) Experimental Set-up With CNC Machine. (B) magnified view with a 2 mm stand-off distance

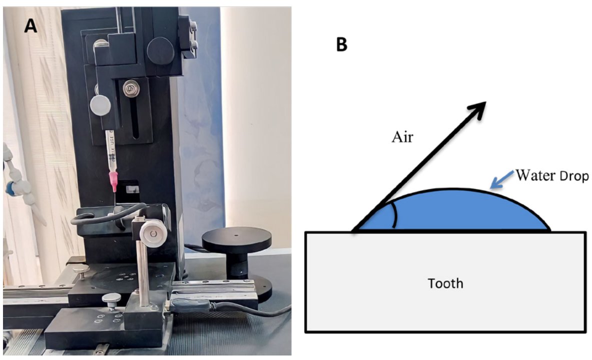

Figure 3. (A) Contact Angle Measurment Device. (B) Diagram shows the contact angle measure

Surface Roughness Examination
Descriptive Statistics and ANOVA tests of all groups showed significant differences in mean surface roughness. The highest mean of roughness change was observed in G3 (Er:Cr:YSGG laser), followed by G2 (phosphoric acid etching). The lowest mean of roughness was seen in G1, as shown in Table 1 and Figure 5.

SEM Examination
Under different magnifications, SEM images showed that the first group's enamel surface had a smear layer with a groove following preparation directions, as shown in Figure 6A. The second group's enamel surface had a honeycomb-like appearance with a lot of smear layer covering it, as shown in Figure 6B, while the third group's enamel surface showed a different range of roughness and irregularities, and it was clean, and free from any smear layer or thermal damage, such as melting, cracks, or carbonization. The surface of the scanned teeth had sharp, jagged projections, and an enamel prism was also visible, as seen in Figure 6C, However, in the third group, the enamel surface (3W) showed a melted enamel surface with decreased surface irregularity, as seen in Figure 6D at different magnifications.

Wettability Test
This test demonstrated that the lased enamel surface had the lowest contact angle compared to that of the other group, as shown in Table 2 and Figure 7.

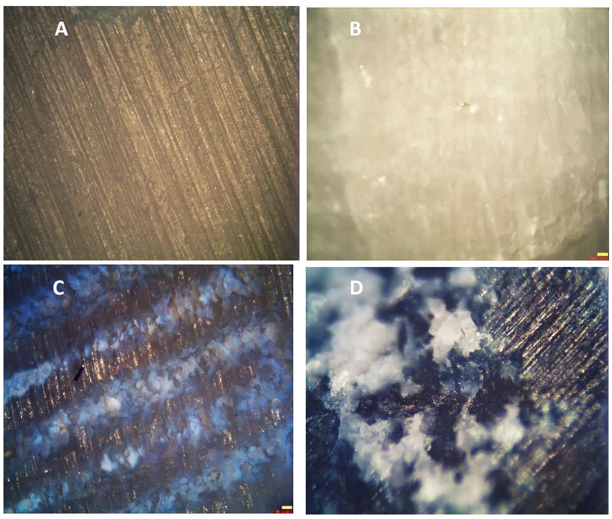

Figure 4. Light Microscopic Images of (A) Untreated Enamel (G1), (B) Acid Etch Enamel (G2); (C) 2W Laser-Irradiated Enamel (G3) and (D) 3W Laser-Irradiated Enamel (G3) at 4X

AFM Examination

Further evaluation of the enamel surface topography of the tested groups was done by using AFM. The results indicated an increase in the enamel roughness of the lased group more than the other groups, as seen in Table 3 and Figure 8.

Discussion

The preparation of the enamel surface for resin bonding or many other treatments is one of the issues that affect the success and longevity of the restoration and the function of the restored teeth; therefore, it is of great concern and attention, which encouraged researchers to look for any modification that could serve these goals.[23] In this study, enamel surface topography and roughness were evaluated after phosphoric acid etching and compared to the effect of Er, Cr: YSGG laser irradiation. The evaluation was made through different tests such as profilometer, AFM, SEM, and Wettability. Research has proposed some disadvantages of using acid in restorative dentistry, such as increased chair time. The conventional etching method includes different steps in sequence, such as acid application, water splashing, and finally drying the field with air, all of which needs 15 seconds. In laser etching, these steps are eliminated, so clinically, the work could be finished in about 5–10 minutes, which would be considerable from a clinical point of view.[24] Hence, in order to save clinical time and improve efficacy of esthetic restoration (5), laser etching was suggested as an alternative to acid. This is may help to overcome the drawbacks, shortages and precautions required during acid usage, furthermore; to benefit from laser as a cavity preparation tool as well as for etching procedure. SEM images showed that the enamel surface of the control and acidic groups was covered with a smear layer; the acid-etched enamel surface showed a honey-combed appearance, which was described for the first time by Silverstone et al.[25] They proposed that SEM images clearly showed that the prism core appeared clearly with the appearance of the prism periphery; this agrees with Luis et al,[26] while Er,Cr: YSGG enamel was clean and highly irregular without the smear layer; this also agrees with Luis et al. and Varsha et al.[26,27] The variation in the appearance of the enamel surface in the different groups is attributed to the mechanism of interaction between acid and laser with the enamel. Acids typically remove about 10 micrometers of the surface of the enamel and leave a microporous layer that is between 5 and 50 micrometers deep to give the characterized appearance of the surface.[28] However, lasers interact with the enamel through energy absorption or interaction due to the creation of microexplosive reaction and superficial ablation shown as craters or rough surfaces.[29] Statistical analysis of surface roughness between different groups showed that an increase in enamel roughness was significantly greater for the laser-irradiated group than for the others; this could be attributed to a lower effect of acid etching on the organic material of the enamel surface. On the other hand, the ablation mechanism of lasers might cause a structural change by melting or changing inorganic tooth structure, expanding the organic matrix, which in turn blocked ion diffusion, channels.[30] This result was in agreement with Lopes et al,[31] who compared phosphoric acid etching and Er,Cr:YSGG laser irradiation influence on enamel surface topography using different powers, and it was in line with Ersahan and Sabuncuoglu[32] who concluded that Er,Cr:YSGG laser irradiation created a microexplosion effect on the enamel surface, converting the enamel surface from smooth to irregular and microfissured structure. However it disagreed with Dilip et al,[33] who evaluated enamel surface roughness after laser and acid etching for orthodontic bracket bonding; the

Table 1. Descriptive Statistics and ANOVA Test of Surface Roughness Between Different Groups

Tested Group	N	Mean ± SD	F-test: Sum of Squares	df	Mean Square	F-value	*P* Value
G1	10	8416 ± 0.14268	18.360	2	9.180	47.523	0.000
G2	10	1.4737 ± 0.14673	5.216	27	0.193		
G3	10	2.7243 ± 0.73324	23.576	29			

Table 2. Descriptive Statistics and ANOVA Test of Contact Angle Between Different Groups

Tested Group	N	Mean ± SD	F-test: Sum of Squares	df	Mean Square	F-value	*P* Value
G1	5	75.8956 ± 4.34613	5348.123	2	2674.061	191.946	0.000
G2	5	42.3588 ± 4.04789	167.175	12	13.931		
G3	5	31.5428 ± 2.55335	5515.2 98	14			

Table 3. Surface Roughness Using Atomic Force Microscopy for Tested Groups

Group	Ra (nm)
G1	44.9 nm
G2	123.4 nm

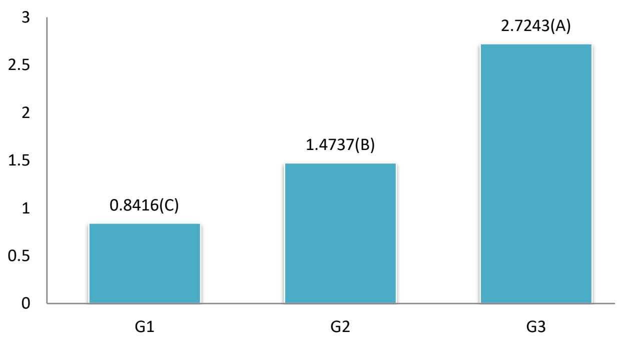

Figure 5. Surface Roughness Between Different Groups. * Alphabetic letters (A, B, and C) represent a significant level respectively

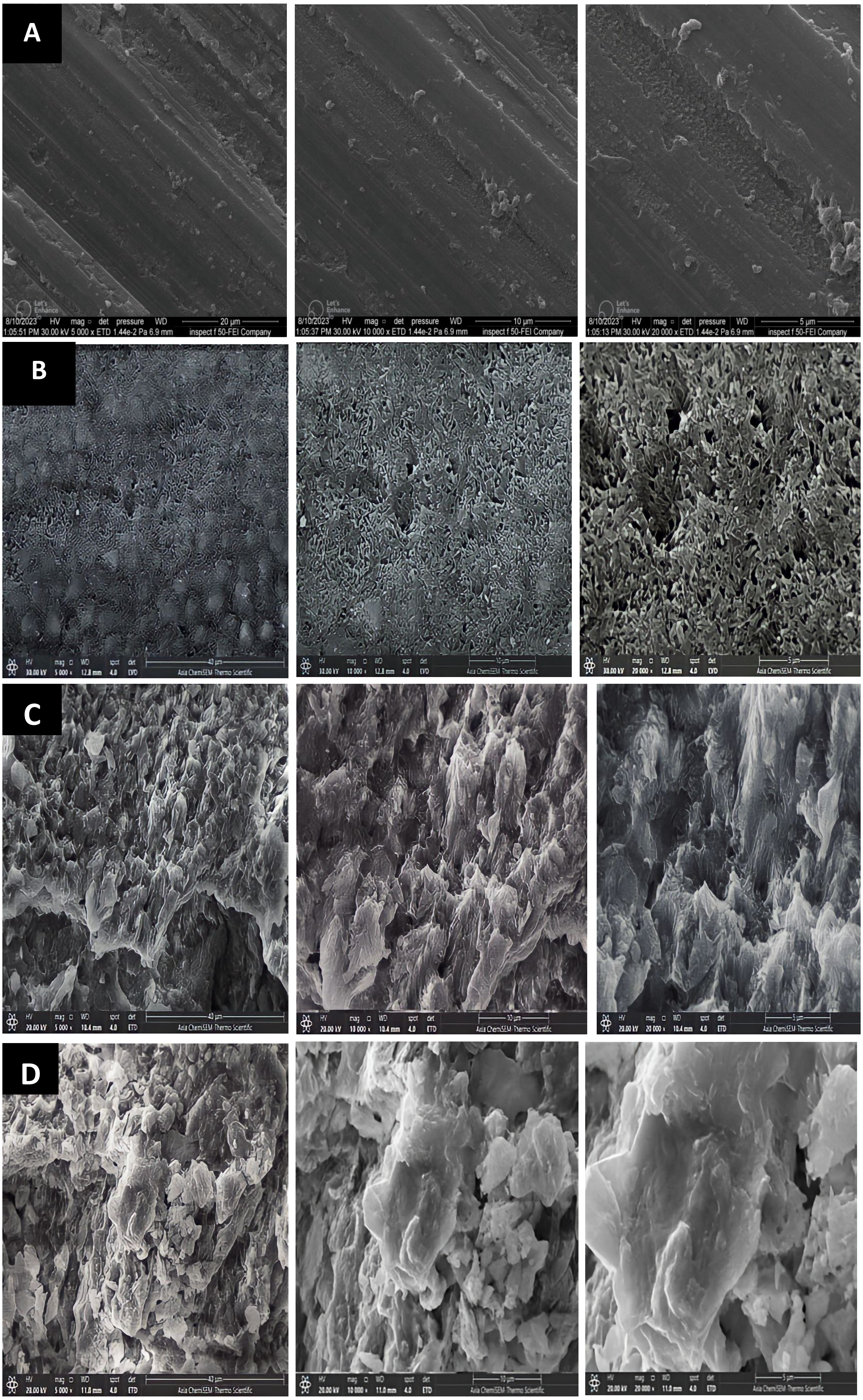

Figure 6. SEM Images Showing (A) Sound Tooth (G1), (B) Acid-Treated Enamel (G2) and (C) 2 W Laser-Irradiated Enamel Surface (G3) and (D) 3 W Laser-Irradiated Enamel Surface (G3) at × 5000, × 10000, × 20000

disagreement could be attributed to the use of untouched enamel surfaces, while in this research, mild preparation and flattening were essential for testing. Laser energy with different powers produced different results; using 3W smoothed the enamel surface and gave a picture of glazing rather than ablation or roughness. This could be attributed to a higher thermal effect, which led to melting rather than ablation. This is agreed upon by Subramanian et al,[5] who proposed that using 3W laser power would produce a flat and smooth surface, as shown by the SEM images. Statistical analysis also revealed a significant increase in surface roughness between the acid-etched group and the control group, which is expected and follows the target of acid use, and this leads to dissolving of hydroxyapatite crystals resulting in an irregular and rough surface as proposed by Gulec et al.[34] The roughness produced by the laser was significantly higher than that of the acid group, which is mainly due to the mode of action, which is photothermal for laser and chemical reaction for acid group. Laser parameters were selected to serve the target of the project which was surface modification to increase surface roughness to serve for enamel etching. Therefore, laser power was low in order not to induce cutting or massive changes in the enamel. Wettability means the tendency of a liquid to spread over a solid surface. Surfaces are considered hydrophobic when the contact angle is greater than 90º and hydrophilic when the contact angle is less than 90º, which is an indicator of the attraction between solid surfaces and liquid drops.[35] In general, wettability determines the interfacial proximity between solid and liquid. In many dental

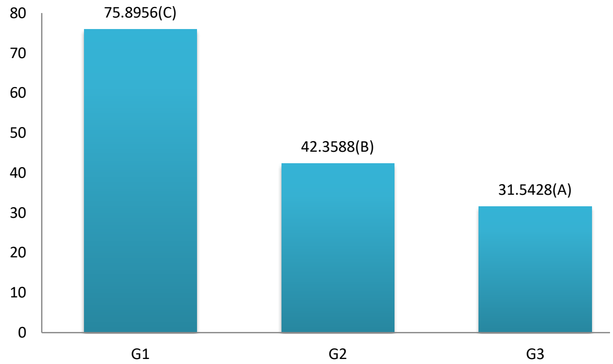

Figure 7. Contact angle between different groups. *Alphabetic letters (A, B and C) represent a significant level respectively

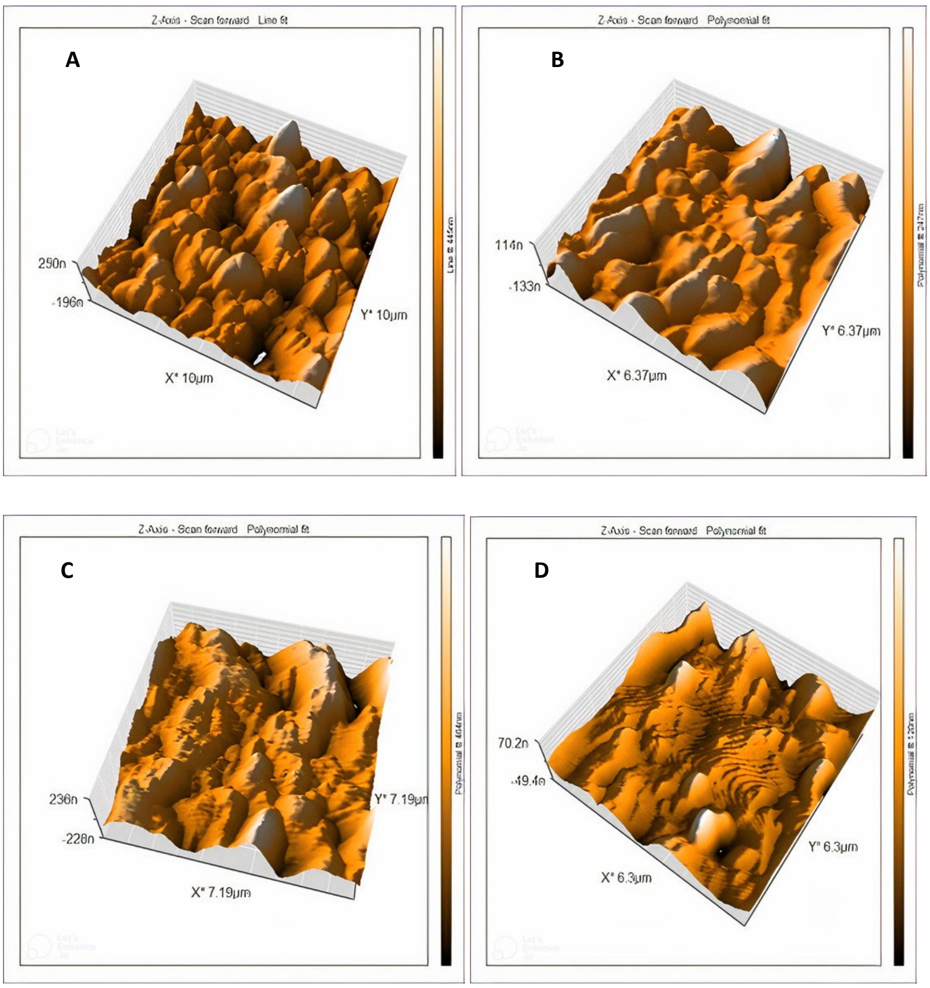

Figure 8. Atomic Force Microscopy Images (A) Untreated Enamel (G1), (B) Acid-Etched Enamel (G2), (C) 2W Laser-Irradiated Enamel (G3), (D) 3W Laser-Irradiated Enamel (G3)

applications, this property is in focus because it affects the next steps as well as the success of the treatment. It can be demonstrated by the contact angle between the applied agent and the substrate.[36] In restorative dentistry, having enamel surfaces with good wettability will improve the adhesion of restorative materials, including bonding and resin materials, and there are three main variables that affect whether a solid surface will become wet or not with a liquid. These variables are the viscosity of the liquid, the surface morphology of the solid, and the surface energies of the solid and liquid.[37] The result of the statistical analysis of the contact angle between different groups showed that the Er,Cr: YSGG laser-irradiated group had the lowest contact angle compared to other groups. This could be attributed to the absence of a smear layer and the increased surface irregularity of the enamel specimen, which supported the spreading of the liquid on the laser-treated enamel surface when compared with other techniques of modification and acid etching, which were covered with a smear layer. Surface irregularity and the existence or extent of the smear layer have a high impact on the wettability of the surface.[38] Two main groups of variables can control the result of laser effect in dental application ; the first one is laser parameters such as power, wavelength, pulse duration, irradiation mode, and time, which are under the control of the dentist, while the second group of variables are the refractive index, absorption coefficient, thermal conductivity, and thermal relaxation time, which represent enamel optical properties that vary between individuals.[39] Although this last group of variables can still be controlled with expert dental staff, they benefit from the flexibility in selecting laser parameters to hit the target or the objective of laser use. It appears that using the Er,Cr:YSGG laser for etching could be helpful and may be considered as an alternative to acid etching.

Conclusion

The use of an Er,Cr:YSGG laser in the etching of dental enamel is helpful in overcoming all the shortages and drawbacks of acid etching. It is able to be used efficiently, and it can produce a good range of roughness and remove the smear layer, which enhances the wettability of the enamel, promising a good prognosis or longevity of the restoration. Laser parameters, specifically the power, are a major contributing factor in surface modification; 2 W showed the best result considering surface roughness and wettability. Further studies could help determine other contributing factors to well-retained restorations.

Authors' Contribution

Conceptualization: Saja Qasim Salman, Basima Mohammed Ali.
Data curation: Saja Qasim Salman, Basima Mohammed Ali Hussein.
Formal analysis: Saja Qasim Salman, Basima Mohammed Ali Hussein.
Funding acquisition: Saja Qasim Salman.
Investigation: Saja Qasim Salman, Basima Mohammed Ali Hussein.
Methodology: Saja Qasim Salman, Basima Mohammed Ali Hussein.
Project administration: Saja Qasim Salman , Basima Mohammed Ali.
Resources: Saja Qasim Salman.
Software: Saja Qasim Salman.
Supervision: Basima Mohammed Ali Hussein.
Validation: Basima Mohammed Ali Hussein.
Visualization: Basima Mohammed Ali Hussein.
Writing–original draft: Saja Qasim Salman.

Competing Interests

Conflicts can arise in situations where someone's judgement may be influenced, by a personal, financial or other interest. Our judgement is not compromise by personal, financial, target or similar measure.

Ethical Approval

Our research study was in vitro completely neither human being nor animal was used clinically and all samples were collected from different iraqi health center

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