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# Evaluation of Microhardness and Surface Roughness of Young Permanent Teeth with White Spot Lesions Treated with Different Techniques

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**Abstract:** In order to get superior therapeutic and cosmetic results, it's important to compare and evaluate the impact of different agents on surface roughness and micro hardness while treating white spot lesions with laser alone, laser with icon and laser with varnish, Icon alone. **Aim:** to evaluate microhardness and surface roughness of young permanent teeth with white spot lesions that will treated with different techniques (ICON, Laser and ICON, Varnish and Laser, Varnish and ICON). **Methods:** The research utilized an in vitro model involving forty intact premolars, cleaned and preserved to simulate natural WSL conditions. After inducing uniform demineralization, various treatment groups were established: ICON® Only: This group demonstrated the ability of ICON® to seal enamel porosities and enhance microhardness by maintaining enamel structure and preventing further decay. Er; YAG Laser with ICON®: This combination proved most effective, significantly increasing microhardness and reducing surface roughness. The laser treatment altered the enamel's microstructure, allowing for deeper resin penetration and improved bonding, thus enhancing the enamel's mechanical properties. Er; YAG Laser with Varnish: Although varnish provided some protective benefits, it resulted in lower microhardness compared to the ICON® group. The varnish may have inhibited effective remineralization due to its barrier properties. ICON® with Varnish: This combination maintained microhardness levels similar to ICON® alone but offered additional aesthetic and fluoride reservoir benefits. The varnish helped correct surface roughness induced by demineralization, contributing to a smoother enamel surface. Microhardness and surface roughness were assessed through rigorous testing, revealing that treatments combining laser and resin not only restore enamel integrity but also enhance its resistance to future demineralization

**Results:** The XRD results indicated that laser treatment improved the crystalline stability of enamel, leading to sharper diffraction peaks, which correlated with increased resistance to acid dissolution. FESEM imaging revealed significant alterations in surface texture, demonstrating that the laser treatment facilitated better retention of restorative materials

**Conclusion:** this research highlights the effectiveness of combining Er; YAG laser treatment with ICON® resin in the management of white spot lesions, promoting both mechanical strength and aesthetic quality. These findings advocate for minimally invasive approaches in dental treatment, potentially transforming the management of early carious lesions into clinical practice. Future studies should focus on long-term outcomes and the clinical applicability of these treatment combinations.

**Keywords:** *Microhardness, Surface Roughness, Young Permanent Teeth, White Spot Lesions*

**Introduction**

The initial carious lesions are the so-called "white spot" lesions, which implies that there is a subsurface area with most of the mineral loss beneath a relatively intact enamel **(1-2).** Surface clinically, early caries lesion in enamel is initially seen as a white opaque spot and is characterized by being softer than the adjacent sound enamel and is increasingly whiter when dried with air. A cross-section of the white opaque spot reveals the features of carious enamel and suggests that dental caries is essentially an enamel defect with a relatively intact surface layer (SL) and some subsurface damage due to acid formed from plaque on tooth surface.

Downer's criteria for detection of caries include: Enamel caries-if there is destruction of the enamel surface or a white area in enamel extending up to, but not including the amelodentinal junction (ADJ), and there is no cavity or discolored area beneath the ADJ. Dentinal caries — if there is destruction of the enamel extending up to and including the ADJ or a cavity or discolored area beneath the ADJ extending into dentine **(3).**

The main types of enamel demineralization include incipient lesions and "surface-softened defect" which are the other names used to describe white spot lesions. It is essential to differentiate incipient lesion from arrested lesions. Incipient lesions are active lesions which continue to progress under acid attack whereas an arrested lesion does not progress. In vivo ultrastructural studies done by Thylstrup and Fredebo concluded that there were wide variations between active and arrested lesions. "Micro-scars" were seen on active lesions while micro-cavitation was usually seen on arrested lesions **(4-5).**

Dental caries is now being increasingly considered as a dynamic disease process wherein equilibrium exists between pathological factors causing demineralization and protective factors causing remineralization. The major pathological factors involve frequent ingestion of fermentable carbohydrates, inhibition of salivary function, and acidogenic bacteria while the protective factors include antibacterial agents which are both natural and applied, composition and rate of salivary flow, fluoride from extrinsic sources and diet. Caries intervention can be natural, or by some mode of treatment or procedure. The disease process is believed to be a continuum beginning with the first atomic level of demineralization, and then the early lesions of the enamel are followed by the dentinal involvement and finally cavitation **(6-7).**

The translucency of enamel is an optical phenomenon that depends on the size of intracrystalline spaces. In early stages, active caries requires air drying to be visible, as the dissolution process of the crystals of the peripheries at the outer surface of enamel begins. Further enlargement of intracrystalline spaces results in a white spot lesion visible without airdrying. **(7).**

The influence of the dehydration state on the translucency of enamel is a result of the water around the enamel prisms being replaced with air. The effect of scattering in a heterogeneous system, such as enamel prisms surrounded by a fluid medium, is a function of the difference in refractive indices of the two involved components. The refractive index of enamel is approximately 1.65. Since the refractive index of water is 1.33 and of air is 1.00, the larger difference, and consequently a greater scattering, are produced at the enamel/air interface **(8).**

The appearance of a white spot lesion is related to its activity, and the consequence of disease control would be seen clinically by a change from chalky and rough (active) to bright and smooth (inactive). Although it has been suggested that white spot lesions could regress or even disappear due to mechanical removal of the affected enamel and remineralization, clinical studies using fluoride toothpaste and gel in long-existing white spot lesions showed no significant enhancement of remineralization. This means that these lesions scarcely disappear clinically **(9).**

Many early non-cavitated lesions will continue to be visible clinically and radiographically after remineralization because most of the detection signal comes from the body of the lesion, where changes in its light scattering and radiolucency are unlikely because of its limited remineralization potential. Thus, although the surface of the lesion may become hard and shiny and the white spot become less obvious, some interior opacity remains. **(9).**

The caries infiltration technique was introduced with the aim of filling the intracrystalline spaces with a low-viscosity resin, to arrest lesions. The caries infiltration is a promising therapeutic method as its approach is between preventive and restorative actions in the treatment of non- cavitated carious lesions. In contrast to the application of sealants, where the diffusion barrier remains on the enamel surface as a covering resin coat, the resin infiltration creates a diffusion barrier within the enamel lesion and enables the strengthening of the demineralized enamel structure with the resin matrix, preventing cavity formation. **(9).** In addition to its action in the control of caries progression, the resin infiltration of white spot lesions produces a positive effect in masking the color of teeth, because the lesions trend to lose their whitish appearance, looking similar to sound enamel. **(10).**

The efficacy of resin infiltration in arresting caries lesions has been investigated in *in vitro*, *in situ*, and *in vivo* studies, and its effect in masking white spot lesions has been shown clinically Nevertheless, the comparison of the masking effect of this technique with the possible remineralization effect of fluoride compounds in the color of demineralized enamel needs to be further studied. **(10).**

Laser is among the new techniques used in treatment of WSLs. whereas, laser interacts photothermally and photo-mechanically with enamel. Laser/Enamel interaction results in recrystallization of enamel crystallites, reduction in enamel permeability, thus increases acids resistance and enhances enamel melting which seal enamel surface. Among the lasers which were used in enamel remineralization either alone or in combination with other topical agents were carbon dioxide, Nd:YAG, Er:YAG and Er,Cr:YSGG laser. Little literature had been reported regarding the effect of Er,Cr:YSGG laser on enamel remineralization either alone or in combination with other topical agents. However, the effect of Er,Cr:YSGG laser in combination with ICON is still not obvious which led to the aim of this study **(10).**

This study aimed to evaluate microhardness and surface roughness of young permanent teeth with white spot lesions that will treated with different techniques (ICON, Laser and ICON, Varnish and Laser, Varnish and ICON).

**Methods**

In present study freshly extracted anonymous maxillary/mandibular premolar teeth extracted for orthodontic reasons will be collected from Outpatient Dental Clinic of Faculty of Dental Medicine for Girls Al-Azhar University. At the end of this study teeth were disposed in medical waste container that will be transferred to incinerator for final disposal.

**Sample size calculation (11-12).:**

The sample size is calculated based on a previous study as a reference. Suppose the mean ± standard deviation of Icon surface roughness is 246.4 ± 123.9. In that case, the estimated difference is 160, minimally the study needed 10 subjects per group when the power was set at 80%, and type I error was set at 0.05. The sample size was performed by using a t-test (Independent) by using P.S. power 3.1.6.

**Samples grouping and intervention (11-13).:**

A total of 40 caries-free human premolars were used in this study. These teeth were taken from patients who had been extracted for orthodontic purposes. They were collected from outpatient dental clinics, hospitals, and the Faculty of Dental Medicine for Girls Al-Azhar University.

All teeth samples were selected according to the following criteria:

- Sound enamel and dentin surface.
- Teeth free from cracks, stains, white spot lesions or caries.
- Teeth without any prior restoration.

Teeth were cleaned from the outside to remove any soft or hard tissue debris by an ultrasonic scaler (Coxo, China), and then washed with water; the teeth were disinfected with 1% NaOCl and stored in a saline solution to preserve the teeth until the day of measurement.

**Samples preparation (11):**

Forty teeth were prepared by sectioning the crown horizontally to separate the coronal portion from the radicular portion of each tooth under water coolant using low-speed double-faced diamond disc (Besqualdia –

disc NY 11373, USA size 22mm), mounted in a grinding machine (Demco alloy grinder, dental maintenance co., INC..BONSALL, CALIF., U.S.A) (Figure 18), the crowns were sectioned into two halves in mesiodistal direction so 80 samples were obtained. A window of 4x4 mm was created on the center of the buccal and lingual surface of the sectioned samples.

**Mounting of teeth in acrylic resin blocks (11)**:

A specially designed plastic mould was designed to fabricate acrylic blocks. Each mould has a diameter of (15 mm X 10mm) to accommodate the acrylic resin (Figure 18). Then the tooth was left fixed in position exposing its buccal or lingual surface until the setting of acrylic resin. The acrylic block was removed from the mould using finger pressure (Figure 18).

**Preparation of white spot lesion (11):**

All the specimens were immersed in demineralising solution for 96 hours in an incubator in distilled water at 37 °C, and the solution was exchanged every 12 hours to keep the pH constant (45). After the induction of artificial carious lesions, all the specimens were rinsed with distilled water for 10 minutes under stirring and then air dried.

**Sample grouping and distribution:**

The prepared premolars were divided into three groups, ten each, according to the treatment protocol:

- **Group (I):** Ten teeth were treated with resin infiltration ICON®.
- **Group (II):** 10 teeth were treated with Erbium LASER and ICON®.
- **Group (III):** 10 teeth were treated with Erbium Laser and Varnish.
- **Group (IV):** 10 teeth were treated with ICON® and Varnish.

➢ **Treatment procedures (14):**

• **Group (I):** After the creation of WSL this group was designed to receive resin infiltration (ICON®) material followed by the manufacturer's instructions for its application as follows:

1. The tooth surface was cleansed. Spray water to eliminate residue. 2. 15% HCl (ICON® Etch) was applied directly on the lesion using a specialised brush applicator for 120 seconds and stirred gently.

3. The tooth was then rinsed with water for 30 seconds and air dry.

4. A syringe containing ICON® Dry was administered directly to the white lesion for 30 seconds. Air dry (Procedures 4-6 can be repeated up to three times until the WSL disappears).

5. The ICON infiltrant was placed and left in place for 3 minutes. Disperse with air. The tooth was exposed to a light cure for 40 seconds.

6. Apply another ICON® Infiltrant. They were left in place for 1 minute and light-cured for 40 seconds.

7. Polishing and finishing with Sof-Lex disc.

• **Group (II) (15-16):**

After WSL was created, this group was designed to receive an Erbium laser followed by resin infiltration (ICON®).

Er: YAG laser (Fotona, LightWalker ST Slovenia), the laser emitted photons at a wavelength of 2.940 μm with the exposure settings of 50 mJ, 10 Hz, using air/water spray. The tip was positioned at 1 mm from the enamel surface (focused mode) and the window was irradiated through scanning movements for 10 seconds. Then resin infiltration (ICON®) material was applied according to the manufacturer's instructions.

**Group (III)(88)**: After the creation of WSL, this group was designed to receive an Erbium laser followed by Varnish (enamelst® Fluoride varnish).

Er: YAG laser (Fotona, LightWalker ST Slovenia), the laser emitted photons at a wavelength of 2.940 μm with the exposure settings of 50 mJ, 10 Hz, using air/water spray. The tip was positioned at 1 mm from the enamel surface (focused mode) and the window was irradiated through scanning movements for 10 seconds. For the V (enamelst® Fluoride varnish), Varnish (ULTRADENT-USA) containing 5% sodium fluoride was applied on the surface by a specialised brush1-2mm thickness for 2 minutes.

**Group (IV):** After the creation of WSL, this group was designed to receive resin infiltration (ICON®), followed by varnish (enamelast®). Application of both materials based on manufacturer instruction as mentioned before.

**Measurement of enamel surface hardness (17-18):**

The interface of the microhardness test was designed to evaluate the hardness of microscopic/thin samples. Microhardness measurements were conducted for **all groups** after demineralization and the application of the treatments. Samples were individually and horizontally mounted on computer-controlled materials testing with a load cell of 300g for 15 seconds **(19)**. The load and time were constant for all samples throughout the study. Measurements after surface treatments of all groups were delayed for 24 hours to allow for a controlled setting that facilitates optimal conditions for enamel recovery and stabilization, this waiting period allows for the completion of any chemical processes, such as remineralization, that may influence the hardness readings. while the teeth were kept in an incubator in distilled water at 37 °C to mimic physiological conditions.

**Image analysis by Scanning Electron Microscope (SEM) (20):**

To determine the different roughness parameters, including average roughness (Ra) and maximum profile peak height (Rp), we performed image analysis on SEM images at a magnification of 1000X using ImageJ software (version 1.53a, National Institutes of Health, USA). The plot profile and surface plot were calculated from six images for each sample to ensure high accuracy. The results were then tabulated and statistically analyzed later.

**X-ray diffraction (XRD) assessment of the crystallinity of the samples (21):**

PANanalytical X-Ray Diffraction equipment model X'Pert PRO with Secondary Monochromator, Cu-radiation (l=1.542Å) at 45 K.V., 35 M.A. and scanning speed 0.04o/sec. were used. The diffraction peaks between 2q =2o and 6*0*o, corresponding spacing (d, Å) and relative intensities (I/Io) were obtained. The diffraction charts and relative intensities are obtained and compared with ICDD files.

**Field Emission Scanning Electron Microscopy (FESM) assessment of surface structure and morphology of crystals (21):**

-Samples were fixed on aluminium stubs with standard diameter using a carbon double sticky tape, Samples were coated with gold using sputter coater modal EMITECH K550X England, A Scanning electron microscope (SEM) (Model Quanta 250 FEG - made in Holland) was employed to investigate the morphological structures of the samples under operating conditions of accelerating voltage 20 K.V, resolution for Gun.1nm, and magnification Representative images of different samples were selected.

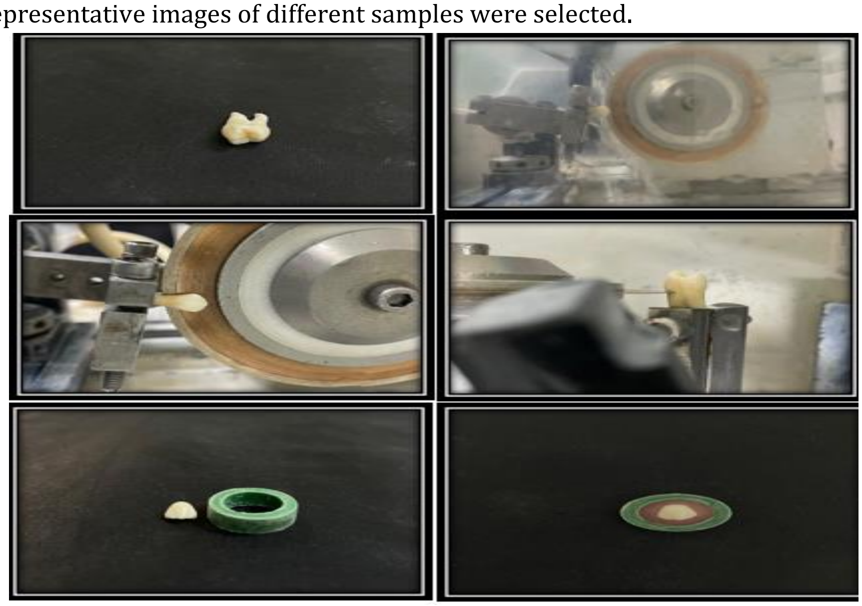


Figure (1): Samples preparation and mounting of teeth in acrylic resin blocks.

(A) (B)

Figure 2: A) Application of Erbium Laser over WSL, B) Application of force over the sample to measure SMH

**Statistical analysis:**

Statistical analysis for comparison between the three groups (Hardness results), and three stages (Roughness results) was performed by applying one-way ANOVA to compare data followed by a Post Hoc test (Tukey) for pairwise comparisons between different parameters. Statistical analysis for comparison between the two groups in roughness results was performed by applying the Independent T-test. Pearson's Correlation test was applied to study the correlation between microhardness results and roughness results, Pearson's correlation is a statistical test used to measure the strength and direction of a linear relationship between two variables. Its value always ranges between -1 (strong negative relationship) and +1 (strong positive relationship) and the test criteria can be classified according to the Likert scale as follows: $r = 0$ No correlation. $0.00 < r < 0.25$ Weak correlation. $0.25 \le r < 0.75$ Moderate correlation. $0.75 \le r < 1.00$ Strong correlation. $r = 1$ Complete correlation. $p \le 0.05$ was considered statistically significant (95% significance level), and $p \le 0.001$ was considered ‘; highly statistically significant (99% significance level). The Shapiro-Wilk test was used for testing the normality of data. Statistical evaluation was performed using the SPSS statistical package (version 25, IBM Co. USA).

**Results**

**I) Assessment of the Microhardness results (Vickers hardness test)**

**1. Basic descriptive statistics**

Table (1): Basic descriptive statistics of microhardness results for the four studied groups.

| | **Mean** | **SD** | **Min** | **Max** |
|---|---|---|---|---|
| **ICON®** | 246.96 | 68.51 | 115.92 | 321.00 |
| **Er: YAG Laser + ICON®** | 311.96 | 48.18 | 210.90 | 346.10 |
| **Er: YAG Laser + Varnish** | 154.36 | 45.42 | 78.00 | 214.50 |
| **ICON® + Varnish** | 250.00 | 62.02 | 121.72 | 327.05 |

**2. <u>Effect of various approaches in the treatment of white spot lesions on the microhardness results (Inter-group comparison)</u>**

The mean microhardness was 154.36 ± 45.42 in the Er: YAG Laser + Varnish group, 246.96 ± 68.51 in the ICON group, 311.96 ± 48.18 in the Er: YAG Laser + ICON group, and 250±62.02 in the ICON + Varnish group. The Er:

YAG Laser + ICON group achieved the highest mean microhardness, surpassing the ICON group, while both treatments showed higher mean microhardness compared to the control group.
According to the Tukey Post Hoc Test for pairwise comparisons between groups, there was a significant difference between the Er: YAG Laser + Varnish group and all other groups, as well as between Er: YAG Laser + ICON group and all other groups. However, there was no significant difference between the ICON and ICON + Varnish groups. The ANOVA test indicated that the overall p-value for inter-group comparisons was statistically highly significant, primarily due to the difference between the Er: YAG Laser + ICON group (which had the highest mean microhardness) and the Er: YAG Laser + Varnish group (which had the lowest mean microhardness).
Table (2): Mean ±SD, and inter-group comparison of microhardness results in the four studied groups.

| | **Mean±SD** |
|---|---|
| **Er: YAG Laser + Varnish** | 154.36±45.42**C** |
| **ICON** | 246.96±68.51**B** |
| **Er: YAG Laser + ICON** | 311.96±48.18**A** |
| **ICON + Varnish** | 250±62.02**B** |
| **P-value*** | **< 0.001HS** |

*- Capital letters for pairwise comparison between different groups (Tukey Post Hoc test) and the means with different superscripts are statistically significant different at P ≤ 0.05.*
*-* **Overall P-value** for Inter-group comparison between the four groups (ANOVA test).*
*- **S**= Statistically significant at P ≤ 0.05* *- **NS**= Non-significant P > 0.05.*
*- **HS** = Highly significant at P ≤ 0.001*

## II) Assessment of the average roughness (Ra) results (Image analysis of SEM images)

### 1. Basic descriptive statistics

Table (3): Basic descriptive data for all groups.

| **Group** | **Stage** | **Mean** | **SD** | **Minimum** | **Maximum** |
|---|---|---|---|---|---|
| **ICON®** | Baseline | 8.48 | 1.23 | 6.18 | 9.84 |
| | After Demin | 25.17 | 6.09 | 16.81 | 34.00 |
| | After Application | 13.85 | 1.75 | 11.89 | 16.49 |
| **Er: YAG Laser + ICON®** | Baseline | 8.48 | 1.23 | 6.18 | 9.84 |
| | After Demin | 25.17 | 6.09 | 16.81 | 34.00 |
| | After Application | 13.85 | 1.75 | 11.89 | 16.49 |
| **Er: YAG Laser + Varnish** | Baseline | 8.82 | 1.27 | 6.43 | 10.24 |
| | After Demin | 28.87 | 5.33 | 20.51 | 37.39 |
| | After Application | 14.41 | 1.82 | 12.37 | 17.15 |
| **ICON® + Varnish** | Baseline | 28.87 | 5.33 | 20.51 | 37.39 |
| | After Demin | 14.41 | 1.82 | 12.37 | 17.15 |
| | After Application | 12.16 | 2.20 | 10.38 | 15.12 |

**2. Change in average roughness (Ra) in different stages under the same major group (Intra-group comparison)**

**In the Er: YAG Laser + Varnish group**, the lowest mean Ra was recorded at baseline, with a value of 8.82 ± 1.27. This increased to 28.87 ± 5.33 after demineralization and then decreased to 14.41 ± 1.82 following ICON application.

**In the ICON group**, the lowest mean Ra was recorded at baseline, with a value of 7.93 ± 1.15. This increased to 15.24 ± 1.79 after demineralization and then decreased again to 14.41 ± 1.82 following varnish application.

**In the Er: YAG Laser + ICON group**, the lowest mean Ra was also observed at baseline, at 8.48 ± 1.23. After demineralization, it increased to 25.17 ± 6.09, and subsequently decreased to 13.85 ± 1.75 after ICON application.

**In the ICON + Varnish group**, the lowest mean Ra was recorded at baseline, at 6.62 ± 2.89. After demineralization, it increased to 17.07 ± 2.93, and subsequently decreased to 12.16 ± 2.20 after varnish application.

*For all groups*, the Tukey Post Hoc Test for pairwise comparisons between stages revealed significant differences among the three stages. The ANOVA test indicated that the overall p-value for intra-group comparisons was statistically highly significant, primarily due to the difference between the baseline stage (which had the lowest mean Ra) and the after-demineralization stage (which had the highest mean Ra).

Table (4): Mean ±SD, and intra-group comparison of average roughness (Ra) in the four studied groups at different stages.

| | **Baseline** | **After Demin** | **After Application** | **P-value**** |
|---|---|---|---|---|
| **Er: YAG Laser + Varnish** | 8.82±1.27**c** | 28.87±5.33**a** | 14.41±1.82**b** | **< 0.001HS** |
| **ICON** | 7.93±1.15**c** | 15.24±1.79**a** | 11.81±2.14**b** | **< 0.001HS** |
| **Er: YAG Laser + ICON** | 8.48±1.23**c** | 25.17±6.09**a** | 13.85±1.75**b** | **< 0.001HS** |
| **ICON + Varnish** | 6.62±2.89**c** | 17.07±2.93**a** | 12.16±2.2**b** | **< 0.001HS** |

*- Small letters for pairwise comparison between different stages (Tukey Post Hoc test) and the means with different superscripts are statistically significant different at P ≤ 0.05.*

*-* Overall P-value for Intra-group comparison between the three stages (ANOVA test).*

*- **S**= Statistically significant at P ≤ 0.05* *- **NS**= Non-significant P > 0.05.*

*- **HS** = Highly significant at P ≤ 0.001*

**3. Effect of various approaches in the treatment of white spot lesions on the average roughness (Ra) under the same stage (Inter-group comparison)**

**At Baseline**:

Although the Er: YAG Laser + Varnish group achieved the highest mean Ra, while the ICON + Varnish group had the lowest, there was no significant difference between the four groups at the baseline stage, and the overall p-value was not statistically significant (P-value = 0.179).

**After Demineralization**: The Er: YAG Laser + Varnish group recorded the highest mean Ra, while the ICON group achieved the lowest. According to the Tukey Post Hoc test, there was no significant difference between the Er: YAG Laser + Varnish and Er: YAG Laser + ICON groups, nor between the ICON and ICON + Varnish groups. However, there was a significant difference between the two groups treated with laser and the other two groups. The ANOVA test indicated that the overall p-value for inter-group comparisons was statistically highly significant, primarily due to the difference between the Er: YAG Laser + Varnish group (which had the highest mean Ra) and the ICON group (which had the lowest mean Ra).

**After Application**: Although the Er: YAG Laser + Varnish group achieved the highest mean Ra and the ICON group had the lowest, there was no significant difference between the four groups at this stage, and the overall p-value was not statistically significant (P-value = 0.064).

Table (5): Mean ±SD, and inter-group comparison of average roughness (Ra) in the four studied groups at different stages.

| | **Baseline** | **After Demin** | **After Application** |
|---|---|---|---|
| **Er: YAG Laser + Varnish** | 8.82±1.27**A** | 28.87±5.33**A** | 14.41±1.82**A** |
| **ICON** | 7.93±1.15**A** | 15.24±1.79**B** | 11.81±2.14**A** |
| **Er: YAG Laser + ICON** | 8.48±1.23**A** | 25.17±6.09**A** | 13.85±1.75**A** |
| **ICON + Varnish** | 6.62±2.89**A** | 17.07±2.93**B** | 12.16±2.2**A** |
| **P-value*** | **0.179NS** | **< 0.001HS** | **0.064NS** |

*- Capitall letters for pairwise comparison between different groups (Tukey Post Hoc test) and the means with different superscripts are statistically significant different at P ≤ 0.05.*

*-** Overall P-value for Inter-group comparison between the two groups (ANOVA test).*

*- **S**= Statistically significant at P ≤ 0.05 - **NS**= Non-significant P **>** 0.05.*

*- **HS** = Highly significant at P ≤ 0.001*

## III) Correlation between Microhardness results and roughness results.

### 1. Correlation between the Microhardness results and the average roughness (Ra) results

The results of the Pearson correlation test between image analysis results (Ra) and microhardness results in the three groups are summarized in Table 6. From the results, we can conclude the following:

- There was a weak positive correlation between Ra and microhardness results in the Er: YAG Laser + Varnish group, which was statistically non-significant (P-value = 0.197).
- There was a moderate positive correlation between Ra and microhardness results in the ICON group, and this correlation was statistically significant (P-value = 0.006).
- There was a moderate positive correlation between Ra and microhardness results in the Er: YAG Laser + ICON group, and this correlation was also statistically significant (P-value = 0.003).
- There was a moderate positive correlation between Ra and microhardness results in the ICON + Varnish group, and this correlation was statistically highly significant (P-value < 0.001).

Table (6): Correlation between average roughness results and Microhardness results in the four groups.

| | **r**** | **P-value** | **Correlation type** |
|---|---|---|---|
| **ICON** | **0.493** | **0.006S** | **Moderate Positive** |
| **Er: YAG Laser + ICON** | **0.518** | **0.003S** | **Moderate Positive** |
| **Er: YAG Laser + Varnish** | 0.238 | 0.197**NS** | **Weak Positive** |
| **ICON + Varnish** | **0.673** | **< 0.001HS** | **Moderate Positive** |

- ** Pearson Correlation value -S Significant (Correlation is significant at the 0.05 level). - NS Non-significant. HS Highly significant (Correlation is significant at the 0.001 level).

Table (6): SEM atmagnification 1000x and 30 μm of study groups samples after demeniralisation

After demineralization

ICON | Er: YAG Laser + ICON | Er: YAG Laser + Varnish | ICON + Varnish

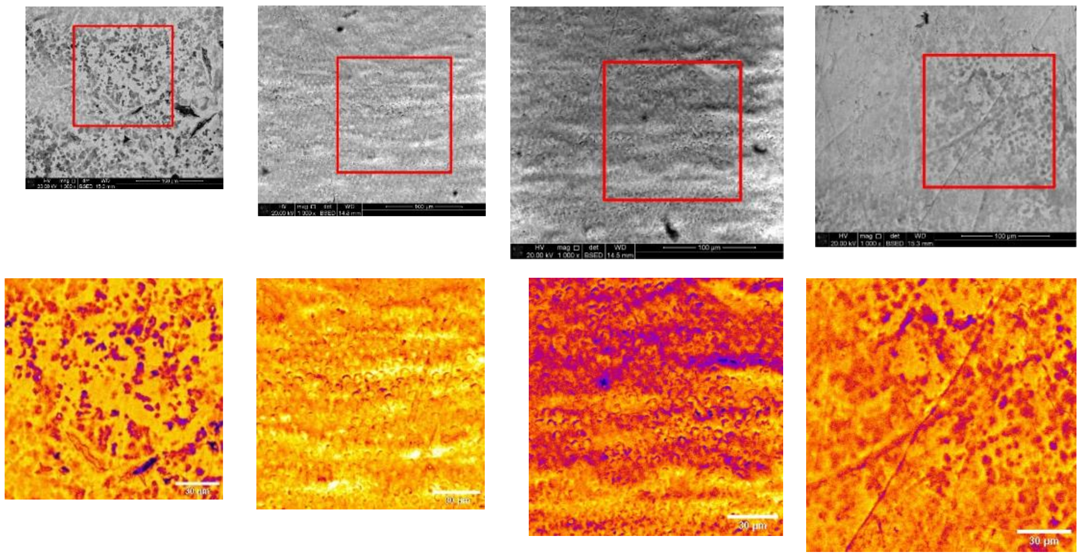


Table (7): profile plot and surface plot after demineralization

ICON | Er: YAG Laser + ICON | Er: YAG Laser + Varnish | ICON + Varnish

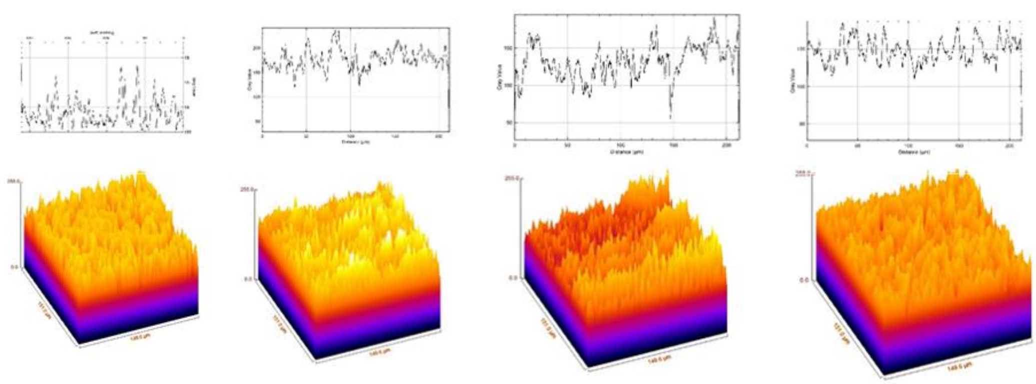


Table (10): SEM atmagnification 1000x and 30 µm of study groups samples after treatment .

After treatment

ICON | Er: YAG Laser + ICON | Er: YAG Laser + Varnish | ICON + Varnish

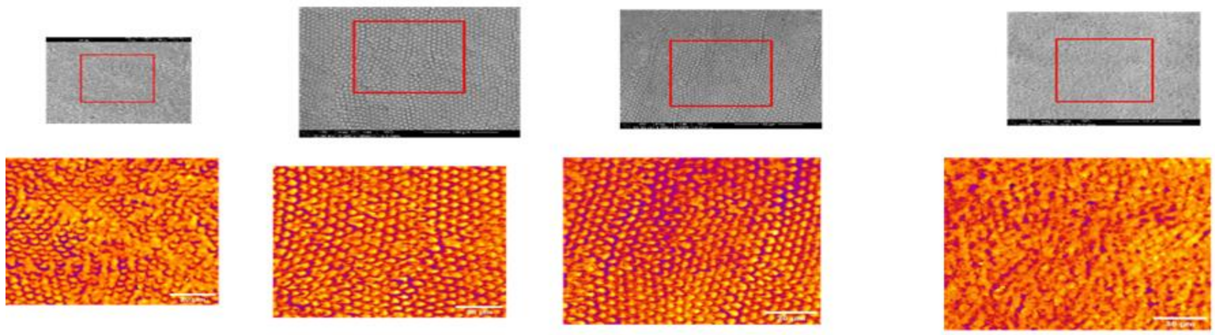
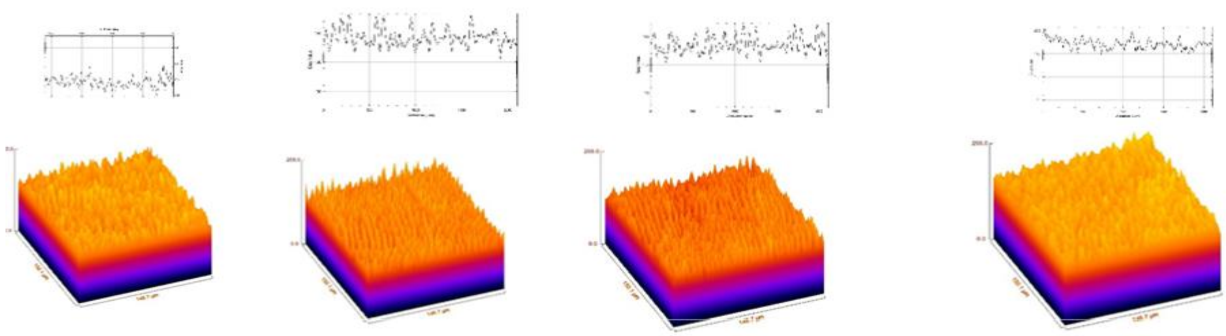
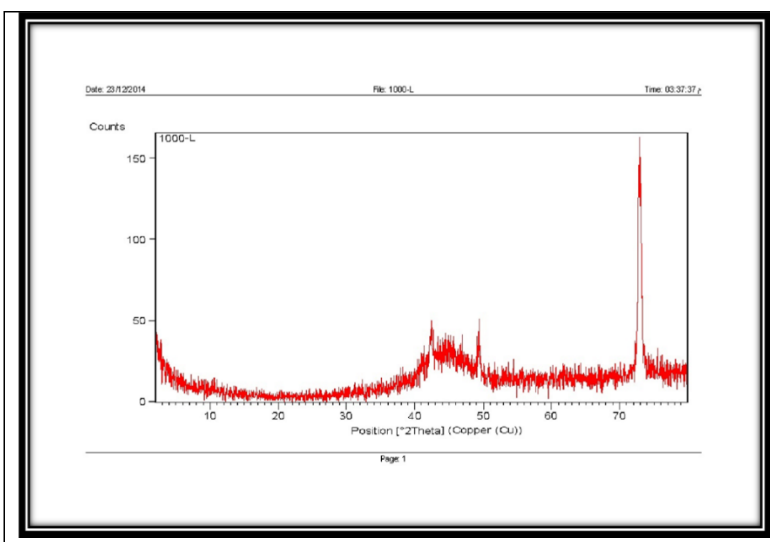


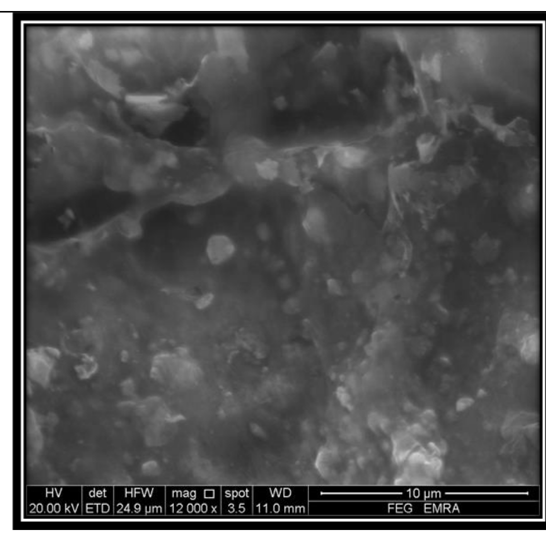
Table (11): profile plot and surface plot after treatment .

After treatment

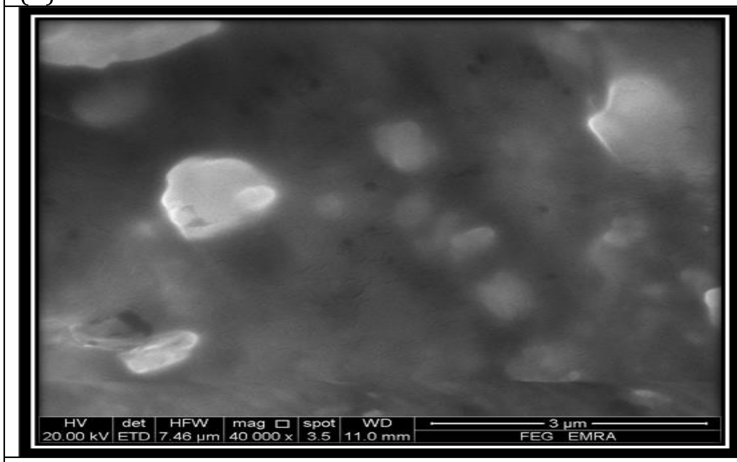
ICON | Er: YAG Laser + ICON | Er: YAG Laser + Varnish | ICON + Varnish



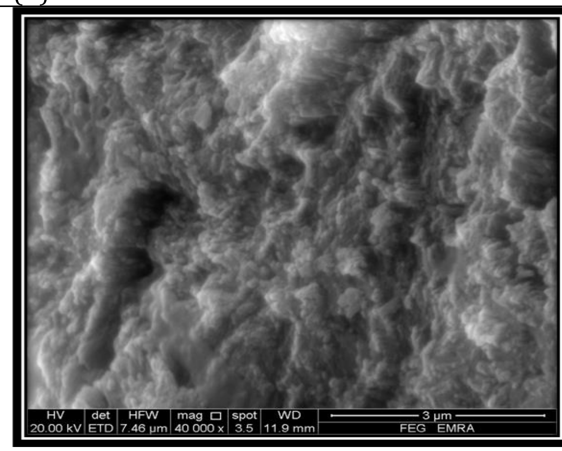


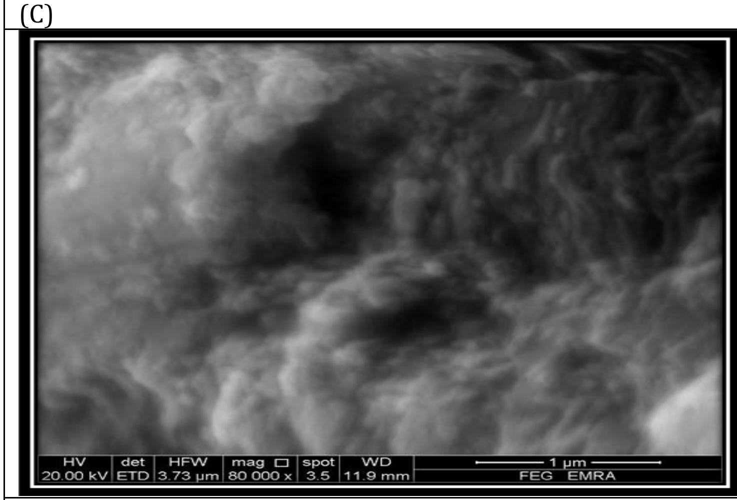
(A)




(B)




(C)




(D)

(E)

Figure 3: A) X-RD spectra of Er; YAG laser, B) FESEM images of crystal on enamel surface before treatment by Er; YAG LASER (12000X), C) FESEM images of crystal on enamel surface before treatment by Er; YAG LASER (40000X), D) FESEM images of crystal on enamel surface after treatment by Er; YAG LASER (40000x), E) FESEM images of crystal on enamel surface after treatment by Er; YAG LAS

**Discussion**

Enamel translucency is a characteristic related to the composition of the inter-crystalline space and can be quantitatively defined by the enamel refractive index (ERI) (22). Any clinical situation that alters the enamel organisation determines a variation of the ERI (1.62). The difference in refractive index between the healthy enamel and the demineralised area generates a lesion with a milky white opaque appearance, clearly distinguishable from the surrounding healthy enamel. (23)

This implies increased porosity within the lesion body (24). The most effective way of managing initial white spot lesions (WSL) with an intact surface is blocking the enamel porosities and remineralising the enamel with elevation of ca/p ions in saliva (22).

Additionally, a current cost-effectiveness analysis indicates that micro-invasive treatment of early WSL by infiltrating instead of postponing restorative treatment can improve oral health care as it lengthens the life cycle of a tooth (25), The surfaces of infiltrated lesions are described as protected enamel surfaces following appropriate polishing, in vitro (26-27). The description of an increase in mechanical strength after resin infiltration by other authors underlines these findings. (28,29)

“In our study, we aimed to treat the white spot lesion with a new approach and the latest techniques. Our goal is not only to treat the white spot lesion and enhance the mechanical aspects but also to promote the remineralisation of the teeth to ensure their long-term preservation.

Despite the growing utilisation of in vivo research data, such studies remain prohibitively expensive, challenging to execute, and subject to stringent ethical regulations. Consequently, this study was designed as an in vitro investigation to evaluate the effects of Erbium laser treatment in conjunction with ICON® material. The aim was to enhance the microhardness and surface roughness of white spot lesions (WSL) to their maximum levels.

This work makes use of forty intact premolars, which are typically extracted during orthodontic procedures to ensure that there are no barriers to experimentation. The teeth were cleaned using an ultrasonic scaler, disinfected with 1% sodium hypochlorite to lower the risk of infection, and preserved in saline to retain structural integrity and moisture. This sort of setup is essential for researching lesions known to be associated with bacterial activity in white spot lesions. In contrast, **Gouda et al. (18),** used fifteen intact third mandibular molars that were cleaned and preserved in a 10% formalin solution and saline.

Additionally, we aimed to create white spot lesions with a uniform depth, as variations in lesion depth could lead to differing results due to variations in laser penetration and, consequently, the depth of material infiltration. **MOOSAVI et al, (30),** In their vitro study, 30 human molars with inactive proximal lesions were selected.

In our study, the forty teeth were prepared by sectioning the crown horizontally to separate the coronal portion from the radicular portion of each tooth under water coolant using low speed double speed-faced diamond disc then the crowns were sectioned into two halves in a mesiodistal direction. A window of 4x4 mm was created on the center of sectioned teeth then the samples were mounted in acrylic resin. The resin helps to stabilise the sample, making it easier to handle and examine without damage. It’s also transparent, which allows for clear observation of the sample’s structure under a microscope. **Gouda et al. (18)**, employed the same technique.

In the current research, all specimens were immersed in a demineralizing solution for 96 hours in an incubator set to 37 °C in distilled water. As previously noted, the teeth were intact before immersion, which was intended to simulate natural (WSL) (19). In contrast, **AL-BLAIHED et al. (31),** investigated treatment options for WSL resulting from orthodontic procedures.

Based on the previous experiments, it is evident that lasers are applied in various methods to treat white spot lesions. Therefore, we choose to test ICON® and Varnish materials in combination with laser treatment, as there is a limited number of studies on this combination. ICON® and Varnish are considered one of the most used materials for treating white spot lesions.

Because ICON® is so good at managing caries in a minimally invasive way**, Group I** used it in our investigation. By stopping early carious lesions without drilling, the resin infiltration procedure preserves the aesthetics and structure of the tooth. ICON® improves enamel microhardness, it’s compatible with contemporary dental procedures that aim to treat carious lesions with efficacy while maintaining natural teeth.

In terms of microhardness, we found that the ICON® group is effective by sealing the tiny pores in the enamel, this resin maintains the structure and prevents additional caries development. ICON® may penetrate deeper into the material, making enamel smoother and harder. Additionally, methacrylate-based ingredients like Bis-GMA and TEG-DMA improve the material's overall strength and smoothness. As a key tool for caries control and remineralization, ICON® alone shows promise for enhancing the microhardness of treated lesions, but not as well as when used in conjunction with Er; YAG laser. According to **IBRAHIM et al. (14)**, the study states that the ICON® adhesive resin performed better in terms of microhardness than the All-Bond Universal adhesive resin, which showed lower microhardness because of insufficient penetration and polymerization. Regarding

ICON®'s efficacy in comparison to universal adhesive systems, the data refute the null hypothesis and support ICON® as a means of enhancing surface microhardness in lesions known as white spots.

On the other hand, The ICON® group also showed minimal roughness at baseline, increasing during demineralization, and then decreasing once more following the ICON® application. The enamel's surface is initially smooth, which leads to low roughness. Mineral loss in response to exposure to a demineralizing solution causes porosities to occur, which in turn compromises the integrity of the enamel and increases surface roughness.

ICON®, a resin infiltrant that fills in these porosities, is applied, and as the resin fills in the gaps, some surface smoothness is restored, improving the mechanical qualities of the enamel. The demineralization-induced roughness is successfully reduced by this technique, leaving the enamel surface smoother after treatment. Although the analysis of **ARNOLD et al (32)** reveals that early enamel caries lesions are usually smoothed by ICON® resin infiltration, several investigations have found no appreciable changes in roughness after therapy. First caries lesions have a pseudo-intact surface layer, which, if left untreated, can exacerbate roughness and encourage the buildup of plaque.

To examine the impact of combining the Er; YAG LASER in particular, the laser group was combined with ICON® in **Group II**.

According to earlier research, Er; YAG LASER performs better in surface ablation than Er, Cr: YSGG LASER, making it especially appropriate for enamel surfaces (90). This focus makes it possible to evaluate in-depth how the material qualities and surface features are impacted by the combination of Er; YAG LASER therapy and ICON®.

Hardness-wise, combining the Er: YAG laser with ICON® resulted in the hardest level of hardness. It is well known that the Er: YAG laser modifies the microstructural characteristics of dental enamel, hence increasing porosity and facilitating the penetration of resins. Because of the improved penetration, the low-viscosity ICON® resin can fill the demineralized areas more successfully, increasing the overall hardness of the treated enamel and encouraging higher resin infiltration.

Furthermore, the heat from the laser may cause modifications that improve the binding between the resin and enamel, which would raise the material's resistance to demineralization even more. Thus, this combination is a useful treatment for caries because it enhances the mechanical qualities of the enamel while also optimizing the remineralization process. On another note, it was found in a study by **MOOSAVI et al. (30),** concluded that the Er; YAG Laser therapy with ICON® resin infiltration combination produced the highest microhardness of all the groups assessed, demonstrating its efficacy in promoting enamel remineralization. Although microhardness was positively impacted by both treatments, the laser's conditioning effects seemed to maximize the resin's penetration and bonding, offering a synergistic advantage.

Regarding roughness, The Er: YAG laser in conjunction with the ICON® group displayed a comparable pattern, with roughness rising during demineralization and then declining following the ICON® application. The Er: YAG laser first efficiently prepares the enamel surface, causing microstructural alterations that could potentially result in a rougher surface after demineralization. This happens because the enamel's porosity may be increased by the laser therapy, increasing its vulnerability to mineral loss.

But after ICON® is applied, these porosities are thoroughly sealed up with resin infiltration producing a smooth surface. By reducing surface roughness after initial deterioration, this resin application lessens the roughness caused by

demineralization, proving that the combination of laser treatment and resin penetration can improve the mechanical characteristics and aesthetics of enamel. While **MOOSAVI et al, (30)**, they are only. looked at the effect of different materials evaluated exclusively from the perspective of microhardness, they noted that Pretreatment by Er; YAG laser before resin infiltration was more effective than other treatments in enhancing microhardness and protecting the tooth against the acidic challenge.

In **Group III,** the laser group was combined with Varnish to examine the effects of this combination. This technique is minimally invasive, which reduces discomfort and promotes quicker healing. The laser enhances the effectiveness of fluoride varnishes by creating microchannels for better penetration, while also reducing bacterial load in carious lesions to support remineralization. This approach allows for immediate assessment of treatment outcomes and improves patient compliance, particularly in children or anxious individuals, ultimately leading to better overall dental health.

Regarding microhardness, the Er: YAG Laser plus Varnish group showed the lowest levels of hardness. There could be several reasons for this decreased hardness. First off, the varnish covering might have created a barrier that prevents fluoride and minerals from penetrating, delaying the laser's planned improvement of the enamel structure. Furthermore, it's possible that the varnish's contact with the laser-treated surface did not promote efficient remineralization.

According to **HAWAS et al. (33)**, light-curable fluoride varnish (Clinpro XT) greatly increased the microhardness of enamel, confirming earlier findings about the protective properties of fluoride against demineralization. The study also found that adding fluoride to Er Cr: YSGG laser therapy increased enamel resistance; however, there was no discernible change between Er; YAG laser treatment and its fluoride mixture.

A comparison of average roughness at several treatment phases shows significant variations within each group. The group that received the Er: YAG laser and varnish had the highest baseline roughness, which increased dramatically after demineralisation and decreased again after varnish application. The group with the greatest roughness sample was still dressed because of the laser's action.

**MAHDI et al (34),** demonstrated that surface microhardness and roughness were greatly enhanced by laser treatment, resulting in microstructures that are favourable to fluoride retention. This suggests that in addition to conventional fluoride treatments, laser technology may be a useful therapeutic option.

In order to investigate the effects of this combination, which seals early carious lesions and provides a concentrated fluoride source that increases enamel hardness, **Group IV** combined the ICON® group with Varnish. By hiding lesions, this combination not only helps with appearance but also stops caries from progressing further. Due to their minimum invasiveness, both treatments are appropriate for patients with anxiety, particularly young ones. It is a comprehensive strategy for controlling early carious lesions and improving oral health because of the potential for enhanced fluoride penetration and overall efficacy resulting from the synergistic effects of ICON® and fluoride varnish.

We are developing this sample without any documented evidence of prior testing by others, indicating that this is a novel approach to our research. This underscores the originality of our work and highlights our commitment to exploring uncharted territory in this area of study.

From the perspective of hardness, the absence of significant differences between the ICON® plus Varnish group and the ICON® group indicates that ICON® alone effectively increases enamel microhardness. No significant changes were identified between the two groups. Although the varnish might offer more protection, it does not considerably increase hardness beyond what ICON® does. This suggests that, in this case, ICON® is sufficient for the elevation of microhardness. Furthermore, the main purpose of adding varnish was to act as a reservoir for the gradual release of fluoride, which could improve the long-term effectiveness of caries prevention without having an immediate effect on hardness readings.

Roughness in the ICON® plus varnish group had a similar pattern. It started low, increased during demineralization, and decreased after varnish application. First, by penetrating the enamel's microporosities, the ICON® treatment smoothes the surface. However, the enamel loses minerals when it is exposed to a demineralizing environment, which causes surface imperfections to emerge and enhance roughness. After that, the varnish is applied, which serves as a barrier of defence, adding more mineral content and improving surface smoothness by blocking any leftover pores and defects. This combination of results demonstrates how varnish can successfully correct some of the demineralization-induced roughness, preserving the integrity and visual appeal of the enamel.

At this point, the SEM generated three different kinds of data: a surface plot, a profile plot, and an image analysis at 1000X magnification. As seen in Figure (), each of these data kinds provides unique insights into the surface properties under investigation.

The correlation between surface roughness and microhardness can be supported by taking into consideration the mineral composition and structural integrity of the enamel. Microhardness, which is frequently associated with the mineral content and arrangement of enamel, measures the material's resistance to wear and deformation. An increase in surface roughness could be a sign of microstructural alterations or flaws that could reduce the overall hardness of the enamel.

In particular, demineralization processes may result in rougher surfaces because of a loss of mineral content that reduces hardness. On the other hand, processes that increase microhardness, such as laser applications with resin infiltration, can enhance surface structure by decreasing roughness and filling microporosities.

Therefore, a positive relationship between decreased surface roughness and microhardness indicates that efficient treatments not only increase hardness but further support the development of a harder, smoother enamel surface. This relationship emphasizes how crucial it is to include both mechanical characteristics when evaluating the success of dental procedures meant to maintain and improve the health of enamel.

In our study, The XRD (X-ray diffraction) analysis reveals distinct differences in the peak intensity and characteristics between the control group and the laser-treated group. In the control group, the peaks may indicate a standard crystalline structure of enamel, with lower intensity suggesting less crystallinity and stability.

Conversely, the laser-treated group shows sharper and more pronounced peaks, indicating an increase in crystallinity and changes in the crystal structure due to the laser application. This enhancement in peak intensity signifies that the laser treatment has led to a more ordered and stable crystalline arrangement within the enamel, which can contribute to improved mechanical properties and acid resistance. Overall, the differences in peak patterns highlight the effectiveness of the laser treatment in modifying the crystallographic characteristics of the enamel compared to the untreated control.

**YANG et al. (21)**, In their study, the findings that XRD analysis indicates that erbium laser etching significantly improves the crystallinity of enamel, with sharper peaks and higher intensity in the Er; YAG group compared to the Er, Cr group. This enhancement correlates with increased levels of Cl, PO43−, and higher Ca/P ratios, contributing to greater structural stability and reduced acid solubility of the enamel. The study highlights the effectiveness of Er; YAG LASER in modifying enamel properties and notes inconsistencies in Ca/P findings across different studies, suggesting further research is needed.

Finally in our study, the analysis of FESEM images following laser treatment reveals significant alterations in the surface morphology of enamel. The erbium laser application appears to enhance the roughness and texture, creating distinct features such as micro-cracks and larger crystal structures. These morphological changes suggest an effective reshaping of the enamel surface, which may contribute to improved acid resistance. The increased surface roughness observed in the FESEM images indicates that the laser treatment may facilitate better retention of dental materials or sealants, enhancing their efficacy. Overall, the FESEM imaging underscores the transformative impact of laser treatment on enamel, highlighting its potential benefits in dental care.

**YANG et al. (21)**, report that the FESEM results indicate that erbium laser treatment significantly enhances the surface morphology of enamel. Specifically, after laser etching, there is an observable increase in the size and crystallinity of enamel crystals, leading to improved structural stability and reduced solubility in acid. The treatment reduces the presence of carbonate and magnesium while increasing the levels of elements such as calcium, phosphorus, and chlorine, contributing to enhanced acid resistance. The increased peak height and sharper diffraction patterns in XRD further support the findings from FESEM, showing higher crystallinity in the enamel structure post-treatment. Overall, these morphological changes underscore the effectiveness of erbium laser treatment in reinforcing enamel's chemical stability and resistance to acid erosion.

## Conclusion

**From the results of this study, it was concluded that:**

This study shows that a variety of materials can be used to treat WSL, both alone and in conjunction with an erbium laser. The use of an ICON® resin infiltration in addition to an erbium laser treatment is very beneficial in treating WSL. The microhardness and surface roughness results show significant improvements, indicating that the laser improves resin infiltration and favourably modifies the enamel structure. The surface roughness results are similar to the group sample using ICON® alone, indicating that the surface roughness final result is based on using ICONE®. Although Erbium Laser treatment with varnish did not significantly increase microhardness, But when combined with ICON® improved appearance. which, when considering surface roughness, seems to be the best category.

The results support the application of minimally invasive dental procedures since they improve enamel's mechanical qualities and encourage the long-term maintenance of dental health. This innovative method may have therapeutic ramifications and open the door to more effective approaches to the management of early carious lesions. The long-term efficacy of these treatments and their suitability in various therapeutic contexts should be investigated in more detail

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