Energy-dispersive X-ray Spectroscopy Analysis of Erbium, Chromium:Yttrium-scandium-gallium-garnet-treated Enamel Surfaces: An In Vitro Study

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ABSTRACT

Aim and objective: The present study evaluated the effects of erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser on the weight percentage of mineral content of enamel when etched at three different power settings.

Materials and methods: Total 20 extracted molar teeth were taken as samples. Enamel slabs were prepared by sectioning the crown from the buccal and lingual aspect with a double-sided diamond disk at slow speed. The 40 specimens were divided into four groups, i.e., control, 1 W, 2 W, and 3 W of 10 specimens each and then irradiation by Er,Cr:YSGG was done. The elements evaluated were calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorus (P). The mean weights of these minerals and the Ca/P ratio in each slab were measured by energy-dispersive X-ray spectroscopy analysis (EDAX). One-way analysis of variance (ANOVA) followed by the Turkey’s test was performed with the help of critical difference (CD) or least significant difference (LSD) at 5 and 1% level of significance.

Results: There was no significant differences among the four groups for the five minerals and for the calcium-phosphorus ratio (p > 0.05). Photomicrographs by scanning electron microscopy observations revealed that the surfaces exposed to a 3 watt irradiation showed more roughness than those of the 1 watt and 2 watt groups.

Conclusion: The Er,Cr:YSGG irradiation of enamel at 1 W, 2 W, and 3 W had no significant effect on the mean percentage weights of Ca, K, Mg, Na, and P or the Ca/P ratio in any group.

Clinical significance: Constriction with conviction is the new motto of restorative dentistry. Er,Cr:YSGG not only fulfills the aim but also is proving to alter the surface properties by recrystallization and change in composition making the prepared surface caries resistant.

Keywords: Calcium:phosphorus ratio, Chromium:yttrium-scandium-gallium-garnet, Energy-dispersive X-ray spectroscopy, Erbium, Laser etching.

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INTRODUCTION

Every part of the human body is a divine creation of God and cannot be replaced by the best possible artificial material. Over 100 years ago, Dr Greene Vardiman Black laid down the basic tenets for operative dentistry. He first described the concept of “Extension for prevention” in 1981 in an article entitled “The management of enamel margins.” The introduction and advances in the field of adhesive dentistry, development of new restorative materials, and a better understanding of the caries process and tooth’s potential for remineralization have led to a paradigm shift from Dr Black’s concept of “Extension for prevention” to a modern concept of “Constriction with conviction.” Since then, there has been a continuous quest to achieve the ideal dental substrate for the purpose of bonding.1–3

Michael Buonocore laid the foundation of intraoral adhesion in 1955 by using 85% phosphoric acid to etch enamel. Phosphoric acid (37%) has remained the material of choice because of the clinically validated predictable and reliable bonding achieved. Phosphoric acid acts on the enamel by selectively dissolving the hydroxyapatite of the prisms, making the enamel surface more permeable and prone to the long-term acid attack and caries.4–8

Hence, various researchers have been looking for an alternative treatment of enamel surfaces instead of acid etching or in conjunction with acid etching. Currently, laser etching is proving to become an alternative to acid etching.9

Erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser with a wavelength of 2.78 µm was cleared by the FDA in October 1998 for caries removal, cavity preparation, and conditioning of the tooth. The enamel consists of 85% by volume carbonated hydroxyapatite with 12% water, 3% protein, and lipids. The mineral matrix of the enamel is composed of a carbonated hydroxyapatite [Ca10(PO4)6(OH)2] predominantly composing of calcium and phosphorus. The wavelength (2,780 nm) is highly absorbed by water and hydroxyapatite together. It generates thermal changes in the enamel, which alters the enamel morphologically and chemically hence affecting its adhesion to the dental materials.10–13

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The Er,Cr:YSGG laser etching causes no pain and vibration, it provides a disinfected surface, tooth isolation is not required and it gives more control over the area that needs to be precisely etched. The laser-etched surface has been proved to be acid-resistant, thereby reducing the susceptibility to secondary caries risk. This acid resistance is attributed to formation of pyrophosphate and β-tricalcium phosphate, which shows reduced solubility.14,15

The mineral structure of enamel in this study has been measured by energy-dispersive X-ray spectroscopy analysis (EDAX). Energy-dispersive X-ray spectroscopy analysis is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analyzing X-rays emitted by the matter in response to being hit with charged particles. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an element’s atomic structure to be identified uniquely from one another. This technique is used in conjunction with chemical microanalysis by scanning electron microscopy (SEM).16

The present study evaluated the effects of Er,Cr:YSGG laser preparation on the mineral content of enamel by the EDAX analysis and the surface irregularities were compared based on the SEM photomicrographs interpretation.

**Materials and Methods**

Twenty nondiseased molars that were extracted due to poor periodontal support were stored in distilled water after cleaning. To prepare the enamel slabs, buccal and lingual surfaces of the crowns were cut with a double-sided diamond disk at slow speed. Thus, a total of 40 enamel slabs were obtained, which were then divided into four groups: group I—control, group II—irradiated at 1 W, group III—irradiated at 2 W, and group IV—irradiated at 3 W. Each group had 10 samples and no treatment was given to control group specimens (Fig. 1A).

The Er,Cr:YSGG laser (Waterlase, Germany) (Fig. 1B) operating at a wavelength of 2.8 μm, fixed repetition rate of 20 Hz and 140 μs pulse duration, coupled with an adjustable air-water spray was used with the following settings—power of 1 W (group II), 2 W (group III), and 3 W (group IV), water 80%, and air 90%. The fiber-optic system delivered laser energy to a sapphire tip of 6 mm length and 600 μm diameter (Fig. 1C). The beam was aligned perpendicularly at 1 mm distance and was moved in a sweeping fashion over the specimens for 15 seconds to achieve an even coverage of the surface by overlapping the laser impacts.

The mineral content (Na, Mg, P, K, and Ca) of the specimens in this study was measured by EDAX equipment, Bose Institute, Kolkata (Fig. 1D), for control group and groups II–IV after laser treatment. In all the groups and for each sample, measurements for each element were done three times and the mean was calculated. The level of the five elements in each specimen was measured. The mineral content was then calculated as weight percentage. The difference in the mineral content was calculated between groups. The specimens were sputter coated with gold and one specimen from each group was observed under SEM at 1000×, 1300×, 2000×, and 5000× magnification and photomicrographs were taken. The weight% of Mg, P, Ca, K, and Na, the Ca/P ratio, and the irregularities of the surfaces were studied.

The means and standard deviation of the weight percentage of the five elements (Na, Mg, P, K, and Ca) and the Ca/P ratio were calculated and the differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by the Turkey’s test with the help of critical difference (CD) or least significant difference (LSD) at 5% and 1% level of significance. p ≤ 0.05 was taken to be statistically significant.

The statistical analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Center for Disease Control and Prevention (CDC).

**Results**

The mean weight% of the elements Na, Mg, P, K, and Ca and the Ca/P ratio in the enamel in each of the 10 samples in each group are presented in Table 1 and Figure 2.

In the study, the atomic analysis of the enamel samples showed a similar content of each element in all the groups. The mean percentage weight of calcium increases from group I to IV whereas the mean percentage weight of phosphorus decreases from group I to IV. The Ca/P ratio of group III is slightly higher but it is not statistically significant (Table 1 and Fig. 2). The results of one-way ANOVA have no significant differences (p > 0.05) in the Na, Mg, P, K, Ca content and the Ca/P ratio in different groups. As per the CD analysis, there were no significant differences between the mean values of Na, Mg, P, K, Ca content and the Ca/P ratio of different groups (p > 0.05).

**Table 1**: Mean percentage weights of the five elements (mean ± standard deviation) and the Ca/P ratio for each group (n = 10)

| Elements | Group E1 (1 W) | Group E2 (2 W) | Group E3 (3 W) | Group E4 (3 W) |
|----------|----------------|----------------|----------------|----------------|
| Na       | 1.06 ± 0.16    | 0.86 ± 0.12    | 0.90 ± 0.11    | 0.96 ± 0.09    |
| Mg       | 0.35 ± 0.05    | 0.35 ± 0.03    | 0.40 ± 0.06    | 0.40 ± 0.06    |
| P        | 30.16 ± 1.13   | 30.09 ± 0.86   | 29.88 ± 1.32   | 29.86 ± 0.55   |
| K        | 0.36 ± 0.03    | 0.36 ± 0.04    | 0.39 ± 0.03    | 0.35 ± 0.04    |
| Ca       | 68.09 ± 1.31   | 68.34 ± 1.01   | 68.42 ± 1.49   | 68.43 ± 0.69   |
| Ca/P     | 2.30 ± 0.14    | 2.30 ± 0.10    | 2.35 ± 0.16    | 2.30 ± 0.07    |
Energy-dispersive X-ray Spectroscopy Analysis of Er,Cr:YSGG-treated Enamel Surfaces

The SEM photomicrographs observations revealed that the surface treated with a 3 W power setting seems to be rougher than those of the 1 W and 2 W groups (Fig. 3). The smear layer was not completely removed on enamel specimens irradiated by 1 W (Fig. 3). In the study, SEM photomicrographs of the irradiated enamel etched at 1 W, 2 W, and 3 W power setting showed a predominant Silverstone’s type II etching pattern (Fig. 3). None of the specimens showed carbonization or melting of the enamel surfaces after irradiation.

**DISCUSSION**

In the present study, the changes in the mineral content of enamel after Er,Cr:YSGG laser preparation were evaluated by EDAX and the surface irregularities were compared based on the SEM photomicrographs interpretation (Fig. 3).

The EDAX is a powerful tool to analyze the mineral content in hard tissues. It is a technique of microanalysis of elements associated to electron microscopy, which is based on the generation of characteristic X-rays that reveal the presence of elements present...
in the specimens. This analysis in the present study was done at Bose Institute Kolkata (Fig. 1D) for the elements Na, Mg, P, K, Ca and the Ca:P ratio.16

The Er,Cr:YSGG laser was used in this study as it can effectively and efficiently interact with enamel for the purpose of etching. The power setting of 1 W, 2 W, and 3 W was used so as to avoid any cavity formation. Higher air to water percentage (90% air and 80% water) was used as it reduces the thermal effect of laser and increases the cleaning and cooling of the substrate.6,17

In the present study, increase in the quantities of calcium (Ca weight%) and decrease in the quantities of phosphorus (P weight%) were seen with an increase in the power setting but the results were statistically insignificant. A similar study done by Secilmis et al. in which enamel specimens from premolar were used showed the same results and the groups (1 W, 2 W, and control) showed no significant differences for Ca, K, Mg, Na, P and the Ca/P ratio (p > 0.05). In the study, the elemental analysis was performed by inductively coupled plasma-atomic emission spectrometry (ICP-AES).6

In a study by Guang et al., the atomic analysis showed no significant difference between the ratio of calcium and phosphorous between lased and unlased specimens. The study also showed that the surface after laser treatment exhibited regular holes having sharp edges, no melting, and carbonization. The observations and results of the above study concur well with the present study in which no samples showed melting of the enamel on exposure to Er,Cr:YSGG (Fig. 3).17

Olivi et al. in his study evaluated the type of etching pattern after exposure to Er,Cr:YSGG and most of the specimens either showed a type II or type III etching pattern. In the present study, the SEM micrographs revealed a predominant type III etching pattern. The differences can be correlated to the inclination of the laser beam and to the ratio of air to water.18

In a recent study by Firdevs Kahecioglu et al. with Er:YAG laser on primary tooth enamel, the Ca, P, F, Mg, K, and Na mean percentage weight was analyzed. The mean weight of Ca, P, and F in the enamel exhibited significant change (p < 0.05) and the wt% of Mg, K, and Na remained unchanged. The results differ from our study, which can be attributed to different Erbium group laser and selection of primary enamel specimens. There was no association of different power setting of laser and changes in wt% of minerals in the enamel in both our study (Table 1 and Fig. 2) and study by Kahveciglu et al.19

Hossain et al. evaluated the compositional changes using SEM/EDAX on the cavity floor. The results showed higher Ca wt% and higher P wt%, which is not in accordance with our study. The difference may be because of the cavity floor been in dentin and in our study the substrate was enamel. The changes in the Ca wt% are thought to result from some componential changes of atoms in the dental hard tissue or by the evaporation of the organic components as a result of laser irradiation. Increased calcium concentrations may affect the permeability, solubility, or adhesive characteristics of dental hard tissues with the restorative procedure. Although same results in relation to the Ca:P ratio were seen, i.e., no significant difference.20

Ana et al. in their studies on Erbium group of laser showed no caries preventive effect of laser on enamel cavities after demineralization, which implies that overall there are no such changes in the content of caries protective minerals like Ca, P and the Ca:P ratio, which even the present study shows.21

The views of many authors are conflicting in relation to the effects of cracks on bonding. Studies conducted by Bor Shunn Lee et al. and Torun et al. showed that occasional cracks enhance retention and are ideal for resin penetration in opposition to the studies by MV Cardoso et al., who showed that microcracks result in weakening of the substrate, thereby decreasing the bonding quality. But the present study showed no such microcracks on irradiation.22,23

The surface roughness was not measured, but by SEM interpretation, the surface treated with laser irradiation at 3 W seems to be rougher than those of the 1 W and 2 W groups. The views of different authors on the implications of increase surface roughness are contradictory. According to Yazici et al. and Hossain et al., higher surface roughness leads to better bonding while Cardoso et al. viewed the increased irregularities as a negative factor that results in zones of defective bond formation.20,24–26

Further correlations among power settings, mineral content, surface irregularities, and roughness need to be investigated. The limitations of the studies are lack of baseline evaluation of all specimens before laser therapy, no measurement of surface roughness, and absence of in vitro and in vivo correlations.

**CONCLUSION**

In conclusion, the present study showed no significant differences in the weight percentage of Ca, K, Mg, Na, and P after irradiation of enamel by Er,Cr:YSGG at 1 W, 2 W, and 3 W. The SEM micrographs showed predominant Type III etching pattern and an increase in surface roughness, which will aid in better bonding to the substrate. However, future in vitro with more parameters and in vivo studies are recommended to assess the caries preventive effect of lasers, which will be a boon for a clinical scenario.

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