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COMPARING THE EFFECT OF SYSTEMP ON THE ULTRA STRUCTURE AND PERMEABILITY OF ENAMEL FOLLOWING TWO DIFFERENT TECHNIQUES OF ENAMEL ETCHING. *AN IN VITRO STUDY*

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ABSTRACT

Background: Acid etching results in an unintentional demineralization of the enamel surface, while laser conditioning might provide microspaces which then remineralize by trapping free ions; thus enhancing resistance to caries. Since, Systemp desensitizer was very successful in reducing the incidence of post-operative pain; consequently, this in-vitro study was designed to evaluate the effect of Systemp on the surface micro roughness and permeability of enamel following two different techniques of enamel etching; acid and laser etching; in premolar teeth.

Methodology: Fifty freshly extracted non-carious premolar teeth were used. The teeth were divided into 5 groups each containing 10 teeth. Group I: control group, group II: teeth exposed to phosphoric acid etching. Group III: teeth exposed to low level laser (LLL) etching. Group IV: Systemp applied to acid etched enamel. Group V: Systemp applied to teeth etched by LLL. The extent of dye penetration was measured using stereo-microscopy and the obtained data were statistically analyzed. The surfaces of the specimens were examined using scanning electron microscopy.

Results: scanning electron microscopic (SEM) examination of group II revealed an obviously porous enamel surface with type III enamel etching pattern. On the other hand, following laser etching, enamel surface showed areas of non removed prismless enamel while most enamel presented type I enamel etching pattern. Systemp application rendered the surface more homogenous especially in the acid etched group. Statistical analysis revealed that the distance travelled by the dye was significantly greater in group II (acid etched group) (mean±SD=1,406.06±0.721), than group III (laser etched group) (mean±SD=1,235.35±0.771). However, group IV (Systemp applied to acid etched enamel) (mean±SD=1,078.47±0.634) showed significant reduction than group V (Systemp applied to LLL etched enaamel) (mean±SD=1,198.44±0.583) as the p-value was less than 0.05.

Conclusion and recommendations: despite laser advantages, laser-etching applications should be improved. Besides, desensitizing agents, like Systemp, could be efficient in sealing etched enamel surface, which could reduce sensitivity resulting from leakage through enamel into the underlying dentin.

KEY WORDS: phosphoric acid etching, laser etching, Systemp, dye penetration, SEM.

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INTRODUCTION

Since 1955, when Buonocore introduced phosphoric acid etching for enamel conditioning, the etching methods have changed considerably and new techniques have evolved. Conventionally, 37% phosphoric acid conditioning for 15 to 60 seconds was the standard procedure with no significant reduction of bond strength [1]. However, acid etching results in an accidental demineralization of the enamel surface [2], a permanent loss of about 10 μm of the mineralized surface and a possible irritation of the adjacent soft tissues [3].

The use of laser in dentistry was first described at 1964[4]. The purpose was to modify the enamel surface and increase its resistance against caries[5]. A laser conditioned enamel surface has altered calcium to phosphorus and carbonate to phosphorus ratios[6]. The percentage of water and organic substances is also reduced [5] which leads to a less acid-soluble enamel surface and hence, caries resistant one [6]. Additionally, laser conditioning might provide microspaces which in turn remineralize by trapping free ions which ultimately enhances resistance to caries [5]. Surface roughness after laser etching is reported to be similar or lower than with conventional acid etching [7&8].

Systemp desensitizer is a protein precipitate type desensitizer based on the Syntac System. It was very successful clinically and its success rate in reducing the incidence of post-operative pain was exceptionally high [9]. Although the exact mechanism of action of desensitizers is still not fully understood, yet, currently used agents probably act by blocking the dentinal tubules through coating, or through coagulation which alters the tubular content, protein precipitation or production of insoluble calcium complexes, or by direct effect on sensory nerves [10]. According to its manufacturer, the polyethylene glycol dimethacrylate in Systemp desensitizer triggers the precipitation of plasma proteins in the dentinal tubules. On the other hand, glutaraldehyde which is the other component of Systemp desensitizer is a cross linking reagent capable of bonding to amine groups of proteins. It was suggested by that glutaraldehyde is responsible for the occlusion of the tubules due to its effect on the serum proteins in the dentinal fluid as this fixative might precipitate plasma proteins from the dentin tubular liquid through coagulation inside the tubules [11, 12].

Consequently, this in-vitro study was designed to evaluate the effect of Systemp on the surface micro roughness and permeability of enamel following two different techniques of enamel etching; acid and laser etching; in premolar teeth.

MATERIALS AND METHODS

Fifty freshly extracted non-carious premolar teeth were used. The teeth were extracted for orthodontic reasons at the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University. The teeth were stored in saline solution at room temperature until experimental procedures. The teeth were randomly divided into 5 groups each containing 10 teeth: Group I: control group to examine the normal enamel surface, Group II: teeth exposed to phosphoric acid etching, Group III: teeth exposed to low level laser (LLL) etching, Group IV: Systemp applied to phosphoric acid etched teeth and Group V: Systemp applied to LLL etched teeth. The materials, description and application techniques used in this study were listed in **table 1.**

Methods of Investigation:

Evaluation of Dye penetration:

All teeth were embedded in acrylic resin blocks, all crown surfaces were covered with two coats of nail polish except for a cervical window on the buccal surfaces measuring 3x3 mm. Teeth were prepared for dye penetration by extra coverage of crowns and roots by blue inlay wax. Then, the teeth were immersed in methylene blue dye solution

TABLE (1) Description and application techniques for the materials used in the current study.

Material	Type	Description	Application
Phosphoric acid	Etching agent	Meta etchant 37 % phosphoric acid semi gel 3gm	Applied by syringe for 30 seconds
Low level diode laser	Etching agent	Wave length 808 nm Power 0.2 watt	Exposure time 2 minutes
Systemp	Desensitizing agent	Polyethylene glycol dimethacrylate 35.0 Maleic acid < 0.01 Glutaraldehyde (50 %) 10.0 Water 55.0	Thin film spread to the surface by brushing for 10 seconds then dried by air syringe.

for 24 hours [13]. Afterwards, the teeth were rinsed under running tap water and then dried. Finally, they were sectioned buccolingually **(Fig. 1 A&B)** into two halves using a low speed saw (Mecatome T201A, Presi, Grenoble, France). All sections were evaluated for dye penetration with a stereo-microscope (Olympus SZ40, Japan) at x200 magnification. The extent of dye penetration was measured from the surface of enamel inwards in an almost horizontal plane **(Fig. 1 C)**.

Scanning electron microscopic examination

The specimens were left to dry in air at room temperature for 3 days then mounted on scanning electron microscope specimen holder and studied with QUANTAFEG 250 SEM (Field Emission Gun) (with accelerating voltage 30 K.V., magnification14x up to 1000000) ***(FEI company, Netherlands)***

Statistical analysis:

The obtained data from stereo-microscopic images for dye penetration were statistically described in terms of mean ± standard deviation (± SD) **(Table 2)**. Comparison between the five studied groups was done using one way analysis of variance (ANOVA) test with post hoc multiple 2-group comparisons, p values less than 0.05 were considered statistically significant. All statistical

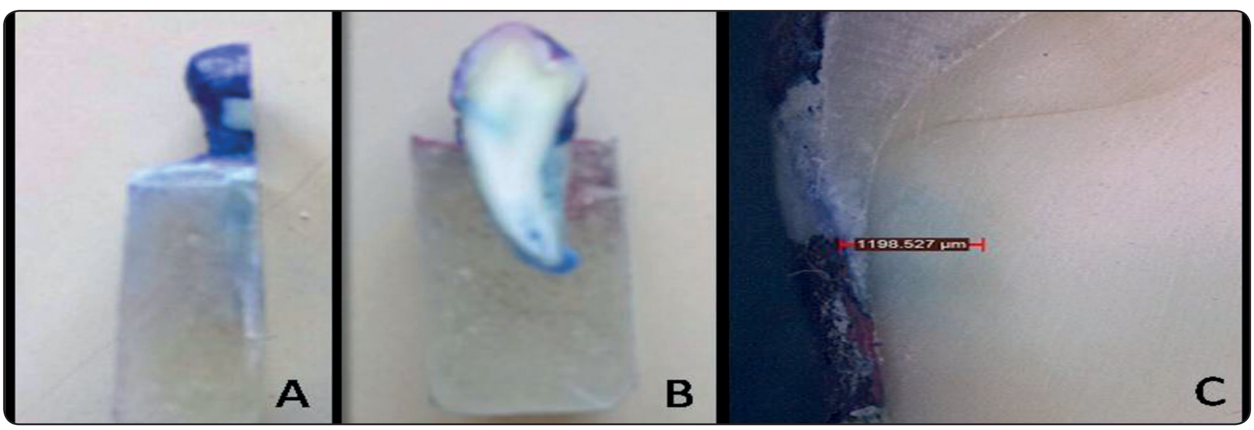

Fig. (1) (A) sectioned tooth in acrylic block covered with blue inlay wax showing buccal window (B) buccolingual sectioning of the tooth for stereo-microscopic imaging (C) A stereo-microscopic image showing the extent of dye penetration through the buccal window (orig. magnification x 200).

calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

TABLE (2) Mean ± standard deviation for the dye penetration results from the five studied groups.

Group	Mean	SD	P value*
I	1,044.00	0.672	0.000
II	1,406.06	0.721	
III	1,235.35	0.771	
IV	1,078.47	0.634	
V	1,198.44	0.583	

***** *The mean difference is significant at the 0.05 level.***

RESULTS

Dye penetration results:

The distance penetrated by the dye was maximum in group II (acid etched enamel) (mean ± SD = 1,406.06 ± 0.721), followed by group III (LLL etched enamel) (mean ± SD = 1,235.35 ± 0.771), group V (Systemp applied to LLL etched enamel) (mean ± SD = 1,198.44 ± 0.583), group IV (Systemp applied to acid etched enamel) (mean ± SD=1,078.47±0.634) and was least in group I (unetched enamel) (mean±SD=1,044.00±0.672).

Scanning electron microscopic (SEM) results:

Group I:

Examination of the normal enamel surface revealed a generally smooth surface with series of transverse wave-like depressions (perikymata) and circular pits corresponding to the Tomes' processes of ameloblasts (Figs. 2 A&B).

Group II:

Scanning electron microscopic examination of the acid-etched enamel surface revealed an obviously porous enamel surface which presented type III enamel etching pattern (mixed etching pattern). Areas of preferential removal of prisms and interprismatic regions could be noticed (Figs. 3 A&B).

Group III:

When the enamel surface was examined by scanning electron microscope after laser application, it showed some areas of prismless enamel which were not removed (fig. 4A) while most enamel presented type I enamel etching pattern with

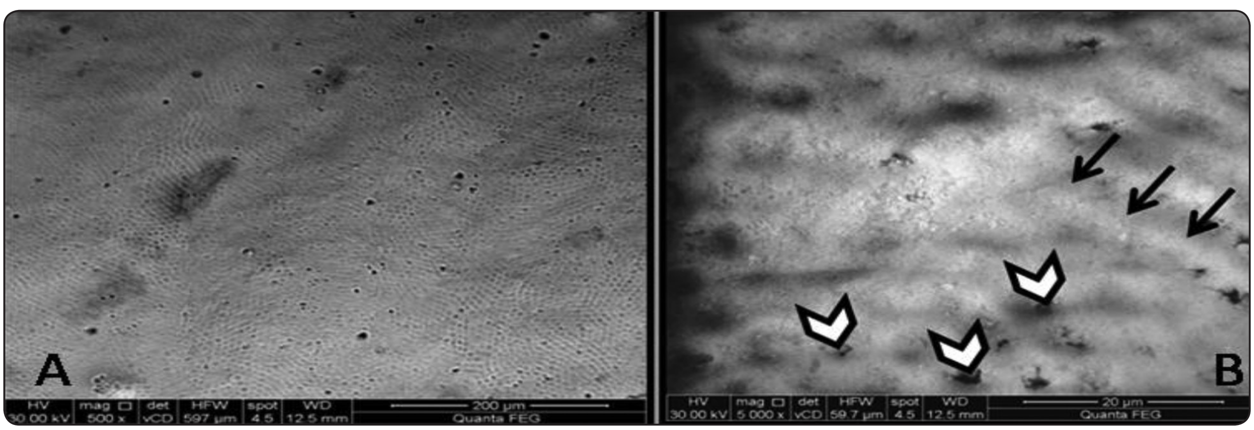

Fig. (2) Scanning electron micrograph of normal enamel surface showing: A) a generally smooth enamel surface with small circular pits, B) a higher magnification showing transverse wave-like depressions (perikymata) (arrows) and circular pits corresponding to the Tomes' processes of ameloblasts (arrow heads) (Original magnification: A-500X &B-5000X).

preferential removal of enamel prisms (Fig. 4B). Occasionally, surface cracks were seen on enamel (Fig. 4A).

Group IV:

In this group, following Systemp application on acid etched enamel, obscured enamel prisms and inter-prismatic areas were evident. Homogeneous, thin and smooth enamel surface deposits were apparent. Isolated pitted areas and fine cracks were also observed (Figs. 5 A&B).

Group V:

Examinatiom of this group showed that the application of Systemp on laser etched enamel appeared to seal the enamel prism as the fish scales pattern of the enamel preims was apparent. Surface deposits in the form of furrows intermingeled with uncovered prismless enamel were seen. Few pitted areas were also observed. However, other areas reflected homogeous covering of the etched enamel surface (Figs. 6A&B).

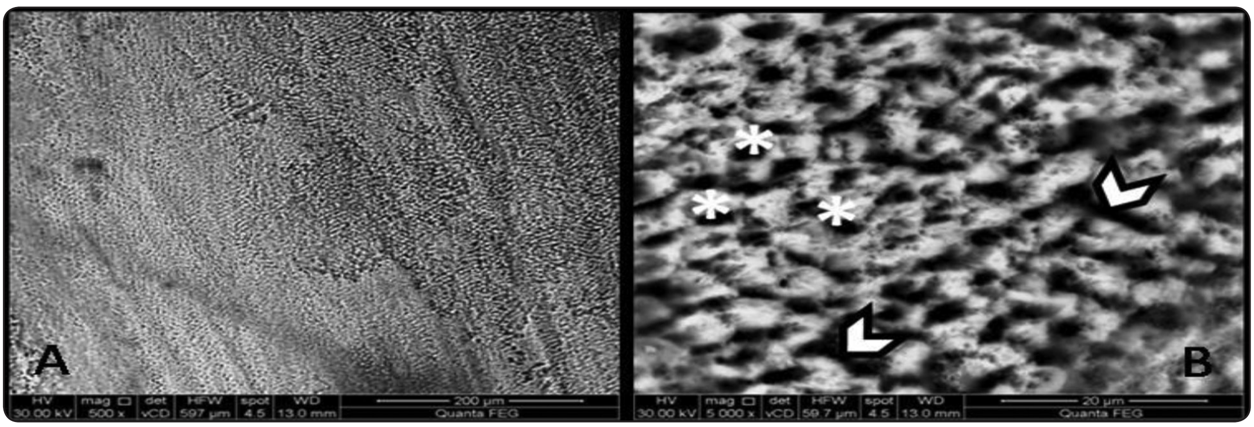

Figure (3) Scanning electron micrograph of enamel surface etched with 37% phosphoric acid for 30 sec. showing: A) a highly porous enamel surface, B) a higher magnification showing type III enamel etching pattern with alternating areas of preferential removal of prisms (asterix) and interprismatic regions (arrow heads) (Original magnification: A-500X &B-5000X).

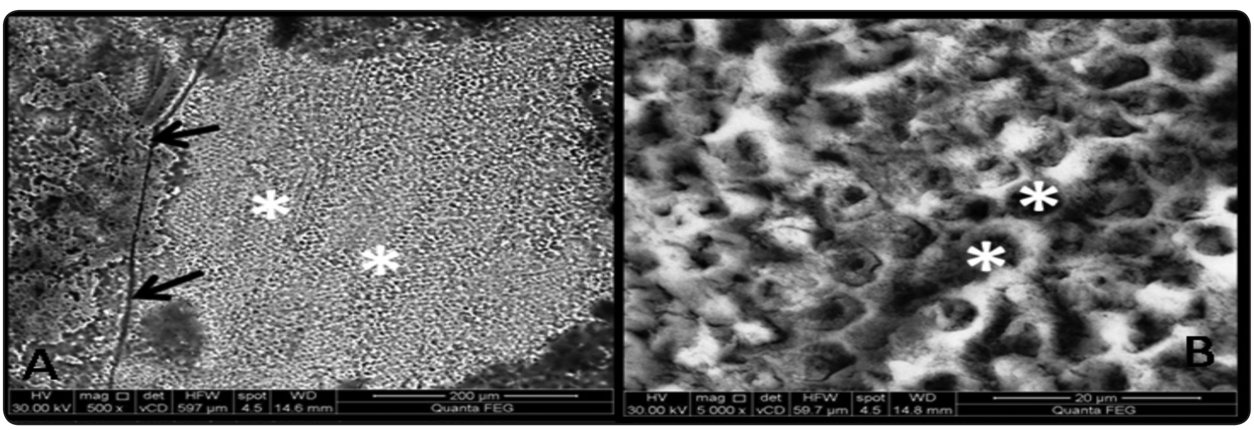

Fig. (4) Scanning electron micrograph of laser etched enamel surface showing: A) the presence of a roughened enamel surface, some areas of prismless enamel were not removed (asterix) and surface cracks were occasionally seen (arrows), B) a higher magnification showing type I enamel etching pattern with preferential removal of enamel prisms (asterix) (Original magnification: A-500X &B-5000X).

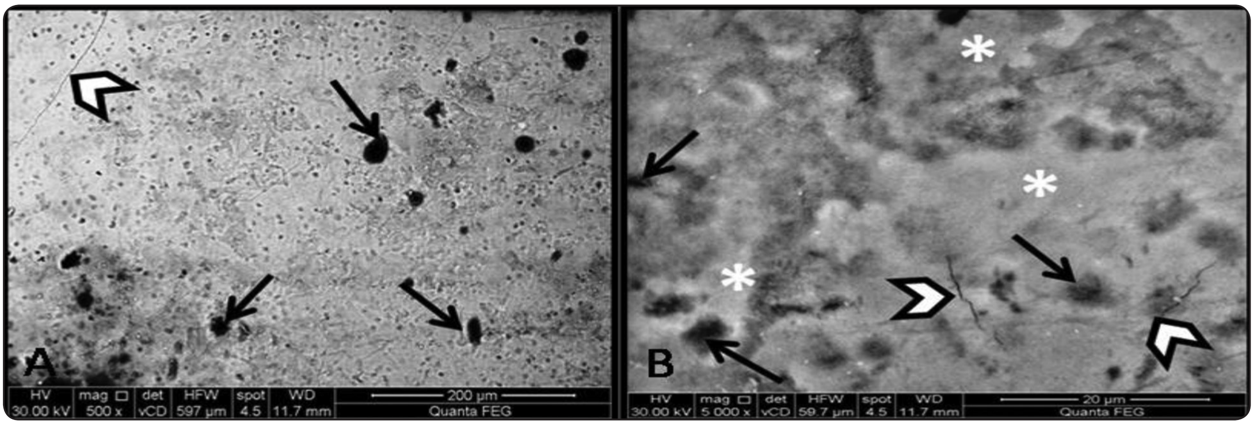

Fig. (5) Scanning electron micrograph of acid etched enamel surface after Systemp application showing: A) areas of smooth thin deposits and few pits (arrows), also fine cracks were observed (arrow heads), B) a higher magnification showing the smooth thin deposits on enamel (asterix), scattered pits (arrows) and fine cracks (arrow heads) (Original magnification: A-500X &B-5000X).

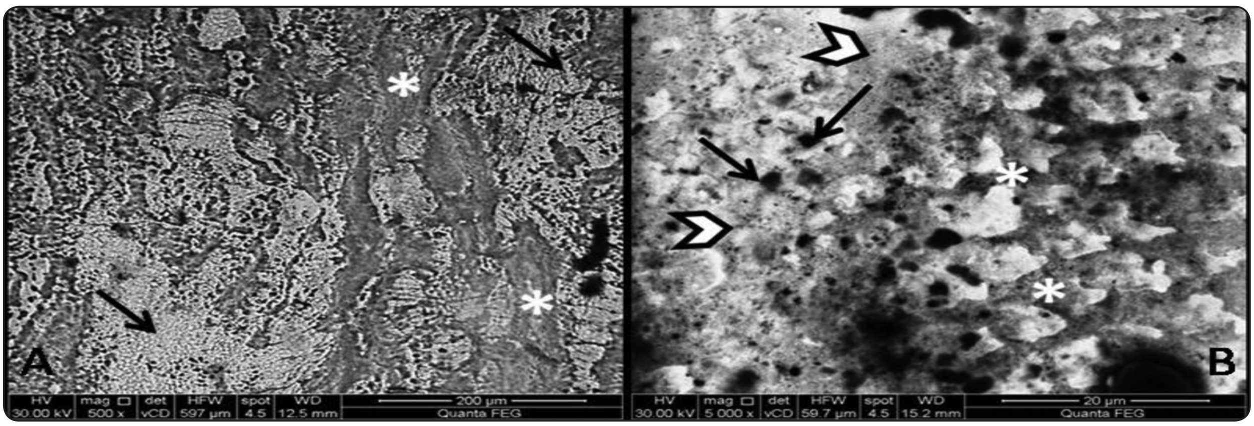

Fig. (6) An electron micrograph of laser etched enamel surface following Systemp application showing: (A) surface deposits in the form of furrows (asterix) intermingeled with uncovered prismless enamel (arrows). (B) a higher magnification showing sealed inter prismatic areas with apparent fish scales pattern of the prisms (asterix), homogeous covering of the etched enamel surface in isolated areas (arrow heads) and few pits were also noticed (arrows).

DISCUSSION

Phosphoric acid is one of the best techniques to bond resins to enamel. The smear layer is removed by acid application on the enamel surface. Microscopic roughness and enamel surface energy are improved by removing prismatic and interprismatic crystals. In general, 10-37% orthophosphoric acid is applied to both enamel and dentin [14]. Acid etching using a 35% orthophosphoric acid gel resulted in the greatest shear bond strength [15]. However, acid etching causes chemical changes that can alter the organic substance and decalcify the inorganic constituent. As a consequence of this demineralization, enamel becomes more prone to caries [16,17].

The literature contains conflicting findings concerning surface treatments and cavity preparations with lasers. For cutting enamel, high-irradiation outputs are used varying from 2.5 to 6 W. The lasers used for the treatment of sensitive teeth may be divided into two groups: a) the middle output power lasers – neodymium-doped yttrium aluminium garnet (Nd:YAG) and CO2 lasers and b) the low-level lasers–helium-neon (He–Ne) and gallium–aluminum–arsenide (GaAlAs) (diode) lasers. The low-level or 'soft' lasers provide cold thermal low energy wavelengths with slight temperature increase of < 0.1 °C. These wavelengths are assumed to stimulate circulation, cellular action and to provide various effects such as anti-inflammatory, vascular, analgesic and tissue healing effects [(18)]**.** Additionally, numerous researches have demonstrated the effect of low-level lasers on dentinal hypersensitivity and most studies have used GaAlAs laser therapy [(19&20)]**.** Moreover, in **2011,** a lower output 2W erbium was used, chromium: yattrium-scandium-gallium-garnet (Er, Cr: YSGG) laser to etch the enamel [(21)].

At present, laser etching of enamel surfaces is well known because of the potential drawbacks of acid etching. Laser etching could be a substitute to acid etching of enamel and dentin. Of the well-known advantages of laser etching is the production of acid-resistant surfaces [(22)]**.**

It is well recognized that physicochemical changes occurring after laser etching made the tooth more resistant to caries. It was reported that remineralization places acting like free-ion traps occurred with laser etching [(5)]. In addition, caries reduction was linked to the altered calcium-to-phosphorous ratio, leading to reduction of carbonate and pyrophosphate formation [(23)]. Additionally, through SEM examination it was observed that Er, Cr: YSGG laser irradiation produced etching patterns similar to those of acid etching [(24)].

In the present work, SEM examination of the acid-etched enamel surface (group II) revealed an obviously porous enamel surface which presented type III enamel etching pattern with alternating areas of preferential removal of prisms and interprismatic regions. On the other hand, following laser etching, enamel surface showed some areas of non-removed prismless enamel while most enamel presented type I enamel etching pattern with preferential removal of enamel prisms.

Regarding the dye penetration results in the current investigation, statistical analysis revealed that the distance penetrated by the dye was noticed to be significantly greater in the acid etched group (mean±SD=1,406.06±0.721), when compared to the laser etched group (mean±SD=1,235.35±0.771), as the p-value was less than 0.05. This, in turn, implies that acid etching was more effective in creating surface roughness than laser etching.

Findings regarding utilizing lasers for enamel etching are conflicting. For example, in 2009, the microtensile bond strength was investigated between enamel and two bonding agents. It was found that the microtensile bond strength was significantly lower in the acid-etched group than the Er, Cr: YSGG and Nd: YAG laser-etched enamel groups for both bonding agents [(25)]. On the other hand, some researchers stated that laser irradiation was not capable of etching enamel. Other investigation found weaker bond forces in an Er: YAG laser-etched enamel surface than in an acid etched enamel surface. This was associated with sub-surface cracks observed in SEM images [(23)]. Additionally, another study declared that Er, Cr: YSGG laser-etching techniques were not a sufficient way to improve the bond between enamel and stainless steel orthodontic brackets when compared to acid-etching techniques [(26)]. Furthermore it was confirmed that Er: YAG laser irradiation didn't eliminate the need to etch the enamel surface with acid before applying sealants. These results are in agreement with the outcomes obtained in the present study [(27&28)]**.**

Clinician's skill to bond a restoration to enamel has subjective changes in prosthetic and cavity preparations, restorative advances for esthetic improvements, bonding techniques for orthodontic devices and the treatment of caries [(29,30&31)]**.** Bonding to enamel has been historically studied for over 50 years and hard work has been made to create a simplified alternative, however; acid etching of enamel remained the most effective method for steady enamel bonding [(32).] Additionally, bleaching treatment is highly praised by patients, and it is a well-known procedure in most esthetic dental treatments. In spite of possessing good esthetic effects;

tooth whitening has some drawbacks including: tooth sensitivity [33-36] and structural changes such as reduction of enamel microhardness [37&38] and increased surface roughness [39,40]. Based on the mentioned facts, the current study was performed in order to explore the effect of Systemp; one of the most commonly used desensitizing agents; on enamel microstructure, enamel surface roughness and enamel permeability following enamel etching either by phosphoric acid or by low level laser.

Concerning the SEM results obtained in the present work, they revealed that Systemp had the ability to change the surface morphology of enamel following etching either by phosphoric acid or LLL. Systemp application rendered the surface more homogenous in texture as a result of sealing the opened prism and/ or the inter-prismatic regions. In addition, the dye penetration results showed that the distance penetrated by the dye was least among groups IV&V where Systemp was applied following acid and laser etching respectively. Furthermore, statistical results have confirmed that the dye penetration in group IV (Systemp applied to acid etched enamel) (mean±SD=1,078.47±0.634) was significantly reduced than that in group V (Systemp applied to laser etched enamel) (mean±SD=1,198.44±0.583) as the p-value was less than 0.05.

Numerous former studies were performed on Systemp as a dentine desensitizer; these studies demonstrated that Systemp desensitizer was valuable in reducing pain resulting from dentine hypersensitivity. These results remain unchanged whether or not the tooth was acid-etched prior to application of the desensitizing agent [41].

Since Systemp desensitizer is often used in conjunction with other temporary and permanent dental restoratives, it is applied after etching to increase the bond strength. This reaction may be attributed to the fact that glutaraldehyde is capable of fixing the smear layer of prepared dentin [42]. As a part of Systemp, polyethylene glycol dimethacrylate is a main constituent of different resin based restorative materials [43,44].

Furthermore, an immunoblotting studyeas done and the authors demonstrated that enamel proteins reacted with the anti-vimentin antibody after fixation with glutaraldehyde. They suggested that the observed immunoreaction is directed against an epitope apparently created by cross linking of enamel proteins during fixation with glutaraldehyde[45]. This finding support our results regarding the glutaraldehyde content in the Systemp which was capable of cross linking the enamel proteins, thus reducing the permeability of enamel in group IV and V as revealed by the dye penetration results.

Conclusively, despite laser advantages and because of the low bond strength reported in many studies[25,28], laser-etching applications should be improved. Besides, desensitizing agents, like Systemp, could be beneficial in efficient sealing of etched enamel surface, which could improve microleakage around restorations involving enamel, and in turn, could reduce sensitivity which may result from leakage through enamel into the underlying dentin.

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