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Research Article

Microhardness, Structure, and Morphology of Primary Enamel after Phosphoric Acid, Self-Etching Adhesive, and Er:YAG Laser Etching

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Background. Phosphoric acid is the traditional etching agent; self-etching adhesives and Er:YAG laser are alternative methods. Knowledge of deciduous enamel etching is required. *Aim*. To evaluate primary enamel microhardness, structure, and morphology after phosphoric acid, self-etching, and Er:YAG laser etching. *Design*. Seventy primary incisors were assigned to five groups ($n = 14$): I (control), II (35% phosphoric acid), III (self-etching adhesive), IV (Er:YAG laser at 15 J/cm^2), and V (Er:YAG laser at 19.1 J/cm^2). Microhardness was evaluated by Vickers indentation. Chemical composition was analyzed by energy dispersive X-ray spectroscopy and morphological changes by scanning electron microscopy. One-way ANOVA, Kruskal–Wallis, Mann–Whitney U, and Pearson bivariate correlation were employed ($\alpha = 0.05$). *Results*. Vickers microhardness showed differences and no correlation with Ca/P ratio. Group II showed differences in carbon, oxygen, and phosphorus atomic percent and group V in Ca/P ratio. Morphological changes included exposed prisms, fractures, craters, and fusion. *Conclusions*. Enamel treated with phosphoric acid showed different chemical characterization among groups. Self-etching and Er:YAG laser irradiation at 19.1 J/cm^2 showed similar microhardness and chemical characterization. Er:YAG laser irradiation at 15 J/cm^2 maintained microhardness as untreated enamel. Er:YAG laser irradiation at 19.1 J/cm^2 enhanced mineral content. Morphological retentive changes were specific to each type of etching protocol.

1. Introduction

Although the adhesion to enamel produced by phosphoric acid etching has been considered strong and highly durable, the value of this technique in recent years has taken a secondary position with the introduction of self-etching adhesive systems as alternative methods [1, 2]. These adhesive systems have simplified bonding procedures in clinical use [3], because they do not require separate phosphoric acid etching, water rinsing, or superficial moist controlling steps [2]. Moreover, self-etching adhesive systems combine primers and bonding agents such that priming and bonding can be achieved by applying either a one- or two-step procedure [3]. However, studies evaluating self-etching adhesives disagree on the efficacy of etching because morphological analyses of enamel surface treated with self-etching primers have shown surfaces that are not very demineralized and unetched areas [2].

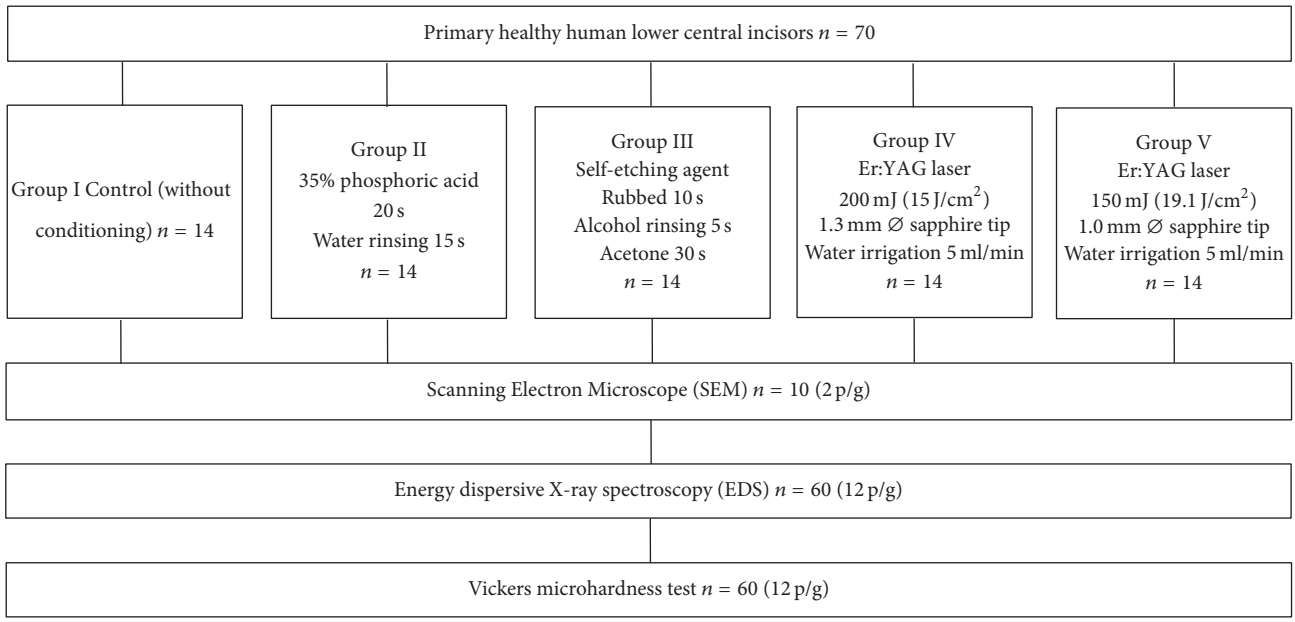

Figure 1: Diagram of the experimental design.

Parallel to advances in adhesive technology, laser in dentistry is gaining popularity. Laser systems are widely used in pediatric dentistry because of compliance in children [4, 5]. The American Academy of Pediatric Dentistry recognizes the judicious use of lasers as a beneficial instrument in providing dental restorative and soft tissue procedures for infants, children, and adolescents, including those with special health care needs [6].

In this regard, the Er:YAG laser has been used for enamel pretreatment, dental caries removal, pulpotomy, and pulpectomy. Laser systems have several important advantages over the traditional approaches of dental treatment for infants. In particular, laser systems may require less anesthetic, allow for painless treatment, and decrease dental fears and anxiety [4].

A number of studies have demonstrated that the microroughened appearance of surface morphology after laser irradiation on permanent enamel is similar to those obtained with conventional acid etching [4]. Thus, lasers have been used for etching permanent enamel and dentine surfaces as an alternative to conventional acid etching methods [7].

However, further studies are required to avoid extrapolating the results from permanent to temporary teeth. This study aimed to evaluate the microhardness, structure, and morphology of the primary enamel surface after etching with phosphoric acid, self-etching adhesive, and Er:YAG laser irradiation.

2. Materials and Methods

2.1. Tooth Selection and Sample Preparation. The study protocol was reviewed and approved by the Research and Ethics Committee of the Autonomous University of the State of Mexico. Seventy human primary mandibular central incisors, exfoliated or extracted because of their persistence in the mouth, were obtained under informed consent. The collected teeth did not present obvious decay or evidence of fluorosis, fractures, or fillings. Immediately after extraction, they were collected in a 0.2% thymol solution and transported to the laboratory. Deionized water was used to clean the teeth; traces of soft tissue were removed with a scalpel. Teeth were gently brushed with a soft brush (Oral-B® Sulcus Brush, Procter & Gamble, Cincinnati, OH, USA) and finally rinsed with deionized water. The collected teeth were stored in a solution of 0.2% (wt/vol) thymol at 4°C until the analyses were performed.

Each tooth was removed from the solution, rinsed with deionized water, and dried with oil-free air from a triple syringe. The teeth were examined with a DIAGNOdent® pen (KaVo, Biberach, Germany), and 70 healthy teeth (lower central incisors) with values between 0 and 13 were selected for characterization.

The incisors were fixed in acrylic resin (Orthodontic Resin, Dentsply Caulk International Inc., York, PA, USA). A mounting jig was employed to align the labial surface of the tooth parallel to the bottom of the mold. The sequence of the procedures and techniques applied is shown in Figure 1.

2.2. Etching. Teeth were randomly distributed into five groups ($n = 14$ per group), and the corresponding etching protocol was applied on the labial surface of the samples. Group I was the control group, in which enamel etching was not conducted. In group II, 35% phosphoric acid (Scotchbond etching gel, 3M ESPE, St. Paul, MN, USA) was applied on the enamel surface for 20 s, rinsed thoroughly with a forceful air-deionized water spray for 15 s, and dried with compressed air for 5 s. In group III, the self-etching adhesive (Adper™ Prompt™ L-Pop™ in a triple lollipop-shaped aluminum foil package, 3M ESPE, St. Paul, MN, USA) was rubbed for 10 s on

Table 1: Comparison of Vickers microhardness among groups.

Group	Vickers microhardness number (H_V)	SD	Post hoc test*
I	198.56	1.92	A
II	**35.54***	0.84	**B***
III	72.34	1.78	C
IV	177.76	6.5	A
V	59.93	4.36	C

*Groups with different letters are significantly different ($p \leq 0.001$) from each other.

the enamel surface, followed by alcohol irrigation to clean the surface. Subsequently, acetone was applied to eliminate the adhesive. Compressed air drying was used to allow visualization of the etching pattern created by the acid monomers. In group IV, irradiation of the specimens was performed using an Er:YAG laser system with a wavelength fixed at 2.94 μm (Lumenis OPUS DUO™ Er:YAG + CO_2, Yokneam, Israel) in noncontact mode, pulse repetition of 15 Hz, and pulse duration of 400 μs. The surface was perpendicularly scanned once by hand using an energy pulse of 200 mJ (15 J/cm^2) with a 1.3 mm ∅ sapphire tip at a working distance of 1 mm. At that tip-sample distance, the exit tip and laser beam had the same diameter, as corroborated with a laminated infrared sensor screen (Lumitek International, Inc., Ijamsville, MD, USA). Deionized water was sprayed (5.0 mL/min) during irradiation to reduce heating. Additionally, energy levels were calibrated using the calipers of the equipment, and the energy delivered was measured periodically with a power meter (LaserMate-P, Coherent Co., Santa Clara, CA, USA). In group V, irradiation of the specimens was performed using the same conditions as group IV samples, except for the energy pulse of 150 mJ (19.1 J/cm^2) and 1.0 mm ∅ sapphire tip.

2.3. Microhardness Test. Microhardness was measured in 60 samples using a Vickers microhardness tester (MXT30-UL™, Matsuzawa, Akita, Japan). A Vickers diamond indenter was loaded at 20 g^f (dwell time = 15 s). Five different indentations for enamel were performed, and the mean was calculated for each tooth. The length of the two diagonals was used to calculate the microhardness value (Vickers microhardness number, H_V); indentations were observed at 40x magnification.

2.4. Energy Dispersive X-Ray Spectroscopy (EDS). Twelve enamel samples per group ($n = 60$) were analyzed by EDS to determine the atomic percent (at%) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca) using an X-ray detector system (Oxford Instruments, 7582, UK) attached to a microscope.

2.5. Scanning Electron Microscopy (SEM) Analysis of the Enamel Surfaces. Ten specimens (2 per group) were covered with a gold filament (Vacuum Desk II, Denton, Moorestown, NJ, USA) for 60 s. Samples were fixed to an aluminum plate with copper tape for morphological analysis by SEM (JSM, 6610LV, JEOL, Pleasanton, CA, USA), and micrographs were obtained at ×500, ×2500, and ×7500.

2.6. Statistical Analysis. All data were analyzed using the SPSS 21 statistical package (IBM, New York, NY, USA). The Kolmogorov–Smirnov test was performed to estimate the data distribution. One-way ANOVA was used to establish statistically significant differences in enamel microhardness groups; when significant differences were found Tukey's post hoc test was employed since Levene's test of homogeneity of variances showed equal variances. In order to determine differences in atomic percentages of the elements among groups the Kruskal–Wallis and Mann–Whitney U tests were applied. The Pearson correlation was calculated to analyze the relationship between microhardness and Ca/P ratio values.

3. Results and Discussion

3.1. Results

3.1.1. Microhardness Test. The experimental microhardness data obtained by the Vickers microhardness tester for all groups are presented in Table 1. When the ANOVA post hoc test was applied, statistically significant differences were found ($p \leq 0.001$). Groups I and IV showed the highest microhardness values, with no significant differences between them ($p = 0.1$). Group II had the lowest microhardness among the groups, whereas groups III and V displayed similar intermediate values.

3.1.2. EDS. The chemical composition of deciduous enamel surface expressed in at% had similar values among the control and experimental groups, except for group II. Statistically significant differences in C, O, and P were noted. Group V showed the highest Ca/P ratio among all the groups (Table 2).

No correlation was found between the Ca/P ratio and Vickers microhardness ($r = 0.094$).

3.1.3. SEM Micrographs. The micromorphological aspects of the five groups are shown in Figure 2. Group I (control) showed a smooth surface with some grooves (a, b, and c). A preferential dissolution at the center of the prisms was observed in the phosphoric acid group (d, e, and f). The self-etching group exhibited dissolution at the center and periphery of the prisms (g, h, and i). Nonselective enamel surface removal with a flaky pattern, exposed prisms, and some microcracks were observed in group IV (j, k, and l). Group V presented enamel surface removal (ablation), exposed prisms, some fractures, and melting (m, n, and o).

Table 2: Atomic percentages (at%) of C, O, P, and Ca and the Ca/P ratio in conditioned human primary enamel.

Group	C	O	P	Ca	Ca/P
I	18.8 ± 3.8 A	55.2 ± 4.9 A	9.9 ± 1.1 A	16.2 ± 2.6 A	1.6 ± 0.1 A
II	**10.7 ± 2.0 B***	**61.4 ± 2.0 B***	**10.9 ± 0.9 B***	17.1 ± 1.8 A	1.6 ± 0.1 A, B
III	16.7 ± 4.6 A	57.1 ± 3.3 A	9.9 ± 1.0 A	16.0 ± 2.0 A	1.6 ± 0.1 A, C
IV	30.8 ± 17.4 A	49.3 ± 8.7 A	7.3 ± 3.4 A	12.6 ± 5.6 A	1.8 ± 0.2 A, C
V	21.1 ± 11.5 A	54.6 ± 6.5 A	8.5 ± 2.4 A	15.7 ± 3.4 A	**1.9 ± 0.4 D***

*Groups with different letters are significantly different ($p \leq 0.05$) from each other.

Undesirable effects were observed in isolated areas of groups IV and V (Figure 2).

4. Discussion

Regardless of the method employed, irregularities are necessary for proper bonding to enamel. However, several methods have been suggested to preserve the integrity of enamel [8]. The etching and irradiation parameters were chosen according to the results of a pilot study conducted in a laboratory and designed after literature review. Water irrigation was used for the Er:YAG laser groups as a cooling agent to limit the temperature rise in dental tissues, as well as avoid the formation of undesired chemical phases [9]. Both high and low energy densities were used to emulate conventional chemical etching and gentle self-etching.

Mechanical properties such as microhardness must be considered when assessing the enamel surface etched. Nonetheless, research in this regard is scarce. Moreover, microhardness of primary teeth has not been adequately studied, whereas several reports have been made on permanent teeth under diverse conditions. This discrepancy may be partly attributed to the difficulty in obtaining primary teeth, especially sound primary teeth, for experiments. Minimal attention has been paid to the mechanical properties of primary teeth compared with those of permanent teeth because primary teeth exist for a limited time in childhood, whereas permanent teeth remain seven to eight times longer [10].

In this study, primary enamel microhardness was tested using a Vickers indenter, as it is more suitable than a Knoop indenter for comparing the variations in mechanical properties of an anisotropic material, such as tooth enamel [11]. This tissue is organized into prisms, and this orientation determines anisotropic performance and affects mechanical properties [12].

Based on the results of this study, enamel microhardness was influenced by the type of etching protocol, but additional studies in this field are suggested. Er:YAG laser irradiation at 15 J/cm^2 was an appropriate agent for primary enamel etching because it provided a stronger enamel structure to be restored (microhardness reduction of 10% compared with the control). Nevertheless, complementary studies are required to evaluate the shear bond strength of adhesive materials under this etching condition.

Although groups III (a strong one-step self-etching agent with pH 0.4) and V (Er:YAG laser irradiation at 19.1 J/cm^2) involved different etching methods [chemical (removing calcium phosphates exposing microporosities) versus photothermal (causing sudden heating and vaporization of inorganic components and water present in enamel)], their microhardness values were similar but lower than those in the control group. A microhardness reduction (64% in group III and 70% in group V) was observed in comparison with the untreated enamel.

As expected, 35% phosphoric acid employed with pH 0.6 resulted in the lowest microhardness value (reduction in 82% compared with the control group), which was associated with completely open prisms, a feature observed solely in this group (Figure 2(d)).

The induced chemical alterations resulting from etching treatment are important for the evaluation and further improvement of adhesion systems [13]. In this study, the chemical composition of the enamel surface was measured by EDS. Enamel surface characterization under several etching alternatives has not been previously reported in the literature.

The results obtained by EDS (Table 2) showed that the control group had very similar values in C, O, P, Ca, and Ca/P at% to those obtained by Zamudio-Ortega et al. [9] for untreated primary enamel. Group II presented the most remarkable and significant differences in chemical composition among all the groups. The C at% decreased, whereas O and P at% increased with respect to the control group. However, the Ca at% and Ca/P ratio remained stable. Phosphoric acid induces a decrease both in the carbonate content of enamel apatite and in the formation of HPO_4^{2-} ions [13]. Additionally, Torres-Rodríguez et al. [14] reported that the degree or mineralization of bovine enamel removed by phosphoric acid remains constant, indicating that mineral and organic components are lost at the same rate. Interestingly, poorly crystalline phosphate and carbonate-rich mineral components were preferentially removed and presumed as the main source of calcium released by acid exposure, as revealed by Fourier transform infrared spectroscopy.

Self-etching and Er:YAG laser irradiation at the low energy density (15 J/cm^2) groups showed similar chemical characterization. Furthermore, both etching protocols are gentler than phosphoric acid etching. Van Meerbeek et al. reported that self-etching adhesives act without demineralizing the tooth surface too profoundly, thereby preserving hydroxyapatite [3]; in addition, microscopic morphological analyses showed less deep etched surfaces and predominantly unetched areas [2]. No changes in oxygen and the Ca/P ratio were observed in group IV, which was consistent with

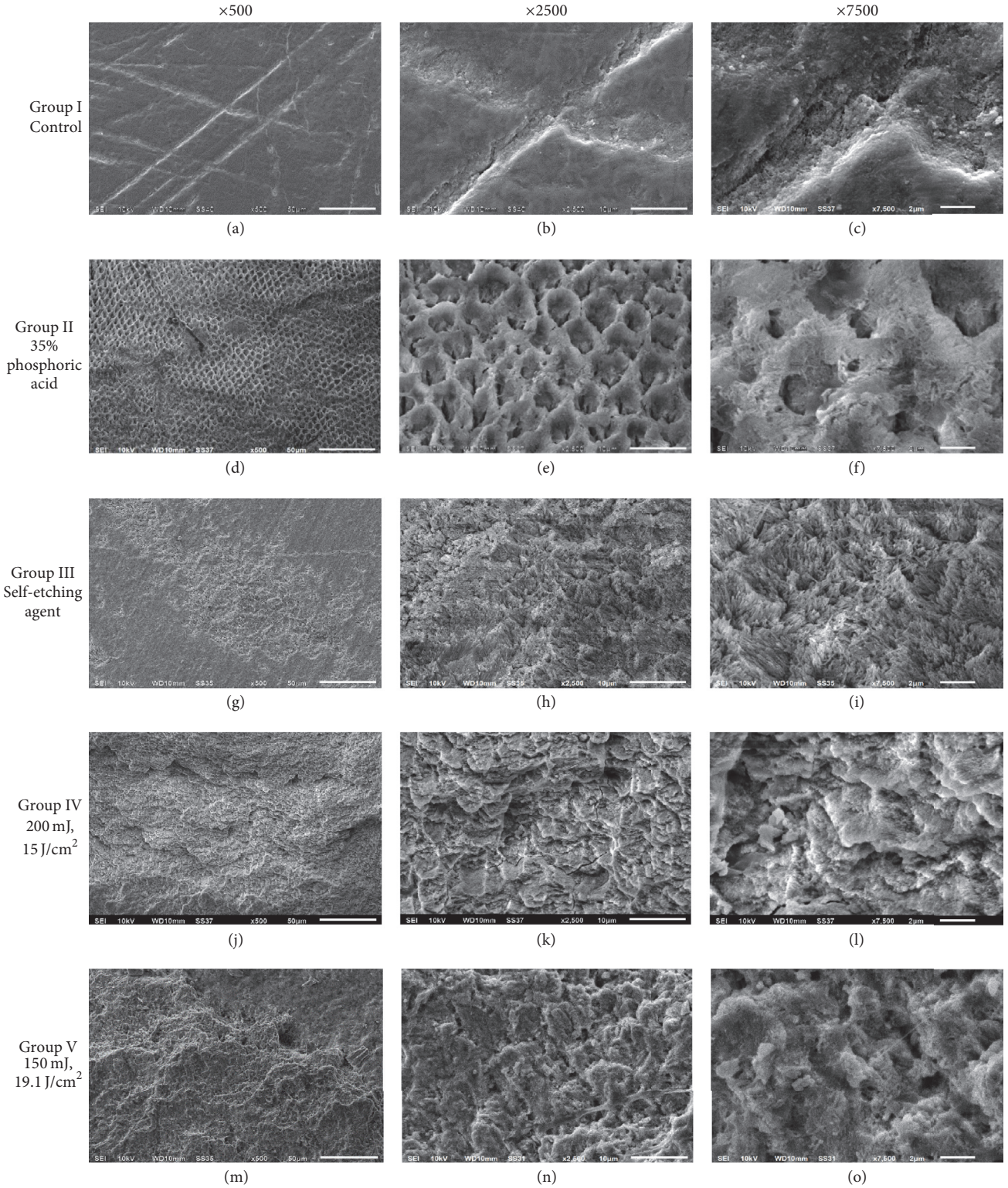

Figure 2: SEM micrographs of untreated (control) and conditioned surfaces of primary enamel. Group I (a, b, c) presented a smooth pattern with some enamel grooves. A traditional honeycomb etching pattern was observed when 35% phosphoric acid conditioning was employed (group II, d, e, f). Group III (g, h, i) exhibited rough zones and exposed prisms produced by self-etching agent. Exposed prisms and flakes, resulting in a rough scaly surface after Er:YAG laser irradiation at 15 J/cm^2 (j, k, l) and 19.1 J/cm^2 (m, n, o) (groups IV and V, resp.), microcracks, and small areas of enamel ablation (original magnifications, ×500, ×2500, ×7500); scale bar = 50 μm, 10 μm, and 2 μm.

the findings of Zamudio-Ortega et al. [9], who employed a close energy density for primary enamel Er:YAG laser irradiation.

Regarding the Er:YAG laser-irradiated group at high energy density (19.1 J/cm^2), the chemical composition was similar to the control group in at% of all elements evaluated. However, the Ca/P ratio increased. Despite no significant reduction in P at%, this value was sufficient to increase the Ca/P ratio close to 1.9, which exceeded the stoichiometric hydroxyapatite value (1.67) [15]. The increment in the Ca/P ratio matched that found by Zamudio-Ortega et al. [9] when high Er:YAG laser irradiation at 39.8 J/cm^2 was applied to primary enamel. This outcome suggested that chemical characterization produced by these irradiation conditions enhanced the mineral content of the enamel structure because the Ca/P molar ratio is considered a reliable mineralization indicator that allows establishment of behavior patterns, independent of variations in other elements in teeth [9].

Although enamel microhardness is positively correlated with calcium concentrations [16], no correlation between the Ca/P ratio and Vickers microhardness was found among the study groups.

In addition to microhardness and chemical composition, morphological evaluation of conditioned enamel is important for the analysis and improvement of adhesive systems [8]. For the control group, untreated primary enamel surface was analyzed by SEM, whereas the experimental groups were observed after etching protocol. The control group showed a "mostly smooth with some grooves" pattern. This pattern is a main morphological type reported by Zamudio-Ortega et al., who explained that healthy untreated enamel is irregular [15], and "prismless enamel" is present in the outermost layer of the deciduous teeth, where crystallites are regularly arranged parallel to each other [17]. Additionally, the well-defined grooves found in some primary teeth have been attributed to the abrasion processes that all erupted teeth undergo, such as the ones caused by toothbrushing, feeding, or certain habits of the child [9, 15].

SEM evaluation revealed preferential dissolution at the center of the prisms in the phosphoric acid group. This finding was consistent with Galil and Wright's (1979) [18] type 1 etching pattern, which is equivalent to Silverstone's (1975) [19] type 1 pattern. However, Boj et al. [18] reported preferential dissolution at the periphery of the prisms as the main pattern (Galil and Wright's type 2 etching pattern, equivalent to Silverstone's type 2 pattern) for primary teeth. In some cases, type 1 pattern was noted either alone or in combination with smooth and level enamel surfaces (Galil and Wright's type 5 pattern) when similar acid etching conditions were employed in primary enamel. Furthermore, the phosphoric acid protocol produced an aggressive enamel etching pattern of the treated surface, with completely exposed enamel prisms.

In group III, a seventh-generation self-etching adhesive Adper Prompt L-Pop was used. This agent has been linked to good etching capacity and produces one of the closest etching patterns to that of phosphoric acid [2]. In group III, the enamel's uppermost layer was less dissolved within and around the prisms, corresponding to Galil and Wright's type 3 pattern. A moderately etched surface compared with the phosphoric acid group was observed, as self-etching primers are a conservative material [1, 3, 20, 21]. In a similar study, Boj et al. [18] found several etching patterns according to Galil and Wright's types 1, 2, and 3, although these patterns were more irregular and less deep in some cases. Nevertheless, some authors have reported that self-etching adhesives are not very effective [1, 3, 20, 22] and have inadequate etching ability and presence of insoluble calcium phosphate, which cannot be removed by irrigation [23]. However, self-etching adhesives are increasingly popular in dentistry [24] because they require fewer application steps [21, 24], eliminate the washing and drying steps, save chairside time, and reduce procedural errors, resulting in low sensitivity [18]. They are considered beneficial [25] and an alternative in certain circumstances [24], such as in the case of pediatric dental treatment, in which children and special patients have difficulty accepting treatment or when speed is crucial [24, 25].

Even though enamel acid etching patterns were established in the mid and late 1970s [18], these patterns do not apply to Er:YAG laser etching, which became known as an etching alternative in the mid-1990s [26]. Er:YAG laser causes morphological changes resulting from a photothermal reaction. However, some studies [4, 7] have reported that the microroughened appearance of the surface after laser irradiation is similar to that obtained by conventional acid etching.

The exposed prisms and flakes observed by SEM in laser groups resulted in a rough scaly surface, which is apparently suitable for retaining adhesive materials. Additionally, microcracks and small isolated areas of enamel ablation were shown as adverse effects produced by enamel laser etching, as previously reported [4, 7]. Given that these surfaces will be covered by an adhesive material, the undesired effects are not risk areas for bacterial adhesion. As expected, etching pattern and adverse effects were slightly more pronounced in the group irradiated at high energy density, although the energy density difference was only 4.1 J/cm^2.

Nevertheless, deciduous teeth remain in the mouth during a short time compared with permanent teeth. The main goals of pediatric dentistry are the maintenance of dentition integrity and proper transition from primary to permanent dentition for the growth and development of an ideal and healthy occlusion.

5. Conclusions

The phosphoric acid group revealed the most aggressive enamel etching agent, according to the etching pattern observed and the lowest microhardness obtained.

Self-etching group showed similar chemical characterization and microhardness value regarding laser irradiation groups (15 J/cm^2 and 19.1 J/cm^2, resp.).

Er:YAG laser irradiation at 19.1 J/cm^2 enhanced the enamel mineral content, as evidenced by the Ca/P ratio.

Er:YAG laser irradiation at 15 J/cm^2 maintained the dental enamel microhardness as untreated enamel.

Morphologically, retentive changes from mild to severe were specific to each type of etching protocol.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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