



# Effect of Er:YAG Laser with or without fluoride on the prevention and progression of erosive tooth wear in primary enamel: an in vitro study

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## Abstract

This study aimed to assess the effect of the Er:YAG laser, either alone or in combination with topical fluoride application, on the prevention and progression of erosive tooth wear in primary enamel. A total of 120 extracted human primary molars were flattened, polished, and divided based on substrate condition: sound or previously eroded with citric acid. The specimens were allocated into 12 experimental groups and received one of the following treatments: no treatment (control), topical application of acidulated phosphate fluoride (APF 1.23%), irradiation with Er: YAG laser using two different protocols (0.20 W and 0.40 W), or combinations of laser followed by fluoride application. All samples were then subjected to a five-day erosive remineralization cycling protocol, involving repeated acid exposure (pH 2.6) and immersion in artificial saliva. Enamel surface loss was assessed by optical profilometry, and morphological changes were evaluated by environmental scanning electron microscopy. The findings demonstrated that all interventions tested, including laser irradiation, fluoride application, and their combinations, were effective in reducing enamel loss when compared to untreated controls. However, no statistically significant differences were found between the various treatment protocols. The results suggest a promising protective effect of Er: YAG laser and fluoride treatments for preventing and controlling the progression of erosive tooth wear in primary enamel, highlighting the need for further research to confirm their efficacy.

**Keywords** Enamel · Erosion · Tooth wear · Laser · Er:YAG laser · Prevention · Fluoride

## Introduction

Dental erosion is a non-bacterial chemical process characterized by the dissolution of the hard tissues of the tooth due to exposure to acids of extrinsic or intrinsic origin [1]. This process leads to surface demineralization, compromising the structural integrity of the enamel and making it more susceptible to routine mechanical forces such as toothbrushing, mastication, and occlusal wear [2]. Although physiological tooth wear is expected over time, erosion becomes pathological when the rate of structural loss exceeds what is considered normal for the patient's age group [3].

Over recent decades, a significant increase in the prevalence of dental erosion has been observed, primarily attributed to changes in dietary and behavioral patterns, including frequent consumption of acidic beverages and ultra-processed foods [4, 5]. Global prevalence estimates range from 30% to 50%, with even higher rates in children, whose primary dentition presents characteristics that favor rapid lesion progression, such as thinner enamel, lower

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mineral content, and greater permeability [6]. Marschner et al. reported a prevalence of 35.6% of erosive tooth wear in primary teeth [7].

In addition to dietary factors, clinical conditions such as gastroesophageal reflux disease, frequent vomiting episodes, and eating disorders exacerbate acid exposure, especially in pediatric populations [8, 9]. If not identified and managed early, erosion in primary teeth may progress to involve dentin and even the pulp, resulting in hypersensitivity, functional impairment, and negative impacts on the child's quality of life [10].

The structure of primary teeth differs from that of permanent teeth, with lower mineralization, reduced microhardness, higher permeability, and incomplete crystalline maturation [11, 12]. These characteristics make primary enamel more susceptible to mineral loss. Moreover, studies indicate that erosive lesions at this stage tend to persist into the permanent dentition, especially when associated with sustained dietary habits and behaviors throughout childhood and adolescence [8].

Given the irreversible nature of dental erosion, early detection and proper management of risk factors are essential for clinical success. Preventive strategies aim to reduce the frequency of acid challenges and strengthen dental tissues against demineralization. In this context, fluorides remain widely used due to their role in enamel remineralization and in forming calcium fluoride (CaF<sub>2</sub>) deposits, which act as chemical barriers and sources of protective ions [13, 14]. However, the high solubility of these deposits in acidic media limits their effectiveness against frequent exposures [15–18].

To overcome these limitations, complementary therapies such as high-power lasers have been proposed. The Er: YAG laser (2,940 nm), due to its high affinity for water and hydroxyapatite, induces morphological and chemical changes in enamel that enhance resistance to acidic demineralization [4]. When combined with fluoride, the laser promotes the formation of fluorapatite and improves CaF<sub>2</sub> retention, while also inducing surface fusion and reducing permeability [19]. Evidence also suggests an increase in resistance to mineral loss in the treated surface [18].

Despite these promising outcomes, clinical protocols involving lasers still lack standardization regarding application sequence, power settings, and the type of dental substrate being treated. Therefore, the present in vitro study aimed to evaluate the efficacy of Er: YAG laser, either alone or in combination with acidulated phosphate fluoride gel (APF 1.23%), in preventing erosion and controlling the progression of erosive tooth wear in human primary enamel. For preventive evaluation, the protocols were applied to sound enamel; for progression analysis, the treatments were

tested on previously eroded enamel. The null hypothesis of the study was that there would be no statistically significant difference in surface loss between the experimental and control groups, regardless of the protocol used.

## Materials and methods

### Ethical considerations

This in vitro study was approved by the Research Ethics Committee of the School of Dentistry, University of São Paulo (CAAE: 63823622.0.0000.0075). A total of 160 samples were obtained from 160 extracted primary molars.

### Experimental design

This in vitro study followed a 6 × 2 factorial design, comprising six treatment protocols and two types of substrate (sound or eroded), resulting in 12 experimental groups. Sample size calculation was performed using G\*Power 3.1 software [20], based on a one-way fixed-effects ANOVA model. The following parameters were adopted: effect size (Cohen's  $f = 0.4$ ), significance level of 5% ( $\alpha = 0.05$ ), statistical power ( $1 - \beta = 0.80$ ), and 12 experimental groups. Based on these criteria, a minimum of 10 specimens per group was estimated, totaling 120 samples for the experiment. The detailed composition of the experimental groups is presented in Table 1.

**Table 1** Study design and experimental groups

Treatments	Type of substrate
1. Negative control: no treatment	1.
2. APF gel: acidulate phosphate fluoride gel (1,23% F, pH 3,6-3,9, DFL, Rio de Janeiro, RJ, Brasil)	Sound primary enamel
3. Er:YAG Laser 1: : 0,20 W, 10 Hz, 20 mJ, 1,44 J/cm <sup>2</sup> , AS7065X tip, diameter of 1,33 mm, 1 mm away from surface, under 50% air cooled with water, pulse width 100 us, for 10s (Lite Touch, Light Instruments, Israel)	
4. Er:YAG Laser 2 : 0,40 w, 10 Hz, 40, 2,88 J/cm <sup>2</sup> , AS7065X tip, diameter of 1,33 mm, 1 mm away from surface, under 50% air cooled with water, pulse width 100 us, for 10s (Lite Touch, Light Instruments, Israel)	2. Eroded primary enamel
5. Er:YAG Laser 1 + APF	
6. Er:YAG Laser 2 + APF	

**Experimental unit:** sound and eroded primary enamel specimens (n=10 each substrate)

**Response variable:** enamel surface loss (in um) evaluated post cycling

**Additional test:** qualitative surface evaluation by environmental scanning electron microscopy (ESEM) post treatment and post cycling

## Specimen preparation

A total of 160 sound human primary molars, free from restorations or visible cracks, were used. All teeth were previously inspected under a stereomicroscope (SZ-PT/SZ40, Olympus, Tokyo, Japan) at 10× magnification. Teeth exhibiting cracks, structural anomalies, or hypomineralized spots were excluded. One specimen was obtained from each tooth, totaling 160 specimens.

Teeth were mechanically cleaned using a slurry of pumice (SS White, Rio de Janeiro, Brazil) and water, applied with Robinson brushes (KG Sorensen, Barueri, SP, Brazil) at low speed. They were then rinsed with distilled water and stored at 4 °C in 0.1% thymol solution until the experimental procedures began.

The roots were separated from the crowns using a metallographic cutter (LabCut, Extec). Enamel fragments were obtained using a double-sided diamond disc (KG Sorensen, Barueri, SP, Brazil) attached to a straight micromotor handpiece (Beltec LB100), producing blocks approximately 4.0×4.0×1.5 mm (height × width × thickness). The fragments were embedded in acrylic resin blocks (VariDur 10, Buehler, Germany), with the enamel surface exposed to allow direct treatment application.

Surfaces were flattened and polished with silicon carbide abrasive papers (#800, #1200, #2400, and #4000 grit, Struers, Ballerup, Denmark) under continuous water irrigation. Each polishing step was standardized: 10 s for the first three grits and 30 s for the last, under a 5 N load at 150 rpm. Between each step, specimens were cleaned in an ultrasonic bath (L100, Schuster Equipamentos Odontológicos, Santa Maria, RS, Brazil) for 3 min with distilled water to remove debris.

To induce initial erosive lesions in half of the specimens (60 samples), non-plasticized UPVC adhesive tapes were applied to the polished surfaces, leaving a 4 mm × 1 mm central window exposed. These specimens were immersed in 1% citric acid solution (Sigma Aldrich, St. Louis, MO, USA; pH~2.4) at room temperature (±25 °C) for 10 min and then rinsed with deionized water.

Next, a baseline surface curvature analysis was performed using 3D optical profilometry to select the 120 most suitable specimens. All specimens were scanned with an optical profilometer (PROSCAN 2100 3D – Scantron, Taunton, UK) to identify those with curvature below 0.3 µm for sound enamel, and with surface loss values between 3 and 5 µm for eroded enamel [21]. The selected specimens were randomly allocated to experimental groups using Microsoft Excel (Microsoft Corporation, 2018) to ensure homogeneous distribution of baseline values.

## Treatments

For treatment application, non-plasticized UPVC tapes were placed on the polished surfaces of sound enamel specimens, leaving a 4 mm × 1 mm window exposed. Both sound and eroded enamel samples received the treatments described in Table 1. No preventive treatment was performed in the negative control groups. For fluoride application groups, acidulated phosphate fluoride (APF) gel at 1.23% (pH~3.5; DFL, Rio de Janeiro, Brazil) was applied passively with a microbrush for 4 min. The gel was then removed using a dry cotton pellet. For the laser irradiation groups, a Litetouch™ device (Light Instruments, Israel) with a wavelength of 2,940 nm was used. Irradiation was performed using a sapphire tip (AS7065X, 1.33 mm diameter), positioned 1 mm from the surface in a focused mode. All laser applications were performed manually by the same operator. For the combined treatment groups (laser+APF 1.23%), the Er: YAG laser was applied first, followed by the fluoride gel, as previously described. Each laser application consisted of four 10-second horizontal sweeping passes covering the full sample area, with the following parameters:

- **Protocol 1:** 0.20 W, 10 Hz, 20 mJ per pulse, 50% air/water spray, energy density of 1.44 J/cm<sup>2</sup>, 100 µs pulse duration, 10 s per irradiation.
- **Protocol 2:** 0.40 W, 10 Hz, 40 mJ per pulse, 50% air/water spray, energy density of 2.88 J/cm<sup>2</sup>, 100 µs pulse duration, 10 s per irradiation.

## Erosive–remineralization cycling

After treatments, all specimens were remounted on their acrylic resin bases using sticky wax (Asfer Indústria Química, São Caetano do Sul, Brazil). They then underwent a five-day erosive–remineralization cycling protocol, totaling 20 erosive challenges. The cycling model was based on the protocol described by Pereira et al. [22]. Each cycle consisted of immersion in 0.3% citric acid solution (Sigma Aldrich, Darmstadt, Germany; pH ~ 2.6) for 5 min, followed by immersion in artificial saliva for 60 min. The saliva composition included: 0.213 g/L CaCl<sub>2</sub>·2 H<sub>2</sub>O, 0.738 g/L KH<sub>2</sub>PO<sub>4</sub>, 1.114 g/L KCl, 0.381 g/L NaCl, and 12 g/L Tris buffer, with pH adjusted to 7.0 using 1 mol/L HCl solution. Four cycles were performed daily at room temperature (~ 25 °C). The acid solution was renewed for each erosive challenge, while the artificial saliva was replaced daily before the first cycle.

During the cycling period, specimens were placed in small plastic containers with perforated bottoms. These containers were inserted into larger ones containing 20 mL

of the respective solution, ensuring full immersion during the 5-minute erosive phase. The holes in the container base allowed rapid drainage of the solution after exposure. Excess solution was removed with absorbent paper after each cycle.

At the end of each day, specimens were rinsed with distilled water and stored under humid conditions using gauze moistened with distilled water, refrigerated at 4 °C until the next cycle. Upon completing the five-day protocol, the adhesive tapes were carefully removed using clinical tweezers.

### Determination of surface loss

Enamel surface loss was assessed using three-dimensional optical profilometry with the PROSCAN 2100 3D device (Scantron, Taunton, UK). Analyses were conducted at three distinct time points: after initial sample preparation and curvature selection (T0 – baseline), after erosion simulation in previously eroded specimens (T1), and after completion of the erosive–remineralization cycling protocol for all experimental groups (T2).

The profilometer sensor was programmed to scan a central area measuring 2 mm in length (X-axis) by 1 mm in width (Y-axis), encompassing the treated region and adjacent untreated reference surfaces on both sides.

Scanning was performed with a step size of 0.01 mm and 200 steps along the X-axis, and 0.1 mm with 10 steps along the Y-axis. Lesion depth (step height) was calculated by subtracting the average height of the treated area from the mean height of the two adjacent reference surfaces, using Proscan Application software (version 2.0.17, Scantron, UK).

Specimens were kept moist during the entire analysis process to avoid dehydration and potential structural changes. Excess moisture was removed immediately before scanning using absorbent paper.

### Environmental scanning electron microscopy (ESEM)

Following treatment and after the erosive–remineralization cycling protocol, three specimens from each experimental group ( $n=3$ ), totaling 36 samples, were randomly selected for evaluation by Environmental Scanning Electron Microscopy (ESEM) to qualitatively assess enamel prism surface morphology.

Analyses were conducted using a Hitachi TM3000 microscope (Hitachi, Tokyo, Japan), operated under Analy observation mode at 15 kV accelerating voltage. Representative micrographs were obtained at 2000× magnification, always captured from the center of each specimen in a pre-defined region to ensure consistency across samples. No prior specimen preparation was necessary to obtain the images.

In the qualitative assessment, the micrographs were evaluated for morphological characteristics of the enamel surface, allowing comparisons among the different experimental groups.

### Statistical analysis

The final surface loss data were tested for normality and homoscedasticity using the Shapiro–Wilk and Levene tests, respectively. As the data showed normal distribution and homogeneous variances, two-way analysis of variance (two-way ANOVA) was applied, followed by Tukey's multiple comparison test. A significance level of 5% ( $\alpha=0.05$ ) was adopted. Statistical analyses were performed using GraphPad Prism software, version 8.0.1 (GraphPad Software Inc., San Diego, CA, USA).

## Results

The values of enamel surface loss for the groups with sound and eroded enamel are presented in Table 2.

### Sound primary enamel

The surface loss values for the groups with sound enamel are presented in Table 2. The negative control group (C) showed the highest mean enamel loss ( $8.13 \pm 3.22 \mu\text{m}$ ), whereas the group treated with 0.40 W laser power combined with fluoride (L2+F) exhibited the lowest loss ( $2.16 \pm 0.91 \mu\text{m}$ ). In general, the combined treatments (laser+1.23% APF) demonstrated better performance in preventing surface loss in sound enamel when compared to isolated treatments.

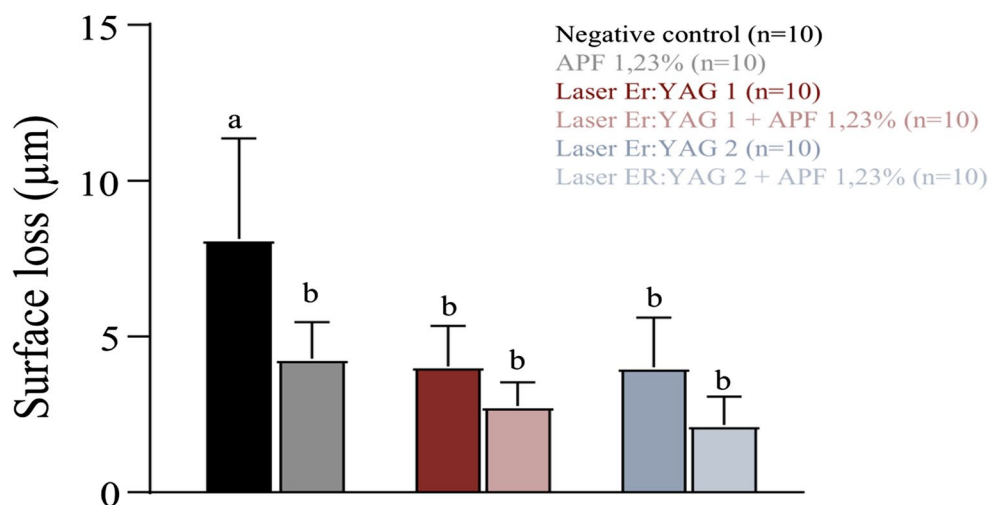
Data normality was confirmed using the Shapiro–Wilk test for all groups ( $p>0.05$ ). Levene's test indicated acceptable variance homogeneity ( $p>0.01$ ), allowing for two-way ANOVA application. The factors considered were the type of substrate (sound) and the different treatments. A significance level of 5% ( $\alpha=0.05$ ) was adopted.

ANOVA revealed a statistically significant effect of treatments on surface loss. Tukey's multiple comparisons

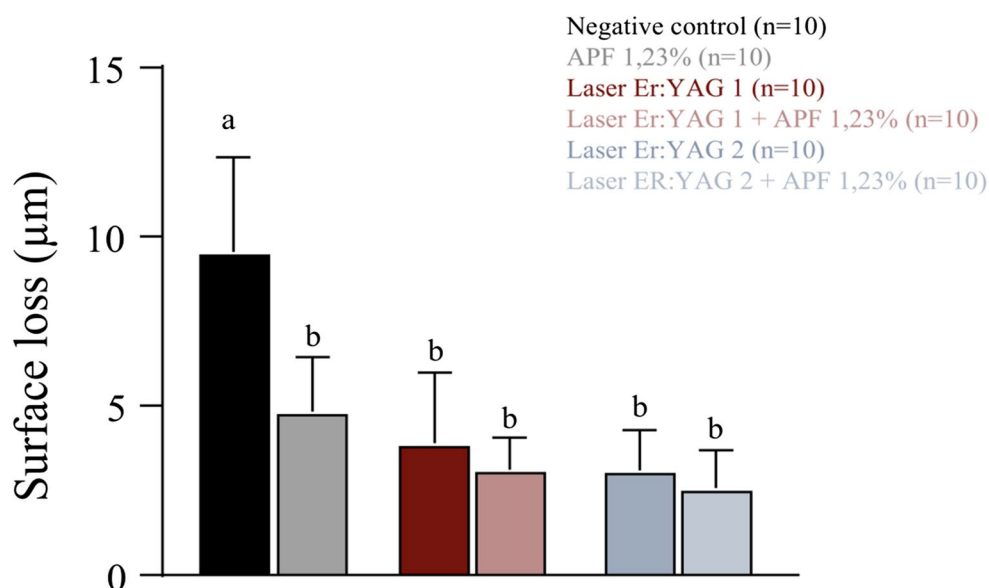
**Table 2** Means and standard deviations (SD) of enamel surface loss ( $\mu\text{m}$ ) for all experimental groups after treatments

Experimental groups	Sound	Eroded
	Mean $\pm$ SD	Mean $\pm$ SD
Negative control	8,13 $\pm$ 3,22	9,54 $\pm$ 2,79
APF 1,23%	4,29 $\pm$ 1,17	4,84 $\pm$ 1,61
Er:YAG 0,20W	4,03 $\pm$ 1,30	3,90 $\pm$ 2,10
Er:YAG 0,20W + APF	2,76 $\pm$ 1,65	3,12 $\pm$ 0,94
Er:YAG 0,40W	4,02 $\pm$ 1,59	3,10 $\pm$ 1,19
Er:YAG 0,40W + APF	2,16 $\pm$ 0,91	2,57 $\pm$ 1,11

**Fig. 1** Surface loss ( $\mu\text{m}$ ) of sound primary enamel: Representative graph showing the mean and standard deviation of surface loss (in micrometers) in sound primary enamel samples. Different letters indicate statistically significant differences between groups ( $p < 0.05$ )



**Fig. 2** Surface loss ( $\mu\text{m}$ ) of eroded primary enamel: Representative graph showing the mean and standard deviation of surface loss in micrometers for previously eroded primary enamel samples. Different letters indicate significant differences between groups ( $p < 0.05$ )



test indicated that the negative control group (no laser or fluoride) differed significantly from the groups treated with fluoride alone, laser alone (both 0.20 W and 0.40 W), and especially from the groups treated with the combined laser and fluoride therapies (L1 + F and L2 + F), with  $p < 0.0001$  in all these comparisons.

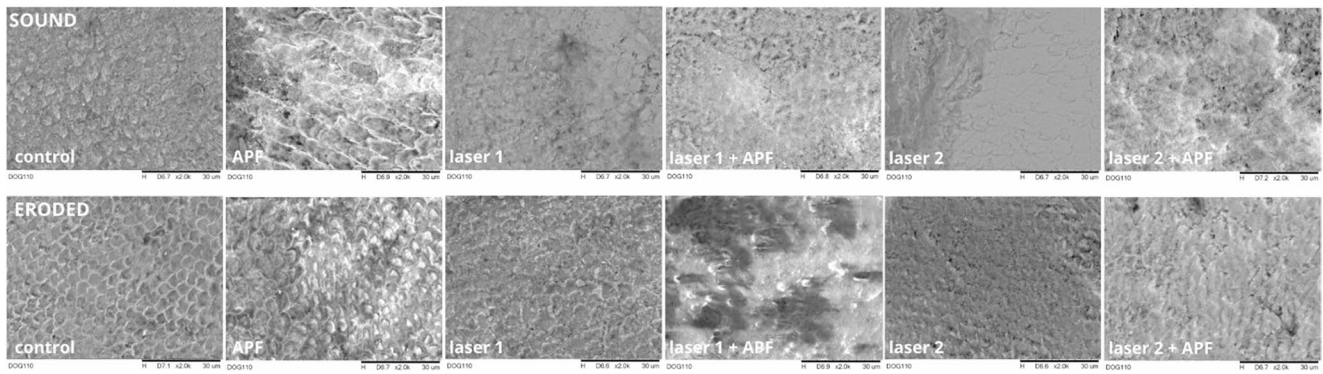
No statistically significant differences were found between the groups treated with laser only or fluoride only, nor between the two combined treatment groups (L1 + F vs. L2 + F) as observed in Fig. 1.

### Eroded primary enamel

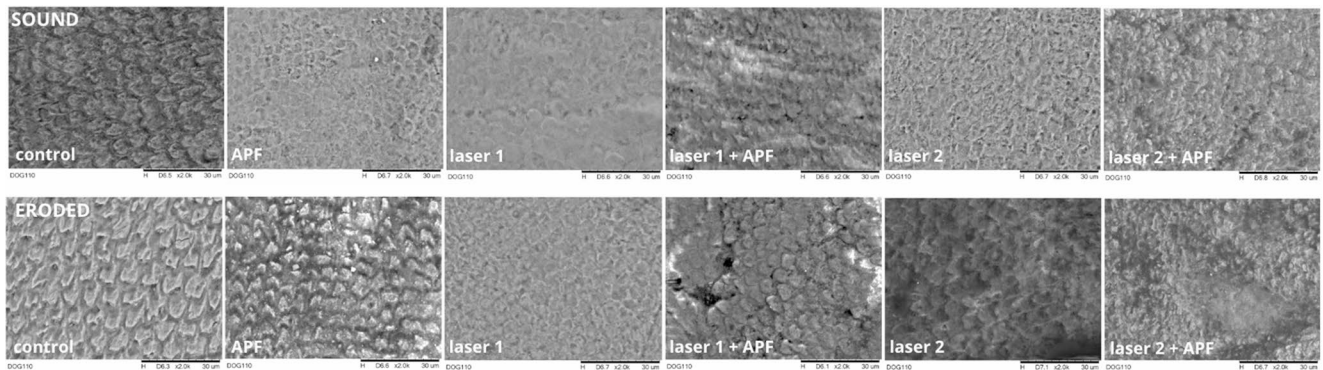
In the groups with previously eroded enamel, the surface loss values (SL) showed a clear protective trend in the treated groups, especially when laser and fluoride were combined. The highest loss was observed in the negative

control group ( $9.54 \pm 2.79 \mu\text{m}$ ), while the lowest was found in the L2 + F group (0.40 W laser + 1.23% APF), with  $2.57 \pm 1.11 \mu\text{m}$ . Normality was confirmed using the Shapiro–Wilk test ( $p > 0.05$ ), and variance homogeneity was verified using Levene's test ( $p > 0.05$ ), allowing for two-way ANOVA.

The ANOVA identified a statistically significant effect of treatments on enamel surface loss ( $p < 0.05$ ). Tukey's post hoc test revealed that the control group differed significantly ( $p < 0.0001$ ) from all other groups treated with fluoride alone, laser alone (L1 and L2), and particularly from the groups receiving combined treatments (L1 + F and L2 + F). No significant differences were found between the individual treatments or between the combined groups, suggesting that all tested protocols were effective in reducing the progression of surface loss compared to no treatment as observed in Fig. 2.



**Fig. 3** Environmental Scanning Electron Microscopy images prior to erosive-remineralizing cycling



**Fig. 4** Environmental Scanning Electron Microscopy images after erosive-remineralizing cycling

### Environmental scanning electron microscopy (ESEM)

In the ESEM images obtained after treatment (Fig. 3), the control group showed a regular enamel surface with no visible alterations. In the eroded control group, a more irregular surface was observed, with features characteristic of acid wear. In the APF-treated samples, scattered particles were seen on the enamel surface, with similar appearance in both substrates.

In the groups irradiated with Er: YAG laser, both at 0.20 W and 0.40 W, fusion of interprismatic spaces and the presence of small surface cavities were observed, along with mild irregularities. The surfaces appeared rougher, with noticeable changes in topography compared to the control.

In the groups subjected to the combined laser and APF treatment, laser-induced modifications were observed together with the presence of surface deposits located within the irregularities. These deposits gave the surface a shinier appearance.

After cycling (Fig. 4), the overall surface pattern was maintained across all groups. An increase in surface irregularities was observed in all conditions, with loss of the previously observed shine in APF-treated surfaces.

### Discussion

Dental erosion is currently considered one of the main causes of tooth structure loss in both children and adults [23]. Given the proven effectiveness of fluoride in preventing dental caries [24], its use has also been recommended for the prevention of dental erosion. Several studies have evaluated the protective effect of fluoride, either alone or in combination with other preventive strategies, against dental erosion [4, 22, 25]. Laser application, whether alone or combined with fluoride, has also been proposed as a means to increase enamel resistance to acid challenges [26, 27]. Research suggests that Er: YAG laser use can improve enamel resistance against acid exposure and caries formation [28, 29].

Considering the need for further investigations regarding the effectiveness of Er: YAG laser in erosion prevention, this study evaluated the effect of Er: YAG laser irradiation combined with topical fluoride application on surface loss in deciduous enamel under erosive challenge. Er: YAG laser application impacts the mineral composition and morphology of deciduous enamel [30], making it a promising tool to increase acid resistance in this substrate.

Our *in vitro* findings demonstrated that all tested treatments, in both sound and eroded enamel, significantly reduced surface loss when compared to the negative control group, which received no intervention.

Given the increasing availability of Er: YAG laser devices, it is relevant to highlight that the parameters used in this study were based on manufacturer recommendations. However, these protocols still lacked scientific validation under specific experimental conditions, reinforcing the importance of this investigation.

Due to the high absorption of erbium lasers by dental tissues and their positive effects observed in previous studies in reducing enamel demineralization [25, 29, 31], Er: YAG laser was employed in this study. To prevent mechanical damage to enamel, subablative parameters were adopted for irradiation.

Other laser types have also been investigated, such as Er, Cr: YSGG (2.78  $\mu\text{m}$ ), which has shown similar effectiveness to Er: YAG in modifying enamel surfaces and improving resistance to demineralization. According to Serdar et al. [32], laser application was as effective as tested remineralizing agents, including neutral sodium fluoride gel, APF gel (1.23%), NaF varnish, and CPP-ACP, with no significant differences in enamel microhardness between laser and fluoride-treated groups. These findings align with our results, which also did not find statistically significant differences between experimental groups, although a trend toward reduced wear was observed in all treated samples. CO<sub>2</sub> lasers, with a wavelength of 10.6  $\mu\text{m}$ , have demonstrated efficacy in forming a fused surface layer on enamel, contributing to increased resistance to erosion and abrasion. Due to their lower absorption, deeper tissue penetration occurs, potentially affecting thicker enamel layers. This feature suggests that the protective effect of CO<sub>2</sub> lasers against demineralization may be longer-lasting. Studies have shown that CO<sub>2</sub> laser treatments significantly reduced surface loss from acidic erosion, with effects lasting up to five days [23].

Studies have also evaluated diode and Nd: YAG lasers in the context of dental erosion. Diode lasers (810–980 nm) are primarily absorbed by melanin and hemoglobin, but their effects on dental tissues have been associated with thermal alterations favoring fluoride retention [33]. The Nd: YAG laser (1064 nm), although less absorbed by enamel, has proven effective in some studies by inducing structural changes in hydroxyapatite crystals, increasing acid resistance [34]. However, more studies are needed to define safe and effective parameters for both.

In the present study, although no statistically significant differences were found between the different treatments, the combination of Er: YAG laser (0.40 W) and fluoride (1.23% APF) in sound enamel resulted in the lowest mean surface loss among all experimental groups (mean =  $1.98 \pm 0.81$

$\mu\text{m}$ ). Although the p-value ( $\sim 0.07$ ) was not statistically significant under conventional standards ( $\alpha = 0.05$ ), it suggests a trend toward beneficial effects. This trend, though not statistically confirmed, may reflect a potential synergistic interaction between laser and fluoride, justifying the need for future studies with larger sample sizes. Furthermore, surface loss values were generally slightly higher in eroded enamel, reinforcing the increased vulnerability of this substrate and the importance of tailored strategies to limit ongoing tissue loss.

The qualitative analyses by ESEM supported profilometry data and provided insight into the mechanisms behind these effects. In control samples, well-defined enamel prisms were seen in sound specimens, while increased interprismatic spacing characterized acid demineralization in eroded ones. APF-treated groups showed spherical surface deposits compatible with calcium fluoride (CaF<sub>2</sub>), as described by Attin et al. [35]. These deposits are known to act as fluoride reservoirs and contribute to protection against acid challenges. In samples irradiated with Er: YAG laser, fusion of interprismatic spaces and the presence of small cavities typical of subablative microablation were noted, producing rougher topography. When laser was combined with APF, CaF<sub>2</sub> deposits accumulated within laser-induced surface irregularities, resulting in a shinier appearance and suggesting enhanced fluoride retention. This synergistic interaction between physical surface modification and chemical fluoride action has also been described by Esteves-Oliveira et al. [36].

After erosive cycling, increased surface irregularities were observed in all groups, consistent with the repeated acid exposures. In the APF-treated specimens, a reduction in surface shine suggested partial dissolution of the CaF<sub>2</sub> layer, confirming its limited stability in acidic environments. This agrees with previous reports emphasizing that fluoride protection decreases under repeated erosive challenges [37].

Topically applied fluoride agents remain among the most recommended approaches for erosion prevention [38, 39]. Monovalent fluorides such as sodium fluoride (NaF) and amine fluoride (AmF) promote CaF<sub>2</sub> deposit formation on enamel surfaces, acting as temporary barriers against acids and releasing fluoride ions upon dissolution, contributing to remineralization. However, this protection is limited, especially with frequent acidic exposures [16, 37].

APF's protective effect is largely attributed to CaF<sub>2</sub> formation, which acts as a fluoride reservoir during acid challenges and facilitates enamel remineralization. Tenuta et al. [24] demonstrated that CaF<sub>2</sub> formation is directly related to fluoride availability and agent pH, highlighting the stabilizing role of CaF<sub>2</sub> in acidic environments. Reviews on dental erosion emphasize that this protective layer's formation depends on agent concentration, pH, and application

frequency, being more effective with agents of  $\text{pH} \leq 5$  and frequent applications [40].

After cycling, a loss of surface gloss was noted in APF-treated groups, along with increased surface irregularities in all samples, indicating the cumulative effect of acid challenges despite prior treatment. This supports previous findings showing that while interventions may attenuate surface loss, they do not eliminate enamel susceptibility to acid wear. Similar conclusions were drawn by O'Toole et al., who highlighted the limitations of fluoride protection under repeated acidic exposure [37].

The application sequence of laser and fluoride has been identified as a critical factor for preventive efficacy. For instance, studies using Nd: YAG lasers showed that irradiation before APF application led to more stable  $\text{CaF}_2$  deposits but also accelerated lesion formation in prolonged demineralization cycles, indicating complexity in sequential effects [22, 34]. In contrast, Dos Reis et al. compared various treatment orders with Er: YAG laser and found that both laser + fluoride (L + F) and fluoride + laser (F + L) reduced demineralization, with L + F yielding lower mineral loss [25].

These data suggest that laser application before fluoride may optimize enamel surface modification, enhancing fluoride retention and effectiveness. In this study, although no statistically significant differences were found, the Er: YAG laser followed by 1.23% APF resulted in the lowest mean surface loss in sound enamel, supporting trends observed in the literature. Thus, this sequence can be considered promising for future preventive protocols.

It is also well established that deciduous teeth are structurally more susceptible to acid wear due to thinner and less mineralized enamel, which intensifies tissue loss under erosive challenges. Rocha et al. reported that agents like stannous fluoride or laser combinations may show limited effectiveness in deciduous substrates [41]. Nevertheless, evidence suggests that combining fluoride with erbium lasers could be a promising strategy for pediatric populations, especially due to laser-induced surface modifications that favor  $\text{CaF}_2$  retention. Contreas-Bulnes et al. also reported beneficial effects of this combination, though results varied depending on parameters and substrate type [42].

Supporting our findings, Asadollah et al. evaluated the resistance of deciduous and permanent enamel to erosion after Er: YAG laser irradiation, with or without APF application, using microhardness as the outcome [43]. Although no statistically significant differences were found, lower microhardness loss was observed in combined treatment groups, particularly when fluoride preceded the laser. These results reinforce the hypothesis that the combination may reduce surface loss even when statistical significance is not reached, potentially due to laser-induced topographic modifications enhancing fluoride retention.

Consistent with this, other investigations have demonstrated that Er: YAG laser irradiation induces morphological changes in enamel that influence the clinical performance of preventive materials. Unal et al. reported that, in primary teeth, Er: YAG used alone led to higher microleakage of fissure sealants, whereas its combination with acid etching reduced leakage and improved marginal sealing [44]. In permanent teeth, the same group observed that higher power settings of Er: YAG, particularly when associated with adhesive systems, significantly enhanced the bond strength of sealants containing amorphous calcium phosphate after aging [45]. Together, these findings indicate that the laser's effects vary according to tooth type, substrate condition, and protocol, underscoring the relevance of further research on deciduous enamel subjected to erosive challenges.

Despite these advances, there is still a gap in the literature regarding the effect of preventive strategies on deciduous enamel, particularly when comparing sound and previously eroded substrates. Most previous investigations have focused on permanent teeth or on single preventive approaches, leaving unanswered questions about how primary enamel responds to combined interventions with Er: YAG laser and fluoride. By addressing both preventive and progression scenarios, the present study contributes to filling this gap and provides a more comprehensive view of erosion control in primary dentition. In spite of the promising outcomes observed, this study has limitations inherent to its *in vitro* design, which does not fully replicate clinical conditions such as salivary flow and composition, dietary and hygiene habits, or individual exposure variability to erosive agents. Additionally, although profilometry is effective for measuring surface loss, it does not provide information on other relevant outcomes, such as changes in enamel microhardness, mineral release, or compositional alterations of the mineral phase, which could be better investigated with complementary techniques like Fourier-transform infrared spectroscopy (FTIR). Future studies incorporating these additional analyses, as well as *in situ* and clinical models, are necessary to confirm and expand the present findings.

It is also worth noting that monovalent fluorides like 1.23% APF form  $\text{CaF}_2$  deposits are highly soluble in acidic environments. Therefore, long-term effectiveness studies are essential, as Er: YAG laser irradiation may represent a more lasting approach by inducing physicochemical changes in enamel, increasing its acid resistance. Future studies should also explore polyvalent fluorides, such as stannous fluoride ( $\text{SnF}_2$ ) or titanium tetrafluoride ( $\text{TiF}_4$ ), which have shown greater stability and resistance to acid challenges in previous research [46]–[47].

Thus, *in situ* and randomized clinical trials are indispensable to validate the efficacy and durability of these interventions and enable individualized protocol adjustments based

on patient risk. Despite limitations, our findings reinforce the potential of the Er: YAG laser combined with fluoride as a safe and promising alternative for erosion prevention, especially in vulnerable pediatric populations.

## Conclusion

Within the limitations of this in vitro study, it can be concluded that treatments using Er: YAG laser, acidulated phosphate fluoride (APF), and their combinations were effective in reducing wear in deciduous enamel, both sound and eroded, when compared to the control group. This suggests that the tested protocols have potential for preventing and controlling the progression of erosive tooth wear. However, no statistically significant differences were observed between the treatments, indicating that no protocol stood out as superior. Further studies are needed to enhance comparisons among different strategies and validate their effectiveness under clinical conditions.

**Author contributions** C.C.C, A.C.C.A designed the project and methodology of the research, L.M.C.C performed the statistical analysis, C.C.C, M.L.Y and L.M.C.C performed the laboratory steps, D.M.Z, A.C.A coordinated the research and C.C.C, A.C.A., L.M. C.C., M.L.Y wrote the main manuscript text. All authors reviewed the manuscript.

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## Declarations

**Consent to participate** Not applicable.

**Competing interests** The authors declare no competing interests.

**Clinical trial number** Not applicable.

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