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Enamel Erosion Reduction through Coupled Sodium Fluoride and Laser Treatments before Exposition in an Acid Environment: An In Vitro Randomized Control SEM Morphometric Analysis

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Abstract: (1) Background: Erosive lesions of dental enamel are steadily increasing owing to both the availability of exogenous acid and the production of endogenous acid. The aim of this study was to investigate the erosion-inhibiting potential of a diode laser irradiation and topical application of fluoride used alone or in combination on the enamel surface of extracted teeth before exposure to an acidic solution. (2) Methods: The four axial enamel surfaces of 40 healthy molars were used for four study groups: (A) no treatment; (B) application of fluoride gel for 120 s; (O) a diode laser application for 120 s; and (X) a combined laser/fluoride for 120 s. Each enamel surface was examined by SEM (scanning electron microscopy). (3) Results: At 700× magnification, it was possible to detect the enamel prisms of the test area of groups A, B, and O, while no structures such as enamel prisms were highlighted for group X because they were covered by an amorphous layer. The mean number of prisms $\times 1000\ \mu m^2$ was 7.2 units with an SD of 0.72 for group A, 8 units with an SD of 0.96 for group B, and 4.8 units with a SD of 0.4 for group O. Student's *t*-test showed no significant difference between group A and B with a $p = 0.054$. Group O showed a significant reduction of prims $\times 1000\ \mu m^2$ compared with group A ($p = 0.0027$) and group B ($p = 0.0009$). Student's *t*-test showed no significant difference between groups A and B with a $p = 0.054$. Group O showed a significant reduction of prims density with respect to group A ($p = 0.0027$) and group B ($p = 0.0009$). (4) Conclusions: This amorphous layer might be correlated with the effect of laser on enamel, which reduces both water and carbonate ion; increases the crystallinity of hydroxyapatite, and improves the mechanical properties of enamel; which is responsible for greater protection expressed by the enamel of group X against acid attacks.

Keywords: enamel erosion; diode laser; fluoride; demineralization

1. Introduction

The goal of modern dentistry is prevention rather than treatment of dental caries. Topical fluoride in conjunction with oral hygiene is the most important means of prevention. Fluoride acts through three main mechanisms: it improves the resistance of the enamel, facilitates remineralization of initial enamel lesions, and interferes with microorganisms by inhibiting bacterial metabolism and the enzymatic process [1].

The most common forms of topical administration of fluoride are toothpaste, gel, varnishes, and mouthrinses. The protective effect of fluoride is due to the formation of a superficial CaF layer that prevents the enamel from being exposed to acids and dissolution [2–4]. However, some studies have shown that the efficacy of fluoride therapy in combating dental erosion is limited because it correlates with the constant presence of fluoride in the oral cavity. This means that, in order to achieve a protective effect, products with a higher fluoride concentration and a longer retention time must be used. For this reason, the use of fluoride to prevent erosive processes would require high patient compliance [5]. Recently, lasers have been proposed as an innovative procedure against the formation of dental caries or to support conventional therapy.

In fact, many researchers propose lasers to improve conventional preventive therapies. Several lasers have been investigated, both alone and in conjunction with fluoride, to induce the formation of more resistant enamel to acid-induced dissolution. In the literature of recent years, there are numerous studies confirming the beneficial effects of diode laser on enamel, either alone or in combination with fluoride, increasing its resistance to acids or favoring the fluoride uptake, making it more resistant to caries [6–9]. The application of laser on dental hard tissues leads to changes in the chemical and mechanical properties in the enamel, increasing the acid resistance of the lasered enamel and decreasing its permeability. The heat developed by the laser light not only prevents the formation of caries on the enamel surface, but also promotes the loss of water and carbon, the decomposition of the organic matrix, and the formation of refractory hydroxyapatite phases such as calcium phosphate or calcium pyrophosphate [10]. Moreover, in combination with topical fluoridation, it promotes the incorporation of fluoride into the hydroxyapatite structure, facilitating the formation of fluorohydroxyapatite and calcium fluoride (CaF_2) both on the surface of enamel and within its crystalline structure, which serves as a fluoride reservoir against caries and facilitates the process of demineralization [11]. The effects of different types of lasers on the ultrastructure of enamel are controversial, and it is difficult to determine which is the best outcome. The variety of these changes is related to the different laser parameters used (modality of laser irradiation emission, wavelength, energy output, and exposure time) and the optical properties of the lasered tissues (hydration state, presence of chromophores, and their absorption coefficient). Vitale M.C et al. (2011) evaluated the treatment with fluoride in combination with a diode laser and studied the laser-induced changes in the composition (contents in F) of enamel after laser irradiation and topical fluoride application [12]. The chemical analyses performed with the fluoride ion-selective electrode showed increased fluoride uptake for the group of teeth treated with diode laser and fluoride gel application compared with the teeth of the untreated group and the group of teeth treated with amine fluoride gel only. The conclusions of the study were that the combination of topical fluoridation and diode laser seems to be a promising preventive measure for dental caries and could be useful for effective and immediate fluoridation of teeth.

The aim of this study was to investigate the erosion-inhibiting potential of a diode laser irradiation and topical application of fluoride used alone or in combination on the enamel surface of extracted teeth before exposure to an acidic solution.

2. Materials and Methods

Scanning electron microscopy (SEM) analysis was performed on the mesial, lingual, distal, and buccal surfaces of the permanent molars. The surfaces were untreated or treated with fluoride gel or laser or coupled fluoride-laser.

2.1. Study Design

Each surface was considered as a single unit and was subjected to a randomization process and further SEM analysis. Four groups were defined according to the clinical procedures to be tested and one control group. Some letters were chosen to identify the study groups, which could not be mistaken after immersing the samples in the acidic environment (A, B, O, and X). Group A: untreated surfaces. Group B: surfaces treated with fluoride-gel. Group O: surfaces treated with laser. Group X: surfaces treated with combined fluoride-gel and laser. Randomization was done in a 1:1:1:1 ratio.

Five investigators were used for the study (three clinical and two non-clinical).

2.2. Sample Selection and Inclusion Criteria

The procedures of this study were in accordance with the institutional and national ethical standards on human experimentation and the Declaration of Helsinki of 1975, as revised in 2000, and they were approved by the Ethics Committee of the AORN of Ospedali dei Colli with N. 117 del 19 February 2020.

The first operator selected 40 healthy permanent molars extracted because of over-retention or lack of space. Inclusion criteria were as follows: absence of both carious lesions and fractures and excessive abrasion, presence of restoration. Samples were collected from patients of both sexes (15 males and 10 females). The age of patients ranged from 20 to 50 years. All patients were treated in the dental clinic of the multidisciplinary department of medical-surgical and dental specialties of the University of Campania "Luigi Vanvitelli" in Naples.

The freshly extracted molars, after being washed with spring water, were immersed and kept in a hermetically sealed glass container containing a 0.1% thymol solution and stored at 4 °C.

At the beginning of the search, each tooth was removed from the container and rinsed abundantly in distilled water for 2 min. The second examiner numbered each specimen by putting a sequential number with a pencil in correspondence with the root tooth and marking the axial enamel surfaces of each tooth crown (vestibular, palatal, mesial, and distal) with a letter (A, B, O, and X) on the corresponding root surface (Figure 1a). In this way, it was possible to obtain four groups for the 40 specimens to test 4 different therapeutic interventions designed to evaluate the protective effect, from acid liquid, on the corresponding enamel surface. The same staff member then delineated a space of approximately 2 mm $\times$ 4 mm (2 mm in height and 4 mm in width) for each tooth by making of an incision performed by a 0.8 mm diameter diamond ball drill (FG) in correspondence with the enamel of each of the four axial surfaces; this window will represent the test area for the clinical trial. The third examiner, who was not a clinician, randomly assigned each area test of each tooth to a specific therapeutic program to be tested using a computerized random distribution program. The fourth examiner, who was not a clinician, performed a preliminary scanning of the spot areas of each sample using a SEM (Zeiss EVO of Carl Zeiss Microscopy, White Plains, NY, USA), before administering a therapeutic protocol.

In this way, photographic areas of the four surfaces of enamel of interest at time zero (healthy enamel) were obtained for a single sample belonging to the four study groups and compared with the same photographic images taken after erosive treatment. The SEM analyses were carried out at the Advanced Materials Laboratory of the Department of Architecture and Industrial Design of the University of Campania "Luigi Vanvitelli". The samples to be analyzed were taken from the preservative fluid, rinsed, placed on bibula paper, and left to dry overnight. At the time of analysis, each tooth was attached on a metal support (grounded) of SEM with graphite-based adhesive tape (Figure 1b). For all study samples, scans of the four spot surfaces were performed in high and low vacuum mode by an electron detector (backscatter) with high sensitivity at different magnifications to identify the structure and morphology of the enamel surface before each type of treatment. In an analysis of the SEM in no spot area of the enamel, an alteration of the gray scale was highlighted that could be traced back to any type of structural change in the enamel. The subdivision of the tooth crown into four areas allowed the identification and formation of four study groups for each tooth.

(**a**) (**b**)

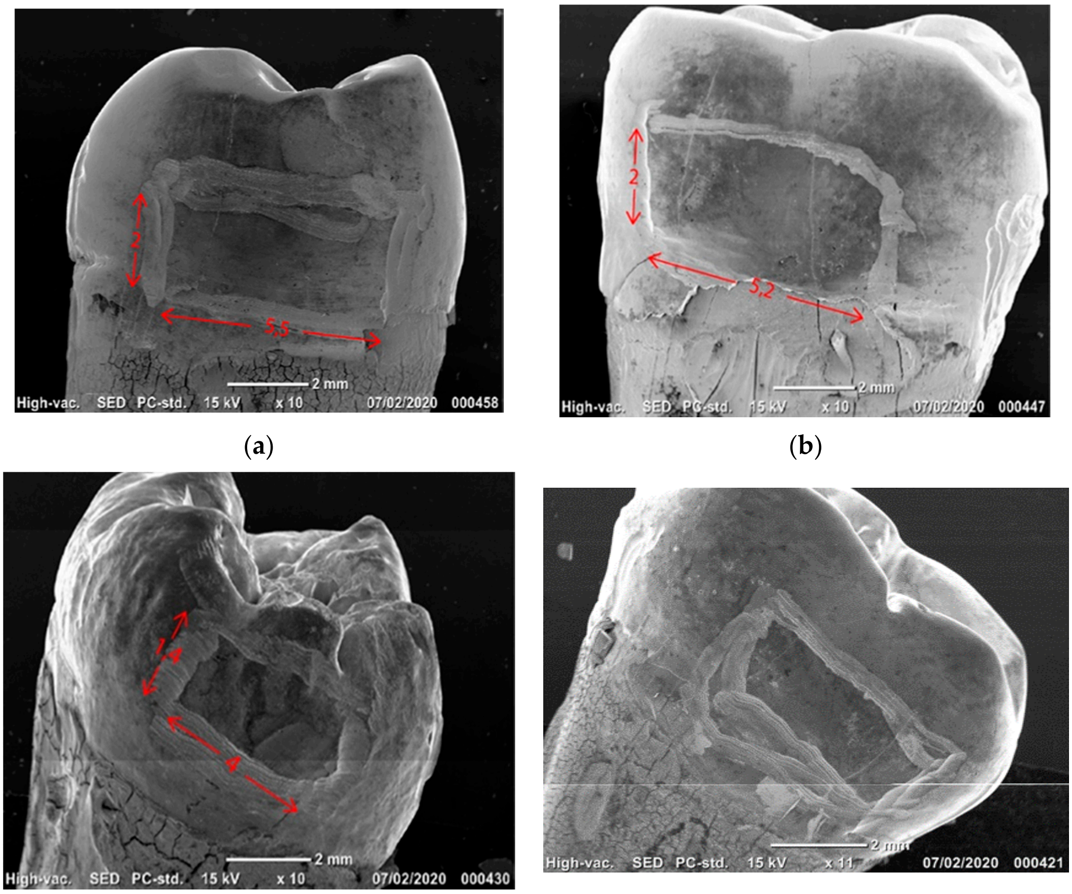
Figure 1. (**a**) Example of specimen numbering and labeling of axial surfaces. (**b**) Preparation of specimen for SEM vision.

The fifth clinical operator was used to administer, in correspondence of the area test, the various treatment protocols to oppose the demineralizing action of an acidic solution according to the following scheme:

- Group A: no pretreatment was administered (control group).
- Group B: a 0.33% acidified sodium fluoride gel (MEDICAL®, Treviso, Italy) was used and applied in contact with the enamel for 120 s.
- Group O: irradiation procedure with a 300-micrometer fiber of a soft touch diode laser (Creation Medical Laser 810 nm, 5 W; Ennebi Elettronica S.r.l. Novedrate—CO; Italy) with a power of 0.2 Watt in continuous mode for 120 s was administered. The tip of the fiber was kept at a maximum distance of about 1–2 mm from the enamel of the areas test by moving it in a circular direction to cover the entire surface of interest (low level laser therapy: LLLT).
- Group X: the procedure used involved a combined action with the application of a 0.33% acidic sodium fluoride gel held in contact with the enamel surface for 120 s and, at the same time, the area test was irradiated with a soft touch diode laser (Creation Medical Laser 5 W) with the same modalities and times as in Group O.

The first clinician, who had already been employed in the selection of the specimens, was again summoned to subject the selected teeth to an erosive treatment based on an acid solution. Indeed, after the completion of pretreatments, all the specimens were rinsed with distilled water for 2 min and, after waiting for about 30 min, they were immersed in a solution of hydrofluoric acid, with a concentration of 1 molar for 30 min, and renewed for each tooth. At the end of the exposure period, all teeth were retrieved from the acid solution and rinsed thoroughly with distilled water for 2 min, and then viewed again with SEM vision by a fourth researcher.

The images coming from four surfaces of each tooth were examined after exposure to the acid solution to evaluate the protective effect provided by each of the procedures used for each single study group. The quantification of the data collected on SEM was carried out using energy dispersion spectrometry (EDS) equipped with a spectrometer consisting of a detector, capable of separating the characteristic rays, and a counter that measures the distribution of the intensity of the X-rays produced by the electron beam on the sample. In this way, the properties of the surfaces could be quantified using the gray values in relation to the total area and compared with the gray values for the same surface before the study. Figures 2–4 show some phases of our research.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. SEM images of each test area for each study sample after immersion in the acid solution. Group A (**a**), Group B (**b**), Group O (**c**), and Group X (**d**).

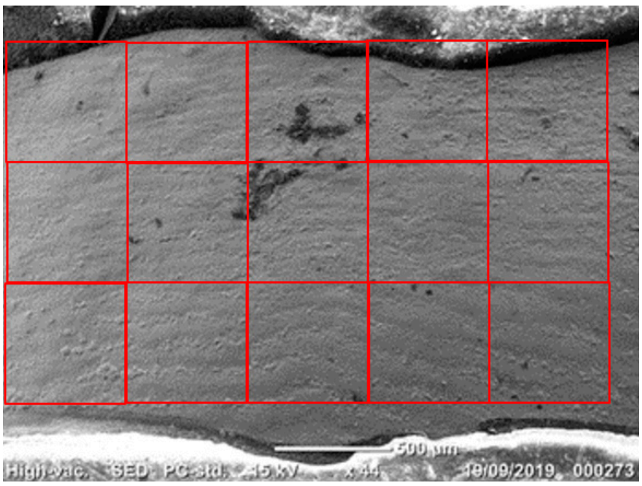
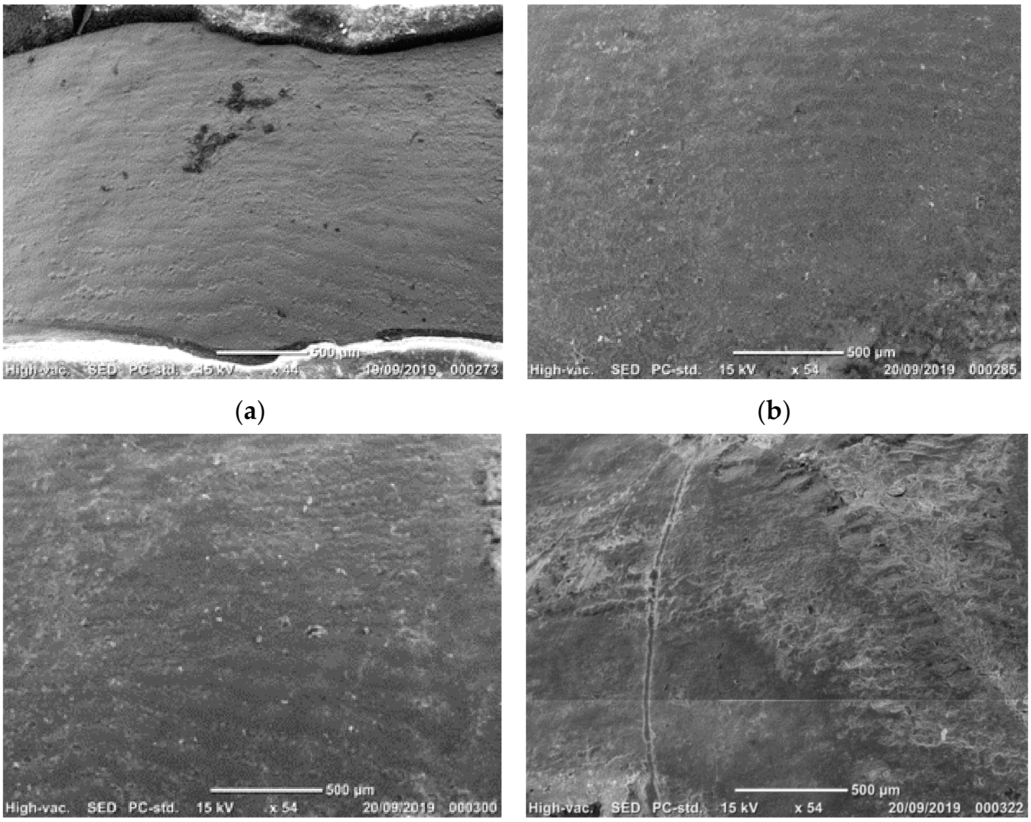


Figure 3. Example of a test area after exposure to the acidic solution and superimposition of a grid used to measure morphological characteristics and quantify surface properties.



(**a**) (**b**)

(**c**) (**d**)

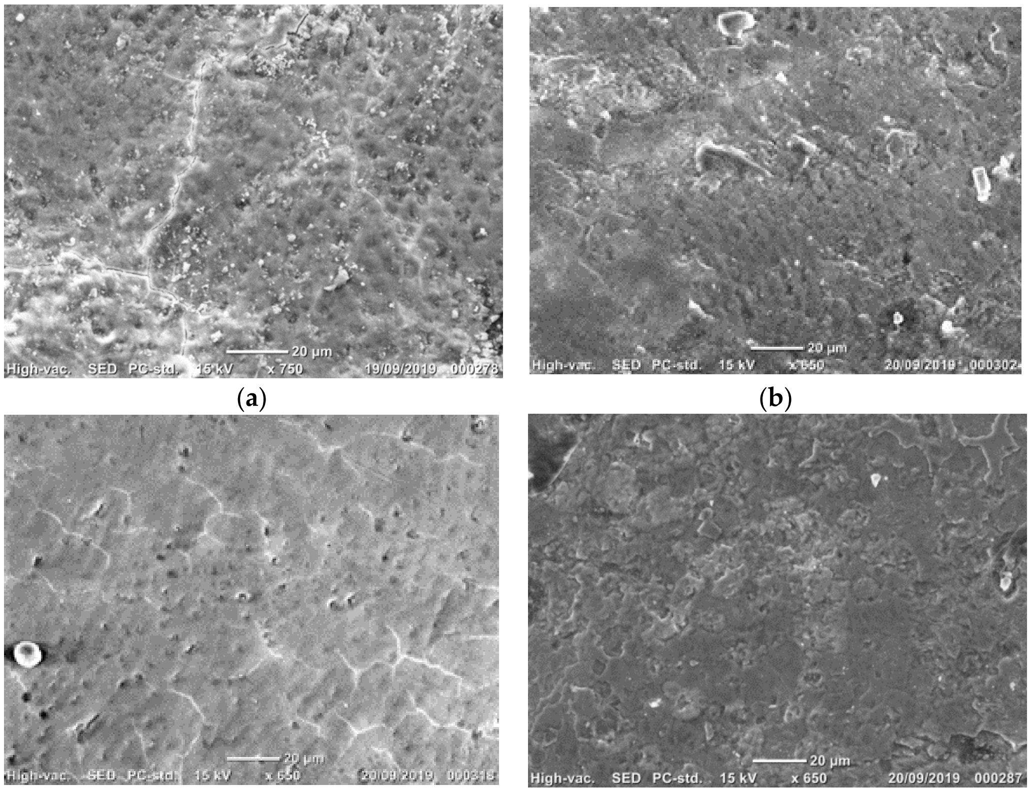
Figure 4. This figure shows the images of the enamel surface of area test of Groups A, B, O, and X obtained by SEM) at 54× magnification. The scale length was set at 500 µm and the images represent an area of 2600 µm × 2000 µm. Debris can be observed on the enamel surface of all the samples. Retzius striae with width ranging from 150 to 200 µm were observed in samples from Groups A (**a**), B (**b**), and O (**c**), although they are less evident with respect to the sample from Group A (**a**). In sample X (**d**), no striae are observed and the enamel appears to be camouflaged by an amorphous layer.

3. Results

At 700× magnification, it was possible to clearly see the enamel prisms on the surface of samples A, B, and O (Figure 5a–c, respectively). In contrast, no structures resembling prisms could be observed on the enamel surface of the area test of Group X (Figure 5d). Although prisms are visible in Figure 5a–c, the enamel surface and prisms appear different in the three images. In sample A, the prisms are evenly spaced and have open concave centers with well-defined peripheral prism outlines and their surfaces are also at the level of the tooth surface; this type of prism is referred as a "well-defined prism" in the text. In samples B and O, smoothly rounded prisms were observed; they are arranged uniformly on the surface with delineated prism outlines, and their surface is in the level with the tooth surface; this type of prism is referred to in the text as a "shallow prism". The enamel surface of sample X appeared with an amorphous layer. Each image covers an enamel area of 19,600 µm^2.

For each group, four surfaces were viewed under 54× and 700× magnification. Prisms' counting was carried out at 700× magnification on each analyzed surface. The resulting prims count on each examined surfaces was expressed as prisms count per surface in µm^2. In Group A, the mean prism number on each surface examined was 7.2/1000 µm^2 with a standard deviation of 0.72/1000 µm^2. The Kolmogorov–Smirnov test for the surfaces of Group A showed a normal distribution of the number of prisms between analyzed surfaces, with D = 0.26 and p = 0.8. In Group B, the mean number of prisms measured by each analyzed surface was 8/1000 µm^2, with a standard deviation of 0.96/1000 µm^2. The

Kolmogorov–Smirnov test for the surfaces of Group B revealed a normal distribution of the number of prisms between the analyzed surfaces, with D = 0.16 and $p = 0.99$. In Group O, the mean number of prisms measured by each analyzed surface was 4.8/1000 μm^2, with a standard deviation of 0.4/1000 μm^2. The Kolmogorov–Smirnov test for the surfaces of group O showed a normal distribution of the number of prisms between the analyzed surfaces, with D = 0.21 and $p = 0.94$. Student's t-test with significance set at $p = 0.01$ revealed no significant difference between Group A and B with a $p = 0.054$, meaning that prism density was similar between Group A and B. Group O showed a significant decrease in prism density compared with Group A ($p = 0.0027$) and Group B ($p = 0.0009$). Statistical analysis was also performed with RM one-way ANOVA test, where F = 458, $p < 0.0001$ with Geisser–Greenhouse's epsilon correction: 0.6846 and R: 0.9892.



(**a**) (**b**) (**c**) (**d**)

Figure 5. This figure shows the images obtained by SEM, under 700× magnification, of an area test from samples of Groups A (**a**), B (**b**), O (**c**), and X (**d**). SEM images represent an enamel surface of 168 µm width and 120 µm height resulting in an area of 19,600 μm^2. A scale length of 20 µm is reported in each image.

4. Discussion

The surfaces of enamel that were untreated, after exposition to the acid solution (Group A), have a greater number of visible prisms than the surfaces of enamel that were treated with the diode laser before being exposed to the acid solution (Group O). On the contrary, the untreated enamel surfaces exposed to the acid solution (Group A) exhibit a number of prisms statistically equivalent to those observed on enamel surfaces treated with fluorine and then exposed to the acid solution (Group B). However, a marked difference is observed between the prisms of enamel of Group A and those observed in Group B. The enamel surfaces of the Group A samples exhibit prisms that are defined as "well defined", and show a central concavity. In contrast, the prisms observed in Group B are defined as "shallow", and these are characterized by a protuberance with respect to the surrounding surface and, apparently, resembling buttons. Such aspects of the enamel surface have previously been identified and described by Akasapu A et al., 2018 [13]. The main difference between "well-defined" and "shallow" prisms lies in the amount and quality of material of which they are made. Well-defined prisms would appear as a surface from which material has been subtracted, exposing the central part of the prism. On the other hand, "shallow" prisms appear as a prism filled with a material added later. This hypothesis would be in line with the nature of the formation of fluorapatite crystals that could fill the concavities of the prisms and make them appear as reliefs with respect to the surrounding surface.

On the other hand, the results obtained from Group X reveal a greater preservation of the structure of the enamel prisms in an acid environment when these are pretreated by a combined procedure using a fluorine gel associated with a diode laser irradiation, compared with the use of laser and topical fluorine individually. This is probably due to presence of amorphous material appearing to cover the enamel surface, preventing the demineralization of underlaying enamel prisms.

This is in agreement with other studies that have used the application of laser and fluorine to protect the surface of enamel when exposed to an acid environment. It became clear that laser treatment significantly increases the deposition of fluoride on the surface of enamel when it is locally associated in gel. This uptake is even greater when fluoride is simply applied topically, and irradiation of laser passes through it before reaching the enamel surface [14–16]. Our results show that irradiation of enamel with diode laser can protect enamel when exposed to the acidic environment with greater efficacy than topical application of fluoride gel alone, but in any case, statistically lower than when combined with topical application of fluoride. Similar results have been reported in the literature with lasers of different wavelengths (CO_2, argon, and Nd-Yag lasers) when applied to the surface of enamel as part of programs that use fluoride in topical form [17–19]. Unfortunately, a comparison with these different wavelengths is not possible because the diode laser has a low absorption coefficient for enamel. In our study, the diode laser was used at low power (LLLT). While there are data in the literature demonstrating the protective effect of fluorine due to the exchange of fluorine with calcium ions, responsible for the transformation of hydroxyapatite into fluoroapatite, which is less soluble in an acidic environment, there are no studies that can demonstrate the protective effect of lasers on the enamel surface, but only hypotheses, especially for lasers used with biostimulation powers [20]. It is hypothesized that laser irradiation affects enamel water content and hydroxyapatite components such as carbonate, phosphate, and calcium ions. In fact, both water and carbonate ion contents decreased significantly, especially with laser irradiation, which would increase the crystallinity of hydroxyapatite and provide a relative improvement in the mechanical properties of enamel [6].

This may justify the presence of this amorphous substance, which is found on the surface of the enamel when irradiated forming a kind of surface layer and has an additional protective value in an acid environment. Our study is in agreement with the research conducted by Vitale M.C. et al., in 2011 [12], which showed that the absorption of fluoride ion in enamel is greater when irradiation with a diode laser is applied after the application of the amine fluoride gel on the enamel surface than when irradiation with a diode laser is applied before the application of the amine fluoride gel on the enamel surface or than when only the fluoride gel is used. This suggests that the diode laser increases the absorption of fluorine when it radiates the enamel through a fluorine gel layer. In studies conducted by Abdallah M et al., in 2019 [21–23], the microhardness induced by diode laser and fluoride ions on the outer layer of enamel was evaluated and tested. The results of the study showed that the application of fluorine paint and diode laser irradiation significantly increased both the microhardness of enamel and fluoride uptake compared with the control group, and this significantly reduced the demineralization of enamel after immersing the samples in an acidic liquid. In addition, the authors hypothesized that increased resistance of enamel to demineralization after diode laser irradiation could be attributed to the surface melting and recrystallization of the hydroxyapatite crystals of the enamel, to the decrease in the permeability and solubility of enamel, and to the greater increase in precipitation of calcium fluoride. Oho T. and Morioka T. in 1990 [24] suggested that the greater absorption of fluorine in enamel treated with a laser may be related to the thermal effects of the laser itself, which may cause changes in the surface layer of enamel (such as roughness) responsible for promoting the retention and absorption of fluoride in enamel. This could explain the results of our research in which the enamel, of the samples under study, pretreated with fluorine gel and then irradiated with a diode laser, limited the demineralizing effects on the enamel prisms after exposure to an acid environment more than the samples pretreated with laser and fluorine used individually. Certainly, the limitations of our study are the in vitro protocol and that the data obtained from our research require studies with a larger number of samples, but we believe that we can advance the possibility of recommending a standardized protocol that can be used for the prevention of enamel demineralization to be used in patients who have a structural alteration of the enamel, who are taking drugs for therapeutic purposes that cause a constant decrease in salivary pH, or who have limited oral hygiene options.

5. Conclusions

The amorphous layer might be correlated with the effect of laser on enamel, which reduces both water and carbonate ion; increases crystallinity of hydroxyapatite; and improves the mechanical properties of enamel, which is responsible for greater protection expressed by the enamel of Group X against acid attacks.

Author Contributions: Data curation, F.F.; Formal analysis, R.F. and L.F.; Investigation, F.F., R.F., L.F., D.A. and M.S.; Methodology, N.S. and V.G.; Project administration, F.F., A.A. and D.A.; Software, L.N.; Supervision, N.S. and R.F.; Visualization, R.A. and V.P.; Writing—original draft, F.F., A.A. and D.A.; Writing—review and editing, F.F. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of AORN of Ospedali dei Colli with N. 117 del 19 February 2020.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study to email: davide.apicella@calabrodental.it and femiano@libero.it.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

SEM	Scanning electron microscopy
LLLT	low level laser therapy
EDS	energy dispersion spectrometry

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