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Original Article

Effect of Nd:YAG Laser and Acidulated Phosphate Fluoride on Bovine and Human Enamel Submitted to Erosion/Abrasion or Erosion Only: An *in Vitro* Preliminary Study

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Abstract

Objective: The aim of the present *in vitro* study was to evaluate, using two different methodologies, the effectiveness of pulsed Nd:YAG laser irradiation associated with topical acidulated phosphate fluoride (APF) for preventing enamel erosion and structure loss under regimes of erosion and abrasion or erosion only. ***Background Data:*** An increased incidence of noncarious lesions (erosion and abrasion) has been observed, consequently new preventative therapies have been proposed. ***Materials and Methods:*** Two different methodologies were performed. For the first, 100 bovine crowns were submitted to four different treatments ($n = 25$): no treatment (control), 4 min application of APF, Nd:YAG laser irradiation (1 W, 100 mJ, 10 Hz, 141.5 J/cm^2), and Nd:YAG laser irradiation + 4 min of APF. After the specimens were exposed to citric acid (2% w/v; 30 min), they were submitted to 5000 brushing cycles. Specimen mass was measured before and after the treatments. For the second methodology, 20 human crowns were embedded in acrylic resin and cut surfaces were exposed and polished. The specimens were divided into four groups ($n = 10$): no treatment (control), APF for 4 min, Nd:YAG laser irradiation (1 W, 100 mJ, 10 Hz, 125 J/cm^2), and Nd:YAG laser irradiation + APF. The samples were then immersed in citric acid (2% w/v; 90 min). Vickers hardness was obtained before and after the treatments. ***Results:*** The Nd:YAG laser irradiation + APF (bovine and human enamel) was more effective and yielded statistically significant results for surface microhardness and enamel wear. ***Conclusion:*** Nd:YAG laser irradiation associated with APF reduced bovine enamel wear and human enamel softening when samples were submitted to a regime of erosion and abrasion or erosion only *in vitro*.

Introduction

Although the incidence of dental caries has declined over the last few years[1] there is an increasing number of other dental lesions that lead to tooth wear, such as erosion.[2–4] The clinical term dental erosion is used to describe the physical results of a pathological, chronic, and localized loss of dental hard tissue, which is chemically etched away from the tooth surface by acid and/or chelation without bacterial involvement.[2] Enamel loss due to erosion is highly prevalent in industrialized countries and among teenagers, because of excessive dietary soft drink consumption as well as an increasing prevalence of gastro-esophageal reflux disease and bulimia.[3–8] Patients with a decrease in saliva flow rate due to medications or diseases also show increased enamel loss, because saliva-buffering capacity plays an important role in minimizing enamel wear in erosive/abrasive attack.[9–11]

An acidic attack leads to loss and softening of the enamel surface and decreases its wear resistance, thus rendering it more susceptible to the effects of mechanical abrasion, such as toothbrushing and friction from the lip, cheek, and tongue.[5,7,12] The consequences can be premature loss of tooth tissue, which may produce sensitivity and eventual pulpal irritation and the need for complex restorative procedures. Up until now, it has not been possible to stop or prevent the progression of the enamel loss caused by acidic erosion. Many manufacturers have been developing products with the specific aim of reducing the effects of erosion.[13] The protective effect of fluoride against erosion is under debate,[14–16] and the treatment of choice has been restoration

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and advice to patients to change their dietary and oral hygiene habits.

Several investigations have demonstrated that different types of lasers such as Nd:YAG, CO_2, Er:YAG, and Ar, with different operating modes and energy outputs, can reduce the rate of enamel surface demineralization and prevent dental caries.[17–22] Different explanations for the increased acid resistance of laser-treated enamel have been suggested, such as decreased enamel permeability, alterations in chemical composition, or combination of both.[19] Some studies also support findings of an increased fluoride uptake by laser-treated dental enamel, increasing its resistance to acid and lessening its susceptibility to acid challenge.[23,24]

The large number of alternatives and efforts suggested in the current literature for preventing enamel wear illustrates the need to find an effective way to stop the progression of enamel loss, considering the increasing prevalence of this condition in the modern population.[14,25,26]

No study could be found in the literature with regard to the effect of high-power lasers in preventing enamel erosion caused by acidic beverages. The aim of the present *in vitro* study was to evaluate, by two different methodologies, the effectiveness of Nd:YAG laser irradiation associated with topical acidulated phosphate fluoride (APF), for preventing enamel softening and structure loss when samples were submitted to regimes of erosion and abrasion or erosion only.

Materials and Methods

Bovine specimens

One hundred recently extracted, cleaned bovine crowns, stored in deionized water, were used as samples. In the center of the buccal surface of each specimen, a window of approximately 5×5 mm was delimited and the area outside this window was covered with 2 layers of 3M Single Bond Adhesive® (3M Espe, São Paulo, Brazil) after acid etching (37% v/v phosphoric acid; FGM, Joinville, Brazil). The specimens were stored in deionized water at a temperature of 4°C during the entire experiment. The initial mass of specimens (baseline) was obtained using an analytic balance (AB204, Mettler Toledo, Greifersee, Switzerland) after standardized dryness, which was performed initially with the aid of absorbent paper to remove excess water, followed drying with compressed air for 1 min to provide a homogeneous dryness. The specimens were randomly divided into four groups ($n = 25$) and submitted to the following experimental treatments. Group 1 received no treatment and served as the control. In Group 2, APF (1.23% NaF w/v; pH 5.3) was topically applied to the surface with disposable brush tips and left undisturbed for 4 min. After the exposure time, the fluoride was removed from the enamel surface with cotton rolls. In Group 3, the surface was coated with a light absorber (triturated vegetable coal diluted in ethanol) and irradiated with a Nd:YAG laser (Pulse Master 1000 IQ; American Dental Technology, San Carlos, CA), 1064 nm wavelength, 100 μs pulse width through an optic fiber 300 μm in diameter. The laser irradiation was done manually without water cooling by one operator. The entire enamel surface was irradiated for 60 sec by scanning (contact) at a slow speed once each in the horizontal and vertical directions to promote homogeneous irradiation and to cover the entire sample area; a He-Ne laser, coaxial with Nd:YAG beam, was used as a guide light. The parameters used were energy per pulse (E_p) of 100 mJ and repetition rate (f) of 10 Hz, with 1 W of average power ($P_a = E_p \times f$). This gives an energy density per pulse of 141.5 J/cm^2 ($D_E = E_p/A_{fiber}$), where A_{fiber} is the area of the laser beam at the fiber exit. In Group 4, the specimens were irradiated with a Nd:YAG laser (the same procedure described in Group 3) and afterwards coated with APF (the same procedure as described in Group 2).

The specimens from all groups were then immersed in a 2% (w/v) citric acid solution (pH 2.6) for 30 min at room temperature, 24 h after treatment. Next, the samples were mounted in an automatic brushing device developed in the Dentistry Department (Dental School, University of São Paulo, Brazil). A frequency of 5000 brushing strokes under a load of 200 g was used. The abrasive slurry was prepared by mixing 200 mL of deionized water with 20 g of toothpaste (1500 ppm sodium fluoride, 0.3% triclosan, and silica; Colgate Total, Colgate-Palmolive CO, São Bernardo do Campo, Brazil). For each specimen a new brush was used. After toothbrushing the samples were washed in running water. The mass measures were obtained again after the treatments, in the same way described previously.

Analysis of variance (ANOVA/Tukey) was applied to the data of the difference between the initial and final mass.

Human specimens

After obtaining approval from the ethics committee of the School of Dentistry/University of São Paulo, Brazil (20/2005), 20 freshly extracted human molar crowns, stored in deionized water, were sectioned mesio-distally, resulting in 40 specimens that were stored in deionized water during the entire experiment, at a temperature of 4°C. The specimens were individually embedded in self-polymerizing acrylic resin in a ring mold, ground flat with a water-cooled mechanical grinder (Buehler, Lake Bluff, IL), and polished with a sequence of 240, 320, 400, 600, and 1200 grit silicon carbide grinding papers (Buehler). After polishing, the samples were then washed in running water. Grounding was performed until the surface was flat, resulting in a smooth and regular area of approximately 4×3 mm, which allowed reading by the surface microhardness tester. The specimens were randomly divided into four groups ($n = 10$) and submitted to the following treatments.

Group 5 was the control group, which received no treatment. In Group 6, the specimens were coated with APF (1.23% NaF; pH 5.3) for 4 min, which was then removed from the enamel surface with cotton rolls (this procedure was repeated three times). In Group 7, the specimens were coated with a light absorber (triturated vegetable coal diluted in ethanol) and irradiated with Nd:YAG laser. The laser irradiation was done manually without water cooling by one operator. The entire enamel surface was irradiated for 3 min by scanning (contact) at a slow speed once each in the horizontal and vertical directions to promote homogeneous irradiation and to cover the entire sample area; a He-Ne laser, coaxial with Nd:YAG beam, was used as a guide light. The parameter used was the same as in Group 3, except the fiber diameter was 320 μm resulting in an energy density (D_E) of 125 J/cm^2. In Group 8, after Nd:YAG laser irradiation (the same procedure described in Group 7), the specimens

Table 1. Mean of Mass Measures (g) of Bovine Specimens Before (Baseline) and After Treatments, Standard Deviation (±SD), and Difference Between Baseline and After Treatments

	Mass (g)			
	Group 1	*Group 2*	*Group 3*	*Group 4*
Baseline	2.5473 (±0.3736)	2.5114 (±0.5032)	2.3199 (±0.4798)	2.3648 (±0.4327)
After treatments	2.5317 (±0.3744)	2.4970 (±0.5032)	2.3060 (±0.4814)	2.3551 (±0.4320)
Difference	0.0156a (±0.0023)	0.0144a (±0.0023)	0.0139a (±0.0033)	0.0097b (±0.003)

Values followed by the same letter are not significantly different.

were coated with APF (the same procedure described in Group 6).

All specimens were then immersed in a 2% w/v citric acid solution (pH 2.6) for 90 min, 24 h after the surface treatments. During this interval they were maintained in deionized water. The initial (baseline) and final (after the treatments) Vickers surface microhardness values for each specimen were obtained through a microhardness tester (HMV 2000 Shimatzu® Corp., Kyoto, Japan), under a static load of 50 g for 45 sec. Three indentations in five different spots in a cross model in the center of the samples were made on each specimen to reach a mean. The length of indentations was measured with an optical analysis system and transferred to a computer.

Analysis of variance (ANOVA/Tukey) was applied to the data of the difference between the Vickers hardness of the specimens before and after the treatments.

Results

Table 1 shows the mean of mass measures of bovine specimens before and after the treatments. All bovine specimens experienced enamel loss, but when laser irradiation was combined with 1.23% APF, enamel loss had a statistically significant reduction in the effect of erosion/abrasion in comparison with the other groups ($p < 0.01$).

All human specimens had a reduction in Vickers hardness after the treatments (Table 2). However, a statistically significant erosion-inhibiting effect was found for the group with laser irradiation and application of APF in comparison with the other groups ($p < 0.05$).

Discussion

This preliminary experimental study verified that Nd:YAG laser and fluoride were together capable of increasing both bovine and human enamel resistance to citric acid. Since many studies have focused on the use of laser irradiation to inhibit dental caries with promising results, the present study tested whether high-power laser irradiation associated with APF could prevent demineralization caused by acid erosion.

Various lasers can reduce the rate of surface demineralization in enamel, when it is submitted to acids (pH 5.5) that cause caries.[17,18] Erosion lesions are caused by acids with pH as low as 2.6, such as in acidic drinks,[27] or in cases of bulimia, when the pH in the oral cavity may be even lower.[28] No studies were found in the literature with regard to the capability of high-power lasers to prevent enamel demineralization caused by beverage-related acid erosion. The only study that verified the association of high-power laser with fluoride in the prevention of tooth softening due to a strong acid was from Vlacic et al.[29] The acid challenge was performed with hydrochloric acid, and Vickers hardness analysis showed promising results for the lased groups. Although promising results were obtained with the same wavelength used in this study (1064 nm), the protocol of irradiation and the sequence of fluoride therapy and acid challenge were different from our protocol. In the study by Vlacic et al.[29] the samples were covered with sodium fluoride gel (1.23%) and immediately after, laser irradiation was performed. In our study topical fluoride therapy was applied after laser irradiation.

The present study performed the acid challenge with citric acid, which is common in beverages. As dietary habits have changed through the years, an increase in consumption of industrially prepared foods and drinks has been noted. As a consequence, several alterations in teeth can be found in the population. The findings of this study suggest that Nd:YAG, within the parameters tested, followed by topical APF application in both methodologies used, can prevent or retard

Table 2. Mean of Vickers Hardness of Human Specimens Before (Baseline) and After Treatments, Standard Deviation (±SD), and Difference Between Values Baseline and After Treatments

	Hardness (VHN)			
	Group 5	*Group 6*	*Group 7*	*Group 8*
Baseline	346.3 (±26.9)	316.4 (±40.2)	333.9 (±59.4)	334.7 (±43.2)
After treatments	153.5 (±15.6)	185.2 (±19.2)	184.3 (±33.2)	298.9 (±32.8)
Difference	192.7a (±28.4)	131.2b (±40.8)	149.6ab (±67.7)	35.8c (±17.4)

Values followed by the same superscript letter were not significantly different.
VHN, Vickers hardness.

the demineralization caused by citric acid erosion and lead to preservation of the enamel.

Preventive strategies for dental erosion are few, and efforts have been made to prevent these lesions.[3,4] Since fluoride is able to reduce caries, its application has been suggested for preventing erosive enamel loss. Several studies have tried to use fluoride to prevent erosion, either by topical fluoride application or even by including it as a component of acidic drinks.[15,16] However, the protective effect of fluoride on erosion is debatable. It seems that erosion cannot be prevented with moderate fluoride concentrations, such as commonly used in toothpastes or applied as a tool in caries prevention.[15,16] Promising results have been obtained with the application of high-concentration fluoride; however, further studies should be conducted in order to determine the optimal treatment regime that provides the highest inhibition, without endangering the health of the patients.[30]

The present hypothesis was tested with bovine enamel initially and then with human enamel, through two different methodologies: enamel wear and microhardness. Different explanations for the increased acid resistance of laser-treated enamel in preventing caries have been suggested in the literature, such as crystalline phase transformation, change in chemical composition, and surface alterations (i.e., fusion and melting of crystallites).[31,32] The melted enamel surface can show a crystal growth that can reduce the interprismatic spaces, and consequently the diffusion of acids during an acid challenge.[18] Studies[23,24,33] support findings of increased fluoride content in enamel treated with acidulated fluoride after laser irradiation. Further studies are necessary to elucidate the mechanisms by which enamel after laser irradiation and topical fluoride applications becomes more resistant to citric acid challenge, as well as to evaluate the effects of laser irradiation, within these parameters, on the pulp temperature.

Conclusion

Within the conditions and limits of this study, it can be concluded that Nd:YAG laser irradiation associated with APF reduced bovine enamel wear and human enamel softening when they were submitted, respectively, to a regime of erosion and abrasion or erosion alone *in vitro*.

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Disclosure Statement

No competing financial interests exist.

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