Potential of CO$_2$ lasers (10.6 µm) associated with fluorides in inhibiting human enamel erosion

Abstract: This in vitro study aimed to investigate the potential of CO$_2$ lasers associated with different fluoride agents in inhibiting enamel erosion. Human enamel samples were randomly divided into 9 groups (n = 12): G1-eroded enamel; G2-APF gel; G3-AmF/NaF gel; G4-AmF/SnF$_2$ solution; G5-CO$_2$ laser (λ = 10.6 µm)+APF gel; G6-CO$_2$ laser+AmF/NaF gel; G7-CO$_2$ laser+AmF/SnF$_2$ solution; G8-CO$_2$ laser; and G9-sound enamel. The CO$_2$ laser parameters were: 0.45 J/cm$^2$; 6 µs; and 128 Hz. After surface treatment, the samples (except from G9) were immersed in 1% citric acid (pH 4.0, 3 min). Surface microhardness was measured at baseline and after surface softening. The data were statistically analyzed by one-way ANOVA and Tukey’s tests (p < 0.05). G2 (407.6 ± 37.3) presented the highest mean SMH after softening, followed by G3 (407.5 ± 29.8) and G5 (399.7 ± 32.9). Within the fluoride-treated groups, G4 (309.0 ± 24.4) had a significantly lower mean SMH than G3 and G2, which were statistically similar to each other. AmF/NaF and APF application showed potential to protect and control erosion progression in dental enamel, and CO$_2$ laser irradiation at 0.45 J/cm$^2$ did not influence its efficacy. CO$_2$ laser irradiation alone under the same conditions could also significantly decrease enamel erosive mineral loss, although at lower levels.

Keywords: Dental Enamel; Fluorides; Lasers; Hardness; Tooth Erosion.

Introduction

Dental erosion is a chemical process characterized by surface dissolution of dental hard tissues, without the involvement of microorganisms.$^1$ In earlier stages, the erosive process involves enamel demineralization, which is characterized by initial softening and increased roughness of the surface.$^2$ This process is of particular clinical importance because studies.$^3$$^4$$^5$$^6$$^7$ have shown that softened enamel can be significantly protected and remineralized by exposure to fluorides.

During a caries challenge, calcium fluoride forms a protective coating on the enamel surface, after exposure to fluoride agents results in their incorporation into the enamel as fluorapatite. Regarding the mechanisms of action of amine and sodium fluoride compounds in erosion prevention, it can be speculated that deposition of CaF$_2$-like precipitates occurs on the enamel surface, preventing loss of enamel and providing some additional mineral to be dissolved in acidic solutions before the underlying enamel is attacked.$^3$
Considering the limited effectiveness of fluoride in preventing dental hard tissue erosion and the effects of high-power lasers on dental hard tissues, previous experiments have shown that CO₂ laser irradiation could be an alternative method to modify the enamel surface and protect it against demineralization. Furthermore, some authors have concluded that laser irradiation associated with fluoride agents could increase the fluoride uptake into the enamel, making the enamel more acid resistant. However, until now, there have been only a few studies evaluating the use of CO₂ lasers to prevent dental erosion, and these studies have been inconclusive.

The effects of different fluoride agents (TiF₄, SnF₂, NaF, AmF, ZnF₂, SnCl₂) on erosive tissue loss in the enamel have also been tested, but the results have not been conclusive. Different product presentations (gel/solution), fluoride concentrations, and recommended times of exposure to enamel can possibly lead to different levels of incorporation of fluoride ions into the dental structure and, consequently, can differently influence their potential to prevent dental erosion.

This in vitro study aimed to evaluate the potential of a pulsed CO₂ laser, associated or not with different fluoride agents, in inhibiting human enamel softening. The hypotheses considered were that pulsed CO₂ laser irradiation associated with fluoride agents would increase the reduction of mineral loss compared to other treatments and that different fluoride agents would have different protective effects on human enamel erosion.

### Methodology

#### Ethical Aspects

This study was approved by the Research Ethics Committee of the School of Dentistry of the Universidade de São Paulo (Protocol#40/11) and by the National Committee for Ethics in Research (Protocol #453/2011).

#### Study Design

Enamel samples were randomly divided in 9 groups (n = 12): G1-eroded enamel (no surface treatment); G2-APF gel; G3-AmF/NaF gel; G4-AmF/SnF₂ solution; G5-CO₂ laser (λ = 10.6 µm); G6-CO₂ laser+APF gel; G7-CO₂ laser+AmF/NaF gel; G8-CO₂ laser+AmF/SnF₂ solution; and G9-sound enamel. The samples were submitted to an erosive challenge, and the effects of surface treatments were quantitatively analyzed for Knoop surface microhardness.

#### Sample Preparation

One hundred eight enamel blocks (5 x 5 x 2 mm), obtained from freshly extracted human third molars, were embedded in epoxy resin (Buehler, Lake Bluff, USA). They were ground flat and were serially polished using silicon carbide papers (#1200, #2400, #4000 grit) and 6µm diamond abrasive paste (Buehler, Lake Bluff, USA) on a polishing machine (EXAKT, Nordersted, Germany). An adhesive tape 2.5 mm in diameter was fixed in the center of the polished surface, and the samples were completely covered with an acid-resistant varnish and left to dry. Subsequently, the adhesive tape was carefully removed, and a round window 2.5 mm in diameter of enamel was exposed. All of the samples were stored in a 100% humidity environment before the beginning of the experiment. Samples from G9 (sound enamel) were submitted to microhardness tests and were not exposed to any of the experimental surface treatments or to erosive challenge.

#### CO₂ Laser Irradiation

A CO₂ laser (λ = 10.6 µm) (Rofin SCx30, Rofin-Sinar Laser GmbH, Hamburg, Germany), emitting a beam with a TEM₀₀ profile, was used. To allow for adequate determination of the energy density, the beam diameter at 1/e² of the intensity level was determined using the knife-edge method. The emitted energy was controlled using an energy/power meter, and the irradiations were performed at a distance of 19.8 cm (focused mode) to obtain a beam diameter at the sample surface of 2.5 mm (coincident with the exposed enamel area). The CO₂ laser irradiation parameters were: 0.45 J/cm²; 15 µs; 128 Hz; 22 mJ; 9 s of irradiation time; and no air-water spray.

#### Fluoride Treatment

The products used in the groups treated with fluoride were the following: G2 and G5-APF gel (DFL® Gel, Nova DFL, Rio de Janeiro, Brazil, 1.23%NaF, pH 3.6-3.9) for 4 min; G3 and G6-AmF/NaF gel (Elmex® solution; and G9-sound enamel. The samples were submitted to an erosive challenge, and the effects of surface treatments were quantitatively analyzed for Knoop surface microhardness.
Gel, GABA International, Basel, Switzerland, 1.25%F, pH 4.8-6.0) for 4 min; and G4 and G7-SnF$_2$ solution (Meridol® Mouthrinse, GABA International, Basel, Switzerland, 0.16%AmF+0.05%SnF$_2$, pH 4.2) for 3 min. All of the products were applied on the enamel surfaces according to the manufacturers’ instructions. After fluoride treatment, the samples were dried with absorbent paper. In G5, G6 and G7, fluoride was applied immediately after laser irradiation.

**Enamel Surface Softening**

Following the surface treatments, the samples were immersed in 20 mL of 1% citric acid (C$_6$H$_8$O$_7$·H$_2$O; M = 210.14 g/mol; E. Merck, Darmstadt, Germany) (pH 4.0) at 30°C under constant agitation in a shaking water bath for 3 min. Then, all of the samples were rinsed with distilled/deionized water for 30 s and were dried for 5 s with absorbent paper. After surface softening, they were stored in a supersaturated mineral solution (1.5 mmol/L CaCl$_2$, 1.0 mmol/L KH$_2$PO$_4$, 50 mmol/L NaCl, pH 7.0) for 24 h. This in vitro experimental model was designed to simulate the clinical conditions present during the early stages of dental erosion.

**Surface Microhardness Measurement (SMH)**

All of the samples had their microhardness measured prior to the beginning of the experiment (SMH average: 355.4). SMH was performed with a Knoop diamond placed perpendicular to the polished surfaces (0.49 N, 20 s). The indentation lengths were measured using a microscope and a specific computer software (DM 4000 M, Leica, Wetzlar, German; and a4i Analysis, Aquinto, Enfield, USA). After the surface softening, SMH was measured for G1-G8, placed 100 µm to the right of the baseline measurements. Six indentations were made at a distance of 50 µm from each other at each measurement time.

**Statistical Analysis**

The data were analyzed using SPSS (SPSS Inc., Chicago, USA) software, version 17.0 for Windows. The results were analyzed by one-way ANOVA with subsequent pairwise comparisons using Tukey’s test (α = 0.05).

**Results**

The results of the microhardness evaluation are shown in Table 1. The softening model chosen for the study was shown to be effective because there was a statistically significant difference between the microhardness values of the eroded (G1) and non-eroded enamel (G9). G2 (407.6 ± 37.3) presented the highest mean SMH after softening, followed by G3 (407.5 ± 29.8) and G5 (399.7 ± 32.9). Among the fluoride-treated groups, G4 (309.0 ± 24.4) had a significantly lower mean SMH than G3 and G2, which were statistically similar to each other. Regarding treatment with CO$_2$ laser (G8), when used alone, the mean SMH (341.2 ± 23.2) revealed that irradiation of enamel with the parameters used was significantly better than in G1 and was not statistically significant different from G9, indicating a preventive effect. However, greater mineral loss was revealed, compared with APF gel application alone (G2) or in combination with CO$_2$ laser irradiation (G5). Regarding the association of laser and fluoride treatments, G6 (373.9 ± 40.2) showed no significant difference in SMH compared to G2 and G3, but it differed from G7 (328.9 ± 25.7).

**Table 1. Mean SMH and SD (standard deviation) for each experimental group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Treatment</th>
<th>KHN ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Eroded enamel</td>
<td>297.0 ± 34.2E</td>
</tr>
<tr>
<td>G2</td>
<td>APF gel</td>
<td>407.6 ± 37.3A</td>
</tr>
<tr>
<td>G3</td>
<td>AmF/NaF gel</td>
<td>407.5 ± 29.8A</td>
</tr>
<tr>
<td>G4</td>
<td>AmF/SnF$_2$ solution</td>
<td>309.0 ± 24.4DE</td>
</tr>
<tr>
<td>G5</td>
<td>CO$_2$ laser + APF gel</td>
<td>399.7 ± 32.9A</td>
</tr>
<tr>
<td>G6</td>
<td>CO$_2$ laser + AmF/NaF gel</td>
<td>373.9 ± 40.2A</td>
</tr>
<tr>
<td>G7</td>
<td>CO$_2$ laser + AmF/SnF$_2$ solution</td>
<td>328.9 ± 25.7CDE</td>
</tr>
<tr>
<td>G8</td>
<td>CO$_2$ laser</td>
<td>341.2 ± 23.2BCD</td>
</tr>
<tr>
<td>G9</td>
<td>Sound enamel</td>
<td>358.5 ± 9.0BC</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significant differences between rows, p < 0.05

**Discussion**

Although different therapies have been reported for tooth erosion, none of them has been able to inhibit completely the mineral loss caused by erosive challenges. Significant studies of initial erosion have used acidic challenges consisting of plain citric acid, soft drinks and fruit-based juices. The focus of our
study was to evaluate the efficacy of different therapies in very incipient erosive lesions, which were created by a single exposure to citric acid for 3 min. Citric acid is commonly found in fruit-based drinks and beverages, and it can provide a strong erosive challenge under certain conditions. Thus, it is considered ideal when testing the potential for fluoridites to prevent enamel erosion. In the mouth, the period for which the pH remains low is usually no longer than 2 min; therefore, the time for exposure to acids should be minimal for initial erosion processes in in vitro models. The CO₂ laser irradiation and fluoridites agents were applied only once to simulate the standard clinical procedure with a single professional application.

Surface microhardness, surface profilometry, microradiography, chemical analysis and SEM have been considered the most established laboratory assessments for enamel erosion. In the present study, SMH was selected because it was reported to have sufficient sensitivity for measuring the very initial stages of erosion, when enamel softening starts, and no quantitative substance loss is suspected to occur.

Some studies have shown the potential of fluoride treatments to prevent or reduce mineral loss during the initial stages of enamel erosion. However, recent studies have shown that its efficacy depends essentially on the nature of the fluoridated compound. Based on this information, the current study investigated the effects of APF gel, AmF/NaF gel and SnF₂ solution on the inhibition of enamel demineralization during an erosive challenge, associating them with an innovative tool: laser.

There has been in vitro evidence that pre-treatment of enamel with stannous fluoride could provide protective effects by inhibiting or reducing the erosive effects of acids. Furthermore, SEM images showed that SnF₂ treatment could protect the enamel surface, possibly forming a coating with very low dissolution rate. Babcock reported that Sn⁺⁺ ions reacted with the pure hydroxyapatite on the surface of the enamel, reducing the enamel’s solubility through the precipitation of Sn₃OHPO₄, SnF₃PO₄, Ca(SnF₃)₂ or CaF₂ salts. In contrast with this literature, the present findings revealed that the treatment that has the least effect on inhibiting enamel erosion was stannous fluoride solution. Some hypotheses could be raised.

One is related to the possible difference in efficacy of the gels compared to aqueous solution, as reported previously by Vieira et al., who evaluated the effects of TiF₁₆, AmF and fluoride varnish on bovine enamel erosion. Differences between the substrates used in both studies (human/bovine enamel) were not considered because the authors showed that bovine enamel was a possible substitute for human substrate in erosion models. Although the enamel surface was cleaned after exposure to fluoride products, the gel products (APF, AmF/NaF) might have remained for a longer time on the enamel surface than the products in the solution (SnF₂). Another hypothesis concerns the fluoride concentration and the exposure time. The SnF₂ product had the lowest fluoride concentration (250 ppm F⁻) and was applied only once to the enamel surface. It is believed that the higher the fluoride concentration is and the longer the exposure time is, the greater the acid resistance of enamel is to erosion. The application time considered in this study (3 min) was possibly not sufficient to allow for the formation of the protective coating formed by some salts. Moreover, studies using different products, with different pH values and/or formulations (gels, solutions or varnishes), make the interpretation of the results difficult with regard to the role of the fluoride compound.

AmF and NaF gels, independent of laser irradiation, were shown to be more effective than the other surface treatments. The anti-erosive effects of these fluoridated agents could be justified by the formation of a layer of spherical CaF precipitates on the enamel surface following topical application, acting as a reservoir of fluoride ions to inducing the formation of fluorapatite or as a physical barrier isolating the enamel surface from further acid attacks, thus preventing demineralization.

Regarding the use of high-power lasers, previous in vitro studies have shown promising results with CO₂ laser in enhancing the acid resistance of enamel during cariogenic challenges. Featherstone et al. reported that CO₂ laser produced radiation in the infrared region, which coincided closely with the phosphate absorption band. This finding indicated that CO₂ laser irradiation – at safe energy levels – could be used to modify carbonated hydroxyapatite
thermally to form a purer hydroxyapatite phase that is more resistant to acid dissolution. In addition to chemical changes, it is also believed that temperature increases on the enamel surface, with consequent alteration in the composition of the mineral phase, can lead to decreased permeability and solubility of the enamel. Recently, authors have shown that a short-pulsed CO₂ laser markedly inhibited enamel mineral loss compared to fluoride varnish alone over 12 months. In the present study, the energy density and pulse duration (0.45 J/cm², 15 µs) used for surface irradiation did not result in a greater reduction in enamel surface mineral loss, compared to APF or AmF/NaF gel application. However, results have shown that laser irradiation indeed had preventive effects because the enamel SMH was statistically significant greater than that of eroded enamel but was not different than that of sound enamel.

Considering the limited efficacy of fluoride in inhibiting enamel mineral loss during erosive challenges and that fluoride alone might not be effective against dental erosion, this study evaluated the additional effects of CO₂ laser irradiation. Some authors have studied the effects of laser irradiation combined with fluorides on enamel demineralization and have concluded that there was some significant synergism between treatments. Although it was expected that CO₂ laser irradiation would increase the efficacy of fluoride, as shown in previous studies performing cariogenic challenges, in the present report, CO₂ laser irradiation did not significantly increase the effects of either fluoride agents (AmF and NaF) on erosion. Corroborating our results, Wiegand et al. reported that AmF was able to decrease enamel erosion, but CO₂ laser irradiation did not improve its efficacy.

Due to the variety of parameters and methodologies employed in the literature, it is difficult to make comparisons with previous studies. Further studies conducting chemical analyses of the enamel surface and the erosive cycling solutions and microscopic evaluations following laser and fluoride treatment should be performed to clarify the mechanisms by which different fluoride products and high-power lasers act on erosion inhibition. In addition, other laser parameters should be tested because other irradiation conditions have already been shown to cause greater protective effects against erosion, and the increase in the resistance of enamel to acid demineralization is highly dependent on laser parameters such as pulse duration, energy density and the number of overlapped pulses.

**Conclusion**

Within the limits of the present _in vitro_ study, it was concluded that AmF/NaF and APF treatment showed the potential to protect and control erosion progression in human dental enamel and that CO₂ laser irradiation at 0.45 J/cm² (15 µs, 128 Hz) did not influence its efficacy. CO₂ laser irradiation alone, under the same conditions, could also significantly decrease enamel erosive mineral loss, although at lower levels.

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