Effect of the CO₂ Laser Combined with Fluoridated Products on the Inhibition of Enamel Demineralization

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Abstract

**Aim:** This *in vitro* study evaluated the combined effects of a 10.6 μm CO₂ laser, fluoridated dentifrice, and mouthrinse on the reduction of lesion progression in carious human enamel.

**Methods and Materials:** Slabs of previously demineralized dental enamel were assigned to nine groups, either treated with/without a CO₂ laser, with/without fluoridated dentifrice, and with/without fluoridated mouthrinse. After a pH-cycling regime, fluoride concentrations were determined in the demin- and remineralizing solutions. A qualitative polarized light analysis was performed on enamel, and enamel mineral loss was determined by cross-sectional microhardness testing.

**Results:** All treatments were able to decrease mineral loss, and the inhibition of demineralization progression ranged from 48% to 60%.

**Conclusion:** The 10.6 μm CO₂ laser irradiation alone or combined with fluoridated products reduced demineralization progression in enamel. However, there was no significant additional demineralization inhibitory effect with the use of the combined laser-fluoride treatments.
**Clinical Significance:** CO₂ lasers have proven to be efficient in reducing subsurface enamel demineralization. Its association with a high frequent fluoride therapy may enhance this protective effect.

**Keywords:** CO₂ lasers, pH cycling, cross-sectional microhardness, fluoride dentifrice, fluoride mouthrinse, dental caries, demineralization

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**Introduction**

Dental caries continues to be an oral health problem in most industrialized countries as it affects 60–90% of school-aged children and the vast majority of adults. In most developing countries dental caries levels have been decreasing until recently, but now there is a trend towards rising caries prevalence rates and increasing dental caries experience. In spite of widespread use of fluoride there is still a high manifestation of this disease in some individuals or groups as demonstrated by studies showing only around 15% of 17-year-old adolescents are caries free. In light of these findings attention should be concentrated on those individuals (‘high-caries-risk’ group) for whom the combination of preventive treatments should be recommended.

Several investigations have demonstrated treatment with various lasers such as the CO₂, Nd:YAG, and Argon lasers can reduce the subsurface demineralization rate in enamel.

CO₂ lasers appear to be the most efficient in this regard due to the enamel absorption coefficient which closely corresponds to the CO₂ laser emission wavelength. Some research groups have found the most efficient wavelengths for caries preventive effects to be 9.3-9.6 μm, 100 μs or less pulse duration, and fluences of less than 4 J/cm².

A recent study tested a continuous 10.6 μm wavelength CO₂ laser with an output power of 2 W on enamel immediately after applying an amine fluoride solution. This approach pointed out the need to focus on a combined laser-fluoride treatment of incipient carious lesions in order to investigate whether lesion progression can be influenced by such treatments. Fluoridated dentifrices and mouthrinses are widely used vehicles that deliver fluoride to the oral cavity, and the use of these products has contributed substantially to the widespread decline in caries incidence in some western countries. However, there is evidence the cariostatic effect of fluoride is related to the sustained presence of low levels of ionic fluoride in components of the oral environment such as in plaque and saliva, therefore, making the effect dependent on the patient’s oral hygiene habits. For effective prevention, therapies not dependent on the patient’s compliance should be more advantageously utilized for high caries risk individuals. Use of a pulsed CO₂ laser at 10.6 μm in combination with/without fluoride might be a good alternative for these patients.

The efficacy of fluoride treatment combined with 10.6 μm CO₂ laser irradiation for caries inhibition has been demonstrated by several investigations. However, none of these studies attempted to investigate the combined effects.
of dentifrice and mouthrinse containing fluoride with a clinical 10.6 μm CO₂ laser for inhibiting the progression of demineralization in carious human enamel. Furthermore, most studies have been carried out with TEA (transversely excited atmospheric pressure) prototype laser technology at a wavelength of 9.6 μm which is not commercially available.

Thus, the purpose of this in vitro study was to assess the combined effects of a pulsed 10.6 μm CO₂ laser with fluoridated dentifrice and mouthrinse on the reduction of lesion progression in human enamel with artificial caries-like lesions.

Methods and Materials

Tooth Selection and Sample Preparation
This study was approved by the Research and Ethics Committee of the Piracicaba School of Dentistry at the State University of Campinas in Piracicaba, SP, Brazil (Protocol No. 58/2001).

Forty-five extracted impacted human third molars, sterilized using gamma radiation, were used to perform this in vitro study.29 These teeth were sectioned without being embedded using a water-cooled diamond saw cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) to obtain 90 unabraded enamel slabs (5 x 5 mm). The slabs were coated with an acid-resistant varnish leaving a window (4 mm²) of exposed enamel surface for the creation of artificial caries-like lesions.

Caries-like Lesion Formation and Grouping
Caries-like lesion formation was performed in all slabs in accordance with the protocol of Paes Leme.29 Early caries lesions were produced by individual immersion of the slabs in an acetate buffer (6.25 mL of solution/mm² of exposed enamel) 0.05 mol/L pH 5.0, 50% saturated with hydroxyapatite for 48 hours at 37°C. The slabs were then randomly assigned to one of the following nine groups (n=10):
• Carious (Ca)
• Control (C)
• Dentifrice (D)
• Mouthrinse (M)
• Laser (L)
• Dentifrice+Mouthrinse (DM)
• Laser+Dentifrice (LD)
• Laser+Mouthrinse (LM)
• Laser+Dentifrice+Mouthrinse (LDM)

The slabs in the first group (Ca) were demineralized using an acetate buffer and were not submitted to the pH-cycling regimen because they provided information about the initial mineral content and on the effectiveness of the other treatments in inhibiting the progress of demineralization.

Laser Treatment
A commercially available Model UM-L30 pulsed CO₂ laser (Union Medical Engineering Co., Yangju-si, Gyeonggi-Do, Korea) at a wavelength of 10.6 μm was used for irradiating the groups L, LD, LM, and LDM with the following parameters:
• 1.6 W of power
• 10 ms pulse duration
• 10 ms of time off
• 50 Hz repetition rate
• 0.3 mm beam diameter

For these conditions, a Model 201 power meter (Coherent Radiation, Palo Alto, CA, USA) indicated a 0.7 W peak power, thus, determining an incident fluency of approximately 10 J/cm² per pulse.29 Irradiation was carried out by scanning the exposed enamel of each slab for approximately 30 seconds from an X-Y positioning platform while maintaining a 10 mm distance from the tip of the handpiece to the slabs in order to provide uniform coverage of each window. The scanning speed was approximately 1 mm/second. These parameters have been previously tested by Steiner-Oliveira29 and showed no possibility of pulp damage.
Fluoride Treatment
After the laser irradiation procedure, the enamel slabs from groups D, DM, LD, and LDM were treated with a 1:3 (w/w) slurry made with deionized and distilled water and fluoridated dentifrice with 1100 ppm F (Crest Cavity Protection®, The Procter & Gamble Company, Cincinnati, OH, USA) before daily immersion in the de- and remineralizing solutions. This was performed twice daily for five minutes under agitation in an orbital shaker (Cientec CT-165, Piracicaba, SP, Brazil). Then the slabs from groups M, DM, LM, and LDM were submitted to a single treatment with mint flavored fluoridated (0.05% of NaF) mouthrinse (REACH®, Johnson & Johnson, São José dos Campos, São Paulo, Brazil) before being immersed for one minute in the undiluted demineralizing solutions and agitated in an orbital shaker. After treatment, all slabs were washed in deionized and distilled water.

The pH-Cycling Process
The pH-cycling model used in this study was based on the model described by Featherstone31 and modified by Klein.32 Each slab was kept in a demineralizing solution (5 mL/mm² exposed enamel) containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer pH 4.6 for three hours. They were then placed in a remineralizing solution (2.5 mL/mm² exposed enamel) containing 1.5 mmol/L calcium, 0.9 mmol/L phosphate, and 150 mmol/L KCl in 20 mmol/L cacodylic buffer pH 7.0 for an average of 21 hours each day. Both solutions were changed daily, and the cycle was repeated for ten days. After five cycles and at the end of the experiment, the slabs remained in the remineralizing solution for two days (37°C). Between the demineralizing and remineralizing stages and at the end of the pH-cycling, the slabs were washed with distilled water for ten seconds and wiped with tissue paper. Both solutions contained thymol to prevent the growth of microorganisms.

Chemical Analysis
Fluoride concentrations in the de- and remineralizing solutions used in the pH-cycling model were analyzed on days one through five and eight through twelve. For this analysis, duplicate aliquots of the solutions were mixed with TISAB III at a ratio of 1:0.1. Fluoride determination was performed using an Orion 96-09 ion-selective electrode (Orion Research Inc., Boston, MA, USA) and an Orion EA-940 digital ion-analyzer previously calibrated with standardized solutions (0.015 to 0.5 μg/mL). Fluoride concentrations measured immediately after preparation of the de- and remineralizing solutions and before pH-cycling were 0.019 and 0.015 μg/mL, respectively.

Polarized Light Microscopy Analysis
Three slabs from each group were cut with a Series 1000 Deluxe Silverstone-Taylor hard-tissue microtome (Sci Fab, Littleton, CO, USA) in the middle of the exposed enamel window to obtain 200 μm sections. These sections were then polished with 600 and 1200-grit abrasive-paper to obtain sections of 100 ± 20 μm thickness. The sections were immersed in water and were observed with a Leica DMLP polarized light microscope (Leica Microsystems, Wetzlar, Germany) coupled to a Leica FFC 280 digital system and standard X10 magnification photomicrographs were taken.

Cross-Section Microhardness Testing
After pH-cycling, the remaining portions of the slabs were embedded in Pre-30 self-polymerized acrylic resin (Arotec SA Ind. E Com, Cotia, SP, Brazil) and then severely flattened and polished. The hardness profile was determined using a Future Tech FM-ARS microhardness testing device (Future-Tech Corp., Tokyo, Japan) with a Knoop diamond under a 25-g load for five seconds. Thirty-six indentations (three rows of 12 indentations each) were made with the long axis of the Knoop diamond parallel to the outer surface, maintaining a 10-μm interval between 10-μm and 80-μm and then a 20-μm interval from 80-μm to 180-μm across the lesion and into the underlying enamel.

The mean Knoop hardness number values at each distance from the surface were obtained and converted into volume percent mineral.33 The volume percent mineral was plotted against the depth for each specimen, and the integrated mineral content of the lesion was calculated relative to underlying sound enamel. The mean sound enamel values to compute the integrated mineral loss were obtained from inner sound enamel under the lesion in the same tooth. To compute ΔZ (integrated mineral loss), the
integrated mineral content of the lesion was subtracted from the value obtained for sound enamel.34

**Statistical Analysis**
First a one-way analysis of variance (ANOVA) model was constructed to assess the enamel mineral loss effects of treatments and the fluoride concentration in de- and remineralizing solutions. Next, the Tukey test was chosen to evaluate the significance of all pair-wise comparisons. Values of p < 0.05 were accepted as statistically significant.

**Results**
Table 1 shows the enamel mineral loss (ΔZ) for each group. The ΔZ values were significantly lower (p<0.05) for the D, M, L, DM, LD, LM, and LMD groups when compared with the C group. Groups D, M, L, and LM did not show a statistically significant difference when compared with the Ca group. In contrast, groups DM, LD, and LDM showed a statistically significant difference when compared with the Ca group. The combination of CO₂ laser and fluoride treatments showed no additional effect (p>0.05) against lesion progression.

Table 2 shows the fluoride concentration in the de- and remineralizing solutions used during pH-cycling to simulate the dynamics of caries development. A significantly higher fluoride concentration was found both in de- and remineralizing solutions for the groups treated with dentifrice (D, LD, DM, and LDM). However, statistically lower fluoride concentrations in the remineralizing solutions were observed for the groups treated with fluoride from mouthrinse (M and LM) which did not differ from those groups that had not received any fluoride treatment (C and L).

In the qualitative polarized light analysis, Figure 1 shows demineralization patterns in nine sections belonging to the nine groups. Moreover, except for the LM group, a remineralization line can be observed in the groups treated with combined therapies (Figure 1.f, g, and i).

**Discussion**
*In vitro* and *in vivo* studies have been carried out to define the optimal fluoride therapy for dental caries prevention.31,35 In high-caries risk situations a combination of preventive measures should be indicated, such as the use of fluoridated agents35,36 or the combination of fluoride with CO₂ laser applications.14,24,25,37

The results of the present study (Table 1) showed all applied treatments were capable of reducing

<table>
<thead>
<tr>
<th>Group</th>
<th>Mineral Loss - ΔZ (vol% x μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carious lesion only (Ca)</td>
<td>3651.3 ± 609.0 b</td>
</tr>
<tr>
<td>Carious lesion + pH cycling (C)</td>
<td>5288.4 ± 971.4 a</td>
</tr>
<tr>
<td>Dentifrice + pH cycling (D)</td>
<td>2497.9 ± 624.1 bc</td>
</tr>
<tr>
<td>Mouthrinse + pH cycling (M)</td>
<td>2764.4 ± 544.6 bc</td>
</tr>
<tr>
<td>Laser + pH cycling (L)</td>
<td>2594.2 ± 634.9 bc</td>
</tr>
<tr>
<td>Dentifrice + Mouthrinse + pH cycling (DM)</td>
<td>2288.1 ± 771.4 c</td>
</tr>
<tr>
<td>Laser + Dentifrice + pH cycling (LD)</td>
<td>2130.3 ± 625.7 c</td>
</tr>
<tr>
<td>Laser + Mouthrinse + pH cycling (LM)</td>
<td>2577.2 ± 308.2 bc</td>
</tr>
<tr>
<td>Laser + Dentifrice + Mouthrinse + pH cycling (LDM)</td>
<td>2364.7 ± 498.9 c</td>
</tr>
</tbody>
</table>

**Note:** Means followed by different letters are statistically different according to the Tukey test (p<0.05).
The finding indicates isolated treatments, as well as the combined laser and mouthrinse regime prevented the additional enamel demineralization promoted by the pH-cycling regime.

With regard to fluoride treatments, the current study results are consistent with Paes Leme and Damato who also found caries-like lesion progression was inhibited by using a fluoridated dentifrice and fluoridated mouthrinse, respectively, in a pH-cycling model. The present study data demonstrated a lack of synergism between the
demineralization and remineralization solutions, as well as the combined laser and mouthrinse regime prevented the additional enamel demineralization promoted by the pH-cycling regime.

Table 2. Fluoride concentration (μg/mL) in the demin- and remineralizing solutions according to the treatments (mean ± SD).

<table>
<thead>
<tr>
<th>Group</th>
<th>Demineralizing Solutions (μg F/mL)</th>
<th>Remineralizing Solutions (μg F/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carious lesion + pH cycling (C)</td>
<td>0.016 ± 0.002 c</td>
<td>0.015 ± 0.001 c</td>
</tr>
<tr>
<td>Dentifrice + pH cycling (D)</td>
<td>0.040 ± 0.012 ab</td>
<td>0.136 ± 0.132 a</td>
</tr>
<tr>
<td>Mouthrinse + pH cycling (M)</td>
<td>0.038 ± 0.025 b</td>
<td>0.028 ± 0.028 bc</td>
</tr>
<tr>
<td>Laser + pH cycling (L)</td>
<td>0.016 ± 0.002 c</td>
<td>0.015 ± 0.001 c</td>
</tr>
<tr>
<td>Dentifrice + Mouthrinse + pH cycling (DM)</td>
<td>0.057 ± 0.003 a</td>
<td>0.131 ± 0.126 a</td>
</tr>
<tr>
<td>Laser + Dentifrice + pH cycling (LD)</td>
<td>0.041 ± 0.009 ab</td>
<td>0.120 ± 0.154 a</td>
</tr>
<tr>
<td>Laser + Mouthrinse + pH cycling (LM)</td>
<td>0.037 ± 0.015 b</td>
<td>0.025 ± 0.028 c</td>
</tr>
<tr>
<td>Laser + Dentifrice + Mouthrinse + pH cycling (LDM)</td>
<td>0.044 ± 0.012 ab</td>
<td>0.109 ± 0.117 ab</td>
</tr>
</tbody>
</table>

Note: Means followed by different letters are statistically different by the Tukey test (p<0.05).

Figure 1. Demineralization Patterns. The qualitative polarized light analysis showing demineralization patterns in nine sections belonging to the nine groups.

caries-like lesion progression in dental enamel (p<0.05) when compared with the control group. The mineral loss inhibition capability of CO₂ laser, in combination with/without fluoride found this study is in line with previously reported results.

The present study data revealed the enamel mineral loss in groups D, M, L, and LM did not show a statistically significant difference (p>0.05) after a high cariogenic challenge from that found in enamel with only a carious lesion (Ca). This finding indicates isolated treatments, as well as the combined laser and mouthrinse regime prevented the additional enamel demineralization promoted by the pH-cycling regime.

With regard to fluoride treatments, the current study results are consistent with Paes Leme and Damato who also found caries-like lesion progression was inhibited by using a fluoridated dentifrice and fluoridated mouthrinse, respectively, in a pH-cycling model. The present study data demonstrated a lack of synergism between the
laser and mouthrinse treatment. Tepper\textsuperscript{24} was also unable to show a synergic effect when using a 2% amine fluoride solution and CO\textsubscript{2} laser therapy in subsurface enamel layers. Similar results were found by Phan.\textsuperscript{29} However, other studies using different irradiation parameters and mainly employing a 9.6 μm CO\textsubscript{2} laser did show a synergistic effect.\textsuperscript{25,27,37} One possible explanation for the lack of synergism between laser application and fluoridated products found in this study is the 10.6 μm CO\textsubscript{2} laser has a lower absorption coefficient and, consequently, is less absorbed by the enamel than a 9.6 μm CO\textsubscript{2} laser. This produced less laser-induced surface change on enamel which may have promoted minor fluoride incorporation. In contrast to the present findings, Hsu\textsuperscript{35} found a synergic effect using 10.6 μm CO\textsubscript{2} laser and a 2% NaF gel with a very low energy density (0.3 J/cm\textsuperscript{2}). However, these authors did not show any morphological laser-induced surface changes on enamel such as melting or large crater formation.

Moreover, when compared with the groups treated with a fluoridated mouthrinse, those treated with a fluoridated dentifrice showed a better remineralizing capacity (Figure 1) as also evidenced by the results of the remineralizing solutions analysis. The groups treated with fluoride from a mouthrinse (M and LM) showed statistically significant lower fluoride concentrations. This did not differ from the groups not receiving any fluoride treatment (C and L). This could be explained by the double\textsuperscript{32} fluoride treatment regime along with a single daily\textsuperscript{40} treatment with the dentifrice and mouthrinse and by using different fluoride concentrations in the vehicles which made the enamel slabs less exposed to fluoride in the second treatment.

When compared with the Ca group, a statistically significant reduction in enamel mineral loss was found for all combined therapies except for the LM group. Thus, it could be suggested these treatments were not only able to prevent the additional enamel demineralization promoted by the pH-cycling regime, but were also capable of remineralizing the softened enamel as evidenced by the recovery of the microhardness of the enamel (Table 1). To corroborate these findings, polarized light (Figure 1) also showed a remineralizing line for the same groups. Since laser treatment does not enhance remineralization in the absence of fluoride,\textsuperscript{41} the remineralizing effect of the combined laser/fluoride therapies found in the present study may be related to the fluoridated dentifrice.

According the polarized light analysis, no remineralization line was observed in M, L, or LM. Consequently, it may be suggested the remineralizing effect found in the DM and LD groups was much more related to fluoride from the dentifrice. In addition, these results can be confirmed considering the data shown in (Table 2). Part of the products formed on the enamel surface was lost during the subsequent cariogenic challenge (pH cycling). This fluoride that was released from the enamel was found in remineralizing and demineralizing solutions. The mean value found for fluoride concentration in both solutions was significantly higher only when fluoridated dentifrice was applied (Table 2). The results of the fluoride concentration values in these solutions are in agreement with those described by Paes Leme,\textsuperscript{29} even though they used a different pH cycling model and daily fluoridated dentifrice exposure.

**Conclusion**

Used alone, or combined with fluoridated products, 10.6 μm CO\textsubscript{2} laser irradiation with a fluence of 10 J/cm\textsuperscript{2} and pulse duration of 10 ms produced an effective protection against the progression of demineralization in enamel. However, combined laser-fluoride treatments showed no additional significant demineralization inhibitory effect.
References
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