Effect of titanium tetrafluoride and amine fluoride treatment combined with carbon dioxide laser irradiation on enamel and dentin erosion

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Abstract

OBJECTIVE: This in vitro study aimed to analyze the influence of carbon dioxide (CO(2)) laser irradiation on the efficacy of titanium tetrafluoride (TiF(4)) and amine fluoride (AmF) in protecting enamel and dentin against erosion. METHODS: Bovine enamel and dentin samples were pretreated with carbon dioxide (CO(2)) laser irradiation only (group I), TiF(4) only (1% F, group II), CO(2) laser irradiation before (group III) or through (group IV) TiF(4) application, AmF only (1% F, group V), or CO(2) laser irradiation before (group VI) or through (group VII) AmF application. Controls remained untreated. Ten samples of each group were then subjected to an erosive demineralization and remineralization cycling for 5 days. Enamel and dentin loss were measured profilometrically after pretreatment, 4 cycles (1 day), and 20 cycles (5 days) and statistically analyzed using analysis of variance and Scheffe's post hoc tests. Scanning electron microscopy (SEM) analysis was performed in pretreated but not cycled samples (two samples each group). RESULTS: After 20 cycles, there was significantly less enamel loss in groups V and IV and significantly less dentin loss in group V only. All other groups were not significantly different from the controls. Lased surfaces (group I) appeared unchanged in the SEM images, although SEM images of enamel but not of dentin showed that CO(2) laser irradiation affected the formation of fluoride precipitates. CONCLUSION: AmF decreased enamel and dentin erosion, but CO(2) laser irradiation did not improve its efficacy. TiF(4) showed only a limited capacity to prevent erosion, but CO(2) laser irradiation significantly enhanced its ability to reduce enamel erosion.
Effect of TiF$_4$ and AmF treatment combined with CO$_2$ laser-irradiation on enamel and dentin erosion

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**Running title:**
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Abstract

Objective: This in vitro study aimed to analyse the influence of CO\textsubscript{2} laser irradiation on the efficacy of TiF\textsubscript{4} and AmF to protect enamel and dentin against erosion.

Methods: Bovine enamel and dentin samples were pretreated with CO\textsubscript{2} laser irradiation only (group I), TiF\textsubscript{4} only (1\%F, group II), CO\textsubscript{2} laser irradiation prior to (group III) or through (group IV) TiF\textsubscript{4} application, AmF only (1\%F, group V) or CO\textsubscript{2} laser irradiation prior (group VI) or through (group VII) AmF application. Controls remained untreated. Ten samples of each group were then subjected to an erosive demineralisation (Sprite Zero, 4x90s/day) and remineralisation (artificial saliva) cycling for 5 days. Enamel and dentin loss was measured profilometrically after pretreatment, 4 cycles (1 day) and 20 cycles (5 days) and statistically analysed by ANOVA and Scheffe’s post-hoc tests. SEM analysis was performed in pretreated but not cycled samples (two samples each group).

Results: After 20 cycles, enamel loss was significantly decreased in groups V and IV, while dentin loss was significantly reduced in group V only. All other groups were not significantly different from the controls. Lased surfaces (group I) appeared unchanged in the SEM images. However, SEM images of enamel but not of dentin showed that the formation of fluoride precipitates was affected by CO\textsubscript{2} laser irradiation.

Conclusion: AmF decreased enamel and dentin erosion, but its efficacy was not improved by CO\textsubscript{2} laser irradiation. TiF\textsubscript{4} showed only a limited capacity to prevent erosion, but CO\textsubscript{2} laser irradiation enhanced its ability to reduce enamel erosion to a significant level.
Introduction

The efficacy of titanium tetrafluoride (TiF$_4$) to prevent enamel and dentin demineralisation is attributed to the formation of an acid-resistant surface coating, an increased fluoride uptake and the titanium incorporation in the hydroxyapatite lattice.\textsuperscript{1} The glaze-like surface layer observed after the application of TiF$_4$ is assumed to be formed from TiO$_2$ and/or from organometallic complexes of titanium and the organic dental matrix and might primary act as a diffusion barrier. However, as the layer is rich of titanium and fluoride it is discussed that the coating might also act as reservoir for fluoride ions which in turn might retard acid dissolution or increase remineralisation of the underlying dental hard tissue. The increased fluoride uptake found after application of TiF$_4$ can be explained by the ability of the polyvalent metal ion to form strong fluoride complexes while simultaneously binding firmly to the apatite crystals.\textsuperscript{1}

However, even though TiF$_4$ was shown to be more effective to prevent erosive demineralisation than sodium, stannous or amine fluoride,\textsuperscript{2-5} most studies found that TiF$_4$ is not able to protect enamel erosion completely.\textsuperscript{5-8}

Previous experiments showed that CO$_2$ laser irradiation induced a melting and recrystallization process resulting in an increased acid resistance of dental hard tissues.\textsuperscript{9-11} Moreover, laser irradiation might be also an approach to increase the efficacy of different fluoride solutions, such as amine\textsuperscript{12} or sodium fluoride,\textsuperscript{13,14} to reduce acid demineralisation. CO$_2$ laser irradiation increased the fluoride uptake in enamel\textsuperscript{12,15} and facilitated the transformation from hydroxyapatite into fluorapatite.\textsuperscript{16}

While laser-induced effects on fluorides commonly used in caries prevention, such as sodium and amine fluoride, were analysed previously,\textsuperscript{13,15} no information about laser irradiation of surfaces treated with TiF$_4$ is available as yet. It is assumed that CO$_2$ laser irradiation might increase the efficacy TiF$_4$ to prevent enamel and dentin erosion, for example by melting and condensing the glaze-like surface layer observed after application of TiF$_4$. 
Thus, it was the aim of the present study to analyse the influence of CO₂ laser irradiation on the effects of TiF₄ compared to AmF solutions to protect enamel and dentin erosion.

**Materials and methods**

Sample preparation

Enamel (4mm x 4mm x 3mm) and dentin (4mm x 4 mm x 2mm) samples were prepared from the labial or root surfaces, respectively, of bovine incisors. The samples were cut using an ISOMET low speed saw cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA), which were separated by a 4-mm diameter spacer. The samples surfaces were ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al₂O₃ papers; Buehler, Lake Bluff, IL, USA), and polished with felt paper wet by diamond spray (1 mm; Buehler). Thereby, the cementum layer of the dentin samples was completely removed. Prior to the experiment, two layers of nail varnish were applied on half of the surface of each sample to maintain a reference surface for lesion depth determination after the experiment.

Each 12 enamel and 12 dentin samples were randomly allocated to 7 test groups and one control: CO₂ laser irradiation (group I), TiF₄ (1% F, group II), CO₂ laser irradiation prior to (group III) or through (group IV) TiF₄ application, AmF (1% F, group V) or CO₂ laser irradiation prior (group VI) or through (group VII) AmF application and control (untreated). Ten samples each group were submitted to erosive cycling after CO₂ and/or fluoride pretreatment, while two samples were only pretreated and left for scanning electron microscopy (SEM) analysis.

CO₂ laser treatment and fluoride solutions

Laser irradiation was performed with a commercially available CO₂ laser (UM-L30, Union Medical Engineering Co, Korea) at 10.6 µm wavelength, 10 µs pulse duration and 50 Hz
frequency. The exposed surface of the samples (free from nail varnish) was irradiated for 13 s by one calibrated dentist by moving the laser probe tip continuously at a standardized distance of 10 mm from the sample surface to guarantee a 0.3 mm spot size. Enamel samples were irradiated with average power input and output of 2.8 W and 2.0 W, respectively (28.6 J/cm²). Irradiation of dentin samples was performed with average power input of 1.5 W and output of 1.05 W (15 J/cm²).

For fluoride pretreatment, equimolar solutions of TiF₄ (1% F, 1.64% TiF₄, pH 1.2, Sigma-Aldrich Chemical Company, Milwaukee, WI, USA) and AmF (1% F, pH: 4.5, Elmex fluid, GABA, Münchenstein, Switzerland, Lot: 86781B) were used. Twenty-five microliters of the respective solutions were pipetted on the samples surface and left undisturbed for 60 s. In groups IV and V, the solutions were applied immediately after laser irradiation. In groups VI and VII, the laser irradiation was performed through the fluoride solutions. Thereby, the laser irradiation started 30 s after the application of the solutions. After treatment, specimens were rinsed with distilled water for 15 s.

Erosive cycling

Ten enamel and 10 dentin samples per group treated as described above were submitted to a 5 day de- and remineralisation cycling. Erosion was performed with Sprite Zero (pH 2.6, 20 ml/sample, unstirred, Coca-Cola, Brazil) four times daily for 90 s. After demineralisation, the samples were rinsed with tap water and transferred into artificial saliva (20 ml/sample) for 2 h. After the last daily erosive treatment, the samples were stored in artificial saliva over night. The artificial saliva (pH 7) was renewed daily and consisted of 1.5 mmol/L Ca(NO₃)₂·4H₂O, 0.9 mmol/L NaH₂PO₄·2H₂O, 150 mmol/L KCl, 0.1 mol/L Tris buffer and 0.03 ppm F.¹⁷
Profilometric measurement

Enamel and dentin loss was quantitatively determined by profilometry (Hommel Tester T1000, VS, Schwenningen, Germany) after fluoride application and/or CO₂ laser irradiation (1. measurement) as well as after 4 erosive cycles/1 day (2. measurement) and 20 erosive cycles/5 days (3. measurement). As no pretreatment was performed in the control group, these samples were measured only after 4 and 20 erosive cycles.

For profilometric measurement, the nail varnish was carefully removed and the samples were dried. The diamond stylus moved from the reference to the exposed area (Length of the profile: 1.5 mm). Five profile measurements were performed in the center of each specimen and averaged. After the 1. and 2. profilometric measurement, the reference area of the specimens was again covered with nail varnish. To assure that the nail varnish was placed over the original reference area, the position of the nail varnish was marked by carving with a scalpel blade at the borders of the sample.

Scanning Electron Microscopy

Two enamel and two dentin samples each group were subjected to SEM analysis (SUPRA 50VP, Carl Zeiss NTS GmbH, Oberkochen, Germany) directly after pretreatment with the fluoride solutions and/or CO₂ laser irradiation. The samples were desiccated for 4 weeks in blue silica gel in a vacuum evaporator, then sputter-coated with gold for 60 s and the examined at 5 to 10 kV.

Statistical analysis

Cumulative enamel or dentin loss, respectively, was analysed by one-way ANOVA followed by Scheffe’s post-hoc tests, separately for the time points “after pretreatment”, “4 erosive cycles” and “20 erosive cycles”. To consider the amount of enamel or dentin loss caused by the erosive cycling only but not by the pretreatment, mean differences between “after pretreatment” and “20
erosive cycles” were computed and analysed by one-way ANOVA and Scheffe’s post-hoc tests. The level of significance was p < 0.05.

Results

Profilometry

Cumulative enamel and dentin loss (mean ± standard deviation [µm]) after pretreatment and after 4 and 20 erosive cycles is presented in Table 1 and 2. For both enamel and dentin, one-way ANOVA revealed significant differences among the groups for each time point.

Table 1

Mean enamel loss (µm) ± standard deviation in the different groups after pretreatment with the AmF or TiF₄ solutions and/or CO₂ laser irradiation, after 4 and after 20 erosive cycles.

<table>
<thead>
<tr>
<th>Group</th>
<th>After pretreatment</th>
<th>4 erosive cycles</th>
<th>20 erosive cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0ᴬ</td>
<td>0.6 ± 0.4ᴬ</td>
<td>3.0 ± 0.4ᴬ</td>
</tr>
<tr>
<td>I  CO₂ Laser</td>
<td>0ᴬ</td>
<td>0.7 ± 0.3ᴬ</td>
<td>2.5 ± 0.6ᴬ,ᴮ</td>
</tr>
<tr>
<td>II TiF₄</td>
<td>1.0 ± 0.3ᴮ,ᴮ,ᴮ,ᴮ</td>
<td>1.3 ± 0.2ᴮ</td>
<td>2.6 ± 0.3ᴬ,ᴮ</td>
</tr>
<tr>
<td>III CO₂ Laser – TiF₄</td>
<td>1.1 ± 0.1ᴬ</td>
<td>1.4 ± 0.4ᴮ</td>
<td>2.7 ± 0.5ᴬ,ᴮ</td>
</tr>
<tr>
<td>IV TiF₄ – CO₂ Laser</td>
<td>0.8 ± 0.3ᴮ</td>
<td>1.4 ± 0.4ᴮ</td>
<td>2.1 ± 0.4ᴮ</td>
</tr>
<tr>
<td>V  AmF</td>
<td>0ᴬ</td>
<td>0.9 ± 0.5ᴬ,ᴮ</td>
<td>2.1 ± 0.4ᴮ</td>
</tr>
<tr>
<td>VI CO₂ Laser - AmF</td>
<td>0ᴬ</td>
<td>0.9 ± 0.4ᴬ,ᴮ</td>
<td>2.5 ± 0.3ᴬ,ᴮ</td>
</tr>
<tr>
<td>VII AmF – CO₂ Laser</td>
<td>0ᴬ</td>
<td>1.0 ± 0.4ᴬ,ᴮ</td>
<td>2.4 ± 0.2ᴬ,ᴮ</td>
</tr>
</tbody>
</table>

As no pretreatment was performed in the control group and possible enamel alterations in the groups I, and IV-VI were below the detection limit, these values were set at zero.

In each column, same letters were used for groups which were not significantly different when compared by Scheffe’s post-hoc tests.
Table 2

Mean dentin loss (µm) ± standard deviation in the different groups after pretreatment with the AmF or TiF₄ solutions and/or CO₂ laser, after 4 and after 20 erosive cycles.

<table>
<thead>
<tr>
<th>Group</th>
<th>After pretreatment</th>
<th>4 erosive cycles</th>
<th>20 erosive cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0⁴</td>
<td>1.2 ± 0.7⁴</td>
<td>2.7 ± 0.8⁴</td>
</tr>
<tr>
<td>I CO₂ Laser</td>
<td>0.5 ± 0.2⁵</td>
<td>1.8 ± 0.3⁴</td>
<td>2.3 ± 0.2⁵,⁶</td>
</tr>
<tr>
<td>II TiF₄</td>
<td>1.6 ± 0.3⁶</td>
<td>1.9 ± 0.3⁴</td>
<td>2.2 ± 0.5⁵,⁶</td>
</tr>
<tr>
<td>III CO₂ Laser – TiF₄</td>
<td>1.3 ± 0.3⁷,⁸</td>
<td>1.8 ± 0.4⁶</td>
<td>2.4 ± 0.4⁵,⁶</td>
</tr>
<tr>
<td>IV TiF₄ – CO₂ Laser</td>
<td>1.2 ± 0.2⁸</td>
<td>1.7 ± 0.4⁶</td>
<td>2.1 ± 0.4⁵,⁶</td>
</tr>
<tr>
<td>V AmF</td>
<td>0.5 ± 0.1⁹</td>
<td>1.3 ± 0.4⁶</td>
<td>1.9 ± 0.5⁶</td>
</tr>
<tr>
<td>VI CO₂ Laser - AmF</td>
<td>0.7 ± 0.3⁴,⁹</td>
<td>1.0 ± 0.4⁶</td>
<td>2.4 ± 0.2⁵,⁶</td>
</tr>
<tr>
<td>VII AmF – CO₂ Laser</td>
<td>0.7 ± 0.2⁴,⁹</td>
<td>1.8 ± 0.6⁶</td>
<td>2.4 ± 0.5⁵,⁶</td>
</tr>
</tbody>
</table>

As no pretreatment was performed in the control group, this value was set at zero.

In each column, same letters were used for groups which were not significantly different when compared by Scheffe’s post-hoc tests.

In enamel, application of TiF₄ (with and without CO₂-Laser irradiation, groups II-IV) caused some surface loss, while samples pretreated with AmF (group V-VII) or CO₂ laser irradiation (group I) were not affected (Table 1). After 4 erosive cycles, cumulative enamel loss was still higher in the TiF₄ groups (groups II-IV) compared to the control, while all AmF groups (groups V-VII) were not significantly different from the control. However, after 20 erosive cycles, cumulative enamel loss was significantly reduced in samples treated by CO₂ laser irradiation through TiF₄ (group IV) and in samples pretreated by AmF only (group V, Table 1). Considering the amount of enamel loss caused by the erosive cycling only (Table 3), samples treated with TiF₄ (groups II-IV) showed the lowest progression of erosion.
In dentin, all pretreatments induced some loss, which was higher for the TiF₄ groups (groups II-IV) than for the AmF groups (groups V-VII) and CO₂ laser irradiation (group I, Table 2). After 20 erosive cycles, cumulative dentin loss was significantly reduced only in the samples pretreated by AmF (group V). Generally, samples treated with TiF₄ (group II-IV) showed a lower increase of dentin loss due to the erosive cycling compared to the AmF groups (groups V-VII) and CO₂ laser irradiation (group I, Table 3).

**Table 3**

Enamel and dentin loss (µm) caused by the erosive cycling only (mean difference ± standard deviation of enamel or dentin loss between “after pretreatment” and “20 erosive cycles”).

<table>
<thead>
<tr>
<th>Group</th>
<th>Enamel</th>
<th>Dentin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.0 ± 0.4A</td>
<td>2.7 ± 0.8A</td>
</tr>
<tr>
<td>I CO₂ Laser</td>
<td>2.5 ± 0.6A,B</td>
<td>1.8 ± 0.2B</td>
</tr>
<tr>
<td>II TiF₄</td>
<td>1.5 ± 0.4C,D</td>
<td>0.6 ± 0.6C</td>
</tr>
<tr>
<td>III CO₂ Laser – TiF₄</td>
<td>1.5 ± 0.5C,D</td>
<td>1.1 ± 0.5B,C</td>
</tr>
<tr>
<td>IV TiF₄ – CO₂ Laser</td>
<td>1.3 ± 0.5C</td>
<td>0.9 ± 0.5B,C</td>
</tr>
<tr>
<td>V AmF</td>
<td>2.1 ± 0.4B,D</td>
<td>1.4 ± 0.5B</td>
</tr>
<tr>
<td>VI CO₂ Laser - AmF</td>
<td>2.5 ± 0.3A</td>
<td>1.7 ± 0.5B</td>
</tr>
<tr>
<td>VII AmF – CO₂ Laser</td>
<td>2.4 ± 0.2A</td>
<td>1.7 ± 0.3B</td>
</tr>
</tbody>
</table>

In each column, same letters were used for groups which were not significantly different.

**SEM images**

The SEM images of enamel and dentin samples are presented in Fig. 1 and 2.

In enamel, CO₂ laser irradiation (group I) produced no visible surface alterations compared to control. The application of TiF₄ (groups II-IV) induced the formation of a granular surface layer. This layer appeared melted and less granular when CO₂ laser irradiation was performed through the TiF₄ solution (group IV) compared to groups II (TiF₄) and III (CO₂ laser irradiation prior to TiF₄). In samples pretreated with AmF (group V), a globular surface layer could be observed.
contrast, samples pretreated with CO₂ laser irradiation prior or through AmF (groups VI and VII) appeared slightly demineralised.

In dentin, CO₂ laser irradiated surfaces (group I) appeared slightly melted compared to the control, with dentinal tubules partly covered by a layer. The application of TiF₄ induced a granular coating (groups II-IV). The superficial layer of this granular coating appeared more condensed in group IV. Surfaces pretreated with AmF (groups V-VII) exhibited globular precipitates.

**Discussion**

AmF and TiF₄ showed only a limited capacity to prevent enamel and dentin erosion under the conditions of the present study. CO₂ laser irradiation alone was not able to decrease erosive enamel and dentin loss, but enhanced the capability of TiF₄ to reduce enamel erosion when it was used during the application of TiF₄.

In the present study, TiF₄ was applied as 1.64% solution to match its fluoride concentration to the fluoride concentration of the commercially available AmF product, thus allowing for a comparison between the solutions. To simulate a realistic application time, the fluoride solutions were applied once for 60 s as done in previous experiments.⁸,¹⁸ As shown previously,¹⁹,²⁰ the application of TiF₄ induced the formation of surface precipitates on both enamel and dentin. The surface precipitates contain high concentrations of titanium⁸ and are assumed to be formed from TiO₂ and/or from organometallic complexes of titanium and the organic dental matrix. This surface layer was shown to be acid-resistant to a certain extent and to protect the underlying dental hard tissue mechanically.¹ On the other hand, the application of TiF₄ induced also some enamel and dentin loss during application as measured by profilometry after pretreatment. It might be speculated that the low pH of the TiF₄ solution causes some demineralisation of the surface while enhancing the formation of an acid-resistant layer on the demineralised surface at the same time.⁸ In contrast to previous studies,⁵,⁶ TiF₄ alone was not effective to protect enamel
and dentin loss significantly when considering the whole experiment (application and erosive cycling). However, considering the amount of enamel and dentin loss induced by the erosive treatment only, TiF$_4$ reduced the progression of dental hard tissue loss significantly. Thus, it is assumed that the TiF$_4$-induced precipitates have the potential to prevent further erosion so that the protective effect of TiF$_4$ might become more apparent under prolonged erosive conditions.

AmF induced the formation of a globular surface coating on enamel and dentin samples, which is known to be composed of CaF$_2$-like precipitates.$^{21-23}$ This CaF$_2$-containing layer acts as a source of free fluoride ions available during the erosive challenge. These are subsequently incorporated into the enamel as hydroxyfluorapatite or fluorapatite, resulting in a decreased susceptibility to further dissolution. Additionally, the CaF$_2$-layer might act as a physical barrier hampering the contact of the acid with the underlying tissue or as a mineral reservoir, which is attacked by the erosive challenge, thus leading to a buffering or depletion of hydrogen ions from the acid. The amount of CaF$_2$ increases with time, concentration, pH-decrease and calcium availability.$^{24}$ As the fluoride solutions were applied only once in the present study, it might be assumed that the efficacy of AmF could be enhanced by a daily application of the solutions.$^{25}$

While several studies found a protective effect of CO$_2$ laser irradiation on caries development and progression,$^{11}$ CO$_2$ laser irradiation alone was not effective to reduce erosive enamel or dentin loss. As yet, only few studies were performed analysing the effects of laser irradiation on erosive dental loss, but show conflicting results. While some studies reported a beneficial effect of a CO$_2$ or a Nd:YAG laser$^{26}$ on enamel erosion, dentin erosion could not be prevented by Nd:YAG laser irradiation.$^{27}$ In the present study, the output parameters for lasing enamel and dentin were chosen according to previous studies showing an increased acid resistance of enamel and dentin$^{10,13}$ or an increased enamel fluoride uptake$^{12,15}$ after CO$_2$ laser treatment. However, pulsed instead of continuous wave lasing was performed to confine possible modifications to a thin surface layer, without affecting the underlying hard tissue or pulp.$^{11}$ The laser energy density applied to enamel (28.6 J/cm$^2$) exceeded the range (10-11.5 J/cm$^2$), which was shown to induce
chemical and morphological changes and, thus, induce an inhibitory effect on demineralisation.\textsuperscript{28} Dentin was lased with 15 J/cm\textsuperscript{2} as it is more sensitive to laser irradiation than enamel so that lower energy densities are recommended.\textsuperscript{11}

A variety of explanations for the caries-protective effect of CO\textsubscript{2} lasers on enamel and dentin were summarized recently. CO\textsubscript{2} lasing is discussed to induce a melting, fusion or recrystallization of enamel and dentin as well as the formation of pyrophosphate, which might decrease the solubility of hydroxyapatite. Moreover, carbonate loss or modifications of the organic matrix might contribute to the decreased demineralisation potential of the substrate.\textsuperscript{11} However, the results of the present study implicate that the laser-induced morphological and crystallographic changes of the surface were not effective to protect enamel and dentin against the erosive demineralisation.

However, as previous studies showed that CO\textsubscript{2} laser irradiation might enhance the efficacy of fluorides in caries prevention,\textsuperscript{14} the present study aimed to analyse whether CO\textsubscript{2} laser irradiation might also increase the protective effects of fluorides, especially TiF\textsubscript{4}, on dental erosion.

While Tepper et al.\textsuperscript{12} and Schmidlin et al.\textsuperscript{15} reported that the protective effect of AmF (same product as in the present study) on enamel demineralisation could be increased by CO\textsubscript{2} laser irradiation, the combined application of AmF and CO\textsubscript{2} laser irradiation failed to reduce erosion in the present study. Enamel samples pretreated with AmF in combination with CO\textsubscript{2} laser irradiation did not exhibit the globular precipitates usually found after application of AmF (groups VI and VII). The surfaces appeared slightly demineralised, but these alterations were below the detection limit of the profilometric device.\textsuperscript{29} It might be speculated that CO\textsubscript{2} laser irradiation enhances the penetration of fluorides in enamel in a way that more structurally bound fluoride instead of loosely-bound CaF\textsubscript{2}-precipitates are formed. The absence of the globular CaF\textsubscript{2} layer in groups VI and VII might account for the lacking protective effect on enamel erosion.

In contrast to AmF, the efficacy of TiF\textsubscript{4} to protect enamel erosion could be enhanced when CO\textsubscript{2} laser irradiation was performed through the solution (group IV). The SEM images showed that CO\textsubscript{2} laser irradiation induced a melting of the granular surface precipitates, which might result in
an enhanced uptake and retention of titanium and/or fluoride, thus leading to an increased acid resistance of enamel. In contrast, CO$_2$ laser irradiation prior to TiF$_4$ application did not lead to visible changes of the surface precipitates, assuming that possible laser-induced surface alterations might not affect the formation of the granular surface precipitates by TiF$_4$.

In dentin, CO$_2$ laser irradiation was not able to enhance the efficacy of the fluoride solutions and to induce a melting of the fluoride precipitates. Only two studies analysed the effects on laser irradiation in combination with fluoride treatment on demineralisation as yet.$^{13,27}$ Hossain et al.$^{13}$ showed that dentin surfaces pretreated with a neutral sodium fluoride solution changed to a melted, smooth and mirror-like appearance when CO$_2$ laser irradiation was applied. Thereby, the combination of CO$_2$ laser irradiation and sodium fluoride was more effective in reducing carious demineralisation than fluoride treatment or laser irradiation alone. In contrast, the efficacy of an acidulated phosphate fluoride gel to prevent dentin erosion could not be enhanced by Nd:YAG laser irradiation.$^{27}$ In the present study, the superficial TiF$_4$-induced layer appears more condense after CO$_2$ laser irradiation. In this group, dentin loss was decreased compared to the control, but failed to reach a significant level. It might be speculated whether CO$_2$ laser irradiation with higher energy might enhance the condensation of the surface precipitation, and, thus, the erosion-protective effect of TiF$_4$. However, it has to be taken into consideration that higher energy levels might be associated with adverse side-effects, such as cracking and fissuring of the surface as well as with thermal changes of the underlying tissue.$^{11}$

**Conclusion**

Under the conditions of the present study it can be concluded that AmF reduced both enamel and dentin erosion, but that its efficacy could not be enhanced by CO$_2$ laser irradiation. TiF$_4$ showed only a limited capacity to prevent erosion, but CO$_2$ laser irradiation enhanced its efficacy to reduce enamel loss when applied during the application of TiF$_4$. 
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Author Disclosure Statement

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Legends for Figures

Fig 1.

SEM images of enamel samples at 60,000x magnification, bar = 300 nm (control, I, II) or 1µm (III-VII) control, I. CO₂ laser irradiation, II. TiF₄, III. CO₂ laser irradiation prior to TiF₄, IV. CO₂ laser irradiation through TiF₄, V. AmF, VI: CO₂ laser irradiation prior to AmF, VII: CO₂ laser irradiation through AmF

Fig 2.

SEM images of dentin samples at 60,000x magnification, bar = 300 nm (II, IV, VI, VII) or 1µm (I, III, IV) control, I. CO₂ laser irradiation, II. TiF₄, III. CO₂ laser irradiation prior to TiF₄, IV. CO₂ laser irradiation through TiF₄, V. AmF, VI: CO₂ laser irradiation prior to AmF, VII: CO₂ laser irradiation through AmF
Fig 1.
Fig 2.