Clinical Oral Investigations
https://doi.org/10.1007/s00784-018-2648-y

ORIGINAL ARTICLE

Randomized in situ study on the efficacy of CO_2 laser irradiation in increasing enamel erosion resistance

K. M. Ramalho[1] · C. P. Eduardo[2] · N. Heussen[3] · R. G. Rocha[1] · H. Meyer-Lueckel[4] · F. Lampert[5] · C. Apel[6] · Marcella Esteves-Oliveira[5]

Received: 7 September 2017 / Accepted: 20 September 2018

Abstract

Objectives The aim of this double-blind, randomized in situ study was to evaluate the erosion-preventive effect of a specific set of CO_2 laser parameters, associated or not with fluoride.

Methods Two hundred forty bovine enamel blocks were prepared for individual palatal appliances ($n = 6$ samples/appliance). The study had four phases of 5 days each, with ten volunteers and the following treatments: CO_2 laser irradiation (L), fluoride treatment (F), combined fluoride and laser treatment (FL), and no treatment, control (C). Laser irradiation was performed at 0.3 J/cm^2 (5 μs/226 Hz/10.6 μm) and the fluoride gel contained AmF/NaF (12′500 ppm F^-/pH = 4.8–6). For erosive demineralization, the appliances were immersed extra-orally in citric acid (0.05 M/20 min/pH = 2.3) twice daily. Analysis of enamel surface loss was done using a 3D-laser profilometer on 3 days. Additionally, fluoride uptake was quantified and scanning electron microscopies were done. Data were analyzed with repeated measures ANOVA and post hoc pairwise comparisons ($\alpha = 0.05$).

Results At all analyzing days, both laser groups caused the lowest means of enamel loss, which were also statistically significant lower than C ($p < 0.05$). At day 5, FL means ± SD (33.6 ± 12.6 μm) were even significantly lower than all other groups (C 67.8 ± 15.4 μm; F 57.5 ± 20.3 μm; L 46.8 ± 14.5 μm). Significantly increased enamel fluoride uptake was observed for both fluoride-containing groups ($p < 0.05$) at day 1.

Conclusion Compared to the control, the CO_2 laser irradiation with a specific set of laser parameters (0.3 J/cm^2/5 μs/226 Hz) either alone or in combination with a fluoride gel (AmF/NaF) could significantly decrease enamel erosive loss up to 5 days in situ.

Clinical relevance Combined CO_2 laser-fluoride treatment has a significant anti-erosive effect.

Keywords Tooth erosion · Fluorides · Lasers · Carbon dioxide · Tooth Wear · Amine fluoride gel

Introduction

Dental erosion is considered a disorder of dental tissues, in which, as opposite to caries, tooth mineral dissolution occurs solely due to the contact of intrinsic and extrinsic acids with the tooth surfaces and without the presence of a biofilm [1]. It has long been considered a purely surface phenomenon, but lately this has changed, as the existence of near-surface demineralization reaching few micrometers within enamel has been experimentally proven [2]. This means that at the first micrometers of the surface, there is a partly demineralized and softened enamel layer [3]. The softened layer represents a challenge for the symptomatic therapy and could be one of the reasons why none of the currently available preventive agents is able to completely eliminate erosive tooth wear progression. The agents with the most promising efficacy for protecting enamel against erosion are products containing

✉ Marcella Esteves-Oliveira
mestevesoliveira@ukaachen.de

1 Department of Stomatology, Integrated Clinic, School of Dentistry, University of São Paulo, São Paulo, Brazil

2 Special Laboratory of Lasers in Dentistry (LELO), Department of Restorative Dentistry, School of Dentistry, University of São Paulo, São Paulo, Brazil

3 Department of Medical Statistics, Medical Faculty, RWTH Aachen University, Aachen, Germany

4 Department of Restorative, Preventive & Pediatric Dentistry, Universität Bern, Bern, Switzerland

5 Department of Operative Dentistry, Periodontology and Preventive Dentistry, Medical Faculty, RWTH Aachen University, Pauwelsstr. 30, 52074 Aachen, Germany

6 Department of Biohybrid & Medical Textiles, Institute of Applied Medical Engineering, Medical Faculty, RWTH Aachen University, Aachen, Germany

Published online: 28 September 2018

both fluoride and polyvalent metal ions [4, 5]. For solutions containing around 800 ppm of Sn^{2+} and at least 500 ppm F^-, 65% reduction in vitro [6] and 67% in situ [7] have been observed. Products having these compounds were recently introduced and are already cleared for clinical use in several countries (i.e., Europe). Another compound that shows high-preventive effect is the TiF_4, but the low pH of the solutions and the open questions about its cytotoxicity still impairs its clinical use [8–10]. As regards, dentifrice as vehicle for the delivery of these substance evidences also shows that AmF/$SnCl_2$ caused significant reduction of erosive loss, which is apparently enhanced, when it is combined with a biopolymer, as for example, 5% chitosan [11, 12]. Nonetheless, the effects get strongly reduced when brushing is also involved, indicating that these compounds are probably not very effective in rehardening previously softened enamel, but rather in preventing further demineralization [4, 9, 13]. Thus, up to now, none of the currently available fluoride compounds could achieve durable protection against erosion and there is still a need for seeking new preventive approaches.

At the same time, the CO_2 laser irradiation (at 10.3, 10.6, 9.3, and 9.6 μm) has shown in several studies a great potential as a new non-invasive therapy [14–16] both against caries and against erosion. The first systematic investigations were focusing on the 9.6-μm wavelength and demonstrated that using low energy densities (below 2 J/cm^2) and low pulse durations (below 10 μs) increase of enamel resistance to acid solubility could be achieved. For energy densities below the ablation threshold, the lower the pulse duration, the higher the preventive efficacy (less energy needed for causing the effect) as well as, the lower the chances of thermal damage to the tissue [17]. For the 9.6-μm wavelength, this effect was even shown clinically [18] and for the 10.6 μm lately, also some similar findings have been obtained after systematical investigations of ideal parameters. A specific set of CO_2 laser parameters has shown to cause both very high reductions of carious [19] as well as erosive demineralization [20, 21]. In the combination with tin-containing fluoride solution ($SnCl_2$/AmF/NaF), even better results have been obtained [22]. Additionally, the use of these parameters caused also no deleterious morphological changes to the surface and avoided a pulp temperature increase above 5.5 °C (threshold for irreversible pulp inflammation), which indicates biological safety and potential for clinical translation.

However, although these previous findings are very promising, the effect of this anti-erosive laser therapy has never been tested before under more clinically similar conditions from an in situ model. Therefore, the aim of the present study was to evaluate for the first time the erosive-protective effect of a specific set of CO_2 laser parameters under in situ conditions, simulating also the influence of natural saliva and the salivary pellicle. The null hypothesis formulated was that there is no significant difference in the protective effect against enamel surface loss caused by solely laser, solely fluoride, combined laser and fluoride treatment, and the negative control at all analyzing times after the therapy.

Materials and methods

The principles of the Declaration of Helsinki were followed and the study was approved by Ethical Committee of Medical School of the RWTH Aachen University (#EK 134/09).

Study design and sample size

This study was a placebo-controlled, double-blind (examiner and volunteer), randomized in situ study composed of two factors. One factor was treatment at four levels (four treatment phases) and the other factor was time in situ at three levels (1, 3, and 5 days). Randomization and blinding were performed by the supervisor of the study using the random sequences function of the Software Excel. The total duration of each treatment phase was 5 days with 1-week wash-out between them and 1-week wash-in at the beginning of the study, as previously described [22]. The sample size was calculated based on the results of a previous study with the same design [23], but in vitro. According to that, mean enamel surface losses after 5 days of 57.8 μm (SD = 5.0) for the negative control and 35.2 μm (SD = 4.0) for the laser group would be expected. In order to obtain a power of over 80%, a sample size of six (volunteers) was calculated (two-sided, SD = 11.8 and $\alpha = 0.05$). However, to account for possible dropouts, ten volunteers per group were included.

Through the whole study and additionally in the wash-in and wash-out phases, individual oral hygiene was performed with fluoride-free products (Weleda toothpaste Calendula, Weleda, Arlesheim, Switzerland), without the appliances in situ. Volunteers were additionally instructed to refrain from eating fluoride-containing food, such as fish, fluoridated salt, and green/black tea. At the beginning of each phase, the volunteers were also extensively trained to follow the study instructions. The intraoral appliances were worn during the whole day except for meals and oral hygiene. After meals, 20 min were elapsed before mouth appliance reinsertion.

Samples

Two hundred forty enamel blocks of 5 × 5 × 2 mm, previously disinfected in 2% formaldehyde (pH = 7) for 30 days [24] were cut from labial central area of bovine incisor crowns (Isomet, Buehler, Illinois, USA). The enamel surface was serially polished using silicon carbide papers (grits #800, #1200, and #4000, Buehler, USA) and a diamond suspension (grain size 1 μm). All samples were examined under a stereo microscope to check for integrity and stored at 100% of humidity throughout the study, except for the time they were in situ. A round window of 2.5 mm diameter on the labial side of the samples was protected with a tape and all other surfaces of the samples were covered with acid-resistant varnish. After that, the tape was carefully removed and the experimental area was checked for remnants of adhesive, under a stereo microscope. If this was the case, the surfaces were cleaned with cotton pellets before the profilometry. Samples were then randomly assigned into the treatment groups.

Volunteers

All volunteers (average age 34 ± 7 years, six females and four males, $n = 10$) have signed a written informed consent and met the following inclusion criteria: good oral health (no active caries or gingivitis/periodontitis), no removable orthodontic appliances or dentures, no sign of hyposalivation (stimulated saliva flow rate > 1 ml/min) [25], saliva pH > 6.8, and normal buffer capacity measured by means of a salivary test (Saliva-Check Test, GC Corporation, Japan). Pregnancy and breastfeeding, general/systemic illness, and the intake of medication reducing saliva secretion were the exclusion criteria.

Palatal appliances

Individual palatal appliances containing two plastic holders of polycarbonate were constructed for each volunteer, as previously described [26]. Three enamel samples were fixed in each of them ($n = 6$ per appliance) using orthodontic wires and wax (Fig. 1). For fixing the sample, holders in the appliance light-curing composite was used. At each examination, day (day 1, 3, and 5) two samples were removed for profilometric analysis.

Surface treatments

The surface treatments in each phase were as follows: (L) laser irradiation (0.3 J/cm^2); (F) fluoride gel treatment (4 min, 12′ 500 ppm F$^-$ as AmF and NaF, pH 4.8–6.0, Elmex® Gel, Gaba, Germany); (FL) fluoride treatment, as previously described followed by laser irradiation; (C) rinsing with distilled water as a negative control.

Laser treatment was conducted with a CO_2 Laser (Rofin SCx 30, Rofin-Sinar Laser, Hamburg, Germany) at 10.60-μm wavelengths. Surface irradiation was carried out with a combination of laser parameters, which has previously shown to significantly increase enamel acid resistance (0.3 J/cm^2/5 μs/ 226 Hz/2036 overlapped pulses/2.5-mm beam diameter/9-s irradiation time/no water cooling). The energy density and beam diameter at 1/e^2 were measured as described previously [19]. Shortly, the knife-edge approach was used and a Gaussian distribution was assured. The emitted energy was controlled during all irradiations (Field Master GS + Detector LM45; Coherent, USA).

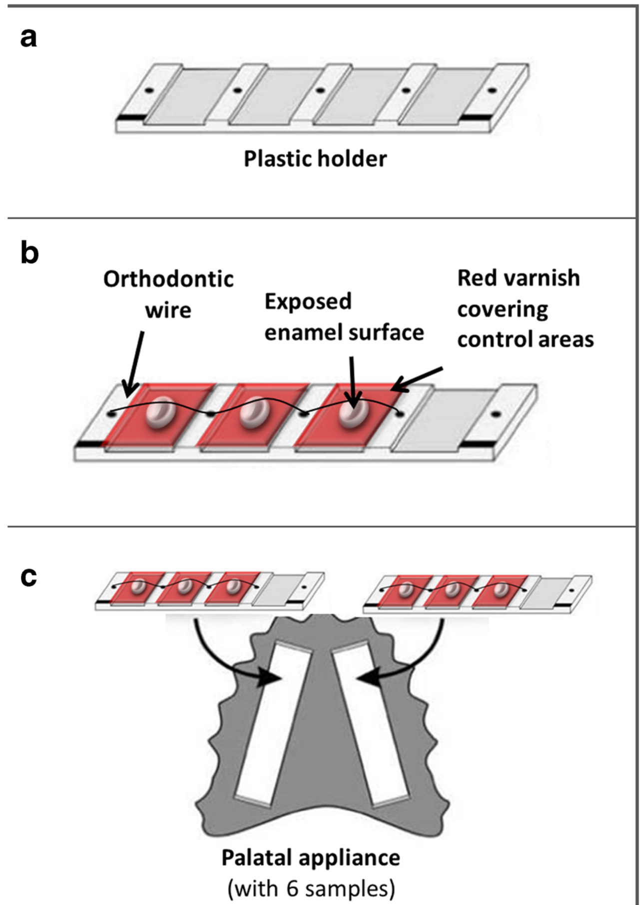

Fig. 1 Schema of the palatal appliances. **a** Plastic holders used to fix the samples. **b** Fixation of three samples per holder with an orthodontic wire. **c** Fixation of two sample holders per appliance using dental wax. Schema modified from Hara et al. 2006

Erosive demineralization

For the erosive demineralization, a protocol previously described by Ganss, Schlueter, Friedrich, and Klimek [27] was chosen. Briefly, the intraoral appliances were gently dried and immersed extra-orally in 0.05 M citric acid (80 ml/20 min/pH 2.3/$C_6H_8O_7$ H_2O, M = 210.14 g/mol) two times a day (morning/evening). Volunteers washed it intensively with tap water before reinsertion [27]. In order to avoid saturation, new demineralization and supersaturated solutions were prepared daily and their pH was monitored.

Profilometric measurements

In order to analyze the enamel surface loss on days one, three, and five, two samples were collected from the appliances on each of those days ($n = 20$/day/treatment, Fig. 2). Using transparent adhesive (Technovit 7230VLC, Heraeus Kulzer

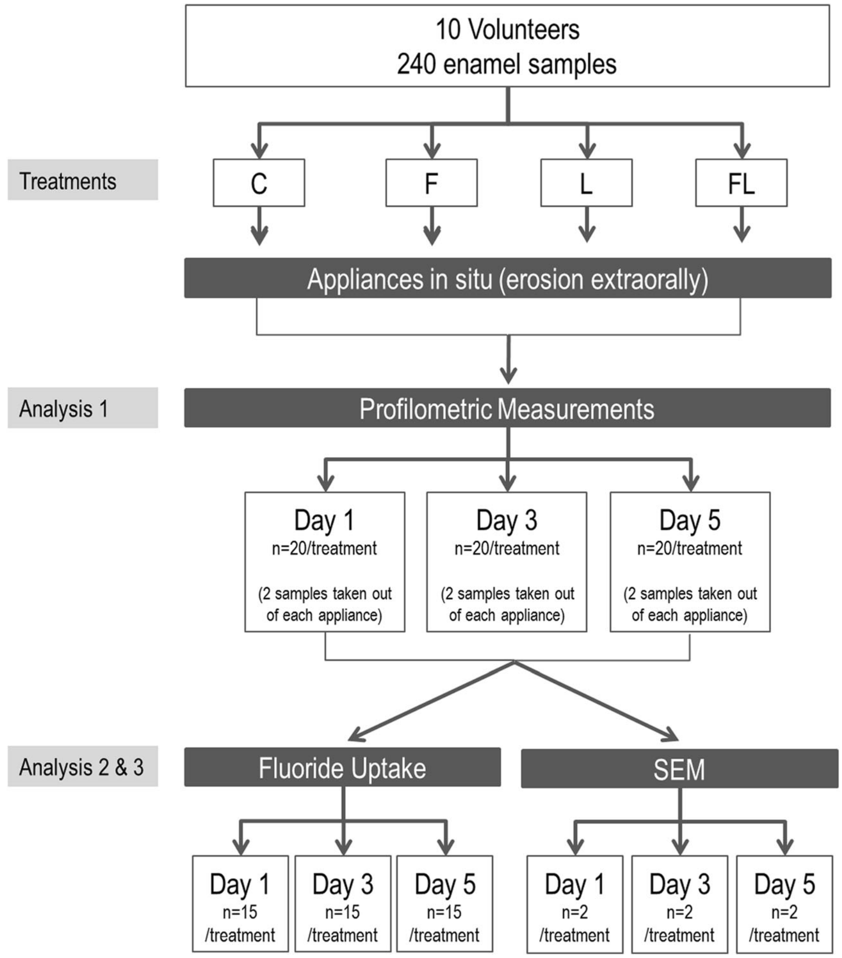

Fig. 2 Flowchart of the study showing the distribution of the samples for the different analyses at days 1, 3, and 5

GmbH, Wehrheim, Germany), the samples were fixed on a plastic slide (50 × 100 × 2 mm, Exakt GmbH, Norderstedt, Germany) keeping the enamel face uppermost. The protecting varnish was removed from the enamel surface leaving the erosion lesion and reference areas exposed. In order to assess enamel surface loss, a contact profilometer (MarSurf XC2, Mahr GmbH, Germany) coupled to an analyzing software (MarSurf XC2, Mahr GmbH, Germany) was used. The scanning was performed with a tungsten carbide stylus with radius of 2 μm at 1-mN force. Assessment of vertical surface loss was done by measuring the difference in height of the reference areas and the erosive lesion as previously described [20]. Shortly, 3 line scans and 18 measurements/line were performed. Measuring ten samples in this way, to assess reproducibility, resulted in a standard deviation of 0.640 μm.

Fluoride uptake analysis

After the profilometric measurements, 180 samples ($n = 15$/day/treatment; Fig. 2) were used for measuring enamel fluoride uptake. The measurements were conducted for a delimited round area of enamel (0.049 cm^2) containing the eroded region. For this, the samples were firstly immersed in 0.5 ml HCl (0.5 M/15 s/under agitation) and subsequently in Tisab (0.5 ml) and NaOH (20 g/l). Fluoride extracted from enamel (μg F/cm^2) was measured in triplicate using an ion-selective electrode and an ion analyzer (Orion 96-09 and Orion EA-940, respectively) [21, 28].

Scanning electron microcopy (SEM)

Twenty-four of the remaining samples ($n = 2$/day/treatment; Fig. 2) were subjected to SEM analysis. Samples were dehydrated in alcohol series, glued to metal stubs, and gold sputtered. The investigation was done using an environmental scanning electron microscope (ESEM XL30 Field Emission Gun, Phillips, Eindhoven, Netherlands) and a secondary electron detector (10 kV/10 mm work distance). The original magnifications were 300, 2000, and 60,000 times.

Statistical analysis

For the analysis of enamel surface loss and enamel fluoride, uptake two-way repeated measures ANOVA analyses were conducted to assess differences between the groups. In the ANOVA model, the repeated factors were either the values of enamel surface loss or the values of enamel fluoride uptake at the different time points. The grouping factors were the different treatments and the interaction term was the time in situ. Adequate contrasts were formulated and tested in order to compare values on two time points for one or two treatments on one time point. Two-sided tests were conducted at a significance level of 5%. The study had an exploratory nature and considering that, no adjustment to the significance level to account for multiple testing was made. The software SAS version 9.1.3 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Profilometric measurements

All volunteers completed the study without reporting any adverse effect and a total of 240 samples were analyzed. The negative control group (C) presented the highest and the groups receiving laser treatment (L and FL) the lowest enamel surface loss means at all experimental days (Fig. 3). At day 1, significantly less enamel surface loss as compared to both negative (no treatment) and the positive control (fluoride application) groups, C and F (C 16.52 ± 5.4 μm, F 13.7 ± 6.3 μm) was observed for the laser groups, FL and L (L 8.6 ± 2.3 μm and FL 4.3 ± 2.6 μm) and the same happened at day 3 (C 40.2 ± 10.5 μm, F 33.7 ± 13.4 μm, L 22.7 ± 6.3 μm, and FL 22.1 ± 11.0 μm; $p < 0.05$). After day 5, group L was significantly better than the control (C, $p = 0.0025$); however, only FL (33.6 ± 12.6 μm) was still statistically significant better than F (57.5 ± 20.3 μm), C (67.8 ± 15.4 μm), and L (46.8 ± 14.5 μm).

The highest percentage of inhibition of enamel surface loss as compared to the negative control was obtained by FL and was of 74% at day 1 and 50% at day 5.

Fluoride uptake analysis

All means and standard deviations of fluoride measurements are presented in Fig. 4. Fluoridated samples (groups F and FL) presented after day 1 statistically significant higher fluoride content, than unfluoridated samples (groups C and L, respectively F × C or L $p = 0.0001$, $p < 0.0001$ and FL × C or L $p = 0.0071$, $p = 0.0004$). After day 3, only F had statistically significant more fluoride than the placebo treatment C ($p = 0.0087$). After day 5, all groups were not significantly different to C ($p > 0.05$), except for L that presented significantly lower fluoride content ($p = 0.0110$).

Scanning electron microcopy (SEM)

At the end of the experiments, samples from all the groups (Fig. 5) showed common characteristics of acid-eroded surfaces. Although the samples from lased groups (L and FL) appeared to be slightly rougher than the others. Especially under 2000 times magnification, FL apparently resulted in more extensive preservation of the enamel prism cores more than the other groups. Even at the highest magnification, no CaF_2-like fluoride deposits could be found for any group.

Discussion

The study design should reproduce the clinical situation as near as possible, while providing outcomes, which helps answering the research question. As regards dental erosion, ideally, the acid demineralization should take place inside the mouth; however, in order to protect the teeth of the volunteers, here, the simulation of the erosive challenge was performed extra-orally. The demineralization time was of 20 min and this is clearly longer than the acid attacks normally occurring clinically, though still in the interval recently described for in situ studies [29]. Besides, this protocol has also the advantage of increasing the compliance of volunteers (longer demineralization period, less times a day), which contributes for standardization. As up to now, no standard model for simulating severe erosion has been described [29, 30] and knowing that the aim was to achieve the highest surface loss possible, we chose to use this study design, previously described [27]. In more mild erosion models with shorter demineralization times, probably less surface loss would have been observed and, presumably, higher preventive effect of the laser and the fluoride therapy could have been seen. However, in this way, an exaggerated erosive challenge was conducted, providing insights about the therapy efficacy in a worst-case scenario and with more clinically relevant substance loss. Moreover, even under these exaggerated conditions, the model was sensible enough to detect significant differences between all laser therapies and the negative control. Nonetheless, the present results must be interpreted with caution and further in situ studies with shorter and more frequent demineralization periods must be conducted.

Another important aspect when designing the study for testing new therapies is the choice of an appropriate gold standard therapy, for which both laboratorial as well as clinical efficacy data are available. This helps assessing the validity of the model. In the present study, an AmF/NaF gel was used as a positive control, because its efficacy for slightly but significantly increasing erosion resistance has been clearly demonstrated not only in vitro [22, 31, 32], but also clinically, if the context of caries is considered [33]. Furthermore, by the time the study was started there was not any fluoride product

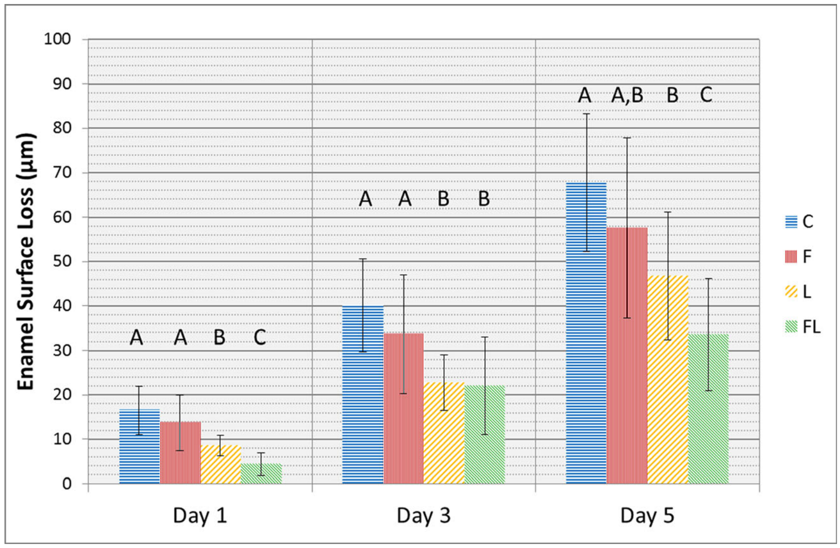

Fig. 3 Mean (± standard deviation) enamel surface loss (μm) after 1, 3, and 5 days in situ. The laser groups (L and FL) caused at all observed times a significant reduction of enamel surface loss as compared to the negative control ($\alpha = 0.05$). FL was additionally significantly more effective than solely amine/sodium fluoride gel application on reducing enamel surface loss at all experimental days. Different letters indicate significant difference between the groups within the analyzed day (repeated measures ANOVA, $\alpha = 0.05$). C, negative control; F, fluoride, L, laser; FL, fluoride + laser

specifically designed for prevention of dental erosion available on the market. However, currently, it is clear that this is not the best fluoride compound against erosion, as polyvalent metal ions and specially tin-containing gels and solutions have been proven to be significantly more effective [4, 34]. However, the use of a AmF/NaF compound still seems meaningful since the majority oral hygiene products contains either amine or sodium fluoride and these are probably the most used from a great part of the population.

The anti-erosive effect of fluoride compounds (AmF/NaF) when used alone is known to be significant but rather low (up to 20% reduction) [7, 22]. This means that the model used here is probably not the best for detecting preventive effects at low range (≤ 20%). However, it did detect effects, which represented erosion reductions of over 30% as compared to the control group. Another important point to be discussed is the fact in the framework of dental erosion, fluoride products work mainly through the formation of CaF_2-like layers over the tooth surface. The amount of formation of these precipitates is highly dependent, though, on the concentration and pH of fluoride preparation (the more acidic, the better) [35], duration of the application, and on the conditions of tooth surface [36]. It has also been shown that CaF_2-like layers forming in situ are significantly more stable than in vitro, presumably due to adsorption of phosphates and proteins [37]. Thus, although we have observed here significantly increased fluoride uptake in enamel, it is not possible to know how much of this fluoride was actually present in the form of CaF_2-like precipitates or fluorohydroxyapatite. Moreover, even that under in situ conditions, CaF_2-like layers are more stable than in vitro, probably, the amount of precipitates formed in our study was not high enough. Specially, because in most of the studies intensive fluoridation, requiring several fluoride gel applications and in combination with daily use of fluoride solution was

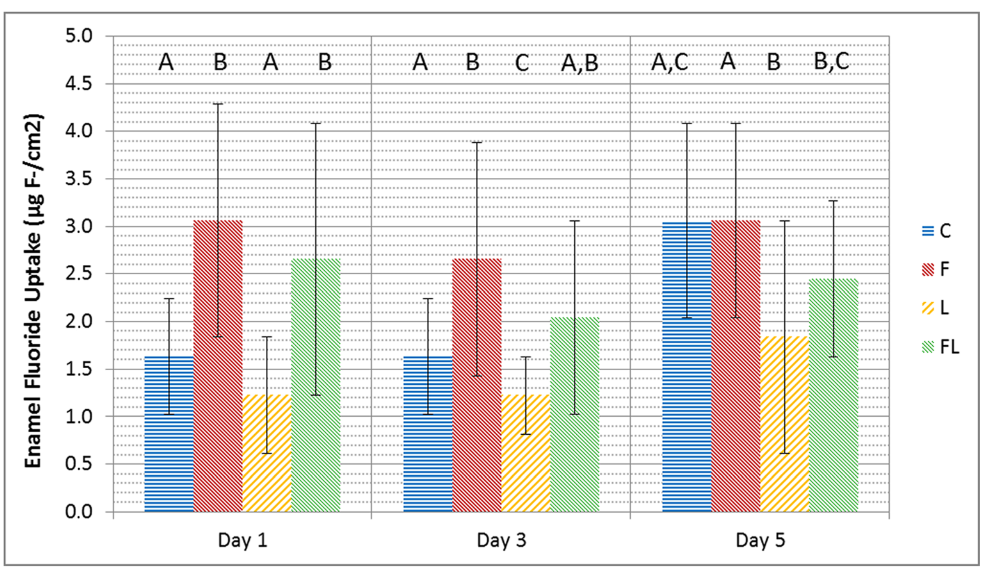

Fig. 4 Mean (± standard deviation) fluoride content ($\mu g\ F^-/cm^2$) in enamel after 1, 3, and 5 days in situ. Different letters indicate significant difference between the groups within the analyzed day (repeated measures ANOVA, $\alpha = 0.05$). C, negative control; F, fluoride, L, laser; FL, fluoride + laser

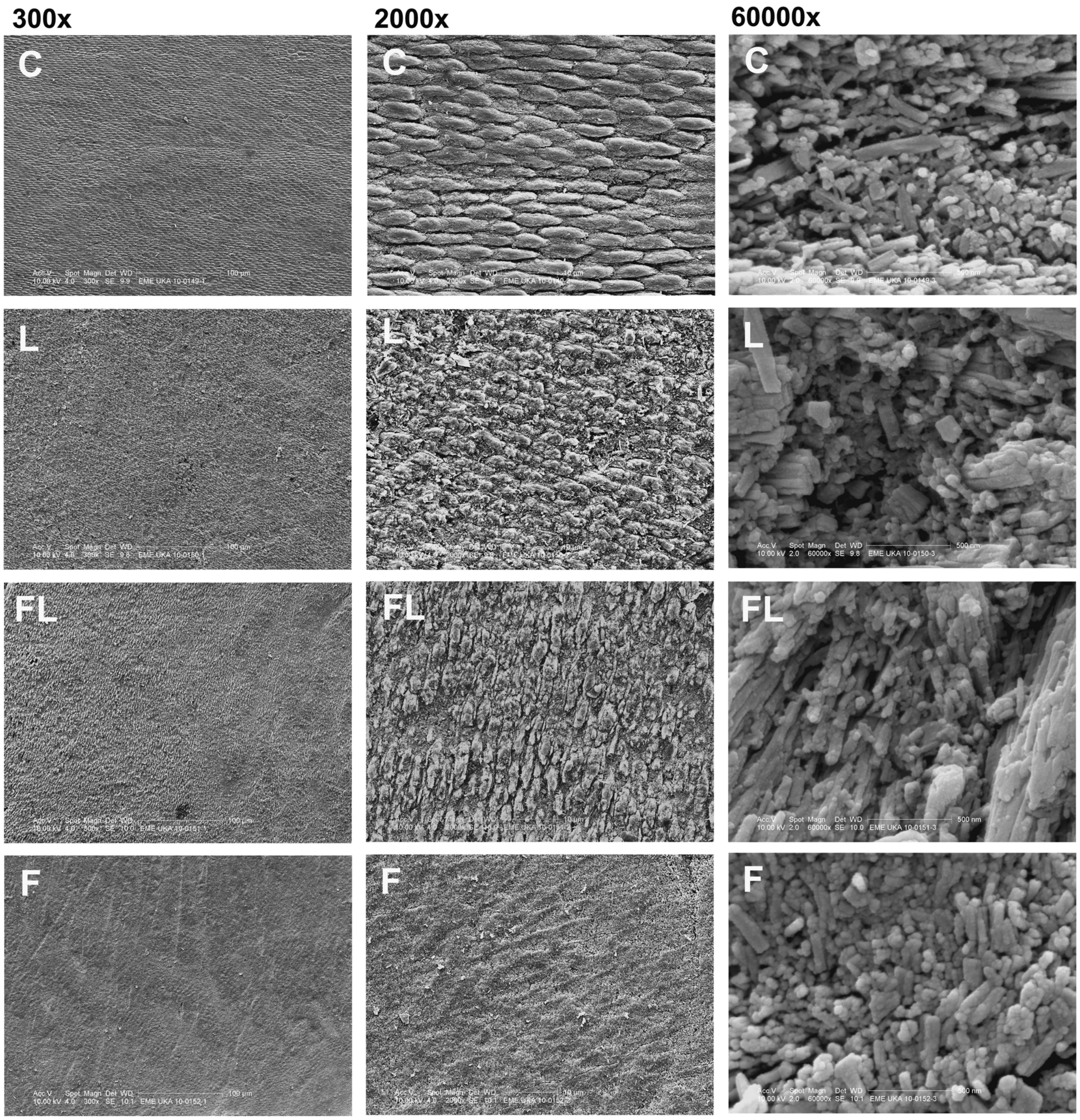

Fig. 5 Representative SEM images of the samples removed from the in situ appliances at day 5 (C, negative control; L, laser; FL, fluoride + laser; F, fluoride). Dissolution of enamel prisms can be observed for all groups, but especially FL shows at 2000 × magnification, preferential dissolution of prism periphery, and stronger preservation of prism cores

needed to cause a limited but significant erosion reduction [22]. Here, the fluoride gel was applied only once before cycling begins, trying to simulate the clinical recommendations of the product for patients to use it once a week, and this was probably not intensive enough.

As regards the laser, in the present study, the erosion preventive effect of a specific set of laser parameters was shown for the first time in an in situ model. A significant reduction of enamel surface loss as compared to the negative control was observed at all cycling days for both laser groups. Moreover, also in comparison to the fluoride therapy (AmF/NaF gel, 12′ 500 ppm F^{-}), a significant reduction of enamel erosive loss until day 3 was observed for both laser groups and up to day 5 for FL. This is in accordance with previous evidence obtained with the 9.6-μm laser wavelength [16–18] as well as with previous findings using exactly the same laser parameters used here [21, 23]. Initially, this set of laser parameters caused 81% of carious demineralization reduction in a pH-cycling model [19]. Further studies in the framework of erosion also showed 99% reduction of enamel softening [21], up to 52% of reduction or enamel erosive loss [23], and 54% reduction of tooth-brushing abrasion [20]. In combination with a sodium fluoride gel, up to 72% of enamel reduction has been obtained, but combined with a tin-containing fluoride solution even almost complete erosion inhibition has been obtained [38]. Although in more than one study, the effect of the CO_2 laser irradiation alone caused significant reduction of enamel erosion, which was not significantly different to the combined laser and fluoride studies. It seems that, depending on the study design, the effect of the combined laser and fluoride treatment [39] is considerably better than the laser irradiation alone.

The preventive effect of amine and sodium fluoride compounds is related to formation of a Ca-F_2-like layer on the enamel surface. The calcium fluoride precipitates work as fluoride reservoirs releasing fluoride, when the pH drops, which is then available for remineralization enamel mineral [40]. In the present study, this preventive effect of amine/sodium fluoride was seen (15–17% reduction, though not significant) and additionally, a synergistic effect when combined with the laser treatment could be detected. At the end of the investigations, the combined laser and fluoride treatment caused a more than three times higher reduction of enamel surface loss than fluoride alone. One can speculate that the reason for that could be an increase of fluoride uptake in enamel after laser irradiation. However, this seems not to be the case here, as FL resulted in a fluoride uptake in enamel, which was not significantly different from the group receiving solely fluoride at days 1, 3, and even significantly lower at day 5. The other possibility might be that there is only a sum of effects, increasing enamel acid resistance in two different ways: the first one through the formation of calcium fluoride-like deposits (precipitates) over the surface, and the second through the increase of enamel crystals acid resistance after laser irradiation. Enamel mineral contains a rather impure form of hydroxyapatite, best described as calcium-deficient carbonate-rich hydroxyapatite [41]. The elimination of impurities like carbonate can be obtained by CO_2 laser irradiation and is known to turn enamel less acid soluble [42, 43]. Considering these two mechanisms of action, it can be speculated that during an acid attack, the acid ions will first dissolve the CaF_2 layer leading to fluoride release and formation of fluorohydroxyapatite and the resulting mixture of these two mineral phases, namely fluorohydroxyapatite and of carbonate-free hydroxyapatite (fluoride-rich and carbonate-poor mineral), is maybe what makes enamel considerably more acid resistant. However, to prove that theory, new studies using X-ray diffractometry and Fourier transformed infrared spectroscopy are still needed.

It is important to notice that, in situ models have a great advantage of allowing the simulation of an important protective factor against erosion, namely the presence of saliva. It allows the formation of an acquired pellicle over the tooth surfaces, which acts as a semi-permeable membrane and normally limiting the diffusion of the acids ions and decreasing enamel erosion [25]. This factor is not easy to simulate in vitro, as the use of artificial saliva formulations or even human saliva in laboratorial experiments does not reflect intraoral erosion adequately [44]. Thus, in order to mimic the clinical situation, in the present study, all volunteers were instructed to wear the appliance at least for 2 h before the erosive challenges, as this contact time with saliva has proven to allow the formation of 20–50 nm acquired pellicle over enamel [45]. Moreover, if clinical translation of the CO_2 laser therapy is desired, more in situ studies investigating the influences of saliva and the acquired pellicle on the efficacy of the laser therapy should be conducted.

Conclusion

In conclusion, in the present study, it was shown for the first time that as compared to the control, the CO_2 laser irradiation with a specific set of laser parameters (0.3 J/cm^2, 5 µs, 226 Hz) either alone or in combination with a fluoride gel (AmF/NaF) can significantly decrease enamel erosive loss up to 5 days in situ. Up to day 3, this reduction was also significantly higher than the observed for the fluoride group, both for laser alone and combined treatment. The highest reduction was though observed for the combined laser and fluoride treatment, which was initially of 74% and at the end of the examination still as high as 50% and significantly higher than fluoride treatment alone.

Acknowledgments This study was part of joint thesis supervision between RWTH Aachen University, Germany and University of São Paulo, Brazil.

Funding This study was funded by FGD (Forschungsgemeinschaft Dental #360303, Germany), START Program of the Medical Faculty of the RWTH Aachen University (#AZ43/09, Germany), and CNPq (#305574/2008–6, Brazil). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by Ethical Committee of Medical School of the RWTH Aachen University (#EK 134/09)

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Lussi A, Carvalho TS (2014) Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci 25:1–15. https://doi.org/10.1159/000360380
2. Shellis RP, Barbour ME, Jesani A, Lussi A (2013) Effects of buffering properties and undissociated acid concentration on dissolution

of dental enamel in relation to pH and acid type. Caries Res 47:601–611. https://doi.org/10.1159/000351641
3. Shellis RP, Featherstone JD, Lussi A (2014) Understanding the chemistry of dental erosion. Monogr Oral Sci 25:163–179. https://doi.org/10.1159/000359943
4. Huysmans MC, Young A, Ganss C (2014) The role of fluoride in erosion therapy. Monogr Oral Sci 25:230–243. https://doi.org/10.1159/000360555
5. Lussi A, Carvalho TS (2015) The future of fluorides and other protective agents in erosion prevention. Caries Res 49(Suppl 1): 18–29. https://doi.org/10.1159/000380886
6. Schlueter N, Duran A, Klimek J, Ganss C (2009) Investigation of the effect of various fluoride compounds and preparations thereof on erosive tissue loss in enamel in vitro. Caries Res 43:10–16
7. Ganss C, Neutard L, von Hinckeldey J, Klimek J, Schlueter N (2010) Efficacy of a tin/fluoride rinse: a randomized in situ trial on erosion. J Dent Res 89:1214–1218. https://doi.org/10.1177/0022034510375291
8. Magalhaes AC, Dos Santos MG, Comar LP, Buzalaf MA, Ganss C, Schlueter N (2016) Effect of a single application of TiF4 varnish versus daily use of a low-concentrated TiF4/NaF solution on tooth erosion prevention in vitro. Caries Res 50:462–470. https://doi.org/10.1159/000448146
9. Wiegand A, Schneider S, Sener B, Roos M, Attin T (2014) Stability against brushing abrasion and the erosion-protective effect of different fluoride compounds. Caries Res 48:154–162. https://doi.org/10.1159/000353143
10. Hove LH, Holme B, Young A, Tveit AB (2008) The protective effect of TiF4, SnF2 and NaF against erosion-like lesions in situ. Caries Res 42:68–72. https://doi.org/10.1159/000112816
11. Schlueter N, Klimek J, Ganss C (2014) Effect of a chitosan additive to a Sn2+−containing toothpaste on its anti-erosive/anti-abrasive efficacy–a controlled randomised in situ trial. Clin Oral Investig 18:107–115. https://doi.org/10.1007/s00784-013-0941-3
12. Ganss C, von Hinckeldey J, Tolle A, Schulze K, Klimek J, Schlueter N (2012) Efficacy of the stannous ion and a biopolymer in toothpastes on enamel erosion/abrasion. J Dent 40:1036–1043. https://doi.org/10.1016/j.jdent.2012.08.005
13. Pini NI, Lima DA, Lovadino JR, Ganss C, Schlueter N (2016) In vitro efficacy of experimental chitosan-containing solutions as anti-erosive agents in enamel. Caries Res 50:337–345. https://doi.org/10.1159/000445758
14. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell G (1992) Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. Caries Res 26:333–339
15. Fried D, Seka W, Glena RE, Featherstone JDB (1996) Thermal response of hard dental tissues to 9- through 11-μm CO_2-laser irradiation. Opt Eng 35:1976–1984
16. Featherstone JDB, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W (1998) CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res 77:1397–1403
17. Gerard DE, Fried D, Featherstone JD, Nancollas GH (2005) Influence of laser irradiation on the constant composition kinetics of enamel dissolution. Caries Res 39:387–392
18. Rechmann P, Fried D, Le CQ, Nelson G, Rapozo-Hilo M, Rechmann BM, Featherstone JD (2011) Caries inhibition in vital teeth using 9.6-mum CO2-laser irradiation. J Biomed Opt 16: 071405. https://doi.org/10.1117/1.3564908
19. Esteves-Oliveira M, Zezell DM, Meister J, Franzen R, Stanzel S, Lampert F, Eduardo CP, Apel C (2009) CO_2 laser (10.6 μm) parameters for caries prevention in dental enamel. Caries Res 43:261–268
20. Esteves-Oliveira M, Pasaporti C, Heussen N, Eduardo CP, Lampert F, Apel C (2011) Prevention of toothbrushing abrasion of acid-softened enamel by CO_2 laser irradiation. J Dent 39:604–611. https://doi.org/10.1016/j.jdent.2011.06.007
21. Esteves-Oliveira M, Pasaporti C, Heussen N, Eduardo CP, Lampert F, Apel C (2011) Rehardening of acid-softened enamel and prevention of enamel softening through CO_2 laser irradiation. J Dent 39: 414–421. https://doi.org/10.1016/j.jdent.2011.03.006
22. Ganss C, Klimek J, Schaffer U, Spall T (2001) Effectiveness of two fluoridation measures on erosion progression in human enamel and dentine in vitro. Caries Res 35:325–330
23. Ramalho KM, Eduardo Cde P, Heussen N, Rocha RG, Lampert F, Apel C, Esteves-Oliveira M (2013) Protective effect of CO2 laser (10.6 mum) and fluoride on enamel erosion in vitro. Lasers Med Sci 28:71–78. https://doi.org/10.1007/s10103-012-1071-x
24. West NX, Davies M, Amaechi BT (2011) In vitro and in situ erosion models for evaluating tooth substance loss. Caries Res 45(Suppl 1):43–52
25. Hara AT, Zero DT (2014) The potential of saliva in protecting against dental erosion. Monogr Oral Sci 25:197–205. https://doi.org/10.1159/000360372
26. Hara AT, Ando M, Gonzalez-Cabezas C, Cury JA, Serra MC, Zero DT (2006) Protective effect of the dental pellicle against erosive challenges in situ. J Dent Res 85:612–616
27. Ganss C, Schlueter N, Friedrich D, Klimek J (2007) Efficacy of waiting periods and topical fluoride treatment on toothbrush abrasion of eroded enamel in situ. Caries Res 41:146–151
28. Cury JA, Francisco SB, Simoes GS, Del Bel Cury AA, Tabchoury CP (2003) Effect of a calcium carbonate-based dentifrice on enamel demineralization in situ. Caries Res 37:194–199. https://doi.org/10.1159/000070444
29. Wiegand A, Attin T (2011) Design of erosion/abrasion studies–insights and rational concepts. Caries Res 45(Suppl 1):53–59
30. Schlueter N, Lussi A, Tolle A, Ganss C (2016) Effects of erosion protocol design on erosion/abrasion study outcome and on active agent (NaF and SnF_2) efficacy. Caries Res 50:170–179. https://doi.org/10.1159/000445169
31. Attin T, Deifuss H, Hellwig E (1999) Influence of acidified fluoride gel on abrasion resistance of eroded enamel. Caries Res 33:135–139
32. Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T (2006) Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 40:148–153. https://doi.org/10.1159/000091062
33. Marinho VC, Worthington HV, Walsh T, Chong LY (2015) Fluoride gels for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 6:CD002280. https://doi.org/10.1002/14651858.CD002280.pub2
34. Carvalho TS, Colon P, Ganss C, Huysmans MC, Lussi A, Schlueter N, Schmalz G, Shellis RP, Tveit AB, Wiegand A (2015) Consensus report of the European Federation of Conservative Dentistry: erosive tooth wear–diagnosis and management. Clin Oral Investig 19: 1557–1561. https://doi.org/10.1007/s00784-015-1511-7
35. Wiegand A, Bichsel D, Magalhaes AC, Becker K, Attin T (2009) Effect of sodium, amine and stannous fluoride at the same concentration and different pH on in vitro erosion. J Dent 37:591–595
36. Saxegaard E, Rolla G (1988) Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res 96: 523–535
37. Ganss C, Schlueter N, Klimek J (2007) Retention of KOH-soluble fluoride on enamel and dentine under erosive conditions–a comparison of in vitro and in situ results. Arch Oral Biol 52:9–14
38. Esteves-Oliveira M, Witulski N, Hilgers RD, Apel C, Meyer-Lueckel H, de Paula Eduardo C (2015) Combined tin-containing fluoride solution and CO_2 laser treatment reduces enamel erosion in vitro. Caries Res 49:565–574. https://doi.org/10.1159/000439316
39. Esteves-Oliveira M, Zezell DM, Ana PA, Yekta SS, Lampert F, Eduardo CP (2011) Dentine caries inhibition through CO_2 laser (10.6 μm) irradiation and fluoride application, in vitro. Arch Oral Biol 56:533–539. https://doi.org/10.1016/j.archoralbio.2010.11.019

40. Rolla G, Ogaard B, Cruz Rde A (1993) Topical application of fluorides on teeth. New concepts of mechanisms of interaction. J Clin Periodontol 20:105–108
41. Featherstone JD, Mayer I, Driessens FC, Verbeeck RM, Heijligers HJ (1983) Synthetic apatites containing Na, Mg, and CO3 and their comparison with tooth enamel mineral. Calcif Tissue Int 35:169–171
42. Fried D, Murray MW, Featherstone JDB, Akrivou M, Dickenson KM, Duhn C and Ojeda OP (1999) Dental hard tissue modification and removal using sealed TEA lasers operating at λ=9.6 μm and 10.6 μm. Book title. SPIE-International Society of Optical Engineering, Bellingham, WA, San Jose
43. Zuerlein MJ, Fried D, Featherstone JDB (1999) Modeling the modification depth of carbon dioxide laser-treated dental enamel. Lasers Surg Med 25:335–347
44. Batista GR, Rocha Gomes Torres C, Sener B, Attin T, Wiegand A (2016) Artificial saliva formulations versus human saliva pretreatment in dental erosion experiments. Caries Res 50:78–86. https://doi.org/10.1159/000443188
45. Hannig M, Hess NJ, Hoth-Hannig W, De Vrese M (2003) Influence of salivary pellicle formation time on enamel demineralization–an in situ pilot study. Clin Oral Investig 7:158–161. https://doi.org/10.1007/s00784-003-0219-2