Screening of CO(2) laser (10.6 m) parameters for prevention of enamel erosion

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Abstract: OBJECTIVE: The aim of this study was to screen CO(2) laser (10.6 m) parameters to increase enamel resistance to a continuous-flow erosive challenge. BACKGROUND DATA: A new clinical CO(2) laser providing pulses of hundreds of microseconds, a range known to increase tooth acid-resistance, has been introduced in the market. Methods: Different laser parameters were tested in 12 groups (n=20) with varying fluences from 0.1 to 0.9 J/cm(2), pulse durations from 80 to 400 s and repetition rates from 180 to 700 Hz. Non-lased samples (n=30) served as controls. All samples were eroded by exposure to hydrochloric acid (pH 2.6) under continuous acid flow (60 L/min). Calcium and phosphate release into acid was monitored colorimetrically at 30 sec intervals up to 5 min and at 1 min intervals up to a total erosion time of 15 min. Scanning electron microscopic (SEM) analysis was performed in lased samples (n=3). Data were statistically analysed by one-way ANOVA (p<0.05) and Dunnett's post-hoc tests. RESULTS: Calcium and phosphate release were significantly reduced by a maximum of 20% over time in samples irradiated with 0.4 J/cm(2) (200 s) at 450 Hz. Short-time reduction of calcium loss (1.5 min) could be also achieved by irradiation with 0.7 J/cm(2) (300 s) at 200 and 300 Hz. Both parameters revealed surface modification. CONCLUSIONS: A set of CO(2) laser parameters was found that could significantly reduce enamel mineral loss (20%) under in vitro erosive conditions. However, as all parameters also caused surface cracking, they are not recommended for clinical use.

Screening of CO_2 Laser (10.6 μm) Parameters for Prevention of Enamel Erosion

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Abstract

Objective: The aim of this study was to screen CO_2 laser (10.6 μm) parameters to increase enamel resistance to a continuous-flow erosive challenge. *Background data:* A new clinical CO_2 laser providing pulses of hundreds of microseconds, a range known to increase tooth acid-resistance, has been introduced in the market. *Methods:* Different laser parameters were tested in 12 groups ($n=20$) with varying fluences from 0.1 to 0.9 J/cm², pulse durations from 80 to 400 μs and repetition rates from 180 to 700 Hz. Non-lased samples ($n=30$) served as controls. All samples were eroded by exposure to hydrochloric acid (pH 2.6) under continuous acid flow (60 μL/min). Calcium and phosphate release into acid was monitored colorimetrically at 30 sec intervals up to 5 min and at 1 min intervals up to a total erosion time of 15 min. Scanning electron microscopic (SEM) analysis was performed in lased samples ($n=3$). Data were statistically analysed by one-way ANOVA ($p<0.05$) and Dunnett's *post-hoc* tests. *Results:* Calcium and phosphate release were significantly reduced by a maximum of 20% over time in samples irradiated with 0.4 J/cm² (200μs) at 450 Hz. Short-time reduction of calcium loss ($\leq$1.5 min) could be also achieved by irradiation with 0.7 J/cm² (300μs) at 200 and 300 Hz. Both parameters revealed surface modification. *Conclusions:* A set of CO_2 laser parameters was found that could significantly reduce enamel mineral loss (20%) under *in vitro* erosive conditions. However, as all parameters also caused surface cracking, they are not recommended for clinical use.

Introduction

I T HAS ALREADY BEEN DEMONSTRATED that several wavelengths of a CO_2 laser may increase enamel acid resistance.[1,2] However, most of the studies were conducted investigating the use of this laser for caries prevention; only a few were for erosion prevention.[3,4] In the latter studies, it was mostly investigated whether laser irradiation might improve the erosion-protective capability of fluorides rather than to analyze laser irradiation effects on the acid resistance of dental hard tissues *per se*. Thereby, different lasers with various parameters were used, but no previous systematic screening was conducted to define appropriate laser parameters.

In previous experiments, we have already shown that the low fluences (<1 J/cm²) when combined with short pulse durations (some tens of microseconds) may be effective in increasing enamel resistance to caries *in vitro*, while reducing

the chances of thermal damage.[5,6] This is possible because pulses of short duration increase the peak temperature caused at the surface, but decrease the heat propagation to the interior of the tooth.[7] For example, irradiating enamel with the same fluence, and decreasing the pulse duration from 500 to 50 μs, causes a 200°C difference in peak surface temperature.[8]

The mechanism through which laser irradiation decreases enamel solubility is apparently not related to melting and fusion. Actually, most of the evidences point to temperature-related changes in enamel structure producing a more pure hydroxyapatite.[9] Recent findings showing decrease or complete carbonate elimination from the surface of enamel after irradiation with parameters causing high temperature increase support this theory.[8,10] Simultaneously, denaturation of organic matrix may also play a role in the observed effects,[11] and the ideal surface temperature to cause these

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changes should be in the range between 600° and 900°C. Currently the studies that showed the best parameters for increasing enamel acid resistance either were conducted with industrial lasers, not available for clinical use, or showed parallel occurrence of side effects, such as surface cracking, or both.[1,5,12,13] Therefore, there is still a need for finding conditions that could decrease enamel solubility with lasers already available for clinical use. This would shorten the time needed for laboratory research becoming a reality for patient treatment. Recently, a new surgical laser has been introduced in the market that emits laser pulses with duration in the range of hundreds of microseconds, the range in which the CO_2 irradiation tends to be safer, and which was not available in earlier equipment. Therefore, the aim of the present study was to find CO_2 laser (10.6 μm) parameters from a new clinical laser, to increase enamel resistance to erosive acid attack.

Methods

Sample preparation

Cylindrical enamel samples (3 mm in diameter, $n = 270$) were obtained from non-damaged crowns of freshly extracted bovine incisors, which were stored in 0.9% NaCl solution until used. The samples were embedded in acrylic resin blocks (6 mm in diameter, Paladur, Hereaus Kulzer, Germany). The labial surface of the specimens were ground flat and polished with water-cooled carborundum discs (1200, 2400, and 4000 grit, Water Proof Silicon carbide Paper, Stuers, Birmensdorf, Switzerland), thereby removing ~200 μm of the outermost layer as checked with a micrometer.[14] After polishing, samples were sonicated for 30 sec (Vitasonic II, Vita, Germany). Only samples presenting no cracks or structural defects were selected for the study and randomly allocated to the 12 treatment groups (each $n = 20$) and one control group ($n = 30$).

Laser irradiation

Enamel irradiation was performed using a CO_2 laser emitting at 10.6 μm (Spectra Denta, Lutronic, Korea). Before the start of the experiments and to allow adequate determination of the energy densities, the beam diameter at $1/e^2$ of the intensity level was determined through the knife-edge method. For all groups, the zoom hand piece, at a fixed ir-

radiation distance of 30 mm, was used and determined a beam diameter of 2 mm at the sample surface. The zoom hand piece allows the operator to choose between five different focus points, and for this experiment the largest setting was used. Both time on and time off for the laser emission were fixed at 0.8 sec. The energy emitted by the laser was checked during the entire irradiation period at a two-sample interval using an energy meter (Coherent Field Master GS+ Detector LM45; Coherent, USA).[15] As, to the best of our knowledge no study on erosion prevention has been published yet with this laser system, it was decided to start the investigations with the set of parameters recommended by the manufacturer. As, in the pilot study, these parameters showed no protection at all against enamel acid dissolution, new parameters were tested in order to search for the most adequate one. Criteria for inclusion were the absence of visible signs of ablation, carbonization, and melting at the surface. In addition, fluences <1 J/cm^2 were preferred, as previous studies have already shown their potential for increasing enamel acid resistance.[16] A detailed description of laser parameters is presented in Table 1.

Erosion experiment

Erosion of the lased samples (each group $n = 20$) and control specimens ($n = 30$) was performed in a small erosion chamber, which has been described earlier by Wiegand et al.[14] Briefly, each sample was fixed in a brass jig, which allowed exposure of the enamel surface to a small erosion chamber of 2 mm in diameter and 0.3 mm in height. Hydrochloric acid (2.5 mmol/L, pH 2.6) was pumped at a flow rate 60 μL/min from a reservoir outside the chamber into the space erosion chamber. The acid was collected via outlet pipes into wells of a microtiter plate. Eight chambers were each connected to a multichannel pump (Ismatec, Glattbrugg, Switzerland). In each run, seven test samples and one control sample were randomly assigned to the eight chambers.

Samples were exposed to acid for a total of 15 min. For the first 5 min, the acid was collected at consecutive 30 sec intervals, then at 1 min intervals up to 15 min erosion.

Calcium and phosphate analysis

Release of calcium and phosphate into the acid was determined colorimetrically using the arsenazo III-method[17,18] or the malachite green procedure,[19] respectively.

TABLE 1. DESCRIPTION OF THE CO_2 LASER PARAMETERS USED FOR THE IRRADIATIONS IN EACH GROUP

Groups	Pulse duration (μs)	Energy per pulse (mJ)	Frequency (Hz)	Energy density (J/cm^2)	Irradiation time (sec)	Pulses overlapped
1	80	4	700	0.1	20	14000
2	100	6	400	0.2	20	8000
3			500		20	10000
4			700		15	10500
5	200	13	250	0.4	20	5000
6			300		20	6000
7			400		10	4000
8			450		7	3150
9	300	21	200	0.7	20	4000
10			250		20	5000
11			300		10	3000
12	400	23	180	0.8	20	3600

Arsenazo-III reacts with calcium in an acid solution to form a blue-purple complex. The intensity developed is proportional to the calcium concentration. Absorption was determined at $\lambda = 650$ nm. For photometric determination of calcium, 10 μL samples were used and mixed with 100 μL reagent, which was composed of 100 mmol/L (imidazole buffer, pH 6.5) and 0.12 mmol/L arsenazo III (Fluitest, Ca-A-II, analyticon, Lichtenfels, Germany). Individual standard curves were obtained with a standardized calcium solution for determination of the calcium concentrations. Precision of the measurements was validated with standard solutions. The lowest standard of the calibration curve (0.4 nmoL Ca/well) was considered as threshold for the detection limit of the procedure.

Malachite green reacts with phosphate to a colored complex which can be determined at $\lambda = 650$ nm. For this purpose, 0.045 g malachite green dissolved in 100 mL aqua bidest was admixed to 12.69 g ammonium molybdate, which was dissolved in 300 mL HCl (4 mol/L). The reagent was stirred for 30 min afterwards and filtered. For colorimetric phosphate determination, 5 μL of each acid fraction was admixed to 200 μL of the reagent. Individual standard curves were obtained by admixing diluted samples of the standardized phosphate solution to the acid. The lowest standard of the calibration curve (0.117 nmoL phosphate/well) was considered as threshold for precision.

Scanning electron microscopy (SEM)

Three samples from each group were analyzed by SEM (SUPRA 50VP, Carl Zeiss NTS GmbH, Oberkochen, Germany) at 5 kV after CO$_2$ laser irradiation. Samples were dehydrated using an ascending ethanol series up to 100%. The width of the microcracks was measured with the software ImageJ version 1.410 (National Institutes of Health, Bethesda, MD).

Statistical analysis

Calcium and phosphate release of lased and control samples into the acid was measured in each acid fraction.

Data were statistically analysed by one-way analysis of variance (ANOVA) at each time point. ANOVA was followed by Dunnett's *post-hoc* tests to compare lased with control samples. Level of significance was set at $p \leq 0.05$.

Results

Calcium and phosphate release

The mean rate of calcium and phosphate release of control samples was stable over time and amounted to 0.362 ± 0.008 and 0.202 ± 0.006 μmol/cm$^2 \cdot$min. For all test groups the means of calcium and phosphate loss (μmol/cm^2) at every 2 min are presented in Tables 2 and 3.

Cumulative calcium and phosphate losses (mean % of control) of lased groups are presented in Figs. 1 and 2. Within each group, coefficient of variation of calcium release varied between 9.1 and 26.4 at the different time points. Regarding the phosphate release, coefficient of variation in each group varied between 11.1 and 32.6 at the different time points.

Generally, the preventive effect of laser irradiation was limited. Calcium and phosphate release was reduced by only up to 20% compared with control, with only few groups reaching statistical significance ($p \leq 0.05$). Over time, calcium release was significantly reduced only in group 8, whereas groups 9 and 11 prevented calcium loss only in the first minute (Fig. 1), group 9 at 1 and 1.5 min ($p = 0.044$ and $p = 0.047$) and group 11 at 0.5 min ($p = 0.008$). Phosphate release was significantly reduced over time only in group 8 (Fig. 2). Group 11 showed significantly less phosphate loss than the control only at 0.5 min ($p = 0.031$) and groups 7 and 3 at 6 min ($p = 0.049$ and $p = 0.030$).

SEM analysis

At high magnification, small nanocracks of < 1 μm were observed at center of the irradiated surfaces, but with different extents. Samples of group 2, 3, 10, and 11 revealed only a few cracks of < 300 nm, whereas surfaces in groups 1, 4, 5, and 12 showed several nanocracks (500–700 nm). Most microcracks could be observed in groups 6–9 (400–600 nm).

TABLE 2. CALCIUM LOSS IN μLMOL/CM^2 (MEAN±SD) FOR ALL GROUPS AT EVERY 2 MIN FROM 1 TO 15 MIN EROSION TIME

Groups	Erosion time (min)							
	1	*3*	*5*	*7*	*9*	*11*	*13*	*15*
G1	0.79 ± 0.2	0.94 ± 0.2	0.96 ± 0.1	0.89 ± 0.2	0.89 ± 0.2	0.92 ± 0.2	0.91 ± 0.2	0.87 ± 0.2
G2	1.08 ± 0.3	1.10 ± 0.3	1.05 ± 0.2	1.07 ± 0.2	1.06 ± 0.2	1.01 ± 0.1	1.03 ± 0.2	1.03 ± 0.2
G3	1.07 ± 0.1	1.08 ± 0.1	1.07 ± 0.1	1.00 ± 0.1	1.07 ± 0.1	1.00 ± 0.1	1.01 ± 0.1	0.96 ± 0.1
G4	0.91 ± 0.2	0.95 ± 0.2	0.96 ± 0.2	0.91 ± 0.1	0.92 ± 0.1	0.93 ± 0.2	0.95 ± 0.1	0.89 ± 0.1
G5	1.05 ± 0.2	1.06 ± 0.2	1.10 ± 0.3	0.99 ± 0.1	1.00 ± 0.2	0.98 ± 0.1	0.95 ± 0.1	0.95 ± 0.2
G6	0.91 ± 0.1	0.95 ± 0.1	0.96 ± 0.2	0.96 ± 0.1	0.96 ± 0.1	0.93 ± 0.1	0.90 ± 0.1	0.86 ± 0.1
G7	0.88 ± 0.2	0.91 ± 0.1	0.94 ± 0.2	0.82 ± 0.1	0.82 ± 0.1	0.89 ± 0.2	0.89 ± 0.2	0.84 ± 0.1
G8	0.73 ± 0.1^a	0.78 ± 0.1^a	0.82 ± 0.1	0.76 ± 0.1^a	0.80 ± 0.1^a	0.79 ± 0.1^a	0.82 ± 0.1^a	0.73 ± 0.1^a
G9	0.79 ± 0.1^a	0.84 ± 0.1	0.86 ± 0.1	0.84 ± 0.1	0.87 ± 0.1	0.86 ± 0.1	0.88 ± 0.1	0.79 ± 0.1
G10	0.87 ± 0.1	0.89 ± 0.1	0.85 ± 0.2	0.88 ± 0.1	0.95 ± 0.1	0.95 ± 0.1	0.86 ± 0.1	0.83 ± 0.1
G11	0.82 ± 0.1	0.87 ± 0.1	0.91 ± 0.1	0.87 ± 0.1	0.88 ± 0.1	0.89 ± 0.2	0.8 5 ± 0.1	0.83 ± 0.1
G12	0.92 ± 0.1	0.92 ± 0.1	0.88 ± 0.1	0.89 ± 0.1	0.93 ± 0.1	0.98 ± 0.1	0.97 ± 0.1	0.89 ± 0.2
GC	0.94 ± 0.1	0.96 ± 0.2	0.96 ± 0.1	0.94 ± 0.1	0.96 ± 0.1	0.94 ± 0.1	0.96 ± 0.1	0.90 ± 0.1

^{a}Statistically significant difference to control group ($p < 0.05$).

TABLE 3. PHOSPHATE LOSS IN μLMOL/CM^2 (MEAN $\pm$ SD) FOR ALL GROUPS
AT EVERY 2 MIN FROM 1 TO 15 MIN EROSION TIME

Groups	Erosion time (min)								
	1	3	5	7	9	10	11	13	15
G1	0.46 ± 0.2	0.59 ± 0.1	0.58 ± 0.1	0.51 ± 0.2	0.52 ± 0.1	0.50 ± 0.1	0.46 ± 0.1	0.51 ± 0.2	0.49 ± 0.2
G2	0.55 ± 0.1	0.54 ± 0.1	0.59 ± 0.1	0.60 ± 0.1	0.58 ± 0.1	0.57 ± 0.1	0.56 ± 0.1	0.58 ± 0.1	0.58 ± 0.1
G3	0.57 ± 0.1	0.58 ± 0.1	0.57 ± 0.1	0.57 ± 0.1	0.56 ± 0.1	0.56 ± 0.1	0.59 ± 0.1	0.51 ± 0.1	0.55 ± 0.1
G4	0.52 ± 0.1	0.52 ± 0.1	0.51 ± 0.1	0.51 ± 0.1	0.54 ± 0.1	0.53 ± 0.1	0.53 ± 0.1	0.53 ± 0.1	0.49 ± 0.1
G5	0.54 ± 0.1	0.62 ± 0.1	0.60 ± 0.1	0.60 ± 0.1	0.55 ± 0.1	0.54 ± 0.1	0.55 ± 0.1	0.53 ± 0.1	0.53 ± 0.1
G6	0.48 ± 0.1	0.55 ± 0.1	0.54 ± 0.1	0.50 ± 0.1	0.51 ± 0.1	0.48 ± 0.1	0.50 ± 0.1	0.49 ± 0.1	0.46 ± 0.1
G7	0.45 ± 0.1	0.45 ± 0.1	0.53 ± 0.1	0.47 ± 0.1	0.47 ± 0.1	0.48 ± 0.1	0.48 ± 0.1	0.49 ± 0.1	0.45 ± 0.1
G8	0.41 ± 0.1[a]	0.45 ± 0.1	0.40 ± 0.1[a]	0.47 ± 0.1	0.48 ± 0.1	0.45 ± 0.1	0.47 ± 0.1	0.47 ± 0.1	0.44 ± 0.1
G9	0.47 ± 0.1	0.49 ± 0.1	0.49 ± 0.2	0.50 ± 0.1	0.53 ± 0.1	0.51 ± 0.1	0.52 ± 0.1	0.53 ± 0.1	0.49 ± 0.1
G10	0.48 ± 0.1	0.50 ± 0.1	0.51 ± 0.1	0.48 ± 0.1	0.51 ± 0.1	0.50 ± 0.1	0.50 ± 0.1	0.49 ± 0.1	0.47 ± 0.1
G11	0.45 ± 0.1	0.49 ± 0.1	0.55 ± 0.1	0.49 ± 0.1	0.49 ± 0.1	0.47 ± 0.1	0.49 ± 0.1	0.48 ± 0.1	0.46 ± 0.1
G12	0.45 ± 0.1	0.47 ± 0.1	0.48 ± 0.1	0.48 ± 0.1	0.47 ± 0.1	0.45 ± 0.1	0.48 ± 0.1	0.47 ± 0.1	0.48 ± 0.1
GC	0.53 ± 0.1	0.54 ± 0.1	0.54 ± 0.1	0.51 ± 0.1	0.54 ± 0.1	0.54 ± 0.1	0.52 ± 0.1	0.52 ± 0.1	0.51 ± 0.1

[a]Statistically significant difference to control group ($p < 0.05$).

A selection of characteristic lased surfaces is displayed in Fig. 3.

Discussion

In the present study, pulse durations from 80 to 400 μs of a clinical CO_2 laser were tested for the first time with the aim of increasing enamel acid resistance. There was, until recently, no clinical CO_2 laser in the market capable of emitting pulses of some hundreds of microseconds; therefore, the conduction of clinical studies has been very limited or almost impossible.[20] Also, there is currently no protocol for a laser treatment of patients at high risk for erosion or caries, although the manufacturer has already included clinical recommendations of irradiation parameters in its treatment guidelines. Therefore, as the increase of enamel acid resistance after CO_2 laser irradiations with low pulse durations ($<100 \mu$s) has already been observed in vitro with the use of industrial lasers, and the manufacturer of this new laser system is already recommending preventive irradiation conditions to be used clinically, it was hypothesized that a high increase of enamel acid resistance could be obtained also with this new clinical device.[1,5, 21–23] However, this hypothesis was not totally confirmed, as the maximum reduction of calcium and phosphate loss was only 20%, which is much less than the already observed 70–81% caries inhibition and the 97% reduction of enamel softening.[22]

Nevertheless, it was possible to obtain some insights about how the different laser parameters may influence the increase or decrease of enamel acid solubility. For the pulse durations, which resulted in the highest inhibitions, namely 200 and 300 μs, it is interesting to observe that in both cases, with the increase of the frequency there was a slight increase of enamel acid resistance. This increase was small (5% reduction of calcium loss when the frequency was increased from 400 to 450 Hz, in groups 7 and 8, and from 250 to 300 Hz in groups 10 and 11) and probably for this study irrelevant; however, as a similar effect has been observed in other studies,[7] it seems worthy to note this tendency, as it may contribute to better understanding of the laser–tissue interaction in future investigations.

Another interesting tendency to be observed is that for all parameters tested, a decrease of the effect with time is seen, and this probably indicates the elimination of the laser-modified layer. For the parameters that caused initially a decrease in mineral loss, there is with the passage of the time a slight increase in the quantity of Ca and PO_4 released. On the other hand, for the parameters that caused initially a slight increase in mineral loss (higher than the control) the opposite occurs and a decrease is observed as time passes. Although these differences in the effect over time were also not statistically significant, it is interesting to note them, as these gradients of effect in the tissue have been well described by Nelson et al. and should be better investigated in further studies.[24]

The increase of enamel acid resistance after laser irradiation is strongly related to the temperature increase caused at the tissue. When enamel is heated to a specific temperature range (600°–900°C), not only less soluble hydroxyapatite may be formed, but also elimination of mineral impurities, such as carbonate, and changes in the organic matrix may occur. However, the positive modification of the tooth mineral solubility is very sensitive and depends on strict heating and cooling conditions. As during the laser irradiation the tooth is not equally heated at the surface and inner layers, the modifications in solubility in inward direction are not the same. Therefore, the degree of acid resistance of lased enamel occurs in gradients and it is possible that over a layer of more acid-resistant enamel, less resistant ones are present, according to the distance from the surface.[9,24]

Although the increase of temperature can be very positive for enamel solubility, it may also have a negative side. This means that if the temperature is not increased only for a short period of time (few microfractions of a second) heat accumulation can happen and cause ablation, melting, or cracking of tissue.[25] Such unwanted effects were also observed here. In addition to the significant increase of enamel resistance to erosion, the laser parameters tested in the present study have shown, under SEM and higher magnification, some surface modification. Surface nanocracks of $<1 \mu$m in width were observed in the samples of almost all
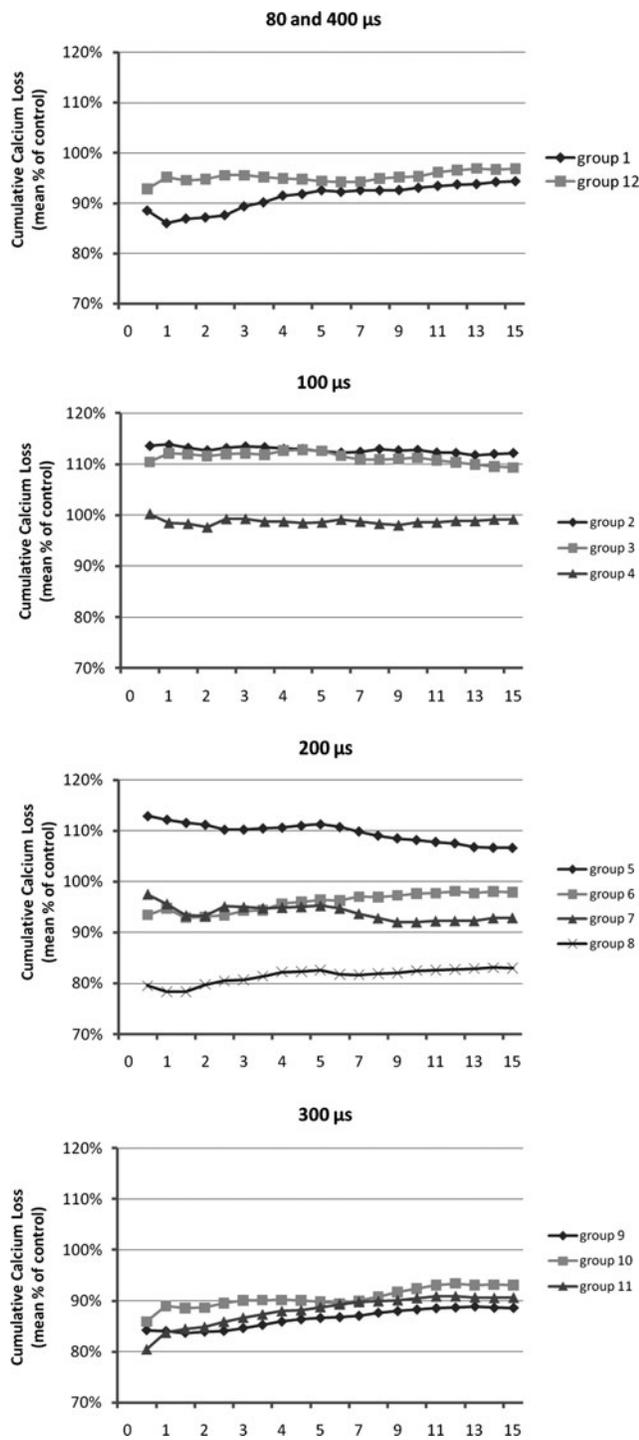
FIG. 1. Cumulative calcium loss (mean, % of control) of enamel in the differently lased groups. Calcium release was significantly reduced in group 8 (all time points except t = 2.5 min, t = 3.5 min, t = 4 min, t = 5 min, and t = 10 min), group 9 (time points t = 1 min, t = 1.5 min) and group 11 (t = 0.5 min).

FIG. 2. Cumulative phosphate loss (mean, % of control) of enamel in the differently lased groups. Phosphate release was significantly reduced in group 8 at t = 0.5 min, t = 1 min, t = 2 min, t = 2.5 min, t = 3.5 min, t = 4.5 min, t = 5 min, and t = 6 min. At t = 0.5 min phosphate release was also significantly reduced in group 11, and at t = 6 min in groups 3 and 7.

the groups. As already expected from previous studies[10,12] the groups with the lowest pulse durations and lowest energy density caused the lowest amount of morphological changes, but caused at the same time a limited increase of enamel acid resistance.
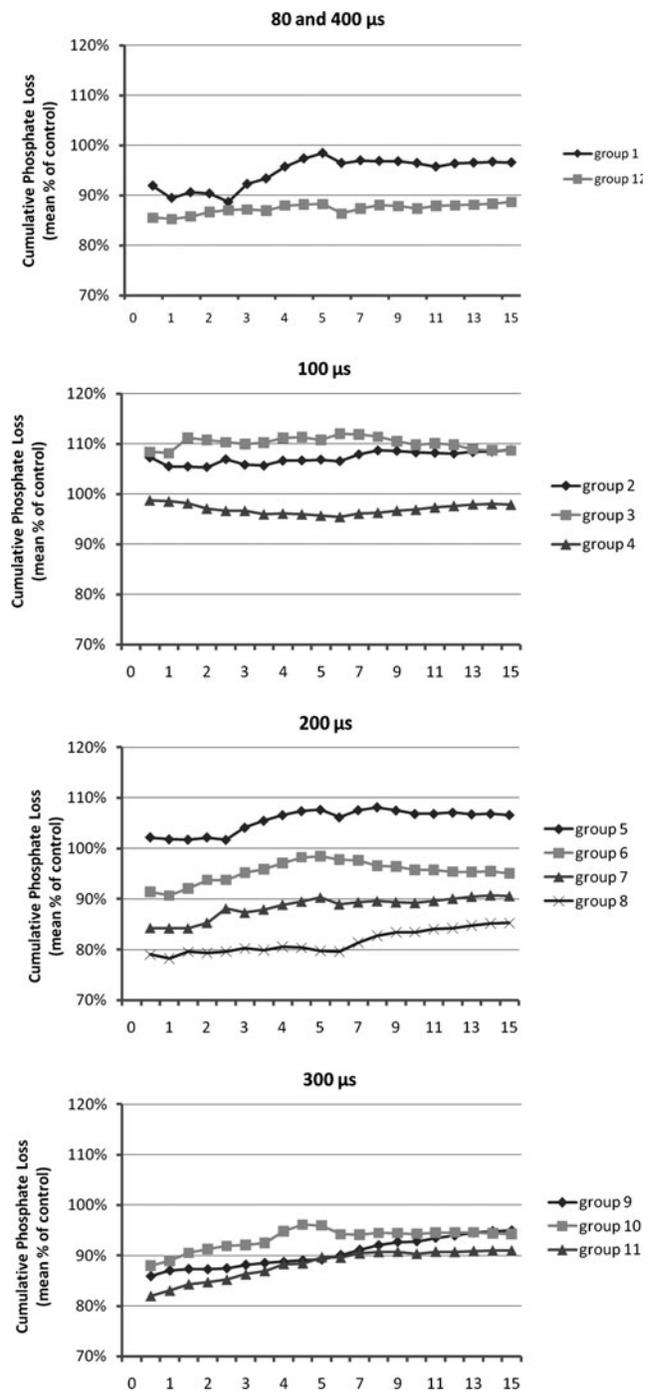
In the present study a relative strong acid attack was reproduced. The erosion model using hydrochloric acid (pH 2.6) simulates the clinical situation of patients with gastroesophageal reflux and frequent vomiting (bulimia), which cause gastric acid penetration into the oral cavity. In

addition, the use of an erosion chamber had two important advantages. The first is that it allowed constant acid flow over the samples, thus preventing acid saturation, normally observed in static conditions; and the second is the possibility of allowing constant acid attack for 15 min, which provides some indications about the duration of the protective effects.[14] The limitation, on the other hand, is the absence of any remineralization solution to simulate the saliva counterbalance on enamel dissolution. However, considering that it is a well-established model that provides standardized and reproducible erosive conditions, it fulfilled the necessities of this initial and explorative study.[26] It must also be kept in mind that this was a a relative strong acid attack and that under clinical conditions an acid challenge results in pH fall below the critical level only for 2 min at the tooth surfaces.[27] These surfaces are additionally normally protected with a protective barrier, the acquired pellicle, and therefore under clinical conditions probably less enamel dissolution should be observed. Even though the maximum effect achieved by the best laser condition was not very great, this effect was in the same range as the protection achieved by several types of fluoride treatment in the same erosion model.[14] Fluoride gel (AmF/NaF, 1.25% F) and tetrafluoride solutions such as titanium tetrafluoride (TiF_4; 0.4 and 1 %), zirconiumfluoride (ZrF_4; 0.4 and 1 %), and hafnium fluoride (HfF_4; 0.4 and 1 %) caused also $\sim$20% erosion reduction.[14] Higher protection was observed by Yu et al., but in samples previously covered with acquired salivary pellicle, formed *in situ*.[28] As the pellicle is known to act as barrier to acid diffusion and provide additional protective effect against erosion, it cannot be stated that the high reduction observed in that study was only caused by fluoride application. Nevertheless other fluoride compounds have already shown higher erosive resistance in other erosion models.[29]

Reduction of carious and/or erosive tooth dissolution has been also observed after enamel irradiation with other types of lasers, such as the diode, Nd:YAG, and erbium lasers.[30–32] However, in most of these studies, significant increase of acid resistance has been only obtained when the irradiation was combined with fluoride compounds. In this case, significant reduction compared with untreated controls has been observed for the combination of Nd:YAG laser with TiF_4 solution,[33] and with acidulated phosphate fluoride (APF).[30,34,35] Additionaly, increase of fluoride uptake at the enamel surface has been also observed for the combination of diode laser irradiation with neutral sodium fluoride (NaF).[36] However, none of them though showed increased enamel resistance to acid solubility after solely laser irradiation, as observed in the present study.

The recommendation of the laser manufacturer in its treatment guidelines provided together with the laser equipment may be misleading for several clinicians. Considering
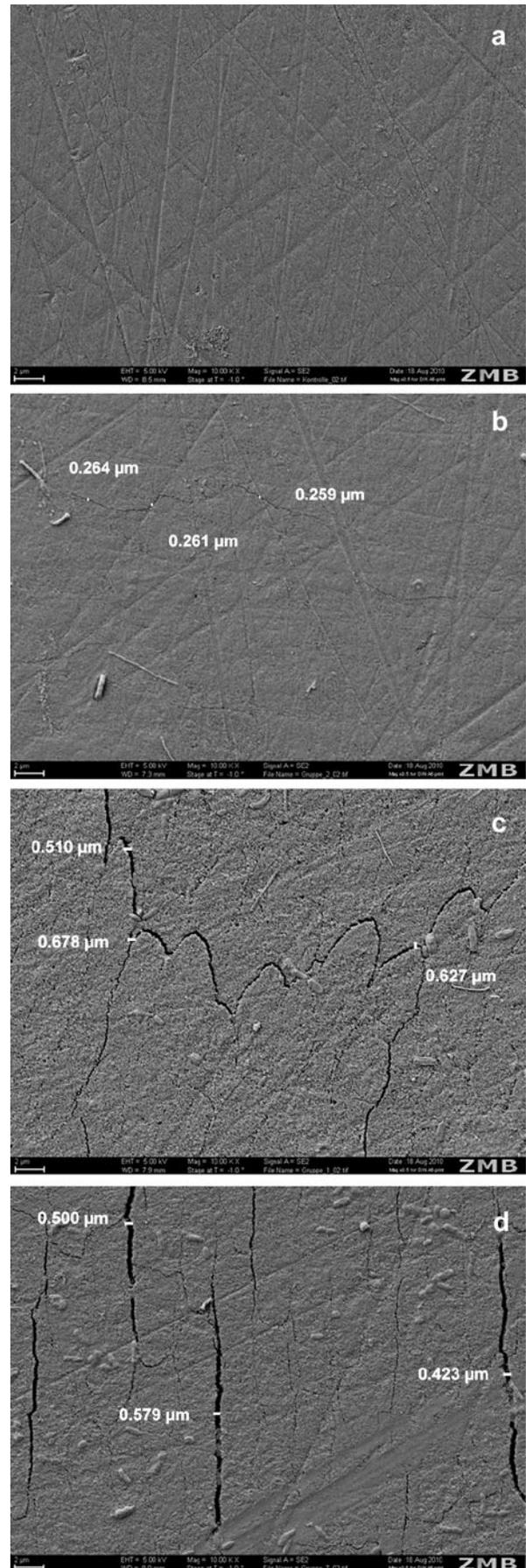

FIG. 3. Characteristic scanning electron microscopic (SEM) pictures of control and lased samples (original magnification $\times$10,000 , bar: 2 μm). **(a)** Control without any surface alterations. **(b)** Few nanocracks (exemplary sample from group 2) having 259–264 nm of width. **(c)** Several nanocracks (exemplary sample from group 1) having 510–678 nm of width. **(d)** Most nanocracks (exemplary sample from group 8) having 423–579 nm of width.

that all the laser conditions tested here caused the occurrence of nanocracks at the surface, a clinical application of this laser in erosion prevention would only make sense in cases in which its combination with fluoride products would drastically increase fluoride retention or form a metal-containing coating layer highly bound to enamel and capable of sealing the nanocracks caused by the irradiation.[28,29,37] However, this hypothesis must be investigated in future studies.

Conclusions

Under the conditions of this study, the maximum statistically significant increase of enamel resistance to erosive mineral loss was obtained after CO$_2$ laser (10.6 μm) irradiation at 0.4 J/cm^2 (200 μs, 450 Hz) and was of 20%. As the irradiation also caused side effects at the surface, even these parameters cannot be recommended for clinical use.

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Author Disclosure Statement

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