Caries Inhibition With a CO_2 9.3 μm Laser: An In Vitro Study

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Background and Objectives: The caries preventive effects of different laser wavelengths have been studied in the laboratory as well as in pilot clinical trials. The objective of this *in vitro* study was to evaluate whether irradiation with a new 9.3 μm microsecond short-pulsed CO_2-laser could enhance enamel caries resistance with and without additional fluoride applications.

Study Design/Materials and Methods: One hundred and one human tooth enamel samples were divided into seven groups. Each group was treated with different laser parameters (CO_2-laser, wavelength 9.3 μm, 43 Hz pulse-repetition rate, pulse duration between 3 μs at 1.5 mJ/pulse to 7 μs at 2.9 mJ/pulse). A laboratory pH-cycling model followed by cross-sectional microhardness testing determined the mean relative mineral loss delta Z (ΔZ) for each group to assess caries inhibition in tooth enamel by the CO_2 9.3 μm short-pulsed laser irradiation. The pH-cycling was performed with or without additional fluoride.

Results: The non-laser control groups with additional fluoride had a relative mineral loss (ΔZ, vol% × μm) that ranged between 646 ± 215 and 773 ± 223 (mean ± SD). The laser irradiated and fluoride treated samples had a mean ΔZ ranging between 209 ± 133 and 403 ± 245 for an average $55\% \pm 9\%$ reduction in mineral loss (ANOVA test, $P < 0.0001$). Increased mean mineral loss (ΔZ between 1166 ± 571 and 1339 ± 347) was found for the non-laser treated controls without additional fluoride. In contrast, the laser treated groups without additional fluoride showed a ΔZ between 470 ± 240 and 669 ± 209 (ANOVA test, $P < 0.0001$) representing an average $53\% \pm 11\%$ reduction in mineral loss. Scanning electron microscopical assessment revealed that 3 μs pulses did not markedly change the enamel surface, while 7 μs pulses caused some enamel ablation.

Conclusion: The CO_2 9.3 μm short-pulsed laser energy renders enamel caries resistant with and without additional fluoride use. The observed enhanced acid resistance occurred with the laser irradiation parameters used without obvious melting of the enamel surface as well as after irradiation with energies causing cutting of the enamel. Lasers Surg. Med. 48:546–554, 2016. Published 2016. This article is a U.S. Government work and is in the public domain in the USA

Key words: CO_2 9.3 μm laser; microsecond short-pulsed; improving caries resistance; laboratory study; pH-cycling; fluoride; cross-sectional microhardness testing

INTRODUCTION

In the early 1970's, shortly after the first laser had been invented, researchers reported experiments *in vitro* that indicated enhanced caries resistance in enamel and dentin using CO_2-lasers [1–10]. Since then, other laser wavelengths that could potentially reduce enamel acid dissolution including Nd:YAG- [11–14], Er:YAG- [15–18], and Er, Cr:YSGG-lasers [19–21] have been explored in laboratory studies. The caries preventive effect of the argon ion lasers [22–28] with and without additional topical fluoride applications has been studied in the laboratory. The argon lasers have also been used in *in vivo* studies around orthodontic brackets [29]. The influence of Nd:YAG-laser treatment combined with initiation dye and acidulated fluoride application on the development of white spot lesions or fissure caries in children also has been assessed [30].

Using considerably lower energy levels than those reported in most of the earlier studies Featherstone and co-workers showed that *in vitro* enhancement of enamel caries resistance was achieved with short-pulsed CO_2-laser irradiation under specific irradiation conditions [9,10,31]. Dental enamel most strongly absorbs 9.3- and 9.6-μm CO_2-laser wavelengths. At these wavelengths the enamel absorption coefficient is ten times higher compared to the 10.6 μm CO_2-laser wavelength [32]. Additionally, operating the laser with microsecond instead of millisecond pulses allows energy applications that facilitate the avoidance of harmful pulpal tissue effects [33].

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and have disclosed the following: This study is a Principle Investigator Initiated Study and was funded by Department of Preventive and Restorative Dental Sciences discretionary funds and by Convergent Dental, Inc. through UCSF Contracts & Grants Division.

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Accepted 19 February 2016
Published online 13 April 2016 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/lsm.22497

In 2003 Rechmann and co-workers [34] showed in an *in vivo*, single blind, prospective clinical trial using an orthodontic bracket model that caries resistance can be improved with short pulsed microsecond CO_2 9.6 μm laser irradiation [35]. Cross sectional microhardness testing quantitatively revealed that the 9.6 μm CO_2-laser irradiation significantly inhibited the formation of carious lesions around orthodontic brackets. In 2013 Rechmann et al performed a single blind, controlled, randomized prospective clinical pilot trial, irradiating molar fissures with a 9.6 μm CO_2-laser emitting 20 microsecond pulses. This *in vivo* study revealed that the laser irradiation with additional fluoride varnish applications significantly inhibited the formation of carious lesions in fissures of molars in comparison to a non-irradiated control tooth in the same arch over a 1-year observational period [36]. In addition, the study also showed that using the CO_2 short-pulsed laser irradiation leads to remineralization of the irradiated enamel. This was proven by ICDAS (International Caries Detection and Assessment System) and SOPROLIFE daylight and fluorescence assessments. Because the absorption characteristics of enamel are very similar at 9.6 and 9.3 μm it can be anticipated that the latter wavelength would preform similarly for caries inhibition.

The objective of this present *in vitro* study was to evaluate whether irradiation with a new 9.3 μm microsecond short pulsed CO_2-laser could enhance enamel caries resistance with and without additional fluoride applications.

MATERIALS AND METHODS

A laboratory pH-cycling model was used, as previously reported [9], to assess caries inhibition in tooth enamel by CO_2 9.3 μm, short-pulsed laser irradiation. Test samples were divided into seven groups. Each group was treated with different laser parameters. One half of each sample served as (laser) untreated control. After laser irradiation, the samples were prepared for pH-cycling. The pH-cycling was performed for some groups with and for some without additional fluoride. After pH-cycling, all samples underwent cross-sectional microhardness testing to determine the mean relative mineral loss values delta Z (ΔZ) among groups using the techniques previously verified and published [9,37–40].

Test Samples

Extracted human molars (UCSF IRB exempt approval for collecting extracted teeth) were stored in 0.1% thymol solution in deionized water and sterilized with gamma irradiation (Cs 137) for 12 hours at a dose above 173 krad. Following sterilization, the collection media was replaced with fresh deionized water and thymol.

Sample preparation was performed as described previously [37–39]. After irradiation the surface of the tooth enamel was covered with acid-resistant nail varnish leaving the irradiated area and the adjacent area of similar size uncovered as control, resulting in a test window of approximately 4×2 mm.

Table 1 gives the number of irradiated samples per group before pH-cycling, the applied laser pulse duration, pulse energy, the resulting fluence, and fluoride use (yes/no) during pH-cycling.

pH-Cycling Model for Study of Caries Progression

The samples were treated according to a validated *in vitro* caries inhibition pH-cycling remineralization/demineralization protocol [40]. The pH-cycling model consisted of alternating periods of demineralization and remineralization to simulate caries in the mouth as measured around orthodontic brackets in a clinical study [34]. The pH-cycling process was repeated for nine, 24-hour periods, with one weekend in the middle with samples in the mineralizing solution. Demineralization in each 24-hour period occurred for 6 hours daily in an acetate/calcium/phosphate buffer at pH 4.4. The buffer contained calcium and phosphate at 2.0 mmol/L, 0.075 mmol/L acetate with 40 ml per specimen used individually.

Remineralization was for 18 hours in a calcium phosphate mineralizing solution at pH 7.0. The mineralizing solution contained 0.8 mmol/L calcium, 2.4 mmol/L phosphate (concentrations similar to saliva), cacodylate 20 mmol/L as a buffer. Samples were suspended in demineralization and remineralization reagents. The solutions were refreshed after the first week to maintain consistent concentrations.

Between each demineralization and remineralization cycle the samples were rinsed in deionized water and then placed in the next solution. The samples receiving fluoride treatment were additionally immersed and shaken on an orbital mixer in a 1:3 Crest Cavity protection (Proctor and

TABLE 1. Irradiated Enamel Samples

Pulse duration [μs] Solea GUI	Pulse energy [mJ]	Fluence [J/cm^2]	Samples (n)	Fluoride (yes/no)	Observed effects during irradiation
3	1.49	3.03	15	Yes	None
4	1.90	3.88	15	Yes	Slight melting
5	2.28	4.64	13	Yes	Smooth, melting
5	2.28	4.64	13	No	Smooth, melting
6	2.51	5.12	15	Yes	Ablation starts
6	2.51	5.12	15	No	Ablation starts
7	2.90	5.92	15	No	Cutting

Gamble, Cincinnati, OH) toothpaste/deionized water slurry for 1 minute, rinsed in deionized water and placed in the next solution. Each tube of slurry was made immediately before use for each sample in each group by vortexing 1 g of the toothpaste with 3 g of double deionized water.

Each tube was masked with colored tape so as to be color coded at the beginning of the study to insure blinding of the laboratory investigator during the pH-cycling and throughout the microhardness measurements. All groups were followed by a color code system until all the results were calculated. The laboratory technicians measuring microhardness did not know the identity of the treatment groups.

Cross Sectional Microhardness Measurements

The analysis method used was cross sectional microhardness by detailed "scatter pattern" of indentation as described previously and verified against cross-sectional microradiography [34,41]. The first indent was placed 15 μm from the resin/lesion interface and 100 μm from the edge of the demineralized/remineralized lesion. Subsequent indents were placed in 5 μm increments to a final depth of 50 μm in the underlying enamel; implementing a V-shaped pattern prevented interaction and interference between the indents.

Additional indents were placed at 25 μm intervals into underlying sound enamel following a straight line perpendicular to the outer surface to a depth of 300 μm. The volume percent mineral for each indent was normalized based on sound underlying enamel (100–300 μm) set at 85% [34,41].

The overall relative mineral loss, ΔZ (vol% × μm), for each sample was calculated as described in detail previously [41]. The individual ΔZ values for each lesion in each group were combined to give a mean ΔZ and standard deviation for each of the test groups.

The color coding was broken only after the measurements had been made, so as to enable the data to be collected into appropriate spread sheets for analysis by group. All labeling was cross-checked after unmasking of the codes at the end of the study.

Laser Settings

The laser utilized in this study was a Carbon dioxide laser (Solea, Convergent Dental, Inc., Natick, MA) emitting a wavelength of 9.3 μm. For this study five different laser pulse durations between 3 μs and 7 μs were used, consequently delivering pulse energies of 1.49 mJ/pulse and up to 2.9 mJ/pulse, resulting in fluences between 3.03 J/cm^2 and 5.92 J/cm^2. The pulse energy was measured with a BeamTrack—Power/Position/Size Thermal Sensor 50(150)A-BB-26-PPS (Ophir-Spiricon, LLC, North Logan, UT) before and after five samples were irradiated. In non-contact mode the beam diameter was set to 0.25 mm (verified by using a 1" FL lens as a relay to magnify the focused spot 5.5× to a Ophir-Spiricon Pyrocam III pyroelectric camera for detection, for measurement BeamGage V5.11 Software was used in pulsed mode w/5mS exposure time, m 90/10 size criteria), with a laser focus length of 4–10 mm.

The originally irradiated sample surface was 4 × 4 mm. The pulse repetition rate was set to 43 Hz. To allow that each spot was irradiated with at least 20 laser pulses (known to be successful for enhancing caries resistance [35]) each sample was irradiated for 2 minutes with overlapping laser irradiation. No air and no water spray were applied.

The laser pulse shape was square with an initial sharp peak. The beam profile was Gaussian. The beam profile was measured with a Ophir-Spiricon Pyrocam III pyroelectric camera with BeamGage V5.11 Software.

Statistical Methods

Each sample exhibits a relative mineral loss value ΔZ (vol% × μm). Means and standard deviations for each group were calculated and the groups were compared statistically by One-way ANOVA, with Newman-Keuls Multiple Comparison Test for significance at $P < 0.05$.

Stereomicroscope Observations and Scanning Electron Microscopy

A stereomicroscope (Fisher Scientific Stereomaster, Fisher Scientific LLC, PA) was used to observe visible effects during and after irradiation (magnification 10x). A maximum of three additional tooth enamel samples were irradiated with each of the five different irradiation conditions as mentioned above in "Laser Settings" for Scanning Electron Microscopy (SEM). For the SEM investigations the samples were desiccated using 100% alcohol, sputtered with gold palladium and then examined with the SEM (JCM 5000, JEOL Ltd., Japan) at different magnifications.

RESULTS

Figures 1 and 2 present the mean ΔZ (vol% × μm) mineral loss and standard deviations for the group of samples with and without additional fluoride use at different laser energies (pulse durations). Table 2 shows the number of samples per test group that were available for cross sectional microhardness testing with 10–15 per group for a total of 183 samples (some samples were lost during the pH-cycling processing).

Relative Mineral Loss ΔZ

Relative mineral loss ΔZ for groups with additional fluoride treatment. The control groups (no laser treatment) with additional fluoride showed a mineral loss ΔZ (vol% × μm) range between 646 ± 215 and 773 ± 223 (mean ± Standard Deviation [SD]). In contrast, the laser treated groups with additional fluoride showed a ΔZ value between 209 ± 133 and 403 ± 245. While the non-laser controls with additional fluoride showed a much larger ΔZ mineral loss, the laser irradiated and fluoride treated samples showed, on average, a $55\% \pm 9\%$ reduction in mineral loss. The ANOVA test indicated that the differences between the laser treated and the control groups, both groups with additional fluoride treatment, were

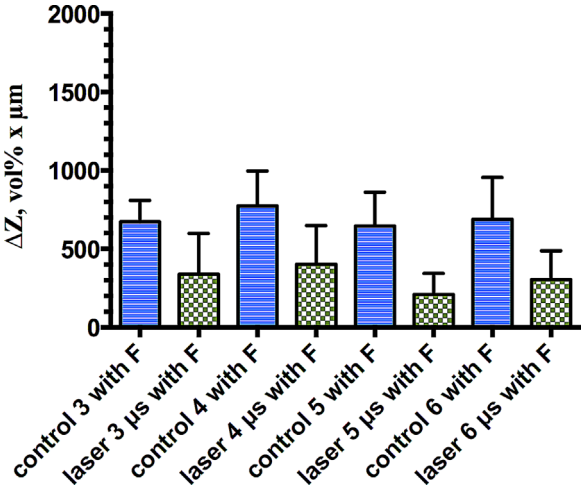

Fig. 1. Mean relative mineral loss ΔZ (vol% × μm) for the laser treated enamel groups and for the control groups for four different laser energies after 9 days of ph-cycling with additional fluoride for an average of 55% reduction in mineral loss for the laser treated teeth. All irradiation energies resulted in statistically significant reduced mineral loss ($P < 0.0001$); Error bars are standard deviations.

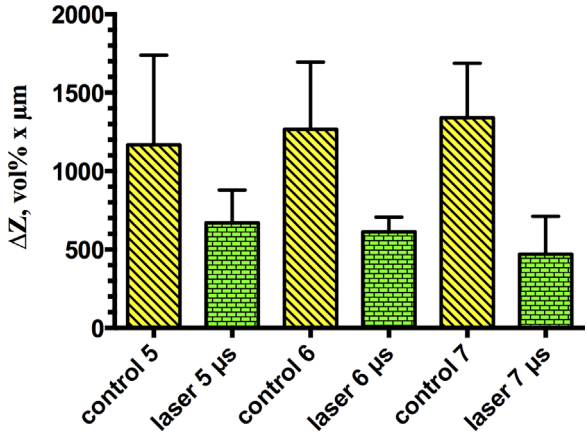

Fig. 2. Mean relative mineral loss ΔZ (vol% × μm) for the laser treated enamel groups and for the control groups for three different laser energies after 9 days of ph-cycling with no additional fluoride treatment for an average of 53% reduction in mineral loss for the laser treated teeth. All irradiation energies resulted in statistically significant reduced mineral loss ($P < 0.0001$); Error bars are standard deviations.

statistically significant ($P < 0.0001$). This was true for all applied energies.

Irradiation of the enamel with a laser pulse duration of 3 μs lead to an almost 50% reduction in mineral loss.

Relative mineral loss ΔZ for groups without additional fluoride treatment. Overall, the groups that did not receive additional fluoride treatment had a higher mineral loss than those that had received additional fluoride. The non laser treated controls in the groups without additional fluoride showed a mineral loss ΔZ (vol % × μm) ranging between 1166 ± 571 and 1339 ± 347 (mean ± SD). In contrast, the laser treated groups without additional fluoride produced a ΔZ between 470 ± 240 and 669 ± 209. Thus again, the controls showed a larger ΔZ mineral loss than the laser-irradiated samples. The irradiated samples showed on average a $53\% \pm 11\%$ reduction in mineral loss compared to the non-irradiated controls. The ANOVA test revealed that the differences between the laser treated and the control groups, both with no additional fluoride treatment, were statistically significant for all applied energies ($P < 0.0001$).

Stereomicroscope and Scanning Electron Microscopical Observations

The stereomicroscopically observed effects resulting from laser irradiation ranged from no visible change of the enamel, through slight melting and melting of enamel to start of ablation and definite cutting of the enamel (see Table 1).

The Scanning Electron Microscope examinations confirmed the visual observations. While there was no visible change using 3 μs pulse duration the SEM revealed only minor changes. Even at the highest magnification (Fig. 3.4) almost no melting became visible. At all magnifications the enamel surface appeared nearly unchanged.

Figure 4 reveals the surface after irradiation with 4 μs pulses. Irradiation with this energy level reveals at all magnifications homogeneous surface melting and no roughness of the surface.

Figure 5 shows the enamel surface after irradiation with 5 μs pulses. Some minor surface roughness can be observed. At higher magnifications a relatively homogenous surface melting can bee seen (Fig. 5.3 and 5.4).

Figure 6.1 gives an overview of the findings after irradiation with 6 μs laser pulses on the upper left side, and with 7 μs laser pulses on the lower left, respectively. 6 μs pulses resulted in a rough surface with little ablation of the enamel (Fig. 6.2–6.4). 7 μs pulses obviously performed ablation of the enamel as already observed during the irradiation.

DISCUSSION

Pure hydroxyapatite [Ca_{10} $(PO_4)_6$ $(OH)_2$] is not created during the formation of the tooth mineral. Instead, the mineral of enamel and dentin is best described as a highly substituted carbonated apatite [42]. The mineral is related to hydroxyapatite but it is much more soluble in acid. It is calcium deficient (calcium replaced by sodium, magnesium, zinc, etc.) and contains between 3% and 6% carbonate by weight, mostly replacing phosphate ions in the crystal lattice [43–45]. Enamel and dentin mineral can be roughly represented by a simplified formula for the carbonated hydroxyl apatite [Ca_{10-x} $(Na)_x$ $(PO_4)_{6-y}$ $(CO_3)_z$ $(OH)_{2-u}$ $(F)_u$].

In the past, several laboratory studies have shown that enhancing enamel demineralization resistance can be achieved by irradiation with microsecond pulsed CO_2-lasers [9,31]. The wavelengths absorbed most strongly in

TABLE 2. Mean ΔZ (vol% × μm) for Each of the Laser and Control Groups With Statistically Significant Difference in Mineral Loss

Pulse duration [μs]	Fluoride (yes/no)	Laser ΔZ (SD) Vol% × μm	Laser (*n*)	Control ΔZ (SD) Vol% × μm	Control (*n*)	ΔZ reduction for laser irradiated enamel in %	Statistically significant difference in mineral loss
3	Yes	338.46 (259.76)	15	672.50 (137.01)	12	49.6	Yes
4	Yes	403.48 (245.37)	13	773.97 (223.44)	13	47.8	Yes
5	Yes	209.62 (133.81)	12	646.17 (215.07)	13	67.6	Yes
6	Yes	304.53 (182.98)	15	688.04 (267.67)	14	55.7	Yes
Average ΔZ all fluoride groups		319.52 (220.09)		670.06 (195.53)			
5	No	669.30 (209.33)	11	1166.92 (571.93)	10	42.6	Yes
6	No	613.08 (92.52)	11	1266.09 (429.42)	15	51.6	Yes
7	No	470.84 (240.74)	15	1339.60 (347.60)	14	64.9	Yes
Average ΔZ all no fluoride groups		572.13 (160.53)		1262.16 (445.63)			

dental enamel are the 9.3 μm and 9.6 μm CO_2-laser wavelengths [32]. The loss of the carbonate phase from the enamel crystals due to the irradiation heat is responsible for the reduction in acid dissolution of enamel [46,47] transforming carbonated hydroxyapatite into the more acid resistant hydroxyapatite. Adding fluoride at this time leads to the formation of fluorapatite, which is even less acid soluble [48].

Fluoride works primarily via topical mechanisms, which include [1] inhibition of demineralization at the crystal surfaces inside the tooth, [2] enhancement of remineralization at the crystal surfaces (the resulting remineralized layer is very resistant to acid attack), and [3] in the mouth additionally by inhibition of bacterial enzymes [49].

With respect to remineralization, fluoride present in solution from topical sources enhances remineralization by accelerating the growth of a new surface on the partially demineralized sub-surface crystals in the carious lesion. The new crystal surface veneer is fluorapatite-like with much lower solubility than the original carbonated apatite tooth mineral [37,50].

In this present study the new 9.3 μm CO_2-laser short-pulsed laser has proven its capability to transform enamel into more acid resistant, less soluble enamel. The non-irradiated control samples in all cases, whether additional fluoride was used or not, showed a higher mineral loss than the laser irradiated samples.

As expected, fluoride application reduced the ΔZ (vol % × μm) mineral loss from around 1,262 ± 446 (mean ± SD) to roughly 670 ± 196 affirming that the use of fluoride is caries preventive on its own. The applied fluoride amount simulates twice daily brushing with a regular over-the-counter (OTC) 1,100 ppm fluoride toothpaste. Treatment of the enamel by a 9.3 μm CO_2-laser short-pulsed laser alone reduced the average mineral loss (ΔZ) to 572 ± 161 resulting in a mineral loss below the lowest mineral loss achieved by fluoride protection alone, which represented a statistically significant 15% reduction in mineral loss ($P = 0.01$). Adding fluoride to the laser treatment group further reduced the mineral loss to the lowest value observed in this study. With a ΔZ of 320 ± 220 the mineral loss is approximately half the mineral loss for laser treatment alone.

The data are comparable to the mineral loss observed in a clinical study where a short-pulsed 9.6 μm CO_2-laser was used to irradiate areas next to orthodontic brackets on bicuspids scheduled for extraction [35]. The difficult-to-clean borders of the brackets served as areas for increased plaque accumulation, resulting in enhanced acid attack on the enamel. In that study the children were provided 1,100 ppm OTC fluoride toothpaste and the bicuspids were extracted after 4 and 12 weeks, respectively. Cross-sectional microhardness testing was performed for the laser treated as well as for a control area and the relative mineral loss ΔZ was calculated. After four weeks, the study reported a mean relative mineral loss ΔZ (vol% × μm) for the controls of 738 ± 131 (mean ± SE) that after 12 weeks reached a greater relative mineral loss ΔZ of 1,067 ± 254. The present *in vitro* study showed a similar high ΔZ value of 1,262 ± 429 (mean ± SD) for the non-laser, no fluoride added enamel group. Thus, it can be stated that the 9-day pH-cycling model simulated natural acid attack of at least 12 weeks around orthodontic brackets, for children with brackets living in a fluoridated water region and presumably using the assigned fluoride toothpaste.

The same *in vivo* orthodontic bracket model study revealed for the laser treated enamel only a ΔZ of 402 ± 85 for the 4-weeks leg and 135 ± 98 (mean ± SE) for the 12-weeks leg, resulting in a 46% and 87% demineralization inhibition, respectively [35]. The average mineral loss of 320 ± 220 (mean ± SD) in the *in vitro* pH-cycling study here was also comparable to the *in vivo* mineral loss ranges after laser irradiation. In the *in vitro* study the observed demineralization inhibition ranged between 43% and 68%.

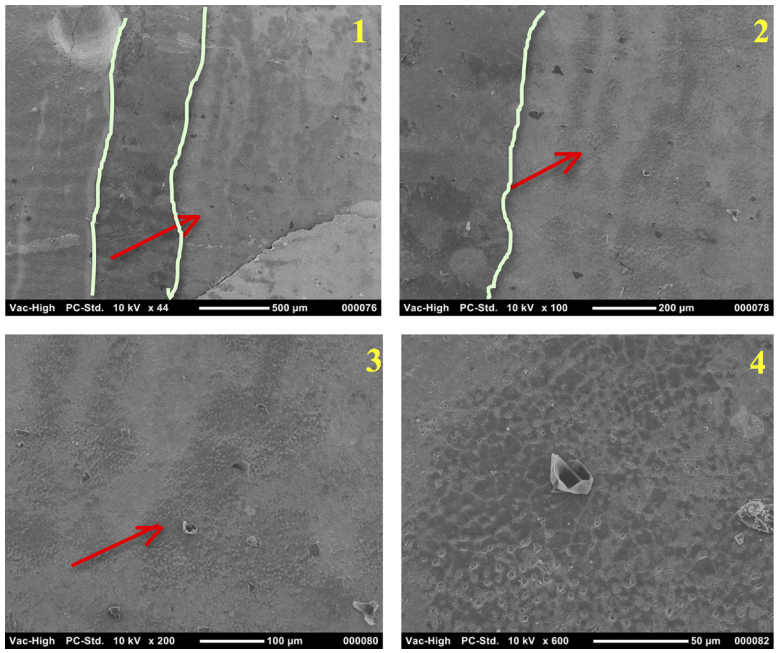

Fig. 3. Enamel surface after irradiation with 3 μs pulse duration; the SEM revealed only minor or no changes; at the highest magnification a few molten areas became visible (arrows point at area showed at the next higher magnification, lines demarcate between irradiated and non irradiated surface).

It has been shown that enamel surface temperatures of 800°C and above, up to 1,200°C, caused the mineral to melt and transform in to less acid soluble mineral when cooled [51–53]. At temperatures above 1200°C ablation of enamel may occur [54]. Other studies have demonstrated that temperatures of only 400°C and above are needed to decompose the carbonate inclusions in the enamel mineral and transform the carbonated hydroxyapatite

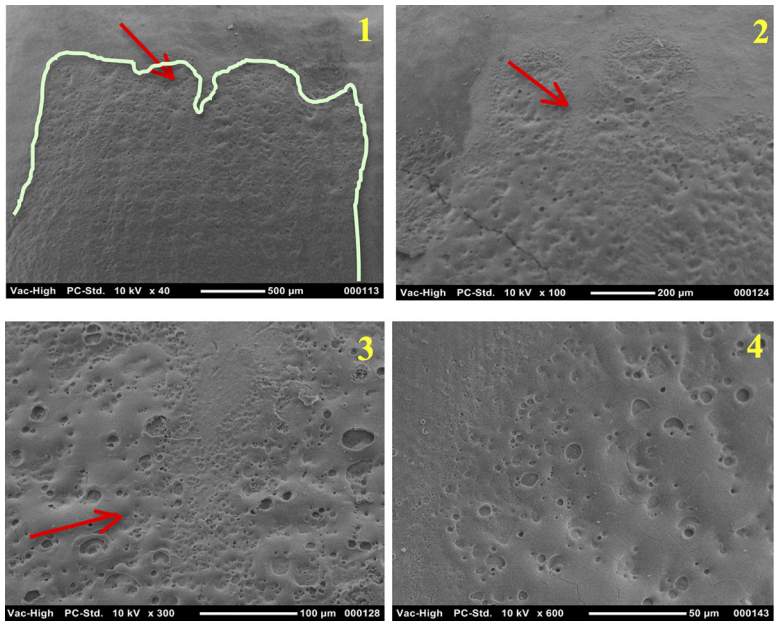

Fig. 4. Enamel surface after irradiation with 4 μs pulse duration; at all magnifications the SEM shows homogeneous surface melting and no roughness of the surface (arrows point at area shown at the next higher magnification, lines demarcate between irradiated and non irradiated surface).

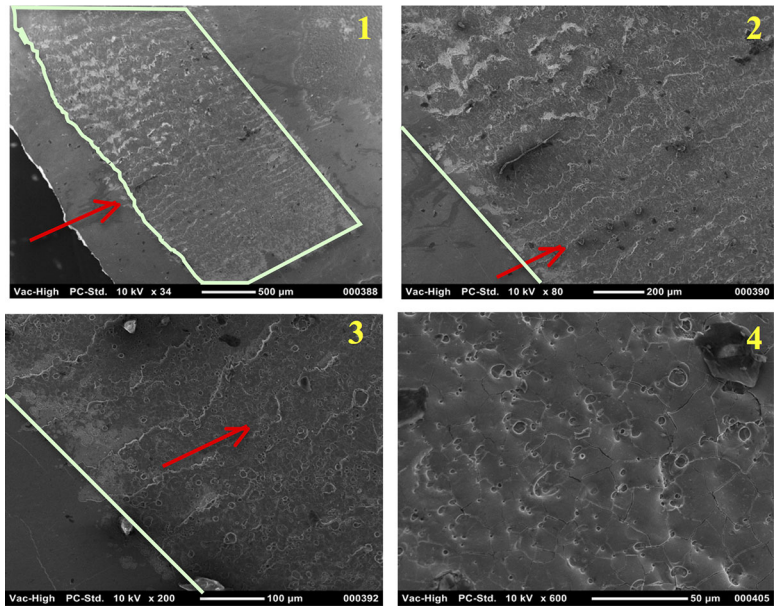

Fig. 5. Enamel surface after irradiation with 5 µs pulse duration; the SEM reveals melting and some minor surface roughness with no surface loss (arrows point at area shown at the next higher magnification, lines demarcate between irradiated and non irradiated surface).

to the much less soluble hydroxyapatite [53,55]. The SEM investigation in the present *in vitro* study revealed that the lowest applied pulse duration did not cause very obvious surface modifications besides some small areas exhibiting slight melting. Nevertheless the cross-sectional microhardness testing after pH-cycling revealed that for caries resistance enhancement enamel melting is not necessary as reported above.

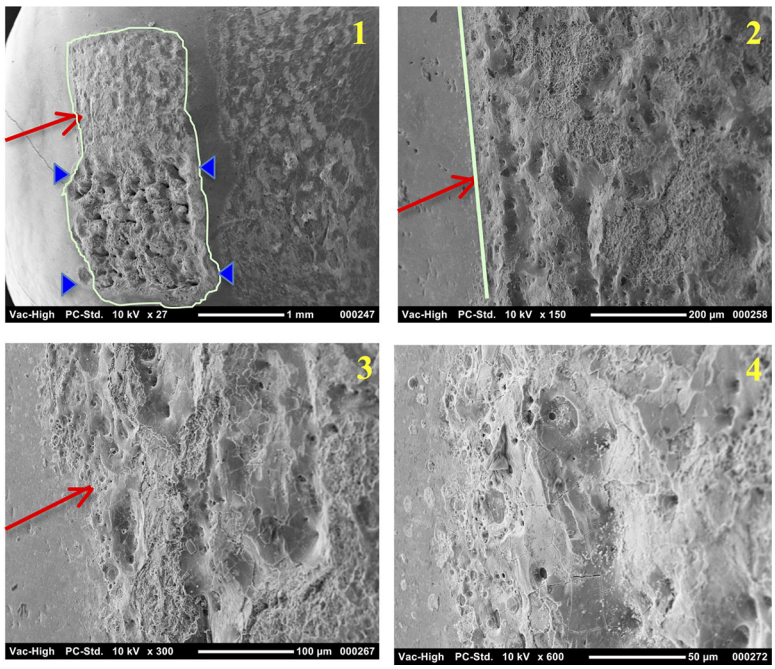

Fig. 6. Enamel surface after irradiation with 6 µs and 7 µs pulse duration; the SEM demonstrates at 6 µs pulses rough surface morphology with slight ablation of the enamel; 7 µs pulses (in 1, between triangles) perform obvious ablation of the enamel (arrows point at area showed at the next higher magnification, lines demarcate between irradiated and non irradiated surface).

Energies, which caused enamel melting but no obvious ablation have shown significant enhancement of caries resistance despite slight roughening of the enamel surface due to the melting. Same energies applied in clinical studies, where surface melting was desired, were successful at enhancing caries resistance in the oral environment in the presence of bacteria from microbial plaque. In a fissure caries prevention study up to 1 year after irradiation with a short-pulsed CO_2 laser with energies causing surface melting, significant caries preventive effects were observed [36].

Furthermore, when 9.3 μm CO_2-laser short-pulsed laser energies are applied, which cause obvious ablation of enamel and are used for cutting teeth, caries resistance of the remaining enamel has been enhanced as shown in this *in vitro* study, leading to a 65% reduction in mineral loss in comparison to the non-irradiated surface. This effect will be advantageous when cavities are drilled with a 9.3 μm CO_2 short-pulsed laser and a restoration is placed. The margins of the restoration should be better protected against recurrent caries and thus a failure of the restoration should be less likely.

A limitation of this study is that only one beam diameter and one pulse repetition rate were tested in a model, which represents approximately 3–6 months natural caries progression conditions. Future clinical trials that explore the caries preventive capabilities of the 9.3 μm CO_2 short-pulsed laser irradiation need to be conducted to confirm the present results *in vivo*.

CONCLUSION

The CO_2 9.3 μm short-pulsed laser energy renders enamel caries resistant with and without additional fluoride use. Enhanced resistance of dental enamel to simulated caries-like acid attack occurred using not only laser irradiation parameters that caused no obvious melting of the enamel surface but also after irradiation with energies that resulted in cutting of the enamel.

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