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ORIGINAL ARTICLE

Influence of irradiation by a novel CO_2 9.3-μm short-pulsed laser on sealant bond strength

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Abstract The objective of this in vitro study was to evaluate whether irradiation of enamel with a novel CO_2 9.3-μm short-pulsed laser using energies that enhance caries resistance influences the shear bond strength of composite resin sealants to the irradiated enamel. Seventy bovine and 240 human enamel samples were irradiated with a 9.3-μm carbon dioxide laser (Solea, Convergent Dental, Inc., Natick, MA) with four different laser energies known to enhance caries resistance or ablate enamel (pulse duration from 3 μs at 1.6 mJ/pulse to 43 μs at 14.9 mJ/pulse with fluences between 3.3 and 30.4 J/cm^2, pulse repetition rate between 4.1 and 41.3 Hz, beam diameter of 0.25 mm and 1-mm spiral pattern, and focus distance of 4–15 mm). Irradiation was performed "freehand" or using a computerized, motor-driven stage. Enamel etching was achieved with 37% phosphoric acid (Scotchbond Universal etchant, 3M ESPE, St. Paul, MN). As bonding agent, Adper Single Bond Plus was used followed by placing Z250 Filtek Supreme flowable composite resin (both 3M ESPE). After 24 h water storage, a single-plane shear bond test was performed (UltraTester, Ultradent Products, Inc., South Jordan, UT). All laser-irradiated samples showed equal or higher bond strength than non-laser-treated controls. The highest shear bond strength values were observed with the 3-μs pulse duration/0.25-mm laser pattern (mean $\pm$ SD = 31.90 $\pm$ 2.50 MPa), representing a significant 27.4% bond strength increase over the controls (25.04 $\pm$ 2.80 MPa, $P \leq 0.0001$). Two other caries-preventive irradiation (3 μs/1 mm and 7 μs/0.25 mm) and one ablative pattern (23 μs/0.25 mm) achieved significantly increased bond strength compared to the controls. Bovine enamel also showed in all test groups increased shear bond strength over the controls. Computerized motor-driven stage irradiation did not show superior bond strength values over the clinically more relevant freehand irradiation. Enamel that is made caries-resistant with CO_2 9.3-μm short-pulsed laser irradiation showed at least equal or significantly higher shear bond strength to pit and fissure sealants than non-laser-irradiated enamel. The risk of a sealant failure due to CO_2 9.3-μm short-pulsed laser irradiation appears reduced. If additional laser ablation is required before placing a sealant, the CO_2 9.3-μm enamel laser-cut showed equivalent or superior bond strength to a flowable sealant.

Keywords CO_2 9.3-μm laser · Microsecond short-pulsed · Human enamel · Bovine enamel · Laboratory study · Shear bond strength · Scanning electron microscopy

Introduction

Multiple laboratory studies [1–3] have shown enhancement of enamel caries resistance using specific short-pulsed carbon dioxide laser irradiation. CO_2 laser wavelengths 9.3 and 9.6 μm are very strongly absorbed by dental enamel. At these specific wavelengths, the absorption coefficient of enamel is ten times higher in comparison to the absorption coefficient at the 10.6-μm emission wavelength of typical current surgical CO_2 lasers [4]. Furthermore, applying microsecond in contrast to millisecond pulses results in lower energy depositions that help to avoid harm to the pulpal tissue [5].

In a single-blind, in vivo prospective clinical trial employing an orthodontic bracket model [6], Rechmann and

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co-workers showed that caries resistance was improved using microsecond pulsed CO_2 9.6-μm laser irradiation. CO_2 laser irradiation was found to significantly inhibit the formation of carious lesions around orthodontic brackets placed on bicuspids scheduled for extraction due to orthodontic reasons. This effect was quantified by cross-sectional microhardness testing on the extracted laser-treated and control bicuspids [7].

In a single-blind, controlled, randomized prospective clinical trial in 2013, Rechmann and co-workers irradiated molar fissures with a 9.6-μm CO_2 laser emitting 20-μs pulses. This in vivo pilot study revealed that the laser irradiation in combination with fluoride varnish applications significantly reduced the amount of newly formed carious lesions in molar fissures compared to a non-irradiated control tooth in the same arch over 1 year [8]. The study also found that CO_2 short-pulsed laser irradiation resulted in remineralization of the irradiated enamel.

Recently, a 9.3-μm microsecond short-pulsed CO_2 laser was introduced to dental clinicians. In a laboratory study, the CO_2 9.3-μm short-pulsed laser produced enamel caries resistance with and without additional fluoride applications. The enhanced acid resistance was observed using laser irradiation parameters that did not result in obvious melting of the enamel surface. In addition, reduced acid dissolution of the remaining enamel occurred after irradiation with energies sufficient for the cutting of enamel [9].

The objective of this present in vitro study was to evaluate whether irradiation of enamel with a novel CO_2 9.3-μm short-pulsed laser with energies enhancing caries resistance influences the shear bond strength of sealants to enamel acting as additional preventive measures. Addition goals were to assess bond strength to enamel, which was cut/ablated with this laser, to evaluate potential differences in bonding to bovine and human enamel, to research potential influence to bonding between "freehand" and computerized motor-driven stage irradiation, and to explore the topography of laser-irradiated enamel after acid etching as a step for bonding.

Materials and methods

Bovine and human enamel samples were irradiated with four different laser energies using two different irradiation patterns. The irradiation was performed "freehand" or the samples were mounted on a computerized motor-driven stage for the irradiation. Composite resin bonding was accomplished using an etch-and-rinse (total etch) bonding system followed by shear bond strength testing after 24 h of storage.

Test samples

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

The tooth roots were removed at the cemento-enamel junction. The human dental crowns were cut into mesial and distal halves so that the enamel of the proximal surfaces could be used for the shear bond strength testing. The proximal surface of each half of the molar crown provided sufficient enamel surface for the bonding of only one sample. The facial surfaces of the bovine incisors were cut into 4 × 4-mm blocks. Acrylic resin (Blue Clear Acrylic, Great Lakes Orthodontics, Tonawanda, NY) was used to create cylindrical blocks with the enamel sample on one side. The sample side was ground to expose the enamel, which was polished with 600 silicon carbide paper. Grinding and polishing continued until an approximately 3-mm circle of human enamel samples and 4 × 4-mm bovine block surfaces, respectively, were exposed. After polishing, the samples were cleaned in an ultrasonic bath for 5 min to remove surface impurities and stored in 0.1% thymol solution at room temperature until bonding. A total of 70 bovine enamel and 240 human enamel samples were used for bonding and shear bond strength testing. For experiments with bovine samples, ten samples were used per group. For the testing with human enamel, each group included 15 samples.

Laser settings and irradiation mode

The laser utilized in this study was a CO_2 laser (Solea, Convergent Dental, Inc., Natick, MA) emitting a wavelength of 9.3 μm. Four different laser pulse durations (3, 7, 23, and 43 μs) were used, which delivered pulse energies between 1.6 mJ/pulse and up to 14.9 mJ/pulse, resulting in fluences between 3.3 and 30.4 J/cm^2 at pulse repetition rates between 4.1 and 41.3 Hz (at the single beam level). 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 15 samples were irradiated.

In the non-contact mode, the beam diameter was set to 0.25 mm with a laser focus length of 4–15 mm. The laser pulse shape was square with an initial sharp peak. The beam profile was Gaussian. The beam profile was measured with an Ophir-Spiricon Pyrocam III pyroelectric camera with BeamGage V5.11 software.

The handpiece of the laser harbors computer-controlled galvos, allowing for different irradiation patterns resulting in different diameters of automatically irradiated surface areas. If the galvos are not engaged, the original beam diameter is 0.25 mm, as mentioned above. In this study, we used two beam diameters, the 0.25-mm and a spiral beam pattern, resulting in a 1-mm irradiated surface area.

Table 1 gives an overview of the different laser settings used in this study showing pulse durations, the laser "speed" as set on the Graphic User Interface (GUI), the laser repetition rate, the beam pattern, and the power and fluence at a single irradiation spot of 0.25 mm. Laser settings with irradiation conditions shown to be successful for enhancing caries resistance [7, 9] and settings allowing enamel ablation were used. For caries prevention, each spot was irradiated with at least 20 laser pulses, and each sample was irradiated for 30–60 s with overlapping laser irradiation. No air or air-water mist was applied. These treatments resulted in no melting (3-μs pulse duration) and slightly molten surfaces (7-μs pulse duration), respectively.

In contrast, irradiation with 23- and 43-μs pulse duration, respectively, while employing 100% air-water mist, resulted in ablation of enamel. Irradiation times were short to avoid major substance loss with deepening of the surface (at 23-μs pulse duration, the irradiation time was 15 s; at 43-μs pulse duration, the irradiation time was 10 s).

For the bovine and one set of human enamel samples, the laser beam was moved in a sweeping fashion by hand over the irradiation area with a working distance of roughly 10–15 mm. One additional human sample set was mounted on a computerized motor-driven stage with a two-axis (*X*–*Y*) linear stage motor (Thor Labs Inc., Newton, NJ). Kinesis software (Thor Labs Inc.) controlled the computerized motor with 0.05-μm precision steps. The irradiation occurred at the 15-mm working distance from the end of the laser handpiece to the enamel. A laser spot overlap (one third or two thirds overlap) was chosen in order to achieve a homogeneous irradiation of the enamel surface. Homogeneous irradiation of the samples was confirmed with a stereomicroscope (Fisher Scientific Stereomaster, Fisher Scientific LLC, Pittsburgh, PA).

Adhesive composite system

Control samples and the laser-irradiated samples were both treated with 37% phosphoric acid etching (Scotchbond Universal Etchant, 3M ESPE) before bonding. The bonding agent Adper Single Bond Plus (3M ESPE) was used, followed by the placement of Z250 Filtek Supreme flowable composite (3M ESPE). Etching and bonding procedures were performed according to the manufacturer's instructions. The enamel surface was dried, the etching liquid was applied with a microbrush, and left in place for 15 s, while stirred to increase its fluidity. For bonding after drying the enamel sample, one drop of adhesive was applied onto the enamel surface and for 15 s agitated with a fully saturated micro-tip applicator. Then, the bonding agent was gently air-thinned over the enamel surface before light curing.

Additional 15 human controls and two laser test sets with 15 samples each were treated with the lowest and the highest laser energy applied in this study (43 and 3-μs pulse durations), respectively, and were consecutively bonded without using phosphoric acid etching.

After the bonding material was applied and cured, the samples were placed into a bonding clamp under a bonding mold insert (Ultradent Products, Inc., South Jordan, UT). The bonding mold has a 2.38-mm wide × 3-mm high hollow tube to bond a 2.38-mm wide composite cylinder on top of the sample surface. After pushing a single increment of composite down through the hollow tube onto the sample surface, the composite was light-cured for 40 s according to the manufacturer's instructions. The bonding agents and the composites were light-cured with a Satelec® Mini LED curing light (Acteon North America, Mount Laurel, NJ) according to the manufacturer-recommended time. The light output of the curing light was verified with a curing radiometer; the Acteon Satelec Mini LED curing light gave consistently an output of >1250 mW cm^{-2} throughout the study. The samples were removed from the mold and stored in clear water at room temperature for 24 h to allow curing of uncured composite.

Shear bond strength testing

After 24 h, the adhesive bonding strength of the 3M Z250 composite to the enamel surface was determined by

Table 1 Laser parameters, energy settings, and intended clinical effect on enamel

Enamel / intended clinical effect	Pulse duration (μs)	"Speed" on GUI (%)	Repetition rate (Hz)	Beam pattern (mm)	Irradiation time (s)	Power (mW)	Fluence/pulse (J/cm^2)
Ablation/cutting	43	100	12.5	1 (spiral)	10	2,604	30.4
Ablation/cutting	23	100	12.5	1 (spiral)	15	1,461	17.0
Melting/caries prevention	7	30	41.3	0.25	60	122	6.0
Melting/caries prevention	7	10	4.1	1 (spiral)	30	171	6.1
No/slight melting/caries prevention	3	30	41.3	0.25	60	73	3.6
No/slight melting/caries prevention	3	30	5.4	1 (spiral)	60	123	3.3

performing a single-plane shear bond test with the UltraTester (Ultradent Products, Inc.) testing device.

The shear bond strength testing machine was calibrated according to manufacturer's instructions. Each acrylic cylinder was secured on a test base clamp. The composite cylinder was placed under the 2.38-mm notched crosshead assembly. Once the test was started, the stage with the bonded sample moved upwards towards the crosshead assembly with a load shell of 1000 lb (453.6 kg) at a steady rate of 1 mm/min (Fig. 1). The display showed the increasing stress in megapascals until the composite cylinder sheared off. At this time, the display showed and recorded the peak shear bond strength in megapascals. Debonded enamel samples and composite stubs were stored in 0.1% thymol solution.

Statistical methods

Means and standard deviations for each group were calculated and the groups were compared statistically by one-way ANOVA, followed by Bonferroni's multiple comparison test for significance at $P < 0.05$ (Prism, GraphPad Software Inc., La Jolla, CA).

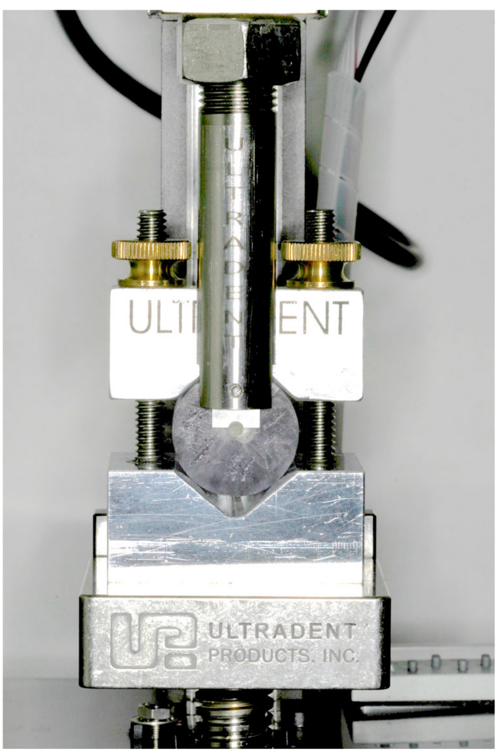

Fig. 1 Shear bond strength testing machine with the acrylic cylinder secured on a test base clamp, test sample placed under the 2.38-mm notched crosshead assembly

Stereomicroscope observations and scanning electron microscopy

A stereomicroscope (Fisher Scientific Stereomaster, Fisher Scientific LLC) was used to observe the debonding failure pattern (adhesive, cohesive—including counts of structural failure in enamel and/or composite; mixed—adhesive and cohesive failure at the same debonding surface) at ×10 magnification.

A maximum of three additional tooth enamel samples were irradiated with each of the four different pulse durations involving the 1-mm diameter irradiation patterns as mentioned in "Laser settings and irradiation mode" for scanning electron microscopy (SEM). Irradiated and non-irradiated surfaces were observed with and without being acid-etched. 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

Table 2 presents the shear bond strength values to bovine and human enamel, respectively, for controls and CO_2 9.3-μm irradiated surfaces with and without phosphoric acid etching using Adper Single Bond Plus as bonding agent and Filtek Supreme as flowable sealant composite.

Bovine enamel shear bond strength

Table 2 shows the shear bond strength results for bovine samples representing the values for the controls and for the samples after laser freehand irradiation. Displayed are the average values for controls and the different treatment groups for the 0.25- and 1-mm beam patterns, respectively.

The laser treatment resulted in increased shear bond strength values for all test groups compared to the non-irradiated controls. The highest bond strength was observed with the 3-μs/1-mm pattern laser treatment (mean ± SD = 29.30 ± 4.44 MPa), which presents a 14% increase over the control group (mean ± SD = 25.68 ± 1.98 MPa). Nevertheless, the differences noted in the shear bond strength values between the control and test groups for the bovine samples were not statistically significant.

Human enamel shear bond strength—"freehand" irradiation

All shear bond strength values after laser treatment with "freehand" irradiation are shown in Table 2. Similar to bovine enamel, human enamel samples in all the test groups revealed

Table 2 Shear bond strength testing results for Adper Single Bond Plus and Filtek Supreme for bovine and human enamel, freehand and computerized motor-driven stage irradiation, with and without acid etching (controls and laser parameters)

Bonding agent/laser conditions/enamel		Control	Control no etch	3 µs/0.25 mm	3 µs/1 mm	7 µs/0.25 mm	7 µs/1 mm	23 µs/1 mm	43 µs/1 mm
Total Etch Adper Single Bond Plus	Bovine enamel (hand)	25.68 ± 1.98	–	29.17 ± 3.24	29.30 ± 4.44	29.05 ± 3.00	26.06 ± 6.15	28.09 ± 3.59	28.02 ± 3.04
	Human enamel (hand)	25.04 ± 2.80	–	$\mathbf{31.90 \pm 2.50}^{***}$	$\mathbf{30.45 \pm 3.42}^{**}$	$\mathbf{29.15 \pm 4.57}^{*}$	26.78 ± 3.38	$\mathbf{28.71 \pm 3.77}^{*}$	25.14 ± 2.52
	Human enamel (motor stage)	25.04 ± 2.80	–	$\mathbf{30.09 \pm 2.74}^{**}$	$\mathbf{28.94 \pm 2.98}^{*}$	27.86 ± 2.30	27.06 ± 4.19	27.02 ± 3.25	25.52 ± 2.43
No etch Adper SBP	Human enamel (hand)	–	3.77 ± 1.76	5.13 ± 1.20	–	–	–	–	5.85 ± 1.30

Results in megapascals (mean ± standard deviation). Statistically significant differences to the control are shown in bold

$^{*}P \leq 0.05$; $^{**}P \leq 0.001$; $^{***}P \leq 0.0001$

increased shear bond strength values in comparison to the control group. Asterisks indicate the significance level.

The highest shear bond strength values were observed in the test group with 3-µs/0.25-mm laser irradiation treatment (mean ± SD = 31.90 ± 2.50 MPa). This is a 27.4% increase in bond strength in comparison to the control group (25.04 ± 2.80 MPa) at a high significance level of $P \leq 0.0001$.

Statistically significant increased enamel shear bond strength values were also observed for the test group using also the 3-µs pulse with the 1-mm laser irradiation pattern (30.45 ± 3.42 MPa, $P \leq 0.001$). The test group using a laser setting of 7 µs/0.25 mm and the group with the 23-µs/1-mm pattern both reached significantly higher bond strength values in comparison to the control group at the $P \leq 0.05$ significance level.

When bonding was attempted to non-acid-etched surfaces, the bond strength values were significantly lower. For the controls without laser application and without prior acid etching, the shear bond strength reached around 3.77 ± 2.80 MPa, and 12 out of 15 samples debonded before actual bond strength values could be determined. For the laser-treated but not acid-etched test groups, the bond strength was slightly higher, with 5.13 ± 1.20 for the 3-µs/0.25-mm and 5.85 ± 1.30 for the 43-µs/1.0-mm pattern, respectively. Nevertheless, without additional acid etching, the shear bond strength values were roughly 80% lower than the laser plus acid-etched values.

Human enamel shear bond strength—computerized motor-driven stage irradiation

The shear bond strength values after laser treatment using a computerized motor-driven stage for moving the samples during irradiation are also listed in Table 2. Similar to the freehand irradiated samples, all test groups showed increased shear bond strength values in comparison to the control group. The highest bond strength values were observed again in the test group with the laser setting at 3-µs/0.25-mm (mean ± SD = 30.09 ± 2.74 MPa, $P \leq 0.001$). Using this laser setting, the shear bond strength increased by 20.2% over the controls. The same pulse energy but with a 1-mm irradiation pattern also resulted in significantly higher shear bond strength (28.94 ± 2.98 MPa, $P \leq 0.05$) in comparison to the control.

Stereomicroscope and scanning electron microscopical observations

The failure mode for debonding of Adper Single Bond Plus and Filtek Supreme sealant after freehand and computerized motor-driven stage irradiation, respectively, from human and bovine enamel is shown in Fig. 2. Adhesive, cohesive, and mixed failures are presented. The results are divided into controls, and the test samples with 3- and 7-µs laser irradiation

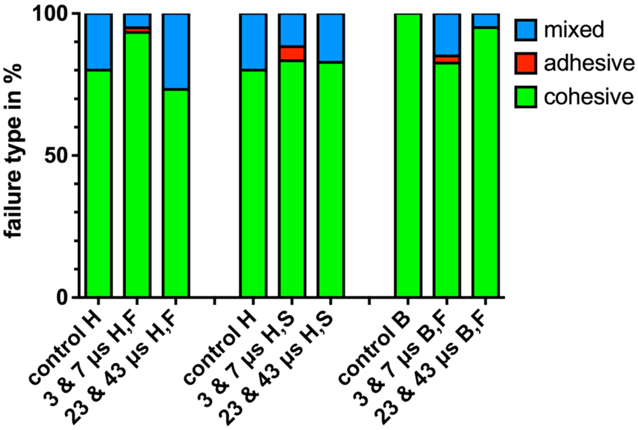

Fig. 2 Bonding failure mode for human (*H*) and bovine (*B*) enamel for Adper Single Bond Plus and Filtek Supreme—freehand irradiation (*F*) and computerized motor-driven stage (*S*) irradiation. Adhesive, cohesive, and mixed failure in percent. Results separated in control, 3- and 7-μs laser irradiation, and 23 and 43 μs combined, respectively

combined as non-ablative caries-preventive irradiation, and 23 and 43 μs combined as ablative laser energies, respectively. For human enamel samples, for both freehand and computerized motor-driven stage irradiation, using the caries-preventive settings caused slightly increased cohesive failures compared to the controls. The cohesive failures occurred in the composite. The application of ablative energies resulted in a slight increase of mixed failures for hand irradiation and a decrease of mixed failures for the motor-driven stage use. For all mixed failures, the surface area of the adhesive failure part was very small (up to 5% of the total surface area). Very similar patterns were observed for debonding failures of the bovine enamel samples. Higher energy applications increased the number of cohesive failure modes, while after lower energy irradiations, slightly more mixed or adhesive failures were observed. Again, cohesive failures occurred in the composite.

Figures 3, 4, 5, and 6 represent the SEM observations of enamel surfaces after laser irradiation with subablative and ablative pulse energies, respectively, with and without additional acid etching. For the 3-μs pulse duration, the controls showed minor changes, revealing at high magnifications a few slightly molten areas. Applying acid etching to such irradiated surfaces resulted in a very homogeneous enamel etching pattern without any obvious signs of remaining melting.

The irradiation with 7-μs pulse durations revealed relatively homogeneous melting and some minor surface roughness with no surface loss. After phosphoric acid etching these surfaces, a relatively homogeneous pattern was observed, still demonstrating obviously confluent molten areas with additional openings enhancing the surface area. Figure 5 represents the situation after 23-μs pulse duration ablative laser energies were applied. The controls reveal homogeneous melting with minor roughness, and acid etching resulted again in a relatively homogeneous pattern, still demonstrating confluent molten areas with additional surface-enhancing openings. The same observations were made after applying 43-μs pulse duration ablative laser energies (Fig. 6).

Discussion

Bond strength to human enamel irradiated with caries resistance-enhancing laser energies

The use of CO_2 microsecond short-pulsed lasers at 9.6- or 9.3-μm wavelength [1–3], respectively, has been reported to enhance caries resistance in laboratory studies as well as in several clinical studies [7, 8]. As an additional caries-preventive step, pit and fissure sealants [10] might successfully be placed on those irradiated fissures. Concerns were related to possible bond strength issues between the irradiated enamel and the fissure sealant.

This present study showed that the shear bond strength to human enamel was consistently higher for the laser-irradiated enamel surfaces in comparison to the non-irradiated enamel serving as the control. Irradiation energy levels that rendered enamel more acid-resistant but only slightly or did not modify the surface resulted in bond strength values that were up to 27.4% higher than the control values (31.90 ± 2.50 MPa for the 3-μs/0.25-mm pattern versus 25.04 ± 2.80 MPa for the control). Both beam size patterns used in this study achieved significantly higher bond strength with laser energy delivered by the 3-μs irradiation pulse width. In addition, the bond strength gain was also significant after applying the 7-μs pulse width using the 0.25-mm beam diameter.

Bond strength to human enamel irradiated with laser energies to cut enamel—freehand and computerized motor-driven stage irradiation

Prior to the placement of pit and fissure sealants, the removal of enamel in some areas of the fissure due to a small existing cavitated carious lesion or a fissure enameloplasty may be necessary. In this study, when higher laser energies were utilized sufficient for enamel cutting, the attainable bond strength to the remaining enamel was at least at the level of the controls. Furthermore, at laser energies just achieving ablation (23-μs pulse duration), shear bond strength values were even significantly higher than those of the controls.

One study by Nguyen et al. in 2011 had shown slightly but not statistically significant lower bond strength values to 9.3-μm irradiated enamel followed by acid etching compared to acid-etched controls [11]. The authors used 3M ESPE single bond and 3M ESPE Z250 composite. They reported bond strength values of 37 MPa (SD = 3.6) for the human enamel

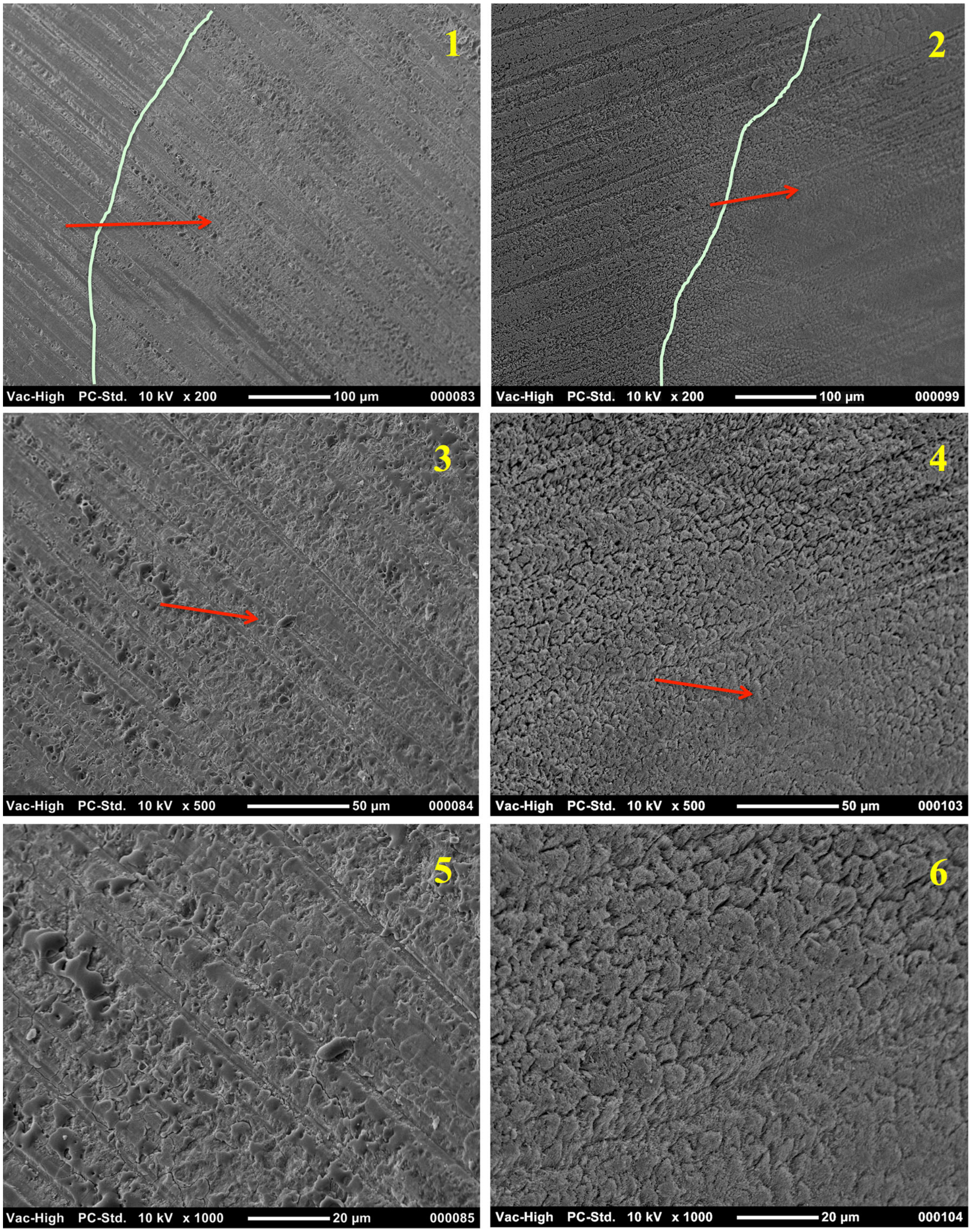

Fig. 3 Human enamel surface after irradiation with 3-µs pulse duration. *Left column*, controls with no acid etching; *right column*, after acid etching. SEM of the controls showed no or only minor changes. At higher magnifications, a few slight molten areas became visible. Acid etching resulted in a very homogenous typical enamel etched pattern (*red arrows* point at area shown at the next higher magnification; *green lines* demarcate between irradiated and non-irradiated surfaces)

control (with acid etching) and 31.2 MPa (SD = 2.5) for laser-treated enamel (fluence = 20 J/cm^2) followed by acid etching. In their study, the use of a fast-scanning computerized motor-driven stage to move the sample under the irradiation beam, resulting in well-controlled laser overlaps, might have presented a limitation of the study. Those uniform irradiation conditions may not be clinically relevant. In contrast, performing "freehand irradiation" may result in bond strength values

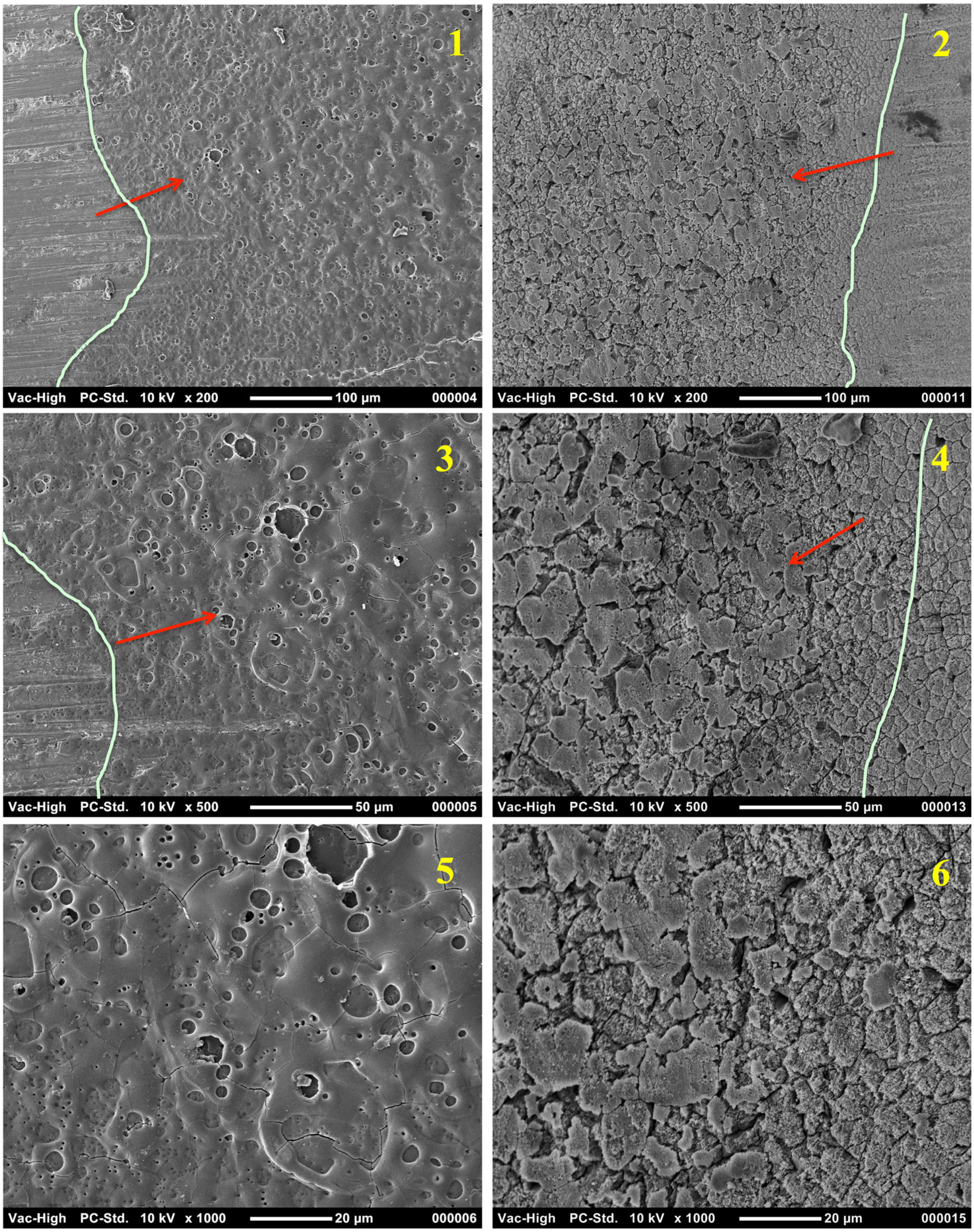

Fig. 4 Human enamel surface after irradiation with 7-µs pulse duration. *Left column*, controls with no acid etching; *right column*, after acid etching. SEM of the controls showed relatively homogenous melting and some minor surface roughness with no surface loss. Acid etching results in a relatively homogenous pattern still demonstrating obviously confluent molten areas with additional surface-enhancing openings (*red arrows* point at area shown at the next higher magnification; *green lines* demarcate between irradiated and non-irradiated surfaces)

which are a better reflection of actual clinical treatment conditions. In this present study, it was shown that there was no important difference between the shear bond strength results using a computerized motor-driven stage scenario or "freehand" irradiation.

An earlier goal in using lasers in dentistry was to replace the acid etching with laser application for bonding of composites. Studies using conventional 10.6-µm CO_2 lasers emitting energy in the milliseconds or seconds range alone achieved typically lower bond strength values on enamel than

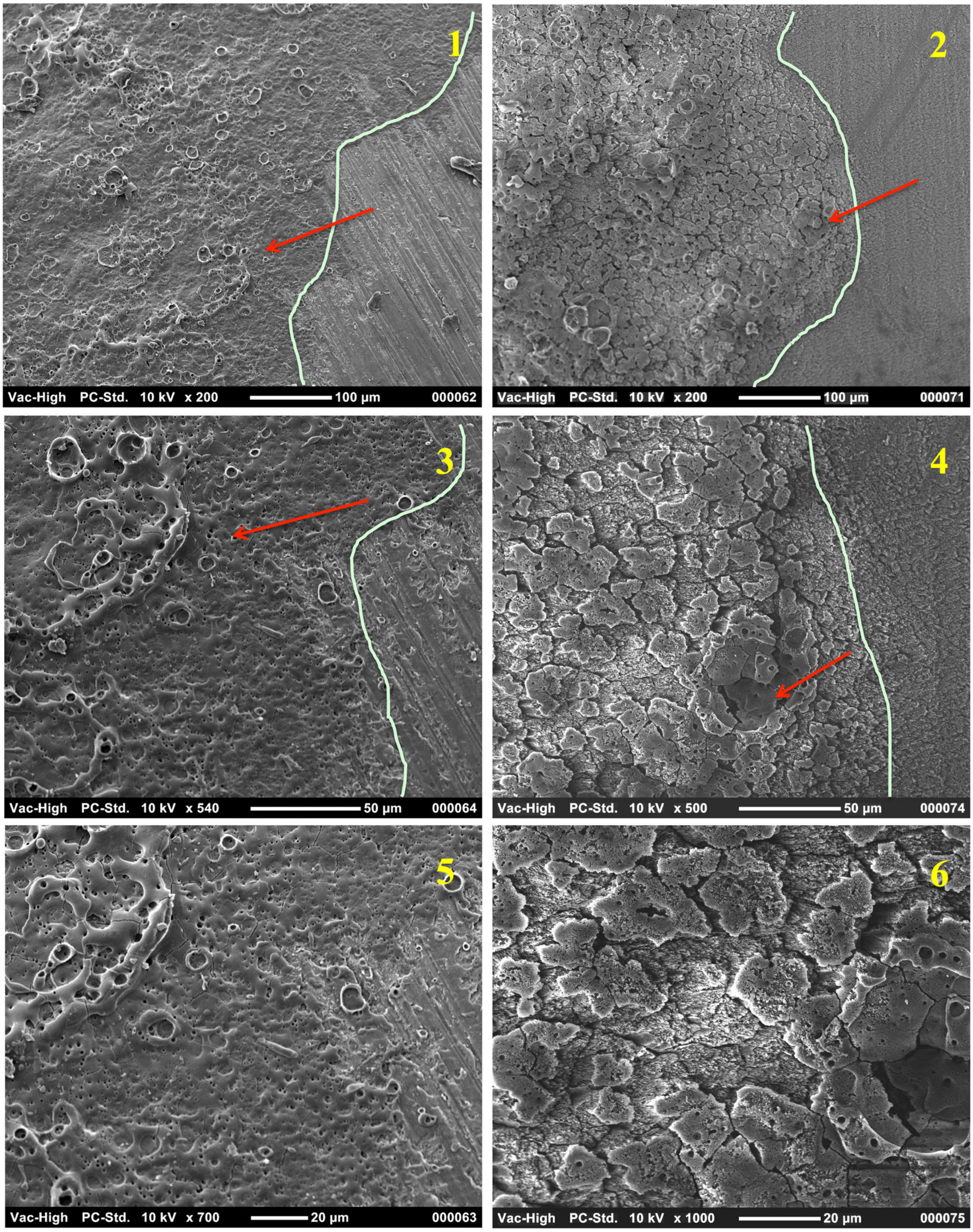

Fig. 5 Enamel surface after irradiation with 23-μs pulse duration. *Left column*, controls with no acid etching; *right column*, after acid etching. SEM of the controls showed homogenous melting with minor roughness. Acid etching results in a relatively homogenous pattern still demonstrating confluent molten areas with additional surface-enhancing openings (*red arrows* point at area shown at the next higher magnification; *green lines* demarcate between irradiated and non-irradiated surfaces)

phosphoric acid application [12–14]. On the contrary, Walsh et al. in 1994 reported superior bonding to enamel over acid etching by creating laser irradiation surface pitting patterns (10.6 μm, 14 W, 10 ms, 10 Hz, 14-s irradiation time, 24 J/cm^2) showing cohesive bonding failures in the composite [15]. Walsh in 1994 also claimed in a split-mouth study that the clinical success rate for laser etching was comparable to that for acid etching, particularly for fissure sealing [16].

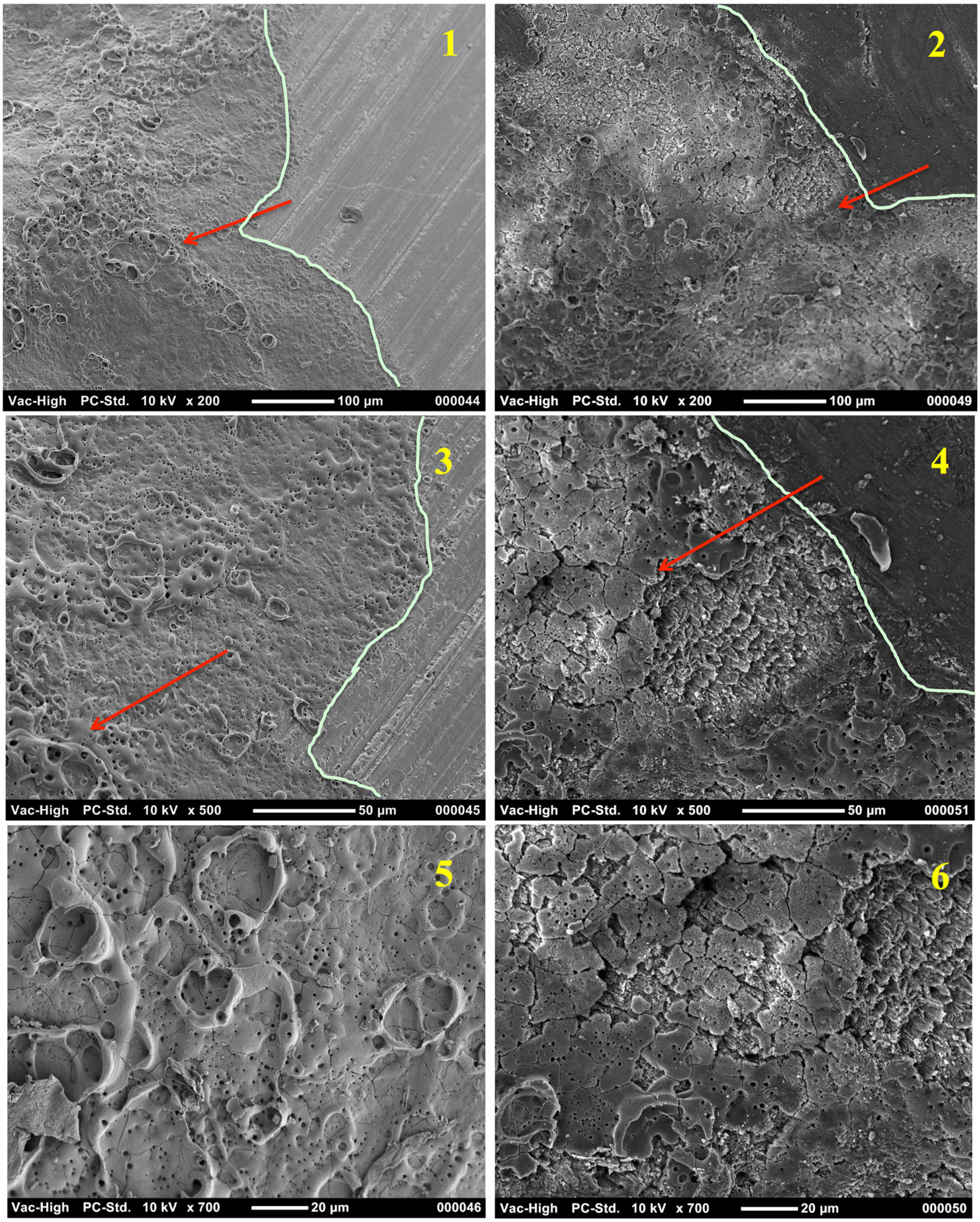

Fig. 6 Enamel surface after irradiation with 43-µs pulse duration. *Left column*, controls with no acid etching; *right column*, after acid etching. SEM of the controls showed homogenous melting with some roughness. Acid etching results in a relatively homogenous pattern with confluent molten areas with additional surface-enhancing openings (*red arrows* point at area shown at the next higher magnification; *green lines* demarcate between irradiated and non-irradiated surfaces)

Nevertheless, irradiation with those conventional 10.6-µm CO_2 lasers emitting millisecond pulses may lead to temperature increase with potential harm to the pulp.

Staninec et al. in 2006, using for the first time a short-pulsed 9.6-µm CO_2 laser (6- to 8-µs pulse duration, 10 J/cm^2), achieved without applying a water spray and without using acid etching roughly 50% of the shear bond strength value of using phosphoric acid alone. They created a very specific laser irradiation pattern on the enamel samples [17]. Nguyen et al. in 2011, applying a 9.3-µm CO_2 laser (10- to

15-μs pulse width, 13 and 42 J/cm^2), again without using acid etching, achieved only 19–51% of the bond strength compared to laser plus acid etching, depending on the irradiation overlap pattern [11]. In our study, when applying the lowest laser energy sufficient to render enamel caries-resistant (3-μs pulse duration), the bond strength without acid etching reached 21%, and using cutting energy (43 μs) resulted in 23% of bond strength for the acid-etched controls. In contrast to the other reports, these levels of bond strength were accomplished without using computer-controlled irradiation patterns, but only by clinically relevant "freehand" irradiation. Nevertheless, with certainty, additional etching of laser-irradiated enamel surfaces resulted in equivalent or even significantly higher shear bond strength values.

Bond strength to bovine enamel

Nakamachi et al. in 1983 reported that acid etching of bovine enamel causes the formation of a rougher surface, and the hydroxyapatite crystals are oval-shaped and narrow, in contrast to the round shape observed with human enamel. However, they found no significant difference in bond strength to human and bovine enamel [18]. A review of the literature done by Yassen et al. in 2011 concluded that inconsistent data existed whether bovine teeth can be considered an appropriate substitute for human teeth in dental research. Also, studies comparing bond strength to human and bovine enamel showed mixed results, with most citing no significant difference between them, while some cited lower bond strength to bovine enamel [19]. In 2015, Teruel et al. reported higher organic matter (bovine enamel 10.90% vs. human 5.70%), similar carbonate content, and lower calcium/phosphate (in mole/mole) ratio in bovine enamel than human enamel. Bovine enamel is described as least mineralized (1.57 Ca/P ratio), followed by human enamel (1.61) and pure hydroxyapatite (1.67) as the most mineralized. Bovine enamel appears to be the closest substitute to human enamel [20].

In this present study, all applied energy settings showed that bovine enamel acted very similarly to human enamel sample, with slightly or even up to 14% higher bond strength values for the laser-treated surfaces. Nevertheless, one-way ANOVA followed by Bonferroni's multiple comparison test did not consider the observed difference as statistically significant.

SEM and failure mode observations

The loss of the carbonate from the enamel crystals due to the irradiation heat is responsible for the reduction in acid dissolution of enamel [21, 22], transforming carbonated hydroxyapatite into the more acid-resistant hydroxyapatite. This transformation is already achieved using 9.3-μm CO_2 laser irradiation at 3-μs pulse duration [9]. SEM showed no or only minor melting of the enamel at this energy level, while acid etching resulted in very homogeneous enamel etching pattern, similar/identical to the non-irradiated enamel. Obviously, the unmelted or slightly molten and then acid-etched surfaces showed higher bond strength, but potential responsible changes were not visible with the applied SEM magnification. It may be speculated that due to the laser irradiation, the protein component of enamel might be reduced, and subsequent etching might allow deeper or wider etching patterns, allowing for deeper resin tag formation, for instance.

Applying higher laser energies led to more melting and additional surface roughness. As expected, this roughness was not sufficient to create adequate bond strength to a composite without acid etching. Following acid etching, the bond strength to a sealant was significantly higher depending on the applied laser energy. After applying those higher laser energies and subsequently performing acid etching, the homogeneous molten surfaces were broken up, but still larger zones of molten enamel remained visible. Additional openings between the molten centers and additional pores became visible. The surfaces appeared covered with the very homogeneous enamel etching pattern, similar to the situation observed after applying the lowest laser energy.

With regard to the observed failure patterns for the irradiation with caries-preventive laser energies, the percentage of the desirable cohesive failure mode slightly increased compared to the controls. The described surface modifications appear to be favorable for this kind of failure pattern, with cohesive failures occurring in the composite. For the higher energies, with similar or even higher bond strength values, the failure mode observation showed increased cohesive failures, especially for the bovine enamel samples.

A limitation of this study is that, in this first shear bond strength study, after using a CO_2 9.3-μm short-pulsed laser irradiation on enamel, only one combination of bonding agent and sealant composite was tested. As comparison to the etch-and-rinse bonding agents, future studies may also include self-etch techniques. Clinical trials that explore the behavior of sealants placed on pits and fissures rendered more caries-resistant with help of the 9.3-μm CO_2 short-pulsed laser irradiation need to be conducted to confirm the present results in vivo. In addition, studies have to show how higher energies for fast cutting of tooth structure for cavity preparation may influence the bond strength of composite fillings to enamel as well as dentin.

Conclusion

With respect to bond strength, enamel in fissure areas rendered caries-resistant by a CO_2 9.3-μm short-pulsed laser appear to offer higher bond strength values to pit and fissure sealants than non-laser-irradiated enamel. The risk of losing sealants from those CO_2 9.3-μm short-pulsed laser-irradiated enamel areas may be reduced, which may have a significant clinical impact.

Furthermore, even if additional laser drilling is required before placing a sealant, the CO_2 9.3-μm laser-cut enamel showed equivalent or even superior bond strength to the flowable sealant composite.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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References

1. Featherstone JD, Nelson DG (1987) Laser effects on dental hard tissues. Adv Dent Res 1(1):21–26
2. Kantorowitz Z, Featherstone JD, Fried D (1998) Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. J Am Dent Assoc 129(5):585–591
3. Featherstone JD, Barrett-Vespone NA, Fried D, Kantorowitz Z, Seka W (1998) CO_2 laser inhibitor of artificial caries-like lesion progression in dental enamel. J Dent Res 77(6):1397–1403
4. Fried D, Zuerlein MJ, Le CQ, Featherstone JD (2002) Thermal and chemical modification of dentin by 9–11-microm CO_2 laser pulses of 5–100-micros duration. Lasers Surg Med 31(4):275–282
5. Goodis HE, Fried D, Gansky S, Rechmann P, Featherstone JD (2004) Pulpal safety of 9.6 microm TEA CO_2 laser used for caries prevention. Lasers Surg Med 35(2):104–110. doi:10.1002/lsm.20043
6. Gorton J, Featherstone JD (2003) In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofac Orthop 123(1):10–14
7. Rechmann P, Fried D, Le CQ, Nelson G, Rapozo-Hilo M, Rechmann BM, Featherstone JD (2011) Caries inhibition in vital teeth using 9.6-μm CO_2-laser irradiation. J Biomed Opt 16(7): 071405. doi:10.1117/1.3564908
8. Rechmann P, Charland DA, Rechmann BM, Le CQ, Featherstone JD (2013) In-vivo occlusal caries prevention by pulsed CO_2-laser and fluoride varnish treatment—a clinical pilot study. Lasers Surg Med 45(5):302–310. doi:10.1002/lsm.22141
9. Rechmann P, Rechmann BM, Groves WH Jr, Le CQ, Rapozo-Hilo ML, Kinsel R, Featherstone JD (2016) Caries inhibition with a CO_2 9.3 μm laser: an in vitro study. Lasers Surg Med 48(5):546–554. doi:10.1002/lsm.22497
10. Beauchamp J, Caufield PW, Crall JJ, Donly K, Feigal R, Gooch B, Ismail A, Kohn W, Siegal M, Simonsen R (2008) Evidence-based clinical recommendations for the use of pit-and-fissure sealants: a report of the American Dental Association Council on Scientific Affairs. JADA 139(3):257–268
11. Nguyen D, Chang K, Hedayatollahnajafi S, Staninec M, Chan K, Lee R, Fried D (2011) High-speed scanning ablation of dental hard tissues with a lambda = 9.3 mum CO_2 laser: adhesion, mechanical strength, heat accumulation, and peripheral thermal damage. J Biomed Opt 16(7):071410. doi:10.1117/1.3603996
12. Anic I, Pavelic B, Vidovic D, Tudja M (1991) Composite retention area produced by CO_2 laser beam. Acta Stomatol Croat 25:157–160
13. Poulsen J, Dhuru V, Ferguson D, Kittleson R, Stenger J (1993) Orthodontic bond strength after etching enamel with CO_2 laser. J Dent Res 72:176
14. MacDonald R, Lobb W, Boran T, Nichols M (1994) The bonding failure characteristics of orthodontic brackets using laser etching. J Dent Res 73:198
15. Walsh LJ, Abood D, Brockhurst PJ (1994) Bonding of resin composite to carbon dioxide laser-modified human enamel. Dent Mater 10:162–166
16. Walsh LJ (1994) Clinical studies of carbon dioxide laser etching. J Clin Laser Med Surg 12(6):311–314. doi:10.1089/clm.1994.12.311
17. Staninec M, Gardner AK, Le CQ, Sarma AV, Fried D (2006) Adhesion of composite to enamel and dentin surfaces irradiated by IR laser pulses of 0.5–35 micros duration. J Biomed Mater Res B Appl Biomater 79(1):193–201. doi:10.1002/jbm.b.30530
18. Nakamichi I, Iwaku M, Fusayama T (1983) Bovine teeth as possible substitutes in the adhesion test. J Dent Res 62(10):1076–1081
19. Yassen GH, Platt JA, Hara AT (2011) Bovine teeth as substitute for human teeth in dental research: a review of literature. J Oral Sci 53(3):273–282
20. Teruel Jde D, Alcolea A, Hernandez A, Ruiz AJ (2015) Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Arch Oral Biol 60(5): 768–775. doi:10.1016/j.archoralbio.2015.01.014
21. Zuerlein MJ, Fried D, Featherstone JDB, Seka W (1999) Optical properties of dental enamel in the mid-IR determined by pulsed photothermal radiometry. Ieee J Sel Top Quant 5(4):1083–1089. doi:10.1109/2944.796333
22. Featherstone JD, Nelson DG (1989) Recent uses of electron microscopy in the study of physico-chemical processes affecting the reactivity of synthetic and biological apatites. Scanning Microsc 3(3): 815–827, discussion 827–818