Title
In Vitro Study of Enamel Bond Strength Following 9.3 µm CO2 LASER Treatment

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In Vitro Study of Enamel Bond Strength
Following 9.3 μm CO_2 LASER Treatment

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

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of the

[redacted]

DEDICATION AND ACKNOWLEDMENTS

I dedicate my work to my loving wife Avani, my parents Kantilal & Hansa Sherathiya and my family & friends whose wishes and endless support have been the foundation of this project.

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This research project would not have been possible without a community of support. It has been a true honor to work with such an amazing team. I am thankful to all the people who offered guidance, critique, encouragement and patience throughout this journey.

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CONFLICTS OF INTEREST DISCLOSURES

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ABSTRACT

In Vitro Study of Enamel Bond Strength Following 9.3 µm CO_2 LASER Treatment

By

Krunal Sherathiya, DDS

BACKGROUND:

Hard and soft tissue LASERs continue to gain popularity in dentistry. Most published literature on enamel and dentin bond strength after LASER use were carried out utilizing Er:YAG, ErCr:YSGG and 10.6µm CO_2 LASER, respectively. The LASER bond strength literature lacks studies using a 9.3 µm short-pulsed CO_2 LASER.

OBJECTIVES:

The objective of this study was to test, whether 9.3 µm short-pulsed CO_2 LASER irradiation has an influence on the bond strength of human enamel following different LASER treatments for pit and fissure sealants as well as for composite restoration placements in a laboratory study in comparison to non-LASER treated enamel by measuring the bond strength of a total-etch adhesive system, using shear-bond strength testing 24 hours after flowable composite placement.

METHODS:

The laboratory study was carried out in four stages. Stage 1 of the study was performed on bovine enamel, and stage 2 was carried out on human enamel samples, respectively. For stage 1 and 2 samples were “hand irradiated” while in stage 3 a computer controlled motor-driven stage was used to move the sample while irradiating with the CO_2 LASER. Stage 4 was used as negative control comparing LASER treatment alone without etching to a no-etched control.

In the first three stages, the control group was treated with 37% phosphoric acid-etch (Scotchbond™ Universal etchant, 3M™ ESPE™, St. Paul, MN) and bonded with Adper Single Bond Plus (3M™ ESPE™, St. Paul, MN) followed by the placement of Z250 Filtek™ supreme flowable composite (3M™ ESPE™, St. Paul, MN). The test group samples were treated with a short-pulsed 9.3 µm CO_2 LASER (Solea, Convergent Dental, Inc., Natick, MA) at different energy settings followed by total-etch bonding. After debonding of the composite with the UltraTester™ (Ultradent™ Products, Inc., South Jordan, UT), the shear-bond strength values were compared between the groups with ordinary one-way ANOVA followed by Bonferroni's multiple comparison tests.

RESULTS:

The bovine treatment groups showed an increase in shear-bond strength in comparison to the non-irradiated control group. However, statistically the increased bond strength values were not significantly different. LASER hand irradiation on human enamel showed an increase in shear-bond strength by 27.4%. LASER irradiation treatment using a stage motor showed an increase in shear-bond strength by 20.2%. Those differences were statistically significant for some of the treatment groups. The negative control groups with no etching after LASER treatment showed a significant, 79.5% reduced shear-bond strength in comparison to the total-etch control group.

CONCLUSION:

The study results suggest that when used with total-etch technique and 3M™ ESPE™ Adper Single Bond Plus, SOLEA 9.3 µm short-pulsed CO_2 LASER treatment increases composite shear-bond strength to enamel by up to 27.4% in comparison to non-LASER treated enamel. Using the LASER settings for caries prevention resulted in the highest shear-bond strength increase. The study also showed that CO_2 LASER treatment by itself is not a substitute for acid etching. In conclusion, when SOLEA 9.3 µm short-pulsed CO_2 LASER irradiation is used to render enamel caries-resistant, the shear-bond strength of the dental sealant to the enamel is increased.

KEYWORDS:

SOLEA 9.3µm short-pulsed CO_2 LASER, enamel, total-etch, 3M™ ESPE™ Adper Single Bond Plus, sealants, 3M Z250 Filtek Supreme, composite shear-bond strength, Ultradent, UltraTester

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INTRODUCTION

Background

Hard and soft tissue LASERs continue to gain popularity in dentistry. Benefits of engaging LASERs include reduced need of local anesthetic injections, less vibration and noise in comparison to an air driven high-speed handpiece, more preservation of tooth structure, a less painful procedure overall, and a less traumatic experience for the patient [1].

Selection of 9.3 μm Short-Pulsed CO_2 LASER

Different LASER wavelengths interact differently with tissues. (Figure **1**) shows the absorption curve of various tissue components like water and hydroxyapatite. Er:YAG LASER at 2.94 μm and ErCr:YSGG LASER at 2.79 μm emission wavelengths are strongly absorbed in water. The absorbed LASER energy converts water into steam, which rapidly expands; the resulting micro-explosions ablate the tooth structure [2-4]. In contrast, 9-11 μm CO_2 LASER wavelengths are well absorbed by the hydroxyapatite crystals, and result in crystal melting and eventually vaporization of the enamel depending on the applied energy levels[5, 6].

Among all CO_2 LASER wavelengths the 9.6 μm wavelength shows the highest absorption in hydroxyapatite crystals, while the 9.3 μm CO_2 LASER wavelength shows slightly less absorption. The reflection coefficient of enamel at the CO_2 LASER wavelengths also peaks at 9.6 μm wavelength. In contrast, reflection and back scattering of LASER irradiation is lower at 9.3 μm [5, 6]. Transmittance at both 9.6 μm and 9.3 μm wavelength is negligible. In conclusion, maximum energy can be transferred to the hydroxyl apatite crystals with the 9.3 μm CO_2 short-pulsed LASER. Thus 9.3 μm CO_2 LASER irradiation produces better and faster ablation and vaporization of enamel [5-9].

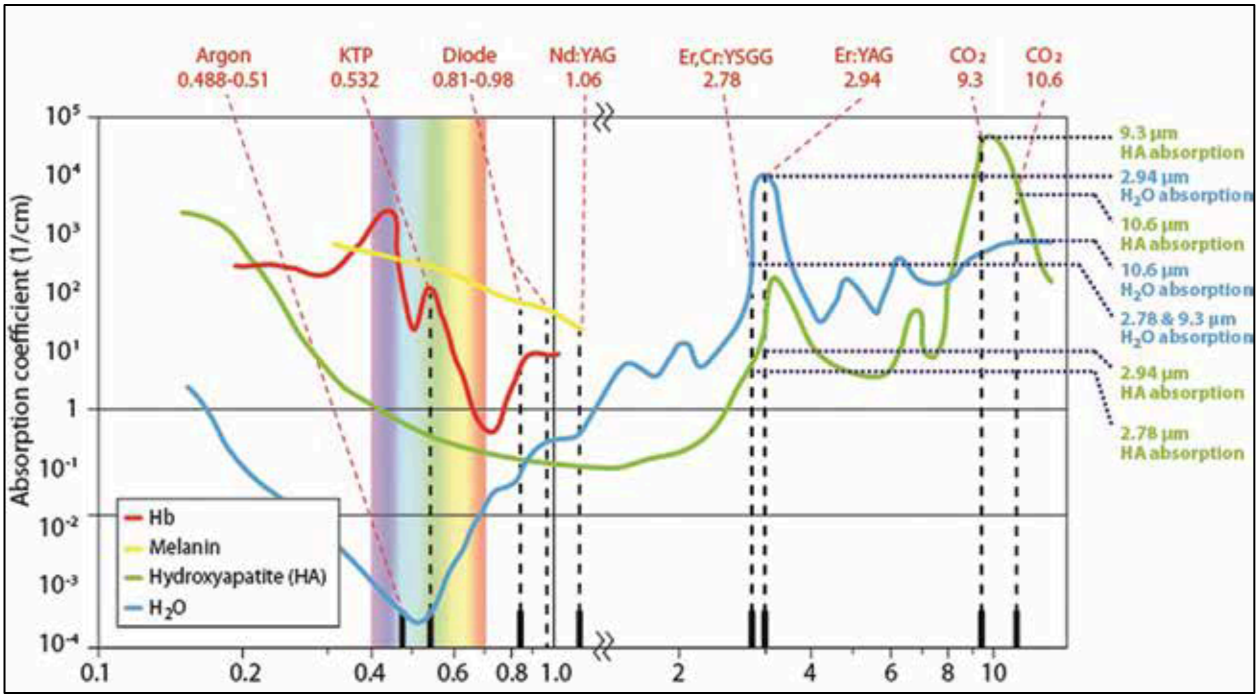


Figure 1: Absorption Curves Of Various Tissue Components

Safety of 9.3 µm Short-Pulsed CO$_2$ LASER

When a CO$_2$ LASER is used to cut the tissue, it creates heat, thus cutting is photo-thermal. As a result, applied to a tooth, a temperature rise of 5.5°C may lead to pulpal necrosis [10]. Several studies have already demonstrated the pulpal safety following the use of 9.3 and 9.6 µm CO$_2$ LASER irradiation for caries removal [11-15]. Already in 2002, Wigdor et al stated that when engaging a 9.6 µm CO$_2$ LASER with 60 µs pulse duration and energy levels of 2 and 3 W (at 90 and 136Hz), no apparent vascular change or pulpal inflammation was observed [16].

Caries Prevention with Short-Pulsed CO_2 LASERs

CO_2 LASER energy is well absorbed in the hydroxyapatite crystals [5, 6]. The caries preventive effect of short-pulsed CO_2 LASERs has been well documented in the literature. It has been demonstrated that 9.6 µm CO_2 LASER treatment can remove the carbonate ion from the carbonated hydroxyapatite crystal, and it can render enamel more resistant to acid dissolution [16-21], thus inhibiting tooth decay. However it might reduce the effectiveness of acid etching by this change, resulting in possibly weaker bonding of composites and sealants to enamel.

Enamel Bond Strength after LASER Treatment

Erbium LASERs emitting at 2.94 µm and 2.79 µm wavelengths are strongly absorbed in water. The absorbed LASER energy converts water into steam, which rapidly expands; the resulting micro-explosions ablate the tooth structure [22]. These micro-explosions also create frail, unsupported enamel prisms in the form of groves, flakes and shelves [2, 4, 23]. Those unsupported enamel prisms can reduce marginal integrity and decrease the enamel bond strength [3, 24-26]. Several studies on enamel bond strength after LASER irradiation were carried out using Er:YAG and ErCr:YSGG LASERs, respectively. These studies showed mixed results with some achieving similar or even higher bond strength after LASER irradiation compared to total-etch technique alone but also some achieved lower bond strength after irradiation.

A few studies showed that Er:YAG or ErCr:YSGG LASER treated surfaces without additional acid-etching produced poor enamel and dentin bond strength in comparison to engaging a total-etch bonding technique [27].

Nevertheless, Erbium LASER irradiation followed by acid etching produced in some studies comparable bond strength, as did acid etching alone [28-38]. The studies showing similar or increased bond strength to enamel following Erbium LASER irradiation recommended the use of Er:YAG and ErCr: YSGG LASER as an alternative to acid-etching for restorative and orthodontic procedures [27, 39-42] . All these studies agree that LASER irradiation of enamel without acid etching will produce lower bond strengths and should not be performed. Lopes et al in 2004 published a review of the literature on dental adhesion to Erbium LASER irradiated tooth structure. They also supported the idea that the acid-etch technique is mandatory after LASER treatment to achieve enamel and dentin bond values comparable to only acid-etched samples. The review also suggested that even though Erbium LASER treatment is supported for dental restorative procedures, more clinical studies are necessary to provide sufficient evidence of adhesive restorations made with Erbium LASERs [43].

Unlike for Er:YAG and ErCr:YSGG LASER irradiation, respectively, only a few studies have been published on the effects of CO_2 and specifically short-pulsed CO_2 LASER treatment on enamel and dentin bond strength. A CO_2 LASER study by Walsh et al in 1994 concluded that 10.6 µm CO_2 LASER irradiation treatment at pulse duration of 10 milliseconds, power density 2380 W/cm^2 and energy density 23.8 J/cm^2 increased the human enamel bond strength in comparison to the acid-etch only group. LASER parameters resulting in energy density settings higher or lower than 23.8 J/cm^2 resulted in significantly lower enamel bond strength value [44].

In 2011 Ngyuen et al published a study showing the effect of enamel bond strength following the use of 9.3 µm short-pulsed CO_2 LASER irradiation on enamel. In this study, Ngyuen et al presented the composite bond strength to enamel following the use of a fast scanned 9.3 µm short-pulsed CO_2 LASER. This study showed lower composite bond strength to enamel following the LASER irradiation but, there was no statistically significant difference in bond strength between control and test groups [11].
Already in 1988 Cooper et al published that 9.3 µm CO_2 LASER irradiation on dentin increased bond strength by 300% over non-LASER treated dentin [45].

Taking the caries preventive properties of 9.3 µm short-pulsed CO_2 LASER irradiation into consideration, a study was warranted to gain more information on enamel bond strength after 9.3 µm short-pulsed CO_2 LASER irradiation. The purpose of this study was to evaluate the bond strength of a flowable composite to enamel after 9.3 µm short-pulsed CO_2 LASER irradiation followed by total-etch technique, using the 3M™ ESPE™ Adper Single Bond Plus bonding agent.

OBJECTIVE

The objective of this study was to test whether 9.3 µm short-pulsed CO_2 LASER irradiation has an influence on bond strength of human enamel following different LASER treatments for pit and fissures sealants as well as for composite restoration placements in a laboratory study in comparison to non-LASER treated enamel by measuring the bond strength of a total-etch adhesive system, using shear-bond strength testing 24 hours after flowable composite placement.

HYPOTHESIS

The use of 9.3 µm short-pulsed CO_2 LASER irradiation (SOLEA, Convergent Dental Inc., Natick, MA) on sound human enamel followed by total-etch technique (Adper single bond Plus) (3M™ ESPE™, St. Paul, MN) produces equivalent or higher composite shear-bond strength to enamel in comparison to a total-etch bonding technique (Adper Single Bond Plus) alone.

RESEARCH AIMS

Aim 1: To test shear-bond strength to bovine enamel after 9.3 µm short-pulsed CO_2 LASER irradiation with different fluences followed by total-etch bonding (Adper Single Bond Plus).

Aim 2: To test shear-bond strength to human enamel after 9.3 µm short-pulsed CO_2 LASER irradiation with different fluences followed by total-etch bonding (Adper Single Bond Plus).

Aim 3: To test whether there is a difference in shear-bond strength to human enamel after 9.3 µm short-pulsed CO_2 LASER irradiation performed by "hand-irradiation" in comparison to irradiation using a computerized motor-driven stage followed by total-etch bonding (Adper Single Bond Plus).

Aim 4: To test whether 9.3 µm short-pulsed CO_2 LASER irradiation with different fluences can be an alternative to acid etching before bonding with Adper Single Bond Plus.

MATERIALS & METHODS

All study procedures were performed in a laboratory setting. The test samples included sound bovine and human enamel (UCSF IRB exempt approval for collecting extracted teeth). First, the irradiation of bovine enamel samples was carried out to achieve preliminary information about possible attainable bond strength values.

Bovine Samples

Young cows' incisors were used as bovine enamel samples. Exclusion criteria consisted of any teeth with craze lines, fractures or surface abnormalities on the enamel side of the teeth.

Extracted bovine incisor teeth were sterilized with gamma irradiation (Cs 137) at a dose above 173 krad overnight (12 hours). Following sterilization, the teeth were cleaned with ivory soap and water, and then kept in a 0.1% thymol solution throughout the entire study in order to keep the samples hydrated and prevent alteration in enamel [46].

The roots of the bovine incisors were removed below the cemento-enamel junction. Incisor crowns were cut into multiple pieces so that the facial surface of the enamel could be used for testing. Each enamel sample was reduced to a 4 mm x 4 mm block. The facial surface of the incisor blocks was polished with a rotating 600-grit silicon carbide paper [47-52]. The final enamel block size dimensions were 4 mm x 4 mm x 2 mm with the facial enamel surface polished until the test surface was flat, smooth and glassy in appearance.

Human Samples

Human maxillary and mandibular 1st, 2nd and 3rd molars were used as enamel samples. Exclusion criteria consisted of any teeth with decalcification, caries, craze lines, fractures or surface abnormalities on the enamel side of the teeth.

As with bovine teeth, extracted human teeth were sterilized, cleaned and stored similar to the bovine teeth.

The roots of the human molars were also removed below the cemento-enamel junction. Tooth crowns were cut into mesial and distal halves, and the proximal surfaces of the enamel were used for testing. Proximal surfaces with the greatest enamel thickness were polished slightly with rotating 600-grit silicone carbide paper under running water to obtain a flat reference point for mounting the samples in the 15-hole mold (Ultradent™ Products, Inc., South Jordan, UT) before embedding the sample in methacrylate (Great Lakes Orthodontics Ltd, Tonawanda, NY) (see below "Embedding of Samples").

All bovine and human enamel samples were cleaned after embedding in an ultrasonic bath for 5 minutes to remove any impurities. Then all samples were stored in different storage containers with 0.1% thymol solution at room temperature until LASER treatment.

Embedding of Samples

Both bovine and human enamel samples were embedded in dental acrylic in a similar manner. The Ultradent 15-hole casting assembly (Figure 2) was used to create methacrylate cylinders. Samples were placed in the casting assembly with the enamel surface facing the later outside of the acrylic cylinder block, then acrylic was poured using the powder-sprinkle technique. The mold produced cylinders measuring 1 inch in diameter and approximately 1 inch in length. Next, the cylinders were ground to produce parallel surfaces using the Ultradent™ grinding mandrel (Ultradent™ Products, Inc., South Jordan, UT) (Figure 3).

Figure 2: Ultradent 15-Hole Casting Assembly

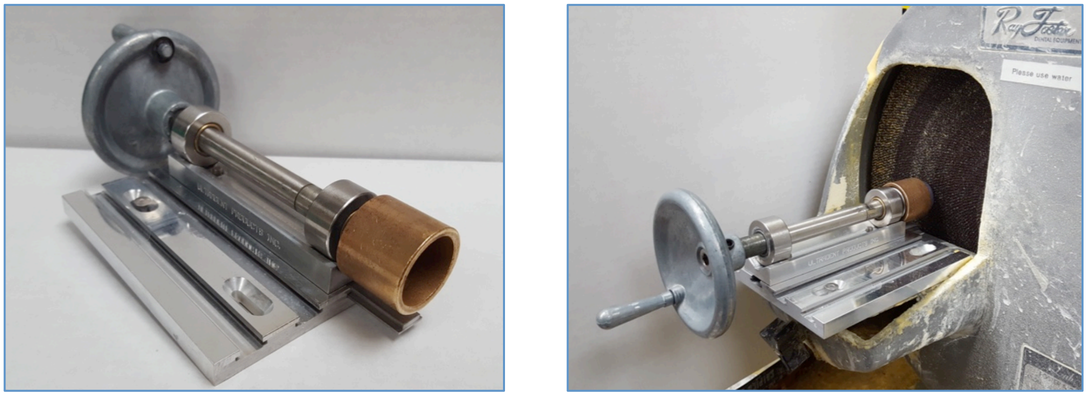


Figure 3: Ultradent Grinding Mandrel

The sample side of the cylinder was polished with rotating 600-grit sandpaper under running water until approximately 3 mm of enamel in diameter was exposed for human enamel samples and all of 4 mm x 4 mm enamel surface for bovine samples, respectively (Figure 4). After polishing, again all samples were cleaned in an ultrasonic bath for 5 min to remove surface impurities and then they were stored in 0.1% thymol solution at room temperature until bonding.

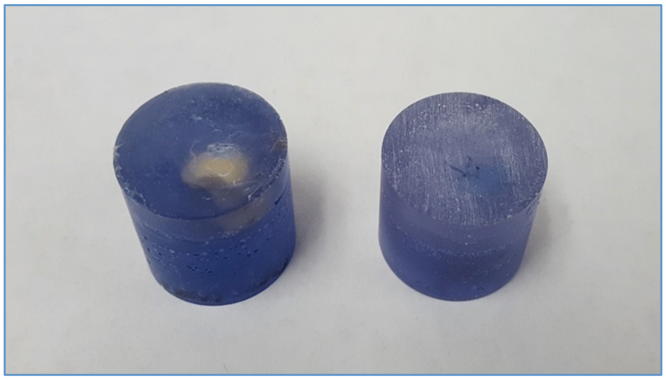


Figure 4: Prepared Enamel Sample in Acrylic Cylinder

LASER

Bovine and human enamel samples were divided into different treatment groups based on different LASER irradiation parameters. LASER treatments were completed using a 9.3 μm short-pulsed CO_2 LASER (SOLEA, Convergent Dental, Inc., Natick, MA). The control group was not LASER irradiated.

LASER Energy and Beam Size/Pattern Settings

Beam Pattern

The SOLEA 9.3 μm short-pulsed CO_2 LASER has a galvo system possibly generating different beam patterns, resulting in different irradiation spot sizes. For this study, the LASER irradiation was carried out using a 0.25 mm and 1 mm beam diameter.

Energy Setting

The SOLEA 9.3 μm short-pulsed CO_2 LASER offers a wide range of pulse repetition rates, energy per pulse settings (pulse duration) and air/water spray settings (mist).
For this study four different LASER pulse durations (3, 7, 23 and 43 μs) were used, consequently delivering pulse energies between 1.6 mJ/pulse and up to 16 mJ/pulse, resulting in fluences between 3.3 J/cm^2 and 32.7 J/cm^2. On the graphical user interface (GUI) of Solea LASER, contrary to the actual pulse durations mentioned above, these pulse durations were displayed as 0, 4, 20 and 40 μs, respectively.

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 ten 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 5.5x 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 17 millimeter.

To cover the most possible and likely conditions for enamel treatments, the following LASER parameters, energy settings and treatment conditions were applied based on the desired ability to cut, "polish", and melt enamel, respectively. Detailed LASER parameters and energy settings can be founded in (Table **1**).

Table 1: LASER Parameters and Energy Settings

Clinical Effect	Pulse duration (µs) GUI	Pulse duration (µs) Real	SOLEA "Speed" (%) GUI	Repetition Rate (Hz)	Beam Pattern (mm)	Irradiation time (sec)	Power (mW)	Fluence / Pulse (J/cm^2)
Ablation/ Cutting	40	43	100	12.5	1(spiral)	10	2,803	32.7
Ablation / Cutting	20	23	100	12.5	1(spiral)	15	1,510	17.6
Moderate Ablation / Polishing	4	7	30	41.3	0.25	60	131	6.5
Moderate Ablation / Polishing	4	7	10	4.1	1(spiral)	30	171	6.1
Melting/ Caries Prevention	0	3	30	41.3	0.25	60	73	3.6
Melting/ Caries Prevention	0	3	30	5.4	1(spiral)	60	123	3.3

Staging of Study Treatments

The entire study was performed in the following 4 stages. The figures 2-4 show the amount of samples used, pulse length, beam diameter, and use of air/water in each study stage.

1. Stage 1: LASER irradiation of bovine enamel (Total of 70 samples)
2. Stage 2: LASER irradiation of human enamel with "hand-irradiation" (Total of 105 samples)
3. Stage 3: LASER irradiation of human enamel while using a computerized motor-controlled stage (Total of 105 samples)
4. Stage 4: LASER irradiation of human enamel using no-acid etching (Total of 45 samples)

   The control group (etch) was the same for stage 2 and 3 of the study. The control group 2 (No etch) was added in stage 4 of the study.

Stage 1. Bovine Enamel – Hand Irradiation

Bovine enamel samples were divided into a control group and 6 different test groups, with 10 samples in each group. The control group samples were not LASER irradiated while the other 6 groups were hand irradiated with different LASER parameters. All groups received acid etching before bonding (details see below "Bonding") (Figure 5). For the detailed LASER parameters including pulse duration, repetition rate, beam pattern, irradiation time and use of water & air see (Appendix 1).

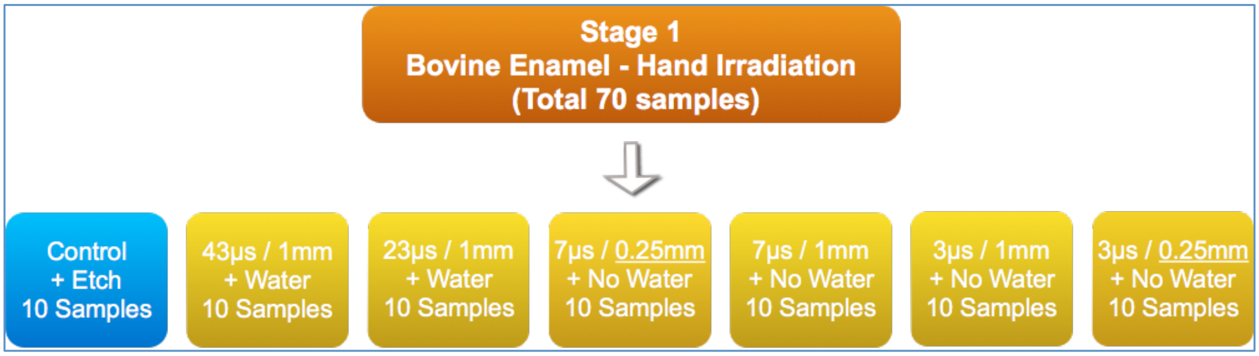


Figure 5: Bovine Enamel Study Protocol

Stage 2. Human Enamel – Hand Irradiation

Human enamel samples were also divided into a control group with acid etching (and no LASER use), and 6 LASER irradiation test groups. Each group consisted of 15 samples. All sampled were bonded with a traditional total etch method using 37% phosphoric acid etch (3M™ Scotchbond™ Universal Etch) (Figure 6) (See below "Bonding"). LASER parameters details are given in (Appendix 2).

For these bovine and human enamel treatments free "hand-irradiation" was performed. "Hand-irradiation" means free-hand LASER treatment by one LASER trained operator, irradiating the desired surface area. The focus optic of this LASER allows irradiation in 10 to 19 mm distance from the hand piece LASER exit point, still being "in focus".

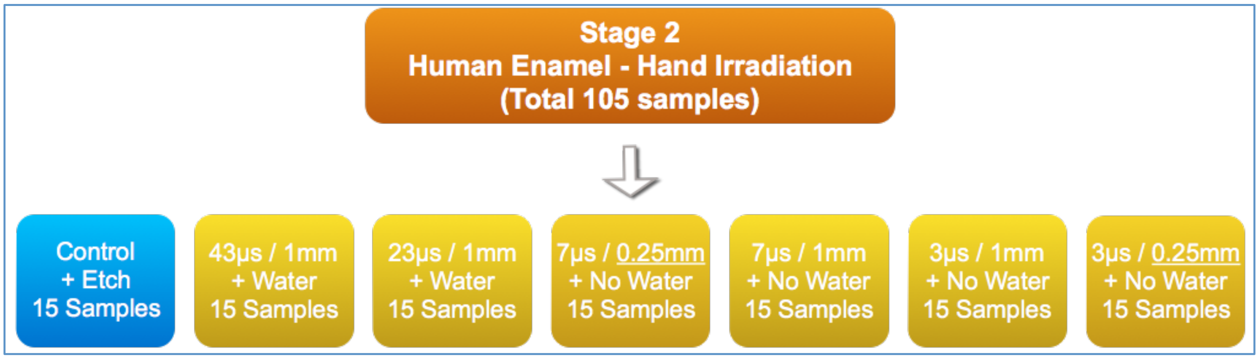


Figure 6: Human Enamel Hand Irradiation Study Protocol

Stage 3. Human Enamel – Stage Motor Irradiation

A second set of human enamel test samples was treated with again 6 different LASER treatment parameters (same as in "Human Enamel – Hand Irradiation"). The previous no LASER use sample group (stage 2) served as control (Figure **7**). In this set, for irradiation the samples were mounted on a computer controlled motor stage with a 2-axis (X-Y) linear stage motor (Thor Labs® Inc., Newton, NJ). Kinesis© software (Thor Labs® Inc., Newton, NJ) controlled the computerized motor with 0.05 µm precision steps.

The irradiation occurred at the 17mm focus distance from the end of the LASER handpiece to the enamel. A LASER spot overlap (1/3 or 2/3 overlap) was chosen in order to achieve a homogenous irradiation of the enamel surface. Homogenous irradiation of the samples was confirmed with a stereomicroscope (Fisher Scientific Stereomaster, Fisher Scientific LLC, PA). Detailed stage motor parameters can be founded in (Appendix 3-4).

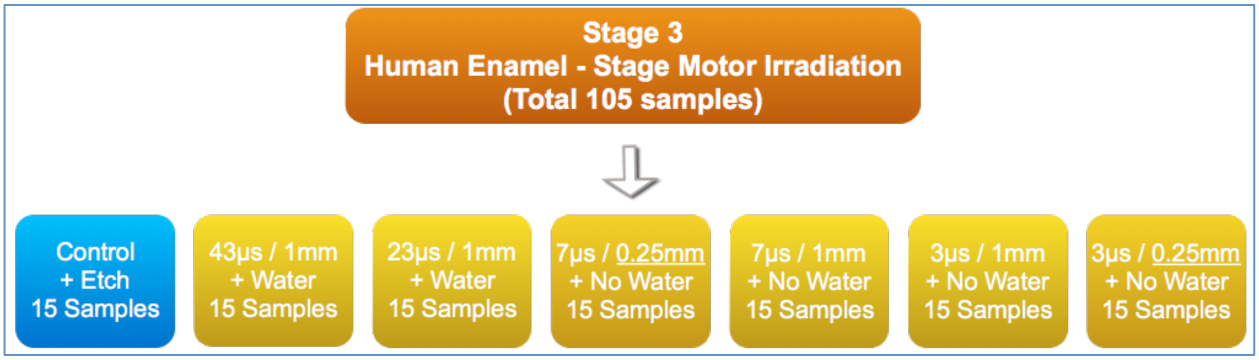


Figure 7: Human Enamel Stage Motor Irradiation Study Protocol

Stage 4. Human Enamel – Hand Irradiation – No acid etching

An additional small human enamel sample group was also divided into a control group without acid etching (and no LASER use), and 2 LASER irradiation test groups. Each group consisted of 15 samples (Figure **8**). All samples were bonded in the same manner, but without using 37% phosphoric acid etch. (See below "Bonding").

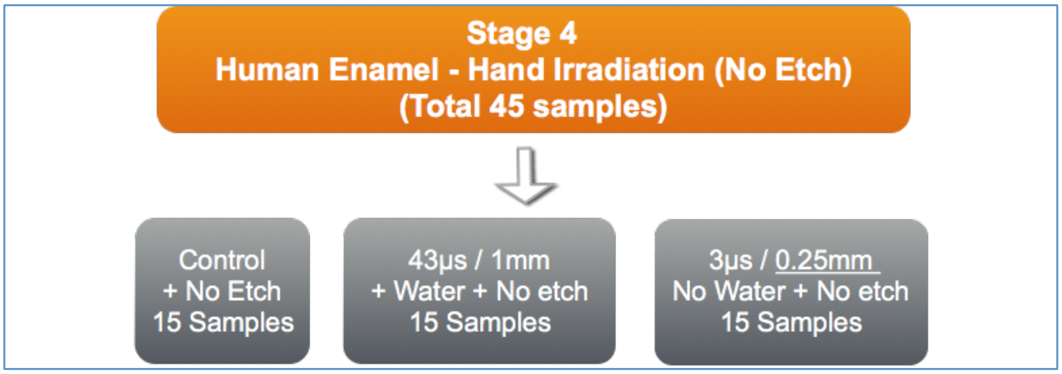


Figure 8: Human Enamel Hand Irradiation - No Etch Study Protocol

Bonding Procedure

All samples were rinsed with water for 5 seconds, dried and then etched with 35% phosphoric acid liquid (3M™ ESPE™ Scotchbond™ Universal etchant) with active scrubbing using a micro-brush for 15 seconds. Then the enamel surface was thoroughly cleaned with air-water spray for 15 seconds followed by air-drying for 5 seconds.

After drying the enamel sample, one drop of Adper Single Bond Plus (3M™ ESPE™, St. Paul, MN) was scrubbed vigorously on to the enamel surface for 15 seconds with a micro-tip applicator. Then the bond was spread thin over the enamel surface using air. Next, the bonding agent was light cured with a Satelec® Mini LED curing lights (Acteon North America, Mount Laurel, NJ) for 20 seconds. 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.

After curing the enamel sample was placed into an Ultradent bonding clamp (Figure 9) under a bonding mold insert (Ultradent™ Products, Inc., South Jordan, UT). The Ultradent bonding mold has a 2.38mm diameter x 3mm in height hollow tube to bond a 2.38 mm diameter composite cylinder on top of the sample surface. 3M Z250 Filtek™ supreme flowable (3M™ ESPE™, St. Paul, MN) was used as composite. After pushing the composite down through the hollow tube onto the sample surface the composite was light cured for 30 seconds.

The samples were removed from the mold and stored in clear water at room temperature for 24 hours to allow curing of uncured composite. The 24-hour time was concluded to be ideal by many other previous studies [53-55].

Figure 9: Ultradent Bonding Clamp and Mold Insert

Debonding and Shear-Bond Strength Testing

After 24 hours, the adhesive bonding strength of the 3M™ Z250 composite to the enamel surface was determined by performing a single plane shear-bond test with the UltraTester™ (Ultradent™ Products, Inc., South Jordan, UT) testing device.

The UltraTester™ was setup according to manufacturer's instructions. Each acrylic cylinder was secured on the Ultradent® test base clamp (Figure **10**). The composite cylinder was placed under the 2.38 mm notched crosshead assembly (Figure **11**). Once the test was started, the stage with the bonded sample moved upwards towards the crosshead assembly with a load shell of 1000 lbs (453.6 kg) at a steady rate of 1mm/min. The display showed the increasing stress in Mega-Pascal (MPa) until the composite cylinder sheared off. At this time the display showed and recorded the peak shear-bond strength in MPa. Debonded enamel samples and composite stubs were stored in the 0.1% thymol solution at room temperature.

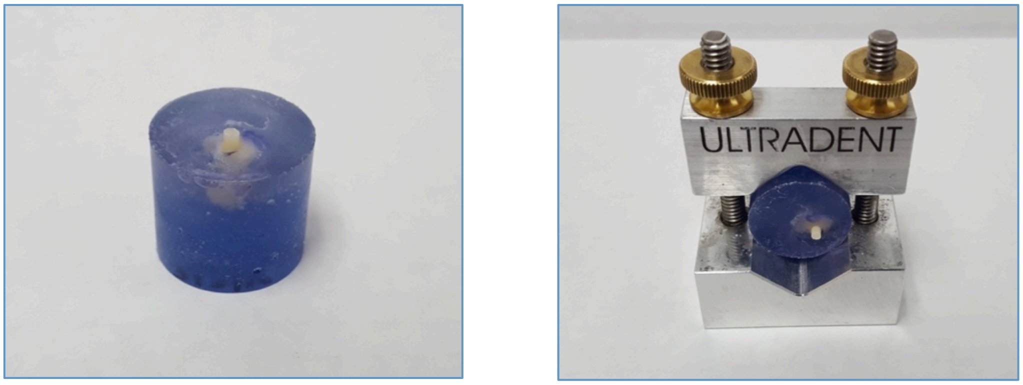


Figure 10: Bonded Sample and Ultradent Base Clamp

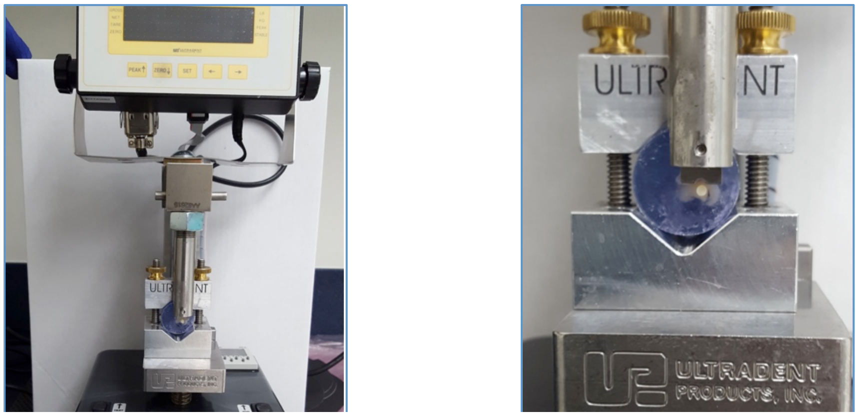


Figure 11: Notched Crosshead Assembly

RESULTS

Shear-bond Strength Testing Results - Overview

Shear-bond strengths values were measured for all bovine and human enamel samples. The results were recorded in Mega Pascal. Mean and standard deviation for all groups were calculated and are shown in Table **2**: Shear-Bond Strength – Results Overview.

Table 2: Shear-Bond Strength – Results Overview

Shear Bond Strength	Control Etch (MPa)	Control 2 No Etch (MPa)	Group 1 43 µs/ 1mm	Group 2 23 µs/ 1mm	Group 3 7 µs/ 0.25mm	Group 4 7 µs/ 1mm	Group 5 3 µs/ 1mm	Group 6 3 µs/ 0.25mm
Bovine (Hand)	25.68 SD 1.98	-	28.02 SD 3.04	28.09 SD 3.59	29.05 SD 3.00	26.06 SD 6.15	29.30 SD 4.44	29.17 SD 3.24
Human (Hand)	25.04 SD 2.80	-	25.14 SD 2.52	28.71 SD 3.77	29.15 SD 4.57	26.78 SD 3.38	30.45 SD 3.42	31.90 SD 2.50
Human (Stage M)	25.04 SD 2.80	-	25.52 SD 2.43	27.02 SD 3.25	27.86 SD 2.30	27.06 SD 4.19	28.94 SD 2.98	30.09 SD 2.74
Human (No etch)	-	3.77 SD 1.76	5.85 SD 1.30	-	-	-	-	5.13 SD 1.20

To compare the shear-bond strengths results after the different LASER treatments, statistical analyses were performed using ordinary one-way ANOVA followed by a Bonferroni's multiple comparisons test for significance at P≤0.05 (Prism, GraphPad software Inc., La Jolla, CA).

Stage 1 – Bovine Enamel – Hand Irradiation – Results

When presenting the results, in Figure 12 to 15 for easier reading two graphs are displayed next to each other, one showing on the x-axis the actual LASER pulse width and the other the resulting fluence applied.

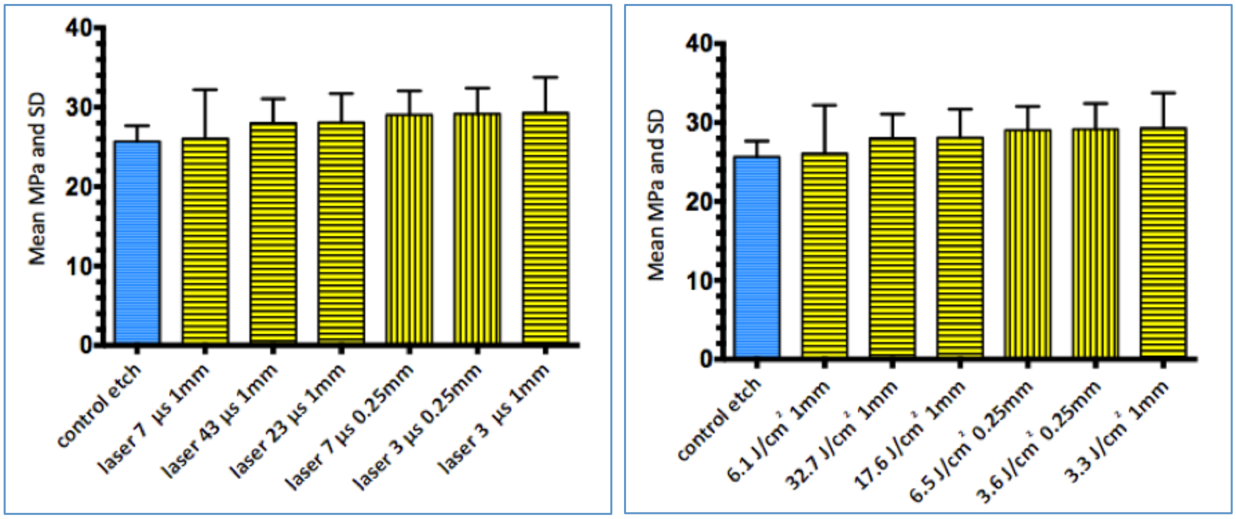
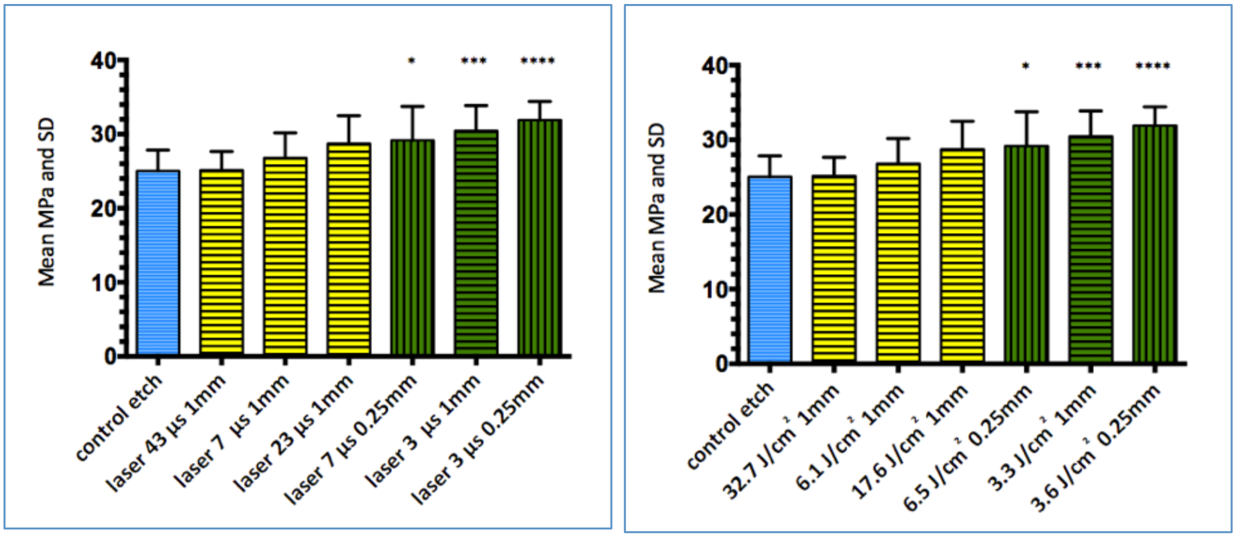


Figure 12: Bovine Enamel Shear-Bond Strength Results

Figure **12** shows the shear-bond strength values for the control samples and for the samples after LASER treatment with "hand-irradiation" for bovine samples. The bar on each column represents standard deviation (SD). Blue and yellow columns represent control and treatment groups, respectively. (Vertical lines mark 0.25 mm beam patterns and horizontal lines 1 mm beam patterns, respectively).

The LASER treatment resulted in increased shear-bond strength values for all test groups. The highest bond strength was observed with the 3 µs / 1 mm LASER treatment (29.30 ± 4.44 MPa, Mean ± Standard Deviation [SD]), which presents a 14% increase over the control group (25.68 ± 1.98 MPa, Mean ± SD). There were no statistically significant differences noted in the shear-bond strength values between the control and test groups for the bovine samples.

Stage 2 - Human Enamel Hand Irradiation – Results



(* Significant at P ≤ 0.05; *** Significant P ≤ 0.001; **** Significant P ≤ 0.0001)

Figure 13: Human Enamel Shear-Bond Strength – Hand Irradiation Results

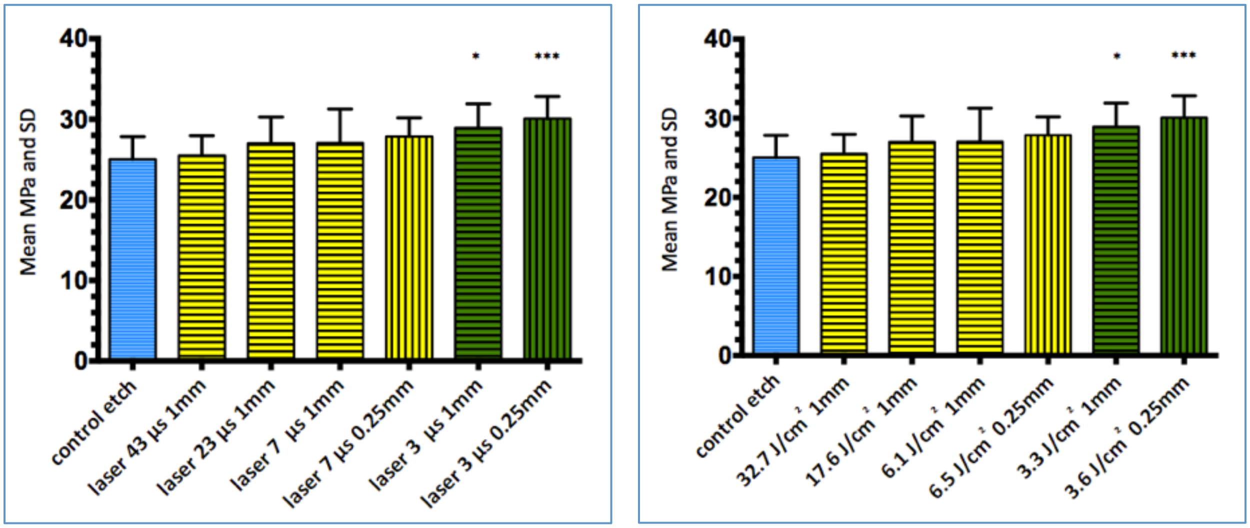
Figure **13** shows the shear-bond strength values after LASER treatment with "hand-irradiation". Similar to bovine enamel, human enamel samples in all the test groups showed increased shear-bond strength values in comparison to the control group. The green columns represent the LASER treatment groups with statistically significant differences in comparison to the control (asterisks mark the significance level).

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

Statistically significant higher enamel shear-bond strength values were also observed for the test group using also the 3 µs pulse duration but the 1 mm LASER irradiation pattern

(30.45 ± 3.42 MPa, $P \leq 0.001$). The test group with a LASER setting of 7 µs / 0.25 mm also reached significantly higher bond strength values in comparison to the control (29.15 ± 4.57 MPa, $P \leq 0.05$).

Stage 3 - Human Enamel – Stage Motor Irradiation – Results



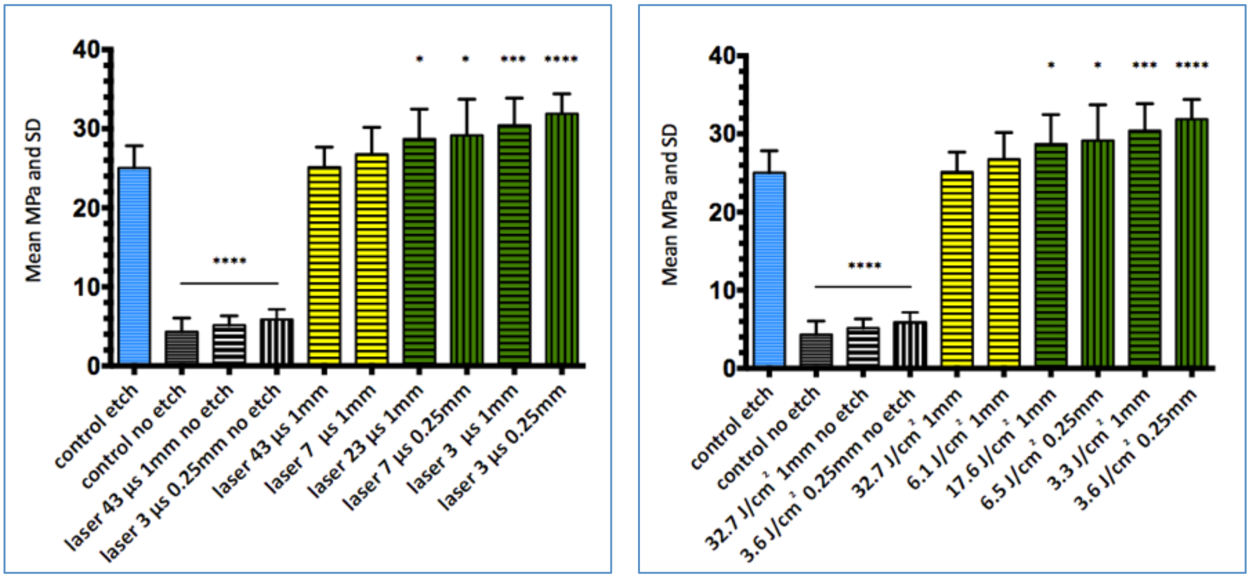
(* Significant at $P \leq 0.05$; *** Significant $P \leq 0.001$)

Figure 14: Human Enamel - Stage Motor Shear-Bond Strength Results

Figure **14** shows the shear-bond strength values after LASER treatment using a computerized motor controlled stage for moving the samples during irradiation. Similar to hand irradiated human enamel samples, all the test groups showed increased shear-bond strength values in comparison to the control group. The highest bond strength values were observed in the test group again with the LASER setting 3 µs / 0.25 mm LASER irradiation pattern (30.09 ± 2.74 MPa, (Mean ± SD), $P \leq 0.001$). Using this setting the shear-bond strength increased by 20.2%.

Using the same pulse energy but a 1 mm irradiation pattern also resulted in significantly higher shear-bond strength in comparison to the control (28.94 ± 2.98 MPa, $P \leq 0.05$).

Stage 4 - Human Enamel – Hand Irradiation - No Acid Etching – Results



(* Significant at $P \leq 0.05$; *** Significant $P \leq 0.001$; **** Significant $P \leq 0.0001$)

Figure 15: Human Enamel - No Acid Etching Shear-Bond Strength Results

Figure **15** represents a comparison between the control group with no LASER irradiation & acid etching (blue column) and the groups without acid etching & different LASER treatments (grey columns), and all LASER treatments (hand-irradiation) with acid etching (as reported above in Figure **13**), respectively.

For the groups when acid etching was not applied, much lower enamel shear-bond strength values were observed. The enamel shear-bond strength values for the negative control without etching was 3.77 ± 1.76, (Mean ± SD, $P \leq 0.0001$), and for the test group with setting of 3 µs/ 0.25 mm LASER irradiation pattern & no etch 5.13 ± 1.20 ($P \leq 0.0001$) and for the test group with setting of 43 μs/ 1 mm LASER irradiation pattern & no etch 5.85 ± 1.30 ($P \leq 0.0001$).

DISCUSSION

Lately a short-pulsed CO_2 LASER became available for use in dental offices. With regards to CO_2 LASERS and absorption in enamel, in the past studies have shown that the CO_2 LASER absorption coefficient in hydroxyapatite crystals is highest using the 9.3 μm and 9.6 μm CO_2 LASERS wavelength [17, 56]. This LASER wavelength can efficiently cut enamel. In addition, delivering LASER energy from such a CO_2 LASER at a fluence around 3 J/cm^2 and above can render enamel surfaces more resistant to caries by removing the carbonate ion, thus changing a very acid soluble carbonated hydroxyapatite crystal into a more acid resistant hydroxyapatite crystal [17, 19, 20, 56-59].

A sufficient bond of the composite to enamel is required for successfully, long-term placement of fillings or sealants [60-62]. While achieving increased acid resistance thermal alterations of the irradiated enamel may occur, such as melting and/or chemical changes. This might render the enamel less receptive for adhesion [63, 64], and consequently might lower the bond strength of a composite material to the altered enamel surface.

Placing a sealant for additional caries prevention into an already CO_2 LASER irradiated fissure might result in the early loss or leakage of the sealant. The goal of this study here was to evaluate potential changes in bond strength of enamel to composite after irradiation with a short-pulsed 9.3 μm CO_2 LASER.

Bond Strength short-pulsed CO_2 Lasers

A study published by Ngyuen et al in 2011 showed no significant difference in bond strength to composite of the 9.3 μm short-pulsed CO_2 LASER treated enamel samples compared to the acid etched control group. In fact, the study showed that LASER treated enamel exhibits slightly lower but not statistically significant different enamel bond strength than the acid etched control group. The authors used 3M™ ESPE™ single bond and 3M™ ESPE™ Z250 composite in the study. They reported bond strength values of 37 MPa (SD 3.6) for the human enamel control and 31.2 MPa (SD 2.5) for LASER treated enamel followed by acid etching (fluence 20 J/cm^2). In this study the use of a fast scanning computer-controlled stage motor to move the sample under the irradiation beam might present a limitation of the study. Those irradiation conditions may not be clinically relevant. Performing "hand irradiation" may result in bond strength values, which are a better reflection of actual clinical treatment conditions. More important, in the study by Ngyuen after treating the enamel surface, in addition, uniformly with different LASER energies, separate retention holes with either a 250 or 500 μm diameter were LASER-drilled, using a fluence of 42 J/cm^2 and 20 LASER pulses per spot before bonding the composite. These additional micro mechanical retention holes might have resulted in a higher bond strength than what might be achieved under clinical conditions [11]. Drilling additional holes might be unrealistic for clinical use.

In the shear bond strength study presented here, CO_2 LASER irradiated human enamel showed increased bond strength values for all treatment groups. LASER treatments with pulse durations of 43 μs and 23 μs, respectively, clinically used for cavity preparation showed an increase in bond strength, as well as treatments with a pulse duration of only 7 μs, clinically used for slow cutting or smoothening the cavity preparation, showed a significant increase in bond strength over the control group ($P \leq 0.05$). Similarly, LASER treatment groups with the lowest pulse duration applied (3 μs), which is clinically used for rendering the enamel acid resistant [59], also showed significantly higher enamel bond strength values ($P \leq 0.0001$ and $P \leq 0.001$, respectively) (LASER "hand-irradiation).

The SOLEA CO_2 LASER generates different beam patterns by using reflecting mirrors, called galvos. The basic beam diameter is 0.25 mm; at the beam delivery pattern of 0.25 mm there is no beam movement by the galvo system. At any other beam pattern setting when using for instance a beam diameter of 1 mm, the 0.25 mm diameter LASER beam is moved by the galvos in a specific pattern to achieve the desired irradiation-beam diameter. This study showed clearly that even using different LASER beam diameters created by different beam delivery patterns, the resulting shear-bond strength of irradiated enamel samples was similar, when keeping the pulse duration thus fluence the same. In summary, similar bond strengths values can be achieved by different beam patterns as long as the fluence delivered is the same.

Comparing LASER irradiation moving the enamel sample with a computer controlled motor stage with free hand-irradiation revealed identical results. It can be concluded that operators using the CO_2 9.3 μm short pulse LASER in a dental office can achieve consistent bond strength results similar to a computer-controlled irradiation.

Bovine versus Human Enamel

The study presented here used bovine as well as human enamel samples. For laboratory bond strength studies the substitution of human teeth by bovine teeth has been studied a few times.

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. Besides this, they found no significant difference in bond strength to human and bovine enamel [65].

A review of literature done by Yaseen et al in 2011 concluded that inconsistent data exist regarding 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 [66].

In 2015 Teruel et al reported higher organic matter (bovine enamel 10.90% vs. human 5.70%), similar carbonate content and lower Calcium/Phosphate (mol/mol) 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 most mineralized. Thus bovine enamel appears to be the closest substitute to human enamel [67].

In the study presented here it could be shown that bovine enamel exhibited an increase in bond strength over the controls in all tested LASER groups, but statistically not significant. Nevertheless, with regards to bond strength bovine and human enamel appear to react similar. Increased sample size numbers in the bovine group might also have shown the statistically significant differences between control and LASER irradiated samples. These results suggest that despite the slight difference in chemical structure of the enamel[66, 67] both bovine and human enamel showed similar trend of increased bond strength following 9.3 µm short-pulsed CO_2 LASER treatment.

The Influence of Etching

Staninec et al in 2003 showed that if acid etch was not performed before a composite was bonded to enamel, 9.6 µm CO_2 LASER irradiation treatment resulted in lower bond strength values (18.52 ± 4.23 MPa; Mean ± SD) than the control group achieved with acid etch and no LASER treatment (31.03 ± 5.26 MPa). They concluded that he addition of a thick water layer (1 mm) during irradiation prevented the formation of undesirable CaP phases, which compromise adhesion to restorative materials. Nevertheless, the bond strength of the laser irradiated surfaces was much lower than that of the acid etched controls [27].

In stage 4 of this study shear-bond strength values were evaluated when no acid etching was used. The controls without LASER use and no etching showed extremely low shear bond strength values. Applying CO_2 9.3 µm short pulsed LASER irradiation, at two different pulse durations with and without water mist, respectively, but no acid etching resulted in slightly increased bond strength values over the non-etched controls, but they were significantly lower than those of the controls with additional acid etching ($P \leq 0.0001$). The study showed that LASER irradiation is not a substitution for acid etching. Any enamel CO_2 9.3 µm short-pulsed LASER treatment should be followed by acid etching prior to bonding to achieve comparable or higher bond strength than acid etching alone.

The total-etch bonding system used in this study was manufactured by 3M™ ESPE™ and is marketed as Adper Single Bond Plus in United States and as Adper Single bond 2 in some other countries. According to the manufacture, there are two distinct advantages of using 3M™ ESPE™ Adper Single Bond Plus. Very few total-etch adhesive systems like the 3M™ ESPE™ Adper Single Bond Plus include nano fillers that do not separate from the solution. In contrast, some other bonding materials like OptiBond™ Solo Plus bond (Kerr™ Corporation, Orange, CA) have micro fillers, which settle down from the remaining solution and the bottle requires shaking before dispensing [68]. The nano-fillers in 3M Adper Single Bond Plus are also intended to reinforce resin tags and the adhesive layer [69]. The low molecular weight does not compromise the inter-diffusion (penetration) in etched enamel [60], and helps to strengthen the hybrid layer in dentin [70, 71]. Multiple studies have shown higher enamel and dentin bond strength with 3M Adper Single Bond Plus (Single Bond 2 / Single Bond) in comparison to other total-etch bond systems [53, 71-74]. Bond strength studies with Erbium and CO_2 LASER irradiation have also shown better strength with 3M Adper Single Bond Plus (Single Bond 2 / Single Bond) in comparison to other total-etch or self-etch bonding materials [49, 75-80].

As part of the bonding process, in this study 37% phosphoric acid etchant liquid (3M™ ESPE™ Scotchbond™ Universal etchant) was used to etch the enamel surface with a rubbing action for 15 seconds and then rinsing with air-water spray for a total of 15 seconds. Studies have shown that there is no difference in etching patterns with the use of either etching liquid or gel. Furthermore, there is no difference reported in fissure penetration between etchant gel and etchant liquid [81-83].

The study by Bates et al showed that the method of 37% phosphoric acid application by dabbing, rubbing, and no agitation has no significant effect in composite bond strength to etched enamel. Furthermore, the tensile bond strength to enamel was not significantly different for enamel etching completed in 30, 60 and 120 seconds [84]. However, due to the lower viscosity of the etchant liquid, it is recommended to rub it on the cavity preparation in order to evenly etch the entire preparation [84].

15 seconds rinsing time after applying the acid etch to the enamel as performed in this study here is recommended by the manufacturer. Summitt et al in 1993 studied the effect of rinse time on micro-leakage between composite and etched enamel. They concluded that a one-second rinse with either water or air/water spray was as effective as a 20-second rinse with air/water spray in preventing micro-leakage at the resin-enamel interface, however higher rinsing time increased bond strength to the enamel [85, 86].

Caries resistance and acid etching

There is a potential concern that acid-etching application to enamel irradiated by LASER might remove the caries resistant layer. Some studies have shown that acid etching on non-LASER treated enamel removes 5-10 µm layer [87-90]. In 2012 Nahm et al published a Polarization Sensitive Optical Coherence Tomography (PS-COT) study showing that the 9.3 µm short-pulsed CO_2 LASER modified acid resistant enamel layer can be removed with 5-10 sec of acid etching with 37.5% phosphoric acid, though acid etching showed large variation in the depth and mineral loss [91]. Contrarily, it has not been studied how deep the LASER modified acid resistance enamel layer actually extends.

Bond strength Testing Machine

The Ultradent UltraTester™ bond strength testing machine was used to measure the shear-bond strength of the composite to the enamel. In 2013 the International Organization for Standardization (ISO) adopted Ultradent's notched-edge testing method as new standard. ISO Standard 29022 specifies a shear test method used to determine the adhesive bond strength between direct dental restorative materials and tooth structure, such as dentin or enamel. The crosshead's thin curve lip edge adapts to the specimen and applies even load to get more accurate shear bond results in comparison to a straight edge, which can give variable results[92].

In 1995 De Hoff et al with 3-dimentional analysis studied that loading with a knife edge exerts a higher stress concentration over a small area, wrongly resulting in less overall stress necessary for bond failure.[93] When evaluating a wire-loop loading device, DeHoff et al reported that this testing method actually applied force over 180 degrees of the circumference of the composite sample. This method leads to a more uniform distribution of stress, while reducing the stress concentration adjacent to the interface [93]. Pakora et al in 2002 compared shear-bond strength results relative to knife-edge and Ultradent's notch edge testing devices. The study showed significantly higher bond strength using Ultradent's notch edge testing device, which is a better representation of the actual shear-bond strength than underestimated shear-bond strength values achieved with a knife edge method [94]. Braz et al in 2010 showed that the chisel/knife edge presented severe stress concentrations near the loading site, giving variable force distribution across the bonded specimen, while orthodontic loop wire and stainless steel tape bond stress did not present peak of stress concentrations, thus giving more accurate presentation of the bond strength [95]. The testing device by Ultradent acts similar to the wire loop by concentrating the load stress 180 degrees around the specimen and distributing the force over a larger area than that of the knife.

Figure 16 shows the bond strength difference between a straight edge (variable peeling strength) and a notched edge (accurate shear-bond strength) bond testing method [92]. Many studies have already started adapting to this new ISO standard for their bond strength testing [96-100].

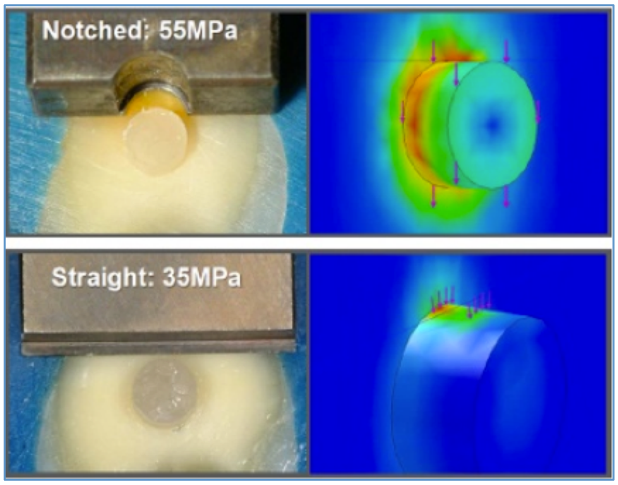


Figure 16: Difference in stress and stress distribution for shear-bond strength testing between a notched edge and straight edge testing device

<u>Clinical Significance</u>

The Clinical significance of this study is that treatment of enamel surfaces with 9.3 µm short-pulsed CO_2 LASER, using settings proven to render enamel more caries resistant, followed by a dental sealant placement may provide enhanced pit and fissure caries resistance and a significantly increased retention of the sealant. Even if a sealant is lost in-between regular patient recall visits, the chance of caries development is still most likely reduced due to the prior CO_2 9.3 μm short-pulsed LASER irradiation.

This is the first study using 9.3 μm short-pulsed CO_2 LASER irradiation that obtained shear bond strength values for the enamel composite bond, which were equivalent or higher than acid-etch treatment alone. Further investigations are required to study the irradiated enamel surface to determine whether structural changes in the hydroxyapatite crystals might contribute to the higher bond strength values following the LASER treatment.

CONCLUSION

The study results suggest that 9.3 μm short-pulsed CO_2 LASER (Solea, Convergent Dental Inc., Natick, MA) treatment followed by total-etch technique with the use of Adper Single Bond Plus bond (3M™ ESPE™, St. Paul, MN) can increase Z250 FilTek™ flowable composite's (3M™ ESPE™, St. Paul, MN) bond strength to human enamel. The shear-bond strength increased by as much as 27 %. Applying LASER irradiation proven to be caries preventive (0 μs / 1 mm or 0 μs / 0.25 mm) resulted in the highest shear-bond strength values of composite to enamel ($P<0.05$). Irradiation with LASER settings for both enamel cutting and polishing, respectively also gave comparable or significantly better shear-bond strength values in the laboratory testing.

The results also suggest that LASER treatment by itself is not a substitute for acid etching. When SOLEA 9.3 μm short-pulsed CO_2 LASER irradiation is used before dental sealant placement, the enamel surface is rendered more caries-resistant and the bond strength of the dental sealant to the enamel is increased, possibly providing longevity to the sealant or restoration.

A randomized, prospective, controlled, blind clinical trial using 9.3 µm short-pulsed CO_2 LASER irradiation and settings applied in this study is required to further prove the hypothesis.

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APPENDICES

Appendix 1: Bovine Enamel – Hand Irradiation

LASER Parameters	Control	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LASER	No	Yes	Yes	Yes	Yes	Yes	Yes
Etchant	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pulse duration GUI (µs)	-	40	20	4	4	0	0
Pulse duration (µs)	-	43	23	7	7	3	3
Repetition Rate	-	100%	100%	30%	10%	30%	30%
Beam diameter (mm)	-	1	1	0.25	1	1	0.25
Irradiation time (sec)	-	10	15	60	30	60	60
Water & Air	-	Yes	Yes	No	No	No	No

Appendix 2: Human Enamel – Hand Irradiation

LASER Parameters	Control 1	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LASER	No	Yes	Yes	Yes	Yes	Yes	Yes
Etchant	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pulse Duration GUI (µs)	-	40	20	4	4	0	0
Pulse Duration (µs)	-	43	23	7	7	3	3
Repetition Rate	-	100%	100%	30%	10%	30%	30%
Beam Diameter (mm)	-	1	1	0.25	1	1	0.25
Irradiation time (sec)	-	10	15	60	30	60	60
Water & Air	Yes	Yes	Yes	No	No	No	No

Appendix 3: Human Enamel – Stage Motor Irradiation

LASER Parameters	Control	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LASER	No	Yes	Yes	Yes	Yes	Yes	Yes
Etchant	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pulse Duration GUI (µs)	-	40	20	4	4	0	0
Pulse Duration (µs)	-	43	23	7	7	3	3
Repetition Rate	-	100%	100%	30%	10%	30%	30%
Beam Diameter (mm)	-	1	1	0.25	1	1	0.25
Irradiation time (sec)	-	10	15	60	30	60	60
Water & Air	Yes	Yes	Yes	No	No	No	No

Appendix 4: Laser Irradiation and Stage Motor Parameters

LASER Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LASER Stage motor	Yes	Yes	Yes	Yes	Yes	Yes
Pulse Duration GUI (μs)	40	20	4	4	0	0
Pulse Duration (μs)	43	23	7	7	3	3
Repetition Rate	100%	100%	30%	10%	30%	30%
Beam Diameter (mm)	1	1	0.25	1	1	0.25
Beam overlap	1/3	1/3	2/3	2/3	2/3	2/3
Stage motor Speed (mm/sec)	2	2	4	2	2	4
Irradiation time (sec)	10	15	60	30	60	60
Water & Air	Yes	Yes	No	No	No	No

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