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Abstract. The aim of this study was to test the hypothesis that in a short-term clinical pilot trial short-pulsed 9.6 μm CO2-laser irradiation significantly inhibits demineralization in vivo. Twenty-four subjects scheduled for extraction of bicuspids for orthodontic reasons (age 14.9 ± 2.2 years) were recruited. Orthodontic brackets were placed on bicuspids (Transbond XT, 3M). An area next to the bracket was irradiated with a CO2-laser (Pulse System Inc, Los Alamos, New Mexico), wavelength 9.6 μm, pulse duration 20 μs, pulse repetition rate 20 Hz, beam diameter 1100 μm, average fluence 4.1 ± 0.3 J/cm2, 20 laser pulses per spot. An adjacent nonirradiated area served as control. Bicuspids were extracted after four and twelve weeks, respectively, for a quantitative assessment of demineralization by cross-sectional microhardness testing. For the 4-week arm the mean relative mineral loss ΔZ (vol% × μm) for the laser treated enamel was 402 ± 85 (mean ± SE), while the control showed significantly higher mineral loss (ΔZ 738 ± 131; P = 0.04, t-test). The difference was even larger after twelve weeks (laser arm ΔZ 135 ± 98; control 1067 ± 254; P = 0.002). The laser treatment produced 46% demineralization inhibition for the 4-week and a marked 87% inhibition for the 12-week arm. This study shows, for the first time in vivo, that the short-pulsed 9.6 μm CO2-laser irradiation successfully inhibits demineralization of tooth enamel in humans.

Keywords: CO2-laser; microsecond pulsed; in vivo caries prevention; orthodontic bracket model; randomized prospective clinical trial.

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1 Introduction

Enhancing caries resistance of enamel with lasers has been reported soon after the invention of the first laser. Besides CO2-lasers, which had originally been used for this purpose1-10 to reduce the acid dissolution of enamel, other lasers have been investigated in laboratory studies including Nd:YAG,11-14 Er:YAG,15-18 and Er:Cr:YSGG-19-21 as well as argon ion lasers22-28 and with and without additional topical fluoride application. There are also reports from small scale in vivo studies using an argon laser around orthodontic brackets29 or Nd:YAG-laser treatment coupled with initiation dye and acidulated fluoride application in children with the effects assessed by following the development of white spot lesions or fissure caries.30

Featherstone et al. have shown in several studies that enhancement of caries resistance of enamel can be achieved in the laboratory by irradiation with short-pulsed CO2-lasers under well-specified irradiation conditions.30,31,32 Nevertheless, a clinical trial to demonstrate that those conditions inhibit dental caries progression in vital teeth in humans has not yet been reported.

In order to investigate the efficiency of specific CO2-laser irradiation, an orthodontic model33 was used in the present study. Orthodontic treatment has been associated with increased enamel demineralization because of increased plaque accumulation around the brackets34 and the development of a more cariogenic bacterial environment.35,36 After bracket placement, the most common place for this demineralization to occur in orthodontic patients is the gingival and middle thirds of the facial surfaces,37 thus shifting the tendency of demineralization from interproximal areas to the facial tooth surface as well as from posterior to anterior regions of the mouth.38,39 For the purposes of the present study this well-established form of dental caries was used as a model system to determine whether the laser treatment inhibits demineralization and/or enhances remineralization in vital teeth in the oral cavity of humans.33,40

In other studies, Featherstone et al. have successfully used the orthodontic bracket model on teeth scheduled for extraction, in order to study means of reducing demineralization or enhancing remineralization.33,41,42 Each of the studies involved four weeks of wearing those appliances in combination with a variety of fluoride delivery systems. In each study, teeth were extracted after four weeks, cross-sectioned and detailed cross-sectional microhardness analyses were done to determine the mineral loss profiles. In the O’Reilly study a measurable demineralization around the brackets was demonstrated even when a 1100-ppm fluoride dentifrice alone was used daily, illustrating that this high bacterial challenge situation overrides the beneficial effect of this clinically proven dentifrice. When a daily 0.05% sodium fluoride (NaF) mouthrinse was added demineralization was eliminated.41

In another study, Gorton and Featherstone33 compared a control group that used a 1100-ppm fluoride dentifrice daily with a...
test group in which the brackets were bonded with a fluoride-releasing glass ionomer cement instead of the conventional composite. The control group demonstrated significantly more demineralization around the brackets in just four weeks even with the use of the fluoride-containing dentifrice. In contrast, in the test group demineralization was, on average, completely inhibited.

Since the orthodontic bracket model had proven to be successful in the O’Reilly as well as in the Gorton study, the identical model was therefore used in the present study with laser treatment being used in place of the F-releasing glass ionomer cement.

The present study was a single blind, controlled, prospective clinical trial assessing treatment effects within-person thereby controlling for genetic, nutritional, hygiene, and oral environment factors. The hypothesis to be tested was that the use of a microsecond pulsed 9.6 μm CO2-laser will significantly inhibit the formation of carious lesions around orthodontic brackets in vivo in comparison to a nonirradiated control area on the same tooth over a short and midterm observation interval.

2 Materials And Methods

2.1 Study Inclusion and Exclusion Criteria

Approval for the study was obtained from the Committee on Human Research at UCSF (approval number H9136–25290-04). Prior to enrollment of each subject into the study, an independent dental examiner, not otherwise involved in the study, conducted a clinical examination to assess caries status and to determine an appropriate orthodontic treatment plan. An intraoral exam, review of intraoral radiographs, medical history, and definitive dental history were also completed.

Inclusion criteria to be eligible for the study were a subject age of 12 to 18 years, being in orthodontic treatment, and scheduled for extraction of bicuspids for orthodontic treatment reasons. The teeth had to be noncarious and not restored on the buccal surface. Subjects had to be willing to comply with all study procedures and protocols. They had to be residents of San Francisco or other nearby local communities with water fluoridation (to eliminate water fluoridation as a potential confounding variable). Subjects had to be healthy and willing to sign the “Authorization for release of personal health information and use of personally unidentified study data for research” form. There were no gender restrictions.

Subjects were excluded from the study if they were suffering from systemic diseases, had a significant past or medical history with conditions that may affect oral health (i.e., diabetes, HIV, heart conditions that require antibiotic prophylaxis), were taking medications that may affect the oral flora or salivary flow (e.g., antibiotic use in the past three months, drugs associated with dry mouth / xerostomia), had in-office fluoride treatment within the last three months prior to being enrolled in the study, or were not willing to stop the use of any mouth rinse during the duration of the study.

Subjects who met the selection criteria were asked to provide verbal assent/consent and their parent/guardian to provide written informed consent.

Twenty-four subjects were recruited for the study, comprising 13 females and 11 males with an average age of 14.9 ± 2.2 years. Twelve subjects were randomly selected for the 4-week and twelve for the 12-week study arm. The average age for the 4-week subjects was 14.6 ± 2.3 years and the average for the 12-week group was 15.2 ± 2.1. The average age for both groups was not significantly different (P > 0.5, t-test).

2.2 Study Procedure

After enrollment, brackets were bonded with a conventional light cured composite resin (Transbond XT, 3M Unitek, REF 712-035), as previously described, onto the buccal surface of the bicuspid scheduled for extraction. An enamel area directly next to the bracket at the cervical area of the tooth was treated according to the laser treatment protocol (see below).

The participants were instructed to brush twice daily with a provided dentifrice containing 1100 ppm fluoride as NaF for one timed minute each brushing. They were asked to fill in a log of their daily tooth-brushing schedule. Free tubes of toothpaste were distributed and weighed before and after the study to crosscheck compliance. Further, the study coordinator called the households twice a week to verify compliance and offer support when necessary.

2.3 Laser Treatment Protocol

The laser used in the study was a CO2-laser, Pulse System, Inc. (PSI) (Model #LPS-500, Los Alamos, New Mexico), wavelength 9.6 μm, pulse duration 20 μs, pulse repetition rate 20 Hz, beam diameter at focus 1100 μm delivered through a straight laser handpiece. The goal was to irradiate each spot of the testing area with 20 laser pulses. The laser fluence per pulse used in this study averaged 4.1 ± 0.3 J/cm2 (range 3.3 to 4.4 J/cm2).

The laser treated area was cervical to the bracket on one side of an imaginary line perpendicular through the slot of the bracket, while the opposite side to this line on the same tooth served as the control side. The area of the surface to be irradiated was measured and the number of laser pulses and the irradiation time, respectively, was calculated (Fig. 1). The laser irradiation was performed using a straight laser handpiece. High volume evacuation was used and a water coolant was not applied. The laser irradiation of the testing area, as described above, occurred only once during the study period.

2.4 Laboratory Microhardness Testing to Evaluate ΔZ Mineral Loss

The bicuspid were carefully extracted four or twelve weeks after irradiation, respectively. They were cut into halves using a custom-made high-speed microtome. The cut was vertically positioned through the bracket separating the laser treated area from the nontreated control area (Fig. 2). Teeth from all 24 subjects were sectioned in this way and embedded in epoxy resin with the cut surface exposed, and serially polished to ensure the tested area was in the laser treated or control, nonirradiated region, respectively.

Prior to microhardness testing (Fig. 3), a technician not directly involved in the study coded each half of an extracted tooth to insure blinding of the laboratory investigator. The overall relative mineral loss, ΔZ, for each sample was calculated by creating a hardness profile curve by plotting normalized volume percent mineral against distance from the enamel surface. The area under the curve that represents ΔZ (vol% mineral × μm) was calculated using Simpson’s integration rule. Also,
the individual ΔZ values for each lesion in each group were combined to give a mean ΔZ and standard deviation.

3 Results
3.1 Mineral Loss Profile for 4- and 12-week Study Arms

In Fig. 4 the volume percentage mineral of enamel is plotted versus the depth from the outer surface resulting in a mineral loss profile for the samples of the 4-week study arm. Each symbol on each curve represents the mean vol% mineral at each depth measured for the 12 laser treated areas and the 12 other nonlaser treated controls. The error bars represent standard error. At a depth of 15 μm, the control teeth (square dots) show an average vol% mineral of only 40%, which increases to an average of 82% at a depth of 45 μm. In contrast for the laser treated enamel (triangular symbols), the average vol% mineral at the 15-μm depth is still 62% and increases to the typical vol% mineral content of sound enamel (85% volume mineral) at the depth of 45 μm.45

Figure 5 presents the mineral loss profile for the 12-week study arm and the controls, respectively. The control group had a mean vol% mineral of only 35% at the outer 15-μm depth, increasing to an average of 72% at a depth of 45 μm and reached 85% at the depth from the surface of 75 μm. In contrast, the laser treated enamel in the 12-week arm (triangle symbols) had a mean vol% mineral of 72% at the 15-μm depth and the mineral content was already 85% at a depth of 25 μm.

Fig. 1 Orthodontic bracket placed on the study bicuspids using a composite resin (Transbond XT); an area to be irradiated cervical to the bracket is marked.

Fig. 2 Four or twelve weeks after irradiation the bicuspids were extracted; for quantitative assessment of demineralization by cross-section microhardness testing to evaluate the relative mineral loss ΔZ (vol% × μm), the teeth were cut into halves separating the laser irradiated area (L) from the nonirradiated control area (C).

Fig. 3 Cross-section microhardness testing: The cross-section of a bicuspid is shown, presenting dentin (D), enamel (E), and the composite (Transbond XT) (C), which was used to glue the orthodontic bracket (B) onto the enamel surface; the lines of micro-indentations (M) were placed right below the enamel surface following a distinct distribution pattern; they are located directly below the area where the metallic orthodontic bracket (B) was fixed to the tooth with a composite (C); this area is where the microbial plaque challenge is most likely to cause demineralization.
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3.2 Overall Relative Mineral Loss, ΔZ, 4- and 12-week Arms

In the 4-week arm the mean relative mineral loss, ΔZ (vol% × μm), for the laser treated enamel group for all subjects was 402 ± 85 (SE) while the control area showed a much higher relative mineral loss of 737 ± 131 (SE). The differences were statistically significant at the P = 0.04 value level (unpaired t-test). The laser treatment produced a 46% demineralization inhibition around the orthodontic brackets in comparison to the nonlaser treated control group (Fig. 6).

For the 12-week arm (Fig. 7), the mean relative mineral loss was ΔZ 135 ± 98 (SE) while the control group showed a comparatively very high relative mineral loss ΔZ of 1067 ± 254 (SE). This difference was statistically significant at P = 0.002 value level (unpaired t-test). For the 12-week arm, the laser treatment produced a marked 87% demineralization inhibition.

4 Discussion

In the past, several laboratory studies have shown that enhancing enamel demineralization resistance can be achieved by irradiation with CO₂-lasers emitting laser pulses in the microsecond range. The wavelengths absorbed most strongly in dental enamel are the 9.3- and 9.6-μm CO₂-laser wavelengths. The loss of the carbonate phase from the enamel crystals due to the irradiation heat is reported to be responsible for the reduction in acid dissolution of enamel.

The orthodontic bracket model used in this study has been proven to present a high caries demineralization challenge to the enamel. It has been shown that this demineralization challenge cannot simply be overcome by using 1100-ppm fluoride toothpaste. Gorton reported in her study using the orthodontic bracket model that the mean mineral loss value (ΔZ) in the control group was 805 ± 78 (SE) vol% × μm demonstrating considerable measurable demineralization in just four weeks even with the use of a fluoride dentifrice.

Comparable to the Gorton Study, in our study the subjects showed a very similar mineral loss in the control regions of the teeth adjacent to the brackets, namely a mean ΔZ of 737 ± 131 (SE) vol% × μm in the 4-week arm and even higher at 1067 ± 254 (SE) vol% × μm in the 12-week arm, respectively. The mean mineral loss for the control groups for both study arms in the present study were not significantly different (P = 0.26 value level, t-test).

As in the Gorton study, the twice per day application of the 1100-ppm fluoride toothpaste could not overcome the demineralization challenge alone. However, the application of the laser irradiation significantly reduced the mineral loss to a mean ΔZ value of 402 ± 85 (vol% × μm) in the 4-week study comparable to, and in, the 12-week arm with ΔZ 135 ± 98 (SE) even slightly lower than Gorton’s test group (glass ionomer...
fluoride-containing cement) with a mean $\Delta Z$ value of 160 ± 80 (SE). The difference in mineral loss between the 4- and 12-week laser treated groups showed a tendency to be statistically significant ($P = 0.052$, t-test). While the mineral loss for the controls for the 12-week group was higher than for the 4-week group, the smaller mineral loss for the treatment group after twelve weeks in comparison to after four weeks might be explained by enhanced remineralization over a longer observation time period.

The quantitative assessment of demineralization by cross-sectional microhardness testing of laser treated enamel revealed that using a 9.6 $\mu$m CO$_2$-laser irradiation (20 $\mu$s pulses) significantly inhibits the formation of carious lesions around orthodontic brackets. Our study showed, to the best of our knowledge, that for the first time in vital teeth in human mouths, this irradiation scheme reduces enamel mineral loss by up to 46% over a time period of four weeks. Evaluating the caries resistance enhancement capacity of the CO$_2$-laser treatment over twelve weeks, at which time there was an 87% reduction in mineral loss in comparison to the control surfaces, might in addition be related to an enhancement of remineralization due to the laser irradiation. At the same time, demineralization for the controls, of course continued to become more severe.

This study showed that caries inhibition demonstrated in numerous models and experiments in the laboratory$^{9,49-51}$ can also be achieved in humans in vital teeth using short-pulsed 9.6-$\mu$m CO$_2$-laser irradiation.

Moreover, this study demonstrates that the orthodontic bracket model can successfully be used to investigate several agents that can inhibit the caries challenge in living teeth in humans.

Using the same laser irradiation conditions in a “pulpal safety study” on teeth in humans, we provided evidence that there is no harm to the pulpal tissue of those irradiated teeth.$^{52}$ Further clinical studies will verify the efficacy of the CO$_2$-laser irradiation on acid resistance of human enamel. Further studies to ascertain the efficiency of treating fissures to reduce demineralization with the short-pulsed CO$_2$-laser are also needed.$^{5}$

5 Conclusion

This study shows, to the best of our knowledge, for the first time in vivo, that the specific short-pulsed 9.6-$\mu$m CO$_2$-laser irradiation can be successfully used for the inhibition of dental caries in enamel in humans.

Acknowledgments

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References


