The effects of argon laser irradiation on enamel decalcification: An in vivo study

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Enamel decalcification is a significant problem in orthodontic patients. The argon laser has been shown to reduce decalcification during an acidic challenge in vitro. The purpose of this study was to investigate the in vivo effects of argon laser irradiation on enamel decalcification during orthodontic treatment. Nine volunteers whose treatment plans included 4 first premolar extractions were enrolled in the study. The 36 extracted premolars were assigned to 1 of the following 4 groups: group 1, control group with no treatment; group 2 (pumice-laser), teeth were pumiced for 3 seconds and treated with a 325 mW, 5-mm diameter laser beam for 60 seconds; group 3 (pumice-etch-laser), teeth were pumiced for 3 seconds, acid-etched with 30% phosphoric acid for 30 seconds, and treated for 60 seconds with laser; and group 4 (laser only), teeth were treated for 60 seconds with laser. A specially designed (oversized) orthodontic band was fitted on each of the premolars to create a pocket for decalcification. The bands were cemented in place for 5 weeks. After extraction, the teeth were sectioned and examined under polarized light microscopy. Images of lesions were digitally analyzed and measured. Average lesion depths were calculated from 3 depth measurements recorded 10 µm apart. Average lesion area was calculated with the aid of imaging analysis software. Data were analyzed with analysis of variance (P < .05) and Student t tests. Significant differences were found in lesion depth (P < .001) and lesion area (P < .01) among the 4 test groups. The average lesion depths were 15.93 \pm 9.31 μ m (control), 6.45 \pm 8.70 μ m (pumice-laser), 1.71 \pm 4.82 μ m (pumice-etch-laser), and 1.34 \pm 3.80 μ m (laser only). The average lesion areas were 1028.67 \pm 725.68 μ m² (control), 555.49 \pm 948.20 μ m² (pumice-laser), 79.91 \pm 226.03 μ m² (pumice-etch-laser), and 55.71 \pm 157.59 μ m² (laser only). The average lesion depth in the laser-only group was reduced by 94.1% and the average lesion area was reduced by 94.4% when compared with the control group. In the pumice-etch-laser group, the average lesion depth was reduced by 89.1% and the average lesion area was reduced by 92.2% when compared with the control group. There were no significant differences in lesion depth and lesion area between maxillary and mandibular teeth (P < .06 and P < .08, respectively) and between the teeth on the right and left sides (P < .08) .68 and P < .55, respectively). These results show that argon laser irradiation is effective in reducing enamel decalcification during orthodontic treatment. Pumicing and etching do not appear to reduce the effect of laser on enamel solubility. (Am J Orthod Dentofacial Orthop 2002;122:251-9)

namel decalcification or formation of white spot lesions during orthodontic treatment presents a significant problem in orthodontic patients.

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Submitted, November 2001; revised and accepted, February 2002.

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0889-5406/2002/\$35.00 + 0 8/1/126596

doi:10.1067/mod.2002.126596

Fixed orthodontic appliances complicate the removal of food debris that results in the accumulation of plaque. Several studies have found an increased amount of plaque around orthodontic appliances.^{1,2} Others have reported an increase in the number of Streptococcus mutans and Lactobacillus species in the oral cavity after the placement of fixed orthodontic appliances.³ Plaque bacteria produce organic acids that cause the dissolution of calcium and phosphate ions from the enamel surface. This dissolution can cause white spots or early carious lesions to form in as little as 4 weeks.⁴⁻⁷ If the diffusion of ions away from the tooth surface continues, cavitation of the enamel surface will result. Patients undergoing orthodontic fixed-appliance therapy are at an increased risk for enamel decalcification, which can present esthetic and restorative problems to both patient and dentist.

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During preparation of a tooth for bonding of orthodontic brackets, pumicing removes 7 to 20 µm of the outer enamel surface, and acid etching removes additional surface enamel from the enamel rods, leaving a roughened surface.⁸⁻¹⁰ Several methods have been implemented to prevent or reduce enamel decalcification during orthodontic treatment, including fluoride application in various forms, enamel sealants, rigorous oral hygiene regimens, and modified appliance designs.^{7,10} The use of the argon laser in dentistry has been proposed for polymerization of resin materials and bleaching of enamel.^{11,12} It has been shown to be safe for the intraoral polymerization of composite materials at low fluence levels.¹³ Recent studies suggest that the argon laser can be used to prevent enamel decalcification by altering the crystalline structure of enamel.¹⁴⁻¹⁶ Blankenau et al¹⁶ reported the first results on in vivo effects of argon laser irradiation on human enamel decalcification. The study found a 29.1% reduction in average lesion depth compared with the control group. It is not clear whether pumicing and acid etching before laser irradiation have any effect on enamel solubility. The objective of this study was to investigate the effects of argon laser irradiation on enamel decalcification during orthodontic treatment in vivo. In particular, we hypothesized that, compared with the control, argon laser irradiation would significantly reduce the amount of decalcification on (1) nonpumiced, nonetched enamel; (2) pumiced enamel; and (3) pumiced and etched enamel.

MATERIAL AND METHODS Sample description

Nine patients who required 4 premolar extractions as part of their comprehensive orthodontic treatment were included in this study. Participants were recruited from the Department of Orthodontics at the West Virginia University School of Dentistry. The criteria for patient selection included teeth without enamel defects or decalcification. The in vivo study received approval by the institutional review board at West Virginia University before the initiation of the project.

Assignment of teeth to treatment groups

The 36 extracted premolars were stored in 10% thymol solution and assigned to 1 of 4 groups as shown in Table I and Figure 1: group 1 (control), no treatment; group 2 (pumice-laser), teeth were pumiced for 3 seconds and exposed to argon laser irradiation with an energy density of 100 J/cm² for 60 seconds; group 3 (pumice-etch-laser), teeth were pumiced for 3 seconds, etched (Gel Etching Agent, Reliance Orthodontic Products, Itasca, III) for 30 seconds, and exposed to argon

Table I. Assignment of premolars to 4 test groups

Patient no.	Location of premolars					
	Upper right	Upper left	Lower right	Lower left		
1	Gp 1	Gp 2	Gp 3	Gp 4		
2	Gp 4	Gp 1	Gp 2	Gp 3		
3	Gp 3	Gp 4	Gp 1	Gp 2		
4	Gp 2	Gp 3	Gp 4	Gp 1		
5	Gp 1	Gp 2	Gp 3	Gp 4		
6	Gp 4	Gp 1	Gp 2	Gp 3		
7	Gp 3	Gp 4	Gp 1	Gp 2		
8	Gp 2	Gp 3	Gp 4	Gp 1		
9	Gp 1	Gp 2	Gp 3	Gp 4		

Gp, group

laser irradiation with an energy density of 100 J/cm^2 for 60 seconds; and group 4 (laser only), teeth were exposed to argon laser irradiation with an energy density of 100 J/cm² for 60 seconds.

Tooth preparation and band fabrication

The facial surface of each tooth was cleaned for 10 seconds with a cotton-tip applicator and rinsed with sterile water for 5 seconds. For each tooth, a specially designed band was fabricated as shown in Figures 2 and 3. The bands were standard plain orthodontic premolar bands (Snap-Fit Bicuspid Bands, GAC International, Islandia, NY). Two sections of 0.040-in stainless steel wire (posts) were welded to the internal surface of the facial portion of each band. The posts created a pocket for the accumulation of plaque and food debris. During cementation, a small piece of cotton was placed between the posts on the facial part of the bands to prevent cement from leaking into the pocket. Each band was cemented with carboxylate cement (Durelon, ESPE America Inc, Norristown, Pa), and the cotton piece was removed. Each band was seated on the tooth, and the gingival border of the facial portion of the band was no more than 1 mm from the gingival margin.

Laser use

A laser (AccuCure 3000, Laser Med, Salt Lake City, Utah) was used with a 5-mm diameter beam directed through a handheld wand. The wand tip was positioned approximately 3 mm from the facial surface of the treated teeth. The 325-mW beam was activated for 60 seconds during each treatment; it delivered an energy density of approximately 100 J/cm². The laser was calibrated with a calibration meter built into the laser before use on each patient. The fluence (energy density) was calculated by using the following equations:

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Fig 1. Flowchart of experimental procedures.



Fig 2. Schematic drawing of band used in this study.

Fluence (J/cm^2) = energy $[(J/s)/cm^2] \cdot$ exposure time(s)

Energy $[(J/s)/cm^2] = watts (J/s)/area (cm^2)$

Area of a circle $(cm^2) = \pi r^2$ where *r* is the radius of the circle in centimeters.

All treatment was recorded in patient charts. For all teeth in each group, the bands remained cemented for 5 weeks. Each patient was given an information form after the treatment appointment. The form included instructions for oral hygiene and contact phone num-



Fig 3. Photograph of band designed for this study.

bers if a band became loose, lost, or damaged. The teeth were then extracted. After the extractions, each tooth was immediately placed in individually labeled glass vials with 10 mL of distilled water.

The specially designed bands were removed and discarded in a labeled biohazard container. The teeth were placed in separate, labeled glass jars with 30 mL of distilled water. The glass jars with the teeth and the



Fig 4. Facial portion of crown was sectioned from roots and lingual portion (*shaded*) of crown.

labeled glass vials were sterilized in a steam autoclave with a liquid cycle (VacoMatic Series 3000, American Sterilizer Corp, Erie, Pa) before handling. After sterilization at 121°C for 45 minutes, the presence of a lesion was confirmed visually by the appearance of a frosty white enamel surface when dried and examined with $10 \times$ magnification. A circle was drawn around the lesion with a red permanent marker to guide the operator while sectioning. The teeth were replaced in the glass vials with 10 mL of distilled water and stored at 4°C until sectioning.

Sectioning of teeth

The roots and lingual cusps were sectioned from the crowns with a high-speed hand piece and a carbide bur to minimize the amount of tooth structure for sectioning (Fig 4). The remaining facial portions (buccal cusp and facial surface) were replaced in the glass vials and stored in a 10% thymol solution and stored at 4°C until final sectioning. A separate 1-in section of 0.5-in diameter copper tube was used for handling each sample while completing the final sectioning. The samples were mounted in the copper tubes with dental sticky wax to facilitate sectioning with a low-speed saw (Beuhler, Lake Bluff, Ill). The samples were mounted with the occlusal portion of the tooth embedded in the wax. The copper tube with the sample was mounted in the low-speed saw with the occlusal portion oriented parallel to the saw blade and clamped into place on the saw chuck. A 4-in diameter diamond-wafering saw blade (No. 11-4244 series 15HC, Beuhler) was used to section the slice of tooth. Each cut was made in a mesiodistal direction starting from the mesial or distal surface of the sample (Fig 5). The cuts were made through the red circle drawn around the lesion. The final samples were thinned to 100 µm for viewing with the polarized light microscopy. The samples were thinned by hand with 600-grit and 400-grit silicon carbide sandpaper (3M Corp, St. Paul, Minn) and



Fig 5. Orientation of tooth section in copper tube and direction of saw cuts. Dark circle represents area of suspected lesion.



Fig 6. Lesion depth measured at first (d_1) , second (d_2) , and third (d_3) lines, 10 μ m apart.

distilled water until each was approximately $100-\mu m$ thick.

Polarized light microscopy

All samples were mounted on microscope slides with distilled water and evaluated at $40 \times$ magnification under a polarized light microscope (Zeiss Axiovert, Thornwood, NY). Digital images (MagnaFire digital microimaging camera, Olympus, Hamburg, Germany) of the lesions were saved to a computer disk. The digital images were examined and measured (Optimas 6.2 software, Optimas Corp, Bothell, Wash). A standard calibration slide was used to construct a 0.7-mm line on the computer screen. The spatial calibration of the software was programmed to correlate 0.1 mm to equal 100 μ m. All lesion depth measurements were automatically converted to micrometers by the software. The measurements were recorded on the lesion data form.

In the samples where the enamel surface was damaged or worn away, a line was drawn on the image that was roughly parallel to the base of the lesion in sound enamel with the edge of the lesion as a guide.



Fig 7. Photomicrograph of control group (Group 1) lesion at $40 \times$ magnification under polarized light.



Fig 9. Photomicrograph of pumice-etch-laser group (Group 3) lesion at $40 \times$ magnification under polarized light.



Fig 8. Photomicrograph of pumice and laser group (Group 2) lesion at $40 \times$ magnification under polarized light.

The lesion depth measurements were drawn perpendicular to this line. Lesion depth was measured by taking the average of 3 depth measurements (Fig 6) that were 10 μ m apart. An acetate template with a 10- μ m grid was used to construct the lines 10 μ m apart. The template was placed on the computer screen with 1 edge on the first line and the second and third lines were drawn according to the grid. All measurements were performed twice by the same examiner



Fig 10. Photomicrograph of laser-only group (Group 4) lesion at $40 \times$ magnification under polarized light.

(A.M.A.) 2 weeks apart. No significant differences were found between measurements. Lesion area was calculated with the software by selecting the area morphometry measurement. A line that followed the border of the lesion and the projected surface outline was drawn with the cursor on the computer screen. The computer was calibrated as stated above. The computer automatically converted the area inside the outline of the lesion to square micrometers. Lesion area was recorded on the lesion data form.

Groups	Average lesion depth (µm)	Reduction of lesion depth compared with control	Average lesion area (μm²)	Reduction of lesion area compared with control
1 (control)	15.69 ± 9.30		1028.67 ± 725.68	
2 (pumice-laser)	6.45 ± 8.70	58.9%	555.49 ± 948.20	46.0%
3 (pumice-etch-laser)	$1.71 \pm 4.80^{*}$	89.1%	$79.91 \pm 226.03^*$	92.2%
4 (laser only)	1.34 ± 3.80*	91.6%	55.71 ± 157.59*	94.6%

Table II. Average and reduction in lesion depths and areas for test groups

*, Groups significantly different from control at P < .05

Table III. Analysis of variance comparison of lesion depth between maxillary and mandibular teeth and right and left teeth

Source	DF	Sum of squares	Mean square	F ratio	Probability >F
Model					
Max vs mand	1	281.97188	281.97188	3.7599	0.0623
Right vs left	1	12.94133	12.94133	0.1726	0.6809
Error	29	2174.8240	74.994		
Cumulative total	31	2469.7372			

DF, Degrees of freedom; Max, maxillary; mand, mandibular.

Data analysis

The mean and SD of the average lesion depth and lesion area were recorded and compared with the treatment method. Analysis of variance (ANOVA) statistic analysis was used to determine significant differences (P < .05) between the average lesion depth and average lesion area of each treatment method. ANOVA was also used to determine significance in relation to the location of the tooth in the maxilla or the mandible and on the left or the right side.

RESULTS

Photomicrographs of a typical lesion in the 4 groups are shown in Figures 7 to 10. The average lesion depth and lesion area for the 4 test groups are shown in Table II. ANOVA showed significant differences among the lesion depths (P < .001) and lesion areas (P < .01) for the 4 test groups. Significant differences were found between the pumice-etch-laser and the control groups (P < .05) and between the laser-only and the control groups (P < .05). No significant differences were found between the pumice-laser and the control groups. The pumice-etch-laser group showed a reduction in lesion depth of 89.1% and a reduction in lesion area of 92.2% when compared with the control group. The laser-only group showed a reduction of 91.4% in lesion depth and lesion area of 94.6% when compared with the control group. In the control group, lesions were found in all samples. In the pumice-laser group, lesions were found in 4 of 8 (50%) samples. In both the pumice-etch-laser group and the laser-only group, lesions were found in only 1 of 8 (12.5%) samples. ANOVA comparisons of lesion depth between the maxillary and mandibular teeth and between the teeth on the right and left sides are shown in Table III. ANOVA comparisons of lesion area between the maxillary and mandibular teeth and the teeth on the right and left side are shown in Table IV. No significant differences were found between lesion depth and lesion area for the maxillary and mandibular teeth (P < .06 and P < .08, respectively) and between the teeth on the right and left sides (P < .68 and P < .55, respectively).

DISCUSSION

In this study, the use of the argon laser alone was found to reduce lesion depth by 91.4% and lesion area by 94.6% when compared with the nonpumiced, nonetched control. In 7 of the 8 samples in the laser-only group, no lesions were found. Pumicing and etching the enamel before lasing reduced the lesion depth by 89.1% and the lesion area by 92.2% when compared with the control group. In 7 of the 8 samples in the pumice-etchlaser group, no lesions were found.

Blankenau et al¹⁶ were the first to report on the in vivo effects of argon laser irradiation on human enamel decalcification. The study found a 29.1% reduction in average lesion depth when comparing the lased and control samples. Several differences exist

Source	DF	Sum of squares	Mean square	F ratio	Probability >F
Model	1				
Max vs mand	1	1553468	1553468	3.3160	0.0786
Right vs left	1	185011	185011	0.3599	0.5531
Error	30	14054317	468477		
Cumulative total	31	15607784			

Table IV. Analysis of variance comparison of lesion area between maxillary and mandibular teeth and left and right teeth

DF, Degrees of freedom; Max, maxillary; mand, mandibular.

between the that study and this one with the main difference being the lesion incidence. In the study by Blankenau et al.¹⁶ lesions were found in all samples. In this study, lesions were detected in all control samples, but, in most of the experimental samples, no lesions were found. Average lesion depths of the experimental groups in this study were less than those in the study by Blankenau et al.¹⁶ This could be related to the diameter of the post wire used in the fabrication of the band-0.032 in for their study and 0.040 in for this studywhich gave a larger pocket size that might have allowed for removal of plaque and food debris during oral cleansing. Blankenau et al¹⁶ used a laser (Model 5, HGM, Salt Lake City, Utah) with a 250-mW and 5-mm diameter beam for 10 seconds. We used a different laser (AccuCure 3000, Laser Med) with a 350-mW and a 5-mm diameter beam for 60 seconds. The calculated fluence for the study by Blankenau et al¹⁶ was approximately 12 J/cm² and for this study was approximately 100 J/cm². These differences might influence the quality of caries resistance.

The results in this in vivo study are supported by several in vitro demineralization studies. Hicks et al¹⁷ found a 31% reduction in lesion depth, and Flaitz et al¹⁸ found a 34% reduction, when comparing laser-treated groups with the control groups; all samples were cleaned with fluoride-free pumice before treatment. Powell et al¹³ and Yu et al¹⁹ found a 50% reduction in lesion depth when comparing lased with control groups; all samples were cleaned with fluoride-free pumice before treatment. Schouten et al²⁰ found an 11.7% reduction in lesion depth when comparing non-pumiced lased with control groups.

According to Stookey,⁸ and Thompson and Way,⁹ pumicing enamel for 3 seconds with a slow-speed hand piece removes the outer 7 to 20 μ m of enamel. Acid etching changes the shape of the dentinal tubules, alters the organic matter, and decalcifies the inorganic component of the surface enamel.²¹ The outer 20 to 30 μ m of enamel have greater mineral concentrations than do deeper layers. This mineral-rich outer layer increases

the enamel resistance to demineralization.²² In this study, prepackaged fluoride-free pumice was used in a rubber prophy cup with a slow-speed hand piece for 3 seconds, and 37% phosphoric acid gel was used for 30 seconds. The study found that despite the removal of these protective outer layers of enamel and the demineralization of these layers, the effect of laser irradiation still made the enamel less susceptible to decalcification than the control. One explanation might be that enamel exposure to argon laser irradiation increases not only the resistance to demineralization but also the uptake of minerals from solution (saliva).^{18,23-27} This theory is supported by the findings of several scanning electron miscroscopy studies.^{21,23,27,28} These studies found that the argon laser alters the surface characteristics of the enamel by creating microspaces that trap ions during an acid attack rather than allowing them to escape.²³ The free ions form globular precipitates that occlude the microspaces.²⁸ The calcium, phosphate, and fluoride ions in saliva might provide a protective effect by being incorporated into the enamel surface.^{17,18,24} This theory is supported by an in vitro study that reported the protective effect of laser irradiation in both caries initiation and progression.²⁹

An optimal fluence or energy for the argon laser has not been established by either manufacturers or research studies. The amount of laser irradiation or fluence used in most of the previously mentioned studies was similar to the irradiation used in this study (approximately 100 J/cm²). The exceptions are the in vitro study by Hicks et al¹⁷ and the in vivo study by Blankenau et al¹⁶; both used a fluence of 12 J/cm² and found a 31% and 29.1% reduction in lesion depth, respectively. Flaitz et al¹⁸ found a 34% reduction in lesion depth compared with controls with 100 J/cm² or argon laser irradiation. Powell et al¹³ used 120 J/cm² of laser irradiation and found a 50% reduction in lesion depth. Likewise, Yu et al¹⁹ found a 50% reduction in lesion depth at 120 J/cm² and a 36% reduction at 60 J/cm2. In 1999, Schouten et al²⁰ used 127 J/cm² and found an 11.7% reduction in lesion depth.

The wavelength of the emitted light determines the effects of the irradiation. The 470 to 488 nm (blue) wavelengths produced by the argon laser are needed for the photoactivator that causes polymerization of resin in light-cured composite restorative materials. The 502 to 514 nm (green) wavelengths interact well with hemoglobin and melanin and are used in surgical lasers for their excellent hemostatic properties.³⁰ Recent studies examining the effects of wavelength on the prevention of demineralization found no differences between lasers produced by the blue and the green wavelengths.²⁹⁻³¹ The studies by Powell et al,¹³ Blankenau et al,¹⁶ Hicks et al,¹⁷ and Yu et al¹⁹ used a laser that produced argon laser irradiation with peaks in the blue-green wavelength of the spectrum (457-514 nm). The study by Schouten et al²⁰ used a laser that did not have the wavelengths at the green end of the spectrum (501-514 nm). Our investigation used the laser that has the blue-green wavelength of the spectrum.

The method of demineralization used also varies among the studies. The in vivo technique used in the current study was similar to that used by Blankenau et al¹⁶ and Ogaard et al³². This technique used specially designed bands that were well adapted to the lingual and proximal surfaces. The bands were held away from the facial surfaces by 0.040-in wire posts welded to the internal band surface. The posts created a pocket to collect plaque and food debris. The bands remained cemented for 5 weeks, which is longer than the 4 weeks needed to create a lesion in vivo.4-7,33 The studies by Powell et al,¹³ Yu et al,¹⁹ and Schouten et al²⁰ used a rotating disk apparatus in the demineralizing process that created a lesion in 24 hours. The studies by Hicks et al¹⁷ and Flaitz et al¹⁸ used an acidified gel to create a lesion in 6 weeks. It is possible that the demineralizing regimens affected the quantity and the quality of the demineralization.

This investigation did not show a significant difference between the pumice-only samples and the control. It was thought that the laser irradiation would protect the pumiced enamel in the same manner that it protected the pumiced, etched enamel. Possible explanations for this contradiction might be (1) the variability of plaque removal and diet among study participants, (2) the inherent resistance of certain teeth to demineralization, (3) salivary buffering capability factors, and (4) fluoride exposures from food, water, and toothpaste.³⁴⁻³⁶

This study did not find a significant difference in the average lesion depth when comparing the locations of the teeth before extraction (maxillary vs mandibular, right vs left). No significant difference was expected because studies have shown that the premolar area generally has a low prevalence of decalcification in vivo. $^{\rm 37-39}$

In the current study, the percentage of samples with lesions differed among the groups. Lesions were found in 100% of the control samples and in only 50% of the pumice-laser samples. Lesions were found in 12.5% of samples of the pumice-etch-laser and laser-only groups. The low incidence might be because the experimental teeth (groups 2, 3, and 4) had smaller lesions that might have been destroyed during the sectioning and thinning process to yield sections that were approximately 100 µm-thick. Teeth in the control group, on the other hand, had large lesions that might not have been affected by the sectioning and thinning process. Another explanation is that the effects of the laser inhibited the formation of lesions by increasing the rate of uptake of minerals including fluoride. This resulted in remineralization of the lesion. In vitro studies showed that fluoridated irradiated enamel and organic matrix significantly reduce demineralization compared with nonfluoridated counterpart. The presence of fluoride and heat from laser improves fluoroapatite formation.^{40,41}

CONCLUSIONS

The following conclusions can be made from this study: (1) argon laser irradiation is effective in reducing enamel decalcification during orthodontic treatment and (2) pumicing and etching before laser treatment do not appear to reduce the effect of laser on enamel solubility.

The authors thank Dr Hera Kim for her organization of the research ideas.

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