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MICROHARDNESS AND SCANNING ELECTRON MICROSCOPY ANALYSIS OF Nd:YAG LASER AND ACID TREATMENT EFFECTS IN DENTIN

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Abstract

Laser irradiation at and above parameters producing the modification threshold for dentin causes structural changes to the dentin surface. This study determined the microhardness of dentin before and after laser modification and acid treatment using a repeated-measures design. Seven dentin sections (4 mm in thickness) were cut from freshly extracted non-carious third molars using a diamond saw. The middle occlusal third was used as the test dentin surface. One section served as a control (C); three received laser modification (L) and then acid treatment (L + A); and three received acid (A) and then laser treatment (A + L). Laser modification was made using a pulsed (120 μs) fiber-optic-delivered (500 μm diameter) Nd:YAG (λ = 1.06 μm) laser at the physical modification threshold of 207 J/cm^2. Acid treatment consisted of 10% nitric acid applied for 45 seconds. Twenty Knoop indentation microhardness measurements (KHN) were obtained using 300 g force engaged for 15 seconds for each section before and after each treatment (n = 400). Knoop microhardness values recorded: C = 62 $\pm$ 3; L = 149 $\pm$ 35; A = 24 $\pm$ 5; L + A = 40 $\pm$ 16; and A + L = 33 $\pm$ 5. Multifactor-repeated measures, with analysis of variance (ANOVA; $p \leq 0.05$), indicated significant differences between all treatment groups. Scanning electron microscopy analysis of dentin surfaces documented unique surface morphology for all treatment conditions. Laser modification of dentin before or after acid treatment increased dentin microhardness.

Key Words: Microhardness, Nd:YAG laser, dentin.

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Introduction

Application of Neodymium:Yttrium-Aluminum-Garnet (Nd:YAG) pulsed infrared laser energy to dentin, using parameters at and above the physical modification threshold, has been documented to produce physical and chemical changes of the surface layer (White *et al.*, 1993a,b). The physical modification threshold is dependent on specific laser parameters (wavelength, pulse duration and energy density) at which the first observable interaction occurs causing macro changes in the dentin structure. This threshold effect has been documented as a function of laser exposure, and laser parameters have been correlated to morphological changes on the dentinal surface (Goodis *et al.*, 1989; Nammour *et al.*, 1992; Shariati *et al.*, 1993). Studies have further determined that laser-modified sound dentin demonstrates increased surface microhardness and that laser modification increases bond strength of composite resin restorations to dentin (Cooper *et al.*, 1988; White *et al.*, 1991a). Goodis and others found that laser modification also reduces bacteria on contaminated dentin surfaces (White *et al.*, 1991b; Marshall, 1993; Goodis *et al.*, 1994). Alternate studies considering the microhardness of demineralized dentin and enamel have determined that a linear correlation exists between volume percent mineral content and microhardness, microhardness decreasing as mineral content decreases (Davidson *et al.*, 1974; Featherstone *et al.*, 1983).

Microhardness

Arends and ten Bosch (1992) reviewed 10 techniques currently in use to measure demineralization and remineralization and found the most practical techniques for quantitative measurement to be microhardness testing (both Knoop and Vickers), light scattering and microradiography. Panighi and G'Sell (1992) measured microhardness and wettability and found positive correlations between the degree of mineralization, dentin compactness and hardness. Panighi and G'Sell (1993) reported strong correlations between microhardness and shear bond strength and found that microhardness is also strongly correlated to calcium concentration.

Laser effects

Laser-produced effects in dentin can be efficacious when they create a protective barrier. Tay *et al.* (1994) examined surface morphology and observed, as a natural phenomenon, the sealing of exposed dentin tubules as a tissue-protective strategy in the inherent process of arresting caries. Using scanning electron microscopy (SEM), they further observed the dynamics of chemical adhesion which imitates the natural protective process by forming "an outer zone" which acts as a sealant. Laser energy has been documented to create this sealing process through the surface melting of dentin. Additionally, researchers found laser-exposed dentin fundamentally altered. Lobene *et al.* (1968) observed the destruction of the organic contents of the dentin tubules following exposure from a CO_2 laser. Scheinin and Kantola (1969) reported increased mineralization and Kantola (1972) described recrystallization of enamel and dentin following CO_2 exposure.

Melcer *et al.* (1984) found that carious dentin demonstrated increased resistance to chemical or physical demineralization. Nelson *et al.* (1986) found the CO_2 laser effective in the inhibition of caries formation. In 1987, Featherstone and Nelson used the CO_2 to effect enamel surface fusion and inhibit the progression of demineralization due to acid attack.

Similarly, the Nd:YAG has been observed to create a sealed tooth surface which resists caries formation (Yamamoto and Sato, 1980). Burkes *et al.* (1992), using an Er:YAG, reported melted enamel surfaces.

Further, Cooper *et al.* (1988) found a 300% increase in shear bond strength in laser-treated samples using a non-contact CO_2 laser, applying laser energy to adhesive-coated dentin and untreated controls. White *et al.*, 1991a,c) obtained similar results using the pulsed contact-delivered Nd:YAG.

Acid effects

Featherstone *et al.* (1985) studied the gradual development of artificial caries-like lesions by simulating the acidic environment corresponding to conditions *in vivo* and documented varying degrees of calcium and phosphorus decline. Fusayama (1991) documented the process of acid etching and provided SEM images of turbid, transparent and normal dentin after etching. Zero *et al.* (1990) studied enamel demineralization and found mineral loss of calcium and phosphorous to be directly proportional to dissolution time. Lewinstein and Rotstein (1992) reported approximately 25% decrease in the microhardness of dentin after a 90-second exposure to trichloracetic acid and documented with SEM an etched tubule surface. Ruse and Smith (1991) documented the effects of acid etching on dentin followed by analysis with X-ray photo-electron spectroscopy and SEM. They reported almost complete demineralization under hydrochloric acid etching; calcium and phosphorus decreased tenfold. Phosphoric acid etching decreased calcium levels even lower, and a phosphorus content approached zero. Their observations indicate the value of additional research on laser-and-acid-exposed dentin to determine specific mineral levels before and after exposure and the degree of mineral conservation achieved through laser exposure.

Ogawa *et al.* (1983) measured the varied microhardness of sound dentin and of carious transparent and inner dentin, documenting crystalline density with SEM and TEM. This provided a foundation for the work of Fusayama (1991) who described the mechanisms involved in demineralization caused by carious acid, which dissolves apatite crystals and eventually the intermolecular cross links of collagen. He recommended the development of mineral conservation strategies through the preservation of the carious inner dentin layer. Baier (1992) pointed out the vulnerability of the organic collagen layer as the foundation for bonding: "This underlayer can dominate a hard biological material's response to environmental stress even in the presence of overlayers of organic integuments. These considerations help explain how adhesive bonds to biological solids fail under stress."

The work of these researchers demonstrated the potential value of alternative strategies for surface treatments and sealants beyond current acid-etching procedures. The purpose of this investigation was to determine if there were changes in the microhardness of dentin treated with the Nd:YAG laser before and after acid demineralization.

Materials and Methods

Specimen preparation

Seven freshly extracted (less than 28 days), non-carious human third molars with complete root formation taken from adults were first sterilized by gamma radiation (cesium-137 source, 16 hours). The teeth were sectioned transversely into discs using a water spray-cooled diamond wheel modified with a thin blade to minimize smear layer (Buehler Limited, Lake Bluff, IL). The specimens were cut into sections 4 ($\pm$ 0.25) mm thick, the first cut being slightly below the dentino-enamel junction at the occlusal surface. The sectioning process produced a uniform dentin surface with little smear layer detectable using SEM techniques. Sections were stored in distilled and filtered water prior to use and during the course of the study. This storage solution causes no significant spectroscopic changes in mineral peak from 1 to 28 days (Strawn *et al.*, 1993).

Procedures

In the experiment, seven of the dentin discs were

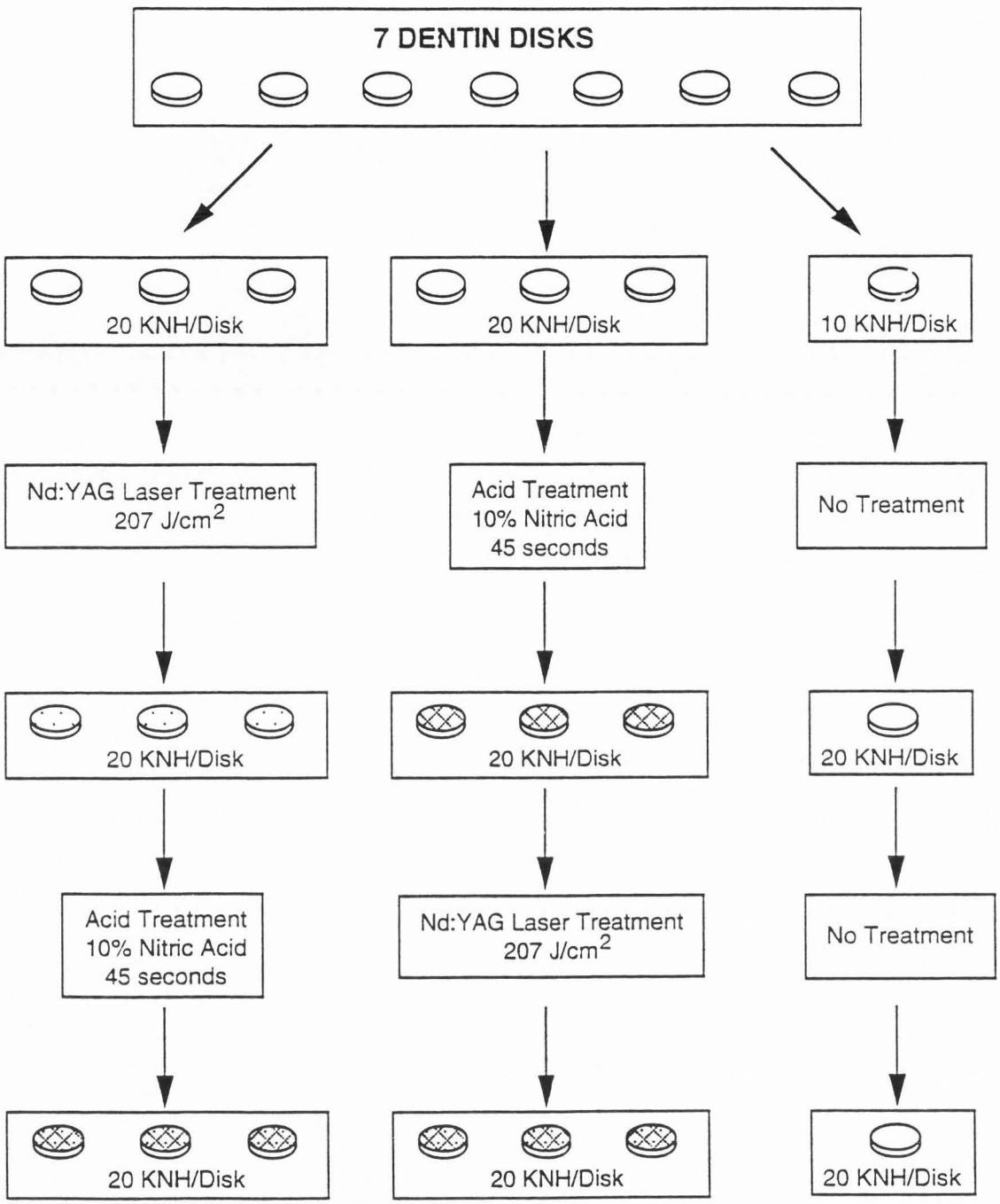

Figure 1. Experimental flow diagram for microhardness tests, laser and acid treatment. KHN: Knoop indentation microhardness measurements.

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used. Of these, one served as a control (C) disc. The control dentin disc received repeated microhardness measurements: 10 pretreatment, 20 when test teeth received laser treatment and 20 when test teeth received acid treatment. This control dentin disc allowed for validation of accuracy of the microhardness measurements over the course of the study. All dentin sections were treated and measured wet. The laser-and-acid treated (L + A) samples were bio-prepared and three of the dentin discs received 20 microhardness pre-treatment measurements. These discs were then laser-treated (L) with the Nd:YAG (207 J/cm^2) and again measured. Finally, the three discs were immersed in 10% nitric acid for 45 seconds, followed by 20 additional microhardness readings.

A second set of dentin discs were microhardness

tested, immersed in 10% nitric acid (A) for 45 seconds, and then laser-treated (207 J/cm^2; A + L). Twenty post-treatment microhardness readings were obtained, following each respective step (Fig. 1).

Microhardness testing

A Kentron microhardness tester (Riehle Testing Machines, East Moline, IL) was used with a highly polished, pointed, rhombic-based, pyramidal diamond which included longitudinal edge angles of 172.5° and 130° (± 0.83°) to measure hardness. The tester was examined using the cross-calibration results of two operators as well as repeatability measures and found to be within 2% error using block standards of 50 g and 300 g. The 300 g force was used for all measurements for consistency and to measure both surface and subsurface microhardness. Measurements were taken in Filar using a measuring microscope at 10x and converted into Knoop (KHN). The accuracy of the measurements was confirmed using a calibrated reference slide and computer image analysis. Any measurement in which one leg of the long diagonal was 20% greater than the other was disregarded. If both ends of the diagonal were not in the same field of focus, the reading was disregarded, also. These procedures were based on a standard test method, ensuring that the surface to be tested was parallel with the table of the hardness (ASTM, 1985). Further criteria used in accepting an indentation value were clearness, absence of cracks, and symmetry.

A 300 g force load applied for 15 seconds was employed for all specimen measurements and converted into Knoop hardness. KHN measurements were read before and after all treatments were performed and compared using multifactor repeated measures analysis of variance (ANOVA; $p \leq 0.05$). Knoop hardness was calculated from the following equation:

$$KHN = C*P/ L^2$$

where:

C = indenter constant relating the projected area of the indentation to the square of the length of the long diagonal (C = 14229)

P = load = gram force (300 g)

L = length of the long diagonal in μm

Scanning electron microscopy

Dentin sections were first reviewed visually for the presence or absence of interactive effects from the respective treatments. Dentin sections were examined by SEM to record the nature of the morphological changes which occurred. Wet SEM examination was performed with a model SX40A ISI (International Scientific Instruments, Inc., Milpitas, CA) SEM fitted with a Robinson scintillator backscatter detector, at specimen chamber pressures of 0.12-0.5 torr according to the methods described by Marshall *et al.* (1989) and techniques previously used for the physical modification threshold determination (White *et al.*, 1993b). The SEM was operated at accelerating voltages of 15-30 kV.

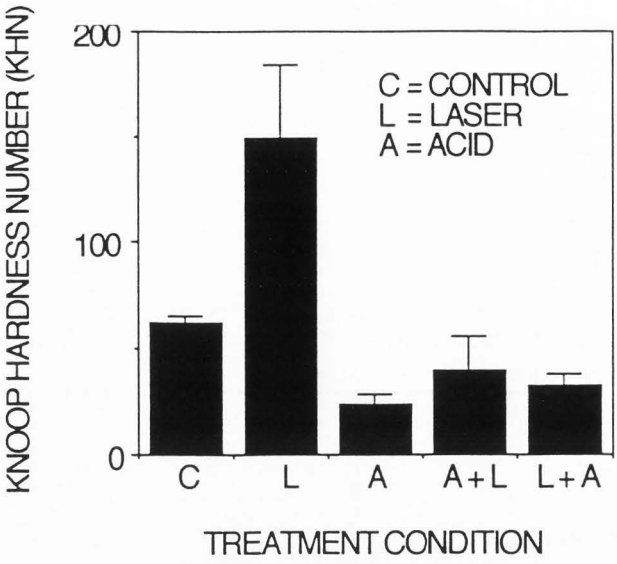


Figure 2. Knoop hardness value as related to treatment condition.

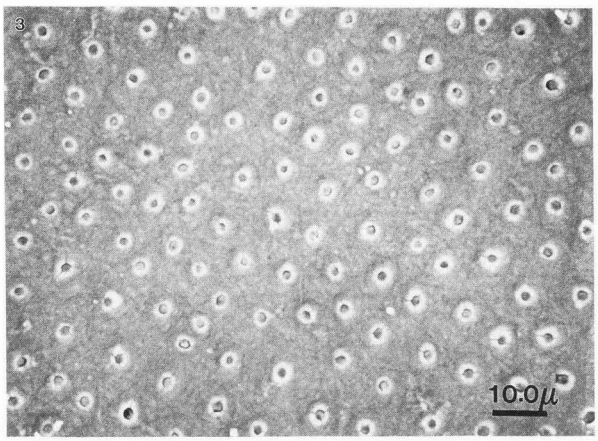
Laser treatment

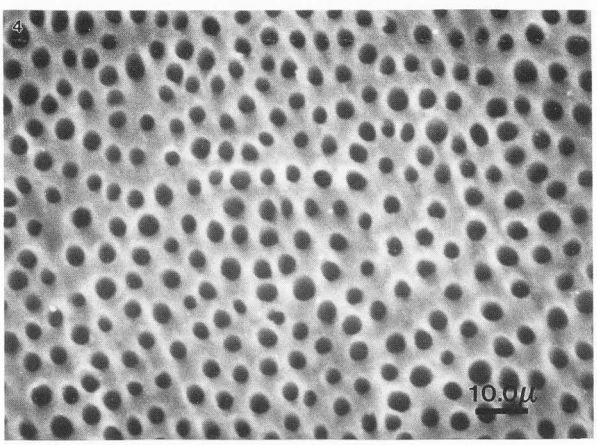
A pulsed fiber-optic-delivered Nd:YAG laser with an emission wavelength of 1.06 μm (Sunrise Technologies, Fremont, CA) was used to modify the dentin surfaces of the specimens. A 120 μs pulse duration was delivered through a circular quartz fiber-optic probe with a diameter of 500 μm. The probe was placed in direct contact with the dentin surface and an energy level of 207 J/cm^2 rendered in single pulses; 207 J/cm^2 has been defined as the parameter of the modification threshold of dentin, the energy parameter at which dentin modification occurs at this emission wavelength (White *et al.*, 1993b). A physical modification in dentin occurred with the delivery of a single pulse at this energy level. An optical detector (Molectron, Portland, OR) was used to confirm the correct energy level before each laser treatment was performed.

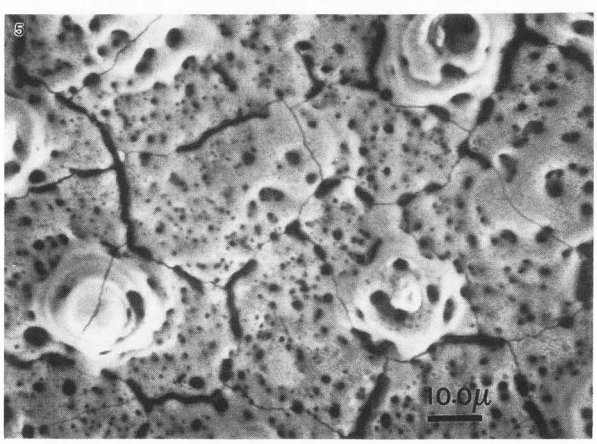
Line exposures were made across the specimen with the probe in contact with the dentin surface, moving at a uniform rate at a laser frequency of 10 Hz. The rate was determined so there would be no overlap of laser modification of dentin and controlled using a mechanical testing machine (Instron Corp., Canton, MA) at a rate of 5 mm/s.

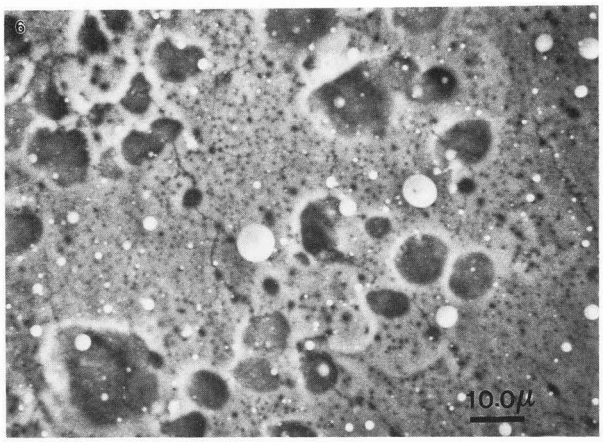
Acid treatment

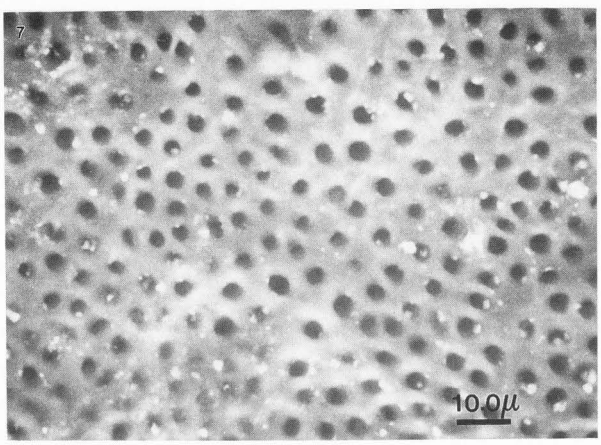
Specimens were immersed in 10% nitric acid for 45 seconds. Based on our preliminary tests, 10% nitric











Figures 3-7. Representative scanning electron micrographs (taken at 20 kV) of: a prepared untreated dentin surface prior to laser or acid exposure (No Tx; **Fig. 3**); a nitric-acid-treated dentin (A; **Fig. 4**); a Nd:YAG laser-modified dentin, exposed at the physical modification threshold (PMT) of 207J/cm^2 (L; **Fig. 5**); an acid and then laser treatment of dentin (A + L; **Fig. 6**); and a laser followed by acid treatment of dentin (L + A; **Fig. 7**). Bars = 10 μm.

acid was used as it was the most convenient way of reducing microhardness by at least half of the original value in a reasonable amount of time.

Results

Significant differences in microhardness were observed for all treatment conditions and untreated control dentin sections (ANOVA, $p \leq 0.05$). The following Knoop microhardness values were recorded: C = 62 $\pm$ 3; L = 149 $\pm$ 35; A = 24 $\pm$ 5; L + A = 40 $\pm$ 16; and A + L = 33 $\pm$ 5. Our results indicated that laser exposure at the threshold of physical modification increased the microhardness of dentin. In addition, laser modification preceding acid treatment presented higher average microhardness values than acid treatment alone. Lastly, laser modification following acid treatment increased microhardness values compared to acid treatment only.

Results are presented in Figure 2.

Scanning electron micrographs

In a representative scanning electron micrograph at 20 kV and 1,000x of prepared, untreated dentin, the morphologic surface is apparent, including tubules,

intertubular and peritubular dentin (Fig. 3). In comparison, a scanning electron micrograph of nitric acid-treated dentin provides visual evidence of acid effects in peritubular dentin, and the widening of the dentinal tubules (Fig. 4).

A clear contrast in morphology is evident in a scanning electron micrograph of dentin laser-modified by the Nd:YAG at the physical modification threshold (PMT) of 207 J/cm^2 (Fig. 5). Melting and resolidification of the surface is present with no remaining unaltered dentin morphology. The surface is irregular in appearance with multiple surface cracks and small holes, presumably from the melting and resolidification process caused by the laser. A lighter color is evident in the micrograph, presumably from the increased mineral content on the surface (White *et al.*, 1994a).

In a scanning electron micrograph of the acid-and-then-laser treatment, the surface is unique and different than untreated dentin, acid-treated dentin, or laser-treated dentin (Fig. 6). There appear to be combustion products on the surface (white spheres), and there is no remaining dentin morphology. Minimal evidence of surface cracking can be observed: all acid-treated dentin has apparently been removed by the laser, with evidence of melting and resolidification of the underlying mineral.

Comparing micrographs of laser-followed-by-acid treatment of dentin (Fig. 7) with acid-treated dentin, dentinal tubules appear to be affected in a smaller surface area. There also appear to be areas of laser-modified collagen which were not removed by the acid. The surface morphology is relatively flat in comparison with that of laser-modified dentin. The acid has removed the majority of the laser-modified collagen but not all of the laser-modified material. Although closest to the acid treated surface, the resulting appearance is unique from all previous treatments. The remaining surface displays representative adherent melting and the resolidification of dentin. The original dentinal tubule morphology is present with slightly widened tubules.

Discussion

This study demonstrated the improvement of the mechanical property of microhardness from pulsed fiber-optic delivered Nd:YAG laser treatment of dentin. Important considerations are the clinical utility and safety at the physical modification threshold. If the findings of this *in vitro* study are confirmed by clinical testing, then there would be clinical use for hardening dentin based on the ease of delivery using the fiber-optic in contact with the dentin. Potential applications would be for hardening root surfaces and dentin prior to adhesive restorations. The laser parameter used (207 J/cm^2, for a single pulse) is not sufficient to cause a pulpal response in shallow cavities where there is greater than 2 mm remaining dentin thickness (White, *et al.*, 1991d, 1992, 1993c, 1994b, 1995a,b,c; Gelskey, *et al.*, 1993). This paper demonstrates resistance of laser treated dentin surfaces to acid attack *in vitro*. Laser treated dentin acid resistance from bacterial sources has not been investigated *in vivo*. It is not known how long this resistance would last in a clinical situation or if other laser parameters would be equally effective. We hypothesize that a more highly absorbing wavelength may be able to produce a similar effect using a lower energy density, with higher temperatures, but this has not been demonstrated for dentin.

SEM techniques used in this study allow for the elucidation of the surface morphology of treated dentin. In order to define possible effects to the underlying dentin, longitudinal sections need be prepared and evaluated. Although surface sealing is observed using SEM, a limitation of the techniques is that it is only descriptive and does not directly measure fluid flow through the dentin. It appears that although surface sealing is present, fluid flow still occurs (White *et al.*, 1991e). The blockage of fluid flow is hypothesized to be caused by both dentinal tubule occlusion and the coagulation of proteins within the tubules (Goodis *et al.*, 1993).

The microhardness values for sound dentin reported in this experiment are in general agreement with the findings of Craig and Peyton (1958) and with the preliminary data of Goodis *et al.* (1994) for laser-modified dentin.

Testing sites for this study were limited to peripheral dentin in an effort to avoid the influence of the pulp horns or chamber and typically were taken at a constant distance from the dentino-enamel junction.

Through experimentation, we found a 300 g load applied for 15 seconds most suitable for this study; this load provides enough force to create a readable measurement in Filar units using the Kentron microhardness tester with a 10x objective on a laser-modified dentin surface. Forces less than this create indentations too small to read accurately; forces larger often cause cracking and distorted readings.

The experimental methods used in this study characterize the physical properties of microhardness and relate this property to surface morphology, as observed utilizing SEM. To fully understand acid and laser effects on the dentin surface, analysis of surface chemistry is needed. We have previously utilized Fourier transform infrared spectroscopy and found that laser-modified surfaces have increased mineral content with the elimination of the organic component of dentin (White *et al.*, 1991f, 1993a,b). Further characterization of the subsurface is needed and characterization of regional differences noted in scanning electron micrographs have yet to be completed.

Conclusions

Research utilizing microhardness as a physical property is relevant to a number of major research issues in contemporary dentistry: the understanding of caries prevention, because it is the reverse process of demineralization which correlates with the process of caries progression; the development of successful bonding agents, adhesive wetting and bond strength through a more complete understanding of surface morphology; the evolution of innovations as a result of laser modification, which has been documented to change the fundamental structure of the dentin and its surface; and the exploration of new bonding agents or processes based on findings related to acid and microhardness (given the role of bioacids in caries progression and of acid treatments in surface preparation for dental bonding). Research findings indicate that the fundamental microhardness of dentin is altered by exposure to laser energy.

In our experiment, we confirmed that pulsed Nd:YAG laser treatment above the physical modification threshold approximately doubles the microhardness of dentin. Additionally, we discovered that laser treatment at the same parameters increases the microhardness of dentin before or after acid treatment, thus creating a surface more resistant to acid attack than untreated dentin.

Acknowledgments

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Discussion with Reviewers

K. Matsumoto: Only one dentin disc was served as a control in this study, but it is too small. You should serve more than 3 discs as a control.
Authors: One dentin disc served as a repeated measure control with multiple (10, 20 and 20) measurements over the course of the study. Microhardness measurements for this control section were within the ASTM standards for accuracy of the testing mechanism.

K. Matsumoto: The microhardness tests with 300 g load is too high for demineralized dentin. Is a 10x objective lens for measurements in this study? Usually a 10x objective lens is not for measurements but for focusing in conventional microhardness machines.
Authors: The 300 g load measures both surface and subsurface microhardness. It is true that lighter loads (50 g) would measure surface effects and small changes in demineralized dentin. We utilized the 300 g load to consistently determine hardness for a wide range of hardness values of dentin, demineralized dentin and laser treated dentin. The repeatability of measurements were within 2% using standard blocks with 50 g and 300 g loads. The 10x objective lens allowed for the accurate measurement of the length of indentation. The accuracy of the Filar measurements using the 10x objective was confirmed using a calibrated reference slide and computer image analysis.

K. Matsumoto: In **Discussion**, there is no mention of thermal effect. Is it possible to use this laser at the parameter (207 J/cm^2) clinically without thermal damage to pulp tissue?
Authors: The laser parameter used (207 J/cm^2) for the physical modification of dentin is not sufficient to cause a pulpal response where there is 2 mm remaining dentin thickness. This laser treatment has been investigated for the potential of light and heat effects, and there are no pulpal effects at this parameter as referenced in the discussion of this paper.

T.D. Myers: In **Introduction**, the authors state: "The physical modification threshold is dependent on specific laser parameters (wavelength, pulse duration and energy density) at which the first observable interaction occurs causing macro changes in the dentin structure." Are the authors referring to micro-alterations or clinically observable macro changes? Also, is this interaction a universally constant change independent of wavelength?
Authors: The physical modification threshold (PMT) is defined as the set of laser parameters where the first observable interaction occurs. This is a macro physical change in the dentin structure. It is theoretically possible that micro alterations such as desiccation or chemical changes would occur before the PMT. However, due to the high peak powers and short interaction times, it is predicable to measure the macro change which is clinically relevant. The interaction is dependent on both the substrate (dentin) and the specific laser parameter, and is not independent of wavelength.

T.D. Myers: In **Results**, the authors state: "A lighter color is evident in the micrograph, presumably from the increased mineral content on the surface ..." What factors may account for the change in color?
Authors: The primary factor effecting the SEM image is the reflection of the beam to the detector. Higher density objects, such as mineralized tissue, reflect more of the beam as compared to less dense tissue, such as organic tissue. The melted and resolidified surface from the laser treatment ablates the organic and leaves a more highly mineralized surface. The results of this paper demonstrate the correlation between hardness and the observed increase in SEM image.

T.D. Myers: Do the authors have evidence that the laser-treated dentin is indeed more resistant to acid attack? If so, how long would they estimate this laser effect to last? Do they believe all wavelengths are capable of this effect?
Authors: This question asks of the relationship between acid treatment and caries attack. This *in vitro* study did not measure the hardness from caries attack, which is a different mechanism than acid treatment. Having the knowledge obtained from this study provides the basis for moving the research direction towards laser effects and caries. The estimated laser effect duration would be dependent upon the pH, buffering capacity, fluid flow and presence of fluoride and can only be determined from clinical trials. We believe that these effects may be able to be reproduced using different wavelengths, assuming they can achieve similar surface temperatures to melt and resolidify dentin.