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Effect of Nd:YAG Laser Irradiation Pretreatment on the Long-Term Bond Strength of Etch-and-Rinse Adhesive to Dentin

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Clinical Relevance

Computer-aided design/computer-aided manufacturing resin composites have different physical properties, and care should be taken when selecting one for clinical use.

SUMMARY

Purpose: To investigate the effect of neodymium-doped yttrium aluminum garnet (Nd:YAG) laser irradiation pretreatment on the long-term bond strength of an etch-and-rinse adhesive to dentin.

Methods: Fifty molars were sectioned parallel to the occlusal plane and randomly divided into two groups (n=25 per group): control group (no treatment) and laser group (dentin surfaces were treated with Nd:YAG laser at a setting of 100 mJ/10 Hz). Afterward, resin was bonded to the dentin surface using a two-step etch-and-rinse adhesive (Adper SingleBond 2), and then 150 beams of each group were produced. Each group was divided into three subgroups (n=50 each group): 24 hours of water storage, thermocycling, and NaOCl storage. The microtensile bond strength (MTBS), failure modes, nanoleakage expression, and Masson's trichrome staining were evaluated. An additional 20 molars were sectioned to obtain 2-mm-thick flat dentin slices. These slices were randomly divided into control and laser-treated groups as mentioned previously. Then slices of each group were examined by scanning electron microscopy, attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffraction (XRD), and the Knoop hardness test.

Results: The results of ATR-FTIR and Masson's trichrome verified that laser irradiation partly removed collagen fibers from the dentin surface; however, no significant difference was found in the Knoop hardness ($p>0.05$). The XRD result showed similar crystalline struc-

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ture regardless of laser pretreatment. There is no significant difference in short-term MTBS between control and laser-treated groups (p>0.05); however, long-term MTBS differed between the groups (p>0.05). Furthermore, the laser-treated group showed less silver deposition than the control group after aging (p<0.05).

Conclusion: Pretreatment by Nd:YAG laser irradiation appeared to have a positive effect on the adhesive-dentin bonding *in vitro* test, and the bonding effectiveness could be preserved after aging.

INTRODUCTION

The promotion of adhesive techniques has become increasingly important with the rising public requirement on esthetics and "minimum intervention" in modern operative dentistry.[1] Dentin bonding is a process in which adhesive resin replaces the hydroxyapatite (HAP) mineral phase removed from the dentin tissue surface through acid etching and then micromechanically interlocks with a network of exposed collagen fibrils to form the so-called hybrid layer. Hybrid layer formation on the surface and within the subsurface of dentin depends on the permeability of dentin and the diffusion of applied monomers. These features determine the durability of the dentin bonding interface over time.[2,3] Compared with bonding to enamel, dentin bonding is more challenging and less predictable.[1] Various bonding strategies, such as laser irradiation, have been developed to maintain resin-dentin bond strength.

Laser technology has been widely applied in clinical trials since the 1960s. Advances in laser technology have led to various dental applications, such as periodontal soft tissue plastic surgery, cavity preparation and treatment of dentinal sensitivity, caries prevention, and bleaching.[4] Moreover, several aspects of lasers can enhance dentin bonding, such as opening dentin tubules without demineralization of peritubular and intertubular dentin, dentin surface sterilization, and a bonding surface with microirregularities without a smear layer.[5-7] Conducting acid etching after laser treatment produces equal or better dentin-resin bond strengths,[8,9] while other aspects, such as the fused denatured collagen fibrils, probably limit resin infiltration into the intertubular dentin and weaken dentin bond strength.[10] Studies on short-term bonding strength after laser irradiation obtained inconsistent results. This inconsistency may be attributed to the different characteristics of the dentin surface resulting from different laser types and laser energy parameters.[11,12] In addition, only a few studies have explored the effects of the properties of laser-irradiated dentin on bond strength.

Neodymium-doped yttrium aluminum garnet (Nd:YAG) laser, which emits a wavelength of 1064 nm, is frequently used in dentin bond studies.[13] It is usually used to melt and recrystallize dentin HAP to occlude dentinal tubules,[14] and collagen loss occurring in this melting process has been reported.[15] In the present study, Nd:YAG laser was applied to melt the dentin surface to remove the smear layer and dentin collagen. Acid etching was subsequently used to form a microscopically rough substrate surface without a demineralized collagen fiber network, possibly strengthening the dentin bond.

Contemporary dentin adhesives present favorable short-term bonding strengths,[16] but the durability of these materials remains limited. To date, resin monomers cannot displace water and infiltrate the collagen network completely. The biodegradation of nonencapsulated collagen fibrils causes hybrid layer degradation, which subsequently results in resin-dentin bond breakdown.[17] Several studies have explored the effects of laser on short-term dentin bond strength. However, the durability of dentin bond strength after laser treatment remains unknown. Laser irradiation vaporizing dentin collagen has been demonstrated by several studies,[10,15] and the loss of dentin collagen may maintain the dentin bond strength by avoiding *in vivo* collagen degradation. Therefore, laser irradiation probably is favorable to dentin bond durability, and the influence of this process warrants further investigation.

This study aimed to investigate the effects of Nd:YAG laser irradiation on short-term and long-term dentin bond strength and on the microstructure of the dentin surface. The null hypothesis was that treating the dentin surfaces with or without laser irradiation before acid etching exerts no significant effect on the microstructure of dentin substrate, microtensile bond strength (MTBS), failure modes, and nanoleakage.

METHODS AND MATERIALS

Specimen Preparation of Field-Emission Scanning Electron Microscopy, Thin-Film X-Ray Diffraction, Fourier Transform Infrared Spectroscopy, and the Knoop Hardness Test

Seventy freshly extracted noncarious human third molars were selected after the patients' informed consent was obtained. The collected teeth were stored in 0.9% (w/v) NaCl containing 0.002% sodium azide at 4°C and used within one month. Twenty molars were chosen to prepare 2-mm-thick flat slices that were cut from the mid-coronal dentin using a water-cooled low-speed cutting saw (Isomet, Buehler Ltd, Evanston, IL, USA), and another 50 molars were reserved for subsequent microtensile bond strength testing and nanoleakage evaluation. The dentin surfaces were polished using 600-grit SiC paper to create a standardized smear layer and then cleaned ultrasonically in deionized water, dehydrated with ethanol, and dried in a critical evaporator.

Treatment with Nd:YAG Laser

The Nd:YAG laser equipment used in this study was the Miracle Laser-3100 (Wuhan Miracle Laser Technologies Inc, Hubei, China) at a wavelength of 1064 nm. The laser irradiation parameters were as follows: 1 W power, 10 Hz repetition rate, within the energy parameters of 100 mJ, and an energy density of 85 J/cm^2 per pulse. Before laser irradiation, black ink was applied to the dentin surfaces to increase the absorption of energy from the pulse into the dentin tissue.[18] To avoid the influence of the measurement of removing the ink and the potential ink remnants, dentin surfaces of the control group also received the same ink, and all the ink was removed as thoroughly as possible before adhesion. The laser was fitted with a quartz fiber tip that was 400 μm in diameter and applied freehand, in noncontact mode, emitting for 60 s/cm^2 two times.[19] During laser application, the laser tip was always perpendicular to the specimen surface at a distance of 2 mm. A special working stage was used to maintain the distance between the laser tip and the dentin surfaces. Samples were moved with a fixed laser beam position using a motorized stage.

Characterization of the Laser Irradiated Dentin Surface

Field-emission scanning electron microscopy—The surfaces and the transverse sections of the acid-etched dentin slices and laser-treated dentin slices followed by acid etching were dried in a drying vessel for 24 hours and then sputter coated with Au-Pd alloy and observed using field-emission scanning electron microscopy (FE-SEM) at 5 kV (Sigma, Zeiss, Jena Germany).

Thin-film X-ray diffraction—Six dentin slices were chosen and randomly divided into control and laser treated groups (n=3). After surface treatment with or without laser, all the slices were examined by thin-film X-ray diffraction (TF-XRD) using an X-ray diffractometer (Bruker AXS D8-XRD, Karlsruhe, Germany) operated under a 40-kV acceleration voltage and 40-mA current with the angle of the incident X-ray beam fixed at 25 degrees and a scanning time of 1 degree/min for a 2θ scan.

Attenuated total reflection Fourier transform infrared spectroscopy—For attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) spectra collection, six dentin slices were prepared as previously described, and each half of every slice was irradiated by laser or left untreated, respectively; three different spots on the surface of each half were randomly chosen and tested. The spectral data were expressed as absorbance. FTIR and ATR spectra of these specimens' surfaces were carried out with a Nicolet 5700 spectrometer (Thermo Scientific Inc, Madison, WI, USA) and a single-reflection ATR setup with germanium (Ge) as an internal reflection element (Smart OMNI-Sampler, Thermo Scientific).

The effect of laser irradiation on collagen depletion was evaluated using the collagen:apatite ratio (the ratio of absorbance of amide I and II peak to phosphate ν_3 peak) and analyzed by one-way analysis of variance (ANOVA) and Tukey's test at the 5% level of significance.

Knoop hardness test—Three dentin slices were divided in half; one-half was irradiated by laser, and the other was left untreated. Microhardness was evaluated using a hardness tester (E. Leitz GmbH, Wetzlar, Germany). A load force of 100 g was used at a hold time of 10 seconds. Six indentations were performed on each half of the sample, and the mean values were statistically analyzed by ANOVA and Tukey's test at the 5% level of significance.

Specimen Preparation of MTBS, Nanoleakage Evaluation and Masson's Trichrome Staining

Fifty freshly extracted intact human third molars were used in this study. A flat surface was prepared using a water-cooled low-speed cutting saw (Isomet, Buehler) to expose the mid-coronal dentin. The dentin surface was polished using 600-grit SiC paper to prepare a standardized smear layer. The teeth were randomly assigned to two groups that were conditioned either with an Nd:YAG laser (experimental group) or with no treatment (control group): a two-step etch-and-rinse adhesive Adper Single-Bond 2 (3M ESPE, St Paul, MN, USA) was used in the current study. Briefly, the dentin surface was first etched with 35% phosphoric acid for 15 seconds, rinsed and blotted dry, coated with two layers of adhesive, gently air thinned for 5 seconds, and light cured for 10 seconds. Afterward, resin composite was built up using 4-mm increments of resin composite (Charisma, Heraeus Kulzer, Hanau, Germany). Each tooth was first sectioned into 0.9-mm-thick slabs after storage in deionized water at 37°C for 24 hours, and three resin-dentin bonded slabs were respectively selected from three teeth of laser-treated and control groups for Masson's trichrome staining. Then the rest of the slabs were sectioned into 0.9 × 0.9-mm beams. Six beams were obtained from the central part of each specimen. In total, 150 beams were harvested for the laser treatment and control groups separately.

Artificial Aging Treatment

The beams with each surface treatment were further divided into the following three subgroups (n=50):

Group 1 (control): Beams without any artificial aging treatment served as the baseline.

Group 2 (thermocycling): Beams underwent a thermocycling test from 5°C to 55°C for 10,000 cycles, with the dwell time set at 30 seconds.

Group 3 (NaOCl storage): Beams were immersed in 10% NaOCl solution for 1 hour at room temperature.

As a matter of convenience, we abbreviated groups 1 to 3 as CC, CT, and CN for no surface treatment and as LC, LT, and LN for laser surface treatment, respectively.

MTBS Test

After different aging treatments, MTBS of 45 bonded beams from each group were tested. Each beam was attached to a universal testing apparatus (Microtensile Tester, Bisco, Schaumburg, IL, USA) with a cyanoacrylate adhesive (Zapit, Dental Ventures of America, Corona, CA, USA) and loaded until failure at a crosshead speed of 1 mm/min. The exact dimensions of fracture region were measured using a digital caliper. The final MTBS values (MPa) were calculated as the maximum load at failure divided by the cross-sectional area.

The MTBS data were analyzed using SPSS 16 (SPSS Inc, Chicago, IL, USA). The correlation between surface treatment and artificial aging methods was evaluated by two-way ANOVA factorial analysis, followed by Tukey's *post hoc* multiple comparison test (α=0.05).

Failure Mode Analysis

After the MTBS test, the dentin side of all the failed modes was evaluated with a stereomicroscope (Stemi 2000-C, Carl Zeiss Jena, Göttingen, Germany) at 50× magnification and classified as A (adhesive failure), M (mixed failure), CD (cohesive failure in dentin), and CC (cohesive failure in composite).[20] Three typical failed samples from each group were examined using FE-SEM (Sigma, Zeiss) after coating with Au-Pd alloy. The general conditions of the fractured surfaces and typical region were captured at 100× and 2500× magnifications.

Nanoleakage Evaluation

Five beams from each group were chosen for nanoleakage evaluation using the method described by Tay and others.[21] The beams were coated with nail varnish applied to within 1 mm of the interfaces. Afterward, the beams were placed in 50 wt% ammoniacal $AgNO_3$ in darkness for 24 hours, thoroughly rinsed in distilled water, and placed in a photodeveloping solution for 8 hours under a fluorescent light to facilitate the reduction of $[Ag(NH_3)_2]^+$ ions into metallic silver grains. After dehydrating, the silver-stained specimens were embedded in epoxy resin and then polished with 600-, 800-, and 1200-grit SiC papers and a soft cloth under running water. The specimens were ultrasonically cleaned and dehydrated for 24 hours at room temperature. After the carbon coating, the resin-dentin interface was analyzed with FE-SEM in the backscattered electron mode (Quanta 450 FEG, FEI, Eindhoven, The Netherlands). Nanoleakage within the adhesive-dentin interfaces was investigated, and four areas of each sample's hybrid layer were randomly photographed at 1000× magnification for quantitative analysis. Thus, 20 images were obtained for each group. Image J (National Institutes of Health, Frederick, MD, USA) was used to calculate the total silver particle percentage in the hybrid and adhesive layers.[22] The data were analyzed using SPSS 16 (SPSS Inc). The correlation between different surface treatments and aging methods was evaluated by two-way ANOVA factorial analysis, followed by Tukey's *post hoc* multiple comparison test (α=0.05).

Masson's Trichrome Staining

Resin-dentin bonded slabs prepared previously of both laser-treated and control groups (n=3) were fixed in a glass holder with a photocuring adhesive (Technovit 7210 VLC, Heraeus Kulzer GmbH & Co, Werheim, Germany) and polished with SiC papers until their thicknesses were approximately 10 μm. Slices were treated with Masson's trichromic acid

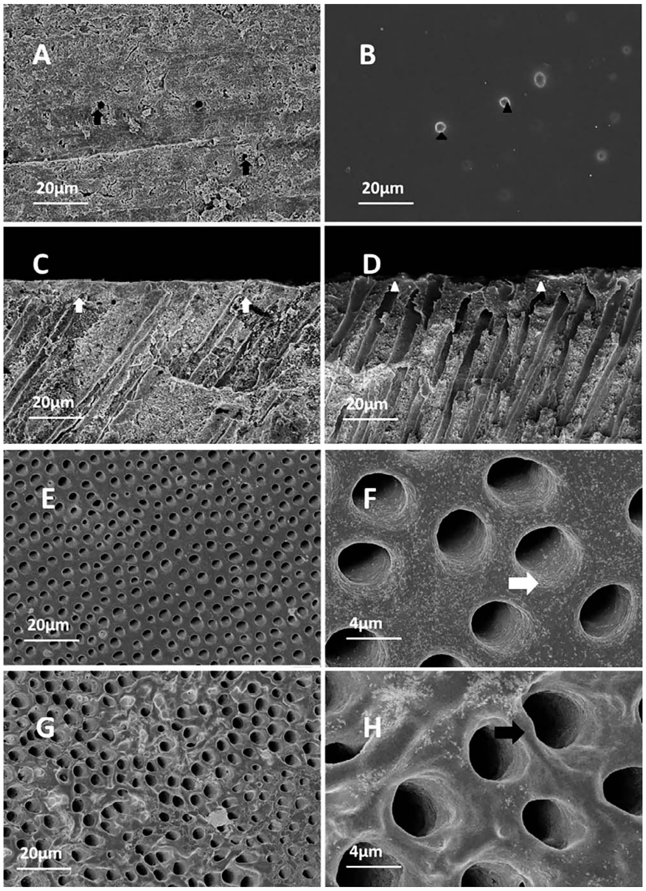

Figure 1. *FE-SEM surface and transverse examination of sound dentin (A and C) and laser-treated dentin (B and D). The sound dentin was covered by smear. The laser-treated dentin tubules were occluded by melting HAP crystallites. Black arrows: occluded dentin tubules by smear. Black triangles: occluded dentin tubules by laser irradiation. White arrows: smear layer. White triangles: laser-irradiated layer. FE-SEM surface examination of sound dentin after acid etching (E and F: the magnification of E) and laser treated dentin after acid-etching (G and H: the magnification of G). Black arrow: cufflike peritubular dentin. White arrow: funnel-shaped dentin tubules.*

staining technique.[23] A cover slip was placed and the specimens were examined by light microscopy (BH-2, Olympus, Tokyo, Japan) at 400× magnification.

RESULTS

Characteristics of Laser-Treated and Sound Dentin Surfaces

The surface and transverse section ultrastructure of sound dentin and Nd:YAG laser-irradiated dentin is shown in Figure 1A-D. The surface of sound dentin showed a widespread smear layer and several blocked dentin tubules (Figure 1A,C). By contrast, the laser-treated dentin slices displayed a flat, smooth, and integrated surface with no smear layer. Dentin tubules were occluded and integrated into the subsurface dentin structure and formed a new unbroken homogeneous modified layer. The transverse section showed that the upper dentin formed a melt layer barely containing collagen fiber and that the walls of dentin tubules became nonporous and smooth after laser treatment. No cracks appeared between the newly formed layer and the dentin substrate (Figure 1B,D).

Figures 1E-H show representative FE-SEM images (low magnification, 1000×; high magnification, 5000×) of sound dentin treated with or without Nd:YAG laser irradiation before acid etching. The characteristics of the dentin surface after acid etching are displayed in Figure 1E,F. The smear layer was completely moved, the surface was flat and regular, and dentin tubules became funnel shaped. As shown in Figure 1G,H, the laser-treated dentin surface became irregular and crater shaped after acid etching with 35% phosphoric acid for 15 seconds. Most of the occlusive dentin tubules were reopened with a cufflike peritubular dentin-protruding dentin surface. The dentin tubules also showed a smooth and nonporous wall in the laser-treated samples.

Mechanical Property and Composition of the Laser-Irradiated and Sound Dentin Surfaces

Sound dentin and laser-modified dentin were characterized using XRD. The spectra are shown in Figure 2A. The peaks at 2θ degrees were 25.9688, 31.6998, 32.0798, and 32.7828 degrees, corresponding to the expected peaks for hydroxyapatite at 002, 211, 112, and 300 planes, respectively (JCPDS No. 09-0432).[24] There was no obvious difference between the two spectra.

The typical ATR-FTIR spectrum of sound dentin is shown in Figure 2B. The $HPO_4 \nu_3$ band (997-1124 cm^{-1}) and the $HPO_4 \nu_1$ band (964 cm^{-1}) indicated the presence of hydroxyapatite crystals,[25] and the bands at 1650 and 1453 cm^{-1} corresponded to the collagen protein amide I and II bands.[15,26] After laser treatment, the collagen:apatite ratio decreased significantly compared with the control group ($p<0.05$) (Figure 2C).

The hardness of the dentin before and after laser irradiation was evaluated using the Knoop hardness test. The Tukey test showed that laser irradiation did not influence dentin hardness significantly (72.91±12.45 vs 77.06±7.38, $p>0.05$).

Optical Microscopy

Representative light micrographs of Masson's trichrome stained sections of the dentin/adhesive

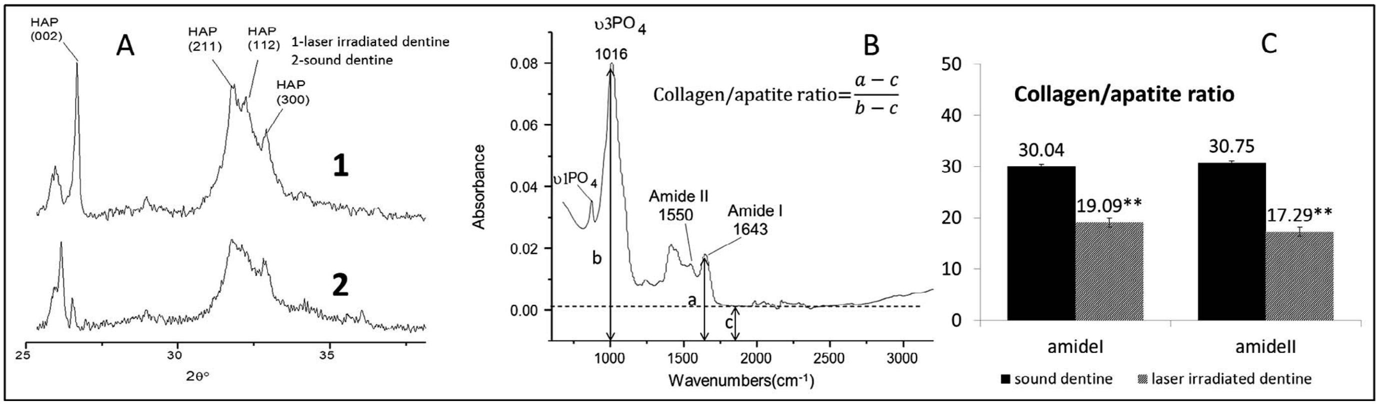

Figure 2. *(A): XRD spectra of dentin samples of the sound dentin surface and laser-irradiated dentin surface. (B): Schematic illustration of how the collagen:apatite ratio was derived from each FTIR spectrum. With the use of laser irradiation, the v_3PO^4 peak of apatite could not be used as a reference for examining changes in height of the amide I and II peaks of intact collagen. Hence, the collagen:apatite ratio was used as the substitute. (C): Column graph of the collagen:apatite ratio of laser-irradiated and control dentin surfaces. The collagen:apatite ratio of laser-treated dentin surfaces decreased significantly compared with the control samples (p<0.05).*

interface are presented in Figure 3. With these trichrome stains, mineralized dentin stained green, exposed demineralized collagen not encapsulated by the adhesive stained red, and adhesive stained beige.[27] Specimens from the control group presented a distinct red zone at the base of the hybrid layer, while a barely red zone was observed in laser-treated specimens.

MTBS

The mean MTBS and standard deviation are summarized per experimental condition in Table 1. The variables dentin surface treatment and aging treatment did not have a significant effect on the bond strength ($p>0.05$). Laser treatment before acid etching had no significant difference on the MTBS ($p>0.05$). Specimens bonded with laser-treated surfaces could preserve the MTBS in both the thermocycling and the NaOCl aging groups. However, the MTBS of specimens only acid etched were significantly decreased ($p<0.05$) after thermocycling and NaOCl treatment (Table 1).

Failure Mode Analysis

The failure modes were analyzed by frequency distribution (Table 1). For all groups, adhesive failure was the predominant fracture pattern. Mixed failure and CD failure was evident in the CN group. Mixed failure percentage also relatively increased in the LN group, but few CD failures were observed. The representative FE-SEM images are shown in Figure 4.

Nanoleakage Observations

SEM interfacial analysis showed that both laser-treated and control samples presented silver uptake along the base of the hybrid layer after 24 hours of aging (Figure 5). Laser treatment had no influence

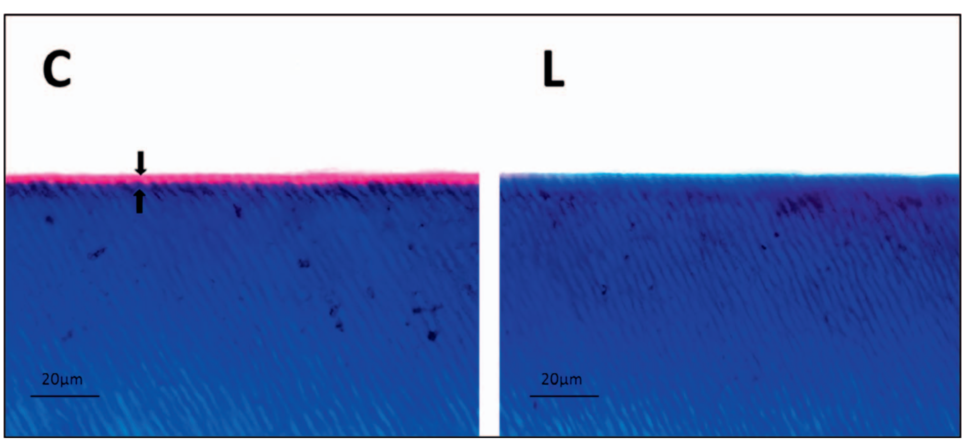

Figure 3. *Representative light micrographs of dentin specimens stained with Masson's trichrome (magnification: 400×). C: Specimen from the control group; the wide red band corresponds to the extent of exposed collagen matrix unprotected by the adhesive resin (space between black arrows). L: Specimen from laser irradiated group; the barely red band was observed in the laser-treated specimen.*

Table 1: *Microtensile Bond Strength (MTBS), Fracture Frequency, and Nanoleakage Expression of Each Group*[a]

Groups	MTBS (MPa)	Failure Frequency (%)				Nanoleakage Expression (%)
		A	CC	CD	M	
CC	20.8 ± 6.4 A	71.1	0	2.2	26.7	9.2(0.8) A
CT	15.1 ± 5.8 B	84.1	2.3	2.3	11.3	24.3(4.5) B
CN	16.1 ± 4.7 B	55.6	0	20	24.4	18.9(1.35) B
LC	21.0 ± 6.4 A	86.7	0	2.2	11.1	9.1(0.8) A
LT	20.6 ± 7.1 A	81.8	4.5	2.3	11.4	12.2(1.5) A
LN	20.8 ± 5.1 A	67.4	0	4.7	27.9	12.4(3.4) A

Abbreviations: A, adhesive failure; CC, cohesive failure in composite; CD, cohesive failure in dentine; M, mixed failure.
[a] *Values of MTBS are mean ± standard deviation. Groups with the same letters are not statistically significant (*$p>0.05$*).*

on silver deposition after 24 hours of water storage ($p>0.05$). Less silver deposition was observed in the laser-treated group than in the control group after aging treatments ($p<0.05$) (Table 1).

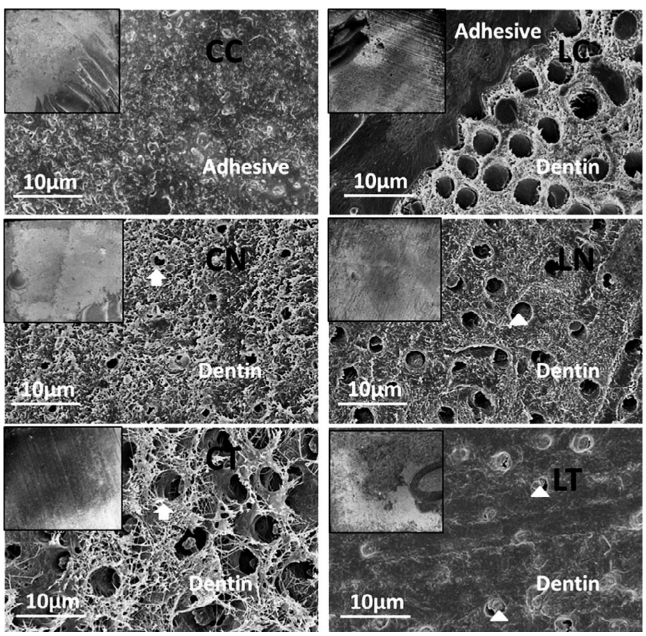

Figure 4. *Representative FE-SEM images (low magnification, 100×; high magnification, 2500×) of dentin sides of fractured specimens in both laser-treated and control groups. The insert indicates general condition of fractured surfaces. CC: Specimens of the CC group showed an adhesive failure. Much adhesive resin covered dentin surface. LC: Specimens of the LC group showed a mixed failure. Dentin tubules were sealed by resin tags, and remnant adhesive could be observed. CN: Specimens of the CN group showed a cohesive failure in dentin. The failure was mainly at the bottom of the hybrid layer. Dentin tubules and a large number of broken collagen fibers were observed. LN: Specimens of the LN group showed a cohesive failure in dentin. The presence of several open dentin tubules showed that the failure was mainly at the bottom of the hybrid layer. CT: Specimens of the CN group showed a cohesive failure in dentin. Wider tubules and the widespread broken collagen fibers showed that the failure was at the bottom of the hybrid layer. LT: Specimens of the LT group showed a cohesive failure in composite. Sealed tubules confirmed that the failure was at the top of the hybrid layer. Triangles, resin tags; arrows, open dentin tubules.*

DISCUSSION

Dentin is a biological composite of ordered mineralization of apatite on the collagen fibril matrix with a fluid-filled tubular structure.[28] Because of this heterogeneous and intrinsically wet substrate, a strong bond between dentin and resin depends on the infiltration of adhesive into the exposed collagen matrix to create micromechanical interlocks.[29,30] The moist dentin environment can prevent intimate contact between the resin and the collagen fibrils, forming incompletely infiltrated zones along the bottom of the hybrid layer.[31] Biodegradation of the nonencapsulated collagen is a primary cause of the decrease in dentin-resin bond durability.[32]

Laser irradiation improves dentin bond strength by removing the smear layer and collagen fibrils of the dentin surface and occludes dentin tubules by melting and recrystallizing the HAP crystallites.[5,33] However, because of the deeper pigment of dentin and the higher penetration depth of the Nd:YAG laser, it is possible that exposure of dentin surfaces to Nd:YAG laser energy may cause pulp damage if the rise in temperature is sufficiently high, so a safe protocol is needed. It was reported that when laser parameters do not exceed 1 W and 10 Hz and the thickness of the dentin exposed to Nd:YAG laser irradiation for 10 seconds is at least 1 mm, no significant pulp damage can be expected.[34] Laser treatment was suggested to replace acid etching as a pretreatment for dentin bonding,[6,35] whereas other research has demonstrated that laser decreased dentin bond strength.[36,37] Instead of replacing the acid etching system, we applied laser irradiation as an adjunctive treatment to modify the dentin surface and subsequently performed acid etching to improve the reliability and durability of dentin bond strength.

In the present study, laser irradiation to the dentin surface did not affect immediate bond

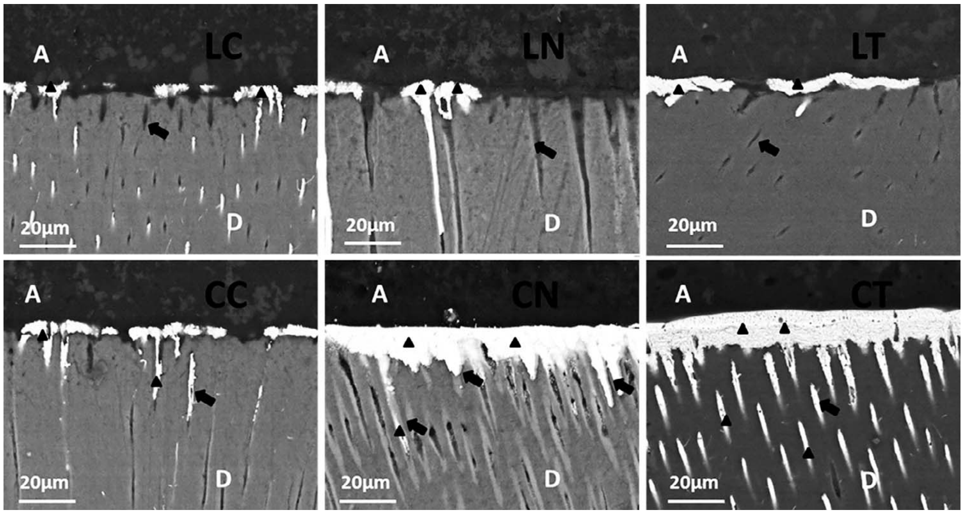

Figure 5. *Representative FE-SEM-BSE images (1000×) of interfacial nanoleakage expression of different groups. A, adhesive; D, dentin; arrows, dentin tubules; triangles, silver deposits; LC/LN/LT, laser-treated groups; CC/CN/CT, control groups. In the LC group, minimal silver deposition was detected along the hybrid layer. In artificial aging groups (LN/LT), more silver uptake occurred at the bottom of hybrid layer but not significantly. In particular, very little silver deposition was detected in the dentin tubules. In the CC group, sparse silver uptake could be observed along the hybrid layer within the adhesive layer. In the CN and CT groups, a large amount of silver uptake was continuously located along the bottom of hybrid layer and in the dentin tubules.*

strength. Aging bond strengths in the laser treatment groups were significantly higher than in the control groups (Table 1). The nanoleakage observations provided concordant results (Figure 5). Given the absence of collagen fibrils in the laser-irradiated dentin surface, resin polymers infiltrate the micropores of HAP created by acid etching instead of demineralized collagen matrices. This phenomenon leads to the formation of a stable dentin-resin bond interface that is insusceptible to the biodegradation *in vivo*. Methods of chemical and mechanical detection need to be further developed to explore the mechanism by which laser irradiation affects the dentin-resin bond strength.

XRD spectra indicated that the main components of both laser-treated and control dentin surfaces were HAP crystals and that the laser-irradiated dentin surface had similar crystallinity to the natural dentin HAP (Figure 2A). Although laser treatment increased the ratio of hydroxyapatite crystal to proteins and water, the microhardness test showed that it did not enhance the hardness of the dentin surface. These results demonstrated that laser irradiation did not influence the mechanical property and crystallographic features of dentin. SEM images showed that the laser-irradiated dentin integrated into the subsurface dentin structure and formed a homogeneous newly modified layer. Moreover, no cracks were observed between the newly formed layer and the dentin substrate (Figure 1). Laser irradiation can maintain the short-term dentin bond strength probably because of these characteristics.

The amide:phosphate ratio of ATR-FTIR spectra indicated that the relative collagen protein amount of the laser-irradiated dentin surface significantly decreased compared with that of the control group ($p<0.05$) (Figure 2C). Moreover, exposed collagen was hardly observed along the laser-pretreated dentin-resin interface as determined through Masson's trichrome staining. This result indicated that laser irradiation can remove the collagen from the dentin bonding interface. NaOCl is a nonspecific proteolytic agent that is widely used in various dental procedures.[38,39] In the present study, NaOCl was used to induce the collagen fiber degradation of the dentin-bond interface. Laser treatment maintained the MTBS and decreased the nanoleakage on the dentin-resin bond surface after immersion in 10% NaOCl solution. This behavior confirmed the speculation that laser irradiation may avoid dentin collagen degradation and increase dentin bond durability by removing the collagen fibers from the dentin-resin bond interface.

The application of acidic agents opens the pathway for the diffusion of monomers into the collagen network. It also facilitates the outward seepage of tubular fluid from the pulp to the dentin surface, deteriorating the bond of some of the current adhesives.[40,41] Wet dentin substrates also decrease the degree of polymerization. Therefore, the hydrophilic nature of the dentin matrix is another crucial factor that affects the durability of dentin bond strength.[42] The results of nanoleakage evaluation revealed less silver deposition in the laser-treated samples than in the control samples. The laser-treated samples barely had silver deposition in the dentin tubules after thermocycling or NaOCl aging (Figure 5). This result demonstrates that laser irradiation can effectively prevent water from invading the dentin-resin bond interface, decrease water leakage on the dentin bond surface, and stabilize the dentin bond strength. These phenomena can be ascribed to three reasons. First, the diameter of the cufflike reopened dentin tubules on the laser-treated dentin surface was lower than that of the funnel-shaped sound dentin tubules after acid etching, confirmed via SEM. This surface morphology may increase dentin bond strength because dentin permeability depends on the size and patency of dentin tubules, and bond strength weakens with increasing dentin moisture content.[43,44] Second, the internal walls of laser-irradiated dentin tubules were smoother and denser, important because collagen fiber loss, porosity change, and denser structure can decrease the permeability of dentin fluid to the bond surface and facilitate the infiltration of bonding monomers into the substrate of dentin tubules. Finally, laser irradiation can vaporize water and other hydrated organic components of the dentin surface, subsequently forming a bond surface without the moist environment of the collagen matrix, and this likely promotes the penetration and polymerization of monomers.

Several approaches to improve long-term bonding have been developed and tested, including inhibition of dentinal endogenous proteases and improved penetration of the adhesive monomers into an ethanol-saturated exposed dentin matrix[3]. However, despite the promising results of *in vitro* research, clinically feasible and commonly accepted methods for improving long-term bonding are still lacking.[45] Lasers have beneficial and promising applications in dentistry. In the present study, laser irradiation combined with acid etching produced similar short-term bond strength and better long-term strength compared with acid etching alone. The pretreatment of the dentin surface with Nd:YAG laser irradiation provides a potential strategy for dentists to obtain the desired bond effectiveness during adhesion, resulting in a longer service life of restorations.

Further research should elucidate the mechanisms by which laser improves dentin bond durability, identify the optimal laser irradiation, and examine the effects of Nd:YAG laser irradiation and the structural transformations of teeth to establish Nd:YAG laser irradiation as a reliable operative technique that improves the durability of dentin bond strength. Most important, it was only demonstrated that this strategy was effective *in vitro*; whether it could achieve the same effect in *vivo* or whether it could be accepted for clinical application still needs to be confirmed in future studies.

CONCLUSIONS

Laser irradiation can partially remove collagen fibers from the dentin surface but not influence its mechanical properties and crystallography. Laser treatment combined with acid etching as a pretreatment of the dentin-resin bond can maintain short-term dentin bond strength, reduce time-related nanoleakage, and increase dentin bond durability.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Ethics Committee for Human Studies, School of Stomatology, Wuhan University, China. The approval code for this study is 067.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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