RESEARCH REPORTS
Biomaterials & Bioengineering

L. Ceballos[1*], M. Toledano[1], R. Osorio[1], F.R. Tay[2], and G.W. Marshall[3]

[1]Department of Dental Materials, University of Granada, Granada, Spain; [2]Conservative Dentistry, Faculty of Dentistry, University of Hong Kong, Hong Kong SAR, China; and [3]Division of Biomaterials and Bioengineering, Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, CA, USA; *corresponding author, C/Emperatriz Eugenia n° 19, 6°D, 18003 Granada, Spain, lauraceballos@hotmail.com

J Dent Res 81(2):119-122, 2002

Bonding to Er-YAG-laser-treated Dentin

ABSTRACT

Er-YAG laser irradiation has been claimed to improve the adhesive properties of dentin. We tested the hypothesis that dentin adhesion is affected by Er-YAG laser conditioning. Superficial or deep dentin from human molars was: (a) acid-etched with 35% H_3PO_4; (b) irradiated with an Er-YAG laser (KaVo) at 2 Hz and 180 mJ, with water-cooling; and (c) laser- and acid-etched. Single Bond (3M ESPE) and Z100 composite (3M ESPE) were bonded to the prepared surfaces. After storage, specimens were tested in shear to failure. Bonded interfaces were demineralized in EDTA and processed for transmission electron microscopy. Two-way ANOVA revealed that conditioning treatment and interaction between treatment and dentin depth significantly influenced shear bond strength results. Acid-etching alone yielded shear bond strength values that were significantly higher than those achieved with laser ablation alone, or in combination with acid-etching. The Er-YAG laser created a laser-modified layer that adversely affects adhesion to dentin, so it does not constitute an alternative bonding strategy to conventional acid etching.

KEY WORDS: laser irradiation, acid etch, shear bond strength, ultrastructure.

Received August 10, 2001; Last revision December 17, 2001; Accepted December 20, 2001

INTRODUCTION

The formation of a hybrid layer and resin tags is essential to the establishment of a strong bond between resin and dentin (Nakabayashi *et al*., 1982; Nakabayashi, 1992; Van Meerbeek *et al*., 1992). One way of achieving this is by the complete dissolution of smear layer and the demineralization of intertubular and peritubular dentin, resulting in an exposed collagen matrix that is subsequently infiltrated by resin that polymerizes *in situ*.

Erbium-YAG (Er-YAG) lasers emit a wavelength of 2.94 μm that coincides with the major absorption band of water. This emitted energy is well-absorbed by hydroxyapatite, and has been shown to remove dental hard tissues more effectively than other laser systems (Hibst and Keller, 1989, 1993; Keller and Hibst, 1989). Little thermal damage has been reported (Keller and Hibst, 1990; Sonntag *et al*., 1996; Li *et al*., 1992), especially when it is used in conjunction with a water spray (Burkes *et al*., 1992; Visuri *et al*., 1996a). The Er-YAG laser has also been used clinically for caries removal and cavity preparation (Matsumoto *et al*., 1996; Pelagalli *et al*., 1997; Keller *et al*., 1998).

Several characteristics of lased dentinal tissue have previously been considered as advantageous for resin bonding. They include the formation of a microscopically rough substrate surface without demineralization, open dentinal tubules without smear layer production, and dentin surface sterilization (Wright *et al*., 1993; Visuri *et al.*, 1996b; Aoki *et al*., 1998; Niu *et al*., 1998). Visuri *et al*. (1996b) reported that Er-YAG laser irradiation of dentin provided shear bond strength results that were better than those achieved with acid-etching. They suggested that laser treatment might replace acid-etching as a pre-treatment procedure for dentin bonding.

The aim of this study was to investigate the effect of Er-YAG laser irradiation on dentin SBS and the ultrastructure of the resin-dentin interface. Superficial and deep dentin samples were studied separately. The null hypothesis tested was that there is no difference in the shear bond strength of a two-step, self-priming adhesive to acid-etched, laser-ablated, or laser-ablated/acid-etched dentin.

MATERIALS & METHODS

Sixty non-carious human third molars were stored in water at 4°C until use. They were obtained with a protocol that was reviewed and approved by the Ethics Committee of the University of Granada and with the informed consent of the donors. Half of the teeth were sectioned below the dentino-enamel junction and ground with 180-grit abrasive paper under running water to provide uniform surfaces of superficial dentin. The remaining 30 teeth were sectioned 1.1 ± 0.1 mm below the original level and ground flat to expose deep dentin.

Superficial and deep dentin surfaces were randomly distributed into three experimental groups:

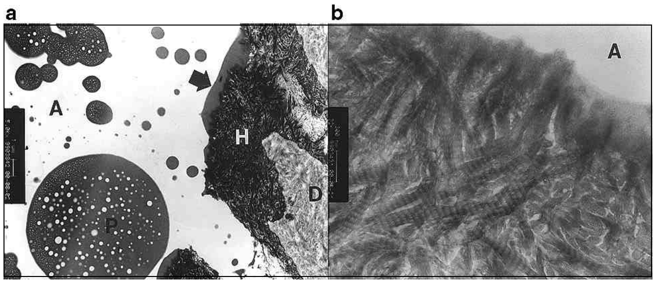

Figure 1. TEM micrographs of bonding of Single Bond to acid-etched, deep dentin. The demineralized, epoxy-resin-embedded sections were stained with phosphotungstic acid and uranyl acetate. **(a)** A low-magnification overall view of the resin-dentin interface. Phase separation of the polyalkenoic acid copolymer (P) component of the adhesive could be seen as globular structures within the adhesive layer (A) and as a continuous layer (arrow) over the surface of the bonding substrate. A 3- to 4-μm-thick hybrid layer (H) could be seen above the laboratory demineralized dentin (D). Bar = 1 μm. **(b)** A high-magnification view showing the presence of intact, banded collagen fibrils within the hybrid layer. A, adhesive layer. Bar = 300 nm.

- Acid-etched: etched with 35% phosphoric acid gel (Scotchbond etchant, 3M ESPE, St. Paul, MN, USA) for 15 sec and rinsed for 10 sec.
- Laser-etched: treated with a pulsed Er-YAG laser (Model 002532, KaVo, Biberach, Germany) with a wavelength of 2.94 μm, a pulse duration of 250 μsec under water cooling. A pulse energy of 180 mJ was used to irradiate the dentin, and the repetition rate chosen was 2 Hz. The laser beam was delivered in a non-contact mode, and the spot size was about 1 mm in diameter. The laser handpiece was mounted in a micro-manipulator (World Precision Instruments Ltd., London, UK) to ensure that the laser beam was delivered perpendicular to the dentin surfaces, to maintain a constant working distance (20 mm), and to allow the handpiece to move freely in a controlled manner.
- Laser-etched and acid-etched: dentin surface was initially treated with the laser, and then acid-etched with 35% phosphoric acid as previously described.

Shear Bond Strength Testing

Specimens were prepared for shear bond strength testing by the Watanabe jig method (Watanabe *et al*., 1996). They were bonded with Single Bond (3M, ESPE) with the use of a wet-bonding technique. Two consecutive coats of adhesive were applied, dried gently for 2-5 sec for evaporation of the solvent, and then light-activated for 10 sec. Z-100 resin composite (3M ESPE) was inserted in 1- to 1.5-mm increments and light-activated separately for 40 sec each. The total composite thickness was approximately 3 mm.

All the specimens were stored in tap water at 37°C for one day, and thermocycled for 500 cycles between 6 and 60°C, with a dwell time of 30 sec each. Shear testing was then conducted by means of a universal testing machine (Model 4411, Instron Corp., Canton, MA, USA) with a crosshead speed of 0.75 mm/min. Fractured specimens were observed with a stereomicroscope for determination of the failure modes, which were classified as cohesive, adhesive, or mixed (Versluis *et al*., 1997).

Statistical Analysis

A two-way analysis of variance (ANOVA) was performed for evaluation of the effects of surface treatment method and dentin depth, and their interactions on SBS. Statistical significance was set in advance at the 0.05 probability level. Multiple comparisons were done by Student-Newman-Keuls tests at $\alpha = 0.05$.

Table. Shear Bond Strengths (MPa) Obtained from Superficial and Deep Dentin after the Different Treatments

	Superficial Dentin	Deep Dentin
Acid-etched	22.5 ± 3.4 (10)*[a]	23.4 ± 5.5 (10)[a]
Laser-ablated	4.0 ± 2.2 (10)[d]	6.3 ± 3.0 (10)[d]
Laser-ablated and acid-etched	16.7 ± 2.9 (10)[b]	13.0 ± 3.2 (10)[c]

* Values are means ± standard deviation (number of specimens). Groups identified with the same letter are not statistically significant ($p > 0.05$).

Transmission Electron Microscopy (TEM)

Twelve caries-free human third molars were used in this part of the study. Two types of dentin surfaces (*i.e.*, superficial and deep dentin) were bonded with Single Bond according to the method described for two teeth in each of three groups and restored with a light-cured, lining resin composite (Protect Liner F, Kuraray Co. Ltd., Osaka, Japan) to facilitate ultramicrotomy. Bonded specimens were fixed, demineralized, dehydrated, and epoxy-resin-embedded according to the TEM protocol reported by Tay *et al*. (1999). Ultrathin sections from 70 to 90 nm thick were double-stained with phosphotungstic acid and uranyl acetate so that the status of the collagen fibrils within the bonded interfaces could be examined by means of a TEM (Philips EM208S, Eindhoven, The Netherlands) operating at 80 kV.

RESULTS

Shear bond strength was not significantly influenced by dentin depth ($p = 0.91$) (Table). However, the type of conditioning treatment ($p < 0.0001$) and the interaction of these two factors were statistically significant ($p = 0.02$).

Multiple comparison tests revealed that acid-etching alone yielded shear bond strength values that were significantly higher than those achieved with laser ablation, regardless of whether the latter was used in conjunction with acid-etching. No statistically significant differences were observed among shear bond results derived from superficial or deep dentin when dentin was acid-etched or laser-ablated. However, significantly higher values were obtained in superficial dentin compared with deep dentin when laser ablation was used before acid-etching.

A mixed-failure mode was predominantly observed in fractured specimens that were etched with phosphoric acid only. Conversely, adhesive failures were exclusively seen in the other groups.

TEM of the acid-etched group in deep dentin showed a 3- to 4-μm-thick hybrid layer (Fig. 1a) that contained intact collagen fibrils with cross-banding (Fig. 1b). A thick surface layer of laser-modified dentin was present in the laser-etched group (Fig. 2a). The superficial part of this layer consisted of electron-dense flakes that exhibited a rippled appearance and were separated by microfissures (Fig. 2b). No collagen fibrils could be identified in this layer. Infiltration of the adhesive was limited to the surface of the superficial part of the laser-modified layer. Dentinal tubules with remnant peritubular dentin were visible. The basal part of the laser-modified layer

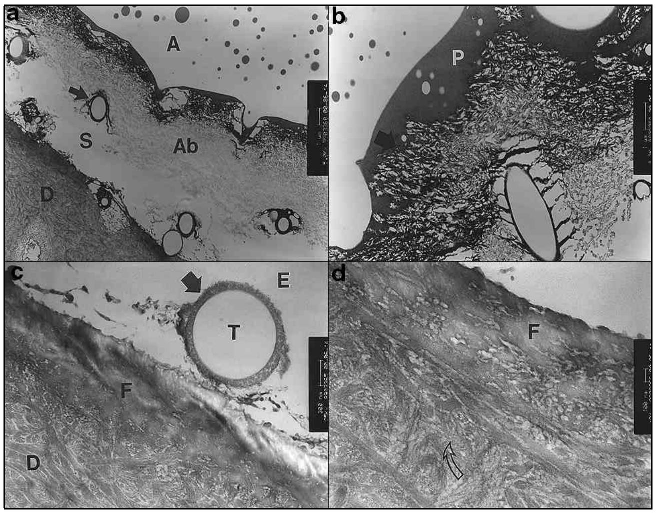

Figure 2. TEM micrographs of bonding of Single Bond to laser-ablated, deep dentin. **(a)** A low-magnification overall view of the resin-dentin interface. A 3- to 5-µm-thick laser-modified layer (Ab) could be observed that had separated from the underlying intertubular dentin (D). The resultant space (S) was infiltrated by the laboratory-embedding resin. Remnant dentinal tubules (arrow) could be seen within the laser-modified layer. A, adhesive layer. Bar = 1 µm. **(b)** A high-magnification view of the superficial part of the laser-modified layer that was partially infiltrated by the polyalkenoic acid copolymer (P). Roughly parallel plates of rippled materials could be identified (arrow). Bar = 1 µm. **(c)** A high-magnification view of the basal portion of the laser-modified layer. Collagen fibrils in this region were fused together to form an amorphous layer (F) that was completely devoid of interfibrillar spaces. This layer probably restricted resin infiltration into the laser-ablated intertubular dentin (D). Above the fused layer, a circumferential layer of mineral-rich peritubular dentin (arrow) could be seen around a dentinal tubule (T). Because of its higher mineral content, peritubular dentin was ablated to a lesser extent than the adjacent collagen-rich intertubular dentin. E, epoxy resin. Bar = 300 nm. **(d)** A very high-magnification view of the base of the laser-modified layer. Beneath the fused layer (F), collagen fibrils from the subsurface intertubular dentin were also partially denatured and appeared as unraveled, microfibrillar strands (arrow). No collagen banding could be seen along the intact collagen fibrils in the subsurface zone for several microns in the underlying dentin. Bar = 100 nm.

consisted of collagen fibrils that were fused together and were devoid of interfibrillar spaces (Fig. 2c). Collagen fibrils from the subsurface intertubular dentin were also partially denatured, and had lost part of their cross-banding (Fig. 2d). In the laser-etched and acid-etched group, the surface of the laser-modified layer was absent after acid-etching (Fig. 3a). However, partial denaturing of the collagen fibrils within the subsurface intertubular dentin could still be observed (Fig. 3b).

DISCUSSION

Previous reports claimed that there are certain advantages in bonding to lased dentin. The adhesive area is enlarged due to the typical scaly and flaky structure that dentin exhibits after Er-YAG laser irradiation (Keller and Hibst, 1989; Visuri *et al.*, 1996b; Aoki *et al.*, 1998; Stiesch-Scholz and Hanning, 2000). This is caused by the micro-explosions the Er-YAG laser produces due to its thermomechanical ablation. The Er-YAG laser initially vaporizes water and other hydrated organic components of the tissue. On vaporization, internal pressure increases in the tissue until the explosive destruction of inorganic substance occurs (Li *et al.*, 1992). Since intertubular

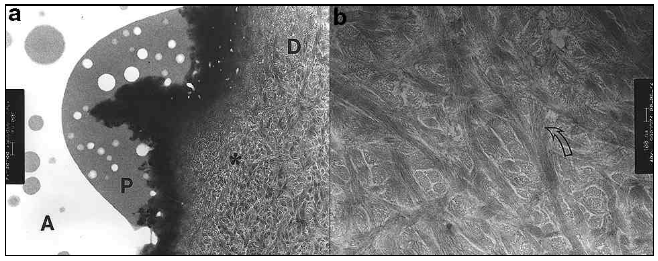

Figure 3. TEM micrographs of bonding of Single Bond to laser-ablated, acid-etched, superficial dentin. **(a)** A low-magnification view showing the absence of the surface laser-modified layer after acid-etching. However, the extent of laser treatment extended into the subsurface intertubular dentin. These partially denatured collagen fibrils could not be removed by phosphoric acid and probably interfered with resin infiltration. A, adhesive layer; P, polyalkenoic acid copolymer; D, demineralized intertubular dentin. Bar = 300 nm. **(b)** A high-magnification view of the region marked with an asterisk in (a). Despite the presence of collagen fibrils with intact microfibrillar architecture (arrow), collagen banding could not be observed from the majority of the fibrils. These subsurface collagen fibrils were probably partially denatured by the heat generated during laser treatment. Bar = 100 nm.

dentin contains more water and has a lower mineral content than peritubular dentin, it is selectively ablated more than the peritubular dentin, leaving protruding dentinal tubules with a cuff-like appearance (Aoki *et al.*, 1998). This may also contribute to an increase in the adhesive area. Patent tubules and the absence of a smear layer are additional factors that may enhance bonding to laser-treated dentin (Visuri *et al.*, 1996b; Aoki *et al.*, 1998; Padrós Fradera and Arroyo Bote, 1999; Stiesch-Scholz and Hanning, 2000).

Adhesion to laser-treated dentin would be explained by the mechanical retention provided by resin tag formation and the infiltration of adhesive resin into the micro-irregularities in lased, mineralized dentin. Our TEM results showed that the superficial part of the laser-modified layer was composed of a scaly surface layer (Aoki *et al.*, 1998) in which collagen fibrils were completely melted and vaporized. It is possible that these parallel plates of flaky materials represented porous layers of melted minerals formed by micro-explosion, forming microfissures that were partially infiltrated by the adhesive. This should be further confirmed with the use of unstained, undemineralized TEM sections. Along the basal part of the laser-modified layer, remnant denatured collagen fibrils were fused and poorly attached to the underlying dentin substrate. The presence of this fused layer in which interfibrillar spaces were lacking probably restricted resin diffusion into the subsurface intertubular dentin, resulting in lower shear bond strength (Table).

Using light and scanning electron microscopy, Aoki *et al.* (1998) reported that the laser-modified layer stained slightly pink with a caries-detecting dye. It is likely that this porous layer was responsible for the staining that was observed in the absence of caries. It is possible that this laser-modified layer is formed by thermal denaturation. Macroscopically, the lased dentin surface revealed no major thermal effects such as carbonization, fusion, or charring (Burkes *et al.*, 1992; Visuri *et al.*, 1996b). However, based on our TEM observations, it is speculated that some degree of heat generation is inevitable with the Er-YAG laser, since it emits in the infrared region (Aoki *et al.*, 1998). Er-YAG laser irradiation produces no demineralization of peritubular dentin, so no funnel-shaped tubule orifices were observed. Resin bonding would form resin tags with parallel walls and with a diameter similar to that of the original tubule lumen. Since hybridization of the lateral walls of dentinal tubules is lacking, lower shear bond strength is expected (Pashley *et al*., 1995).

When acid-etching was used after Er-YAG laser irradiation, a substantial increase in shear bond strength was observed, although it did not reach the control values. It has been reported that microleakage was not improved after acid-etching (Ceballos *et al*., 2001). The adjunctive use of phosphoric acid following by water-rinsing appeared to have eliminated the surface laser-modified layer. However, the thermomechanical effects produced by laser irradiation probably extend into the subsurface dentin and undermine the integrity of the resin-dentin interface (Stiesch-Scholz and Hanning, 2000). This probably accounts for the decreased shear bond strength values when compared with acid-etched dentin without laser treatment. In conclusion, the null hypothesis is rejected. Er-YAG laser irradiation adversely affects adhesion to dentin and does not constitute an alternative to acid etching.

ACKNOWLEDGMENTS

This research project was supported by Grant MAT98-0937-CO2 from the Comisión Interministerial de Ciencia y Tecnología, Spain. This work was also partially supported by NIH/NIDCR Grant DE11526 from the US Public Health Service, Bethesda, MD. The authors thank Gertrudis Gómez-Villaescusa for assistance in specimen preparation.

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