Journal of Dentistry (2003) **31**, 127–135

Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Dentistry

www.elsevier.com/locate/jdent

SEM evaluation of the interaction pattern between dentin and resin after cavity preparation using ER:YAG laser

Marcelo Thomé Schein[a,*], Jorge Sadivar Bocangel[b], Gesse Eduardo Calvo Nogueira[c], Patrícia Alessandra Limas Schein[a]

[a]*Abdon Batista 121, sala 904 Centro, 89201-010 Joinville, SC, Brazil*
[b]*Departamento de Dentística, Faculdade de Odontologia, Universidade de São Paulo, Av. Prof. Lineu Prestes, 2227, Cidade Universitária, Butantã, 05508-900 São Paulo, SP, Brazil*
[c]*Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares—IPEN, Universidade de São Paulo, Travessa 'R', n° 400, Cidade Universitária, Butantã, 05508-900 São Paulo, SP, Brazil*

Received 28 August 2002; accepted 12 December 2002

KEYWORDS
Scanning electron microscopy; Hybrid layer; Dentin; Laser; Er:YAG laser

Summary *Objective*. The aim of this study was to describe the interaction pattern formed between dentin and resin on cavities prepared with an erbium laser (Er:YAG). The morphological aspect of the irradiated dentin after acid etching was also observed.

Methods. Ten dentin disks were obtained from fresh extracted third molars. Each disk received two cavities, one prepared with a conventional high-speed drill, while the other cavity was obtained by the use of an Er:YAG laser (KaVo KEY Laser, KaVo Co.). The laser treatment was performed with 250 mJ/pulse, 4 Hz, non contact mode, focused beam, and a fine water mist was used. Five disks were prepared for morphological analysis of the acid etched dentin. The other five disks had their cavities restored with Single Bond (3M) followed by Z100 resin (3M). The specimens were observed under scanning electron microscopy after dentin–resin interface demineralization and deproteinization.

Results and conclusions. It was observed that the morphological characteristics of the acid-etched irradiated dentin were not favorable to the diffusion of monomers through the collagen network. The dentin–resin interfacial aspect of irradiated dentin, after acid etching, showed thin tags and scarce hybridization zones, which agreed with the morphology of the irradiated and acid-etched dentin substrate observed.

Introduction

The search for techniques and materials able of restoring teeth, recovering esthetics and function, with the least discomfort for the patient is

*Corresponding author. Tel.: +55-47-422-8796; fax: +55-47-426-0385.
E-mail address: mpschein@uol.com.br

0300-5712/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0300-5712(03)00003-4

a constant in dentistry. In the last few years, new techniques have become available due not only to the advances in dentistry itself, but also as a consequence of the development of other areas of knowledge.

In 1955 new concepts of restorative dentistry came up as Buonocore[1] gave the first steps towards adhesion in dentistry. The years passed by and the micro mechanical retention of resin based materials, due to enamel acid etching and subsequent penetration of polymerizable monomers into the spaces created forming resin tags, became an established and predictable clinical procedure.[2,3]

Conversely, bonding to dentin has been more difficult to obtain. The complex composition of its hydrated biological structure, especially evident when acid-etch technique is used on dentin surface, has made harder to find a way of obtaining an intimate association of adhesive and substrate.[4,5]

Dentin etchants are used to remove the smear layer, open dentin tubules, and to demineralize the dentin surface, exposing a dense filigree of collagen fibrils.[4-6] The present concept of dentin bonding is based on the micro mechanical retention of the restorative material upon infiltration of monomers with hydrophilic radicals through the spaces of the collagen web. This dentin-resin interdiffusion zone, known as hybrid layer,[7] has been under investigation by many workers, since the hybrid layer and the resin tags formed in the dentinal tubules are responsible for stability and durability of the adhesive procedure.[8-12]

As well as dentistry had many advances after the second half of the 20th century; other areas also developed a lot. Many of the technological advances were embraced by the dental society contributing to the evolution of it.

The creation of the first laser in 1967, by Theodore Maimann,[13] using the principle of the stimulated emission of radiation postulated by Einstein in 1917,[14] is one example of it. The word *laser* is an acronym for light amplification by stimulated emission of radiation.

As soon as the first laser was created, studies searching for laser applications in dentistry were conducted. The first results of laser dental hard tissue removal were not promising; in order to remove dental structure a great deal of thermal damage to the surrounding tissues and to the pulp was observed.[15-17]

In 1989, the yttrium-aluminum-garnet doped with erbium (Er:YAG) laser was described as being able of removing dental hard tissues without causing thermal undesirable effects, such as cracking or charring, to the remaining dental tissues.[18,19] These first studies were promising and the Er:YAG laser was considered as a promise for replacing the dental drill.

The morphological aspect of irradiated dentin left after cavity preparation with Er:YAG laser has been reported as presenting an irregular surface with open dentinal tubules, and lack of smear layer.[20-25] These aspects, considered favorable for dentin bonding, are a consequence of the thermo-mechanical ablation pattern of Er:YAG laser due mainly to its wavelength (2940 nm) which is highly absorbed by water molecules present in the crystalline tooth structure.[26,27]

In order to evaluate the quality of Er:YAG irradiated dentin surface for bonding of commercially available adhesive systems, several studies have been conducted. These studies have shown the possibility of using such materials on laser-irradiated dentin.[23,28-33] Although some authors have discussed about the possible denaturation of collagen fibrils,[22] and others[21,34] have reported that the use of Er:YAG laser adversely affected the formation of the hybrid layer.

The aim of this study was to observe, under SEM, the interaction pattern formed between dentin and adhesive when Er:YAG laser was used for cavity preparation. The morphological aspect of the demineralized irradiated dentin matrix was also assessed because of the importance of the exposed collagen network for hybridization.

Materials and methods

The methodology of the present study, which is now being described, is presented diagrammatically in Fig. 1.

Specimen preparation

Ten caries-free extracted human third molars, kept in 0,9% physiologic solution at 4 °C after extraction, were used in this study. The roots, at cementum-enamel junction, and the oclusal enamel were removed, and ten dentin disks of approximately 2 mm thick were obtained by slow-speed sectioning with a diamond saw (Labcut 1010-Extec). The disks were fixed on utility wax squares (Wilson-Polidental Ind. e Com. Ltda.), and in each disk two similar cavities, next to each other, were prepared, one with a round diamond bur (1012 KG Sorensen) through a high-speed drill (Roll Air 3, KaVo, Brazil), with 350.000 rpm, under air-water spray; and the other with an Er:YAG laser 2051 hand piece

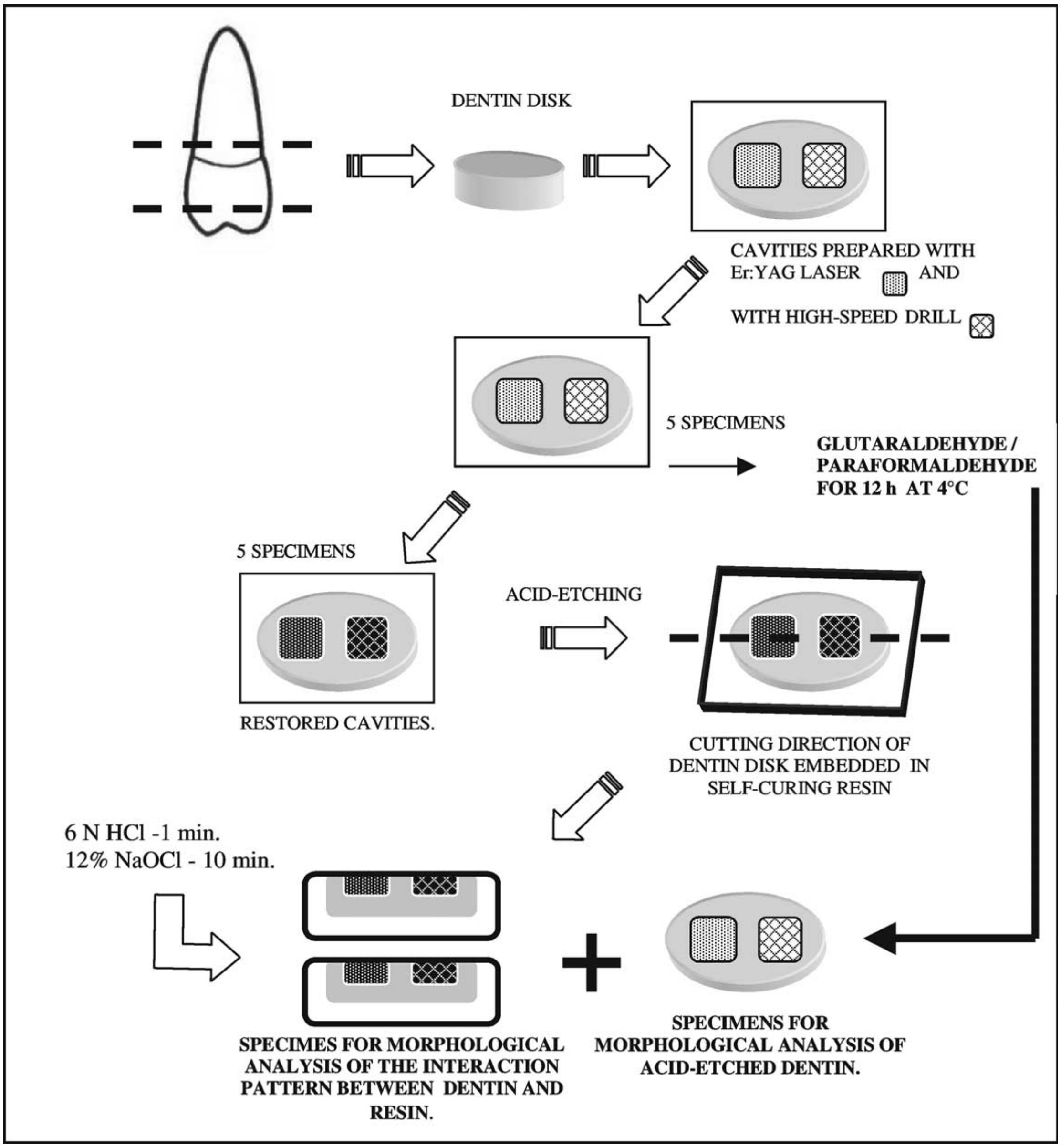

Figure 1 Diagram showing the methodology used for preparation of the samples for morphological analysis of acid-etched dentin and the interaction pattern between dentin and resin.

(KaVo KEY laser, KaVo, Germany). The cavities were 2×2 mm^2 and 1 mm deep.

KaVo KEY Laser consists of a laser, which has an active medium of a solid crystal of yttrium–aluminum–garnet doped with erbium. This laser presents a wavelength of 2940 nm, energy levels varying from 60 to 500 mJ, repetition rate from 1 to 15 Hz, and pulse length from 250 to 500 μs. The irradiation, in the present study, was performed with an energy level of 250 mJ per pulse, repetition rate of 4 Hz, energy density of 83.3 J/cm^2, The cooling system consisted of a water spray set for 8 ml/min. The laser beam was kept perpendicular to the target area during irradiation and the delivery system kept within 12–15 mm from the target area, where the spot size is considered to be 0.63 mm^2.

The 10 disks were divided in two groups ($n = 5$). *GROUP* 1—directed to morphological analysis of acid-etched dentin surface; *GROUP* 2—directed to morphological analysis of the interaction pattern between dentin and adhesive.

Specimen preparation for morphological analysis of acid-etched dentin

After cavity preparations the group 1 disks had their cavities etched with 35% phosphoric acid for 15 s, and washed with air–water spray for 10 s. Afterwards, these specimens were immersed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 h at 4 °C. After fixation they were rinsed with 0.1 M sodium cacodylate buffer at pH 7.4 for 1 h with three changes, followed by distilled water for 1 min.

Specimen preparation for morphological analysis of the interaction pattern between dentin and resin

Group 2 disks received a coat of nail varnish (Colorama Ltda.) on the pulpal side of each disk to prevent organic solvents to scape through dentinal tubules. The specimens from group 2, after etching with 35% phosphoric acid for 15 s, were restored with Single Bond (3 M) and resin Z 100 (3 M) according to manufacture directions.

Group 2 disks were embedded in self-curing epoxy resin (Redefibra Ltda.). After curing, the casts were sectioned exposing the dentin–resin interface of both cavities—laser and conventionally prepared—with a single cutting direction with a diamond saw (Labcut 1010-Extec).

Each half was polished with carbide papers of decreasing abrasiveness (up to 4000-grit).

The specimens were demineralized in 6N HCl for 1 min, rinsed in distilled water, and deproteinized in 12% NaOCl for 10 min, followed by distilled water.

Specimen preparation for scanning electron microscopy (SEM)

After rinsing in distilled water all the samples (groups 1 and 2) were dehydrated in ascending grades of ethanol (25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, and 98.93% for 60 min). The specimens were ultra-sonicated in ethanol 98.93% for 10 min. Then, the samples were immersed in hexametildisilazane (HMDS) for 10 min, placed on a filter paper inside a covered glass vial, and air dried at room temperature. The specimens were then mounted on aluminum stubs, so that they could be observed in a perpendicular direction to the cross-sectional interfaces. After sputtering with gold-palladium the specimens were observed under a JXA-6400 (JEOL, USA).

Dentin–resin interaction pattern assessment

For the morphological investigation of the interaction pattern between dentin and adhesive resin the adhesive penetration inter-, peri-, and intratubular were assessed.

Results

Morphological analysis of acid-etched dentin

Fig. 2 presents the morphological aspects of the dentine surface and sub-surface after etching with phosphoric acid when the cavities were performed conventionally (rotatory instrument). A porous surface, with increased permeability, characterized by the loss of the mineral phase within the collagen matrix, leaving nanometer-sized porosities available for resin infiltration was observed. The tubules were free of smear plugs with widened tubule orifice due to removal of peritubular dentin inorganic phase at the opening of the tubules.

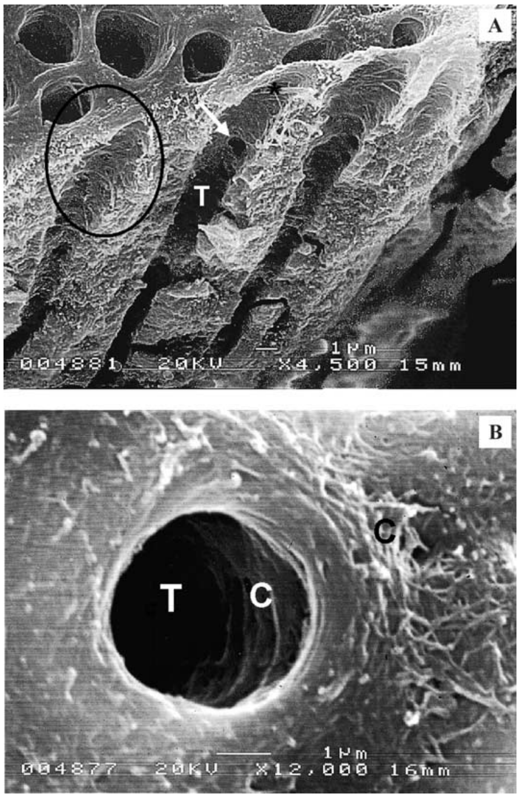

Figure 2 SEM of dentin prepared using high-speed drill and acid-etched with 35% phosphoric acid. (A) Open dentinal tubules (at the top of the picture), longitudinally fractured open tubules (T) and collagen fibrils (*) are evident. Note the tubules are wider for superficial dentin (oval), and also the presence of lateral tubule anastomosis (arrows)(4500*X*; bar = 1 μm). (B) Oclusal view showing open tubule (T) with collagen fibril network present (C) (12000*X*; bar = 1 μm).

On the contrary, in the specimens where the laser was used to prepare the cavities, depicted in Fig. 3, an open collagen matrix was not visible after dentin acid etching. The collagen fibrils were not found forming a porous network responsible for the increased porosity of dentin surface and subsurface. The dentinal tubules were open and they were lined by peritubular dentin, which the acid etching was not able of removing.

Morphological analysis of the interaction pattern between dentin and resin

As can be seen in Fig. 4, in the cavities prepared, prior to acid etching and adhesive application, with the high-speed drill, signs of hybridization were evident along all the interface assessed. Resin tags, of different length, were also reported. Triangular-shaped hybridization at the transition between peri- and intertubular dentin, known as resin tag hybridization, was also found, as well as resin tags anastomosis, which were evident.

In the cavities prepared with Er:YAG, Fig. 5, hybridization zones were hard to find, due to scarcity and discontinuity of such interdiffusion area along of resin–dentin interface. Tags were observed, though fewer and thinner than those found in the conventional group. Tag hybridization, although not frequently, was found and related to the presence of hybridization in intertubular dentin.

Discussion

Bonding resin composites to dental hard tissues was one the most significant contributions for restorative dentistry.[3] Initially to the enamel,[1] and afterwards to dentin,[7] the micro mechanical retention of resin based materials within the porosities, created

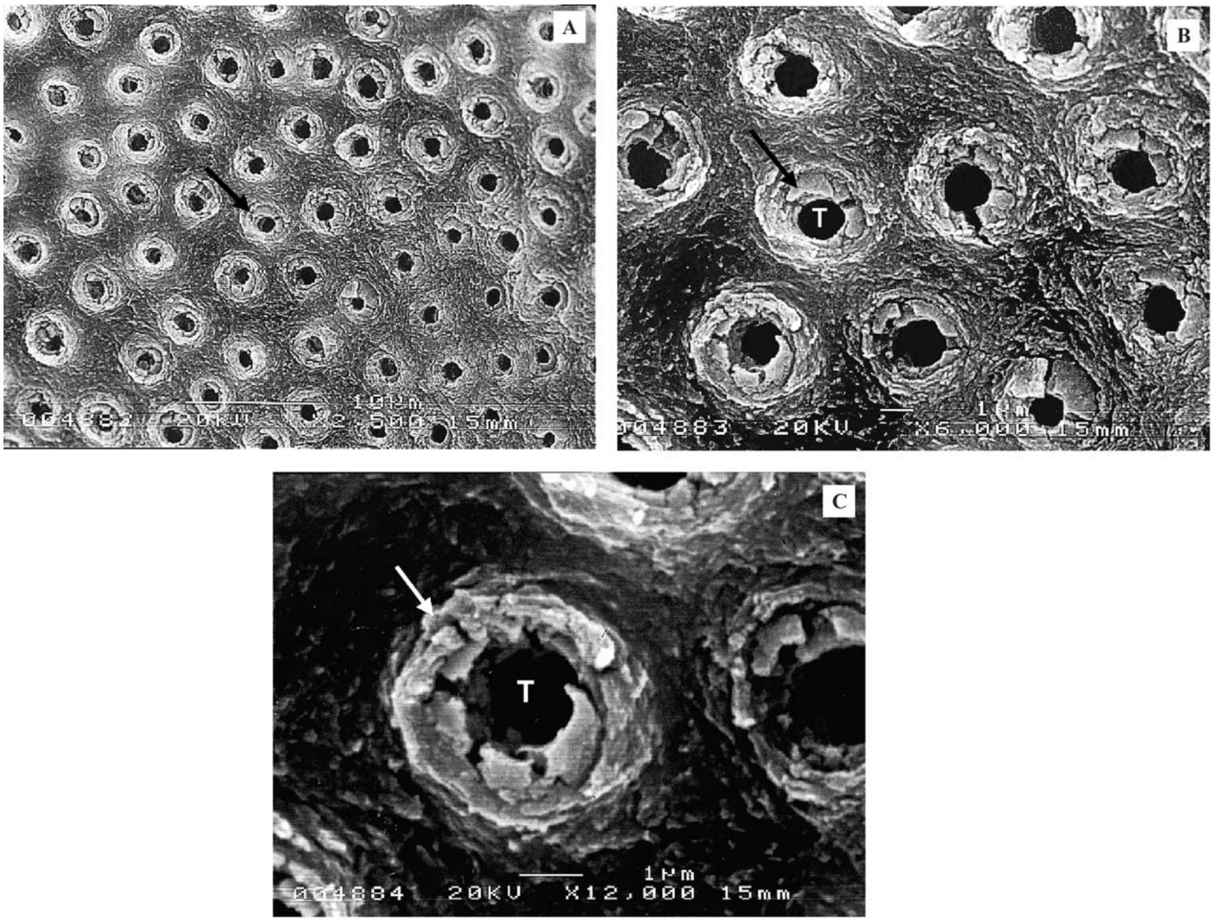

Figure 3 SEM of Er:YAG laser prepared and 35% phosphoric acid etched dentin. (A) Open tubules lined with peritubular dentin are observed (arrow), even after phosphoric acid-etching been used (2500*X*; bar = 10 μm). (B) and (C) Higher magnifications showing that peritubular dentin remained after acid etching (arrow). Open tubules (T). The open collagen matrix is no longer observed and the collagen fibrils are structurally different from the group, which a rotatory instrument was used for cavity preparation (6000*X*; bar = 1 μm).

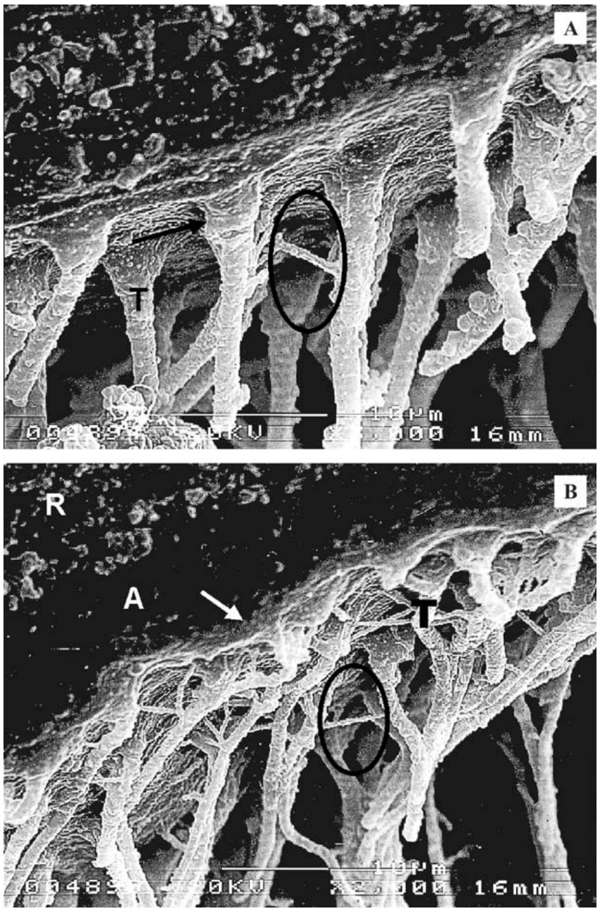

Figure 4 SEM showing the bonding interfaces between dentin and resin when the cavity was prepared conventionally (rotatory instrument) and it was restored with Single Bond (3M) and Z 100 (3M) resin. (A) Resin tags (T), infiltration of resin into secondary lateral tubule (oval). Note the triangular-shaped hybridization visible around the neck of the tags (arrow) (3000*X*; bar = 10 μm). (B) Another specimen from the same group showing Resin tags (T), resin infiltrated lateral tubule anastomosis (oval), resin–dentin interdiffusion zone, known as hybrid layer (arrow), adhesive layer (A), Z 100 resin (R) (2000*X*; bar = 10 μm).

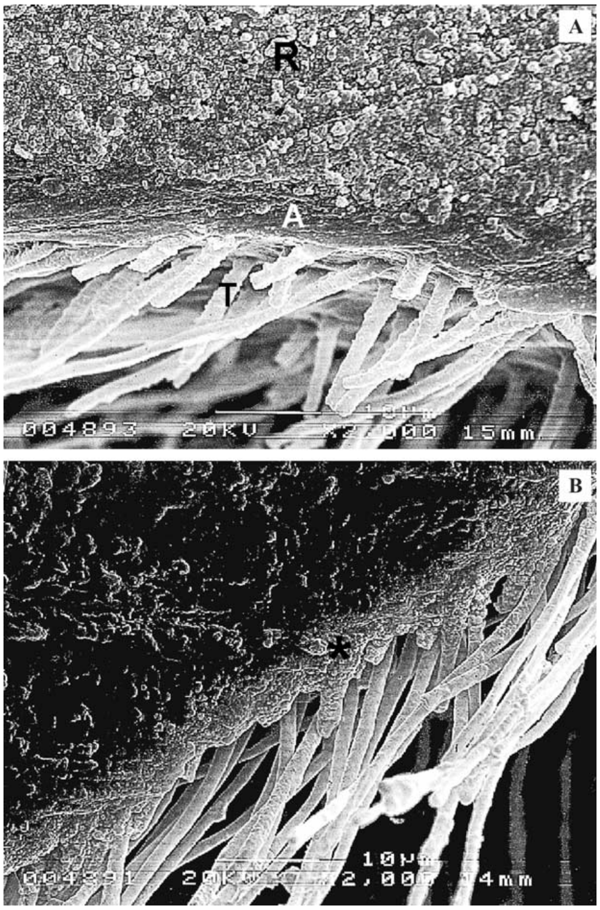

Figure 5 SEM showing the interaction pattern between dentin and resin when the Er:YAG laser was used for cavity preparation followed by dentin acid-etching with 35% phosphoric acid, Single Bond (3M) and Z100 (3M) resin. (A) Z 100 resin (R), adhesive layer (A), resin tags (T) with no signs of hybridization were frequently seen, the tags presented a similar width along their length (2000*X*; bar = 10 μm). (B) We can notice signs of hybridization, along the bonding interface (∗), which were seldom observed and when seen did not show continuity along the bonding interface (2000*X*; bar = 10 μm).

by acid etching dental hard tissues, is currently the most successful approach for dental bonding.[2,3,8–12,35] This has been responsible for preserving healthy tooth structure, as well as has expanded the possibilities of restoring teeth.

In the present study, the interaction pattern between dentin–resin, in cavities prepared with a high-speed drill and Er:YAG laser, was assessed through SEM. These findings were related with the morphological aspects of acid-etched dentine after cavity preparation.

The use of Er:YAG laser on dental hard tissues has been considered effective and efficient, being able of not causing thermal damage to the adjacent tissue nor to the pulp.[18,19,36–41]

The morphological aspect of dentin after cavity preparation with Er:YAG laser has been characterized as an irregular surface with no cracking, or fissuring, lack of smear layer, and open tubules.[20–25] These characteristics were responsible for considering this surface adequate for resin bonding.

This morphological aspect is a consequence of the high water absorption wavelength of Er:YAG radiation, which among other factors, related to laser and to tissue, is a very important determinant for the kind of interaction the laser energy is going to have with the target tissue.[42] The water molecules that are bond to the crystalline structures of the tooth absorb the laser energy readily and easily. The vaporization of the water within the mineral substrate causes volume expansion, and this causes the surrounding tissue literally to explode away. This thermo-mechanical tissue removal process is responsible for the possibility of ablating dental hard tissue with minimal thermal damage to the surrounding tissues.[26,27]

In the present study the cavities, prepared with high-speed drill and etched with 35% phosphoric acid, showed a morphological aspect that is in accordance with previous studies, which reported about the demineralized open dentin matrix role in dentin adhesion.[4-6,35,43-46]

On the other had, when the Er:YAG laser was used to prepare the cavities, the aspect of the dentinal substrate after acid conditioning was quite different. The open tubules were present, representing the space to where adhesive could penetrate, since could not be observed spaces around the collagen fibers for it. The presence of peritubular dentin after acid-etching, as reported by other authors,[21,23] was also another finding in our study that might be due to increased acid resistance of dentin by the laser, since it has been reported the possibility of Er:YAG laser diminishing the solubility of irradiated dentin when immersed in acid solution.[46-48]

When the restorative procedures were conducted, in the specimens prepared conventionally, the hybrid layer formed in a similar way to previously reported.[11,21,49-56] Hybridization of tags,[11,43,44,51,57-59] sealing the entrance of the tubules, and resin tag anastomosis were also observed due to penetration of monomers through the branching of dentinal tubules, which can be important for the infiltration of adhesive towards the deepest demineralized dentin zone.[12,59]

The Er:YAG laser, on the other hand, seems to have had some adverse effects on dentin hybridization, agreeing with previous reports[21,22] on the effect of Er:YAG laser on a possible denaturation of proteins. In our study this ill-defined hybridization was already expected due to the morphological characteristics of the acid etched irradiated dentin surface.

Resin tags were present, in both groups, but the laser group tags showed to be less pronounced than those found in the conventional group, with scarce areas of tag hybridization. These findings are similar to those obtained when applied adhesive on non-acid etched open tubule dentin.[50]

Indirect methods for assessing the bonding quality between resin and irradiated dentin, through micro leakage, tensile and shear bond strength tests, have been carried and have not found differences between irradiated and non irradiated dentine surface.[29-33]

Another study[28] demonstrated that the laser-treated teeth bonded with composite better than laser/acid-, hand piece-, or hand piece/acid-treated teeth. This was not in agreement with other authors[60,61] who justified the low tensile bond strength values obtained in Er:YAG laser treated dentin group as a consequence of physio-chemical changes of the tissues due to laser energy.

The thermal damage of dental tissues caused by laser treatment has been assessed through the morphological aspect of the remaining tissue after irradiation, and also, through the use of temperature measurements done at the pulp chamber and on the surface. Theses studies have proved the possibility of obtaining a surface with no fissures, cracking, or melting aspects, as well as other signs of thermal damage to the mineral component of the tissues and to the pulp.[18,19,36-41] These findings are corroborated by the clinical comfort the patient experiences when using laser for caries removal and cavity preparation.[62-65] However, there is a lack of studies about the effect of Er:YAG laser on the organic components of dentin, especially collagen fibers, which, at present, play a major role in bonding of resin materials.

Recently, one study[66] demonstrated, through light microscopic evaluation, the collagen denaturation promoted by dentin irradiation using an yttrium-scandium-gallium-garnet doped with erbium and chromium (Er,Cr:YSGG) laser, which presents a wavelength of 2790 nm.

At present little is known about the bonding mechanism on laser-irradiated surfaces. The formation of an interdiffusion zone, similar to the one described when the dentin is prepared conventionally and acid etched, seems difficult to be created.

Even knowing the efficiency of Er:YAG laser for caries removal and cavity preparation and the comfort proportioned by the laser, the quality of remaining dentin, obtained in this study, was not favorable to the bonding mechanism based on dentin hybridization, a mechanism developed for a dentin surface cut by rotatory instruments. Further studies should be conducted in order to bring to light the superficial and sub-superficial characteristics of Er:YAG irradiated dentin. Such findings are important for the development of materials able to interact properly with this irradiated surface forming then a new pattern of interaction for bonding of resin-based materials.

Conclusions

The results obtained showed that the pattern of interaction between dentin–resin in cavities prepared with Er:YAG laser and acid-etched was different from that typically found in cavities prepared conventionally.

Inside the limits of our study we can conclude that:

1. The morphological aspect of acid-etched dentin in cavities prepared by Er:YAG did not show the presence of an open collagen matrix necessary for the interdiffusion of the adhesive.
2. The pattern of interaction between dentin and resin when dentin was treated with Er:YAG laser was characterized by ill-defined hybridization signs.

Acknowledgements

The authors wish to thank Prof Flávio Demarco (Faculdade de Odontologia de Pelotas, UFPel, RS, Brazil) (Faculdade de Odontologia de Pelotas, UFPel, RS, Brazil) for his valuable contribution in present work.

References

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *Journal of Dental Research* 1955;**34**:849–53.
2. Barkmeier WW, Cooley RL. Laboratory evaluation of adhesive systems. *Operative Dentistry* 1992;**5**(Suppl.):50–61.
3. Leinfelder KF. Dentin adhesives for the twenty-first century. *The Dental Clinics of North America* 2001;**45**:1–6.
4. Marshall Jr. GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. *Journal of Dental Research* 1997;**25**:441–58.
5. Pashley DH. The effects of acid etching on the pulpodentin complex. *Operative Dentistry* 1992;**17**:229–42.
6. Pashley DH, Carvalho RM. Dentin permeability and dentin adhesion. *Journal of Dentistry* 1997;**25**:355–72.
7. Nakabayashi N, Kojima K, Mashura E. The promotion of adhesion by resin infiltration of monomers into tooth structure. *Journal of Biomedical Materials Research* 1982;**16**:265–73.
8. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. *Journal of Esthetic Dentistry* 1991;**3**:133–8.
9. Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization. *American Journal of Dentistry* 1993;**6**:7–9.
10. Perdigão J, Lopes M. Dentin bonding-state of the art. *Compendium of Continued Education in Dentistry* 1999;**20**: 1151–63.
11. Schneider H, Fröhlich M, Erler G, Merte K. Interaction pattern between dentin and adhesive on prepared class V cavities in vitro and in vivo. *Journal of Biomedical Materials Research* 2000;**53**:86–92.
12. Nakabayashi N, Pashley DH. *Hybridization of dental hard tissues*. Tokyo: Quintessence; 2000. 129 p.
13. Maiman TH. Stimulated optical radiation in ruby. *Nature* 1960;**187**:493–4.
14. Sulewski JG. Historical survey of laser dentistry. *Dental Clinics of North America* 2000;**44**:717–52.
15. Goldman L, Hornby P, Meyer R, Goldman B. Impact of the laser on dental caries. *Nature* 1964;**203**:417.
16. Stern RH, Sognnaes RF. Laser effect on dental hard tissues. *Journal of South California. State Dental Association* 1965; **33**:17–19.
17. Adrian JC, Bernier JL, Sprague WG. Laser and the dental pulp. *Journal of the American Dental Association* 1971;**83**: 113–7.
18. Hibst R, Keller U. Experimental studies of the application of the Er:YAG laser on dental hard substances: I. Measurement of the ablation rate. *Lasers in Surgery and Medicine* 1989;**9**: 338–44.
19. Keller U, Hibst R. Experimental studies of the application of the Er:YAG laser on dental hard substances: II. Light microscopic and SEM investigations. *Lasers in Surgery and Medicine* 1989;**9**:345–51.
20. Tanji EY. Alterações morfológicas do esmalte e dentina de cavidades classe I preparadas com laser de Er:YAG-estudo in vitro. M.D.S. dissertation, University of São Paulo, São Paulo, SP; 1998.
21. Kataumi M, Nakajima M, Yamada T. Tensile bond strength and SEM evaluation of Er:YAG laser irradiated dentin using dentin adhesive. *Dental Materials Journal* 1998;**17**: 125–38.
22. Aoki A, Ishikawa I, Yamada T, Otsuki M, Watanabe H, Tagami J, Ando Y, Yamamoto H. Comparison between Er:YAG laser and conventional technique for root caries treatment in vitro. *Journal of Dental Research* 1998;**77**:1404–14.
23. Burnnet LH, Conceição EN, Pelino JE, Eduardo CP. Comparative study of influence on tensile bond strength of a composite to dentin using Er:YAG laser, air abrasion, or air turbine for preparation of cavities. *Journal of Clinical Laser Medicine and Surgery* 2001;**19**:199–202.
24. Hossain M, Nakamura Y, Yamada Y, Kimura Y, Nakamura G, Matsumoto K. Ablation depths and morphological changes in human enamel and dentin after Er:YAG laser iradiation with or without water mist. *Journal of Clinical Laser Medicine and Surgey* 1999;**17**:105–9.
25. Armengol V, Jean A, Rohanizadeh R, Hamel H. Scanning electron microscopic analysis of diseased and healthy dental hard tissue after Er:YAG laser irradiation: in vitro study. *Journal of Endodontics* 1999;**25**:543–6.
26. Burkes EJ, Hoke J, Gomes E, Wolbarsht M. Wet versus dry enamel ablation by Er:YAG laser. *Journal of Prosthetic Dentistry* 1992;**67**:847–51.
27. Coluzzi DJ. An overview of laser wavelengths used in dentistry. *The Dental Clinics of North America* 2000;**44**: 753–66.
28. Visuri SR, Wright DD, Widgor HA, Walsh TJ. Shear strength of composite bonded to Er:YAG laser-prepared dentin. *Journal of Dental Research* 1996;**75**:599–605.
29. Niu W, Eto JN, Kimura Y, Takeda FH, Matsumoto K. A study on microleakage after resin filling of class V cavities prepared by Er:YAG laser. *Journal of Clinical Laser Medicine and Surgery* 1998;**16**:227–31.
30. Khan MFR, Yonaga K, Kimura Y, Funato A, Matsumoto K. Study of microleakage at class I cavities prepared by Er:YAG laser using three types of restorative materials. *Journal of Clinical Laser Medicine and Surgey* 1998;**16**:305–8.

31. Blankenau RJ, Moses KD, Cherubini ML, Latta MA. Effects of Er:YAG laser on enamel and dentin microleakage. *Journal of Dental Research* 1999;**78**:393. Abstract 2299.
32. Roebuck EM, Whitters CJ, Saunders WP. The influence of three Erbium:YAG laser energies on the in vitro microleakage of Class V compomer resin restorations. *International Journal of Paediatric Dentistry* 2001;**11**:49—56.
33. Ceballos L, Osorio R, Toledano M, Marshall GW. Microleakage of composite restorations after acid or Er:YAG laser cavity treatments. *Dental Materials* 2001;**17**:340—5.
34. Latta MA, Blankenau RJ, Ellis RW. Hibrid zone microstructure of Er:YAG treated dentin. *Journal of Dental Research* 1999; **78**:110. Abstract 36.
35. Carvalho RM, Ciucchi B, Sano H, Yoshiyama M, Pashley DH. Resin diffusion through demineralized dentin matrix. *Revista de Odontologia da Universidade de São Paulo* 1999;**13**: 417—24.
36. Widgor H, Abt E, Asharif S, Walsh Jr. JT. The effect of lasers on dental hard tissues. *Journal of the American Dental Association* 1993;**124**:65—70.
37. Sonntag KD, Klitzman B, Burkes EJ, Hoke J, Moshonov J. Pulpal response to cavity preparation with the Er:YAG and Mark III free electron lasers. *Oral Surgery Oral Medicine and Oral Pathology* 1996;**81**:695—702.
38. Pelagalli J, Gimbel CB, Hansen RT, Swett A, Winn II DW. Investigational study of the use of Er:YAG laser versus dental drill for caries removal and cavity preparation—phase I. *Journal of Clinical Laser Medicine and Surgey* 1997;**15**: 109—15.
39. Glockner K, Rumpler J, Ebelesender K, Ständler P. Intra-pulpal temperature during preparation with the Er:YAG laser compared to the conventional bur: an in vitro study. *Journal of Clinical Laser Medicine and Surgery* 1998;**16**:153—7.
40. Takamori K. A histopathological and immunohistochemical study of dental pulp and pulpal nerve fibers in rats after the cavity preparation using Er:YAG laser. *Journal of Endodontics* 2000;**6**:95—9.
41. Jayawardena JA, Kato J, Moriya K, Takagi Y. Pulpal response to exposure with Er:YAG laser. *Oral Medicine Oral Surgery and Oral Pathology* 2001;**91**:222—9.
42. Gimble C. Hard tissue laser procedures. *The Dental Clinics of North America* 2000;**4**:931—53.
43. Van Meerbeek B, Dem A, Goret-Nicaise M, Lambrechts P, Vanherle G. Comparative SEM and TEM examination of the ultrastructure of the resin—dentin interdifusion zone. *Journal of Dental Research* 1993;**72**:495—501.
44. Titley K, Chernecky R, Chan A, Smith D. The composition and ultrastructure of resin tags in etched dentin. *American Journal of Dentistry* 1995;**8**:224—30.
45. Walshaw PR, McComb D. Microscopic features of clinically successful dentin bonding. *Dental Update* 1998;**25**:281—6.
46. Luz MAAC, Garone Netto N, Arana-Chavez VE, Sobral MAP, Singer JM. Evaluation of chemical and/or mechanical treatments of the smear layer as revealed by scanning electron microscopy—a blind comparative study. *Pesquisa Odontológica Brasileira* 2000;**14**:101—6.
47. Arimoto N, Suzakl A, Katada H, Senda A. *Acid resistance in lased dentin. Proccedings of the International Congress on Lasers in Dentistry, Maui*, **vol. 6**. Maui: International Society of Lasers in Dentistry; 1998. p. 61—2.
48. Hossain M. Caries-preventive effect of Er:YAG laser irradiation with or without water mist. *Journal of Clinical Laser Medicine and Surgery* 2000;**18**:61—5.
49. Van Meerbeek B, Conn LJ, Duke ES, Eick JD, Robinson SJ, Guerrero D. Correlative transmission electron microscopy examination of nondemineralized and demineralized resin—dentin interfaces formed by two dentin adhesive systems. *Journal of Dental Research* 1996;**75**:879—88.
50. Ferrari M, Goracci G, García-Godoy F. Bonding mechanism of three one bottle systems to conditioned and unconditioned enamel and dentin. *American Journal of Dentistry* 1997;**10**: 224—30.
51. El Kalla IH, García-Godoy F. Bond strength and interfacial micromorphology of four adhesive systems in primary and permanent molars. *Journal of Dentistry for Children* 1998;**8**: 169—76.
52. Abdalla AI, Davidson CL. Bonding efficiency and interfacial morphology of one-bottle adhesives to contaminated dentin surfaces. *American Journal of Dentistry* 1998;**11**:281—5.
53. Prati C, Chersoni S, Mongiorgi R, Montanari G, Pashley DH. Thickness and morphology of resin-infiltrated dentin layer in young, old, and sclerotic dentin. *Operative Dentistry* 1999; **24**:66—72.
54. Krejci I, Schüpbach P, Balmelli F, Lutz F. The ultrastructure of a compomer adhesive interface in enamel and dentin, and it's marginal adaptation under dentinal fluid as compared to that of a composite. *Dental Materials* 1999;**15**:349—58.
55. Perdigão J, May Jr. KN, Wilder AD, Lopes M. The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer. *Operative Dentistry* 2000; **25**:186—94.
56. Han L, Abu-Bakr N, Okamoto A, Iwaku M. WDX study of resin—dentin interface on wet vs. dry dentin. *Dental Materials Journal* 2000;**19**:317—25.
57. Perdigão J, Swift Jr. GE, Wefel JS, Donly KJ. In vitro bond strengths and SEM evaluation of dentin bonding systems to different dentin substrates. *Journal of Dental Research* 1994;**73**:44—55.
58. Perdigão J, Ramos JC, Lambrechts P. In vitro interfacial relationship between human dentin and one-bottle dental adhesives. *Dental Materials* 1997;**13**:218—27.
59. Perdigão J, Marshall SJ, Kinney JH, Balooch M. The effect of a re-wetting agent on dentin bonding. *Dental Materials* 1999;**15**:282—95.
60. Martinez-Insua A, Dominguez LS, Rivera FG, Santana-Penín UA. Differences in bonding to acid-etched or Er:YAG-laser-treated enamel and dentin surfaces. *Journal of Prosthetic Dentistry* 2000;**84**:280—8.
61. Ceballos L, Toledano M, OSorio R, Tay FR, Marshall GW. Bonding to Er:YAG-laser-treated dentin. *Journal of Dental Research* 2002;**81**:119—22.
62. Matsumoto K, Nakamura Y, Mazeki K, Kimura Y. Clinical dental application of Er:YAG laser for class V cavity preparation. *Journal of Clinical Laser Medicine and Surgey* 1996;**14**:123—7.
63. Cozean C, Archoria CJ, Pelagalli J, Powell GL. Dentistry for the 21st century? Erbium: YAG laser for teeth. *Journal of the American Dental Association* 1997;**128**:1080—7.
64. Keller U, Hibst R, Geursten W, Shilke R, Heidemann D, Klaiber B, Raab WHM. Erbium:YAG application in caries therapy, Evaluation of the patient perception and acceptance. *Journal of Dentistry* 1998;**26**:649—56.
65. Dostálová T, Jelínková H, Kucerová H, Krejsa O, Hamal K, Kubelka J, Procházka S. Noncontact Er:YAG laser ablation: clinical evaluation. *Journal of Clinical Laser Medicine and Surgey* 1998;**16**:273—82.
66. Farmakis ET, Konyakiotis E, Kouvelas N. *Effect of erbium Cr:YSGG laser on human dentin collagen-a preliminary study. Proccedings of the Congress of the European Society for Oral Laser Applications, Viena*, **vol. 1**. Viena: European Society for Oral Laser Applications; 2001. p. 13.