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Effect of ultrashort laser microstructuring of enamel and dentin surfaces on bond strengths in orthodontics and conservative dentistry

Einfluss der Mikrostrukturierung von Zahnschmelz- und Dentinoberflächen mittels ultrakurz gepulster Laserstrahlung auf die Haftfestigkeit – Bedeutung für die Kieferorthopädie und die konservierende Zahnheilkunde

Abstract: The improvement of adhesion properties in orthodontics and conservative dentistry still remains an open issue. In this work, dentin and enamel surfaces have been totally conditioned by means of ultrashort pulsed laser microstructuring (wavelength: 795 nm, pulse duration: 120 fs, repetition rate: 1 kHz, maximum mean power: 1 W) in order to assess the procedure as an alternative to conventional techniques (acid etching and Er:YAG processing) for clinical practice. Molar dentin surfaces and premolar specimens were used for the study. Adhesive bond strengths were evaluated by means of microtensile bond strength (µTBS) and shear bond strength (SBS) tests of a total etch adhesive system to microstructured dentin and enamel, respectively. The results were related to scanning electron microscope (SEM) observations of the processed and failure surfaces. Bonding strengths were found to be comparable to other conditioning techniques and sometimes even higher. This makes femtosecond laser conditioning of dental tissues a suitable procedure for clinical practice of orthodontics and conservative dentistry.

Keywords: femtosecond laser; acid etching: Er:YAG laser; microtensile bond strength test; shear bond strength test.

Zusammenfassung: Die Verbesserung der Adhäsionseigenschaften stellt in der Kieferorthopädie und der konservierenden Zahnheilkunde ein immer noch offenes Thema dar. In der vorliegenden Studie wurde die Mikrostruktur von Dentin- und Zahnschmelzoberflächen mittels ultrakurz gepulster Laserstrahlung (Wellenlänge: 795 nm, Pulsdauer: 120 fs, Repetitionsrate: 1 kHz, maximale mittlere Leistung: 1 W) mit dem Ziel verändert, die Prozedur als Alternative zu konventionellen Techniken (Ätzen, Er:YAG-Laser) für die klinische Praxis zu evaluieren.

Dentinproben von 15 frisch extrahierten humanen Molaren und 20 humane Prämolaren wurden in der Studie untersucht. Die Haftfestigkeit von Total-Etch-Adhäsiven auf den mikrostrukturierten Dentin- und Zahnschmelzoberflächen wurde mittels Mikrozugfestigkeits- und Scherfestigkeitstests geprüft. Die Ergebnisse wurden mit Rasterelektronenmikroskop-Aufnahmen der bearbeiteten Oberflächen korreliert.

Es zeigte sich, dass die beobachteten Haftfestigkeiten vergleichbar mit anderen konventionellen Verfahren sind und sogar darüber liegen. Dies lässt den Schluss zu, dass die Mikrostrukturierung dentaler Oberflächen mittels Femtosekundenlasern ein geeignetes Verfahren für die Kieferorthopädie und die konservierende Zahnheilkunde darstellt.

Schlüsselwörter: Femtosekundenlaser; Ätzen; Er:YAG-Laser; Mikrozugfestigkeitstest; Scherfestigkeitstest.

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1 Introduction

Over the last few years, new techniques and procedures for hard dental tissue removal have been developed as alternatives to the conventional mechanical procedures. The family of erbium lasers, which brings together a number of lasers sharing an yttrium garnet doped with erbium as active media, was introduced into dentistry specifically as an alternative to traditional mechanical instrumentation for tooth structure preparation [1–6]. The advantages of using erbium lasers for hard tissue preparation include bactericidal effects and less noise, vibration, and discomfort for the patient than a rotary handpiece [7]. By the way, dentin and enamel surfaces structured with erbium lasers exhibit a great similarity to acid-etched ones, motivating clinicians to use such laser systems as an alternative to chemical etching [2, 6, 8].

Despite the outstanding performance of erbium lasers for removing hard dental tissue, reported bond strengths of composite resin to tooth substrates prepared by erbium lasers are confusing and contradictory. Some studies have reported higher bond strengths to laser-prepared than to acid-etched dentin [2, 6]. Others have reported significantly lower bond strengths [4, 9–14] and some others have reported no significant differences [15]. Although some studies have suggested that the irradiation of dentin with the erbium: yttrium aluminium garnet (Er:YAG) laser might replace acid etching as a pretreatment procedure for dentin bonding, most studies have shown that phosphoric acid etching after Er:YAG irradiation is necessary for improving adhesion [4, 10, 16–21].

Concerning orthodontics, bonding brackets to enamel by means of resins has been a common procedure in dentistry long ago [22–25]. Nowadays, acid etching is the usual bonding technique to attach brackets to the enamel surface [11, 26]. Despite the overall spreading of the technique in orthodontics, a potential drawback of acid etching is the demineralization of the most superficial enamel layer [27]. As a result of demineralization, the surface becomes more sensitive to long-term acid attack and caries, especially if resin impregnation is incomplete or defective [11, 28]. The bonding procedure needs to be improved as well, to maintain clinically useful bond strengths while minimizing enamel loss [26, 29].

From the 1960s, researchers have shown that exposing enamel to laser irradiation imparts some degree of protection against demineralization under acid attack [30]. However, as it was previously mentioned for dentin, the results of previous studies on the application of erbium lasers to enamel for increasing bond strengths of restorative materials have been controversial. Some studies reported significantly lower bond strengths for laser-structured as compared to acid-etched teeth [31, 32], whereas comparable results were also stated [33] and even better results were reported for laser-structured enamel in some other works [34]. To our knowledge there is no study comparing the performance of femtosecond (fs) laser-microstructured enamel surfaces with regard to other conventional techniques to improve the bonding of different orthodontic attachments.

From the end of the 1980s ultrashort pulsed laser sources have attracted increasing interest, due to their remarkable performance in materials processing. Sapphire crystals doped with titanium (Ti:Sa) [35], are the most common source to produce laser pulses with duration in the range of the tens and hundreds of femtoseconds (10^{-15} s). These laser pulses, amplified up to energies of the order of millijoule [36] and conveniently focused on the surface of materials, allow the ablation of thin layers with extreme precision and reproducibility, and cause much less collateral damage to the adjacent material than any other thermal, chemical or mechanical process [37–40]. Femtosecond lasers have been already used in dentistry on dental hard tissues [41–45]. It has been demonstrated that the irreversible damage to dental pulp tissue – which is particularly sensitive to such thermal effects – as well as microfractures or "cracks" in hard dental tissues produced by conventional laser sources becomes almost negligible. However, Rego Filho et al. [46] have recently observed that very high powers and long exposure times to ultrashort pulses induce thermal and mechanical damage to the dental surfaces.

Up to date, there is very scarce available research discussing the usefulness of fs laser structuring as enamel or dentin conditioner to improve the adhesion properties of orthodontic attachments or restorative dentistry. Recently, Gerhardt-Szep et al. [47] used this laser source to provide a surface structure in dentin that favours, in some cases, the mechanical retention of the adhesive. Our aim is to use fs laser pulses to fit up the whole surface of enamel and dentin for the application of a total etch adhesive system and afterwards, orthodontic attachments or restorative materials. The mechanical resistance of the adhesion zones was compared with the corresponding ones in samples conditioned by acid etching or erbium laser and in some cases combined with acid etching. This is a preliminary attempt to establish the prospects of this promising technique as a tool to substitute other laser-based, chemical or mechanical techniques currently used in the clinical practice.

2 Materials and methods

2.1 Dentin specimen preparation

Fifteen caries-free human third molars, freshly extracted within a 6-month period and stored in distilled water at 4°C, were selected and cleaned with an ultrasonic system (Cavitron R; Dentsply, Brazil). After this, a sodium bicarbonate device (Profident R; Dabi Atlante, Brazil) was used to remove calculus and adherent tissues from the tooth surface.

The specimens were sectioned transversely at a distance of 4 mm from the occlusal surface in order to remove the enamel and expose a large surface of dentin. A precision cutting machine (Isomet 5000; Buehler, USA) and grinding diamond discs (Struers 330 K; Struers, Denmark) were used with abundant water coolant. Afterwards, the exposed dentin surfaces of each sample were ground with granulated sandpaper at 300, 400 and 600 in a polishing machine (Phoenix Beta; Buehler, USA) under flowing water to induce the formation of a standardized smear layer.

Dentin surfaces were controlled by means of a stereoscopic zoom microscope (SMZ800; Nikon, Japan) to check the presence of remains of enamel and/or pulp tissue.

The dentin specimens were randomly distributed into five experimental groups as many as performed conditioning procedures: acid etching; Er:YAG laser; Er:YAG laser plus acid etching; fs laser and fs laser plus acid etching.

2.2 Enamel specimen preparation

Twenty human premolars, divided in two groups of n=10, were stored in distilled water for a maximum of 6 months after extraction. Exclusion criteria included previously restored premolars and premolars with enamel defects or cracking and delamination of the enamel. The teeth were embedded in a self-cure acrylic block and examined with the stereoscopic zoom microscope (SMZ800; Nikon, Japan). The buccal crown surface of each premolar were rinsed and dried after a 15 s polish with fluoride free pumice.

Before laser irradiation, the buccal enamel surfaces were pumiced, washed for 30 s, and dried for 10 s with a moisture-free air spray.

2.3 Femtosecond laser processing

The laser system consists of, first, a commercial Ti:Sa oscillator (Tsunami; Spectra Physics, USA), which provides pulses in the near infrared ($\lambda = 795$ nm) and duration of approximately 120 fs but energies too low (around 10 nJ) to produce massive ablation of the materials. In order to increase the energy of some of the seed pulses, the system includes a regenerative amplifier (Spitfire; Spectra Physics, USA) based on the chirped pulse amplification technique [36]. Finally, the pulses are 120 fs long with a repetition rate of 1 kHz, and the maximum pulse energy is 1 mJ.

The pulse energy is finely controlled by a half-wave plate and a linear polarizer. Neutral density filters were used when further energy reduction was required. The average power of the beam was measured with a thermopile detector (407A; Spectra Physics, USA). The transversal mode is nearly a Gaussian TEM00 with a 9 mm beam diameter (at $1/e^2$). The laser pulses were focused by means of an achromat doublet lens (f=100 mm) both on dentin and enamel surfaces. With this focusing system, the spot size have a diameter of approximately 12 μm.

The specimens were fixed on a computer-controlled XYZ motorized stage (Micos ES100; Nanotec, Germany). The laser pulses impinged vertically on the dentin surfaces and laterally in the case of enamel surfaces. Therefore, in the first case, XY are the scanning axes and Z allows an optimum focalization of the pulses whereas for enamel surfaces, the latter is provided by Y motion and scanning by XZ motion.

Dentin surfaces exposed to laser pulses were flat, so that a conventional rectangular grid was the selected scanning pattern. The reason to scan bidirectionally is that the surface should be processed not only to check adhesion properties but to condition it for clinical treatment. So far, the superficial layer needs to be completely removed to erase any rest of contaminated tissue or impurities. The laser beam was defocused by elevating 1 mm the samples, in order to obtain a more uniform pattern across the surface minimizing the depth of the grooves generated by laser ablation.

For enamel surface processing, a computer code was developed which drives the three motors in a way that the three-dimensional (3D) surface of the premolars could be homogeneously processed across the region of interest which is in the range of 15–40 mm² depending on the specimen. Since our system does not allow beam motion, the angle between the sample surface and the beam axis must be minimized in order to maximize the absorption of the pulse energy. Otherwise there would be a substantial difference between the structuring at the apex and at the slopes of the surface. So far, the sample is tilted so that the laser pulses face the flatter surface possible. The laser was

not defocused and the scanning pattern was bidirectional as well.

The processing parameters were selected according to our previous works on fs laser processing of hard dental tissues [45]. The focal length of the lens, pulse energy, scanning velocity and pitch between adjacent scans were chosen to generate smoothly overlapping and swallow microstructures.

For dentin processing, the pulse energy was 0.045 mJ, the scanning velocity 0.5 mm/s and the pitch 0.03 mm. In the case of enamel, the same scanning velocity was arranged whereas the pulse energy was switched to 0.03 mJ and the pitch to 0.015 mm. It is important to bear in mind that the ablation fluence threshold (energy density per unit of area) is larger for enamel, and on the other side, enamel was processed in tight focusing conditions.

The specimens were processed in a saturated vapor atmosphere to preserve the tissues from drying. Prior to laser processing and after the treatment, the specimens were stored in a water dilution.

2.4 Acid etching

The surfaces were etched with a 37% orthophosphoric acid gel (3M™ ESPE™ Scotchbond™; 3M Espe, USA) for 15 s (dentin) and 30 s (enamel) and rinsed for 10 s.

2.5 Erbium laser processing

The Er:YAG laser used in this study for dentin irradiation was a Fidelis Plus III (Fotona, Slovenia), which emits at $\lambda = 2.94$ μm with a pulse duration ranging from 150 μs to 250 μs. A pulse energy of 300 mJ was used and the repetition rate was 9 Hz. The laser pulses were delivered in a non-contact mode with a working distance of 15 mm, and the diameter of the spot size was about 1 mm. A water spray was used as coolant. The irradiation was performed with handpiece R14 until a visual inspection confirms that the whole surface is processed. Thus, the processed area was not a priori fixed but depended on the specimen itself.

2.6 Bonding procedures

In the case of dentin-processed surfaces, after 24 h water storage at 37°C to allow adequate water absorption and equilibration, the roots and pulp tissue were removed from their coronal parts using a diamond bur. Apical resection was performed with retrograde obturation with resin and adhesive technique.

The total-etch adhesive (Adper Scotchbond™ 1 XT; 3M Espe, USA) was bonded to the dentin surfaces according to the manufacturer's instructions. After photopolimerization of the adhesive, a resin-based composite crown was constructed with 1.5 mm layers of Filtek Z 250 composite (3M Espe, Brazil), to reach a height of approximately 4–5 mm. Each layer was photocured for 20 s with a LED light-curing unit (Bluephase G2; Ivoclar Vivadent, Liechtenstein). Light intensity output was monitored with a curing radiometer (Model 100; Demetron Research Corporation, USA) to be at least 600 mW/cm^2.

Twenty orthodontic metal brackets (Victory Series; 3M Unitek, USA) were bonded with a total etch adhesive system to enamel consisting of a primer and a composite (Transbond™ XT; 3M Espe, USA) according to the manufacturer's instructions.

Composite was applied to the bracket base (approximately 12 mm^2) and the bracket itself was positioned on the tooth surface and firmly pressed with a Hollenback carver to expel the adhesive in excess. Each bracket was subjected to a 300 g compressive force using a force gauge (Correx, Switzerland) for 10 s, after which excess bonding resin was removed using a sharp scaler. Then, composite was light-cured for 20 s from the incisal edge and 20 s from the gingival bracket edge. The light-curing equipment was the same as for bonding to dentin surfaces.

2.7 Morphological analysis

Representative fractured dentin samples were dehydrated for 48 h in a desiccator (Sample Dry Keeper Simulate Corp., Japan) and then observed with a variable pressure scanning electron microscope (SEM) (Zeiss EVO MA25; Carl Zeiss, Germany) to examine the morphology of the debonded interfaces and determine the failure mode. Failure modes were classified as adhesive, cohesive or mixed in dentin or composite. Obviously, failure mode is a qualitative indication of the relative strength of the adhesion. Usually, the fracture takes place in the adhesion interface, often involving some dentin or resin detachment. Cohesive fractures might indicate that the adhesion region is mechanically more resistant than the dentin or resin themselves. However, following Scherrer et al. [48], cohesive failure samples were discarded because they are not representative of an outstanding interfacial bond strength but of some mechanical weakness inherent to the structure of dentin or resin, or to some misuse of the testing machine.

After debonding, the bracketed teeth were also examined with the same SEM to identify the location of the bond failure. The residual resin remaining on the premolar was assessed by using the adhesive remnant index (ARI), to include a score for enamel fracture where each specimen was scored according to the amount of material remaining on the enamel surface as follows: 0=no adhesive remaining; 1=<50% of the adhesive remaining; 2=more than 50% of the adhesive remaining, and 3=all adhesive remaining with a distinct impression of the bracket base.

2.8 Mechanical analysis

The bond strength of adhesive systems is one of the major factors to be considered when placing a restoration. A common method to evaluate adhesion to a dentin or enamel surface is to determinate the tensile or shear stress applied to a bonded specimen.

2.8.1 Microtensile bond strength test

The dentin specimens were serially sectioned longitudinally to obtain 1 mm thick slabs using a low speed diamond saw under water cooling. Each slab was sectioned into beams with a cross-sectional area of approximately 1 mm^2 using again a low speed diamond saw, following the method described by Shono et al. [49]. Approximately 35–43 beams resulted from each group and microtensile bond strength (μTBS) evaluation was carried out with a universal testing machine (Instron 3345; Instron Corp., USA), running at a cross-head speed of 0.5 mm/min and 500 N load until fracture. The bond strength values were measured in MPa.

2.8.2 Shear bond strength test

The bracketed teeth were immersed in sealed containers of deionized water and placed in an incubator at 37°C for 24 h to allow adequate water absorption and equilibration. To carry out the shear bond strength (SBS) test, the specimens were secured in a jig attached to the base plate of a universal testing machine (Autograph AGS-X 10KN; Shimadzu, Japan). A chisel-edge plunger was mounted in the movable crosshead of the testing machine and positioned so that the leading edge was aimed at the enamel-resin interface before being brought into contact. A cross-head speed of 0.5 mm/min was used.

3 Results

3.1 Morphological analysis: before bonding procedure

SEM observations are useful to examine the surfaces before and after mechanical tests. Additionally, they help to explain and support the mechanical behaviour of the materials in the adhesion region.

In Figure 1 micrographs are shown of (A) raw dentin surface after diamond milling, (B) dentin surface after acid etching, (C) dentin surface after fs microstructuring and (D) dentin surface after fs microstructuring and late acid etching. Dentin surface after diamond milling exhibits a directional macropattern (Figure 1A). As a result of the thermal load, a layer of debris, deformed and resolidified material constitutes the exposed surface of dentin. The dentinal tubular structure can be observed as soon as the acid is applied on the surface (Figure 1B).

After fs microstructuring (Figure 1C), the surface exhibits again a directional pattern that wipes out the previous mechanically induced structure. A grid was expected, but the pitch is small enough to conceal almost any remainder of the first scan and preserves just the second one. The surface presents smooth undulation as a result of the scanning procedure and a micro- and nanoroughness structure typical of fs laser processing (see, for instance [45]).

By the way, the surface is completely different to the one obtained by means of acid etching (Figure 1B) or Er:YAG processing (see, for instance [50]). Microscopically, the most remarkable difference is that most of the dentinal tubules are obliterated, what should be crucial to adhesion performance. Both after acid etching and Er:YAG processing, the surfaces exhibit a large number of open dentinal tubules with protruded peritubular dentin distributed on the surface.

After acid etching, the overall structure of fs processed surfaces is preserved (Figure 1D), but looking further, a large number of dentinal tubules become open with larger diameters than the ones observed in acid etching conditioning or Er:YAG processing. This alveolar structure is radically different to the one obtained by the aforementioned procedures.

Concerning enamel conditioning, Figure 2 shows a series of micrographs of enamel surfaces: (A) boundary between the raw and the fs laser-processed enamel surface, (B) the same region after acid etching, and the fs laser microstructures magnified before (C) and after (D) acid etching.

After fs laser conditioning, the enamel surface presents – as dentin surfaces – a directional pattern provided
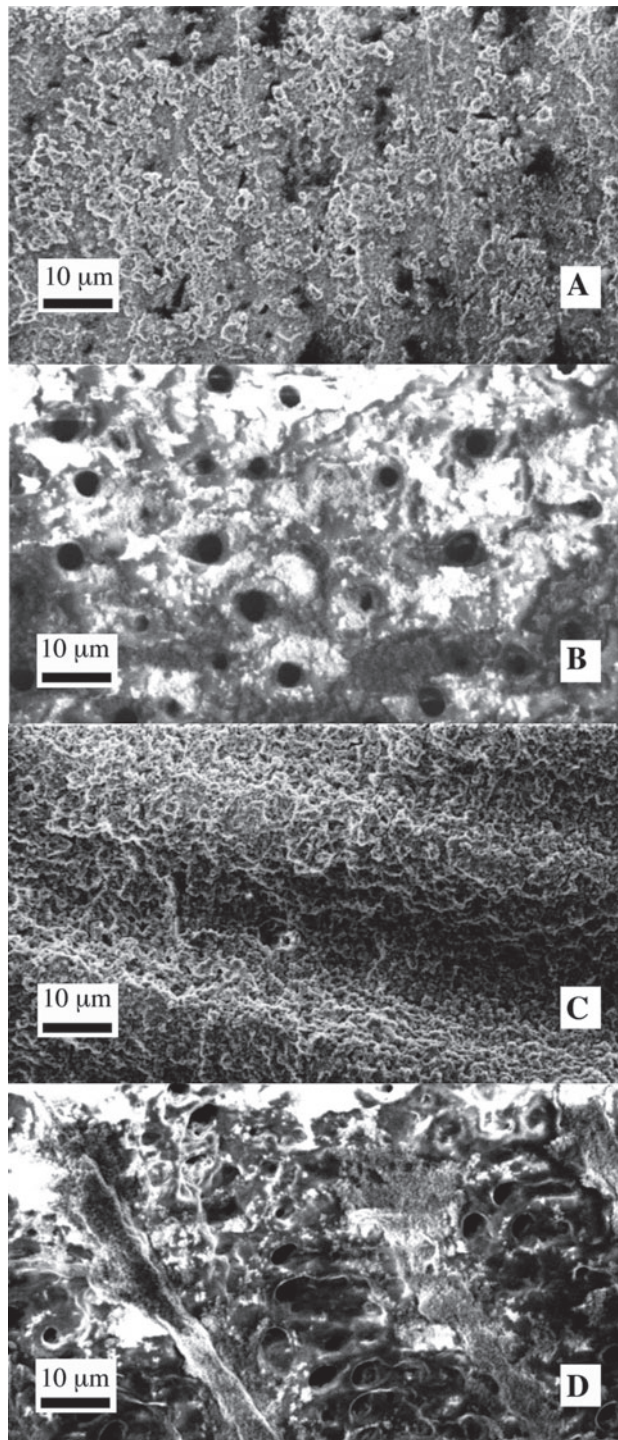
Figure 1 SEM images of dentin surface. (A) The raw surface after preparation by diamond milling, (B) after acid etching, (C) after fs laser microstructuring and (D) after fs laser microstructuring plus late acid etching.

Figure 2 SEM images of enamel surface. (A) Boundary between the raw surface and the fs laser-microstructured surface, (B) the same zone after acid etching, (C) magnification of the fs laser-processed region and (D) magnification of the boundary in (B).

by the scanning procedure (Figure 2A). The microstructured surface boundaries have sharp edges and the original tissue out of bounds is not affected by laser processing. Figure 2C shows a detail of the micro- and nanoroughness which resembles the results obtained for dentin processing with the same laser source.
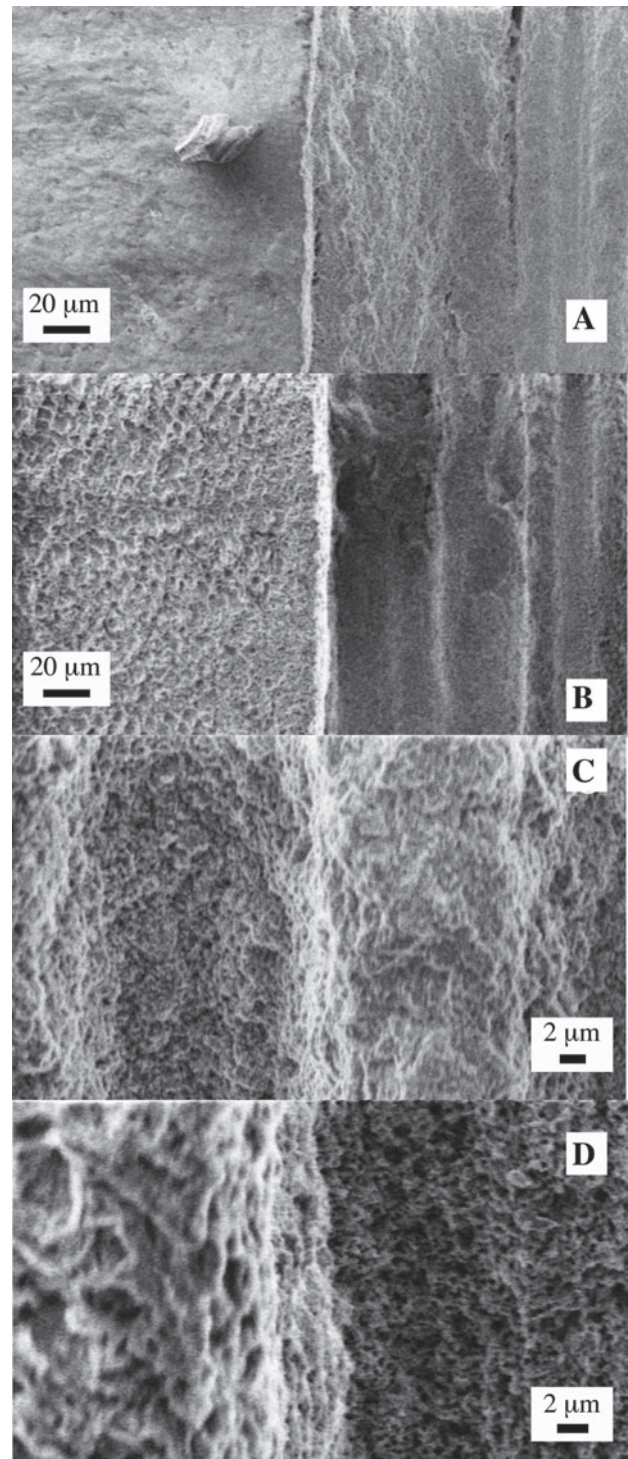
After acid etching, the raw enamel surface becomes rough and plenty of micropores (Figure 2B and D).

Application of acid to the previously fs laser-processed surface preserves the directional pattern whereas the induced roughness is very similar to the acid-etched enamel but the micropores are now more homogeneous and smaller in size (Figure 2D). Anyway, the aspect of the surface in Figure 2D has nothing to do with the aspect of the dentin surface undergoing the same process.

No traces of thermal load can be identified in any of the two tissues, such as cracks or charring which are observed, for instance, in Er:YAG-processed surfaces.

3.2 Morphological analysis: after mechanical testing

After tensile bond strength tests, the mixed failure mode was predominantly observed in acid-etched dentin specimens (Figure 3A) whereas fs laser-processed samples tend to fail within the adhesion zone regardless acid etching was applied after laser processing or not (Figure 3B and C, respectively). In fact, in both cases the pattern produced by irradiation with fs laser pulses is still visible after failure. However, one can appreciate some remarkable differences at the micrometer scale. In Figure 3B remains of adhesive can be identified all across the fracture surface, while in the case that acid etching was not applied after laser treatment, some islands of free dentin can be observed. In these zones, the dentin structure is very similar to the one observed before bonding procedure (Figure 1C).

To analyze the bond failure in the case of brackets on enamel, ARI was used. This index gives a semiquantitative evaluation of the amount of residual adhesive on the tooth surface after debonding and provides an indication of the failure mode and location. The most common failure mode was ARI1 (around 50%) for fs laser-processed enamel, which means that the failure is preferently adhesive in nature and located at the enamel/adhesive interface. In Figure 4 the failure surface for a specimen corresponding to mode ARI1 is shown. In the first picture, Figure 4A, with low magnification, the bracket footprint on the remnant resin still adhered to enamel together with resin-free zones are observed (Figure 4B). In the latter, the microstructures produced by fs laser irradiation are still visible.

When acid etching is applied after fs processing, ARI2 becomes the predominant failure mode (around 70%). This means that the failure surface is essentially localized within the resin/bracket interface. In Figure 5 the failure surface is shown together with a magnification of the region where resin was absent after mechanical test. In this region, fs laser structuring may still be appreciated.
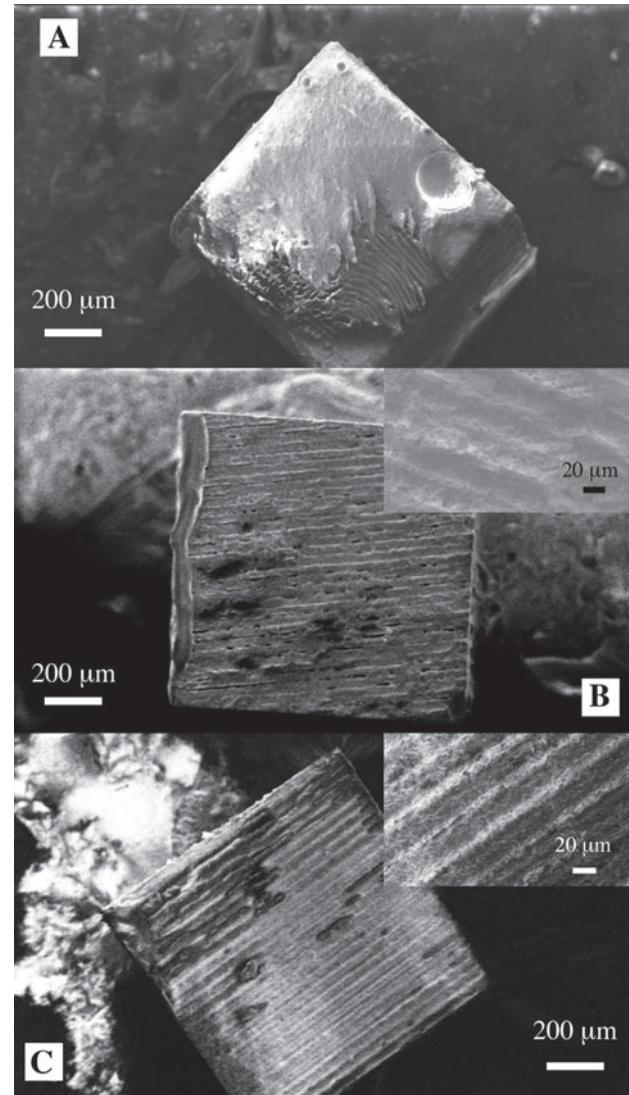

Figure 3 SEM images of the failure surfaces of conditioned dentin beams after μTBS test. (A) Acid-etched, (B) fs laser-microstructured plus late acid etching and (C) fs laser-microstructured.

3.3 Tensile bond strength analysis

Dentin beams debonded before microtensile testing were discarded for the analysis as well as the results corresponding to cohesive failure either in dentin or the resin. The mean values of μTBS and standard deviations are summarized per experimental group in Table 1.

3.4 Shear bond strength analysis

Table 2 shows the results of SBS tests performed on fs laser-processed enamel together with the range of values obtained from recent studies corresponding to acid-etched
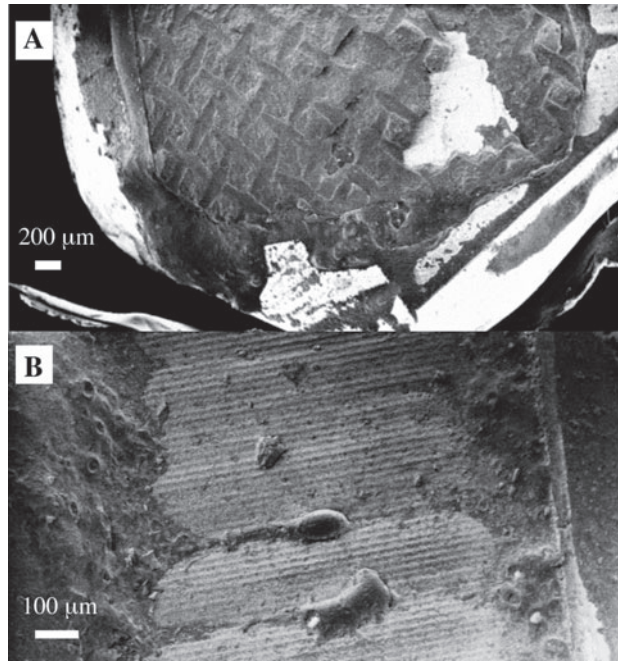
Figure 4 SEM images of the failure surface of a fs laser-processed specimen after SBS test. The sample belongs to ARI1 failure mode group. (A) Overall view showing the footprint of the bracket on the remnant resin. (B) Magnification of a zone free of resin where remains of fs laser structures are shown.

Figure 5 SEM images of the failure surface of a fs laser-processed and acid-etched specimen after SBS test. The sample belongs to ARI2 failure mode group. (A) Overall view and (B) magnification of a zone free of resin where fs laser structuring may be appreciated.

and Er:YAG laser-processed surfaces for the sake of comparison. In all the cases, the adhesive system to bond the bracket was the same.

4 Discussion

Our interest in this study was to check the ability of fs laser microstructuring to condition the surfaces of dentin and enamel for the clinical practice of restoration and orthodontics. Up to date, fs laser microstructuring of hard dental tissues has been the object of a few *in-vitro* studies [41–45], but to our knowledge, the research in this topic has never been so close to the boundaries of the clinical practice.

For restorative and orthodontical purposes, it is essential to remove completely the outer layer of the dental tissue to eliminate any remainder of contaminated tissue or impurity, and at the same time to preserve both the chemical composition of the new surface and provide it with a microstructure that could foster mechanical retention of the adhesive. Morphological analysis by means of SEM shows that total conditioning is achieved both for dentin and enamel with the processing parameters used (Figures 1 and 2). Other recent works [47] were focused to
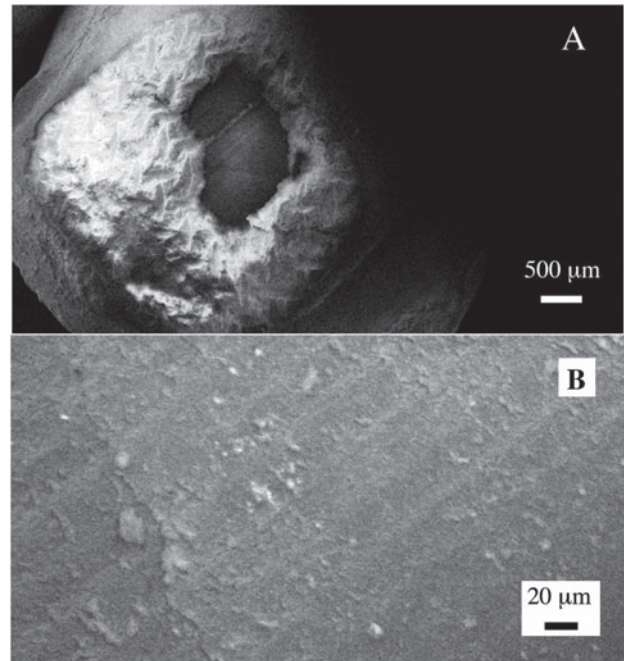
evaluate the adhesion properties of surfaces microstructured with ultrashort laser pulses but they fail to process the whole surface what raises doubts about the validity of their conclusions for clinical practice.

Most of the literature on dentin conditioning for restorative purposes agree in pointing out that acid etching provides the best bonding strengths among the existing procedures, including Er:YAG processing. As described by Perdigao et al. [57] it seems that the bonding mechanism of resin to acid-etched dentin is well understood to be micromechanical, but little is known about the mechanism of resin adhesion to Er:YAG laser-processed dentin. The formation of an interdiffusion zone similar to that described for acid-etched dentin seems to be unlikely. The peritubular dentin seems to be more resistant to acid etching than to Er:YAG processing and the intertubular dentin is highly demineralised and possibly not completely impregnated by monomers, creating a hybrid layer more susceptible to hydrolysis [7, 58].

The use of Er:YAG laser produces a surface layer on the dentin substrate with a particular morphological pattern and possibly formed by thermal denaturation. The laser-processed dentin surface revealed no thermal effects like carbonization, fusion or charring, at least macroscopically, but as a result of the laser wavelength and the duration of the pulses, some effects due to heat transfer should

	Acid etching	Er:YAG laser processing	Er:YAG laser processing+acid etching	fs laser processing	fs laser processing+acid etching
μTBS (MPa)	28.9±9.0	26.5±7.1	24.2±7.8	21.7±5.7	26.0±7.6
N	39	35	43	36	35

Table 1 Mean values of microtensile bond strengths (in MPa) obtained for dentin specimens, prepared from 15 caries-free human third molars, after different conditioning procedures and the number of specimens tested.
μTBS, microtensile bond strength; N, number of tested specimens.

be expected [59]. According to [4] this layer is composed of a superficial part, which consists of a scaly surface where the collagen fibrils are completely melted and vaporized and where the adhesive may infiltrate through micro-cracks or micropits. The basal part contains the rest of the denatured collagen fibrils that were fused and weakly attached to the underlying dentin with reduced interfibrillary spaces. The presence of this layer avoids deep infiltration of the adhesive, resulting in lower bond strength values. Although additional application of acid etching appeared to remove this layer, the thermomechanical effects produced by laser irradiation probably spread down into the dentin, undermining the integrity of the resin-dentin interface [15]. This statement could explain why similar bond strength values are obtained for Er:YAG laser-processed dentin independently of the application of acid etching (Table 1).

The challenge was to determine whether fs laser irradiation is a valid procedure for conditioning dentin and at the same time the evaluation of the performance of fs laser-processed specimens as compared to acid-etched and Er:YAG laser-processed ones. The results obtained in μTBS tests show that fs laser-processed specimens exhibit bond strengths lower but comparable to the other groups. When acid etching follows fs laser treatment, the bond strengths increase in opposition to what happens for Er:YAG laser-processed surfaces and this suggests that the mechanisms involved in adhesion in both laser treatments should be different. The most important difference between both procedures resides in the nature of the

ablation mechanisms and, consequently, the collateral effects around the ablated zones. Thermal load on the samples is very important during Er:YAG ablation whereas it is almost negligible when the surface is ablated with fs laser pulses. Since heat transfer is responsible for modifications in the chemical composition of the materials and in the roughness features on the surfaces, one should expect the previous explanation to fail for fs laser-processed surfaces [44–47] and the mechanical resistance of the outer layer of dentin to be higher than for Er:YAG-processed surfaces. By the way, the micro- and nanoroughness induced by fs laser treatment (Figure 1C), should theoretically improve the adhesion of the resin since the effective surface is much larger than in the case of acid-etched or Er:YAG-processed surfaces. However, the values of bond strengths are smaller.

Our explanation has to do with the tubular structure within the surface. When fs laser pulses irradiate the dentin surface, most of the dentinal tubules are obliterated (Figure 1C) because of the smear layer produced by ablation, unlike to what happens when acid etching or Er:YAG laser is applied (Figure 1B). This morphological landscape represents a serious drawback to adhesive penetration. It has been previously suggested [60] that most of the bond strength could be attributed to the formation of resin tags and the previously mentioned hybrid layer. In the case of fs-processed dentin, only the micro- and nanoroughness can be responsible for mechanical retention. In this sense, the results after μTBS tests indicate that this mechanism should be quite

	Literature data			Experimental data	
	Acid etching [51–54, 56]	Er:YAG laser processing [51–53]	Er:YAG laser processing+acid etching [51, 55]	fs laser processing	fs laser processing+acid etching
SBS (MPa)	<20	≤11	≤17	22.9±8.3	24.6±5.6
N				10	10

Table 2 Comparison of the mean values of shear bond strengths (in MPa) obtained for enamel specimens after different conditioning procedures. The results for acid-etched and Er:YAG laser-processed surfaces were taken from recent studies in the literature [51–56].
SBS, shear bond strength; N, number of tested specimens.

powerful providing bonds strong enough to be used in clinical practice. The application of late acid etching on the fs-microstructured surface improves the performance of the specimens but not in a really significant way. Looking at Figure 1D, one can observe how acid etching opens dentinal tubules superimposed to the fs-induced microstructures. This tubules are morphologically different to those observed after acid etching or Er:YAG processing, exhibiting larger diameters. Obviously this favours adhesive penetration, but at the same time some degree of mechanical weakening arises since peritubular dentine surface is reduced.

Most of the failures of the fs laser-processed groups are adhesive in nature. For what it is important, the fs laser microstructures remain practically unaltered after debonding in those regions where some dentin merges in the failure surface (Figure 3B and C). This is an indication of the swallow penetration of the adhesive in the dentin tissue and the absence of tags, and supports the previous statements.

Concerning enamel, the scenario changes unexpectedly. Bond strengths after SBS tests (Table 2) increase substantially with regard to acid etching or Er:YAG data in the literature [51–55]. Up to our knowledge there is no work reporting on the mechanical behaviour of enamel surfaces after fs processing. Therefore, this will be the first time fs lasers merge as an advantageous tool as compared to state of the art procedures, to improve the mechanical performance of enamel surfaces in bracket bonding.

Anyway, the results of SBS tests for surfaces undergoing other conventional procedures are very contradictory and the explanation of these results really motley. Many of them appeal to roughness and microcrack formation that could favour retention and impregnation as the reason for a better performance of Er:YAG-processed surfaces as compared to acid-etched ones [2, 20]. Those who found opposite results, either argue that microcracks constitute weak regions on the surface that give rise to fractures and contaminant filtrations to the tissue [11, 55] or blame on inappropriate or defective use of the laser tool [52, 53]. Er:YAG is an alternative to acid etching depending on the irradiation parameters. Bonding strengths values are at most similar regardless the acid etching is applied or not after laser irradiation (see Table 2).

The performance of fs-processed enamel surfaces can be attributed to the mechanical retention of the resin provided by the micro and nanoroughness produced by fs laser ablation. In this case, the practical absence of thermal load on the remaining tissue precludes the formation of microcracks. So far, any explanation involving this superficial feature is no longer valid for fs-processed surfaces. By the way, the chance of caries formation for specimens processed by means of fs laser should decrease substantially.

However, assuming that mechanical retention is, under any enamel conditioning procedure, provided by roughness, fs laser-processed enamel exhibits a far rougher surface than Er:YAG surfaces and, what makes the real difference, the typical size of the porous structure is much smaller than for Er:YAG and even acid-etched structures (see Figure 2C). This homogeneously distributed nanoroughness is, in our opinion, responsible for the improved bond strengths of fs laser-processed specimens. Acid etching contributes to increase the number of nanopores of the fs-processed surface (Figure 2D), the mechanical retention and so, the bond strengths. Figures 4 and 5 show that the adhesion of resin to enamel is strong enough to make ARI1 and ARI2 failure mode, respectively, the predominant ones. In those islands where enamel surface becomes visible after mechanical test, the presence of the undulated surface produced by fs laser processing can be still observed but flattened as compared to the pictures taken before the mechanical tests. In fact, the nanoroughness pattern has almost disappeared, pulled out with the resin.

5 Conclusions

Total conditioning of dentin and enamel surfaces by means of fs laser ablation alone or followed by acid etching was demonstrated to be a valid procedure to prepare surfaces for restorative intervention or orthodontics. The bond strengths obtained for the adhesion of composite resins were enough to meet the requirements of clinical practice.

In the case of bracket bonding to enamel surfaces, the performance of fs laser-processed surfaces is better than the conventional procedures, namely, acid etching and Er:YAG laser processing. For dentin surfaces, the values of bond strengths are slightly lower. The reason for this uneven behaviour has to do with the mechanism of mechanical retention. In the case of dentin, it has been previously reported that the major contribution to bond strength comes from the adhesion provided by the infiltration of the resin in the tubular structure of conditioned dentin whereas retention due to micro and nanoroughness is a secondary mechanism. On the contrary, in absence of such tags, roughness should be the leading mechanism of retention for enamel surfaces. And the surfaces processed

with fs laser pulses present a much larger effective area for retention than the surfaces conditioned by other means as a result of the process of ultrafast ablation.

More research is needed to understand the mechanisms of adhesion whatever the technique used to condition the surfaces, but especially in the case of fs lasers. However, the way to the implementation of this technique to the clinical practice is open.

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