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Ultra-structure characterization of self-etching treated cementum surfaces

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Abstract

Objectives: to evaluate the effect of different conditioning treatments on surface roughness and topography of dental cementum. **Study Design:** Extracted human canines were used for the present study. The mesial surface from the cervical third of the roots were ground flat with wet 600-grit silicon carbide paper. They were polished (up to 1/4 μm diamond paste) and treated as follows: 1) No treatment, 2) 35% H₃PO₄ during 15 s, 3) Clearfil SE Bond primer (SEB), 4) One-Up Bond F (OUB). The adhesive systems were applied following manufacturer's instructions. SEB primer and OUB were removed from surfaces by washing and ultrasonic agitation with ascending ethanol solutions. Digital images of treated surfaces (5x5 and 15x15 μm) were obtained by means of an atomic force microscope (AFM) analysis. The average surface roughness (Ra nanometers) of the scanned areas was assessed. Data were analyzed by ANOVA and SNK multiple comparisons tests ($p < 0.05$). **Results:** phosphoric acid treatment produced the highest mean roughness value, at all scan sizes. At 5x5 μm AFM images, for self-etch adhesive systems no differences in roughness were detected. At 15x15 μm , when One-Up Bond F was employed the lowest value was obtained. **Conclusions:** When phosphoric acid treatment was applied, cementum surface roughness increased and a strong demineralization with exposed collagen fibers could be observed.

Key words: *Cementum, AFM, topography, roughness, phosphoric acid, self-etching.*

Introduction

Developments in adhesive dentistry and in periodontology have enabled tooth lesions to be treated more conservatively, and thus lengthen their lifespan in the oral cavity (1, 2). In addition, elderly people are at high risk to have sub-gingival and root caries (3).

Adhesion process depends not only on the adhesive system, but also on the dental substrate (4). Clinically, the margins of many adhesive restorations on the root surface are thought to be positioned in cementum or cervical outer dentin (2). While the bond to dentin is important, that to cementum is also critical to prevent microleakage

and bacteria gaining access to the underlying dentin (5). The root cementum has high organic content and predominantly consists of cross-linked collagen structure. This tissue is less hard and more permeable to a variety of materials compared with enamel and dentin (6). Dental cementum has been defined as a composite of non-collagenous proteins and collagen fibrils reinforced with a 65 % on weight inorganic phase (apatite crystals) (7). There are three fundamentally types of cementum: Acellular afibrillar cementum, which covers minor areas of the enamel; acellular extrinsic fiber cementum is mainly found on the cervical and middle portions; and

cellular intrinsic fiber cementum, that cover the furcations and the apical root portions. (1).

New adhesive systems have been developed in an attempt to obtain a reliable bonding to all tooth substrates (8). Two different approaches are the most frequently used. The etch-and-rinse technique is applied by removing the smear layer with phosphoric acid. This is followed by the application of a primer and an adhesive in two different steps or in a single step. To make this process even simpler and more reliable new adhesives that do not need an acid etching step prior to its application were created, called self-etching adhesive systems. In the self-etching approach, the acid and the primer are combined into one solution to form an acidic monomer (9), prior to the application of a subsequent bonding step. One-step self-etching adhesives have been introduced and contain all components in either a two-bottle set or in a single bottle (10).

Several self-etching systems are available today, but little is known about their ability to interact with the dental cementum. Surface treatments of dental substrate increase the roughness and create microporosities, enhancing the potential for mechanical retention of the adhesive system (11, 12). The surface roughness of the cementum can be characterized by topographic measurements (Atomic Force Microscope, AFM), because it provides a real topographical three-dimensional image

of a sample surface with vertical resolution from 0.1 nm and lateral resolution from 0.1 nm (13).

The objective of this study was to evaluate the effect of different conditioning treatments on surface roughness and topography of dental cementum. The null hypothesis is that the surface treatments do not change the surface roughness and topography of dental cementum.

Materials and Methods

-Specimen preparation

Twelve canines stored in 0.5% chloramine T at 4 °C for less than one month were employed. The crowns of the teeth and the apical third of the root were perpendicularly removed by using an Accutom-50 (Struers, Copenhagen, Denmark) under copious water irrigation. Flat cementum regions from the mesial surfaces of the cervical root third were used as bonding substrates, after grinding with wet 600-grit silicon carbide paper with the initiated point 3 mm below the cement-enamel junction. The presence of cementum was previously verified under a stereomicroscope, using a 40x objective (Olympus/De Trey, Konstanz, Germany). Surfaces were metallographically polished (up to 0.25 µm diamond paste). Adhesive systems were applied on the surfaces of the acellular cementum. Composition, manufacturers, and application protocol of the tested adhesives are displayed in table 1. The pH of

Table 1. Information about tested adhesive systems.

Components	Principle ingredients	Mode/steps of application
Clearfil SE Bond (SEB) – Kuraray Co. Ltd., Osaka, Japan (pH=2.1)		
Primer	10-MDP; HEMA; hydrophilic dimethacrylate; dl-camphorquinone; N,N-diethanol-p-toluidine; water.	Apply Primer for 20 seconds. Mild air stream. Apply Bond.
Bond	10-MDP; Bis-GMA; 2-hydroxyethyl methacrylate; hydrophobic dimethacrylate; di-camphorquinone; N,N-diethanol-p-toluidine; silanated colloidal silica.	Gentle air stream. Light cure for 10 seconds.
One-up bond F (OUB) – Tokuyama Europe GmbH, Dusseldorf, Germany (pH=1.3)		
Bonding Agent A	Phosphoric monomer, MAC-10, multi-functional methacrylic monomers, photo-initiator	Mix Bonding Agent A and Bonding Agent B until the mixed turns homogeneously pink. Apply the mixture. Leave the surface undisturbed for 20 seconds.
Bonding Agent B	Fluoroaminosilicate glass filler, water, mono-functional monomers, dye-sensitizer, borate derivate	Light cure for 10 seconds. The pink colour should turn to a pale brown after light irradiation.

Abbreviations: 10-MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bis-phenol A diglycidylmethacrylate; MAC-10: Methacryloyloxy-alkyl acid phosphate.

the bonding agents were assessed using pH indicator strips (Merck KgaA, Darmstadt, Germany).

Roughness analysis by AFM

AFM observation of the conditioned surfaces was performed. The cementum surface treatments were: 1) No treatment, 2) 35% phosphoric acid (Scotchbond Etchant, 3MESPE, St. Paul, MN, USA) was applied for 15 s and washed out with distilled water for 30 s, 3) Clearfil SE Bond primer was applied for 20 s, and 4) One-Up Bond F was applied for 20 s. When self-etching adhesives were applied, resin remnants were removed by washing and ultrasonic agitation with different alcohol solutions in ascending series of ethanol (50%, 60%, 70%, 80%, 90%, 96% and 100%) for 1 min each, and further sonicated in absolute ethanol for 1 min to dissolve the self-etching primer or adhesive (14). The cementum surfaces, including the phosphoric acid treated surfaces, were rehydrated with distilled water to determine the nanoroughness values.

The topographical features of the surfaces were scanned using an AFM in tapping mode (Multimode Nanoscope IIIa, Digital Instruments, Veeco Metrology group, Santa Barbara, CA, USA). Three digital images 5x5 µm and three images 15x15 µm under wet conditions were obtained from each specimen. The tapping mode was performed using a 1-10 Ohm-Cm phosphorus (n) doped Si tip (at 50 µm). Changes in vertical position provided the height of the images, registered as bright and dark regions. A data scale of 1504 µm and recorded with a slow scan rate (0.1 Hz) was employed. For each image, ten randomized boxes (five at 3x3 µm and five at 10x10 µm, respectively) were created to examine the surface roughness of the treated cement (n=120). The topography and the average surface roughness (Ra nanometers) of the scanned areas were qualitatively and quantitatively evaluated, respectively, using Nanoscope Software V7.

-Statistical analysis

Nanoroughness values (Ra –nm-) were analyzed with ANOVA and Student Newman Keuls multiple comparisons tests. Statistical significance was set in advance at the 0.05 probability level.

Results

Mean roughness (Ra) are shown in table 2. In 5x5 µm size images, mean roughness values were higher after using H3PO4 than non-treated surface and when self-etching adhesive systems were employed (F=7.30; P<0.01). No differences exist between both self-etching adhesives. In 15x15 µm images, roughness values increased significantly after using phosphoric acid (F=102.70; P<0.0001). The SE Bond primer group was similar to the polished group. The lowest roughness values were obtained when One-Up Bond F was employed. Attained pH values for each adhesive are displayed in table 1.

Figures 1 and 2 are AFM images of polished and conditioned cementum. Morphological differences are encountered. Height variations in the sample are represented by differences in gray scale, where white represents the highest features and black the lowest features of the scale. The gray level at each position on the surface represents a different depth on the sample surface. The non-treated specimens (Fig. 1a) showed a lamellar pattern corresponding of acellular extrinsic fiber cementum (Notice: A point or locus of periodontal ligament insert can be seen). Figure 1b exhibits cementum surface after phosphoric acid etching: the most aggressive removal of the mineral phase is observable. When the cementum surface was treated with SEB primer or OUB (Figs. 1 c, d) the surfaces appeared rougher than polished group, but morphological differences were not found between both conditioning treatments. At figure 2a, non-treated specimen at 15x15 µm, smear layer covers the surface. After H3PO4 etching (Fig. 2b) the cementum surface appeared rougher than the other studied surfaces, and valley-like regions could be observable. When SEB primer was used (Fig. 2c) the image showed a mildly cementum etched surface. Surface after being treated with OUB is shown in figure 2d: remaining homogeneous surface of cementum with no lamellar pattern is denoted.

Table 2. Mean (SD) surface roughness (Ra) (nm) after different cementum treatments in 5x5 µm and 15x15 µm size images (n=120).

Surface treatment	5x5 µm	15x15 µm
No treatment	32.77 (11.5) B	72.13 (7.2) b
35% H ₃ PO ₄ etching	51.79 (15.4) A	230.78 (37.8) a
CSEB Primer	36.02 (4.6) B	61.35 (11.4) b
OUB	20.84 (7.2) B	24.87 (2.5) c

In columns, different letters are significantly different at a 95% confidence level.

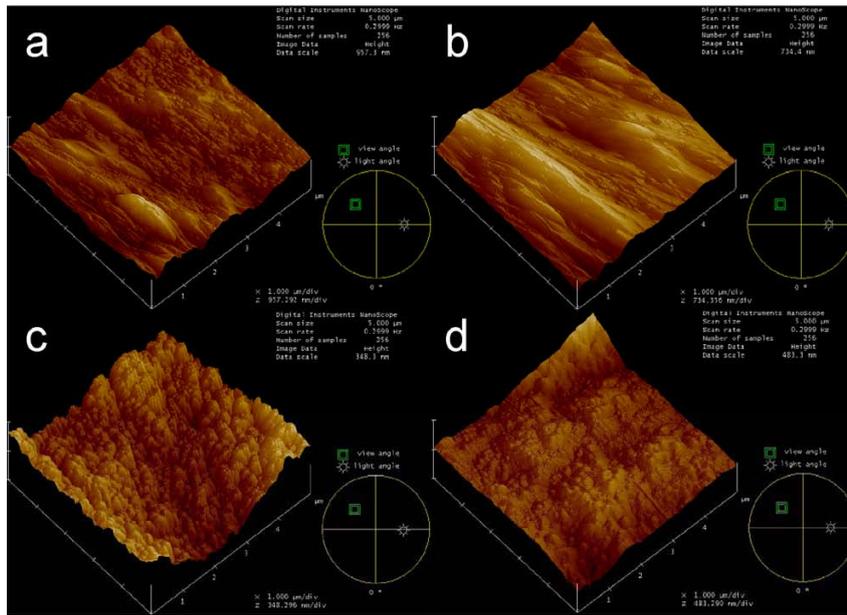


Fig. 1. AFM images of 5x5 μm area of non-treated (a) and treated cementum surface with H3PO4 (b), SEB (c) and OUB (d).

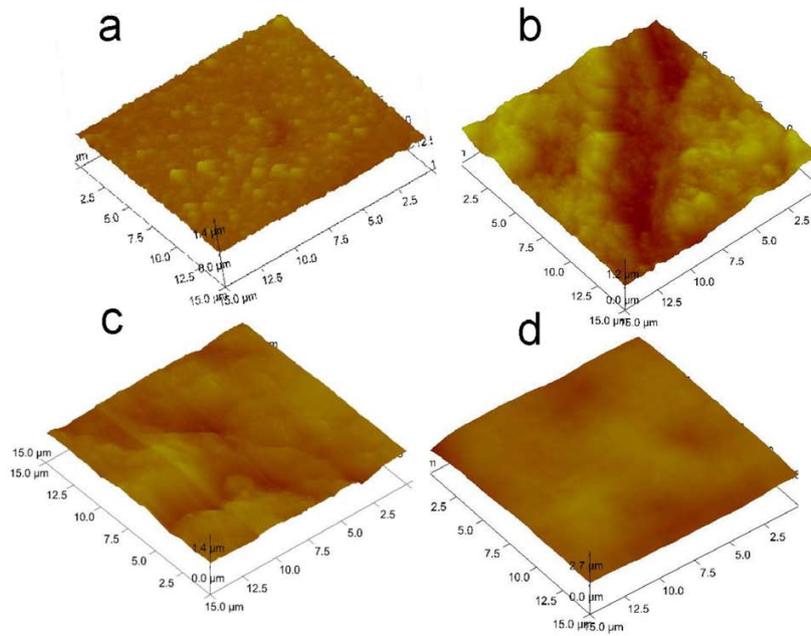


Fig. 2. AFM images of 15x15 μm area of non-treated (a) and treated cementum surface with H3PO4 (b), SEB (c) and OUB (d).

Discussion

In this study, the results require the rejection of the null hypothesis that surface treatments do not change the surface roughness and topography of dental cementum. Despite morphological differences between cementum, dentin and bone, they are composed mainly of collagen and hydroxiapatite (5). Generally, cementum is least mineralized when compared to the others hard tissues: enamel, dentin and bone (15), and is less hard and more permeable compared with enamel and dentin (6).

In Adhesive Dentistry, a rough substrate offers an increased contact surface that means the best micromechanical retention of the system substrate-adhesive (16). In this study, roughness of cementum was increased with phosphoric acid treatment (Table 2). The strong etching effect of phosphoric acid totally removes mineral crystals and exposes denude collagen fibers (3). The minute size of the mineral crystals compared with enamel results in a much larger specific surface area of the mineral component (1). As a consequence, cementum has a more readily to be decalcified in the presence of acidic conditions (1). Also, the lower mineral content and the higher porosity of cementum compared with that of dentin contributed to deeper penetration of the acidic solutions (6). In addition, it appears that the collagen fibers in the cementum may in part accelerate the penetration of etching solutions (2). These reasons indicate that the cementum was more susceptible to acid etching than other dental tissues (1, 2).

When self-etching adhesive systems were used as surface treatment, the roughness values were lower than the phosphoric acid group. It is possible that the penetration of the resin components was not homogeneous within the whole thickness of the demineralized cementum layers (6). The adhesive agents used in this study (SEB primer and OUB) contains HEMA. This molecule promotes homogeneous diffusion of various hydrophilic and hydrophobic adhesive monomers within the adhesive itself and into the etched substrates (17). Nevertheless, resin penetration usually occurs around the circumference of the collagen fibers (2). The coarse collagen bundles in the strongly etched cementum may have hampered the penetration of the resin monomers (18). In this treated substrate some water is left even after the drying process (2), which is due in part, to the presence of proteoglycans in the cementum structure (7).

In our study, the AFM images of 5x5 μm and the 15x15 μm have shown similar roughness results. Both of them have demonstrated that H3PO4 etching group exhibit the highest roughness, but only 15x15 μm images was able to detect differences between the other groups. The most recent high-resolution microscopy tool, AFM, in theory, allows imaging up to atomic level (19). The roughness is scale-dependent. The differences appear

when a larger area is studied (20, 21). Therefore, depending on the field size discrepancies would be different.

The differences in the roughness of cementum surfaces treated for the two self-etching adhesive systems (at 15x15 μm scan size) reflected the demineralization ability produced by different composition, application mode and pH values of each material. Even when cementum surface roughness, after SEB primer application (pH=2.1), was lower than in the phosphoric etched group, the treated cementum with this primer showed an intermediate etching pattern (Fig. 2c) between H3PO4 and OUB. These moderate roughness values are compatible with a higher performance, as has been previously reported (3, 4, 22), by the chemical interaction between SEB and human dentin that is able to enhance the bonding mechanism (23). Roughness was drastically decreased after OUB was used, at 15x15 μm scan size (Table 2). The lowest roughness of this one-step self-etch bonding agent (pH=1.3) may be associated with: (a) the combination of acids, hydrophilic and hydrophobic monomers into a single solution may compromise the function of each one of these components (8, 24); (b) the differential infiltration due to the phase separation of sparingly water-soluble resin components (10, 25), more important when the substrate may contain high concentrations of water remaining (2, 7); and (c) the fluoroaminosilicate glass filler contained in the blend might affect the depth of demineralization in a region with thick collagen fibers (3).

Conclusions

This study demonstrated that phosphoric acid etching enhances the roughness values of the cementum surface. The nanoroughness means of Clearfil SE Bond and One Up Bond F is scale-dependent and increases when a larger area is studied. Only the 15x15 μm images was able to detect that OUB shown the lowest roughness.

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