Article published online: 2021-11-01

ORIGINAL ARTICLE

Dental enamel roughness with different acid etching times: Atomic force microscopy study

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ABSTRACT

Objective: An important characteristic of human dental enamel not yet studied in detail is its surface roughness in mesoscopic scale. This study evaluated quantitatively and qualitatively the surface topography of acid etched enamel with different etching times. **Materials and Methods:** Ninety-six human maxillary bicuspids were randomly distributed into three groups (n=32): T0 (control), pumiced; T15, 35% phosphoric acid etched enamel for 15 s; T30, 35% phosphoric acid etched enamel for 30 s. Roughness measurements Ra, Rz and root mean square (RMS) and 3D images of enamel's topography were obtained with atomic force microscopy (AFM), which is a powerful technique to obtain direct measurements on microscale features. **Results and Conclusions:** Roughness variables Ra, Rz and RMS presented statistically significant differences to all groups (P<0.000), with values increasing with etching time. This increase was greater from T0 to T15 than from T15 to T30. Enamel surface alterations T15 to T30 occur mainly due to increase in height and deepening of prisms central region.

Key words
Acid etching, atomic force microscopy, dental enamel, scanning electron microscopy, topography

INTRODUCTION

With the advent of acid etching to dental enamel, in the 1950s,[1] adhesion was enabled to dentistry. Acid etching causes a selective demineralization that increases the free surface energy, enamel porosity, and increase in surface area.[2] Adhesion to enamel is dependent on the resins capacity to penetrate between rods and crystals,[3] resulting in micromechanical retention. Infiltrated resin encapsulates individually hidroxyapatite crystals creating micro-tags[4] and constitute the hybrid layer which promotes a nanoretention mechanism between dental structure and resinous material.[5,6] Micro-tags probably contribute more to adhesion effectively than macro-tags that fill the space surrounding the enamel prisms.[4]

Retention characteristics of etched enamel surface depend on enamels chemical composition, acids type and concentration, and etching time.[7-9] Studies demonstrated that variating etching time from 15 to 90 s, with 35-37% phosphoric acid, do not present significant impact on shear bond strength to orthodontic brackets.[10-12]

Access this article online	
Quick Response Code:	Website: www.ejgd.org
	DOI: 10.4103/2278-9626.105385

Although lots of studies have been devoted to acid etched enamel surface characterization (Galil and Wright, 1979; Oliver, 1987; Gardner and Hobson, 2001; Hobson *et al.*, 2002; Hobson and McCabe, 2002), the majority used scanning electron microscopy (SEM) and, therefore, provide only qualitative data of dental enamel topography. The etched patterns observed with SEM[13] were classified in five types: (1) Preferential dissolution of the prism cores, resulting in a honeycomb-like appearance; (2) preferential dissolution of the prisms peripheries, giving a cobblestone-like appearance; (3) a mixture of type 1 and type 2 patterns; (4) pitted enamel surfaces as well as structures that look like unfinished maps or networks; and (5) flat, smooth surfaces.

Roughness is defined as a complex role of irregularities, or, little projections and indentations that characterizes a surface and influence on wetting, quality of adhesion, and brightness. Despite micro-mechanical roughness being pointed out as primordial to obtain efficient adhesion to enamel,[14,15] the precise etched enamel characteristics involved, and in which metrical scale adhesion occurs are not known. The effect that surface roughness exerts on adhesion is not completely understood.[16] However,[17] asserted that if a surface is roughened, producing more surface area, and if intimate contact between the adhesive and the adherent is established, the actual adhesive bonding will be stronger because of the increase in surface area.

Since the introduction of acid etching to aid adhesion to enamel, there has been much research into dental materials to improve bond strength, but little into the surface topography of etched enamel.[18] An important characteristic of human dental enamel not yet studied in detail is its surface roughness in microscopic scale.[19] Studying dental structures and surfaces from a nanoscale perspective may lead to better understanding of the structure-function-physiological relationship of dental surfaces.[20]

Traditionally, surface roughness is expressed in a measurement that represents an averaged and macroscopic measurement of the overall surface topography. Microscopic surface details may be neglected because of instrument limitations. The atomic force microcopy (AFM), with high lateral and vertical resolutions, allows the exploration of this roughness in a low scale that is little influenced by macroscopic components as surface waviness, which are less relevant for studying processes involving microscopic particles.[21] This micro-probe technique does not require conducting samples or special sample preparation, with addition of an extralayer (gold sputtering, for example) modifying the original surface. It is a direct way to experimentally evaluate the surface roughness quantitatively. In[22] study, quantitative measurements with AFM provided a comparative assessment between groups. Therefore, the aim of this study was to compare and analyze the enamel roughness alterations that occur with acid etching for 15 and 30 s.

MATERIALS AND METHODS

Enamel specimens preparation

Ninety-six human intact maxillary bicuspids, extracted for orthodontic reasons, proceeding from Tooth Bank of the Pontifical Catholic University of Paraná, were disinfected in a 0.5% cloramine-T solution for 7 days, and then stored in distilled water at 4°C, which was changed every 7 days, accordant to ISO/TS 11405. All procedures were approved by the Ethic Committee of Research of the Pontifical Catholic University of Paraná.

The specimens were obtained by cutting the buccal surface of all maxillary bicuspids, with flexible perforated diamond disc 7015 (KG Sorensen, Barueri, SP, Brazil), maintaining the middle third of the buccal face parallel to the cutted plane. Pumice prophylaxis slurry was applied for 10 s, rinsed with distilled water for 20 s, and air dried for 20 s. Teeth were randomly divided into three groups: (*n*=32): T0 (control), pumiced enamel; T15, 35% phosphoric acid etched enamel (3M Dental Products Division, St. Paul, MN, USA) for 15 s, rinsed with distilled water for 20 s, and air dried for 20 s; T30, 35% phosphoric acid etched enamel for 30 s, rinsed with distilled water for 20 s, and air dried for 20 s. Thirty specimens of each group were analyzed with AFM and additionally two with SEM.

Atomic force microscopy

Ninety samples (is it the previously prepared) were stored in a desiccator for 48 h. All images were obtained with a Shimadzu SPM-9500J3 AFM (Shimadzu Co., Tokyo, Japan) using a silicon nitrate pyramidal contact tip (Olympus, Tokyo, Japan) in constant force mode, scanned area sized 30 μm × 30 μm, maximum vertical amplitude (*Z*) 5 μm, frequency of 1 Hz, 512 lines taken per image and operating point of 2 V.

Three images of the buccal face middle third of each specimen were selected to apply to the surface analysis. Images that presented type I etch pattern were selected since this is the most common etching pattern observed in this surface enamel area.[13] The etching pattern selection should avoid that the macro-geometric topography of other etching patterns misunderstood the evaluation and comparison of the micro-roughness between groups. The data were minimally modified, and only the flatten command was applied with the software SPM Manager v2.11 (Shimadzu) to compensate any tilt of the samples during the AFM measurements. After that, using the same software, surface analysis was performed to obtain the following data: Ra (mean arithmetic roughness), Rz (mean distance between five peak maximums and five valley minimums), and root mean square (RMS) roughness and profile analysis to illustrate the results.

Scanning electron microscopy

Six samples were gold coated with 15 nm and stored in a desiccator for 48 h. Photomicrographs with ×4000 magnification were taken with a Jeol JSM 6360-LV SEM (Tokyo, Japan) operated with 15 kV to illustrate the results.

Statistical analysis

The mean of the variables Ra, Rz, and RMS were obtained from 270 images, three of each of the 90 specimens.

The variance homogenity test of Levene indicated that the groups presented heterogeneous variances to all variables. ANOVA and the Games–Howell multiple comparisons test was used to evidence the differences among the variables mean values to the different groups. Pearson's correlation test was used to correlate the variables.

RESULTS

Atomic force microscopy

Table 1 describes the variables Ra, Rz, and RMS to the three groups. All variables presented significantly statistical difference among groups ($P<0.0000$). The roughness increased, not linearly, with increase in etching time [Figure 1].

Ra, Rz, and RMS measurements presented high correlation among each other ($R>0.959$ and $P<0.0000$).

Three-dimensional images of type 1 etch pattern of acid etched enamel for 30 s [Figure 2e] presented greater mineral removal of prisms core region than the enamel etched for 15 s [Figure 2c].

Scanning electron microscopy

Pumiced enamel presented a regular surface with eventual wear [Figure 2b], and 35% phosphoric acid etched enamel for 30 s [Figure 2d] presented the type 1 etch pattern better defined than for 15 s [Figure 2f].

DISCUSSION

As reviewed, the demineralization patterns, when statically analyzed, can be differentiated and classified.[13] Dissolution does not occur equally throughout all enamel surface with etching time due to apatite cristallites orientation, initiating in core/wall interfaces of rods and developing anisotropically along the *c*-axis.[23] In the present study, the enamel etched with 35% phosphoric acid for 30 s [Figure 2e and f] revealed a type 1 etching pattern better defined and deepened in prisms cores than for 15 s [Figure 2c and d]. In agreement with,[16] using 37% phosphoric acid encountered the type 1 and 2 patterns with 30 s better defined than 15 s.

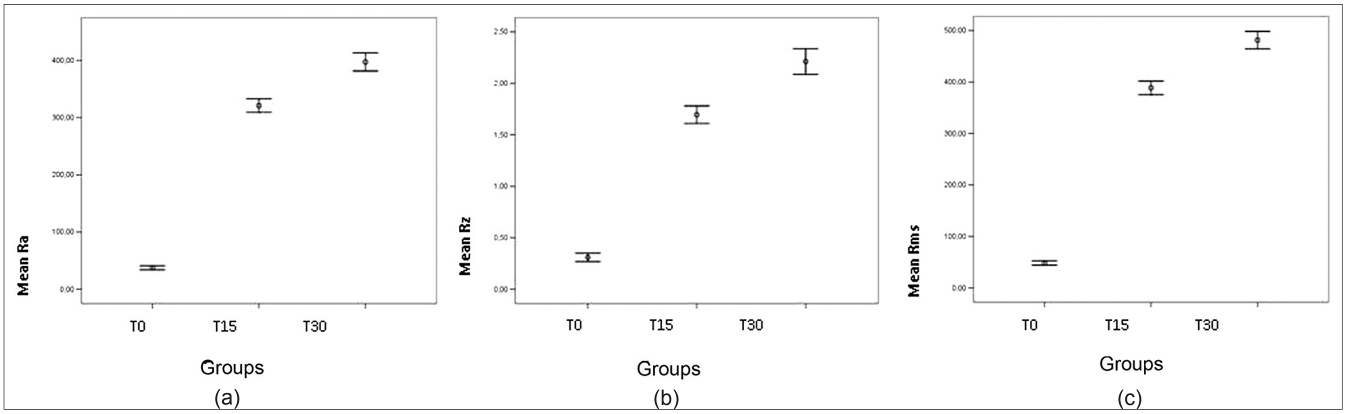
In agreement with a study made by Carstensen,[8] the relationship between etch pattern and bond strength indicates that the type 1 and 2 patterns promote maximum adhesion. In addition, the longevity of bonding is influenced by etch pattern.[24] However, Nakabayashi and Pashley[5] suggested that resin-enamel bond strength is the result of the cumulative cross-sectional area of the resin tags that infiltrate the etched enamel surface. Therefore, exposure of enamel crystallites is more important than well-defined etch patterns.[24] Orellana *et al.*[25] suggested that enamel porosity is more important than a defined etch pattern using pioneerly the BET method (gas adsorption) which did not find correlation between specific surface area and the different etch patterns.

As presented here, the increase in roughness related to acid etching time, although progressive was not linear, increasing in lower proportion with time. There was a greater increase of all roughness variables from T0 to T15, than from T15 to T30. However, the quantity of removed mineral at demineralization process was linear with etching time,[23,26] pointed out the same behavior to depth using 2% phosphoric acid to etch enamel with times up to 3 min, and mean roughness (Ra) presented lower increases with etching time.

Roughness measurements Ra and RMS represent, respectively, the mean arithmetic and quadratic deviation of roughness related to the mean profile of the surface. The measurement RMS is, therefore, more

Table 1: Descriptive statistics of roughness variables Ra, Rz E Rms (μm) to etching time groups T0, T15 and T30 (sec)

Variable	Groups	*n*	Mean	Standard deviation
Ra	T0	30	0.037	0.009
	T15	30	0.321	0.031
	T30	30	0.397	0.042
Rz	T0	30	0.309	0.108
	T15	30	1.695	0.232
	T30	30	2.212	0.337
Rms	T0	30	0.049	0.011
	T15	30	0.389	0.036
	T30	30	0.481	0.046



(a) (b) (c)

Figure 1: Mean values difference of variables (a) Ra, (b) Rz and (c) root mean square between groups T0, T15 and T30

sensitive to extreme values[27], which are represented in the roughness measurement Rz. Despite the measurement Ra being employed many times as the sole indicator of surface texture, it cannot differentiate the depth of irregularities[28], so peaks and valleys are registered in an identical manner.[29] Among the groups, the measurements Rz and RMS increased proportionally more than Ra [Figure 1]. Therefore, the results suggest that in type 1 etch pattern, from 15 to 30 s, the vertical difference between maximum peaks and valleys increased, corresponding respectively to the periphery and to the core of the prism. So, the etch pattern presented a better definition, with deepening in the core of the prisms. The profile analysis allow to visualize that the difference in height between peaks and valleys increased while the prism walls width were maintained regular [Figure 3].

The results of this study suggest that the difference of roughness between T15 and T30 may not represent surface alterations that aid to form micro-tags, enhancing hybrid layer formation. The better definition of etch pattern in T30, is determined by the increase of the great vertical differences expressed by Rz, and is favorable to form macro-tags. However, the contribution of these to adhesion effectiveness is still questionable.[4]

Adhesion is usually measured by shear bond strength tests that evaluate the impact of variables on clinical performance. Barkmeier *et al.*,[10] Wang and Lu,[11] Triolo *et al.*[12] concluded that 15 s of acid etching provides bond strength similar to 30 s. In the present study, the increase in etching time caused alterations on enamel surface that probably do not promote significant enhancement in adhesion. Therefore, the increase in etching time may lead only to greater loss of dental material,[23,26] without a real increase in retention of resinous material to enamel.

In conclusion, AFM enabled quantitative evaluation of enamel roughness, which increased with increasing etching times. This increase is greater from 0 to 15 s when compared from 15 to 30 s. Enamel surface alterations from T15 to T30 occurred mainly due to increase in height (Rz) and deepening of prisms central region.

Quantitative studies as presented here may help to evaluate the real contribution of dental enamel roughness to adhesion values in future work.

ACKNOWLEDGMENTS

The authors acknowledge the Electronic Microscopy Center and the Atomic Force Microscopy Laboratory at Physics Department, both at Federal University of Paraná, for support.

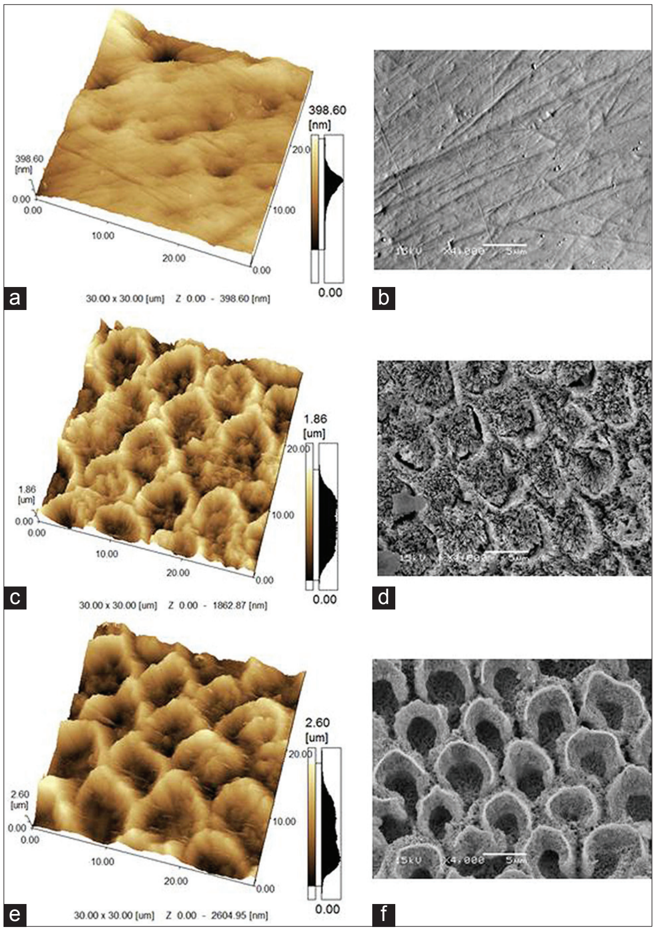


Figure 2: (a, c, e) Three-dimensional images, sized 30 μm ×30 μm (AFM). (b, d, f) Photomicrographs in ×4000 magnifications (SEM). (a, b) Dental enamel pumiced, presented a regular surface with eventual wear, due to prophylaxis with pumice slurry. (e, f) The 35% phosphoric acid etched enamel for 30 s revealed a better defined type 1 etch pattern, and deepened prim's cores than (c, d) for 15 s. Thus, the honeycomb structure could be observed in etched enamel (c-f)

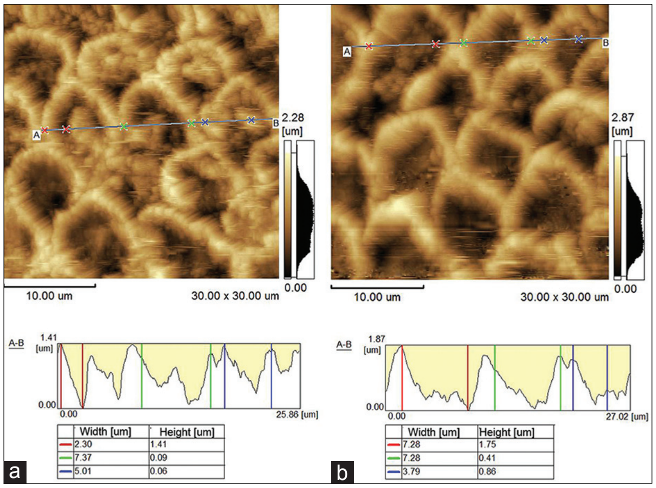


Figure 3: AFM profile analysis of 35% phosphoric acid etched dental enamel. Profile lines A-B comparison between T15 (a) and T30 (b). Peak to valley height was higher to T30 (red), prisms width and walls did not alter significantly between T15 and T30 (green), and prisms peak to core height was higher to T30 (blue) which presented greater demineralization and prism core depth

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How to cite this article: Cerci BB, Roman LS, Guariza Filho O, Camargo ES, Tanaka OM. Dental enamel roughness with different acid etching times: Atomic force microscopy study. Eur J Gen Dent 2012;1:187-91.

Source of Support: Nil. **Conflict of Interest:** None declared.