

# The Morphological Effect of the Acquired Pellicle on Acid-etched Enamel: A Scanning Electron Microscopy Analysis

Elena Marchetti<sup>1</sup>, Andrea Guida<sup>2</sup>, De Carolis Carlo<sup>3</sup>, Lomurno Giuseppe<sup>3</sup>, Stefano Eramo<sup>3</sup>

<sup>1</sup>Conservative Department, University of Perugia, Via Sartena 9, 00122 Rome, Italy. <sup>2</sup>Materials Science and Technology Department, University of Limerick, Castletroy, Co. Limerick, Ireland. <sup>3</sup>School of Dentistry, Department of Surgical Sciences and Experimental Medicine, University of Perugia, Piazza dell'Università 1, 06121 Perugia, Italy.

## Abstract

**Purpose:** The aim of this study is to determine the effect of the acquired pellicle on enamel etching.

**Materials and Methods:** One-hundred teeth were sectioned in the buccal-lingual direction at the half crown. One-hundred half-crowns were polished with pumice powder and rotating brushes (group B) and one-hundred half-crowns were not polished on the buccal surface (group A). The samples were etched by 37% orthophosphoric acid gel for 30 seconds in a 2 mm diameter circular area at the middle upper section of the crown, rinsed with distilled water for 20 s, air dried for 20 s and observed by Scanning Electron Microscopy.

Three independent operators determined the quality of enamel etching on a four-point scale using 200 images, which were computed by digital Image Analysis software. It analyses the grey values and converts them to three-dimensional graphics to value the surface extension. Analysis Of Variance test was used for comparisons between and within groups.

**Results:** The mean of the operator values for the A samples is 2 ("Poorly defined etch") and for the B samples is 3 ("Defined etch"). The Image Analysis showed a significant difference (Analysis of Variance test) in the extension of the etched areas between groups A and B. The samples from group B had 11.7% more effectively etched enamel surfaces than the samples from group A.

**Conclusion:** Acquired pellicle removal by dental prophylaxis is necessary before enamel etching because it improves the effectiveness of the etching procedure.

*Key words: Orthophosphoric acid, Acquired pellicle, Enamel, Etching, Image analysis, Scanning Electron Microscopy (SEM)*

**Abbreviations:** ANOVA: Analysis of Variance; e.g.: exempli gratia; s.: seconds; SEM: Scanning Electron Microscopy; SPSS: Statistical Package for Social Science; 3D: 3D Dimensions

## Introduction

Effective enamel cleaning before acid etching is necessary to prepare direct and indirect restorations and fissure sealants [1,2]. Plaque and discoloration are removed by dental prophylaxis with pumice powder or paste and a rotating brush or small rubber cup [3]. There are other prophylaxis techniques, such as air-flow and bicarbonate jet polishers, which are faster and as effective, but they can damage tissues and contaminate surfaces [4-6]. Even in patients with a good oral hygiene status (e.g., with a patient who has performed dental prophylaxis in the preceding days), it is always necessary to remove the invisible acquired pellicle with dental prophylaxis. The acquired pellicle is a shapeless and organic pellicle, without cells, which covers cleaned dental surfaces in a few minutes [7].

Acquired pellicle is important in tooth decay [8], especially in enamel demineralization/remineralization process [9,10]. The acquired pellicle is important with respect to the enamel surface response to no-bacterial acid exposition.

There is considerable information in the literature about the corrosive effects on enamel by acidic drinks and substances [11]. An acquired pellicle could be a barrier for diffusion or a semi-permeable membrane between acids and dental surfaces [12], but it has also been shown that an acquired pellicle has an inhibitory effect on enamel demineralization [13-15].

It has been stated that "In clinical conditions the

effectiveness of acquired pellicle ability to protect dental surface is unknown, and the dental surface response to different acids exposure is unknown as well" [16].

Numerous studies have been published on acid etched enamel surface characterization [17] and the majority used Scanning Electron Microscopy (SEM) and provided only qualitative data on dental enamel topography. The etched patterns observed with SEM were classified into five types: (1) preferential dissolution of the prism cores, resulting in a honeycomb-like appearance; (2) preferential dissolution of the prism peripheries, resulting in a cobblestone-like appearance; (3) a mixture of type 1 and type 2 patterns; (4) pitted enamel surfaces and structures that look like unfinished maps or networks; and (5) flat, smooth surfaces [18].

In agreement with a study by Carstensen [19], the relationship between etch pattern and bond strength indicates that type 1 and 2 patterns promote maximum adhesion. The exposure of enamel crystallites is more important than well-defined etch patterns [20]. Orellana et al. [21] suggested that enamel porosity is more important than a defined etch pattern.

This *in vitro* study evaluates the difference on acid-etched enamel between the same tooth surface with and without acquired pellicle.

## Material and Methods

One-hundred healthy human permanent molars were extracted for periodontal reasons from patients ranging 40 to 60 years of age. This study was conducted in accordance with the guidelines established by the Italian Ministry of Health's Code of Bioethics for Dentists, in the Official Italian Standard, and

in the bioethics regulations enforced by the University of Perugia. Patients who agreed to participate in the study gave their written authorization.

Each patient had a professional cleaning including pumicing. The subjects were asked to refrain from eating and drinking. After 2 hours, the teeth were extracted, cleaned and sterilized in 2% glutaraldehyde to remove organic remnants. A two-hour pellicle is virtually free from plaque accumulation. Roots were amputated with a low-speed double sided diamond disk (Shofu #S23-1164 Japan), under continuous water spray irrigation. The teeth without roots were sectioned in buccal-lingual direction at half-crown using the same flexible perforated diamond disk. For each tooth, the buccal surface of half-crown (sample B) was polished with pumice, rinsed with distilled water for 15 seconds utilizing a rotating brush (Voco GmbH, Germany) and the buccal surface of the other half-crown (sample A) was not polished. A total of 100 samples were taken, which retained the acquired pellicle, from group A (A1 to A100); and, another 100 samples were taken from group B (B1 to B100) which were without acquired pellicle.

All of the samples were examined by SEM (Philips XL 30, Philips, Eindhoven, Netherlands). In all of the samples from group A, there was acquired pellicle, which was not a homogeneous film covering the enamel samples. The young 2-hour pellicle at SEM analysis has been identified as a 100 to 500 nm thick uneven and incomplete organic coating of enamel. The surface of the *in vivo*-formed pellicle is characterized by a sponge-like network of spherical particles. In all of the samples from group B, the acquired pellicle had been removed.

To obtain enamel samples comparable among themselves and with uniform physical and chemical characteristics, the observation zone for all of the samples was standardized at the middle upper section (2-mm diameter) of the tooth, between the cusp and the equator of the clinical crown.

The samples from both groups were etched by 37% orthophosphoric acid gel for 30 seconds in a 2-mm diameter circular area, rinsed with distilled water for 20 s, and air-dried for 20 s.

All of the specimens were given a 15-nm gold coat in a vacuum evaporator (SCD 050, Balzers, Schaan, Liechtenstein) to provide a conformal conductive coating and stored in a desiccator for 48 h.

The observation zone for all of the samples was standardized at the middle upper section (2-mm diameter) of the tooth, between the cusp and the equator of the clinical crown.

The treated enamel surfaces were examined completely under a SEM and photomicrographs were taken with 5000X magnification. To illustrate the results one photomicrograph for each half crown as a representative surface area was taken at magnification 5000X.

The 200 images were at first subjectively valued and compared in counterpart and randomized pairs by three independent operators who determined the quality of the enamel etching based on the following four-point scale [22]:

4. Well defined etch: the outline of each individual enamel prisms was clearly visible and continuous.

3. Defined etch: the outline of the each individual enamel

prisms was clearly visible but not continuous.

2. Poorly defined etch: a spectrum of surface irregularities was indisputably visible, but not markedly distinctive.

1. Unetched: a relatively smooth enamel surface that was, apparently unaffected by the acid etching was visible.

The identical image were computed by digital image analysis software (ImageJ, Scion Corporation, USA), which calculates the grey values [23]. This programme allows a quantitative analysis, and the grey values of two-dimensional SEM enamel images were converted in colored three-dimensional graphics. In these graphics, the third parameter is the depth. ImageJ calculates the extension of the three-dimensional surface in the image of the etched enamel with acquired pellicle and of the etched enamel without the acquired pellicle. The areas notched by acid etching are deeper orange-yellow peaks in the images of the etched enamel without the acquired pellicle than the ineffective etched area (dark surface) because there is more quantity of enamel for adhesion.

SPSS software (SPSS 11.0, SSPS Inc., USA) was used for the statistical analysis, considering the etched enamel surfaces as statistical units. ANOVA was used for the comparison between the groups and within group.

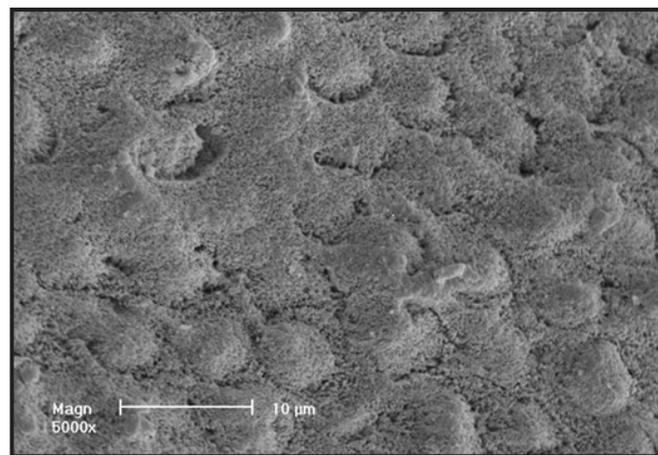
## Results

The results are visible in 200 microelectronic images and the corresponding 3D graphic elaborations, which allow the operators to value the available adhesion areas on the sample images and to calculate the consequent significant differences by the SPSS software. The authors provide the complete images of sample 1 as follows: the SEM image of sample A1 (*Figure 1*) and its 3D graphic elaboration (*Figure 2*) and the SEM image of sample B1 (*Figure 3*) and its 3D graphic elaboration (*Figure 4*).

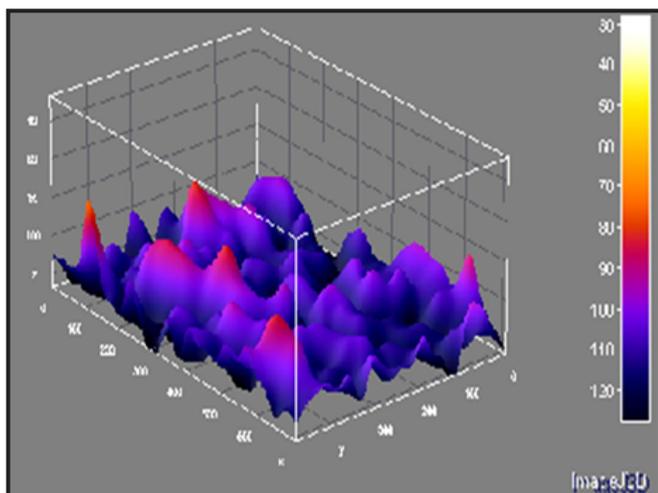
*Figure 1:* This image shows the buccal surface of the A1 sample (with the acquired pellicle), which has been etched. There is no orthophosphoric acid etching around the enamel rods (rod sheath) and only a few rod heads have been exposed.

*Figure 2:* The 3D graphic elaboration of *Figure 1*. This image shows the buccal surface of the A1 sample (with the acquired pellicle), which has been etched. The blue valleys show minor effects of the orthophosphoric acid on the enamel with the acquired pellicle.

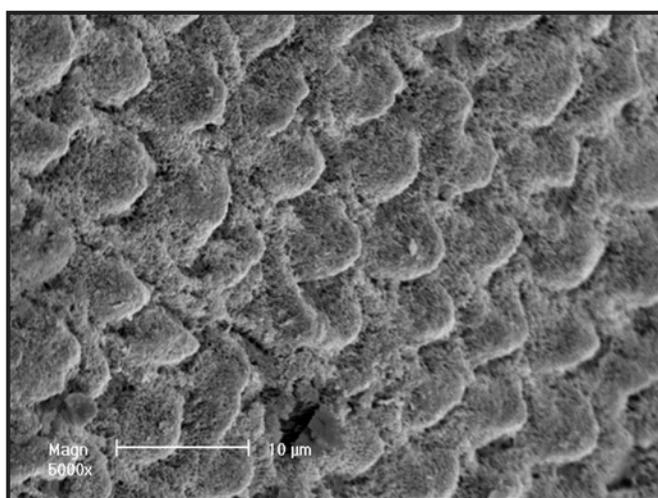
*Figure 3:* (SEM 5000X) This is the buccal surface of B1



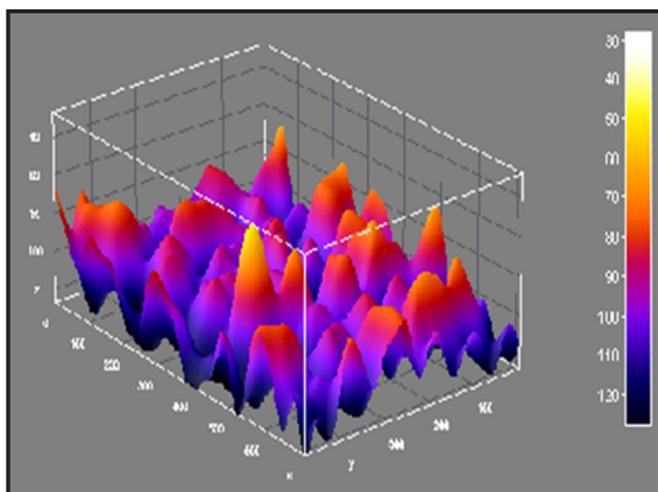
*Figure 1.* (SEM, 5000X)-Enamel surface with the acquired pellicle (sample A2), etched.



**Figure 2.** 3D graphic elaboration of Figure 1.

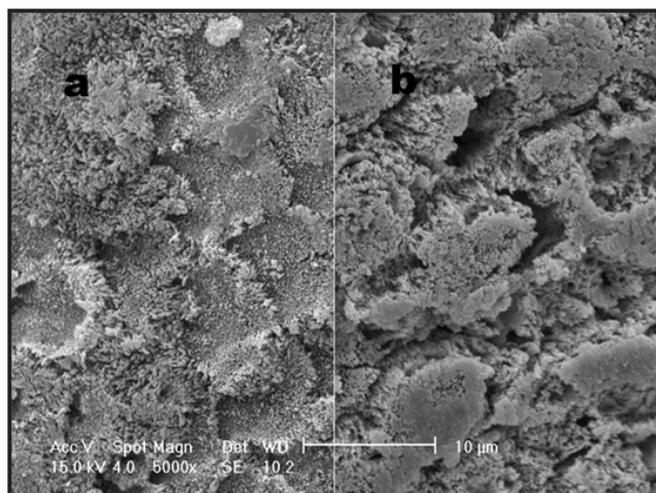


**Figure 3:** (SEM 5000X) - The identical enamel surface without the acquired pellicle (sample B1), etched.



**Figure 4.** 3D graphic elaboration of Figure 3.

sample (without the acquired pellicle). This sample belongs to the same tooth of Figure 1. There has been a distinct orthophosphoric acid etching than sample A1. In this sample there is an evident orthophosphoric acid etching around the enamel rods and the interrod substance and the prisms' heads have been well exposed. Preferential dissolution of the prisms peripheries gives a cobblestone-like appearance (type 2 of etched patterns observed with SEM).



**Figure 5.** (SEM 5000X) Sample 3, surface A (with the acquired pellicle) and B (without the acquired pellicle). On side a is shown the buccal surface of the A3 sample (with the acquired pellicle), which has been etched. There is a moderate orthophosphoric acid etching on rod cores.

On side b is shown the buccal surface of the B3 sample (without the acquired pellicle), which has been etched. The acid penetration is more evident in the side b. There has been a similar orthophosphoric acid action on the rod cores of both sides but acid orthophosphoric etching is evident on the interrod substance on the side b image.

**Figure 4:** 3D graphic elaboration of Figure 3. This graphic shows the buccal surface of the B1 sample (without the acquired pellicle) and better orthophosphoric acid etching than sample A1. The orange-yellow peaks level shows major effect of orthophosphoric acid on the enamel without acquired pellicle.

The orange-yellow peaks give a better definition of the surface, determined by the increase of the vertical differences in the height between the peaks and valleys.

ImageJ calculates the extension of the three-dimensional surface in this image of the etched enamel without the acquired pellicle. The areas notched by acid etching (orange-yellow peaks) are bigger than the ineffective etched A1 valleys (dark points). In this image, the extension of the three-dimensional surface of the etched enamel without an acquired pellicle is bigger than the surface of the etched enamel with an acquired pellicle, which has been ineffectively etched.

The other images are divided into side A, comprising the images with the acquired pellicle, and side B, comprising the images of the same sample without the acquired pellicle.

The mean of the values of the subjective valuations of the three independent operators for the A samples, which have been etched without acquired pellicle removal, is 2. This value is related to a "Poorly defined etch". The mean of the values for the B samples, which have been etched after acquired pellicle removal, is 3. This value is related to "Defined etch". The B sample areas were different from the A sample areas for only 0.5-1 mm of the surface. These results agree with Hoepfner et al. [24], who proposed the same line of reasoning.

The image analysis of the 200 photomicrographs with x 5000 magnification and the corresponding 3D graphic elaborations exhibit significant differences (ANOVA test) in the enamel areas that have been effectively etched. These

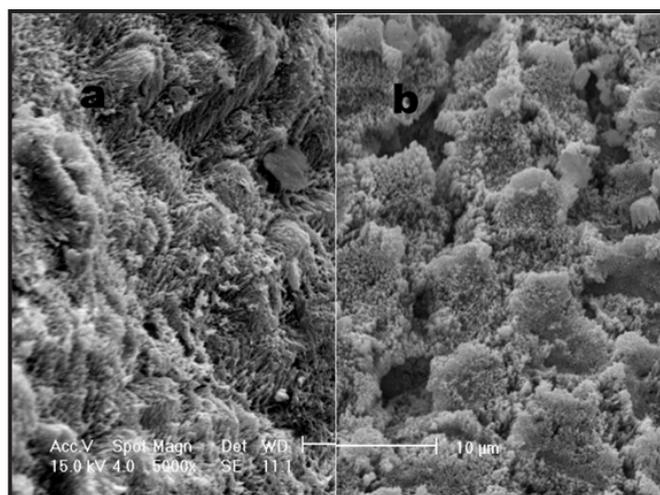
areas in the B samples were larger than the corresponding A samples (intra group), and the mean of all the B samples is larger than that of the A samples (inter group). The B samples have 11.7% more effectively etched enamel surface than the A samples.

### Discussion

The comparison between the value of etched enamel with and without removal of acquired pellicle shown by the images and their calculated three-dimensional surface extension illustrate that the acquired pellicle removal significantly improves the extension and depth of enamel etching. ImageJ-software assigns different color values to the stored grey scale values. On the right of *Figures 2 and 4* is the color scale. The analyzed area is 700 microns x 500 microns (X x Y) and the third dimension, Z, is the depth.

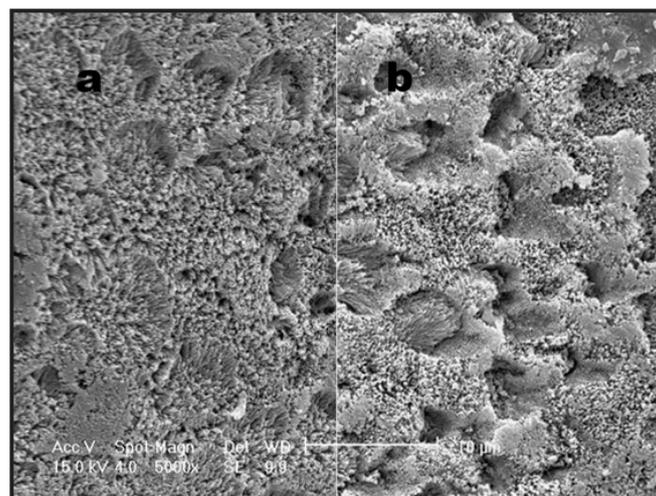
In the previous image, orange-yellow peaks represent the points of major effect of the orthophosphoric acid on the enamel and dark valleys represent the points of less effect of the orthophosphoric acid on the enamel. Typical images are stored with 256 grey levels. Humans can rarely discern more than approximately 20 grey levels in an image. This graphic is the result of histograms, which are plots of the number of pixels in an image with each of the possible brightness levels. For a typical "8 bit" grey scale image there are 256 possible stored values, but in this study ranging from 130= black to 0= white was sufficient. Human vision is insensitive to gradual brightness variations, and tends to judge grey levels in the context of their surroundings rather than absolutely by brightness. The histogram indicates whether an image covers the entire contrast range and the degree of definition and uniformity of the various regions.

The following SEM images (divided into side A and side B) include two half -crowns images of each tooth (*Figures 5-9*).The strength of this study is that in each image of one-



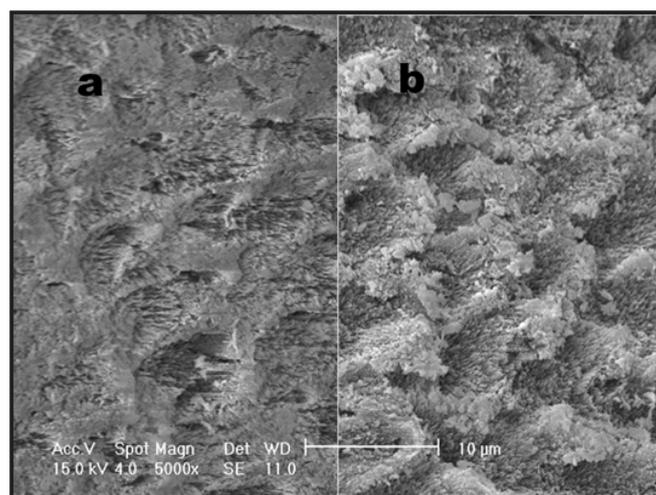
**Figure 6.** (SEM 5000X) Sample 4, surface A (with the acquired pellicle) and B (without the acquired pellicle).

On side a there is shown the buccal surface of the A4 sample (with the acquired pellicle), which has been etched. An irregular surface is evident, which can be a consequence of simultaneous etching on the rod core and on the interrod substance. On side b is shown on the buccal surface of the B4 sample (without the acquired pellicle), which has been etched, and orthophosphoric acid etching is evident on the interrod substance.



**Figure 7.** (SEM 5000X) Sample 7, surface A (with the acquired pellicle) and B (without the acquired pellicle).

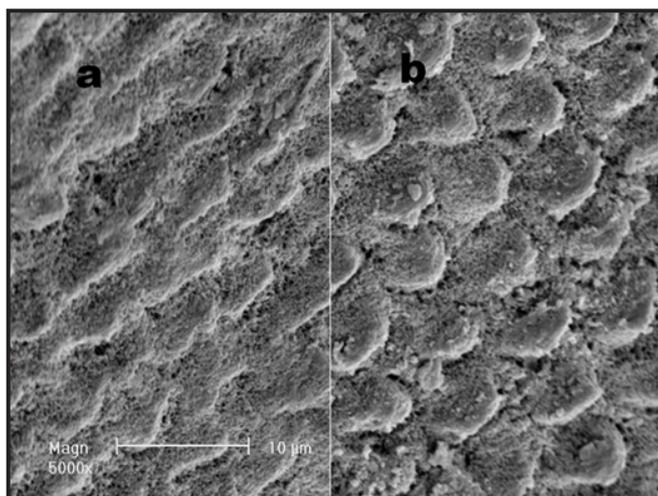
On side a is shown the buccal surface of the A7 sample (with the acquired pellicle), which has been etched. There is moderate orthophosphoric acid etching especially on the rod cores. On side b there is shown the buccal surface of the B7 sample (without the acquired pellicle), which has been etched; there is a deeper orthophosphoric acid etching than the previous sample especially on the interrod substance. Preferential dissolution of the prism cores results in a honeycomb-like appearance (type 1 of the etched patterns observed with SEM).



**Figure 8.** (SEM 5000X) Sample 8, surface A (with the acquired pellicle) and B (without the acquired pellicle). On side a is shown the buccal surface of the A8 sample (with the acquired pellicle), which has been etched. There is scarce orthophosphoric acid etching on the rod cores and the interrod surface. On side b there is shown the buccal surface of the B8 sample (without the acquired pellicle), which has been etched; there is a moderate orthophosphoric acid etching, especially on the rod cores. Preferential dissolution of the prism cores results in a honeycomb-like appearance (type 1 of etched patterns observed with SEM).

hundred teeth the areas notched by acid etching are bigger in sample A, that has been etched after pumice powder and rotating brush cleaning, than the half-crown etched without acquired pellicle removal (sample B).

Several studies demonstrate that dental film may produce a type of interference with the acid etching process when this step is performed, and this hypothesis agrees with the criteria of Burrow and Makinson [25] to explain the reduction of ion diffusion and protection of the enamel surfaces against



**Figure 9.** (SEM 5000X) Sample 10, surface A (with the acquired pellicle) and B (without the acquired pellicle). On side a is shown the buccal surface of the A10 sample (with the acquired pellicle), which has been etched. There is scarce orthophosphoric acid etching. On side b is shown the buccal surface of B10 sample (without the acquired pellicle), which has been etched and there is moderate orthophosphoric acid etching. Preferential dissolution of the prism peripheries produces a cobblestone-like appearance (type 2 of the etched patterns observed with SEM).

demineralization during the exposure to acids in general, when they are covered by the dental film.

The results of the previous study are in agreement with those of Gwinnett [26] with regard to the necessity of eliminating residues including dental film, from the enamel surface to increase the area that will be in contact with the acid etching and subsequently with the resin. The procedure ensures the possibility of achieving an adequate bonding of the materials to the tooth structure [27].

Adhesion to enamel depends on achieving the maximum retentive capacity of the surface from the effect of acid etching. Our study showed that polishing the enamel surface eliminates

the organic components that hinder effective enamel etching. Incomplete removal by cleaning and acid etching of some of the organic material is a factor that compromises the findings of the study. It is highly likely that, despite our best efforts, the organic layer cannot be entirely removed without considering the proteins immersed in the crystals forming the enamel.

In patients who have good oral hygiene status (e.g., if they have performed dental prophylaxis in the previous days or previous hours) and need aesthetic fillings, enamel cleaning could be neglected.

The subjective valuations of these images and the objective results of image analysis have confirmed that the removal of acquired pellicle by dental prophylaxis is necessary before enamel etching procedures. Acquired pellicle removal significantly improves (by approximately 12%) the extension and depth of enamel etching [28].

The acquired pellicle removal increases the area that would be in contact with the acid during the etching and subsequently in contact with the resin. Adhesion to the enamel depends on the retentive capacity of the surface for the effect of the acid etching. These results have influenced filling and veneer bonding techniques and orthodontic bracket bonding.

## Conclusion

By conducting this study the following conclusions were drawn:

1. The removal of acquired pellicle by polishing with pumice powder and rotating brushes significantly increased the quality of enamel etching according to the mean of the operator values expressed on two-hundred SEM images.
2. The removal of acquired pellicle significantly increased (11.7% more effectively etched enamel surfaces than not polished enamel surfaces) the extension of the etched areas according to the Image Analysis.

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