

THE EFFECT OF ACID ETCHING TREATMENT ON SURFACE PROPERTIES OF DENTAL HARD TISSUE

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ABSTRACT

Our study aims to investigate the acid etching treatment effects applied on dental structures, considering their surface properties. We have used a liquid PA in a concentration of 36.1% wt. (36.1% PA) and a gel PA with 35% wt. (35% PA) active compound as etching agents. These substances were applied to the human teeth for a maximum of 15 seconds. As investigation methods, the surface roughness was determined based on atomic force microscopy (AFM), and we analyzed the surface morphology in detail using scanning electron microscopy (SEM). Then, we analyzed the hydrophile character of the dental structure by experimentally measuring the contact angle (CA) and computed the surface energy (SFE). Following this study, we can say that both investigated etching treatments produce the demineralization of both enamel and dentine structures, with a higher degree in the case of liquid PA (36.1%). Increasing the values for the surface free energy will favor better infiltration and polymerization of the bonding systems.

Keywords: Dental structures, etching, structure, roughness

INTRODUCTION

Enamel acid-etching based on phosphoric acid (PA) was adopted in 1995 and completely modified the traditional ideas of restorative dentistry [1]. It is well known that etch-and-rinse (ER) adhesive materials require first the application of certain acidic substances on the teeth enamel and dentin to obtain a so-called mechanical interlocking phenomenon [1]. The optimal concentration of PA for this type of treatment was established to be between 30% and 40%, and the application time was not lower than 15 seconds and did not exhibit 60 seconds. It was noticed that when a concentration of 37% PA was involved, the interprismatic and prismatic crystals on the tooth enamel were removed through their dissolution, and a porous surface occurred. In the case of an inferior PA concentration (e.g., lower than 27%), the apparition of dicalcium phosphate dihydrate was reported. This chemical compound is usually hard to remove on the enamel surface. On the other hand, for

concentrations higher than 40% PA, the calcium dissolution process becomes very slow, and a shallow etching pattern is present [2]. The commercially available solutions exhibit a 37% PA concentration and are applied on human teeth between 15 and 20 seconds. Applying the PA reveals a demineralized collagen network of a maximum thickness of 9 μm [3], [4]. After the rising step, it is occupied by water, being adequate for the polymeric resin diffusion procedure [5]. Studies [6], [7], [8] revealed that a perfect polymeric resin diffusion in the case of dentin pre-etched with PA is difficult to obtain because of the porosities, which occurred due to the presence of demineralized resin-sparse dentin area. These places are characterized by collagen degradation that has an important effect on resin-dentin durability [9], [10]. Clinical solutions such as multiple adhesive coat use [11], [12], vigorous rubbing [13], [14], and the use of protease inhibitors [15], [16], [17], [18] were proposed.

One of the most important etchants

based on PA meet in practice are Kerr etching liquid (Kerr Europe, Karlsruhe, Germany – 37% wt. H_3PO_4), Vococid (VOCO-Chemie, Cuxhaven, Germany – 36.1% wt. H_3PO_4), Esticid (Kulzer-Heraus, Weinheim, Germany – 35% wt. H_3PO_4), Vivadent (Vivadent, Schaan, Liechtenstein - 37% wt. H_3PO_4), ESPE (ESPE, Seefeld, Germany - 36.1% wt. H_3PO_4) [19]. The lowest viscosity value was obtained for Kerr etching liquid (284 mPas), while the highest value was noticed in the case of ESPE etching gel (22165 mPas). All the etching agents had a hydrophilic surface with a minimum contact angle of 49° (surface tension of about 91.7 mJ/m^2 (Vococid)) and a maximum value of 61° (surface tension of 89.8 mJ/m^2 (ESPE)) [19].

We have identified some important studies in the literature. Lee et al. [20] investigated the influence of PA etching on the bond strength of epoxy resin-based compared to calcium silicate-based sealers. They found that in the case of the human third molars, the PA-pretreated surfaces exhibited lower bond strength compared to control (no PA treatment applied) and calcium silicate-based sealer groups. Anastasiadis et al. [21] studied the effect of various conditioning agents such as phosphoric acid (32%), nitric acid (3%), phytic acid (20%), citric acid (20%), and ethylenediaminetetraacetic acid (EDTA) (17%) on human dentin. The authors analyzed the surface roughness and the collagen structure. They noticed that all surface treatments were linked to an increase in roughness, with a most important value obtained for PA. Regarding the collagen structure, it was observed that after PA-pretreatment etching, there was a reduction in β -turns and α -helices and an increase of β -sheets. The authors concluded that understanding and comparing the effect of different acids is of utmost importance in establishing some guidelines for resin-based restoration applications. Sahadi et al. [22] investigated the dentin micro tensile bond strength, failure modes (after 24 h and 1-year post-restoration), demineralization of the surface, in situ metalloproteinase activity, and the antibacterial effect of different experimental dentin etchants compared to PA

(35%). The authors observed that PA exhibited the most durable and the highest bone strength after 1-year post-treatment. In addition, it was characterized by adequate antibacterial activity against *S. Mutans*. The main conclusion of this recent study was that although there are many options for dentin treatment today, the use of PA must not be neglected in cases where the patient's situation permits its application. Burrer et al. [23] analyzed the effect of adhesive application methods on bond strength in over-etched dentin. They isolated dentin samples from the human third molar and divided them into 6 groups. Group 1 was selected as a control with a PA etching treatment for 30 seconds, and then an etch-and-rise adhesive was applied for 20 seconds. For groups 2 to 5, the adhesive was applied by actively rubbing the dentin, applying a second adhesive layer, heating at about 68°C the adhesive before its application, and by ultrasonic activation in the case of group 5. Group 6 was considered positive control, and an acid-etching operation was applied for 15 seconds, and then adhesive was applied for 20 seconds. It was concluded that no noticeable differences occurred in the case of active, preheated, double, or ultrasonic applications compared to control and positive groups. The authors noticed that over-etched dentin could hardly be stabilized because adhesive failure modes were detected in all groups.

Our study aims to investigate the acid etching treatment effects applied on dental structures, considering their surface properties. We have used a liquid PA in a concentration of 36.1% wt. (36.1% PA) and a gel PA with 35% wt. (35% PA) active compound as etching agents. These substances were applied to the human teeth for a maximum of 15 seconds. As investigation methods, the surface roughness was determined based on atomic force microscopy (AFM), and we analyzed the surface morphology in detail using scanning electron microscopy (SEM). Then, we analyzed the hydrophile character of the dental structure by experimentally measuring the contact angle (CA) and computed the surface energy (SFE) (Figure 1).

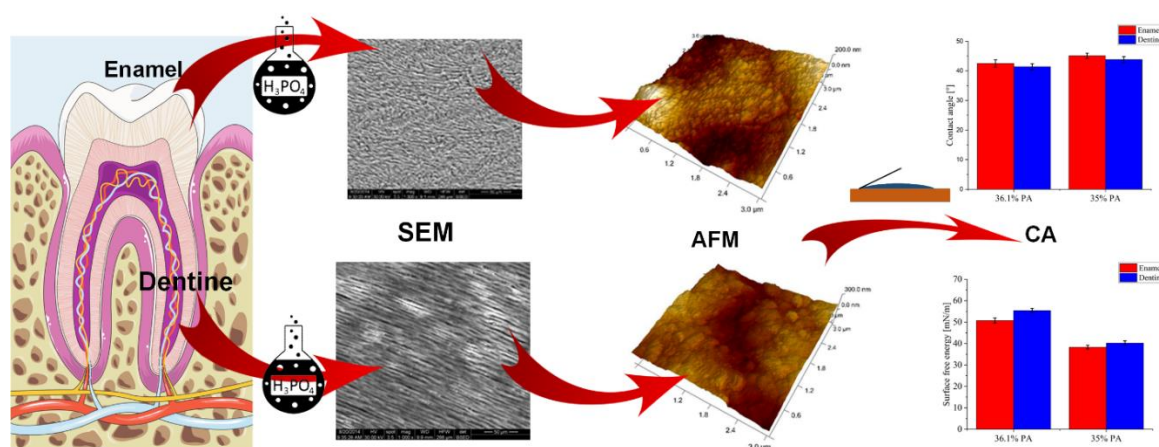


Figure 1. Tooth surface analyses after acid etching treatment. The figure was generated using images assembled from Servier Medical Art, which are licensed under a Creative Commons Attribution 3.0 unported license (<https://smart.servier.com>, accessed on 14 July 2024).

MATERIAL AND METHODS

Three extracted and caries-free pluriradicular teeth were used. They were ultrasonically cleaned, the remaining soft tissue was removed based on dental cures, once again cleaned with a special toothbrush, and then longitudinally cut in the buccal-to-lingual directions at the mesial-to-distal diameter half value based on a slow-speed saw (Isomet, Buehler, Lake Bluff, USA) to obtain a number of six samples. The samples were acid etching treated using orthophosphoric acid in liquid (36.1%) or gel (35%) form prepared in our laboratory with rough matters bought from Sigma Aldrich, St. Louis, USA. The application time was set at 15 seconds to replicate actual treatment conditions. Then, the samples were washed under high pressure for 30 seconds. The etched teeth surface must not be contaminated with foreign agents and must be thoroughly dried. To accomplish this step, a professional medical air dryer was involved and used for 5 minutes to keep the dentin still wet. After that, the specimens were inserted and kept in 0.9% saline solution at 23°C.

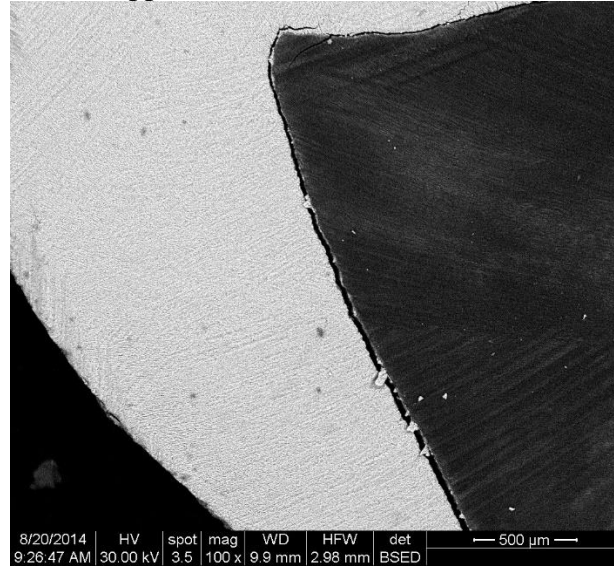
The sample surface morphology was evaluated using a Philips XL 30 ESEM TMP scanning electron microscope (SEM) (FEI/Philips, Hillsboro, OR, USA). The samples were sputtered with gold in a vacuum evaporator (SCD 050, Balzers,

Schaan, Liechtenstein). To obtain high-quality images, a voltage of 30 kV and a pressure of 0.7 torr were set for the microscope. The teeth' wettability was analyzed through contact angle determinations. It was used a Kruss Drop Shape Analyzer – DSA100 (A. Kruss Optronic GmbH, Hamburg, Germany). This device allows measurements with water, ethylene glycol, and diiodomethane, making possible not only the contact angle experimental determination but also the computation of surface free energy based on Owens, Wendt, Rabel, and Kaelbe (OWKR) method [24]. The measurements were conducted in laboratory conditions (temperature of 23 ± 5°C, humidity of 45 ± 5%), and an average of 3 determinations per sample was considered adequate for this study. All the images given by the drop shape analyzer were manually investigated with ImageJ 1.50 software (National Institutes of Health, Bethesda, MD, USA). The atomic force microscope Veeco Multi-Mode VS-AM (Veeco, New York, USA) was used to investigate the surface roughness. It permits the measurements of spatial roughness in tapping mode with a crystalline Si probe on an area of 70 × 70 μm². The arithmetic (R_a) and root mean square (R_q) average values of the roughness were determined and analyzed.

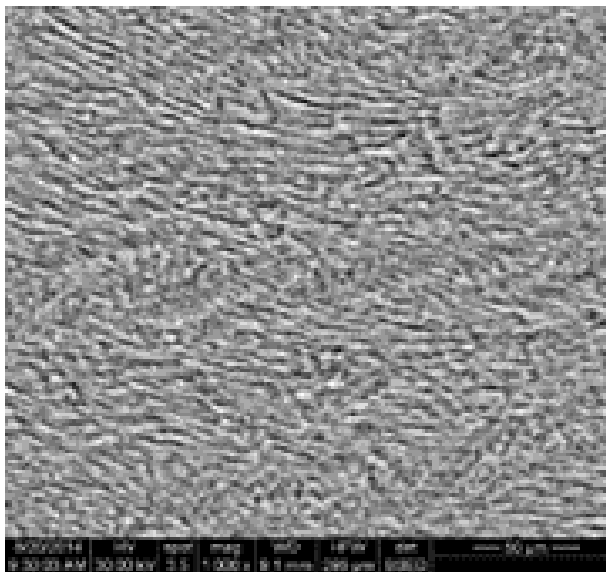
RESULTS AND DISCUSSIONS

Scanning electron microscopy (SEM) analysis

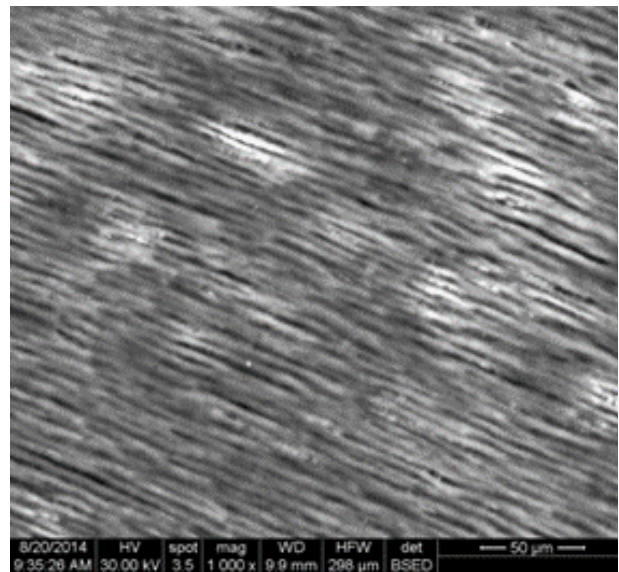
Figures 2 and 3 show the effects of acid etching treatment for the gel and liquid forms. In the case of gel PA-treated samples, an incomplete demineralization process can be noticed because the gel form of the acid cannot penetrate the dental canaliculi and does not create a surface beneficial to adhesive substance applications.



(a)

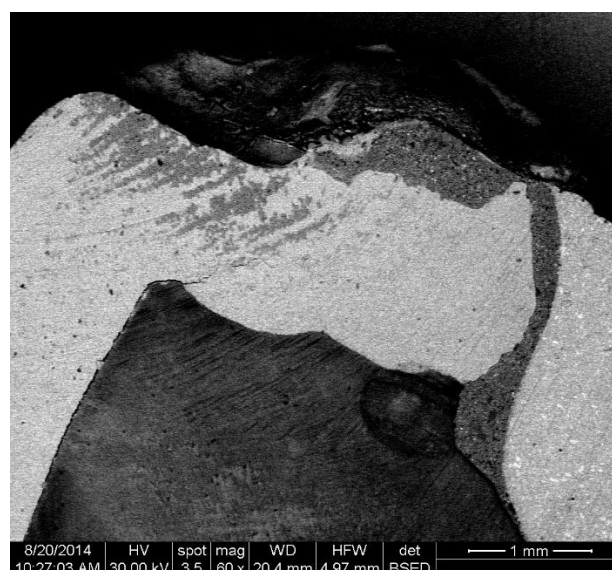


(b)

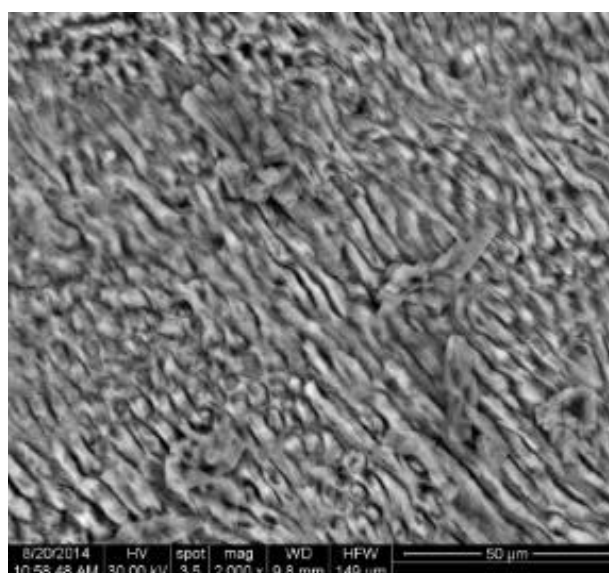


(c)

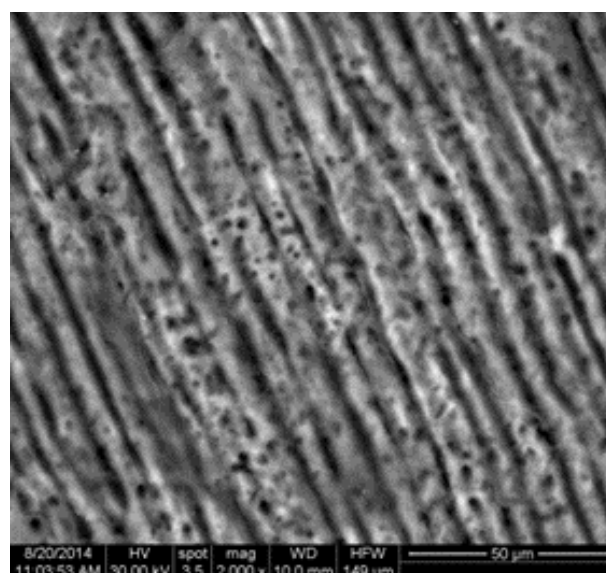
Figure 2. SEM images on samples etched with 36.1% PA: (a) tooth; (b) enamel; (c) dentin



(a)



(b)



(c)

Figure 3. SEM images on samples etched with 35% PA: (a) tooth; (b) enamel; (c) dentin

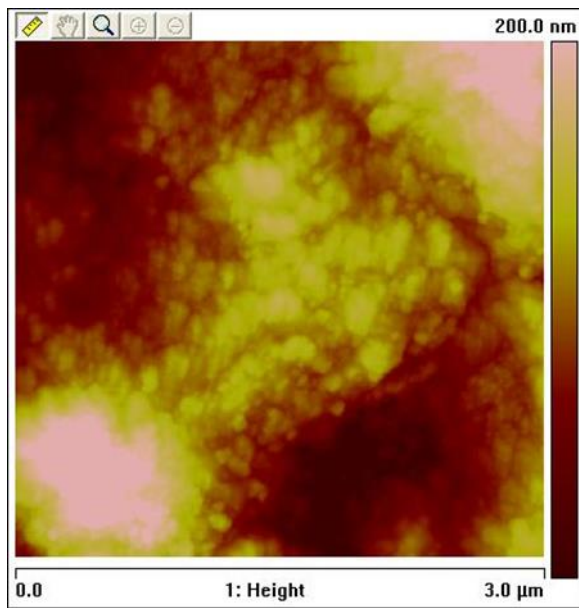
Better results were observed for samples etched with liquid PA because small holes with a diameter of about 4-5 μm were created. These spaces were left undamaged after the water jetting cleaning procedure was applied. It can be concluded that, in this case, the adhesive can fill the demineralized canaliculi. As a general conclusion, it can be noticed that all the acid-etched samples are characterized by an irregular surface that can be directly linked to important damage to the prismatic structure of the teeth enamel. The 37% PA is considered

the gold standard for pit bonding, orthodontic brackets, and fissure treatment, as established in [25], [26], [27]. The etched surface area and the number of microporosities that are on the enamel surface represent two major sources of adhesive failure. The retentive morphology must be considered homogenous. Silverstone et al. [28] classified the microporosities into five types and concluded that types 1 and 2 are adequate for good adhesion and retention. We also found that this behavior was obtained in the case of our samples.

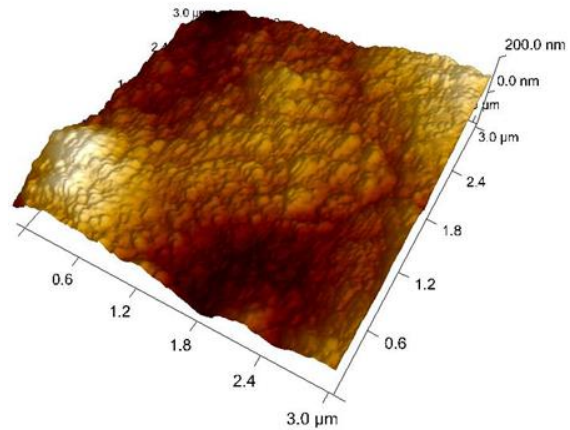
Atomic force microscopy (AFM) analysis

The roughness of the enamel and dentin samples was assessed by atomic force microscopy (Figures 4 and 5). The 2D and 3D topography images highlight surfaces with higher roughness in the case of dentin samples

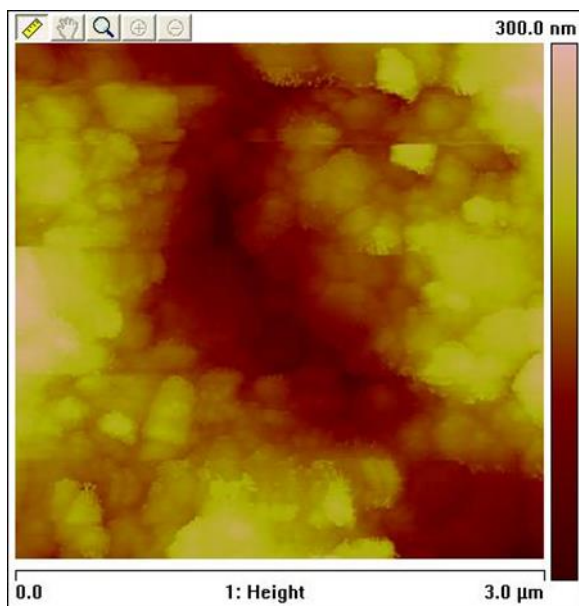
etched with liquid PA. Regarding the type of the etching agent used a rougher surface was highlighted in the case of liquid-PA-treated samples that penetrated better into the dental canaliculi.



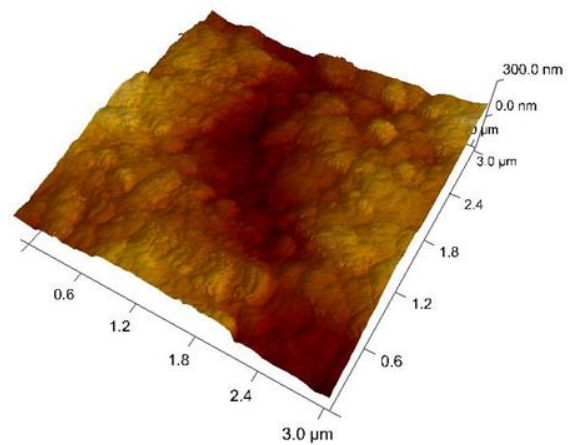
(a)



(b)

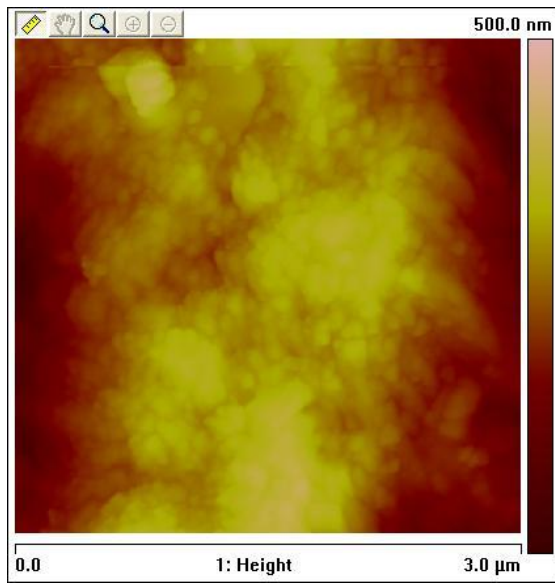


(c)

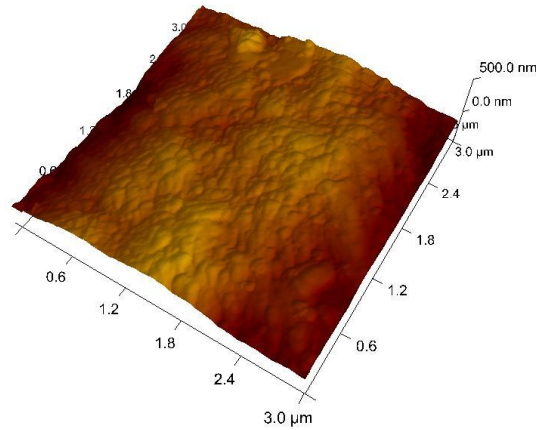


(d)

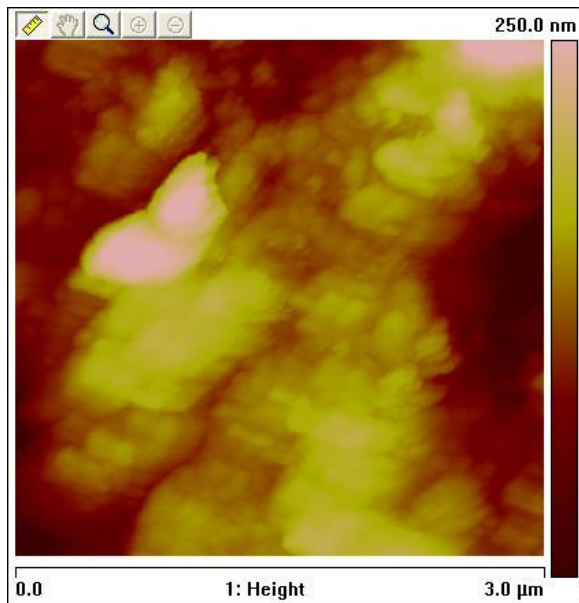
Figure 4. AFM microscopy on samples etched with 36.1% PA: (a), (b) enamel; (c), (d) dentin



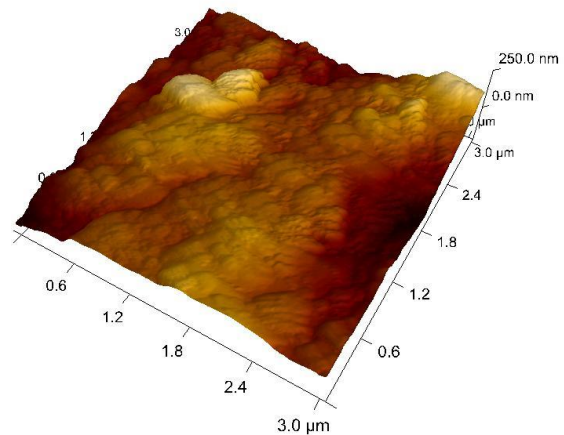
(a)



(b)



(c)



(d)

Figure 5. AFM microscopy on samples etched with 35% PA: (a), (b) enamel; (c), (d) dentin

Surface roughness represents a very important property when adherence investigation is considered. The surface topography of our samples was analyzed using atomic force microscopy. We determined the arithmetic average deviation from the mean line (R_a) and the root mean square average of the

profile heights over the evaluation length (R_q) values. Based on the Wenzel model [29], a direct correlation with the CA values can be established. This model sustains that for hydrophilic surfaces, an increase in roughness parameters immediately decreases the CA value. Table 1 presents the obtained results.

Table 1. Roughness measurement results.

Samples	R _a [nm]		R _q [nm]	
	Enamel	Dentin	Enamel	Dentin
36.1% phosphoric acid etching	110	121	139	149
35% phosphoric acid etching	107	113	86.6	112

In both PA treatments, dentin is much rougher than enamel. The etching agent's liquid state led to increased roughness values compared to its gel formulation (Table 1). The roughness values are in good agreement with the contact angle ones, associating an increased value of R_a or R_q with a decreased value of CA.

Contact angle analysis.

Usually, in dentistry, interfacial contact is established through wetting property. A good adherence between dental materials and the surface of the tooth is highly influenced by the contact angle (CA) value. In practice, low values for the CA are preferred as a measure of the liquid tendency to spread on a solid surface. An increased wettability of the etched surfaces can be achieved through the acid etching procedure. In the case of our investigated samples, it was used the sessile drop measurement method and drops with a volume of 4 μ L were released on the material surface. To calculate the surface free energy (SFE), as described in [24], contact angle values were measured for three different liquids (water (W), diiodomethane (DIM), and ethylene glycol (EG)) purchased from Sigma Aldrich, St. Louis, USA.

We have noticed that the dentine exhibits a more pronounced hydrophilic character compared to enamel (e.g., 41.4 – dentine and 42.5 – enamel for 36.1% PA acid etching; 43.8 – dentine and 45.1 – enamel for 35% PA acid etching) (Figure 6).

It can be concluded that the liquid form of the etching agent led to a better surface quality with enhanced dental material adherence. We can estimate that the adhesive will be uniformly distributed on the etched surface in this case.

In addition, a lower CA value is directly related to a higher SFE value. For the

liquid-etched surface, the SFE value of 55.4 mN/m was obtained in the case of dentine treatment, and 50.7 mN/m was reached for enamel treatment, which was compared to 40.2 mN/m—dentine and 38.3 mN/m—enamel in the case of gel-etched samples (Figure 7). These results are in good agreement with those obtained through SEM investigations.

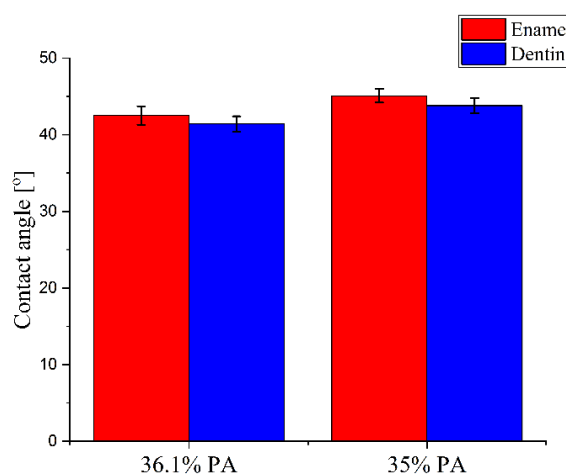


Figure 6. Contact angle values for the investigated samples in the case of two types of etching agents.

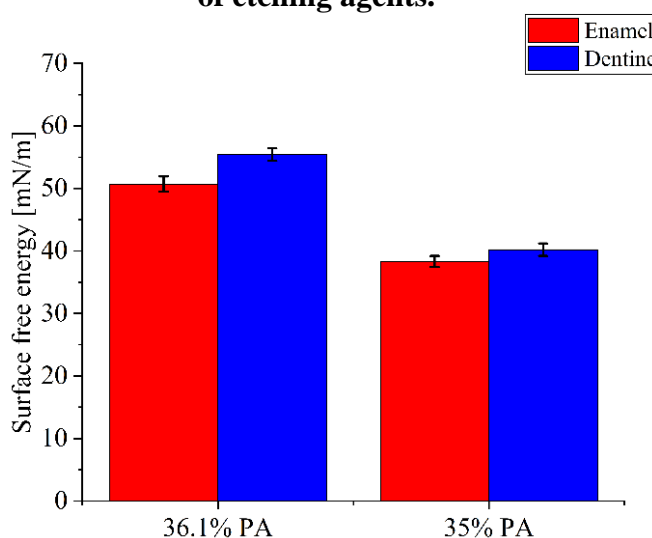


Figure 7. Surface free energy values for the investigated samples in the case of two types of etching agents

CONCLUSIONS

Some protocols are used to apply PA at a concentration between 30% and 40%. Most of them recommend an acid etching duration of about 15 seconds, which is considered adequate for mild enamel etching, while the dentin is demineralized over a distance between 5 and 8 μm , making the teeth ready for medical treatment applications [30]. It is worth mentioning that in our research, the optimal time of 15 seconds was chosen, as well as a concentration of PA in accordance with the present indications. In our case, the PA concentration of 36.1% proved to have a better effect on increasing the surface roughness,

which is correlated with improved hydrophilic behavior and a high value of SFE. It is expected that rough enamel and dentin surfaces prepared with PA etching due to decreased contact angle values will permit a good adhesion of the restorative resins used in dentistry. This beneficial action can be directly correlated with a reduced pH and enzymatic activity and with the fact that the phosphoric acid diminishes the dentin gelatinolytic activity until 14 h, as stated by Trevelin et al. [31]. We can conclude that the acid treatment is adequate for the teeth surface etching step, but the PA concentration must be chosen according to the medical application, patient age, and health state of the dentition.

REFERENCES

1. Buonocore MG, A Simple Method of Increasing the Adhesion of Acrylic Filling Materials to Enamel Surfaces. *J Dent Res* 1955, 34:849–853, doi:10.1177/00220345550340060801.
2. Silverstone LM, Fissure Sealants: Laboratory Studies. *Caries Research* 2009, 8:2–26, doi:10.1159/000260090.
3. Kanca J, Resin Bonding to Wet Substrate. 1. Bonding to Dentin. *Quintessence Int* 1992, 23:39–41.
4. Yousry MM, Effect of Re-Etching Oxalate-Occluded Dentin and Enamel on Bonding Effectiveness of Etch-and-Rinse Adhesives. *J Adhes Dent* 2012, 14:31–38, doi:10.3290/j.jad.a22744.
5. Marshall GW, Marshall SJ, Kinney JH, Balooch, M. The Dentin Substrate: Structure and Properties Related to Bonding. *J Dent* 1997, 25:441–458, doi:10.1016/s0300-5712(96)00065-6.
6. Nakabayashi N, Takarada K, Effect of HEMA on Bonding to Dentin. *Dent Mater* 1992, 8:125–130, doi:10.1016/0109-5641(92)90067-m.
7. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H, In Vivo Degradation of Resin-Dentin Bonds in Humans over 1 to 3 Years. *J Dent Res* 2000, 79:1385–1391, doi:10.1177/00220345000790060601.
8. Reis A, Carrilho M, Breschi L, Loguercio AD, Overview of Clinical Alternatives to Minimize the Degradation of the Resin-Dentin Bonds. *Oper Dent* 2013, 38:E1–E25, doi:10.2341/12-258-LIT.
9. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, Tezvergil-Mutluay A, Carrilho M, Carvalho RM, Tay FR et al. Strategies to Prevent Hydrolytic Degradation of the Hybrid Layer-A Review. *Dent Mater* 2013, 29:999–1011, doi:10.1016/j.dental.2013.07.016.
10. Perdigão J, Reis A, Loguercio AD, Dentin Adhesion and MMPs: A Comprehensive Review. *J Esthet Restor Dent* 2013, 25:219–241, doi:10.1111/jerd.12016.
11. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, Van Meerbeek B, Pashley DH, In Vitro Effect of Nanoleakage Expression on Resin-Dentin Bond Strengths Analyzed by Microtensile Bond Test, SEM/EDX and TEM. *Biomaterials* 2004, 25:5565–5574, doi:10.1016/j.biomaterials.2004.01.009.

12. Ito S, Tay FR, Hashimoto M, Yoshiyama M, Saito T, Brackett WW, Waller JL, Pashley DH, Effects of Multiple Coatings of Two All-in-One Adhesives on Dentin Bonding. *J Adhes Dent* 2005, 7:133–141.
13. Dal-Bianco K, Pellizzaro A, Patzlaft R, de Oliveira Bauer JR, Loguercio AD, Reis A, Effects of Moisture Degree and Rubbing Action on the Immediate Resin-Dentin Bond Strength. *Dent Mater* 2006, 22:1150–1156, doi:10.1016/j.dental.2005.10.010.
14. Reis A, Pellizzaro A, Dal-Bianco K, Gones OM, Patzlaft R, Loguercio AD, Impact of Adhesive Application to Wet and Dry Dentin on Long-Term Resin-Dentin Bond Strengths. *Oper Dent* 2007, 32:380–387, doi:10.2341/06-107.
15. Stanislawczuk R, Amaral RC, Zander-Grande C, Gagler D, Reis A, Loguercio AD, Chlorhexidine-Containing Acid Conditioner Preserves the Longevity of Resin-Dentin Bonds. *Oper Dent* 2009, 34:481–490, doi:10.2341/08-016-L.
16. Carrilho MR, Can Exogenous Protease Inhibitors Control Dentin Matrix Degradation? *J Dent Res* 2012, 91, 1099–1102, doi:10.1177/0022034512462399.
17. Zhou J, Tan J, Chen L, Li D, Tan Y, The Incorporation of Chlorhexidine in a Two-Step Self-Etching Adhesive Preserves Dentin Bond *in Vitro*. *Journal of Dentistry* 2009, 37:807–812, doi:10.1016/j.jdent.2009.06.011.
18. Hass V, de Paula AM, Parreiras S, Gutiérrez MF, Luque-Martinez I, de Paris Matos T, Bandeca MC, Loguercio AD, Yao X, Wang Y, et al. Degradation of Dentin-Bonded Interfaces Treated with Collagen Cross-Linking Agents in a Cariogenic Oral Environment: An *in Situ* Study. *Journal of Dentistry* 2016, 49:60–67, doi:10.1016/j.jdent.2016.02.009.
19. Bottenberg P, Gräber HG, Lampert F, Penetration of Etching Agents and Its Influence on Sealer Penetration into Fissures *in Vitro*. *Dent Mater* 1996, 12:96–102, doi:10.1016/S0109-5641(96)80075-3.
20. Lee JY, Shin SJ, Park JW, Influence of Phosphoric Acid Etching on Bond Strength for Calcium Silicate-Based Sealers. *J Endod* 2023, 49:514–520, doi:10.1016/j.joen.2023.03.008.
21. Anastasiadis K, Nassar M, The Effect of Different Conditioning Agents on Dentin Roughness and Collagen Structure. *J Dent* 2024, 148:105222, doi:10.1016/j.jdent.2024.105222.
22. Sahadi B, Sebold M, André CB, Nima G, Dos Santos A, Nascimento FD, Tersariol ILDS, Giannini M, Effect of Experimental Dentin Etchants on Dentin Bond Strength, Metalloproteinase Inhibition, and Antibiofilm Activity. *Dent Mater* 2024, 40:e12–e23, doi:10.1016/j.dental.2024.02.017.
23. Burrer P, Simoni S, Zwicky P, Attin T, Tauböck TT, Effect of Different Adhesive Application Approaches on Bond Strength in Over-Etched Dentin. *Am J Dent* 2023, 36:118–122.
24. Annamalai M, Gopinadhan K, Han SA, Saha S, Park HJ, Cho EB, Kumar B, Patra A, Kim SW, Venkatesan T, Surface Energy and Wettability of van Der Waals Structures. *Nanoscale* 2016, 8:5764–5770, doi:10.1039/C5NR06705G.
25. Eidelman E, Shapira J, Haupt M, The Retention of Fissure Sealants Using Twenty-Second Etching Time: Three-Year Follow-Up. *ASDC J Dent Child* 1988, 55:119–120.
26. Johnston CD, Burden DJ, Hussey DL, Mitchell CA, Bonding to Molars--the Effect of Etch Time (an *in Vitro* Study). *Eur J Orthod* 1998, 20:195–199, doi:10.1093/ejo/20.2.195.
27. Gandhi M, Lakade L, Davalbhakta R, Patel A, Chaudhary S, Jajoo S, Scanning Electron Microscope Analysis to Evaluate the Effect of Pretreatment with Ozone and Sodium Hypochlorite on Pits and Fissures of Primary Teeth. *Journal of Indian Society of Pedodontics and Preventive Dentistry* 2023, 41:258, doi:10.4103/jisppd.jisppd_352_23.
28. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O, Variation in the Pattern of Acid Etching of Human Dental Enamel Examined by Scanning Electron Microscopy. *Caries Res* 1975, 9:373–387, doi:10.1159/000260179.
29. Sigmund WM, Hsu SH, Wenzel Model. In *Encyclopedia of Membranes*; Drioli, E., Giorno, L., Eds.; Springer: Berlin, Heidelberg, 2015; pp. 1–2 ISBN 978-3-642-40872-4.

30. Retief DH, Middleton JC, Jamison HC, Optimal Concentration of Phosphoric Acid as an Etching Agent. Part III: Enamel Wettability Studies. *J Prosthet Dent* 1985, 53:42–46, doi:10.1016/0022-3913(85)90062-9.
31. Trevelin LT, Villanueva J, Zamperini CA, Mathew MT, Matos AB, Bedran-Russo AK, Investigation of Five α -Hydroxy Acids for Enamel and Dentin Etching: Demineralization Depth, Resin Adhesion and Dentin Enzymatic Activity. *Dental Materials* 2019, 35:900–908, doi:10.1016/j.dental.2019.03.005.