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Influence of Different Etching Times on Dentin Surface Morphology

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A B S T R A C T

The aim of this study is to investigate the influence of different etching times on demineralized dentin surface morphology using scanning electron microscopy and qualitative line microanalysis of chemical structure. Two sample groups, consisting of 30 first premolar teeth in each group, were established. Teeth were cut at the half-distance between the enamel-dentin junction and the pulp. The first group of specimens was etched for 10 seconds and the second group for 30 seconds. 37% ortophosphoric acid was used. SEM (scanning electron microscopy) was utilized to observe the following parameters: number and diameter of dentinal tubules, dentinal and intertubular dentinal surface percentage, appearance of the dentin surface porous zone containing smear layer and demineralized residual collagen particles with dentin demineralization products in acid globules, and dissolved peritubular dentin cuff. After calculating measurements of central tendency (X,C, Mo, SD), Kolmogorov-Smirnov and Student t-test were performed to confirm the quantitative results, and the χ^2-test was run to produce qualitative data. In contrast to the 10-second etching time, the increased etching time of 30 seconds resulted in the following findings: (1) an increased number of dentinal tubules ($p<0.05$), (2) an increase in dentinal tubule diameter ($p<0.05$), (3) an increase in dentinal tubule surface percentage ($p<0.001$), (4) a decrease in intertubular dentinal surface percentage ($p<0.001$), (5) appearance of dentin surface porous zone containing smear layer and demineralized residual collagen particles with dentin demineralization products in acid globules ($p<0.001$), and (6) completely dissolved peritubular dentin cuff ($p<0.001$). Therefore, different etching times using the same phosphoric acid concentration result in different morphological changes in demineralized dentin surface. Moreover, based on a comparison with current studies, prolonged etching time causes morphological changes to dentin surface. Such changes, have, in turn, negative effects on the dentin hybridization process.

Key words: *dentin adhesives, dentin surface morphology, dentin hybridization, dentinal tubule diameter, etching time, ortophosphoric acid, scanning electron microscopy*

Introduction

Dentin hybridization is a modern dental adhesion procedure, which was first described by Nobuo Nakabayashi et al. (1982) at the Institute for Medical and Dental Engineering in Tokyo, Japan[1]. It involves the creation of hybrid dentinal layers, which, in turn, results in a bond between biological material, dentin and the restorative material[2]. Hybridized dentin begins under the dentin surface after surface und subsurface demineralization and adhesive monomer infiltration into exposed collagen network. Thus, the result of the revolutionary discovery by Nakabayashi and colleagues has opened new horizons of restorative dentistry.

After cavity preparation, exposed intact mineralized dentin surface is not found to be clinically suitable for sufficient adhesive monomer infiltration because it is covered with a smear layer of dentin saw-dust[2]. It was discovered in the 17th century by Leewenhoek In 1975, McCombe and Smith, using scanning electron microscopy (SEM), also discovered a dentinal microstructure

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composed of anorganic calcified particles, odontoblastic processes, hemathogenic cells, and saliva, sometimes contaminated by microorganisms [3]. It measures from 0.2 to 2.0 μm in thickness, but could measure up to 40 μm, depending on the type of cavity preparation technique, instruments used and substrate properties[4]. Covering the dentinal surface with smear plugs in tubulus orifices does decrease dentin permeability and protect the pulp, but only temporarily, because saliva dissolves it, and the dentin liner is also insufficient. Removing the smear layer increases dentin permeability more than twenty times. Smear layer, however, is still not an ideal bonding mediator because shear bond strength to underlying dentin is only 5 MPa, and that is not a sufficient anchor of restoration[5].

For a sufficient bonding to dentin, the smear layer must be modified or removed, and the dentin underneath demineralized. One modification technique utilizes self--etching primers that contain weak acids for a one-step simultaneous smear layer and underlying dentin demineralization with exposed collagen penetration. The demineralization process is controlled by a biological buffer of dissolved dentin minerals and smear layer particles that are integrated into the adhesive layer[2]. The total removal of the smear layer is an older and more conventional adhesion restoration procedure. In 1979, Fusayama first started with a »total etch« concept that was revolutionary because he overcame old fears of pulp inflammation caused by acids[6]. The first step of his method uses stronger acids, but only for smear layer removal and underlying dentin deminearlization. The second step is a demineralization products and acid particles water removal and collagen network infiltration after application of primer monomers. Both smear layer procedures involve changing the chemical and physical surface properties favorable for micromechanical and chemical adhesive bonding. The most important properties considered are exposure of dentinal tubule openings, increased tubule diameter, and changing the ratio of peritubular to intertubular dentin. In other words, these processes increase dentinal intertubular and intratubular permeability[2].

Two terms, which are not synonymous, are used to describe dentin chemical treatment: conditioning and etching. Jendresen and Glantz determined that etching includes substrate dissolving, while conditioning involves cleaning, structural alteration and increasing substrate adhesiveness[7]. Conditioning, after smear layer removal, can also increase dentinal tubule diameter by dissolving acid-sensitive peritubular dentin and exposing some collagen fibrils. Conditioning is a less aggressive procedure because it uses only weak to mild (i.e., 10% acids); on the other hand, etching is more aggressive since it uses acids in the range of 30–40% in strength.

During dentin etching, surface and subsurface demineralization occurs in the depth range of 3–7 μm and dissolving of anorganic dentin phase [8]. Thus, the hybridization process is preceded by etching, not conditioning. After the phase where complete anorganic interfibrilar dentin is removed, collagen is destabilized and exposed to enzymatic degradation. Nonetheless, some enclosed intrafibrilar hydroxylapatit crystals, which cannot otherwise be removed, do act to stabilize it. Furthermore, all acidic solutions for dentin etching are water-based solutions because water ionizes acid particles and removes dissolved minerals. Cleaning the surface with water again after etching removes all minerals, and the demineralized surface is water-impregnated. The final result of this process should be hybrid layer formation with monomer impregnated water spaces, although collagen collapse could still interfere. The mechanism underlying collagen collapse, which also occurs in water and is always present after demineralization in amounts from 0,3–0,5 μm, remains unknown. However, considering the great amount of collagen collapse that happens, even under dry conditions, the issue is only academic[8].

In 1996, Nakabayashi discovered the connection between acid concentration and monomer infiltration depth. Specifically, concentrated acids were found to consist of weakly dissolved dihydrogenic phosphate which, in turn, creates undissolved calcium salt, all leading to a weak etching effect[9]. Etching time is also important. In 1996, Hamid demonstrated a correlation between the shallowness of penetration with 2-hydroxyethyl methacrilate (HEMA) as a monomer and the length of dentin etching time. This could have resulted from the longer precipitation of the insoluble demineralization products and possible collagen collapse because of drying[10].

In 1982, Nakabayashi patented the famous 10–3 etching solution that satisfied the requirements for monomer infiltration and bonding. It was made of 10% citric acid and 3% ferric chloride that prevents collagen collapse[11]. Currently, the choice of stronger acids (30–40 %) for total etching is based on the simultaneous treatment of enamel and dentin, which allows the shorter etching time in the range of 10–15 seconds, for dentin etching. Phosphoric acid (37%) is now the most commonly used etchant and is most commonly applied in gel form. The aqueous preparations mixed with polymer thickeners etch more deeply than those in silica gel. The chemical compositon of silica gel provides higher pH value, thus weakening it such that it is sometimes insufficient for adequate hybridization because of the correlation between depth of etching and the pH of the etchants[12]. Also silica particles can contaminate demineralized surface, although that in itself has no influence on hybridization[13]. Today, it is common to incorporate some type of antibacterial product, such as benzalchonium chloride (BAC) or cetylpyridinium chloride (AB), into etchants. Neither the addition of antibacterial products nor the addition of colors harms hybridization. It should be noted that calcium is often added, and cupric ions, which link collagenous or noncollagenous proteins, act to stabilize interfibrilar spaces for further monomer infiltration[14,15].

Dentin chemical treatment procedures are performed as independent processes using conventional one- or two-step smear layer-dissolving dentin adhesives (DA) of the third or fourth generation. Such procedures may also

be conducted simultaneously with monomer infiltration using a one- or two-step smear layer modification or self-etching dentin adhesives (SDA) of the fifth generation. It is also irreplaceable using the newest preparation procedures as a laser or air abrasion units. It is unchanged and important as it was in 1979. when it started.

The aim of this study is to examine the influence of different etching times on the morphological changes of demineralized dentin surface during removal of the smear layer as a part of the dentin hybridization procedure and then to compare our findings to those of other current studies involving the effect of increased etching time on hybrid layer quality.

It is considered that the preponderant risk to the durability and firmness of hybrid layer lies within unprotected collagen fibrils, which, under the thermal and occlusial stress during polymerization, relation failure[16]. These unprotected fibrils remain as a consequence of incomplete monomer infiltration into demineralized dentin matrix. The main reason for this occlusion is excessively deep dentin demineralization, collagen network collapse and the residue created by various demineralization products during the increased etching period.

Materials and Methods

Our investigation was divided into a clinical part, involving the collection and preparation of specimens, and a laboratory part, using SEM sample comparative observation.

The clinical part includes the collection of 60 extracted human upper and lower intact first premolar teeth, which were extracted for orthodontic reasons in patients ranging in age from 14–21 years to avoid the dentin structural variability that tends to correspond to age and pathologic processes. After the extractions, periodontal tissue was removed from the teeth, and the teeth were desinfected and stored in physiologic dissolve at 37 Celsius degrees. Two sample groups, each consisting of 30 teeth, or a total of 60 specimens, were established. Teeth were cut by carbon disc and water cooling sagital in mesiodistal direction in the coronal dentin region and then cut again horizontally at the half-distance between the enamel dentin junction and the pulp. Dentinal surfaces on horizontal cross sections of the first sample group (S1) and second sample group (S2) were treated for 10 and 30 seconds, respectively, with 37% ortophosphoric acid (Total Etch, Ivoclar Vivadent). The mixture consisted of 37% ortophosphoric acid, water, polyvinil alcohol and coloring. After conditioning, surfaces were rinsed with water for 5 seconds and then lightly dried with compressed air, as they are prepared for restoration.

Laboratory SEM investigation consisted of comparative observations of all specimens on a scanning electronic microscope JOEL, JSM-5800 (JOEL, Tokyo, Japan) 15 kV, which has a magnifying capability of 50 000 ×. To obtain the best electrical conduction, the specimens' observed surfaces were steamed with a layer of gold with a thickness of 10–15 nm. This was done in a vacuum of 10-1 TORR in a device S 150 Sputter Coater Edwards. Microimages were then made on the SEM screen, which was mounted with a photocamera (Figures 1–3).

SEM analysis is very suitable for surface morphology and structure research. With this technique, the reflected and secondary electrons are transformed into electrical signals with certain detector. Because they are electrically charged, they can be diverted in a focus with an electromagnet, and they are brought over in a cathode pipe where we get a picture. To avoid any potential atmospheric effects, these experiments were carried out in a vacuum. The amount of reflected and secondary electrons depends on the tension we use, the position of the detector, and the surface of the specimens. The specimens' surface images, which are based on the electrons, have a deep sharpness and display in very clear relief. In

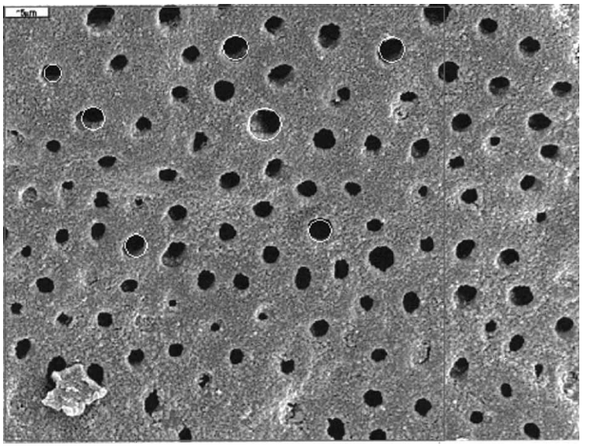

Fig. 1. Microphotography of dentinal surface etched for 10 seconds with 37% ortophosphoric acid, enlargement 2000 ×, bar=5 μm, square 50 × 50 μm.

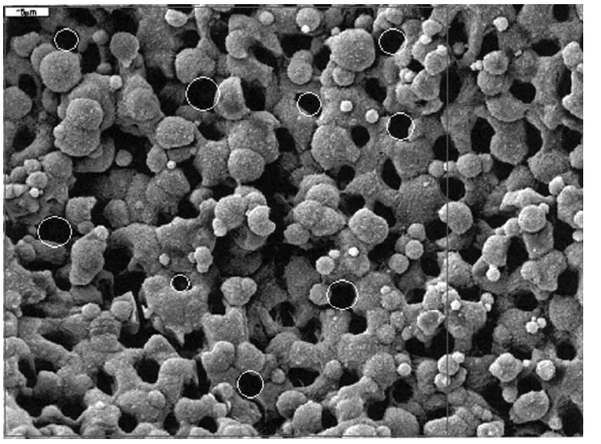

Fig. 2. Microphotography of dentinal surface etched for 30 seconds with 37% ortophosphoric acid enlargement 2000 ×, bar=5 μm, square 50 × 50 μm.

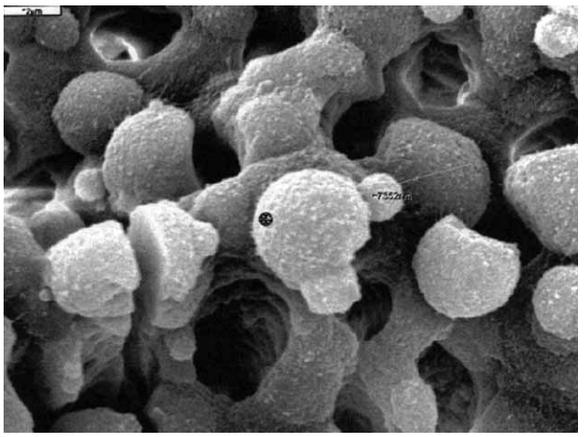

Fig. 3. Microphotography of dentinal surface segment etched for 30 seconds with 37% ortophosphoric acid on which qualitative line microanalysis of chemical structure was performed, enlargement 4000 ×, bar=2 μm, line 7352 nm.

addition, SEM equipment also has the capacity to produce qualitative microanalyses of chemical structure. Qualitative microanalysis principles are based on the characteristics of each chemical element in the composition of a given surface. These elements correspondingly emit different wavelengths, which the instrumentation registers, analyses and displays on the SEM screen. Specifically, if the electron bundle and specimen are still, we get spot microanalysis; however, when the electron bundle crosses over the specimen's surface, we get line microanalysis. In this investigation, we used line microanalysis (Figures 3 and 4).

SEM comparative analysis of the spacimens was carried out for the following parameters:

1. Number of openings of the exposed dentinal tubules (N/mm^2) were counted in a square on the dentinal surface measuring 50×50 μm. This number was then divided by 2500 to get the number of the openings of the dentinal tubules in a square micrometer ($N/\mu m^2$).

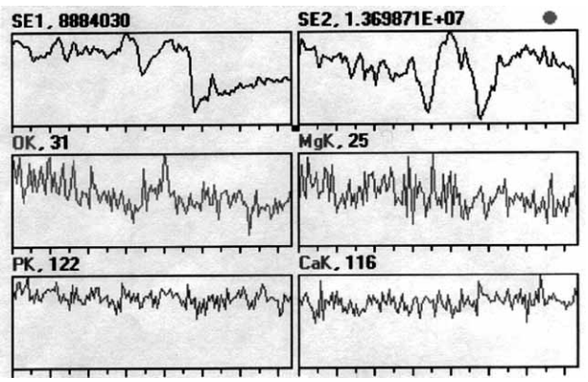

Fig. 4. Graphical review of surface pourosity and chemical structure of dentinal surface segment etched for 30 seconds with 37% ortophosphoric acid.

The resulting number was, finally, multiplied by 10^6 to get the number of the openings of the dentinal tubules in a square millimeter (N/mm^2) (Figures 1–2).

2. Diameter of the openings of the exposed dentinal tubules (2r) was measured in μm as the greatest diameter of irregularly shaped tubules in a square on the dentinal surface measuring 50×50 μm and calculated as an average value of each specimen (Figures 1–2).
3. The percentage of surface area that is occupied by dentinal tubules (Pdt) was calculated based on tubule number and diameter by the following formula: $N \times r^2\pi/2500\ \mu m^2 \times 100\%$. Although dentinal tubules are irregularly shaped, for our calculation, they were idealized as regular circles, and our diameter also included peritubular dentin that was removed by acid etching (Figures 1–2).
4. The percentage of surface area that is occupied by intertubular dentin (Pitd) was calculated based on tubule number and diameter by the following formula: $100\%\text{-}N \times r^2\pi/2500\ \mu m^2 \times 100\%$ (Figures 1–2).
5. Appearance of the dentin surface porous zone containing smear layer particles and demineralized residal collagen particles with dentin demineralization products in acid globules was detected by SEM line qualitative microanalysis (Figures 3–4).
6. Peritubular dissolved dentin cuff was detected by SEM (Figures 1–2).

Statistical results analysis consisted of calculation, distribution, comparison and interpretation of variables found in a both groups of samples (S1 and S2), using adequate univariant statistical methods by SPSS 3.0 (Statistical Package for Social Science). For quantitative records of number (N), diameter (2r), percentage of surface area that is occupied by dentinal tubules (Pdt) and by intertubular dentin (Pitd), the following analyses were performed:

a) descriptive statistical analysis represented by measurements of central tendency, including mean (X), median (C), mode (Mo) and standard deviation (SD);
b) table review of calculated measurements of central tendency;
c) Kolmogorov-Smirnov test for standard distributions; and
d) Student t-test.

For qualitative records, 2×2 tables of the observed variables were made and χ^2–test for independent samples was performed to confirm the appearance of the dentin surface porous zone containing smear layer particles and demineralized residual collagen particles with dentin demineralization products in acid globules and dissolved peritubular dentin cuff.

Results

Results are presented by table reviews.

Table 8 represents Student t-test of the first four parameters of the observed criteria. Statistical significant

difference between two sample groups, with particular confidence level α and significance p, exists if: $t<t\alpha/2$ or $t>t(1-t\alpha/2)$[17].

Figure 3 represents scanning electron microscopic images of dentin surface segments etched for 30 seconds with 37% ortophosphoric acid for which qualitative line microanalysis of chemical structure was also performed. Figure 4 shows the results. The yellow line in Figure 3 indicates the direction of microanalysis which was perfomed through one of the bubble-like structures and surrounding dentin area to detect their chemical structures and differences. Such morphological differences are not present on the SEM images of the first sample group (Figure 1). The first two rows of the table graph in Figure 4 represent microanalysed surface porosity detected by secondary electrons (SE 1, SE 2). Higher values represent higher surface density, which, in our case, is localized in the bubble-like structures. The second two rows represent the so-called K or L α values that correspond to the particular atomic shell level of the detected element, which is the numerical concentration of the detected element. Values for oxygen, magnesium and phosphorus are lower in the dentin area, but for calcium, they are equal.

Table 9 represents the χ^2-test of the last two parameters of the observed criterion. Statistically significant difference between two sample groups, with particular degrees of freedom d.f. and significance p, exists if $\chi^2>\chi^2$ border[17].

Discussion

These findings confirmed that the different etching times with the same phosphoric acid concentration result in different morphologic changes of demineralized dentin surface. This was very evident in the striking changes in the number, diameter and surface area of dentinal tubules, intertubular surface area, appearance of the dentin surface porous zone containing smear layer and demineralized residual collagen particles with dentin demineralization products in acid globules, and the completely dissolved peritubular dentin cuff that happened after prolonged etching time (Tables 1–7).

Significant statistical difference ($p<0.05$) in the number of opened dentinal tubules was observed in the second group (S2), with a prolonged etching time, which demonstrates that the increased etching time, with the same phosphoric acid concentration, does result in the increased number of opened dentinal tubules of treated dentinal surface[18–20]. The reason for this is that the longer etching treatment removes more of the smear layer, which has a different thickness, and, thus, more smear plugs in tubulus orifices, which might otherwise be closed under the condition of shorter etching time, are removed[21]. Significantly, the increased number of den-

TABLE 1
NUMBER OF EXPOSED DENTINAL TUBULES (N/2500 μm^2)

Specimens	X	C	Mo SD
Sample group 1 (10s 37% H_3PO_4)	81	73.5	74 6.78
Sample group 2 (30s 37% H_3PO_4)	86	92.5	78 7.96

TABLE 2
NUMBER OF EXPOSED DENTINAL TUBULES ($N\times10^3/mm^2$)

Specimens	X	C	Mo SD
Sample group 1 (10s 37% H_3PO_4)	32.4	29.4	29.4 2.712
Sample group 2 (30s 37% H_3PO_4)	34.4	37	31.2 3.184

TABLE 3
DIAMETER OF THE OPENINGS OF THE EXPOSED DENTINAL TUBULES (μm)

Specimens	X	C	Mo SD
Sample group 1 (10s 37% H_3PO_4)	2.4	2.75	2.3 0.33
Sample group 2 (30s 37% H_3PO_4)	2.65	3	2.5 0.41

TABLE 4
THE PERCENTAGE OF SURFACE AREA THAT IS OCCUPIED BY DENTINAL TUBULES (Pdt/%)

Specimens	X	C	Mo SD
Sample group 1 (10s 37% H_3PO_4)	14.4	17.4	12.3 2.59
Sample group 2 (30s 37% H_3PO_4)	18.3	26.1	15.3 5.90

TABLE 5
THE PERCENTAGE OF SURFACE AREA THAT IS OCCUPIED BY INTERTUBULAR DENTIN (Pidt/%)

Specimens	X	C	Mo SD
Sample group 1 (10s 37% H_3PO_4)	85.6	82.6	87.7 2.59
Sample group 2 (30s 37% H_3PO_4)	81.7	73.9	84.7 5.90

TABLE 6
APPERANCE OF THE SURFACE POUROS ZONE CONTAINING SMEAR LAYER PARTICLES AND DEMINERALIZED RESIDUAL COLLAGEN PARTICLES WITH DENTIN DEMINERALIZATION PRODUCTS IN ACID GLOBULES

Specimens	Yes	No	Total
Sample group 1 (10s 37% H_3PO_4)	0	30	30
Sample group 2 (30s 37% H_3PO_4)	30	0	30
Total	30	30	60

TABLE 7
PERITUBULAR CUFF DISSOLVE

Specimens	Yes	No	Total
Sample group 1 (10s 37% H_3PO_4)	0	30	30
Sample group 2 (30s 37% H_3PO_4)	30	0	30
Total	30	30	60

tinal tubules also influences the dentin hybridization process in the sense of forming more resin tags that, in turn, contribute to bond strength.. The bond strength to sclerotic dentin is lower compared to normal dentin due to the absence of resin tag formation[22]. Different dentin adhesives have different bonding mechanisms, and some of them depend on the fact that intratubular dentin permeability can form more quality hybrid layers in a dentin that has more dentin tubules[23,24,25]. On the other hand, an increased number of tubules, which may be caused by over-etching, might also be antagonistic to dentin hybridization because of possible collagen network collapse and longer precipitation of the insoluble demineralization products that could harm monomer diffusion, which could also be a reason for the discrepancy of demineralization depth and monomer infiltration[10,26]. Another contributor to low hybrid layer quality is the pattern of nano- and micro-leakage forming. Also, an increased number of poorly hybridized dentinal tubules can result in dentinal sensitivity and permit bacterial assault[27].

Significant statistical difference ($p<0.05$) in the diameter of dentinal tubules was also determined between the two sample groups, and the prolonged etching time was also shown to benefit the second group. This gives evidence that the increased etching period with the same phosphoric acid concentration results in an increased diameter of dentinal tubules of the treated surface. This results from a deeper demineralization, the collapse of collagen network, and, most importantly, the completely dissolved peritubular dentin cuff that is linearly correlated with etching time without considering dentin depth[28,29]. Such over-etching effect on lateral tubule walls is, however, bad for hybridization because, for adequate lateral wall hybridization of dentinal tubules, an expanded collagen network is more important than tubule diameter.[2,21] The balance between the demineralization depth and monomer penetration [until underlying mineralized dentin] is the essence of hybrid layer quality[30].

The percentage of surface area that is occupied by dentinal tubules (Pdt) is greater in the second sample group ($p<0.01$), and that was expected because it is a product of the number and diameter of exposed dentinal tubules. In contrast, the percentage of surface area that is occupied by intertubular dentin (Pitd) is smaller in the second sample group ($p<0.01$) because it is disproportional to the number and diameter of dentinal tubules [31,32]. It is statistically significant ($p<0.01$) that prolonged etching time causes the increase of Pdt and the decrease of Pitd. The surface area of intertubular dentin represents the biolgical substrate for hybrid layer formation and, thus, its reduction has a negative influence on hybridization[33].

The appearance of the dentin surface porous zone containing smear layer particles and demineralized residual collagen particles with dentin demineralization

TABLE 8
STUDENT-T TEST OF SIGNIFICANT DIFFERENCES BETWEEN TWO SAMPLE GROUPS
(SAMPLE GROUP 1: 10s 37% H_2PO_3; SAMPLE GROUP 2: 30s 37% H_2PO_3)

Variable: Parameter of observed criterion	t	$t\alpha/2$	$t\ (1-\alpha/2)$	α	p
1.) Number of exposed dentinal tubules	–2.623	–2.018	2.018	0.05	0.975*
2.) Diameter of exposed dentinal tubules	–2.604	–2.018	2.018	0.05	0.975*
3.) Surface of exposed dentinal tubules	–3.313	–2.664	2.664	0.01	0.995**
4.) Surface of intertubular dentin	3.313	–2.664	2.664	0.01	0.995**

Legend: * = significance 95 %; ** = significance 99 %

TABLE 9
χ^2-TEST OF SIGNIFICANT DIFFERENCES BETWEEN TWO SAMPLE GROUPS
(SAMPLE GROUP 1: 10s 37% H_2PO_3; SAMPLE GROUP 2: 30s 37% H_2PO_3)

Variable: Parameter of observed criterion	χ^2	χ^2 border	d.f.	p
5.) Appearance of the surface pouros dentin zone containing smear layer and demineralized residual collagen particles with dentin demineralization products in acid globules	30	10.83	1	< 0.001***
6.) Peritubular dentin cuff dissolve	30	10.83	1	< 0.001***

Legend: *** = significance 99.9 %

products in acid globules is a pure qualitative property for comparative SEM observation in our two sample groups. Under SEM investigation, the second sample group with the prolonged etching time showed a rough surface texture ($p<0.001$). Surface roughness in the first sample group was unlike that of the second group and therefore could be the product of loose water and substrate collapse in a vacuum[28]. Qualitative microanalysis of chemical structure of the over-etched dentinal surface showed that K α values for Mg, P and Ca are fundamentally equal (Figures 3–4). This proves that demineralization products and mineral salts settle in the demineralized substrate and residual acid drops[10]. However, the known chemical composition of our aqueous echant (Total Each, Ivoclar Vivadent) without silica particles could not have left a silica-contaminated surface[13]. Regardless of chemical composition, all etchants leave mineral salts in demineralized dentin that increase as the etching period increases[34]. Higher oxygen (O) with higher surface density values (SE1, SE2) in the examined bubble-like structure is evidence of the liquid consistency that includes the acid drops. Somewhat higher values of Mg and P occur in acid drops, which is expected because the Mg and P salts are already in the etchant's chemical composition. Bubble-like, organized acid drops are present in the second sample group, probably as a result of the short water rinsing time of 5 seconds. Again, such uneven and unclean surface morphology, which derives from over-etching treatment, has a negative influence on the hybridization process in the sense of physical and chemical interference with monomer infiltration[9,21].

Finally, the presence of peritubular dentin cuff, observed as a light halo, was seen around dentinal tubule orifices in the first sample group, but not seen in the second group. Prolonged etching time easily accomplished the complete dissolving of the dentin peritubular cuff ($p<0.001$)[29]. For hybrid layer quality and resin tag formation, it is important to remove only a part of peritubular dentin from tubular orifices in order to enhance apropriate demineralization of underlying substrate[35].

Currently used hydrophilic resin monomers are rarely able to completely infiltrate the demineralized zone, even given an adequate etching time, and it is speculated that this failure could contribute to microleakage and influence the long-term durability of the bond. More diluted acids than those available commercially are shown to reduce both the degree and depth of demineralization and result in a thinner layer that can lead to more complete resin infiltration of the collagen[30]. Depth of demineralization increased by both acid concentration and conditioning time follows a logarithmic relationship[36]. Pioch et al. established that the highest tensile bond strengths are achieved after 15 s of etching, followed by 30 s and 60 s. Under these etching conditions, and irrespective of the bonding agent, bond strengths were significantly higher than without etching, or after 120 and 180 s of etching. There was no linear correlation between the thickness of the hybrid layer and the bond strength[37]. Area percentage of the hybrid layer increases with the increase in etching time, whereas the bond strength and the integrity of the hybrid layer decreases with the increase in acid-conditioning time, especially the top part, has an effect on bond strength[38]. There is a direct correlation between etching time and the depth of demineralized zone. The hybrid layer thickness correlates directly to the etching time. Increased etching time demineralizes dentin surface to a depth greater than that to which resin monomers can penetrate, producing a thick, poorly infiltrated hybrid layer. Therefore, reducing etching time reduces the depth of the demineralized zone and may be effective for achieving complete penetration and for sealing the dentinal surface[39].

Conclusions

Based on statistical distribution of investigation results of influence of different etching times (10 seconds and 30 seconds) on demineralized dentin surface morphology, we conclude that increased etching time causes:

1. the uncovering of a large number of dentinal tubules that had been covered by the smear layer ($p<0,05$);
2. dentinal tubule diameter to increase ($p<0,05$);.
3. dentinal tubule surface percentage increase over the entire exposed dentinal surface ($p<0,001$);
4. intertubular dentin surface percentage decrease over all exposed dentinal surface ($p<0,001$);
5. appearance of the dentin surface porous zone containing smear layer and demineralized residual collagen particles with dentin demineralization products in acid globules ($p<0,001$); and
6. complete dissolving of peritubular dentin cuff ($p<0,001$).

Based on these statistically significant conclusions, we can confirm the hypothesis that the different etching times with the same phosphoric acid concentration result in different morphologic changes of demineralized dentinal surface. Moreover, based on comparison with current studies, these morphological changes are negatively correlated with increasing etching time and negatively influence the hybridization process in terms of durability and firmness of the hybrid layer.

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UTJECAJ RAZLIČITOG VREMENA JETKANJA NA MORFOLOGIJU POVRŠINE DENTINA

SAŽETAK

Svrha ovog istraživanja je utvrditi utjecaj različitog vremena jetkanja na demineraliziranu morfologiju površine dentina korištenjem scanning elektronskog mikroskopa (SEM) i kvalitativne linijske mikroanalize njezine kemijske strukture. Pripremljene su dvije skupine uzoraka, svaka od po 30 prvih premolara. Zubi su rezani na polovini udaljenosti između caklinsko-dentinskog spojišta i pulpe. Prva skupina uzoraka je jetkana 10, a druga 30 sekundi 37%-tnom ortofosfornom kiselinom. SEM je korištena za određivanje sljedećih parametara: broja i promjera dentinskih tubulusa, postotka površine pod dentinskim tubulusima i intertubularne dentinske površine, pojavnost porozne dentinske površinske zone koja sadrži zaostatni sloj i demineralizirane rezidualne čestice kolagena s produktima demineralizacije u česticama kiseline, i pojavnost otopljene peritubularne dentinske manžete. Nakon određivanja mjera centralne tendencije (X, C, Mo, SD), Kolmogorov-Smirnovim i Student t-testom su obrađeni kvantitativni, a χ^2-testom kvalitativni podaci. U usporedbi s jetkanjem od 10 sekundi, jetkanje od 30 sekundi rezultiralo je sljedećim promjenama: (1) povećanim brojem dentinskih tubulusa ($p<0,05$), (2) povećanjem promjera dentinskih tubulusa ($p<0,05$), (3) povećanjem površine pod dentinskim tubulusima ($p<0,001$), (4) smanjenjem površine intrtubularnog dentina ($p<0,001$), (5) pojavom porozne dentinske površinske zone koja sadrži zaostatni sloj i demineralizirane rezidualne čestice kolagena s produktima demineralizacije u česticama kiseline ($p<0,001$), i (6) pojavnost potpuno otopljene peritubularne dentinske manžete ($p<0,001$). Za zaključiti je da korištenje iste koncentracije ortofosforne kiseline za jetkanje dentina u različitim vremenskim intervalima rezultira različitim morfološkim promjenama demineralizirane dentinske površine. Prema usporedbi s dosadašnjim studijama, produženo vrijeme jetkanja uzrokuje morfološke promjene dentinske površine. Takve promjene, naime, imaju negativan utjecaj na proces hibridizacije dentina.