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The effect of multiple enamel conditioning on enamel micro-hardness

Parisa Salehi[1], Mojgan Shavakhi[2], Sepideh Nazari[1], Shabnam Ajami[1]

[1]Department of Orthodontics, Orthodontics Research Center, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran, [2]Department of Orthodontics, Dental Research Center, Dental Research Institute, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Background: White spot formation is one of the common side effects in orthodontic treatments and multiple enamel conditioning might happen even during on session of fixed orthodontic treatments. The aim of the present study was to evaluate the impact of multiple enamel conditioning with different methods on enamel micro-hardness (MH).

Materials and Methods: In this *In vitro* experimental study, the buccal surfaces of 105 extracted premolars were evaluated in seven groups: One control and six experimental groups. The enamel conditioning was performed in three ways: Etching with phosphoric acid 37%, etching with phosphoric acid 37% followed by primer application and conditioning with self-etch primer. The conditioning process in each way was also performed twice consecutively. The specimens were submitted in pH cycling model with demineralization and re-mineralization solutions for 14 days. Afterward Vickers MH test was applied with 0.981N force on the teeth for 10 s indentation time. Data were analyzed using One-Way ANOVA and Tukey HSD (honestly significant difference) test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Results: MH analysis showed statistically significant differences between the control group and the other conditioned groups ($P < 0.05$). The groups conditioned with acid-etch and primer, particularly twice, showed the lowest amount of MH in comparison to other groups. Self-etch primer had the least effect on MH of the enamel. Single time etching without using primer, made no considerable difference when compared to multiple etching.

Conclusion: Etching process and covering the enamel with primer decrease enamel MH. Using self-etch primer is a more conservative method of enamel conditioning.

Key Words: Dental caries, dental tissue conditioning, hardness, tooth demineralization

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Address for correspondence:
Dr. Shabnam Ajami,
School of Dentistry,
Qom Abad, Ghasrodasht St. Shiraz, Shiraz, Iran.
E-mail: dr.ajami.sh@gmail.com

INTRODUCTION

Fixed orthodontic appliances need to be bonded to the enamel surface. This process is a preliminary step of fixed orthodontic treatments. The conventional method of bonding procedure includes surface treatment of the enamel by etching with phosphoric acid to achieve micro-retentions.[1] Another method for bonding the brackets is using self-etch primers which consists of conditioner and priming agents.[2] The phosphate group in self-etch primers dissolves the calcium bonded to hydroxyapatite. The dissolved calcium bonds to the phosphate group and forms the network during polymerization.[3] One of the flaws of these methods is bracket attachment failure. It can be due to inappropriate forces exerted to the

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brackets by patients during masticatory functions, or because of the poor bonding technique. Moreover, bracket repositioning might be needed at bonding appointments or later. Therefore, re-etching and re-bonding of the enamel surface might be mandatory several times.[4]

White spot lesion formation is a common clinical problem in orthodontic patients.[5] These lesions are the first manifestations of enamel decalcification and can affect the esthetic aspects of the orthodontic treatments,[6] and cause dissatisfaction, particularly for those who demand facial and dental esthetics.[7] Moreover, white spot lesion is the primary stage of enamel demineralization and in case of poor oral hygiene can lead to enamel caries which necessitates restorative treatments.[8] White spot lesions can also be initiated iatrogenically; Mechanical or chemical damages to enamel increase the permeability of enamel and consequently can increase susceptibility to demineralization and caries.[9] It has been shown that increasing the etching duration and exceeding the area beyond the bracket bases[10] trigger the white spot formation. Furthermore, etching the enamel particularly more than five times increases surface permeability significantly.[9] On the other hand, nonrinse conditioners prepare a smooth and adequately rough surface on enamel[11] and produce more conservative retentive patterns in enamel structure than conventional etch and rinse agents.[12]

Multiple enamel etching due to poor bracket position or bonding failures is common in fixed orthodontic treatments and this process may increase the susceptibility of enamel to white spot lesion formation which decreases enamel micro-hardness (MH). Although etching the whole enamel surface seems to be harmful, covering the surplus etched area with adhesive is recommended.[10] On the other hand, it has been claimed that under the clinical circumstance where fluoride is used regularly, extending the etched area would not be a problem.[13] In the present study, we aimed to evaluate the effect of multiple conditioning of the enamel with self-etch and conventional etch-and-rinse method on the MH of enamel sites beyond the bonding area beneath the brackets.

MATERIALS AND METHODS

In this *in-vitro* experiment, a total of 105 premolar teeth were collected after extraction for orthodontic purposes. The exclusion criteria were as follows: Existence of carious lesions, history of fixed orthodontic treatment, etching or restorative treatment, presence of white spot lesions or any developmental enamel defects, cracks due to the extraction procedure or exposure to any chemical solutions like hydrogen peroxide. To prevent demineralization the specimens were stored in tap water (21°C) for 2 weeks before the trial.

The roots of the teeth were cut from the cemento-enamel junction by diamond disc (Jota, Germany). The remained crowns were mounted in the 1 cm × 1.1 × cm 1 cm^3 cube of self-cure acrylic blocks (Akropars, Iran). They were polished by fine handpiece acryl rubber (Jota, Germany) and followed by fine silicon carbide polishing paper (Matador-Germany-P: 1000), preparing a flat surface for the Vickers test.

The samples were allocated to seven trial groups (n = 15 each). The groups were as follows: (1) No procedure was applied on the enamel surface (control group). (2) The enamel surfaces were etched once. The samples of group 2 of the enamel conditioning process were etching with 37% phosphoric acid (3M ESPE Etching Liquid 1923-USA) for 15 sec, rinsing with water for 30 s, and drying with oil-free dry air for 20 s. (3) The same etching procedure as Group 2 was performed two times consecutively. (4) The teeth were etched the same as Group 2 and then primer (Transbond XT, 3M, Unitek) was applied as a thin uniform coat and cured for 10 s from the buccal surface direction with an LED curing unit (Elipar S10, 3M-ESPE, Seefeld, Germany, 1200 mw/cm^2, 430–480 nm) immediately. (5) The same procedure as the fourth group was done for two times. The buccal surface of the teeth was polished with adhesive removal finishing bur (G and H orthodontics, USA) after the first etch and primer applying procedure. (6) The enamel surfaces were conditioned with self-etch primer (Transbond Plus self-etch system, 3M, Unitek) for 5 s. Then, it was cured for 10 s from the buccal direction with an LED curing unit immediately. (7) The same procedure as the sixth group was done two times. The buccal surfaces of the teeth were polished with adhesive removal carbide bur (G and H orthodontics, USA) after the first self-etch primer application.

Then, all specimens were submitted to the dynamic demineralization and re-mineralization cycling model

proposed by O`reilly,[14] with the solutions proposed by Argenta.[15] The pH-cycling challenge consists of immersing the specimens in a demineralization solution (2.0 mmol/L of calcium, 2.0 mmol/L of phosphate, and 75 mmol/L of acetate in pH 4.5) for 6 h, followed by rinsing in deionized water, drying with air-jet and immersing in a re-mineralization solution (1.5 mmol/L of calcium, 0.9 mmol/L of phosphate, 150 mmol/L of potassium chloride, and 20 mmol/L of Tris buffer at pH 7.0), for 18 h at 37°C. The entire procedure was repeated daily for 14 days, and the solutions were changed on a 5-day cycle period.

After pH cycling, the samples were tested for transversal MH measurements. Hardness is defined as the resistance to permanent surface indentation or penetration.[16] The Vickers hardness test uses a square-based diamond indenter. The indenter is a 136° diamond-shaped indenter which forces into the material with a definite load application.[16] Transversal Vickers hardness (MHV 10002, SCTMC, china) was performed with 0.981 N forces on the teeth for 10 s indentation time. Each measurement was conducted three times with 50 μm distance from each other. Indentations were evaluated with infinite focus microscope (Alicona Imaging, Grambach, Austria) measuring longitudinal and transverse axes of the indents (d_1, d_2 [μm]). Vickers hardness number (HV) was calculated using the following equation: $HV = 0.102 \times 2\times \sin(136/2) \times (F/d^2)$, with $d = (d_1 + d_2)/2$.

Statistical analysis was performed using SPSS 19 software (SPSS, Chicago, Ill). To evaluate the impact of method types and times of enamel conditioning on Log_e MH (MH = MH) a repeated measures One-Way ANOVA was performed. For multiple comparisons between groups Tukey HSD test was used as the *post Hoc*. The significance level was subset at $\alpha = 0.05$.

RESULTS

Seven specimens were lost during MH test and data of 98 specimens were subjected to statistical analysis. The means and standard deviations for MH of all groups are presented in Table 1 and Figure 1. The maximum mean of MH was detected in Group 1 and then in Group 6; and Group 5 attained the minimum of MH.

The result of multiple comparisons is presented in Table 2. The comparison of Group 2 with Group 3 and Group 6 with Group 7 did not show considerable difference ($P > 0.05$). Furthermore, Group 2 did not show significant differences in comparison with Group 6 and 7. The other comparisons between groups revealed significant differences ($P < 0.05$).

DISCUSSION

Etching enamel surface around the bonded brackets removes the fluoride-rich surface of the enamel and makes the enamel susceptible to demineralization during orthodontic treatment.[17] Artificial white spot formation on the enamel surface affects its MH.[18] So, we used MH test to evaluate the mineral loss that occurred among experimental groups. The results of this study indicate that any type of enamel conditioning decreases surface MH significantly, as it was suggested by previous studies.[19-21] Then, it is better not to condition the whole enamel surface for bonding the brackets.[10]

Conditioning with self-etch primers even two times, had minimum effect on MH. Applying self-etch primer only once showed the highest MH among the non-control groups. It has been shown that self-etch primers preserve the resistance of enamel to demineralization[22] and enamel conditioning with the conventional acid-etch method leads to more enamel loss than the self-etch primer technique.[23] Furthermore, Masahiro Iijima *et al.*[24] have shown that self-etch primers have the minimal effect on enamel hardness-evaluated by nanoindentor-and enamel elastic modulus compared to conventional bonding technique. These results are in agreement with the present study based on the higher MH in self-etch primer groups in comparison to conventional etch-rinse conditioning. Transbond self-etch primer does not affect enamel MH as much as the conventional etching technique. This bonding technique is based on preserving the hybrid

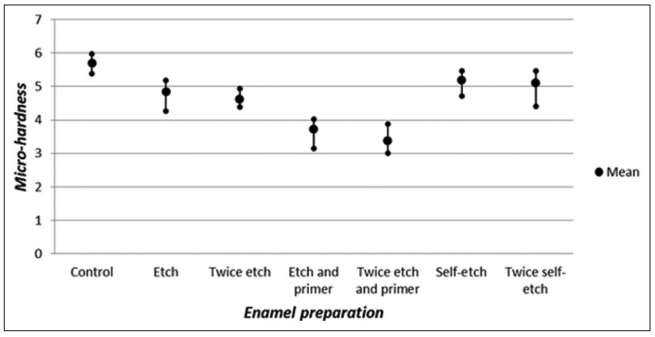

Figure 1: The micro-hardness value of different groups of enamel preparation.

Table 1: The mean and standard deviation of micro-hardness in different groups of enamel preparation

Group	*n*	Micro-hardness (HV)	SD	Minimum	Maximum
Control	15	5.69	0.18	5.38	5.97
Etch	12	4.85	0.22	4.26	5.18
Twice etch	15	4.62	0.18	4.38	4.93
Etch and primer	14	3.72	0.27	3.14	4.02
Twice etch and primer	14	3.38	0.25	3.01	3.88
Self-etch	14	5.19	0.22	4.71	5.46
Twice self-etch	14	5.11	0.26	4.41	5.46
Total	98	4.66	0.80	3.01	5.97

HV: Hardness value; SD: Standard deviation

Table 2: Two by two comparison of micro-hardness between the groups with different methods of enamel preparation

Group	Control	Etch	Twice etch	Etch and primer	Twice etch and primer	Self-etch	Twice self-etch
Control	-	S	S	S	S	S	S
Etch	S	-	NS	S	S	NS	NS
Twice etch	S	NS	-	S	S	S	S
Etch and primer	S	S	S	-	S	S	S
Twice etch and primer	S	S	S	S	-	S	S
Self-etch	S	NS	S	S	S	-	NS
Twice self-etch	S	NS	S	S	S	NS	-

S: Significant; NS: None significant

layer which leads to more intact enamel surface. In groups which had two times of conditioning and primer bonding resembling the situation where bracket repositioning is needed in the same session of treatment, the cured primer was removed with polishing bur (G and H orthodontics, USA) each time. The polishing process affected those samples treated with the conventional method of enamel conditioning more than the self-etching primer method. This was stated by Ingrid Hosein *et al*.[23] that following enamel clean-up with different methods, conventional etching system causes more enamel loss in comparison to the self-etching system. Furthermore, it has been shown that self-etching primers cause less damage to the enamel in the secondary bonding process and have adequate bonding strength.[25]

After self-etch primer, etching the enamel without applying the primer had the least effect on MH. It seems that even two times of etching (each time for 15 s) made no differences in MH, although it has a nonsignificant declining trend. Therefore, if the clinician etches the enamel again in the re-bonding procedure, it will be better to restrict the area of primer application when using the conventional etch-and-rinse method.

Etching the enamel (once or twice) followed by primer application had the lowest MH values. Acid etch removes about 10–20 micrometers of the outer layer of enamel. Based on the result of the present study, it could be implied that when the enamel surface is etched and covered with the primer bonding agent, it would prevent the proper re-mineralization process. Our results showed that not only simple primers (Transbond XT) bonding did not have preventive effect on acid-etched enamel but even it might prevent the re-mineralization process of buffering cycle of saliva. This is also stated in a review article by Øgaard and Fjeld,[26] that etched enamel which was not covered by resin material undergoes re-mineralization relatively fast, following the salivary exposure. Although a study conducted by Yap *et al*.[27] declared that using resin-based sealant on enamel surface had physical protective effect around the bracket, this is implied only to sound enamel and it cannot be generalized to etched surfaces of the enamel. Furthermore, another study evaluated the effect of light-cured sealants on enamel demineralization and declared that unfilled sealants have no preventing effect.[28] The resin-based sealant may be protective in case of sound enamel surface by preventing micro-leakage in the surrounding margins of attachments. In the present study, repeating the conventional bonding procedure twice had the most effect on enamel hardness. On the other hand, it has been shown that etching the enamel is not necessary in

the process of bracket re-bonding or repositioning.[29] Therefore, it can be suggested not to etch the enamel surfaces which have been etched and primed before.

Although this study tried to imitate the saliva buffering cycle (de-mineralization and re-mineralization), it did not include oral hygiene, fluoride supplements, and bacterial impact. Furthermore, in clinical conditions often there is a time interval between the initial bonding and the rebonding, and during this time the enamel might be remineralized. Therefore, it is suggested that these factors be considered in future studies. As a consequence, this study could not extrapolate the *in-vivo* situation completely.

CONCLUSION

As the clinical relevance, it can be concluded that clinicians could affect the enamel MH during orthodontic treatments iatrogenically. Trying to prevent multiple surplus etchings or not to covering the etched surface of enamel that exceeds the bracket area can be beneficial. It is also recommended to use self-etching primers instead of conventional etching systems for more conservative treatments.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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