

## Effects of Three Different Topical Agents on Enamel Demineralization around Orthodontic Brackets: A Clinical Study

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### Abstract

**Background:** One of the serious side effects of fixed orthodontic appliances is the development of white spot lesions.

**Aim:** The aim of this study was to evaluate the *in vivo* effects of three topical protecting agents on enamel demineralization around orthodontic brackets at two time intervals.

**Material and methods:** Twenty-eight patients, 13-16 years (mean: 14.06 +/- 1.73 years), scheduled to have four first premolar teeth extracted, were divided into four groups: three experimental and one control. SeLECT-Defense™, (Lubbock, TX, USA), Clinpro fissure sealant (3M ESPE-USA) and White Varnish with TCP (3M ESPE-USA) were applied to tooth surfaces around brackets in the experimental groups. After one month, two premolars of each patient; (14 premolars from each group) were extracted. The teeth were stored in a refrigerator in flasks containing gauze dampened with saline. After two months, the other fourteen premolars from each group were extracted and treated similarly. Demineralization of enamel around the brackets was evaluated by a cross-sectional microhardness method with an indentation at two positions (occlusal-cervical), 10 µm depth.

**Results:** There was no significant difference between the microhardness of either the occlusal or the cervical halves in the two times tested. Statistically significant differences were determined between each of the treatment and control groups ( $p < 0.001$ ). However, no statistically significant differences were detected between the experimental groups ( $p < 0.05$ ).

**Conclusion:** The findings from the present study highlight the value of the usage of any of the three protecting agents to decrease demineralization around orthodontic brackets.

**Keywords:** Demineralization, Organoselenium, Fissure sealant, Varnish

### Introduction

Placement of fixed appliances is still unfortunately linked with a high risk of developing white spot lesions, in spite of advances in orthodontic materials and treatment techniques [1,2]. During orthodontic treatment, plaque accumulation is frequently inevitable around the brackets because of inadequate oral hygiene which is very common in pubertal people [2]. Dental plaque is a biofilm produced by a bacterial community that may be composed of over 700 species. One of the main components of this plaque is *Streptococcus mutans* (*S. mutans*), which is also considered to be the primary etiologic agent of plaque formation and human dental caries by interacting with other *streptococci* [1-3]. Consequently, patients with fixed orthodontic appliances have an elevated risk of tooth caries, and enamel lesions can occur within a month, despite mechanical plaque control trials and whether or not fluoridated dentifrice is used [3,4]. The incidence of white spot lesions in patients treated with fixed appliances was reported to be up to 50% [5,6].

Many methods can decrease or prevent white spot lesions as improving oral hygiene, modifying diet (low carbohydrate), and treating with topical fluoride. Most of these methods, however, rely on patient compliance, which is unreliable, and is seen only in 13% of orthodontic patients. Attempts have been made to use compliance-free methods [7]. Prevention of metabolic activity of plaque bacteria, forming fluoro-apatite crystals, and stimulating remineralization are well-known caries control potential of fluoride-releasing agents [8-10].

A study by Yap J, et al. 2014 was conducted to evaluate and compare the relative

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efficacy of a resin fissure sealant, nano-filled self-adhesive protective coating, resin infiltrant, glass ionomer cement (GIC), and GIC containing casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) in preventing the formation of subsurface lesions of enamel (SLE) adjacent to orthodontic brackets by acting as an enamel surface sealant (ESS). The results concluded that the use of GIC alone or incorporating CPP-ACP significantly reduced demineralization compared with other materials. Backscattered SEM images showed no measurable demineralization for enamel treated with either GIC material in contrast with other groups, which showed statistically significant demineralization levels [11].

Another study was conducted to compare the effect of pH cycling on the microhardness of the enamel of primary human teeth treated with a conventional brown Sodium Fluoride (5% NaF) Varnish to those treated with a white Fluoride Varnish (5% NaF) enhanced with functionalized tricalcium phosphate (fTCP). The results of this study suggest that the use of an additive such as fTCP to a fluoride varnish significantly improves the protective ability of the varnish on primary teeth in vitro [12].

It has been shown that an organo-selenium compounds covalently attached to different biomaterials inhibited bacterial biofilms [13]. The catalytic mechanism by which selenium generates superoxide radicals has been previously described [14]. The study confirmed that organo-selenium polymerized into dental sealant is effective in inhibiting bacterial attachment and biofilm formation by the two main oral pathogens, *S. mutans* and *S. salivarius*. Moreover, it was claimed that this sealant is very durable, and can withstand the abrasion from daily tooth brushing [13].

The aims of this study were to evaluate the effect of three topical agents; SeLECT-Defense™, organo-selenium dental sealants, Clinpro fissure sealant (3M ESPE dental products-USA) and White Varnish with TCP (Tri-Calcium Phosphate-3M ESPE-USA) in reducing enamel demineralization around orthodontic brackets and to compare with a control group.

## Materials and Methods

Twenty eight orthodontic patients, aged 13-16 years (mean: 14.06 +/- 1.73 years), scheduled to have four first premolar teeth extracted for orthodontic reasons were invited to participate in the study. The inclusion criteria were: normal salivary flow rate (>1.0 mL/min) and buffer capacity (pH: 6.8-7.5), good general and oral health with no active caries lesions or periodontal treatment needs, ability to comply with the experimental protocol and not using fixed or removable orthodontic devices.

This study was organized as a parallel group design with three groups receiving the experimental protocols and a control one.

The patients were selected and divided into four groups; each group was consisting of 7 patients. Block randomization was used in each group. For group standardization, before starting the procedure, all the patients' teeth were evaluated clinically and radio-graphically to determine the baseline caries risk. Eleven male participants (39.29%) and 17 females (60.71%) were randomly distributed among the groups. This study was approved by the Research Ethical Committee of Faculty of Dentistry, Mansoura University, Egypt. Signed consents (from parents or legal gradients) were obtained for each patient.

The salivary flow rate and buffer capacity were recorded. Before collecting saliva for the different proposed tests, the patients were asked not to eat or drink for at least an hour. Unstimulated saliva was collected in a sterilized clear container and the flow rate calculated through 5 min time. Buffering capacity of saliva was calculated using the CRT Buffer Test [CRT® Buffer Test (Vivadent Ets., Lichtenstein, Australia) using colorimetric test strips. Each strip was wetted with saliva using a pipette and the resulting color change determined according to the manufacturer's instructions. High, medium and low salivary buffer capacities are indicated by blue, green and yellow test fields, respectively.

Twenty-eight brackets (Dyna-Lok series, 100-gauge mesh, 3M Unitek) were bonded for each group (14 maxillary and 14 mandibular first premolars). Brackets were applied after surface preparation with a 37% phosphoric acid gel (3M Dental Products; St Paul, Minnesota, USA), liquid primer of the Transbond XT (3M Unitek; Monrovia, California, USA) was applied to the etched surface and the stainless-steel orthodontic premolar brackets were bonded to teeth with Transbond XT (3M Unitek). Any excess resin was removed with an explorer before the resin was polymerized.

Patients were divided into four groups (n=7 patients): three experimental and one control received no treatment. SeLECT-Defense™, organo-selenium dental sealants, Clinpro fissure sealant (3M ESPE dental products-USA) and White Varnish with TCP (Tri-Calcium Phosphate-3M ESPE-USA) were applied to the tooth surfaces of patients in the experimental groups. Agents formed were left undisturbed for five minutes on the enamel surfaces. No agent was applied to the tooth surfaces of the control group. During the experimental period and three weeks prior, all subjects brushed their teeth with a non-fluoridated dentifrice. They received no specific instructions regarding oral hygiene, kept their usual habits, and were instructed not to use any antibacterial substance

The 28 patients were treated on the same time by the same operator (Hammad.S). After one month, two premolars of each patient of the four groups; one upper and one lower (14 premolars from each group; 7 upper and 7 lower premolars) were extracted. The teeth were stored in a refrigerator in flasks containing gauze dampened with saline. After two months, the other 14 premolars from each group were extracted and treated as previously mentioned. Demineralization in enamel around the brackets was evaluated by a cross-sectional microhardness method. The evaluation of the microhardness was blindly done by the two evaluators. The roots were removed 2 mm apical to the cemento-enamel junction, and the crowns were hemi-sectioned vertically into mesial and distal halves with a large wafering blade on a low-speed saw (Buehler, Lake Bluff Illinois, USA) directly through the slot of the bracket leaving a gingival and incisal portion. The teeth were embedded in self-cure epoxy resin (Buehler), leaving the cut face exposed. The half-crown sections were polished with three grades of abrasive paper discs (320, 600, and 1200 grit). Final polishing was undertaken with a 1 µm diamond-spray and a polishing-cloth disc (Buehler). A Shimadzu (Kyoto, Japan) HMV-700 microhardness tester under a 2 N load was used for the microhardness analysis.

An indentation was made in each half crown, from two positions and one depth. On the buccal surface, from the occlusal and cervical region, indentation was made at the edges (0 μm) of bracket. At these positions, indentations were made at a depth of 10 μm from the external surface of the enamel. The values of micro-hardness numbers found in the two half-crowns were averaged.

### Statistical Analysis

Data analyses were performed using the Statistical Package for Social Sciences (SPSS, Version 13.0, SPSS Inc; Chicago, Illinois, USA). The Shapiro-Wilk normality test and the Levene's variance homogeneity test were applied to the microhardness data. The data showed normal distribution, and there was homogeneity of variances between the groups.

The teeth within individual patients were used in the analysis. The tooth surface is not an independent unit for statistical purposes because it is subject to similar conditions as the surrounding teeth. However, a multilevel design statistical test [15] showed the same results with a non-clustering comparison. Therefore, (ANOVA) was used to evaluate the effects of all topical agents on enamel surface. For multiple comparisons, the Bonferroni post hoc test was used. The statistical significance level was set at  $p < 0.05$ .

To evaluate the intra- and inter observer agreements, microhardness measurements were under taken by the investigators using the same instrument at two separate times and Cohen's Kappa scores were determined.

### Results

The intra- and inter-examiner Kappa scores for assessment of microhardness were high with all values exceeding 0.80, 0.82 and 0.79 which implies substantial agreement between the observers (Table 1).

Descriptive statistics and comparisons of microhardness between the occlusal and cervical regions of the four groups in the times testing are shown in Table 2. Comparisons of occlusal and cervical microhardness for all specimens showed no statistically significant side differences ( $p > 0.05$ ) in both one month and two months' time. There was no significant

Observation type	Kappa score (K)
Intra-examiner agreement (Examiner 1)	0.80
Intra-examiner agreement (Examiner 2)	0.82
Inter-examiner agreement	0.79

K < 0.40 poor agreement  
 K = 0.41-0.60 moderate agreement  
 K = 0.61-0.80 substantial agreement  
 K > 0.80 almost perfect agreement

Table 1. Intra- and inter-examiner agreement in measurement of microhardness scores quantified by Cohen's Kappa.

Group	One month		T- test	P- value	Two months		T- test	P- value	P- value between occlusal s.	P- Value between cervical s.
	Occlusal	cervical			Occlusal	cervical				
1. SeLECT	289.85±11.20	289.14±10.22	0.418	0.950	283.42±10.53	287.42±10.53	0.47	0.81	0.59	0.94
2. White V.	285.71±9.82	284.85±10.18	0.160	0.950	285.85±10.22	283.42±10.01	0.26	0.96	0.49	0.92
3. Clinpro S.	285.28±12.39	283.57±11.60	0.267	0.267	284.12±12.00	282.22±11.46	0.31	0.83	0.95	0.93
4. (Control)	206.14±12.36	203.57±12.69	0.384	0.384	204.42±12.05	202.11±12.66	0.37	0.87	0.95	0.99

Table 2. Descriptive statistics and comparison of the occlusal and cervical microhardness values of the four groups after one month and two months of topical agent's application.

Group	Microhardness
	Mean ±SD
1 SeLECT	288.64±10.43 <sup>a</sup>
2 White V.	284.14±9.75b <sup>b</sup>
3 Clinpro S	283.00±11.56 <sup>c</sup>
4 Control	203.21±11.94 <sup>abc</sup>
ANOVA	89.01
P-Value	0.001*

Table 3: Multiple comparisons among the four groups (ANOVA test)

difference between the microhardness values of either the occlusal or the cervical halves in the two times testing. Therefore, occlusal and cervical microhardness scores for each specimen were pooled and the microhardness scores for each group were obtained by calculating the mean of occlusal and cervical microhardness scores of the "one" months scores. The results of multi-comparison statistical tests for microhardness among the four groups are shown in Table 3. ANOVA showed statistically significant differences among the investigated groups ( $p < 0.001$ ). According to multiple comparisons, statistically significant differences were determined between the SeLECT-Defense™; organo-selenium dental sealants and the control ( $p < 0.001$ ), the Clinpro fissure sealant and the control ( $p < 0.001$ ), and the White Varnish with TCP and the control group. However, no statistically significant differences were detected between the experimental groups ( $p < 0.05$ ).

### Discussion

The results of this study showed that the application of SeLECT-Defense™; organo-selenium dental sealants, Clinpro fissure sealant and White Varnish with TCP topical agents to tooth surfaces around orthodontic brackets were able to prevent demineralization of enamel, thereby suggesting its usefulness in the prevention of white spot lesions. To the best of our knowledge, this study is the first comparison of these topical agents against enamel demineralization around orthodontic brackets in an in vivo condition by microhardness testing.

The present in vivo study evaluated the effect of three different preventive topical agents on enamel demineralization around orthodontic brackets. Mineral loss was assessed by in vitro cross-sectional microhardness, a recognized analytical method. Cross-sectional microhardness was used to evaluate demineralization/caries because of the strong correlation ( $r = 0.91$ ) between enamel microhardness scores and the percentage of mineral loss in caries lesions [16]. Previously, the cariostatic effect of fluoride releasing materials were investigated by using a split-mouth design [17,18]. However, in the present study, the subjects in the experimental groups were randomly divided into three equal groups each received only 1, because the baseline clinical, radiological, salivary and laser fluorescence examinations showed that the patients were equivalent in

regards to caries risk or demineralization activity. As suggested by Pascotto, et al. [19] the current experimental design was chosen instead of the split-mouth technique to avoid the carry-across effect due to fluoride, calcium and phosphate or antibacterial release by the agents on enamel around the brackets. Pascotto, et al. [19] observed reduced enamel hardness in the cervical region of the bracket compared with that in the occlusal area. In vivo, the explanation for this observation is greater dental plaque accumulation and difficulty in cleaning the area. In vitro, the explanation would be the less mineralization and the higher carbonate on the cervical surface than in the occlusal region [19]. Interestingly in the present study, in contrast to previous findings [16,19-21], similar mineral loss was observed at the cervical and occlusal region at 0.1 m positions<sup>13</sup>. Statistically significant microhardness differences were determined between tested materials and the control at the same position. The control group showed less hardness values that indicated more mineral loss than the tested materials that result in accordance with Uysal, et al. [13].

The results of the study comes in accordance with other studies revealed that fluoride varnish containing tri-calcium phosphate inhibit progression of initial primary dental lesions [16]. Also, it was concluded that remineralization agents containing different Ca-Ph formulas and fluoride have increased remineralization potential [17].

Regarding Clinpro fissure sealant results, fluoride release property of the material could stand behind the increased microhardnes values of the enamel treated by the sealant. It has been suggested that fluoride release from sealants may help remineralizing incipient enamel caries and providing a fluoride-rich layer that should be more caries resistant. Although, trapping bacteria beneath the sealants is inevitable, and inadvertent sealing of initial carious lesions may occur. Neither of these processes increases the chance of caries developing or caries growing beneath the surface. The ability of bacteria to survive under sealant is considerably impaired because ingested carbohydrates cannot reach them. Several investigators have found that the number of bacteria in sealed carious lesions decreases dramatically with time [18,19]. Also, several previous studies indicated an antimicrobial effect for fluoride-containing dental sealants against oral bacteria [20,21].

Also, organo- selenium polymerized into dental sealant is effective in inhibiting bacterial attachment and biofilm formation by the two main oral pathogens, *S. mutans* and *S. salivarius*. In addition, the organo- selenium dental sealant is very durable in that it is still completely effective in inhibiting *S. mutans* attachment after 2 months of soaking in aqueous solution. Therefore, the use of organo-selenium dental sealants has the potential to prevent dental caries and plaque formation by oral pathogens. In addition, bacteria cannot live under the dental sealant, while they can live under a control sealant [21].

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