Scanning Electron Microscopy Evaluation of the Protective Effects of Fluoridated Dental Materials on Enamel Surface Morphology under Artificial Demineralization

İdris Onur YALÇIN*, İbrahim Erhan GELGÖR

Department of Orthodontics, Faculty of Dentistry, Uşak University, Cumhuriyet Mahallesi, Kolaj Sokak No:3, 64200 Uşak Merkez, Türkiye

http://doi.org/10.5755/j02.ms.45785

Received 10 August 2026; accepted 22 October 2026

The primary aim of this in vitro study was to compare enamel surface morphology following standardized artificial demineralization among five protective dental materials and an untreated control. Specifically, scanning electron microscopy (SEM) was used to characterize acid-exposed and wax-protected enamel regions and to compare five-point ordinal surface-preservation scores across the six experimental groups. Fifty-four human premolars extracted for orthodontic reasons provided 108 enamel surfaces. A 2 × 3 mm window was prepared on each surface, and specimens were randomly assigned to six groups (n = 18): control, APF gel containing 1.23 % fluoride ions, Enamel Pro Varnish containing 5 % sodium fluoride (NaF), Enamel Pro Prophy Paste with ACP, fluoride-releasing light-cured Pro Seal, and Duraphat containing 5 % sodium fluoride (NaF). Specimens were stored in artificial saliva and exposed to an artificial caries solution at pH 4.4 twice daily for 1 h over 30 days. After cycling, the surfaces were examined by SEM at ×500 and ×1500 magnifications. Images were morphologically classified and rated using a five-point ordinal surface-preservation scale, in which lower scores indicated better preservation. Data were analysed using the Kruskal–Wallis test followed by Holm-adjusted Dunn comparisons. Mean ordinal SEM scores were 5.00, 3.72, 2.06, 3.06, 3.50, and 3.56 for Groups A–F, respectively, with an overall group difference ($H(5) = 68.32$, $p < 0.001$). Enamel Pro Varnish had the lowest mean ordinal SEM score (2.06), although its difference from Group D was not statistically significant after Holm adjustment ($p = 0.115$). Although all treated groups had more favourable ordinal SEM scores than the control, none reproduced the SEM appearance of untreated sound enamel.

Keywords: fluoride, enamel demineralization, SEM, white spot lesion, dental material.

1. INTRODUCTION

Fixed orthodontic appliances such as brackets, bands, archwires, ligatures and auxiliary attachments increase plaque retention, complicate oral hygiene and elevate the risk of enamel demineralization [1]. These early carious lesions appear as matte, opaque white areas and are associated with subsurface mineral loss, increased porosity and disruption of the enamel prism structure [2]. White spot lesions are a significant manifestation of enamel demineralization during fixed orthodontic treatment [3].

Because enamel is composed predominantly of hydroxyapatite crystals, acidic conditions promote calcium and phosphate loss, resulting in surface dissolution and roughening [1,3]. Although mechanical plaque control remains the principal preventive approach, limited patient compliance may necessitate adjunctive protective materials [4]. Topical fluoride-containing materials can reduce enamel solubility, promote acid-resistant fluoridated mineral phases and contribute to remineralisation of early demineralised areas [5].

Fluoride varnishes, topical fluoride applications, fluoride-releasing resin-based sealants and calcium-phosphate-supported preparations are among the main approaches evaluated for preventing white spot lesions during fixed orthodontic treatment [1, 4, 6]. Their effects depend on material form, contact time, mineral supplementation, barrier effect and persistence on the enamel surface [4, 6, 7]. Scanning electron microscopy permits high-magnification examination of enamel surface roughening, porosity and morphology under demineralising conditions [1].

Clinical studies have investigated the preventive effects of topical fluoride preparations during fixed orthodontic treatment. A systematic review reported that professional fluoride varnish application may reduce the occurrence of white spot lesions, although the overall certainty of the available evidence was very low [8]. Furthermore, a randomized clinical trial comparing a calcium phosphate-containing fluoride varnish with a resin-based enamel sealant found no statistically significant difference in white spot lesion development between the two interventions [6]. These findings emphasize the clinical relevance of protective dental materials while indicating that their relative effectiveness may depend on material properties and experimental or clinical conditions.

Beyond clinical outcomes, experimental investigations have examined enamel responses to protective materials using complementary analytical techniques. Abufarwa et al. evaluated fluoride-containing varnish formulations using surface microhardness testing and SEM, demonstrating the value of assessing both mechanical properties and surface morphology [9]. Similarly, Ding et al. investigated enamel demineralization using SEM in combination with

* Corresponding author: İ.O. Yalçin
E-mail: *idris.yalcin@usak.edu.tr*

fluorescence-based, elemental, and microhardness analyses [1]. Although these techniques provide complementary information, SEM enables direct characterization of surface architecture, enamel prism-related alterations, and morphological changes following acidic challenges.

Although the protective effects of fluoride-containing dental materials have been extensively investigated, comparing their performance remains scientifically relevant because different formulations may interact with enamel surfaces through distinct mechanisms. Fluoride gels, varnishes, calcium-phosphate-containing preparations, and resin-based sealants differ in their modes of application, physical characteristics, and potential persistence on enamel. Previous experimental investigations have employed different treatment protocols and outcome measures, including surface microhardness and SEM-based morphological assessment [9]. Consequently, differences observed across individual studies cannot necessarily be attributed to material composition alone. A standardized experimental comparison is therefore valuable for determining whether different protective formulations exhibit distinct enamel surface morphologies when exposed to the same acidic challenge. The present investigation addresses this question at the level of surface morphology, without assuming that morphological preservation is equivalent to mineral retention or clinical caries-preventive efficacy. In this context, the contribution of the present study lies in the standardized morphological comparison of five protective dental materials representing different formulations, including an APF gel, two fluoride varnishes, an ACP-containing prophylaxis paste, and a fluoride-releasing light-cured resin-based sealant. All experimental groups were subjected to the same 30-day artificial demineralization protocol, allowing SEM-based comparisons of acid-exposed and protected enamel regions. In addition to descriptive morphological classification, a five-point ordinal surface-preservation scale was used to provide semi-quantitative comparisons between the groups. This approach was intended to identify differences in enamel surface appearance under controlled conditions rather than to establish direct measurements of mineral loss or clinical caries-preventive efficacy.

The aim of the present in vitro study was to comparatively evaluate the effects of five dental protective materials on human enamel surface morphology following repeated artificial demineralization using scanning electron microscopy. Specifically, the study sought to characterize material-associated differences in enamel surface patterns and to compare semi-quantitative ordinal surface-preservation scores under standardized experimental conditions.

2. EXPERIMENTAL DETAILS

The sample size for this in vitro study was calculated using the G*Power programme based on data from similar demineralization and fluoride varnish application studies. Kooshki et al. evaluated the effects of conventional fluoride varnish and various varnish-containing materials on enamel surface microhardness of extracted human premolars following artificial demineralization [10]. Using the post-treatment microhardness values from that study, Cohen's f effect size for one-way analysis of variance was calculated as 0.424. With test family "F tests", statistical test "ANOVA: fixed effects, omnibus, one-way", $\alpha = 0.05$, power = 0.90 and number of groups = 6, the required total sample size was estimated at approximately 98 enamel surfaces [11]. To ensure balanced distribution among six groups and to allow for potential sample loss during the experimental procedures, a total of 108 enamel surfaces were planned for use in the present study.

The study protocol was reviewed and approved by the Uşak University Non-Interventional Clinical Research Ethics Committee (decision no. 1301-1301-12, dated 25 June 2026). The human premolar teeth used in this investigation had been extracted for orthodontic indications rather than for research purposes.

Accordingly, fifty-four human premolar teeth extracted for orthodontic reasons and stored in 0.1 % thymol solution were used. Two enamel surfaces were prepared from each tooth, yielding a total of 108 enamel surfaces for experimental evaluation. Prepared surfaces were coated with an insulating wax layer and a 2 × 3 mm window was opened near the cervical region of each specimen. The tooth surfaces were randomly allocated into six groups, each containing 18 enamel surfaces.

Group A served as the control and received no protective preparation. Surfaces in Group B were treated with APF gel containing 1.23 % fluoride ions (Enamel Pro Gel, Premier, North Carolina, USA). Surfaces in Group C were treated with Enamel Pro Varnish, an ACP-containing fluoride varnish formulated with 5% sodium fluoride (NaF) (Premier Dental Products Co., Plymouth Meeting, PA, USA). Group D specimens received Enamel Pro Prophy Paste with ACP, an ACP-containing professional prophylaxis paste (Premier Dental Products Co., Plymouth Meeting, PA, USA). Group E specimens received the fluoride-releasing light-cured Pro Seal sealant (West Thorndale Ave, Itasca, USA). Group F specimens were treated with Duraphat varnish containing 5 % sodium fluoride (NaF) (Colgate, Wiirngen, Germany).

All specimens were stored in artificial saliva. The artificial saliva solution was prepared to contain 20 mmol/L $NaHCO_3$, 3 mmol/L NaH_2PO_4 and 1 mmol/L $CaCl_2$. To simulate demineralization, specimens were subjected to an artificial caries solution containing 2.2 mmol/L Ca^{2+}, 2.2 mmol/L PO_4^{3-} and 50 mmol/L acetic acid, adjusted to pH 4.4. Specimens were immersed in this solution twice daily for 1 hour each time over a 30-day period. After each demineralization period, specimens were returned to artificial saliva. Following completion of the 30-day cycle, the insulating material and protective preparations were removed from the tooth surfaces.

Morphological evaluation of enamel surfaces was performed using a scanning electron microscope (JSM-5600, JEOL Ltd., Tokyo, Japan) operated at an accelerating voltage of 20 kV. For each specimen, SEM images were obtained from the centre of the 2 × 3 mm acid-exposed window and the wax-protected enamel region at magnifications of ×500 and ×1500, corresponding to scale bars of 50 µm and 10 µm, respectively.

SEM was selected as the primary analytical method because the study was specifically designed to compare enamel surface morphology following repeated acidic

challenges, rather than to quantify mineral content or mechanical properties. Examination of both the acid-exposed window and the wax-protected enamel region at two magnifications enabled a direct within-specimen morphological comparison, particularly regarding enamel prism-related features, surface irregularities, and preservation of surface architecture. The SEM-derived ordinal scores were therefore interpreted as semi-quantitative assessments of surface morphology rather than direct measurements of mineral loss, surface roughness, or microhardness.

The SEM images were interpreted using two distinct approaches: descriptive morphological pattern classification and ordinal surface-preservation scoring. First, enamel surface architecture was characterized by reference to the etching patterns originally described by Silverstone et al. [12] and subsequently extended by Galil and Wright [13]. The distinguishing morphological characteristics of Types 1–5 are summarized in Table 3. Because these categories were originally established through acid-etching investigations, their application to the present repeated demineralization model was descriptive, and observed similarities were interpreted as Type-like appearances rather than evidence of identical dissolution mechanisms. The morphological categories were not used as numerical severity rankings.

Second, overall morphological preservation of each specimen was assessed using a separate five-point ordinal SEM scale based on surface regularity and smoothness relative to the non-acid-exposed enamel appearance. The rating categories are provided in Table 1, with lower scores indicating more favourable surface preservation. The mean of the ordinal ratings obtained for each specimen was recorded as its final SEM surface-preservation score. This ordinal scale was evaluated independently of the descriptive Type 1–5 etching-pattern classification.

For descriptive analysis, the mean, standard deviation, and median of the ordinal SEM surface-preservation scores were summarized for each experimental group. The number and percentage of specimens receiving favourable preservation scores (1 or 2) were also calculated. These percentages represent the distribution of ordinal SEM ratings and should not be interpreted as measurements of preserved enamel surface area or mineral loss.

Descriptive statistics were calculated for each experimental group. Because the outcome was a five-level ordinal SEM surface-preservation score, intergroup differences were evaluated using the Kruskal–Wallis test. When the overall comparison was significant, pairwise differences were assessed using Dunn's rank-based test with Holm correction for 15 multiple comparisons; ties in ordinal scores were accounted for. The Kruskal–Wallis epsilon-squared effect size was estimated as $(H - k + 1)/(N - k)$, where $k = 6$ groups and $N = 108$ surface-level observations. All tests were two-sided, and adjusted $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Evaluation of SEM scores

Descriptive statistics and the distribution of all ordinal SEM surface-preservation ratings are presented in Table 1. All 18 control surfaces were assigned a score of 5. Mean ordinal SEM scores were 3.72 for Group B, 2.06 for Group C, 3.06 for Group D, 3.50 for Group E, and 3.56 for Group F. Lower ordinal scores indicated more favourable surface preservation; the lowest mean was observed in Group C and the highest in the control group.

The distribution of ordinal SEM surface-preservation scores provided an additional numerical description of the intergroup differences. In Group C, 12 of 18 specimens (66.7 %) received scores of 1 or 2, indicating very good or good morphological preservation. The corresponding proportion in Group D was 6 of 18 specimens (33.3 %). No specimens in Groups A, B, E, or F received scores of 1 or 2. All 18 control specimens (100 %) received a score of 5, whereas all specimens in Group C received scores between 1 and 3. These findings indicate that the more favourable mean score observed in Group C was also reflected in the distribution of individual ordinal ratings.

Table 1. Distribution and descriptive statistics of ordinal SEM scores

Group	Scores 1/2/3/4/5, n	Mean ± SD	Median	Scores 1–2, n, %
A	0/0/0/0/18	5.00 ± 0.00	5	0 (0.0)
B	0/0/8/7/3	3.72 ± 0.75	4	0 (0.0)
C	5/7/6/0/0	2.06 ± 0.80	2	12 (66.7)
D	0/6/5/7/0	3.06 ± 0.87	3	6 (33.3)
E	0/0/9/9/0	3.50 ± 0.51	3.5	0 (0.0)
F	0/0/8/10/0	3.56 ± 0.51	4	0 (0.0)

Note. Each group contained 18 enamel surfaces (total N = 108). A, control; B, APF gel; C, Enamel Pro Varnish; D, Enamel Pro Prophy Paste with ACP; E, Pro Seal; F, Duraphat. Score frequencies indicate the number of surfaces assigned scores 1, 2, 3, 4, and 5, respectively. Scores were defined as 1 = very good, 2 = good, 3 = moderate, 4 = poor, and 5 = very poor surface preservation. Lower scores indicate more favourable morphological preservation. The scores 1–2 column represents favourable ordinal ratings, not the percentage of preserved enamel area or mineral retention. SD, standard deviation. This ordinal scale is distinct from the Type 1–5 morphological etching-pattern classification.

The Kruskal–Wallis test showed an overall difference in ordinal SEM surface-preservation scores across the six groups ($H(5) = 68.32$, $p < 0.001$; $\varepsilon^2 = 0.621$). Holm-adjusted Dunn comparisons are reported in Table 2. The control group differed significantly from all treated groups (adjusted $p \leq 0.002$). In post-hoc comparisons, Group C differed significantly from Groups B, E, and F (Holm-adjusted $p \leq 0.003$), but not from Group D (adjusted $p = 0.115$). Groups B and D did not differ significantly after correction (adjusted $p = 0.400$). No significant differences were detected among Groups B, E, and F or between Group D and Groups E or F (Table 2). Group C nevertheless had the lowest descriptive mean score.

3.2. Evaluation of SEM images

SEM examination revealed heterogeneous surface changes following artificial demineralization, with differences in the appearance and distribution of irregularities among the experimental groups. In the untreated control group, acid-exposed enamel exhibited pronounced nonuniform roughening and irregular surface-associated deposits (Fig. 1).

Group B showed a heterogeneous surface with porous and granular features (Fig. 2).

Table 2. Holm-adjusted dunn pairwise comparisons of ordinal SEM surface-preservation scores

Groups	Mean score difference	Adjusted *p*
A–B	1.28	0.002
A–C	2.94	<0.001
A–D	1.94	<0.001
A–E	1.50	<0.001
A–F	1.44	<0.001
B–C	1.67	<0.001
B–D	0.67	0.400
B–E	0.22	1.000
B–F	0.17	1.000
C–D	−1.00	0.115
C–E	−1.44	0.003
C–F	−1.50	0.002
D–E	−0.44	0.929
D–F	−0.50	0.829
E–F	−0.06	1.000

Note. Adjusted p values were calculated using Dunn's rank-based post-hoc test with Holm correction for 15 pairwise comparisons (two-sided). Mean score differences are descriptive values calculated as the first-listed group minus the second-listed group, whereas statistical significance was determined from ranks. Negative differences indicate lower mean ordinal scores in the first-listed group. Adjusted $p < 0.05$ was considered statistically significant. Group definitions are provided in Table 1.

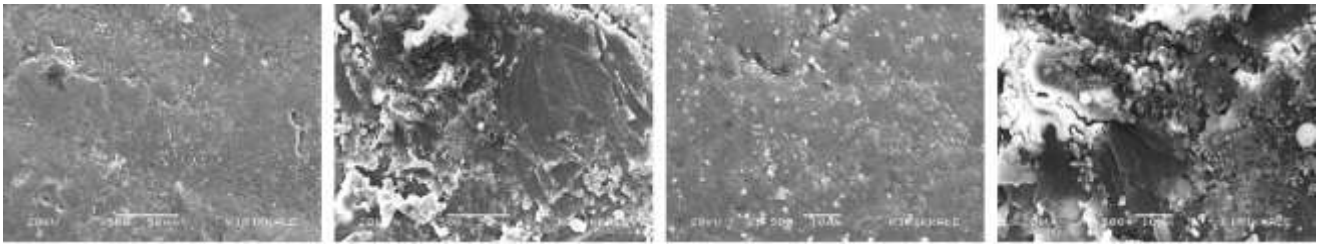
Group C demonstrated a roughened, granular morphology with localized areas of comparatively smoother appearance (Fig. 3). Groups D, E, and F exhibited variable degrees of surface irregularity, pitting, and surface-associated deposits (Fig. 4 – Fig. 6).

These observations showed morphological similarities to the irregular Type 3 and Type 4 etching patterns described in the established classification system, while localized smoother regions in Group C were considered Type 5-like. However, the characteristic preferential dissolution of prism cores or peripheries required for identifying classical Type 1 and Type 2 patterns could not be clearly distinguished in the selected representative micrographs. Therefore, the etching-pattern categories were used as descriptive morphological references rather than definitive identification of identical dissolution mechanisms.

Table 3. Enamel etching-pattern classification and corresponding SEM findings

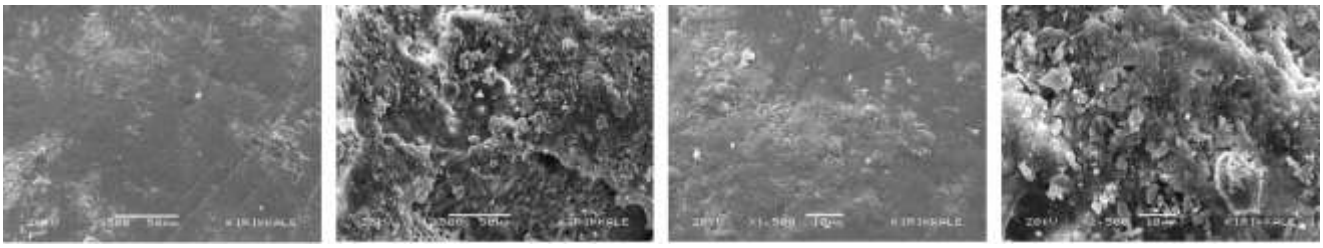
Type	SEM morphology	Present SEM figures
1	Preferential dissolution of prism cores; honeycomb-like appearance	Not distinctly identified
2	Preferential dissolution of prism peripheries; relatively intact prism cores	Not distinctly identified
3	Mixed Type 1 and Type 2 prism-dissolution features	Type 3-like irregularities: Fig. 1, 2, 4, 5, 6
4	Irregular pitted or porous enamel without a well-defined prism pattern	Type 4-like features: Fig. 2 – Fig. 6
5	Relatively smooth enamel without a recognizable etching pattern	Localized Type 5-like areas: Fig. 3

Note. Types 1–3 were originally described by Silverstone et al. [12]. The five-type classification and corresponding reference SEM micrographs (Fig. 1 – Fig. 5) were presented by Galil and Wright [13]. References to figures from the present study indicate descriptive Type-like appearances rather than definitive identification of all five etching patterns. This morphological classification is independent of the ordinal surface-preservation scores reported in Table 1.



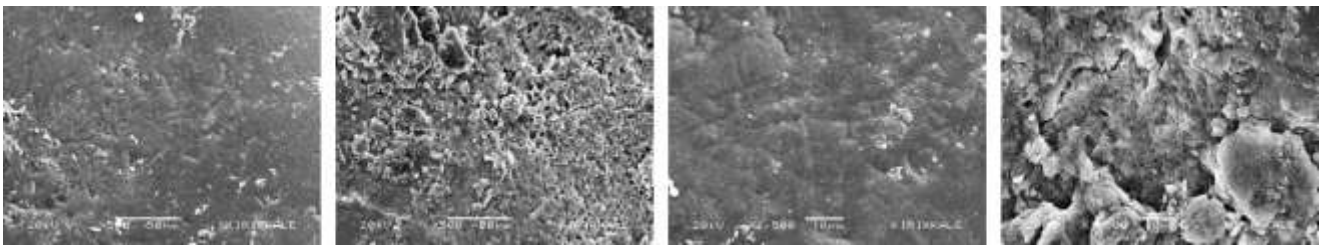
a b c d

Fig. 1. SEM images of the control group (Group A): a, c – non-acid-exposed enamel surfaces; b, d – acid-exposed enamel surfaces. Acid-exposed panels show heterogeneous roughening described as Type 3-like



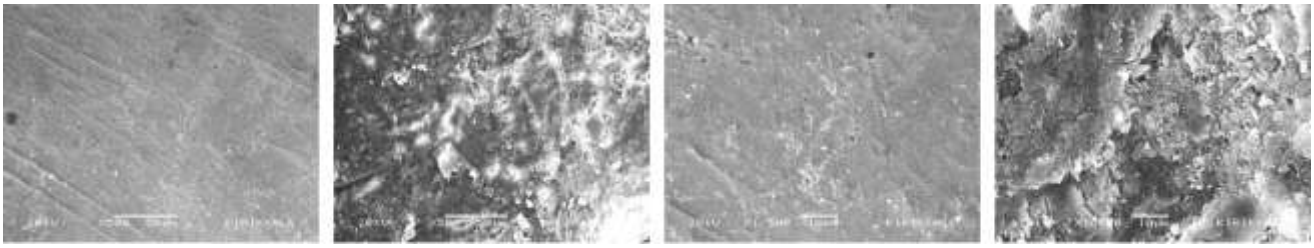
a b c d

Fig. 2. SEM images of the APF gel group (Group B): a, c – non-acid-exposed enamel surfaces; b, d – acid-exposed enamel surfaces. Acid-exposed panels illustrate mixed irregular surface features described as Type 3/4-like



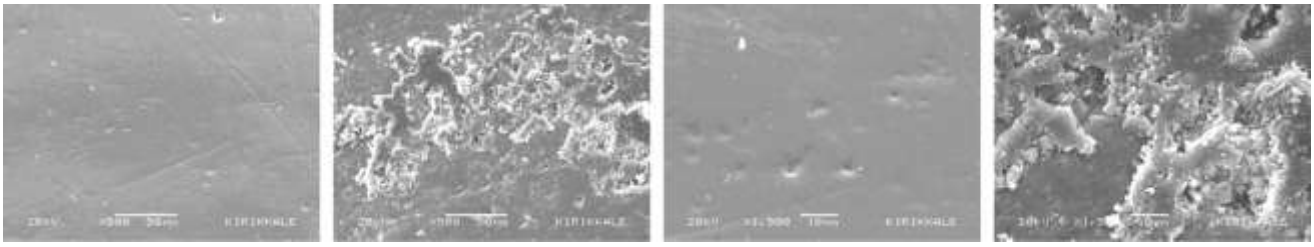
a b c d

Fig. 3. SEM images of Enamel Pro Varnish (Group C): a, c – wax-protected enamel; b, d – acid-exposed enamel. Acid-exposed surfaces display granular irregularities and localized smoother, Type 5-like regions; this appearance alone does not establish mineral recovery



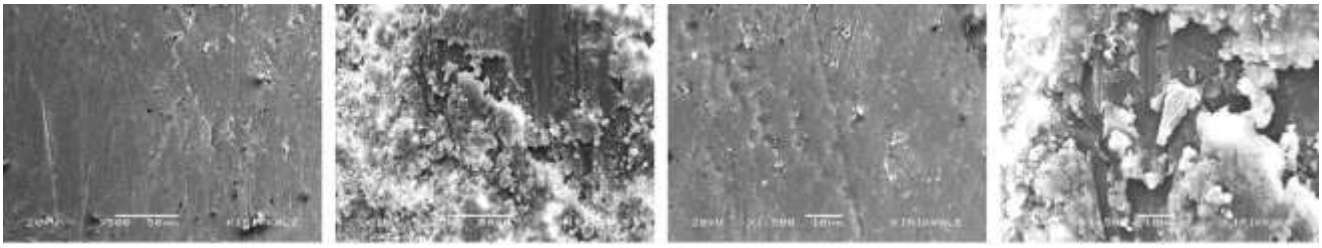
a b c d

Fig. 4. SEM images of the Enamel Pro Prophy Paste with ACP group (Group D): a, c – non-acid-exposed enamel surfaces; b, d – acid-exposed enamel surfaces. Acid-exposed panels illustrate heterogeneous roughening described as Type 3/4-like



a b c d

Fig. 5. SEM images of the Pro Seal group (Group E): a, c – non-acid-exposed enamel surfaces; b, d – acid-exposed enamel surfaces. Acid-exposed panels show heterogeneous roughening described as Type 3/4-like



a b c d

Fig. 6. SEM images of the Duraphat group (Group F): a, c – non-acid-exposed enamel surfaces; b, d – acid-exposed enamel surfaces. Acid-exposed panels illustrate heterogeneous and irregular roughening described as Type 3/4-like

When all groups were considered together, the control group had the highest ordinal SEM score, whereas Group C had the lowest ordinal SEM score. Because lower ordinal scores indicated better surface preservation, Group C showed the lowest descriptive mean score, although the difference between Groups C and D was not statistically significant after adjustment for multiple comparisons.

Nevertheless, no experimental group completely reproduced the SEM appearance of untreated enamel that had not been exposed to the artificial caries solution.

4. DISCUSSION

In the present study, all five treated groups exhibited more favourable ordinal SEM surface-preservation scores than the untreated control following a standardized 30-day artificial demineralization protocol. Enamel Pro Varnish (Group C) demonstrated the lowest mean score, although its difference from the ACP-containing prophylaxis paste group (Group D) was not statistically significant after correction for multiple comparisons. None of the treatments reproduced the morphological appearance of unchallenged enamel. These findings suggest formulation-associated

differences in surface morphology while highlighting the distinction between favourable SEM appearances and directly measured mineral preservation.

Retentive areas created by attachments during fixed orthodontic treatment increase biofilm accumulation and exposure to acidic attacks around brackets and in cervical enamel regions. Professional preventive applications have been reported to limit the development of white spot lesions and caries during fixed orthodontic treatment [14]. The significant difference between the control group and all experimental groups in the present study indicates that enamel surfaces without protective material exhibited more pronounced morphological changes following artificial demineralization. Similarly, in vitro studies that examined protective materials around orthodontic brackets by SEM, microhardness and elemental analysis have reported better enamel protection on material-treated surfaces compared with control surfaces [15].

The favourable SEM appearance in Group C may relate to prolonged enamel contact and fluoride release from the varnish formulation. Fluoride varnishes have been reported to exert protective or remineralising effects on orthodontic and post-orthodontic white spot lesions [14, 16]. A particularly informative finding was the difference between Enamel Pro Varnish (Group C) and Duraphat (Group F), both formulated with a nominal sodium fluoride (NaF) concentration of 5 %. Despite this similarity, the mean ordinal SEM scores were 2.06 and 3.56, respectively, representing a difference of 1.50 score points. This comparison suggests that the observed enamel surface morphology may be influenced by differences in material formulation and behaviour under acidic conditions, rather than nominal fluoride concentration alone. However, because fluoride release, surface retention, and chemical composition were not directly quantified, the underlying mechanisms cannot be established from the present data. This formulation-specific morphological comparison adds useful comparative evidence without implying that one material necessarily produces greater mineral retention or clinical caries prevention.

Likewise, the efficacy of fluoride applications versus biomimetic remineralising approaches may vary according to material composition and application protocol [16, 17].

Group B, treated with APF gel, showed lower ordinal SEM scores than the control group but higher scores than Group C. APF-based fluoride applications have been reported to contribute to prevention of white spot lesions during fixed orthodontic treatment [18].

Group D, treated with an ACP-containing prophylaxis paste, demonstrated a mean ordinal SEM score of 3.06, compared with 3.72 in the APF gel group and 2.06 in the Enamel Pro Varnish group. Calcium-phosphate-containing preparations have been investigated as adjunctive approaches for the management of enamel white spot lesions [19]. Previous studies have also examined biomimetic systems for improving mineral recovery in early enamel lesions [20]. Importantly, both Enamel Pro Varnish (Group C) and Enamel Pro Prophy Paste (Group D) incorporate ACP technology, but differ in their physical formulation and intended mode of application. Consequently, the observed morphological differences between these groups cannot be attributed to the presence or absence of ACP alone. Although Group C had a lower mean ordinal score than Group D, this difference was not statistically significant after Holm adjustment (adjusted $p = 0.115$). Differences in material retention, surface interaction, and formulation-related behaviour may contribute to the observed appearances; however, these properties were not independently measured in the present investigation.

Group E treated with Pro Seal demonstrated lower ordinal SEM scores than the control group. Resin-based surface protectants can form a physical barrier that isolates the enamel surface from acidic challenge. Sealants used around orthodontic brackets have been reported to protect enamel from demineralization in vitro [21]. Enamel surface sealants with antimicrobial properties have also been shown to support prevention of white spot lesions [22]. The efficacy of such materials depends on factors including surface adaptation, polymerisation, marginal integrity and mechanical durability. Brushing simulation studies have demonstrated that the persistence of orthodontic sealants on the surface may change following simulated toothbrushing [23].

Comparison with previous investigations requires consideration of the different outcome measures used to evaluate enamel protection. Abufarwa et al. reported an 89% reduction in surface microhardness in untreated control specimens, compared with reductions of 24 – 58 % in fluoride varnish-treated groups under simulated oral conditions [9]. Vicente et al. combined SEM with elemental analysis and detected fluoride on 65 % of the enamel surfaces in one experimental group, demonstrating the additional compositional information obtainable by energy-dispersive X-ray spectroscopy [24]. In a systematic review, Sonesson and Twetman reported a pooled risk ratio of 0.64 (95 % confidence interval: 0.42 – 0.98) for white spot lesion development following fluoride varnish application, although the certainty of evidence was very low [8]. These findings provide quantitative context but cannot be directly equated with the ordinal SEM scores in the present investigation, because microhardness changes, elemental detection, clinical lesion occurrence, and morphological ratings represent different outcomes.

Previous SEM-based investigations also help define the distinct contribution of the present study. Nelson et al. characterized surface coatings and morphological alterations associated with topical fluoride agents [25]. Brar et al. investigated changes in enamel surface topography following the application of different topical fluoride preparations during prolonged storage in artificial saliva [26]. Flynn et al. examined protective dental materials using clinical white spot lesion outcomes, representing a different level of assessment from laboratory-based surface morphology [6]. In contrast, the present investigation compared five protective material formulations under the same 30-day artificial demineralization conditions, examining acid-exposed and wax-protected enamel regions at two magnifications. The principal contribution is the standardized comparison of material-associated morphological changes supported by ordinal score distributions, rather than the introduction of a novel microscopy technique or the identification of a new remineralization mechanism.

SEM was selected because the primary objective was to characterize enamel surface architecture, including prism-related changes, irregularities, and material-associated deposits. Nevertheless, visual observations and SEM-derived ordinal ratings cannot directly determine mineral loss, fluoride uptake, surface roughness, or mechanical resistance. Previous research has demonstrated the complementary value of surface roughness and microhardness measurements when evaluating treated enamel lesions [27].

Furthermore, residual material deposits and comparatively smooth, Type 5-like appearances should not independently be interpreted as evidence of remineralization. The in vitro model also cannot reproduce the complex effects of salivary flow, biofilm formation, dietary challenges, toothbrushing, and material wear. Therefore, the present findings should be considered comparative morphological evidence, and future studies integrating SEM with elemental analysis, quantitative profilometry, and microhardness testing are needed to establish whether the observed surface differences correspond to measurable mineral preservation or mechanical benefits.

5. CONCLUSIONS

Within the limitations of this in vitro SEM study, Group C (Enamel Pro Varnish) showed the lowest mean ordinal preservation score (2.06 ± 0.80), followed by Group D (3.06 ± 0.87), Group E (3.50 ± 0.51), Group F (3.56 ± 0.51), Group B (3.72 ± 0.75), and the untreated control Group A (5.00 ± 0.00). The overall group difference was statistically significant ($H(5) = 68.32$, $p < 0.001$; $\varepsilon^2 = 0.621$). Holm-adjusted Dunn comparisons showed significant differences between Group C and Groups B, E, and F (adjusted $p \leq 0.003$), but not between Groups C and D (adjusted $p = 0.115$). Favourable preservation scores (1 or 2) were recorded in 12 of 18 surfaces (66.7 %) in Group C and 6 of 18 surfaces (33.3 %) in Group D, whereas none of the remaining groups had scores in these categories.

All treated groups showed lower ordinal SEM scores than the untreated control, but none reproduced the SEM appearance of unchallenged enamel. The findings support formulation-associated differences in enamel surface morphology under standardized artificial demineralization conditions. However, they do not directly quantify mineral retention, mechanical properties, or clinical caries-preventive efficacy.

REFERENCES

1. **Ding, L., He, D., Zheng, S., Zhou, X., Li, H., Xi, Y., Wang, X., Sun, X.** In-Vitro and in-Vivo Comparative Studies of Treatment Effects on Enamel Demineralization during Orthodontic Therapy: Implications for Clinical Early-Intervention Strategy *Clinical Oral Investigations* 28 (10) 2024: pp. 545. https://doi.org/10.1007/s00784-024-05944-4
2. **Yagci, A., Seker, E.D., Demirsoy, K.K., Ramoglu, S.I.** Do Total or Partial Etching Procedures Effect the Rate of White Spot Lesion Formation? A Single-Center, Randomized, Controlled Clinical Trial *The Angle Orthodontist* 89 (1) 2019: pp. 16 – 24. https://doi.org/10.2319/013018-84.1
3. **Kashash, Y., Hein, S., Göstemeyer, G., Aslanalp, P., Weyland, M.I., Bartzela, T.** Resin Infiltration Versus Fluoride Varnish for Visual Improvement of White Spot Lesions during Multibracket Treatment. A Randomized-Controlled Clinical Trial *Clinical Oral Investigations* 28 (6) 2024: pp. 308. https://doi.org/10.1007/s00784-024-05695-2
4. **Sonesson, M., Brechter, A., Abdulraheem, S., Lindman, R., Twetman, S.** Fluoride Varnish for the Prevention of White Spot Lesions during Orthodontic Treatment with Fixed Appliances: A Randomized Controlled Trial *European Journal of Orthodontics* 42 (3) 2020: pp. 326 – 330. https://doi.org/10.1093/ejo/cjz045
5. **Moraes, S.M., Koshino, L.A., Carvalho, T.S., Souza, B.M., Honorio, H.M., Magalhães, A.C., Garib, D.G., Buzalaf, M.A.R.** Effectiveness of Fluoride Varnishes for White Spot Lesion Prevention and Remineralization during Orthodontic Treatment: A Randomized Controlled Trial *Caries Research* 58 (6) 2024: pp. 589 – 603. https://doi.org/10.1159/000540375
6. **Flynn, L.N., Julien, K., Noureldin, A., Buschang, P.H.** The Efficacy of Fluoride Varnish Vs a Filled Resin Sealant for Preventing White Spot Lesions during Orthodontic Treatment *The Angle Orthodontist* 92 (2) 2022: pp. 204 – 212. https://doi.org/10.2319/052521-418.1
7. **Bergamo, A.Z.N., Miguel, L.P., Delgado, R.Z.R., Palma-Dibb, R.G., Faraoni, J.J., Gatón-Hernández, P., da Silva, R.A.B., da Silva, L.A.B., Nelson-Filho, P.** Efficacy of Clinpro™ XT Varnish Surrounding Brackets on the Enamel Surface of White Spot Lesion *Brazilian Dental Journal* 34 (4) 2023: pp. 143 – 149. https://doi.org/10.1590/0103-6440202305405
8. **Sonesson, M., Twetman, S.** Prevention of White Spot Lesions with Fluoride Varnish during Orthodontic Treatment with Fixed Appliances: A Systematic Review *European Journal of Orthodontics* 45 (5) 2023: pp. 485 – 490. https://doi.org/10.1093/ejo/cjad013
9. **Abufarwa, M., Noureldin, A., Dziak, R., Covell, Jr.D.** Efficacy of Cpp-ACP Fluoride Varnish Applied with and without Acid Etching in Preventing Enamel Demineralization Compared to Light-Curable Fluoride Varnish *The Angle Orthodontist* 92 (2) 2022: pp. 213 – 219. https://doi.org/10.2319/050121-345.1
10. **Kooshki, F., Fatemi, S.M., Darvishghaderi, S., Vahedi, P.** Comparison of the Effects of Fluoride Varnish Containing Silver Nanoparticles and Conventional Fluoride Varnish on the Surface Microhardness of Tooth Enamel *Dental and Medical Problems* 61 (2) 2024: pp. 241 – 247. https://doi.org/10.17219/dmp/151702
11. **Faul, F., Erdfelder, E., Lang, A.G., Buchner, A.** G* Power 3: A Flexible Statistical Power Analysis Program for the Social, Behavioral, and Biomedical Sciences *Behavior Research Methods* 39 (2) 2007: pp. 175 – 191. https://doi.org/10.3758/BF03193146
12. **Silverstone, L., Saxton, C., Dogon, I.L., Fejerskov, O.** Variation in the Pattern of Acid Etching of Human Dental Enamel Examined by Scanning Electron Microscopy *Caries Research* 9 (5) 1975: pp. 373 – 387. https://doi.org/10.1159/000260179
13. **Galil, K.A., Wright, G.Z.** Acid Etching Patterns on Buccal Surfaces of Permanent Teeth *Pediatric Dentistry* 1 (4) 1979: pp. 230 – 234.

14. **Grocholewicz,K., Mikłasz, P., Zawiślak, A., Sobolewska, E., Janiszewska-Olszowska, J.** Fluoride Varnish, Ozone and Octenidine Reduce the Incidence of White Spot Lesions and Caries during Orthodontic Treatment: Randomized Controlled Trial *Scientific Reports* 12 (1) 2022: pp. 13985. https://doi.org/10.1038/s41598-022-18107-w
15. **Elshenawy, E.A., Alawy, S.B., Alghonemy, W.Y., El Dosoky, A.I.** Preventing White Spot Lesions around Orthodontic Brackets: Efficacy of Pre-Reacted Glass-Ionomer Barrier Coat Versus Silver Diamine Fluoride: An in Vitro Study *BDJ Open* 11 (1) 2025: pp. 45. https://doi.org/10.1038/s41405-025-00332-w
16. **Güven, E., Eden, E., Attin, R., Fırıncıoğulları, E.C.** Remineralization of Post-Orthodontic White Spot Lesions with a Fluoride Varnish and a Self-Assembling P 11− 4 Peptides: A Prospective in-Vivo-Study *Clinical Oral Investigations* 28 (8) 2024: pp. 464. https://doi.org/10.1007/s00784-024-05865-2
17. **Shaalan, O., Fawzy El-Sayed, K., Abouauf, E.** Evaluation of the Remineralization Potential of Self-Assembling Peptide P11-4 with Fluoride Compared to Fluoride Varnish in the Management of Incipient Carious Lesions: A Randomized Controlled Clinical Trial *Clinical Oral Investigations* 28 (8) 2024: pp. 438. https://doi.org/10.1007/s00784-024-05822-z
18. **Pilli, L.N., Singaraju, G.S., Nettam, V., Keerthipati, T., Mandava, P., Marya, A.** An Extensive Comparison of the Clinical Efficiency of Acidulated Phosphate Fluoride (APF) and Neutral Sodium Fluoride (NaF) Oral Rinses in the Prevention of White Spot Lesions during Fixed Orthodontic Treatment: A Randomized Controlled Trial *BioMed Research International* 2022 (1) 2022: pp. 6828657. https://doi.org/10.1155/2022/6828657
19. **Ciribè, M., Cirillo, E., Mammone, M., Vallogini, G., Festa, P., Piga, S., Ferrazzano, G.F., Galeotti, A.** Efficacy of F-ACP-Containing Dental Mousse in the Remineralization of White Spot Lesions after Fixed Orthodontic Therapy: A Randomized Clinical Trial *Biomedicines* 12 (6) 2024: pp. 1202. https://doi.org/10.3390/biomedicines12061202
20. **Jablonski-Momeni, A., Nothelfer, R., Morawietz, M., Kiesow, A., Korbmacher-Steiner, H.** Impact of Self-Assembling Peptides in Remineralisation of Artificial Early Enamel Lesions Adjacent to Orthodontic Brackets *Scientific Reports* 10 (1) 2020: pp. 15132. https://doi.org/10.1038/s41598-020-72185-2
21. **Leal, T.P., Navarro, R.S., Freitas, P.M., Shitsuka, C., Souza, H.M.M., Corrêa, M.D., Ortolani, C.L.F.** Effects of Sealants on in Vitro Demineralization of Enamel Surrounding Orthodontic Brackets *Research, Society and Development* 10 (11) 2021: pp. e560101119923. https://doi.org/10.33448/rsd-v10i11.19923
22. **Amaechi, B.T., McGarrell, B., Luong, M.N., Okoye, L.O., Gakunga, P.T.** Prevention of White Spot Lesions around Orthodontic Brackets Using Organoselenium-Containing Antimicrobial Enamel Surface Sealant *Heliyon* 7 (3) 2021: pp. e06490. https://doi.org/10.1016/j.heliyon.2021.e06490
23. **Lorenz, J., Schidtmann, I., Morawietz, M., Kiesow, A., Wehrbein, H., Sarembe, S., Erbe, C.** In Vitro Surface Analysis of the Brushing Resistance of Orthodontic Sealants Using Two Different Profilometric Evaluation Methods *Scientific Reports* 12 (1) 2022: pp. 16133. https://doi.org/10.1038/s41598-022-19702-7
24. **Vicente, A., Ortiz Ruiz, A.J., González, Paz, B.M., García López, J., Bravo-González, L.A.** Efficacy of Fluoride Varnishes for Preventing Enamel Demineralization after Interproximal Enamel Reduction. Qualitative and Quantitative Evaluation *Plos One* 12 (4) 2017: pp. e0176389. https://doi.org/10.1371/journal.pone.0176389
25. **Nelson, D., Jongebloed, W., Arends, J.** Morphology of Enamel Surfaces Treated with Topical Fluoride Agents: SEM Considerations *Journal of Dental Research* 62 (12) 1983: pp. 1201 – 1208. https://doi.org/10.1177/00220345830620120501
26. **Brar, G.S., Arora, A.S., Khinda, V.I.S., Kallar, S., Arora, K.** Topographic Assessment of Human Enamel Surface Treated with Different Topical Sodium Fluoride Agents: Scanning Electron Microscope Consideration *Indian Journal of Dental Research* 28 (6) 2017: pp. 617 – 622. https://doi.org/10.4103/ijdr.IJDR_675_16
27. **Chabuk, M.M., Al-Shamma, A.M.** Surface Roughness and Microhardness of Enamel White Spot Lesions Treated with Different Treatment Methods *Heliyon* 9 (7) 2023: pp. e18283. https://doi.org/10.1016/j.heliyon.2023.e18283



© Yalçin et al. *2027* Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.