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Evaluation of Microleakage and Remineralization Potential of a Fissure Sealant Containing Microcapsules on Artificial Enamel Lesions: With and Without Adhesive Application

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Abstract

Within the concept of minimal invasive dentistry, the progression of initial caries lesions can be inhibited with preventive measures. This study evaluated the microleakage and remineralization potential of a bioactive fissure sealant (FS) on artificial caries lesions. For microleakage assessment, 40 extracted human molars were demineralized and randomly assigned to four groups (n=10). Specimens were sealed with either bioactive FS (BioCoat®; Premier Dental Co, USA) or a conventional resin-based FS (Clinpro™; 3M ESPE, USA), with half of each group pretreated with a universal adhesive system. After thermocycling, microleakage was evaluated using a dye penetration test. For remineralization analysis, 10 molars were divided into BioCoat and Clinpro groups (n = 5 each). Four standardized windows were prepared on each tooth to represent sound enamel, demineralized enamel, and demineralized enamel treated with either sealant, with or without prior adhesive application. After a 30-day dynamic pH cycling process, Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDX) was performed at depths of 0, 5, 10, 15 and 20 µm from the outer enamel surface toward dentin. Microleakage scores were analyzed using the chi-square test, while SEM/EDX measurements were evaluated using the Kruskal–Wallis test and repeated-measures ANOVA ($p < 0.05$). No significant differences in microleakage were observed between the bioactive and conventional FS groups. SEM/EDX analysis demonstrated greater mineral-related changes across the evaluated depths in the bioactive FS group, with increased Ca, P, and F levels ($p<0.001$). In contrast, the conventional FS showed limited remineralization at depths of 0 and 5 µm ($p<0.05$). The use of an adhesive system under FS did not significantly influence microleakage or remineralization. These findings suggest that bioactive fissure sealants may enhance enamel remineralization in initial caries lesions more effectively than conventional sealants. However, further clinical studies are required to support these findings.

Keywords

Fissure sealant; demineralized enamel; remineralization; microleakage; bioactive; dental adhesive; SEM/EDX

Introduction

Dental caries, one of the most prevalent chronic diseases worldwide and a significant public health issue, particularly affects the occlusal surfaces of molars in young individuals [1,2]. These surfaces are highly susceptible to caries formation due to their plaque-retentive morphology, the difficulty of plaque removal, and the limited efficacy of other caries-preventive agents such as fluoride varnish in these areas. Fissure sealants are commonly indicated for the prevention of occlusal caries in teeth with deep pits and fissures, particularly in newly erupted molars and in individuals with high caries risk. When applied to the occlusal surfaces of teeth, sealants are typically placed as a single procedure and act as a physical barrier against food impaction and plaque accumulation, thereby playing a protective role against the demineralization of dental hard tissues [3].

To enhance the effectiveness of pit and fissure sealants, various remineralizing compounds, such as bioactive glasses, amorphous calcium phosphate, nanohydroxyapatite particles, and zinc oxide, along with ions like fluoride, calcium, and phosphate, are incorporated into resin-based fissure sealants [4-8]. Bioactive materials induce biological effects on host tissues by promoting hydroxyapatite re-precipitation on enamel and dentin or by releasing biologically active ions [9,10]. These materials, which respond to pH changes by releasing and recharging ions, are categorized into two types: those with ion-releasing glasses (e.g., Activa™, Giomer™) and those utilizing semipermeable microcapsules combined with special monomers (BioCoat™). This semipermeable microcapsule technology, often described as “smart capsule technology,” involves embedding microcapsules within the resin matrix to enable controlled ion release. The rechargeable polyurethane microcapsules, containing bioactive compounds such as calcium carbonate, sodium fluoride, and potassium hydrogen phosphate, have demonstrated the ability to prevent enamel demineralization during acidic conditions. Moreover, these microcapsules provide sustained ion release over extended periods, significantly enhancing the protective properties of sealants [10-12].

The protective effect of fissure sealants is mainly achieved through their ability to penetrate deep into the pits and fissures, forming strong micromechanical adhesion with the etched enamel surface. However, the relatively high resin content in resin-based fissure sealants may lead to polymerization shrinkage, which can negatively impact their retention. Moreover, the complex morphology of deep pits and fissures increases the C-factor, which amplifies stress caused by shrinkage. To address this, the application of a material with a low elastic modulus such as a dental adhesive prior to sealant placement may act as a stress reliever and improve the material's retention. Adhesive application may enhance the bonding strength between the sealant and the tooth surface, reducing the likelihood of microleakage at the tooth-sealant interface [13,14]. However, the effectiveness of adhesive systems is highly dependent on proper isolation, which may be challenging to achieve in pediatric patients. By improving retention, the risk of secondary caries, discoloration, and fissure sealant detachment can be significantly minimized [15].

Limited research has investigated the performance of the recently introduced bioactive resin-based fissure sealant incorporating microcapsule technology [10,12,16]. To the best of our knowledge, only two studies have evaluated the remineralizing ability of the BioCoat fissure sealant [10,12], but neither assessed the impact of applying an adhesive system before sealant placement. Furthermore, no studies have examined microleakage at the interface between the BioCoat fissure sealant and the enamel surface. Therefore, the aim of this in vitro study was to evaluate the remineralizing efficacy of a bioactive fissure sealant containing microcapsules on the enamel surface using Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDX) and to assess the presence and extent of microleakage on surfaces treated with this bioactive fissure sealant. The null hypotheses were as follows: (I) the bioactive fissure sealant would exhibit similar remineralizing capacity and microleakage severity to a conventional fluoride fissure sealant; (II) the use of adhesive systems prior to sealant placement has no impact on its remineralizing capacity or microleakage severity.

Materials and Methods

The ethical approval was obtained from the Clinical Research Ethics Committee of the Faculty of Dentistry, Istanbul University (Reference No: 2020/39-484). Extracted human third molars, including both maxillary and mandibular teeth and free from caries, fractures, or cracks, were collected from systemically healthy individuals aged between 20 and 40 years. The teeth were extracted for routine therapeutic reasons such as pain, sensitivity, space limitations, or periodontal issues, independent of this study. They were immersed in 4% formaldehyde solution for 48 hours, rinsed with saline, and stored in 1% thymol solution at 4°C for up to two months prior to the experiment [17,18].

A priori sample size calculation was performed using G*Power software (version 3.0). For the microleakage analysis, the calculation was based on the data reported by Prabakar et al. [19]. Assuming an effect size of d = 1.168, a minimum of 10 specimens per group was required to achieve 80% power at a 5% significance level. For the remineralization analysis, the sample size calculation was based on the study by Memarpour et al. [8]. Assuming an effect size of d = 0.845, a minimum of 5 specimens per group was determined under the same statistical conditions.

Microleakage Assessment

The laser fluorescence (LF) device, the DIAGNOdent (KaVo, Biberach, Germany), was used to assess changes in the occlusal surfaces of extracted teeth before and after the demineralization process. Following device calibration, the DIAGNOdent tip was moved over the dried occlusal surfaces to measure their initial LF values. Based on the DIAGNOdent user manual, 40 teeth with LF values between 0–14, indicating the absence of enamel caries, were included in the study for microleakage analysis.

Artificial carious lesion creation:

To induce enamel surface demineralization, the teeth were immersed for 72 hours at 37°C in an acidic solution prepared as previously described in the literature [20], containing 8.7 mmol/L $CaCl_2$, 8.7 mmol/L KH_2PO_4, 0.05 ppm fluoride from NaF, and 75 mmol/L acetic acid at pH 4. After the incubation process, the teeth were removed from the solution, rinsed thoroughly under running water, and dried using an air spray. The demineralization of the occlusal surfaces was verified using the DIAGNOdent device, which recorded LF values between 15–20, corresponding to the presence of early enamel caries detectable at a histological level.

Group allocation: The 40 demineralized teeth were randomly assigned to two main groups based on the type of fissure sealant used: a bioactive resin-based pit and fissure sealant (BioCoat®; Premier Dental Co, Plymouth Meeting, PA, USA) and a conventional resin-based pit and fissure sealant (Clinpro™; 3M ESPE, St. Paul, MN, USA, Lot No.NA86550). Each main group was further divided into two subgroups, with and without adhesive system application, resulting in a total of four groups (n=10). The study groups were defined as follows: Group 1 (B3): BioCoat fissure sealant, Group 2 (B4): BioCoat fissure sealant with prior adhesive system application, Group 3 (C3): Clinpro fissure sealant, Group 4 (C4): Clinpro fissure sealant with prior adhesive system application. The compositions and application procedures of the fissure sealants and the adhesive system are provided in Table 1.

Table 1. The materials used in the study, their manufacturers, and compositions.

Materials	**Code**	**Composition**	**Application**	**Manufacturer**
BioCoat® Bioactive Resin Pit & Fissure Sealant	B	Calcium donor, Phosphate donor, Fumed Silica, Barium aluminoborosilicate, Triethylene glycol dimethacrylate, photoinitiator	Clean, rinse and dry the pit and fissures, etch for 20 s, rinse and dry, apply BC and light cure for 20 s.	Premier Dental Co, Plymouth Meeting, PA, USA

3M™ Clinpro™ Sealant	C	Bisphenol A-glycidyl methacrylate (BIS-GMA), Triethylene glycol dimethacrylate (TEGDMA), Silane Treated Silica, Tetrabutylammonium Tetrafluoroborate, Diphenyliodonium Hexafluorophosphate, Titanium Oxide, Triphenylantimony, Ethyl 4-Dimethyl Aminobenzoate (EDMAB), Hydroquinone	Clean, rinse and dry the pit and fissures, etch for 15-60 s, rinse and dry, apply CP and light cure for 10 s.	3M ESPE, St. Paul, MN, USA
3M™ Single Bond Universal Adhesive	SB	Methacryloyloxydecyl dihydrogen phosphate (MDP) Monomer, Dimethacrylate resins, Hydroxyethyl methacrylate (HEMA), Vitrebond Copolymer, Filler, Ethanol, Water, Initiators, Silane	Apply bond and rub it for 20 s. Gently air dry for 5 s. Light cure for 10 s.	3M Deutschland GmbH, Seefeld, Germany

The pits and fissures of all specimens were etched with 35% phosphoric acid (3M Scotchbond Universal Etchant, Germany) for 30 seconds, rinsed with water for 30 seconds, and air-dried for 10 seconds. In groups B4 and C4, 3M™ Single Bond Universal Adhesive (3M Deutschland GmbH, Seefeld, Germany) was applied to the fissures according to the manufacturer’s instructions and light-cured for 20 seconds to standardize the curing procedure between materials, using an LED curing unit (Elipar Deep Cure-S LED Light, 3M ESPE, St. Paul, MN, USA). BioCoat bioactive fissure sealant was applied in groups B3 and B4, while Clinpro fissure sealant was applied in groups C3 and C4. The sealants were applied following the manufacturer’s instructions and light-cured for 20 seconds using the same LED curing unit. After polymerization, the specimens were stored in distilled water at 37°C for

24 hours in an incubator, followed by thermocycling for 5000 cycles between water baths maintained at 5±2°C and 55±2°C. Each cycle included a dwell time of 30 seconds in each bath with a 15-second transfer interval [21].

Dye penetration test: Prior to the dye penetration test, the roots were sealed with a nanohybrid composite resin (Filtek Z550 Universal Restorative, 3M ESPE, St. Paul, MN, USA) to prevent possible dye infiltration. The specimens were then coated with nail polish, leaving a 1 mm margin around the occlusal surfaces, and immersed in 0.5% basic fuchsin dye solution for 24 hours at room temperature. After immersion, they were rinsed under running tap water to remove any excess dye.

Each specimen was sectioned buccolingually with two parallel cuts using a low-speed diamond saw (IsoMet 1000, Buehler Inc., UK), resulting in three sections and four evaluable internal surfaces per sample for microleakage evaluation. The cuts were performed through the sealed fissure area to obtain approximately equal sections among specimens. A total of 160 surfaces were examined under a stereomicroscope (Olympus SZX-7, Evident corp., Tokyo, Japan) at 20x magnification and scored by a single investigator following the Williams and Winter scoring system (Table 2). The average score of the four surfaces for each tooth was then calculated and recorded for further analysis.

Table 2. Williams and Winter Scoring for dye penetration [22]

Score	**Dye penetration**
0	No dye penetration between the tooth surface and fissure sealant
1	Dye penetration into less than one-third of the entire length of the surface between the sealant and the tooth surface
2	Dye penetration into one-third to two-thirds of the entire length of the surface between the sealant and the tooth surface

3	Dye penetration into more than two-thirds of the entire length of the surface between the sealant and the tooth surface

Remineralization Assessment

Specimen preparation: For remineralization assessment, separate specimens were used from those allocated to the microleakage evaluation. The buccal and lingual surfaces of the specimens were polished using a grinding and polishing machine (MetaServ 250®, Buehler Inc, IL, USA) to ensure a standardized surface. Ten specimens were then coated with nail polish, leaving four exposed windows (2 mm x 2 mm) per specimen, two on the buccal and two on the lingual surfaces. Each window was numbered 1 to 4 for identification. The windows were defined as standardized intra-sample measurement areas within each specimen to evaluate depth-dependent mineral changes and were not considered separate samples. These measurements were used to assess within-sample variations rather than to increase the number of independent observations. Windows labeled ‘1’ were additionally covered with a different-colored nail polish before demineralization as they served as the sound enamel groups (B1,C1) (Table 3).

Demineralization: Windows numbered 2, 3 and 4 were subjected to demineralization. The specimens were immersed in an acidic solution at 37ºC for 72 hours in an incubator, following the same demineralization procedure used in the microleakage experiments. After incubation, the specimens were removed from the solution, rinsed under running water, and dried with an air spray. Windows numbered ‘2’ were then coated with nail polish after demineralization and received no further treatment (B2, C2).

Group allocation: A total of 10 specimens were randomly assigned to two groups: B (BioCoat) and C (Clinpro) with five specimens per group. Windows numbered ‘3’ and ‘4’ were treated with either the microcapsule containing bioactive fissure sealant (BioCoat) or the conventional resin-based fissure sealant (Clinpro), applied alone (B3, C3) or in combination with an adhesive system (B4, C4), as described in the microleakage assessment section.

Table 3. Group allocation for remineralization experiments

Group (window)	**Application**	**Group (window)**	**Application**
B1	Sound enamel (no demineralization, no treatment) (n=5)	C1	Sound enamel (no demineralization, no treatment) (n=5)
B2	Demineralized enamel (n=5)	C2	Demineralized enamel (n=5)
B3	Bioactive fissure sealant (after demineralization) (n=5)	C3	Conventional fissure sealant (after demineralization) (n=5)
B4	Adhesive + bioactive fissure sealant (after demineralization) (n=5)	C4	Adhesive + conventional fissure sealant (after demineralization) (n=5)

pH cycling: All experimental groups underwent a pH cycling process to simulate alternating demineralization and remineralization conditions as previously described in the literature [20]. The specimens were immersed in a demineralization solution (3 mmol/L $CaCl_2$, 1.8 mmol/L K_2HPO_4, 0.1 mol/L lactic acid, 1% carboxymethylcellulose, pH:4) for 1 hour, followed by immersion in a remineralization solution (1.2 mmol/L $CaCl_2$, 0.72 mmol/L K_2HPO_4, 2.6 µmol/L F, 50 mmol/L HEPES, pH:7) for 23 hours at 37^{o}C in an incubator. This cycle was repeated daily for 30 days, resulting in a total of 30 cycles, with solutions renewed every 24 hours. Specimens were rinsed with distilled water between solution changes.

SEM/EDX evaluation: The specimens were sectioned mesiodistally into buccal and lingual segments using a low-speed diamond saw (Isomet 1000, Buehler, IL, USA) under water cooling. To obtain slices with a minimum thickness of 1 mm, additional buccolingual incisions were made separately from the buccal and lingual segments. Elemental analyses were performed using SEM/EDX (FEI, QUANTA FEG 250, FEI Technologies Inc., Oregon, United States) at depths of 0, 5, 10, 15, and 20 μm, measuring from the outer enamel surface toward the dentin. Three different points were analyzed and the mean value was calculated at each depth to ensure accuracy.

The weight percentages (%wt) of Calcium (Ca), Phosphorus (P), and Fluoride (F) were determined, along with the Ca/P ratio, as indicators of mineralization. For SEM imaging, one specimen was randomly selected from each group, and images were captured separately from four windows on each specimen at 1000× magnification.

Statistical analysis: All data were analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Normality was assessed using the Kolmogorov–Smirnov test. Descriptive statistics were reported as mean and standard deviation.

Differences in microleakage score distributions between groups were analyzed using the chi-square test. For SEM/EDX analysis, between-group comparisons of elemental measurements were performed using the Kruskal–

Wallis test, whereas within-group depth-dependent changes were analyzed using repeated-measures analysis of variance (RM–ANOVA), since measurements obtained from different depths within the same specimen represented repeated observations rather than independent samples. Spearman correlation analysis was performed to evaluate the relationship between the distance from the material and the elemental levels (Ca, P, and F). A p-value of < 0.05 was considered statistically significant.

Results

Microleakage Assessment

In the microleakage assessment, 7 out of 160 surfaces (3 from Group B and 4 from Group C) were excluded based on predefined criteria, including insufficient sealant penetration (n = 5) and dye contamination (n = 2), as these conditions could compromise the validity of the measurements. The distribution of microleakage scores for each group is presented in Table 4. In all groups, a microleakage score of 0 was observed in 10% of the samples. In the BioCoat (B3) and adhesive + BioCoat (B4) groups, 70% and 60% of the samples, respectively, had a score of 1. In the Clinpro groups (C3 and C4), microleakage scores were distributed across scores 1, 2, and 3. No statistically significant differences were found between the groups (p ▯ 0.05). Additionally, the application of an adhesive system did not significantly impact microleakage in either fissure sealant group.

Table 4. Distribution of microleakage scores among the experimental groups.

Scores	**B3**	**B4**	**C3**	**C4**	***p***

	n (%)	n (%)	n (%)	n (%)	
0	1 (10)	1 (10)	1 (10)	1 (10)	0.56
1	7 (70)	6 (60)	3 (30)	3 (30)	
2	1 (10)	3 (30)	3 (30)	3 (30)	
3	1 (10)	0 (0)	3 (30)	3 (30)	

Chi-square test, $p < 0.05$. B3: BioCoat, B4: adhesive + BioCoat, C3: Clinpro, C4: adhesive + Clinpro

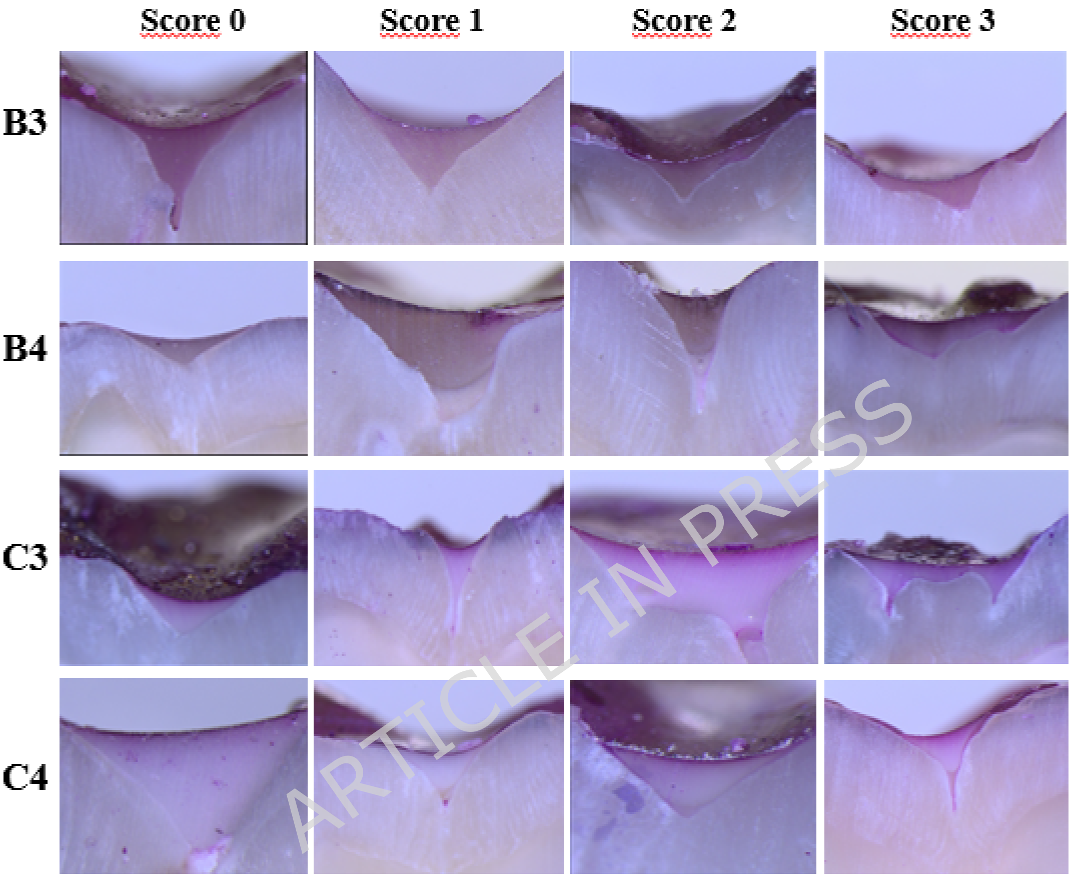

Figure 1. Representative stereomicroscope images showing dye penetration scores in each experimental group. B3: BioCoat, B4: adhesive + BioCoat, C3: Clinpro, C4: adhesive + Clinpro

Remineralization

SEM/EDX Elemental Analysis

The mean weight percentages (%wt) of Ca, P, and F elements obtained from all cross-sectional depths were compared among the subgroups within both main groups. Following demineralization, specimens exhibited a statistically significant decrease in Ca, P, and F levels compared with baseline values ($p < 0.001$ for each element).

After the dynamic pH cycling process, a noticeable increase in Ca, P, and F levels was detected in the specimens treated with the sealant materials (Figure 1). Compared with Group B2, both B3 and B4 showed highly significant increases in all three elements ($p < 0.001$ for Ca, P, and F) across all evaluated depths. Furthermore, the Ca/P ratios of Groups B3 and B4 tended to be lower than that of Group B2 across most depths. Statistically significant differences were observed at 15 µm and 20 µm ($p = 0.003$ and $p = 0.015$, respectively). In the conventional sealant groups, C3 and C4 exhibited significantly higher Ca, P, and F levels than C2 ($p = 0.004$, $p < 0.001$, $p < 0.001$ for C3; $p = 0.004$, $p = 0.001$, $p < 0.001$ for C4, respectively). In contrast, no statistically significant differences were observed in the Ca, P, or F levels between C3 and C4, or between B3 and B4, indicating that the application of an adhesive system did not substantially affect the elemental composition in either the bioactive or the conventional sealant groups.

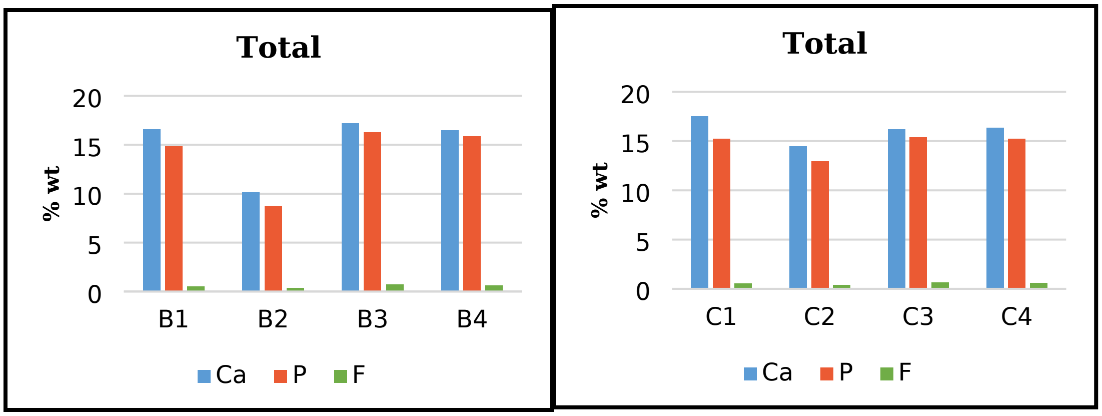

Figure 1. Mean weight percentages (%wt) of Ca, P, and F elements obtained by cross-sectional SEM/EDX analysis at depths of 0–20 µm. (a) Bioactive fissure sealant (Group B) (b) Conventional fissure sealant (Group C)

The weight percentages (%wt) of Ca, P, and F elements and the Ca/P ratio at specific depths (0, 5, 10, 15, and 20 µm) from the outer enamel surface toward the dentin in the bioactive fissure sealant groups and their corresponding control groups are presented in Table 5.

The remineralization efficacy of the microcapsule-containing bioactive fissure sealant applied to demineralized enamel showed higher elemental levels across all evaluated depths up to 20 µm. The weight percentages of Ca, P, and F, which are indicative of remineralization, were higher than those of demineralized enamel and were either comparable to or higher than the values observed in sound enamel. Although the elemental levels were slightly lower in the group where the bioactive sealant was applied after an adhesive system than in the non-adhesive group, this difference did not reach statistical significance (Table 5).

For the conventional resin-based fissure sealant applied to demineralized enamel, remineralization activity was most prominent at the outermost enamel surface. In all groups, Ca, P, and F levels at the 0–5 µm depth were significantly higher than those of demineralized enamel; however, this significance diminished progressively toward the 20 µm depth. The Ca/P ratios showed a statistically significant difference only at the outer enamel surface (0 µm) ($p = 0.013$), whereas no significant differences were observed at deeper levels (5–20 µm) (Table 6).

		B1 **Sound enamel**	**B2** **(Demineralized enamel)**	**B3** **(BioCoat)**	**B4** **(Adhesive + BioCoat)**	**p**
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Ca	**0 µm**	$9.35 \pm 2.23^{A,a}$	$6.44 \pm 2.10^{B,a}$	$13.82 \pm 2.70^{C,a}$	$12.66 \pm 3.18^{C,a}$	**<0.001**
	5 µm	$17.63 \pm 5.31^{A,b}$	$9.18 \pm 4.86^{B,b}$	$17.19 \pm 1.74^{A,b}$	$16.26 \pm 1.61^{A,b}$	**<0.001**
	10 µm	$18.20 \pm 3.24^{A,b,d}$	$9.97 \pm 4.32^{B,b}$	$18.14 \pm 1.37^{A,b}$	$17.78 \pm 2.02^{A,c,e}$	**<0.001**
	15 µm	$18.64 \pm 2.87^{A,b,e}$	$12.27 \pm 5.48^{B,c,e}$	$18.60 \pm 2.52^{A,b}$	$18.09 \pm 1.78^{A,d,e}$	**<0.001**
	20 µm	$19.25 \pm 3.41^{A,c,d,e}$	$12.90 \pm 4.55^{B,d,e}$	$18.30 \pm 1.65^{A,b}$	$17.75 \pm 2.33^{A,b,c,d}$	**<0.001**
		<0.001	**<0.001**	**<0.001**	**<0.001**	
P	**0 µm**	$8.67 \pm 1.75^{A,a}$	$5.65 \pm 1.77^{B,a}$	$13.10 \pm 2.76^{C,a}$	$12.37 \pm 2.62^{C,a}$	**<0.001**
	5 µm	$14.99 \pm 3.31^{A,B,b}$	$7.57 \pm 3.78^{C,a}$	$16.26 \pm 1.29^{A,b}$	$15.48 \pm 1.45^{B,b}$	**<0.001**
	10 µm	$16.35 \pm 1.74^{A,c}$	$10.13 \pm 5.59^{B,b}$	$17.29 \pm .70^{A,b}$	$17.04 \pm .99^{A,c}$	**<0.001**
	15 µm	$16.89 \pm 1.47^{A,c,e}$	$10.04 \pm 4.84^{B,b}$	$17.29 \pm 1.06^{A,b}$	$17.22 \pm 1.08^{A,c}$	**<0.001**
	20 µm	$17.41 \pm 1.72^{A,d,e}$	$10.52 \pm 4.66^{B,b}$	$17.42 \pm 1.10^{A,b}$	$17.16 \pm 1.07^{A,c}$	**<0.001**
		<0.001	**0.010**	**<0.001**	**<0.001**	
F	**0 µm**	$.47 \pm .11^{A}$	$.31 \pm .10^{B}$	$.70 \pm .16^{C}$	$.69 \pm .10^{C}$	**<0.001**
	5 µm	$.45 \pm .22^{A}$	$.39 \pm .20^{A}$	$.75 \pm .16^{B}$	$.71 \pm .26^{B}$	**<0.001**
	10 µm	$.48 \pm .13^{A}$	$.37 \pm .11^{B}$	$.64 \pm .18^{C}$	$.59 \pm .19^{C}$	**<0.001**
	15 µm	$.53 \pm .21^{A}$	$.40 \pm .20^{B}$	$.79 \pm .34^{C}$	$.63 \pm .28^{A,C}$	**0.001**
	20 µm	$.58 \pm .20^{A}$	$.37 \pm .10^{B}$	$.62 \pm .18^{A}$	$.59 \pm .20^{A}$	**<0.001**
		0.183	0.486	0.210	0.354	
Ca/P	**0 µm**	1.08 ± .18	1.15 ± .20	1.07 ± .17	1.03 ± .14	0.203
	5 µm	1.20 ± .34	1.18 ± .23	1.06 ± .14	1.05 ± .11	0.188
	10 µm	1.13 ± .26	1.07 ± .32	1.05 ± .09	1.04 ± .11	0.912
	15 µm	$1.11 \pm .19^{A}$	$1.25 \pm .22^{B}$	$1.08 \pm .20^{A}$	$1.05 \pm .10^{A}$	**0.003**

20 µm	1.11 ± .23^{A}	1.31 ± .31^{B}	1.05 ± .09^{A}	1.03 ± .13^{A}	**0.015**
	0.881	0.169	0.937	0.994	

Table 5. SEM/EDX elemental analyses of Ca, P, and F elements and Ca/P ratio at different depths (0–20 µm) in sound, demineralized, and BioCoat treated enamel groups, with or without adhesive application.

Between-group comparisons were performed using the Kruskal–Wallis test, whereas within-group depth-dependent changes were analyzed using RM–ANOVA. Different superscript capital letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Different superscript lowercase letters within the same column indicate statistically significant differences between depths ($p < 0.05$).

Table 6. SEM/EDX elemental analyses of Ca, P, and F elements and Ca/P ratio at different depths (0–20 µm) in sound, demineralized, and Clinpro treated enamel groups, with or without adhesive application.

	C1 **(Sound enamel)**	**C2** **(Demineralized enamel)**	**C3** **(Clinpro)**	**C4** **(Adhesive + Clinpro)**	**p**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Ca (0 µm)	11.67 ± 4.92^{A,a}	7.22 ± 3.76^{B,a}	9.59 ± 2.63^{A,a}	10.86 ± 3.19^{A,a}	**0.013**
Ca (5 µm)	16.77 ± 4.28^{b}	14.08 ± 4.09^{b}	16.8 2± 2.28^{b}	16.82 ± 2.64^{b}	0.099
Ca (10 µm)	19.95 ± 4.00^{A,c}	15.74 ± 5.24^{B,c}	17.19 ± 2.51^{B,C,b}	18.24 ± 2.21^{A,C,b,d}	**0.016**
Ca (15 µm)	19.15 ± 3.30^{c}	17.4 5± 4.59^{d}	18.31 ± 2.91^{b,d}	17.31 ± 2.52^{b,e}	0.246
Ca (20 µm)	19.93 ± 3.18^{c}	17.70 ± 4.18^{d}	19.14 ± 2.28^{c,d}	18.43 ± 3.19^{c,d,e}	0.136
	< 0.001	**< 0.001**	**< 0.001**	**< 0.001**	
P (0 µm)	9.45 ± 3.98^{A,a}	5.22 ± 3.24^{B,a}	9.88 ± 2.24^{A,a}	9.84 ± 3.30^{A,a}	**<0.001**
P (5 µm)	13.74 ± 3.53^{A,b}	11.88 ± 2.99^{B,b}	15.98 ± 1.91^{C,b}	15.77 ± 2.29^{A,C,b}	**<0.001**
P (10 µm)	16.89 ± 1.88^{c}	15.57 ± 2.13^{c}	16.25 ± 1.49^{b,d}	16.16 ± 1.49^{b}	0.194
P (15 µm)	17.60 ± 1.34^{c}	15.32 ± 2.99^{c}	17.15 ± 1.65^{c,d}	16.86 ± 2.20^{b,d}	0.139
P (20 µm)	18.54 ± 1.00^{d}	16.74 ± 2.56^{d}	17.73 ± .95^{c}	17.36 ± 2.64^{c,d}	0.094
	< 0.001	**< 0.001**	**< 0.001**	**< 0.001**	
F (0 µm)	.57 ± .17^{A,a,c,d}	.38 ± .13^{B}	.77 ± .20^{C,a}	.70 ± .18^{C,a}	**<0.001**
F (5 µm)	.44 ± .13^{A,B,C,b,d}	.37 ± .14^{A}	.57 ± .25^{B,b}	.52 ± .18^{C,b}	**0.009**

F (10 μm)	.64 ± .25^{A,c}	.45 ± .18^{B}	.57 ± .20^{A,B,b}	.55 ± .16^{A,B,b}	**0.043**
F (15 μm)	.48 ±.15^{d}	.43 ± .16	.58 ± .24^{b}	.57 ± .18^{b}	0.088
F (20 μm)	.49 ± .13^{A,d}	.36 ± .13^{B}	.60 ± .21^{A,b}	.51 ± .14^{A,b}	**0.001**
	0.007	0.268	**0.027**	**0.009**	
Ca/P (0 μm)	1.27 ± .25^{A,a,e,f}	1.74 ± 1.47^{A}	.97 ± .18^{B,C}	1.19 ± .49^{A,C}	**0.013**
Ca/P (5 μm)	1.26 ± .29^{a}	1.21 ± .32	1.06 ± .17	1.08 ± .14	0.079
Ca/P (10 μm)	1.19 ± .27^{a,c}	1.02 ± .31	1.06 ± .13	1.14 ± .20	0.118
Ca/P (15 μm)	1.08 ± .13^{b,c,e}	1.14 ± .23	1.07 ± .15	1.04 ± .18	0.251
Ca/P (20 μm)	1.07 ± .12^{b,f}	1.05 ± .11	1.08 ± .13	1.07 ± .12	0.909
	0.035	0.237	0.202	0.238	

Between-group comparisons were performed using the Kruskal–Wallis test, whereas within-group depth-dependent changes were analyzed using RM–ANOVA. Different superscript capital letters within the same row indicate statistically significant differences between groups ($p < 0.05$). Different superscript lowercase letters within the same column indicate statistically significant differences between depths ($p < 0.05$).

Correlation analysis between the distance from the material and the Ca, P, and F levels was also performed for the sealant-only groups. In Group B3, significant negative correlations were observed for Ca (r: –0.346, p: 0.001), P (r: –0.232, p: 0.028), and F (r: –0.254, p: 0.016). As the distance from the material increased, the increases in Ca, P, and F levels progressively decreased. In Group C3, the negative trend was statistically significant for P only (r: –0.469, p: < 0.001), while the correlations for Ca (r: –0.200, p: 0.059) and F (r: –0.135, p: 0.204) did not reach statistical significance.

When compared with demineralized enamel, the bioactive fissure sealant containing microcapsules—applied without an adhesive system—showed greater increases in Ca and P at all depths (0–20 μm) than the conventional resin-based sealant applied without adhesive. These findings are consistent with greater mineral uptake in the enamel treated with the microcapsule-containing bioactive fissure sealant.

When compared with sound enamel, the microcapsule-containing bioactive fissure sealant applied without adhesive demonstrated higher increases in Ca, P, and F levels at 0 and 10 µm than the conventional resin-based sealant applied without adhesive; however, this difference disappeared at depths of 15 and 20 µm.

Scanning electron microscopy (SEM) analysis revealed distinct morphological differences among the experimental groups. In sound enamel specimens, the surface morphology appeared relatively smooth and compact, with minimal surface irregularities. In contrast, demineralized enamel specimens exhibited increased surface roughness, evident porosity, and pronounced structural deterioration. In the BioCoat applied group, a more homogeneous surface layer was observed on the enamel surface, suggesting partial surface coverage. In the group where BioCoat was applied in combination with a bonding agent, no additional notable morphological changes were detected compared with the BioCoat-only group (Figure 2). In the Clinpro treated group, surface irregularities and microstructural defects remained. Similarly, in the adhesive system + Clinpro group, a comparable surface morphology was observed, and no marked improvement in surface characteristics was identified (Figure 3).

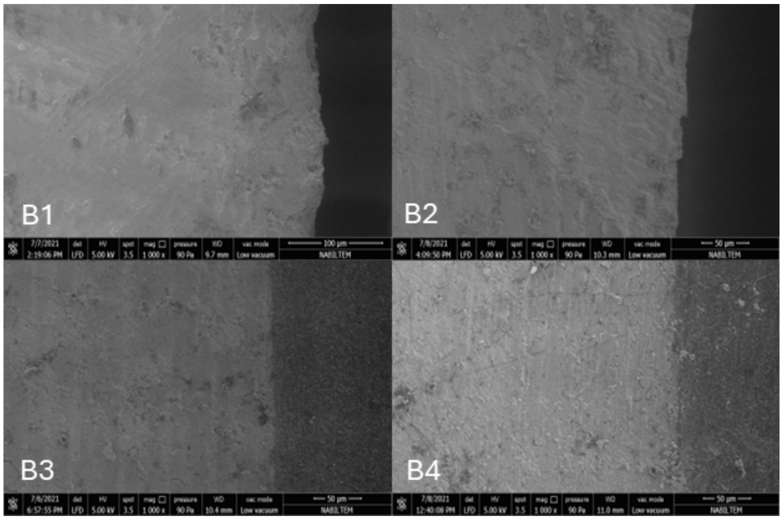

Figure 2. SEM images of Group B. B1: Sound enamel, B2: Demineralized enamel, B3: BioCoat, B4: Adhesive BioCoat.

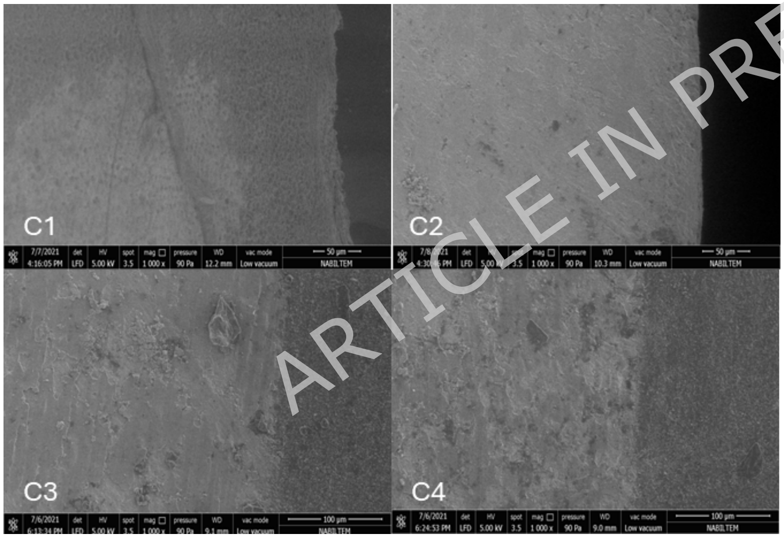

Figure 3. SEM images of Group C. C1: Sound enamel, C2: Demineralized enamel, C3: Clinpro, C4: Adhesive + Clinpro.

Discussion

To the best of our knowledge, this is the first in vitro study to evaluate the effects of a conventional resin-based fissure sealant (Clinpro™, 3M ESPE, MN, USA) and a bioactive sealant containing polyurethane microcapsules with Ca, P, and F (BioCoat®, Premier Dental Products, USA), used either alone or in combination with a dental adhesive, on microleakage and remineralization in demineralized enamel surfaces.

Many studies have recommended the use of pit and fissure sealants, which form a physical barrier between the tooth and bacteria through micromechanical bonding, even in the presence of demineralization or carious lesions in enamel. These studies have reported that resin-based sealants penetrate the porous enamel structure, providing mechanical support and preventing the progression of enamel caries by isolating bacteria from the substrate [19,21,23]. In addition, recent research has increasingly focused on the development of dental materials capable of neutralizing the acidic environment at the tooth-material interface and contributing to enamel remineralization as a preventive strategy against caries formation. The release of calcium and phosphate ions from bioactive materials may help reduce acid-induced demineralization at the tooth–material interface [18,24]. The efficacy of fluoride ions, which can reduce demineralization and promote remineralization, is further enhanced in the presence of calcium and phosphate ions at concentrations that do not compromise their solubility. Therefore, the incorporation of Ca, P, and F ions into dental materials such as fissure sealants is expected to contribute positively to enamel remineralization [25]. This study evaluated the effects of a resin-based, filler-containing bioactive fissure sealant (BioCoat, Premier Dental Products, USA), formulated with polyurethane microcapsules that release Ca, P, and F ions, on the microleakage and remineralization of demineralized enamel, and compared its performance with that of a fluoride-releasing, filler-free conventional resin-based fissure sealant (Clinpro, 3M ESPE, MN, USA).

To effectively prevent caries development, fissure sealants must adequately fill the pits and fissures and establish a durable bond with the enamel surface. Inadequate adaptation or incomplete sealing may result in microgaps at the tooth-sealant interface, leading to microleakage, which facilitates bacterial penetration and increases the risk of secondary caries. Thus, the long-term success of fissure sealants is strongly associated with their bond strength to the enamel and the quality of their marginal adaptation. One factor proposed to enhance the bonding performance of fissure sealants is the application of adhesive systems prior to sealant placement. While several studies have reported that the use of adhesives may improve sealant retention and reduce microleakage, the evidence remains inconclusive, with findings often varying depending on the material combinations used. The effectiveness of fissure sealants placed after prior adhesive application may depend on the specific material properties and formulations involved [18,26,27]. Accordingly, the present study aimed to evaluate the effects of applying fissure sealants in combination with an etch-and-rinse adhesive system (3M ESPE Single Bond Universal Adhesive) on microleakage and enamel remineralization, although the manufacturer’s application protocol for the bioactive fissure sealant does not include a separate adhesive application step.

In the present study, the majority of microleakage scores in the bioactive fissure sealant groups were concentrated at score ‘1’, suggesting effective marginal sealing. This finding may be attributed to their low viscosity and high bonding efficacy to enamel, allowing deeper penetration into fissure morphology [28]. In contrast, microleakage scores in the resin-based fissure sealant exhibited a wide distribution; however, no statistically significant difference was observed between the two groups. Therefore, both fissure sealants appear to have acceptable sealing ability. Both groups showed higher microleakage scores compared to previous studies [28,29]. This may be due to the altered content of demineralized enamel. The reduced inorganic composition and the increased porosity of early carious lesions may have compromised resin penetration and bonding strength. Dye penetration, although widely used for microleakage assessment in in vitro studies, represents an indirect method and should be interpreted with caution [28].

Adhesive systems have been reported to improve micromechanical retention and enhance the sealant–enamel interface, potentially increasing the retention of the material [14]. In the present study, no statistically significant difference was observed between groups with and without adhesive application, which is in agreement with the findings of Vijayakumar et al. [15], while differing from the outcomes reported by Nahvi et al. [27]. Such variations among studies may be attributed to differences in experimental design, including the use of sound versus demineralized enamel, variations in microleakage evaluation protocols, and discrepancies in sample size. Overall, the current body of evidence indicates a lack of consensus regarding the impact of adhesive systems on the microleakage performance of fissure sealants. Given the influence of multiple clinical variables, such as the condition of the enamel surface, isolation quality during sealant placement, and patient cooperation, the decision to incorporate an adhesive system prior to sealant application may be best guided by the clinical judgment of the practitioner and tailored to the specific clinical scenario.

Previous studies have typically evaluated the remineralization potential of fissure sealants by assessing changes limited to the enamel surface. In contrast, the present study is the first to assess remineralization by scanning from the enamel–material interface to a depth of 20 µm in 5-µm increments, allowing for a more detailed characterization of subsurface mineral changes. In line with the findings of Ibrahim et al. [12], the present study demonstrated that the weight percentages (%wt) of Ca, P, and F at the enamel surface (0 µm) treated with the BioCoat bioactive fissure sealant were significantly higher than those in sound and demineralized enamel groups. Similarly, the enamel surface (0 µm) treated with the Clinpro conventional fissure sealant also showed significantly higher Ca, P, and F content compared to the negative control group. However, in contrast to Ibrahim et al. [12], the percent changes in elemental composition at the enamel surface (0 µm) revealed that the increase in Ca content was significantly greater in the BioCoat group compared to the Clinpro group. Although F levels were also higher in the BioCoat group, no statistically significant differences were found between the groups with respect to phosphorus or fluoride content. SEM/EDX results at the material-enamel interface were also consistent with the findings of Salma et al. [10] and Balkan et al. [30], indicating continuous ion release from microcapsules. The potential advantages of bioactive fissure sealants over conventional materials are mainly attributed to their ion-releasing properties. Smart capsules may help maintain calcium and phosphorus ions in a bioavailable form, which may facilitate the incorporation of fluoride ions into enamel. Although the pH cycling model provides calcium and phosphate ions in the remineralization solution, the higher elemental levels detected in the bioactive sealant groups suggest an additional contribution from the microcapsule-containing formulation. However, these findings should be interpreted with caution, as the model does not allow direct isolation of the material’s intrinsic remineralization effect. In addition, Ca/P ratios were generally lower in the bioactive sealant groups compared with the demineralized enamel group, although statistically significant differences were observed only at deeper levels (15 and 20 µm). This finding may suggest alterations in mineral composition during the remineralization process.

In this study, the bioactive fissure sealant (BioCoat) produced significantly greater increases in the % wt of Ca, P, and F compared with the conventional resin-based fissure sealant (Clinpro) when evaluated relative to demineralized enamel. Previous in vitro studies have reported similar findings. Chen et al. [31] reported that the Clinpro fissure sealant released Ca, P, and F ions, while Şişmanoğlu [32] similarly demonstrated fluoride release from the material and observed an increase in fluoride levels within demineralized enamel following dynamic pH cycling. In the present study, the Ca, P, and F levels between 0–5 μm depth in the Clinpro group were higher than those in the demineralized enamel group, but no significant differences were observed in deeper sections, suggesting that the remineralization effect was mainly limited to the enamel surface. These results indicate that the microcapsule-containing bioactive fissure sealant may allow greater mineral penetration into demineralized enamel compared with conventional fluoride-releasing sealants, whose effects appear to be largely limited to the enamel surface. Consistent with the findings of Lobo et al. [33] and Abdelsalam et al. [34], this study demonstrated that Clinpro, when applied alone, could facilitate fluoride uptake in demineralized enamel; however, this effect was limited to the outermost 5 μm. This localized action may be attributed to the theory that fluoride forms a lamination-like barrier within the subsurface lesion, thereby restricting its ability to penetrate deeper layers [35]. Similarly, Ca/P ratios in the conventional sealant groups differed significantly only at the enamel surface, whereas no significant differences were detected at greater depths. Conventional fissure sealants, although effective, may present certain limitations, including limited ion release capacity and reduced effectiveness in promoting subsurface remineralization compared to bioactive materials [12,35,36].

Previous research has suggested that fluoride can exert its remineralizing effect only up to approximately 30 µm from the enamel surface in early carious lesions [35,37]. In this regard, studies that assess the remineralization capacity of dental materials solely at the surface–material interface may present limitations and lead to misinterpretation of outcomes. Although in the present study the degree of remineralization was evaluated via SEM/EDX, which offers a reliable depth-based analysis, supporting these findings with additional remineralization assessment methods would provide a more comprehensive understanding of material performance.

SEM images confirmed mineral loss in demineralized enamel, showing surface irregularities and increased porosity compared to sound enamel. Both fissure sealants demonstrated a reduction in pore size after the pH cycling process, particularly at the material-enamel interface. In this study, consistent with the SEM findings of Garcia et al., Salma et al. and Balkan et al. [10,30,36], the SEM images of the BioCoat bioactive fissure sealant revealed more uniform mineral deposition. These SEM observations were further supported by the EDX analysis results, reinforcing the consistency between the morphological and elemental findings. While SEM/EDX provides valuable information on elemental composition and depth-dependent mineral changes, it does not directly reflect structural or mechanical recovery of enamel and therefore should be interpreted as an indirect indicator of remineralization [10,30]. The results of this study are supported by the findings of Burbank et al. [11], who evaluated ion release from microcapsule-containing fissure sealants, as well as Burbank et al. [38], who investigated the ion release and fluoride recharging capability of microcapsules incorporated into orthodontic cements using ion-selective electrodes.

In the present study, SEM/EDX analysis demonstrated that calcium, phosphate, and fluoride ions released from the microcapsule-containing formulation were able to integrate into the structure of demineralized enamel even under dynamic pH cycling conditions involving an aggressive acidic solution with a pH of 4. In studies investigating the combined use of fissure sealants and adhesive systems on remineralization, it has been reported that acid etching prior to sealant application significantly increases the depth of demineralized enamel lesions. When fissure sealants are applied without an adhesive system, fractures may occur in areas lacking adequate bonding. By contrast, following acid etching, the adhesive system can infiltrate and fill the micro-irregularities of both sound and demineralized enamel [39]. In the present study, the use of an adhesive system before fissure sealants did not demonstrate any significant effect on mineral composition. Although enamel samples treated with fissure sealants alone showed slightly greater elemental uptake indicative of remineralization, the difference was not statistically significant. Variations among studies may be related to the use of different adhesive and sealant brands with differing chemical compositions.

In this study, a fluoride-free adhesive system (Single Bond Universal Adhesive) was used together with the fissure sealants. One limitation is that only a single brand of adhesive was evaluated, and the remineralization capability of the selected bonding agent itself was not assessed. Some studies have suggested that adhesive systems may contribute to remineralization or release fluoride; however, the fluoride concentrations reported are often below the lower detection limits of calibration curves [40,41]. In addition, SEM/EDX analyses were performed on buccal and lingual surfaces of the teeth, and the behavior of the materials in irregular fissure morphologies was not investigated. While SEM/EDX provides detailed assessment of elemental composition at different depths, future studies combining this approach with techniques such as micro-CT or microhardness analysis may provide a more comprehensive evaluation of remineralization. Future research should investigate the effects of bioactive, fluoride-releasing, or conventional fissure sealants applied to demineralized or intact enamel surfaces, in combination with adhesive systems with or without fluoride, on both demineralization and remineralization outcomes. The in vitro nature of this study limits direct clinical extrapolation, and further in vivo studies are required to confirm these results.

Conclusions

Fissure sealant applications may promote remineralization in artificial enamel lesions. In the present study, the microcapsule-containing bioactive fissure sealant demonstrated greater mineral-related changes across the evaluated depths compared with the conventional sealant, while the use of an adhesive system did not produce any significant difference in either microleakage or remineralization outcomes. Therefore, the first null hypothesis was rejected, whereas the second was accepted.

The decision to use adhesive systems for preventing microleakage may thus be left to the clinician’s preference, considering the clinical condition of the tooth. Additionally, the influence of moisture contamination, which was not assessed in this study, should not be overlooked, particularly in pediatric patients where achieving ideal isolation may be challenging.

Although the remineralization of early carious lesions requires the elimination of several caries-related risk factors, the bioactive fissure sealant containing microcapsules with Ca, PO_4, and F ionic solutions appears promising due to its enhanced remineralization potential. Further studies are recommended to investigate the depth-dependent remineralization capacity of bioactive fissure sealants and to evaluate the effects of combining fissure sealants with adhesive systems on microleakage and remineralization, in order to support and expand upon the findings of the present research.

List of abbreviations

BIS-GMA: Bisphenol A-Glycidyl Methacrylate

Ca/P: Calcium-to-Phosphate Ratio

EDMAB: Ethyl 4-Dimethyl Aminobenzoate

EDX: Energy Dispersive X-ray Spectroscopy

FS: Fissure Sealant

GIC: Glass Ionomer Cement

HEMA: Hydroxyethyl Methacrylate

LF: Laser Fluorescence

MDP: Methacryloyloxydecyl Dihydrogen Phosphate

RM–ANOVA: Repeated-Measures Analysis of Variance

SEM: Scanning Electron Microscopy

TEGDMA: Triethylene Glycol Dimethacrylate

µm: Micrometer

Declarations

Ethics Approval and consent to participate: The ethical approval was obtained from the Clinical Research Ethics Committee of the Faculty of Dentistry, Istanbul University (Reference No: 2020/39-484). The study was conducted in accordance with the Declaration of Helsinki and relevant national regulations. Extracted human teeth used in this in vitro study were collected anonymously after obtaining informed consent from the patients and/or their legal guardians for their use in research. No personal data were recorded. The teeth were extracted for routine therapeutic reasons independent of the present study.

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Author contributions: APE. and BP conceived the ideas; BP collected the data; BP, APE and ES analyzed the data; and YG, BP, APE wrote the manuscript. All authors read and approved the final manuscript.

Data availability: The data that support the findings of this study are available from the corresponding author (Parlak B.), upon request.

Consent for Publication: Not applicable

Conflict of interest: The authors declare that they have no conflict of interest.

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