

The Remineralizing Effect of Toothpaste Containing Hydroxyapatite on Initial Interproximal Caries Adjacent to Different Restorative Materials: An In Vitro Study

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Abstract

Objectives

To compare the effects of fluoride (F), fluoride toothpaste containing hydroxyapatite (F + HA), and hydroxyapatite-containing (HA) toothpastes on artificial initial interproximal carious lesions adjacent to Class II resin composite and glass ionomer cement (GIC) restorations.

Methods

Eighty human maxillary premolar specimens with standardized 1 × 1-mm artificial carious lesions were randomized into eight groups (n = 10) according to restorative material (resin composite or GIC) and toothpaste (F, F + HA, HA, or no-toothpaste control). Specimens underwent 14-day pH cycling at 37°C. Mineral density (MD), lesion depth (LD), surface roughness, morphology, and elemental composition were assessed by micro-CT, profilometry, SEM, and EDS. Data were analyzed using two-way ANOVA and Tukey's test ($\alpha = 0.05$).

Results

GIC produced greater MD gains and smaller LD increases than resin composite ($p < 0.05$). In composite groups, HA significantly reduced LD versus control ($p < 0.05$). In GIC groups, all toothpastes significantly reduced LD versus control ($p < 0.05$); F + HA showed lower MD change than F and HA, although none differed from control. Toothpaste significantly affected surface roughness ($p < 0.01$), whereas restorative material did not. HA produced lower roughness than F, and SEM showed smoother surfaces with F + HA and HA. EDS detected fluorine in all GIC groups; the GIC/HA group showed the highest Ca/P ratio and calcium content.

Conclusions

GIC provided a more favorable remineralizing environment than resin composite in all toothpaste conditions. HA-containing toothpaste improved enamel surface characteristics in both resin composite and GIC.

Clinical relevance:

GIC may provide a favorable restorative environment for remineralization of initial interproximal carious lesions. Conventional 1500-ppm fluoride toothpaste remains recommended for caries prevention. HA-containing toothpastes may offer an alternative or adjunctive option, particularly for improving enamel surface characteristics.

1. Introduction

Dental caries is a biofilm-mediated disease characterized by a dynamic imbalance between demineralization and remineralization of dental hard tissues. Acidic metabolites produced by cariogenic bacteria reduce the local pH and induce mineral dissolution, while protective factors such as saliva, fluoride, and calcium-phosphate availability support remineralization. When demineralization predominates, mineral loss exceeds the natural repair capacity of enamel, leading to lesion progression. In its initial stage, however, caries remains non-cavitated and potentially reversible if remineralization is enhanced (1–4).

Fluoride toothpaste remains the primary strategy for caries control, promoting remineralization, inhibiting further mineral loss, and increasing enamel resistance to acid challenges through sustained fluoride availability in saliva and plaque fluid (5). In recent years, synthetic hydroxyapatite has been introduced as a biomimetic remineralizing agent. Hydroxyapatite particles can release calcium and phosphate ions and act as nucleation sites for mineral deposition, forming an apatite layer that resembles natural enamel (6). *In vitro* and *in situ* studies have demonstrated that hydroxyapatite-containing toothpaste enhances mineral deposition, improves surface microhardness, and reduces enamel surface roughness (7, 8). Its remineralization efficacy has been reported to be comparable to low-fluoride toothpaste formulations, while higher hydroxyapatite concentrations show effects similar to those of conventional high-fluoride toothpaste (9–11). Toothpaste formulations combining fluoride and hydroxyapatite do not appear to compromise remineralization outcomes when compared with fluoride toothpaste alone (12).

Most remineralization studies have focused on smooth enamel surfaces, whereas evidence regarding proximal enamel surfaces remains limited. Initial interproximal carious lesions are clinically relevant due to their high prevalence and limited accessibility for mechanical plaque removal. These lesions are frequently present adjacent to Class II restorations, where the restorative material itself may influence the mineral balance of nearby enamel. Fluoride-releasing restorative materials have been shown to reduce mineral loss and enhance remineralization in adjacent early carious lesions. Glass ionomer cement (GIC) has demonstrated superior inhibitory effects on mineral loss compared with resin-modified GIC and resin composite materials (13). More recently, alkasite restorative materials and nano-calcium-phosphate composite restorations have exhibited remineralization effects comparable to glass ionomer cement and superior to conventional resin composite, as evidenced by significantly greater recovery of surface microhardness in adjacent proximal enamel lesions under pH-cycling conditions (14, 15).

Despite these findings, resin composite remains the most used material for interproximal restorations due to its favorable mechanical properties and durable adhesion. Interestingly, hydroxyapatite-containing toothpaste has been shown to induce calcium- and phosphate-rich precipitates on resin composite surfaces, suggesting a potential indirect remineralization effect through mineral deposition at the tooth–restoration interface (16). However, the influence of hydroxyapatite-based toothpaste—alone or in

combination with fluoride—on remineralization of initial interproximal caries adjacent to different restorative materials has not been adequately investigated.

Therefore, the aim of this in vitro study was to compare the remineralization effects of fluoride toothpaste, fluoride toothpaste containing hydroxyapatite, and HA toothpaste on artificial initial interproximal carious lesions adjacent to different Class II restorative materials. The null hypotheses were that fluoride toothpaste, fluoride toothpaste containing hydroxyapatite, and HA toothpaste showed no statistically significant differences in mineral density (MD) or lesion depth (LD) of artificial initial interproximal caries adjacent to restorations. And there was no statistically significant difference in MD and LD of artificial initial interproximal caries after contact with resin composite or GIC restorations.

2. Materials and Methods

The overall experimental design, procedural sequence of the study, and specimen preparation are illustrated in Fig. 1.

2.1 Sample size calculation

Sample size calculation for the micro-computed tomography (micro-CT) analysis was performed using G*Power software (version 3.1) based on MD data reported by Akkuc et al. (17), whose study design closely resembled the present investigation. With a 95% confidence level, 95% statistical power, and an effect size of 0.61, a minimum of nine specimens per group was required. To compensate for potential specimen loss or experimental error, ten specimens per group were included. For the surface roughness analysis, the sample size was determined with reference to Mielczarek et al. (18). Using a 95% confidence level, 95% statistical power, and an effect size of 0.83, a minimum of five specimens per group was required. To account for possible experimental variability, six specimens per group were included.

2.2 Specimen preparation

The study protocol was approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2024-031). Eighty extracted human maxillary premolars, obtained for orthodontic reasons, were used. Teeth were disinfected in 0.1% thymol solution for 7 days and used within 2 months of extraction (19). Only sound teeth free from white spot lesions, cracks, defects, or restorations were included, as confirmed under a stereomicroscope at $\times 20$ magnification (OLYMPUS SZ61, Japan).

To standardize the evaluation area, proximal enamel surfaces were flattened and polished with 600-grit silicon carbide paper to remove the fluoride-rich surface layer. Teeth were sectioned into enamel slabs measuring $2 \times 2 \times 3$ mm using a slow-speed cutting machine (Buehler, IL, USA). Each enamel slab was embedded in acrylic resin, and reference points were created on both the enamel surface and the resin

block. Specimens were sequentially polished with 600-, 800-, 1000-, and 1200-grit abrasive papers and ultrasonically cleaned in deionized water for 15 min.

2.3 Artificial caries formation

Specimens were coated with acid-resistant nail varnish (Revlon, New York, USA), leaving a central window of 1×1 mm for lesion formation. Each specimen was immersed individually in a demineralizing solution containing 2.0 mM calcium, 2.0 mM phosphate, and 75 mM acetic acid adjusted to pH 4.4 with 1 M KOH at 37°C for 48 h to induce artificial initial carious lesions (15). After demineralization, specimens were rinsed with deionized water for 20 sec and stored in artificial saliva for 24 h. Lesions were confirmed under a stereomicroscope ($\times 20$); specimens showing surface erosion were excluded. Baseline MD and LD were assessed using micro-CT (SKYSCAN 1272, Bruker, Germany), after which specimens were stored at 4°C under 100% relative humidity until further use.

2.4 Class II restorative procedure preparation

Eighty acrylic resin tooth-model blocks with standardized Class II cavities ($4 \times 4 \times 2$ mm³) were fabricated (Fig. 1B). Cavities were restored according to the manufacturers' instructions. Group C received a bulk-fill resin composite (Filtek™ One Bulk Fill Restorative; 3M ESPE, St. Paul, MN, USA), and Group G received a high-viscosity glass-ionomer cement (EQUIA Forte®; GC Corporation, Tokyo, Japan) using a Tofflemire matrix system. As acrylic resin models were used, no etching, conditioning, or bonding procedures were performed.

2.5 Experimental grouping and intervention

Enamel slabs were allocated to eight experimental groups using permuted-block randomization and attached to the designated restorative materials to simulate a natural interproximal contact point, as previously described (14) (Fig. 1C). Specimens were categorized based on the restorative material adjacent to the enamel slabs, with **C** indicating resin composite and **G** indicating GIC. Each experimental toothpaste group was accordingly divided into composite (C) and GIC (G) subgroups.

Group 1 (C1 and G1) included enamel slabs treated with fluoride (F) toothpaste; Group 2 (C2 and G2) included enamel slabs treated with fluoride toothpaste containing hydroxyapatite (F + HA); Group 3 (C3 and G3) included enamel slabs treated with hydroxyapatite-containing (HA) toothpaste; and Group 4 (C4 and G4) included enamel slabs treated without toothpaste (Control). Toothpaste slurries were prepared by mixing toothpaste with deionized water at a 3:1 (w/w) ratio (20). Composition of the tested toothpaste, batch numbers, and expiration dates in detail was shown in Table S1.

2.6 pH cycling

Specimens underwent a 14-day pH-cycling protocol at 37°C in an orbital shaker incubator (ES-20, Biosan, Latvia), adapted from previous studies (15, 21). The demineralizing solution contained 2.2 mM CaCl₂, 2.2 mM NaH₂PO₄, and 0.05 mM acetic acid (pH 4.7), while the remineralizing solution contained 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, and 0.15 M KCl (pH 7.0). Each daily cycle consisted of two 3-h demineralization

periods separated by a 2-h remineralization phase. Specimens were immersed in toothpaste slurry for 2 min twice daily, before the first and after the second demineralization period, and stored overnight in remineralization solution. All solutions were freshly prepared daily. Specimens were rinsed with deionized water between steps. After completion of pH cycling, specimens were stored in artificial saliva for 24 h prior to post-cycling micro-CT analysis.

2.7 micro-CT analysis

The MD and LD of enamel specimens were measured by micro-CT. For bone-mineral-density (BMD) calibration, Bruker micro-CT phantoms containing calcium-hydroxyapatite (CaHA) disks of 0.30 and 1.25 g cm⁻³ were scanned. The X-ray source was operated at 80 kV and 125 µA with a rotation step of 0.4°. A 1-mm aluminum filter was used. The images were set to 10 µm with a resolution of 1,024 x 1,024 pixel. NRecon reconstruction software (SkyScan Bruker, Germany) was used to reconstruct the scanning images and image planes were aligned using DataViewer software.

The MD was calculated and plotted against the depth within a centrally positioned volume of interest (100 × 100 × 250 µm³) using CTAn software (Bruker, Germany). The MD profile was converted to a relative MD profile by assuming sound enamel with a maximum MD (22, 23).

LD of each specimen was determined from the cross-sectional images. 10 cross-sectional images per enamel specimen were randomly selected and analyzed with ImageJ (National Institutes of Health, USA). An image area with a grayscale value of more than 95% was defined as sound enamel (24). The mean percentage change in MD and LD were calculated as previously described (25).

2.8 Surface roughness analysis

Six enamel specimens from each group were randomly selected for surface roughness analysis. Following 15 days of pH cycling and micro-CT evaluation, surface roughness (Ra) was measured at the central area of each specimen using a non-contact optical profilometer (Alicona, Graz, Austria) at 50× magnification over a 0.4 × 0.4 mm measurement area. Five scans were obtained from regions surrounding the center of each specimen, and the mean Ra value was calculated and recorded in nanometers (nm) to represent the surface roughness of each sample.

2.9 Surface characteristic evaluation

Surface characteristics were evaluated using scanning electron microscopy (FEI, Hillsboro, USA). Three specimens per subgroup were randomly selected, sputter-coated with gold, and examined at magnifications of ×1,000 and ×5,000. The most representative image of the enamel surface was captured.

Elemental analysis was performed using energy-dispersive X-ray spectroscopy (EDX) under high-vacuum conditions at an accelerating voltage of 20 kV to quantify the elemental composition, including calcium (Ca), phosphorus (P), and fluorine (F), at a magnification of ×500. Three measurements were obtained

from different regions of each specimen, and the mean value was calculated and recorded to represent the elemental composition of each sample.

2.10 Statistical analysis

Statistical analysis was performed using SPSS version 29.0.1 (SPSS Inc., Chicago, USA), with the level of significance set at 0.05. Data normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene’s tests, respectively. MD and LD within each group were compared before and after pH cycling using a paired t-test, with statistical significance defined as $p < 0.05$. The percentage changes in MD, LD, and surface roughness were analyzed using two-way analysis of variance (ANOVA), followed by Tukey’s post hoc test for multiple comparisons at a 95% confidence level.

3. Results

3.1 Mineral density

The MD data are presented in Tables 1. The mean baseline MD of artificial initial carious lesions across all specimens ranged from 19.95 to 21.75 gHAp/cm³, with no significant differences among groups at baseline ($p = 0.942$). Within the resin composite groups, no significant change in MD was observed following treatment with F toothpaste ($p = 0.548$). In contrast, significant mineral loss was detected in specimens treated with F + HA, HA toothpaste, and in the composite control group ($p = 0.017$, $p < 0.01$, and $p < 0.01$, respectively). For the GIC groups, no significant change in MD was observed with F + HA ($p = 0.918$), whereas significant mineral gain was observed in the F toothpaste, HA toothpaste, and control groups ($p < 0.01$, $p < 0.01$, and $p = 0.010$, respectively).

Table 1
Mineral density of artificial initial carious lesions before and after pH cycling.

Restorative material	Toothpaste	n	Baseline MD (gHAp/cm ³)	MD after pH cycling (gHAp/cm ³)	Change in MD (%)
Resin composite	Fluoride	10	19.953 ± 2.715	19.737 ± 2.694	-0.911 ± 5.620 [¶]
	Fluoride + HA	10	21.004 ± 3.094	19.968 ± 2.928 [†]	-4.785 ± 4.807 ^{¶¶}
	HA	10	21.044 ± 3.514	18.343 ± 3.325 [†]	-12.866 ± 5.285 [¶]
	No toothpaste	10	21.387 ± 2.738	17.695 ± 3.285 [†]	-17.333 ± 10.435
GIC	Fluoride	10	21.752 ± 3.089	22.875 ± 3.133 [†]	5.291 ± 3.090 ^{¶*}
	Fluoride + HA	10	21.013 ± 3.425	21.054 ± 3.276	0.371 ± 4.125 ^{¶*}
	HA	10	21.468 ± 3.242	22.950 ± 3.204 [†]	7.116 ± 3.884 ^{¶*}
	No toothpaste	10	21.429 ± 2.299	22.090 ± 2.754 [†]	2.915 ± 2.927 ^{¶¶*}
Data are presented as mean ± standard deviation (SD). HA, hydroxyapatite; GIC, glass ionomer cement.					
†Significant difference between baseline and post-pH cycling values within the same experimental group ($p < 0.05$; paired t -test).					
Within each restorative material, different lowercase superscript letters in the percentage-change column indicate significant differences among toothpaste groups ($p < 0.05$; Tukey post hoc test following two-way ANOVA).					
*Significant difference between resin composite and GIC for the corresponding toothpaste group ($p < 0.05$).					

Two-way ANOVA revealed a significant interaction between toothpaste type and restorative material ($p < 0.01$), indicating that changes in MD were dependent on the combination of toothpaste and restorative material. Toothpaste type had a significant main effect on percent MD change ($p < 0.01$), and restorative material also showed a significant main effect ($p < 0.001$). Within the resin composite groups, F + HA did not differ significantly in percent MD change compared with F or HA toothpaste ($p = 0.599$ and $p = 0.060$, respectively) but demonstrated a significantly greater percent MD change than the control group ($p = 0.001$). HA toothpaste produced the lowest percent MD change among the toothpaste groups and showed a significantly lower percent change compared with F toothpaste ($p = 0.002$). Within the GIC groups, F toothpaste containing hydroxyapatite differed significantly in percent MD change compared

with both F toothpaste ($p = 0.017$) and HA toothpaste ($p < 0.001$). However, none of the toothpaste groups differed significantly from the control group ($p = 0.449$, $p = 0.372$, and $p = 0.055$, respectively).

When restorative materials were compared within each toothpaste type, resin composite specimens exhibited significantly lower percent MD change than glass ionomer cement specimens for F, F + HA, and HA toothpaste ($p = 0.014$, $p = 0.041$, and $p < 0.001$, respectively) (Tables 1).

3.2 Lesion depth

The LD data are presented in Table 2. The mean baseline LD of artificial initial carious lesions ranged from 110.70 to 112.40 μm , with no significant differences among groups at baseline ($p = 0.999$). Paired t-test analysis demonstrated a significant increase in LD in all resin composite groups following treatment with F toothpaste, F + HA, HA toothpaste, and in the control group (all $p < 0.001$). In contrast, the GIC groups showed a significant reduction in LD after treatment with F, F + HA, and HA toothpaste ($p = 0.010$, $p = 0.007$, and $p = 0.011$, respectively). However, a significant increase in LD was observed in the GIC control group ($p < 0.001$) (Table 2).

Table 2
Lesion depth of artificial initial carious lesions before and after pH cycling.

Restorative material	Toothpaste	n	Baseline LD (gHAp/cm ³)	LD after pH cycling (gHAp/cm ³)	Change in LD (%)
Resin composite	Fluoride	10	111.767 ± 8.408	134.833 ± 14.096 [†]	20.459 ± 5.587 [Ⓜ]
	Fluoride + HA	10	111.167 ± 7.101	132.200 ± 12.859 [†]	18.800 ± 6.313 [Ⓜ]
	HA	10	111.066 ± 6.625	128.266 ± 10.057 [†]	15.581 ± 7.476 [Ⓜ]
	No toothpaste	10	110.700 ± 4.517	138.300 ± 11.458 [†]	24.398 ± 8.606 [Ⓜ]
GIC	Fluoride	10	111.667 ± 7.445	108.067 ± 8.225 [†]	-3.234 ± 3.161 ^{Ⓜ*}
	Fluoride + HA	10	112.400 ± 8.362	109.333 ± 8.038 [†]	-2.693 ± 2.337 ^{Ⓜ*}
	HA	10	112.266 ± 7.359	108.500 ± 5.013 [†]	-3.213 ± 3.112 ^{Ⓜ*}
	No toothpaste	10	111.633 ± 5.734	115.533 ± 5.994 [†]	3.501 ± 1.491 ^{Ⓜ*}
Data are presented as mean ± standard deviation (SD). HA, hydroxyapatite; GIC, glass ionomer cement.					
†Significant difference between baseline and post-pH cycling lesion depth within the same experimental group ($p < 0.05$; paired t -test).					
Within each restorative material, different lowercase superscript letters in the percentage-change column indicate significant differences among toothpaste groups ($p < 0.05$; Tukey post hoc test following two-way ANOVA). Groups sharing a superscript letter are not significantly different.					
*Significant difference between resin composite and GIC for the corresponding toothpaste group ($p < 0.05$).					

Two-way ANOVA revealed significant main effects of restorative material and toothpaste type on percent LD change (both $p < 0.001$), while no significant interaction between these factors was detected ($p = 0.553$), indicating that the effect of toothpaste on LD change was independent of the restorative material. Within the resin composite groups, only the HA toothpaste group showed a significantly smaller increase in percent LD compared to the control group ($p = 0.028$). F + HA did not differ significantly from F or HA toothpaste ($p = 0.505$ and $p = 0.235$, respectively). Within the GIC groups, all toothpaste-treated specimens exhibited significantly lower percent LD change compared with the control group (all $p < 0.001$). Although F + HA showed a numerically lower percent LD change than F and HA toothpaste, these differences were not statistically significant ($p = 0.967$ and $p = 0.970$, respectively). Across all toothpaste types, specimens restored with resin composite exhibited a significantly greater

increase in percent LD change compared with those restored with GIC ($p < 0.001$). Representative micro-CT cross-sectional images of the enamel specimens at baseline and after the 14-day pH-cycling protocol are presented in Fig. 2.

3.3 Surface roughness evaluation

Normality of the data was assessed using the Shapiro–Wilk test, which confirmed a normal distribution. The results of the two-way analysis of variance, presented in Table S2, demonstrated a statistically significant effect of toothpaste type on surface roughness ($p < 0.01$). In contrast, neither the type of restoration nor the interaction between restoration type and toothpaste had a statistically significant effect ($p = 0.682$ and $p = 0.156$, respectively).

Post hoc comparisons were conducted using Tukey’s test. The mean and standard deviation of the surface roughness values was presented in Table 3. Within each restorative material (resin composite and GIC), a consistent pattern was observed. The no-toothpaste control group exhibited the highest surface roughness values, with statistically significant differences identified among the other toothpaste groups ($p < 0.01$). This was followed sequentially by the F toothpaste, F + HA, and the HA toothpaste group. The HA toothpaste demonstrated significantly lower surface roughness compared with the F toothpaste ($p < 0.01$). However, no statistically significant difference was observed between the HA toothpaste and the F + HA in either restorative material ($p > 0.05$). Additionally, comparisons within the same toothpaste type across different restorative materials revealed no statistically significant differences ($p > 0.05$).

Table 3
Mean and standard deviation of surface roughness (Ra) values (nm)

Restoration	Tested Groups				
	No toothpaste	Fluoride toothpaste	Fluoride + HA	HA toothpaste	P - value
Composite	2771.98 ± 265.69 ^{Aa}	1826.99 ± 159.63 ^{Ab}	1493.17 ± 289.44 ^{Abc}	1203.18 ± 251.99 ^{Ac}	< 0.01
GIC	2494.66 ± 315.96 ^{Aa}	1996.87 ± 326.34 ^{Ab}	1585.17 ± 255.25 ^{Abc}	1345.61 ± 229.74 ^{Ac}	< 0.01
P - value	0.079	0.276	0.553	0.360	
Different superscript lowercase letters within the same row indicate statistically significant differences between groups ($p < 0.05$), as determined by two-way ANOVA. Different uppercase letters within the same column indicate a statistically significant difference between restoration types ($p < 0.05$).					

3.4 Surface morphology and elemental analysis

The SEM photomicrographs (Fig. 3) of enamel specimens in contact with resin composite and GIC in the no-toothpaste group demonstrated greater surface porosity compared with the other toothpaste groups. This observation was particularly evident at 5000× magnification, with pronounced porosities clearly visible. In the F toothpaste group, signs of enamel remineralization and a relatively smoother surface were observed; however, localized surface irregularities remained evident. In contrast, specimens treated with F + HA and HA toothpaste exhibited a more homogeneous and smooth surface morphology, without apparent irregularities or porosity, in both restorative materials at 1000× and 5000× magnifications.

EDS analysis showed variations in fluorine, calcium, phosphorus, and the Ca/P ratio among the experimental groups (Table 4). In specimens adjacent to resin composite, fluorine was not detected in the no-toothpaste and HA toothpaste groups, whereas low fluorine levels were detected in the fluoride (0.55 wt%) and F + HA (0.28 wt%) toothpaste groups. Calcium and phosphorus contents ranged from 27.11 to 28.29 wt% and from 12.51 to 13.25 wt%, respectively, with Ca/P ratios ranging from 2.11 to 2.22.

Table 4
Elemental composition (wt%) of fluorine, calcium, and phosphorus and calcium-to-phosphorus (Ca/P) ratio in enamel specimens after pH cycling.

Restorations	Conditions	Elemental analysis (%wt)			Calcium/phosphate (Ca/P) ratio
		Fluorine (F)	Calcium (Ca)	Phosphate (P)	
Resin composite	No toothpaste	0.00	27.89	13.25	2.11
	Fluoride toothpaste	0.55	27.82	12.51	2.22
	Fluoride + HA	0.28	28.29	12.87	2.20
	HA toothpaste	0.00	27.11	12.62	2.15
GIC	No toothpaste	0.58	29.53	13.97	2.11
	Fluoride toothpaste	0.73	21.91	10.94	2.00
	Fluoride toothpaste + HA	0.37	24.45	11.70	2.09
	HA toothpaste	0.70	29.83	12.64	2.36

In specimens adjacent to GIC, fluorine was detected in all groups, including the no-toothpaste control (0.58 wt%), with values ranging from 0.37 to 0.73 wt%. The highest fluorine content was observed in the fluoride toothpaste group (0.73 wt%), followed by the HA toothpaste group (0.70 wt%). Calcium content ranged from 21.91 to 29.83 wt%, phosphorus content from 10.94 to 13.97 wt%, and Ca/P ratios from 2.00 to 2.36. The highest Ca/P ratio was observed in the GIC/HA toothpaste group (2.36), which also showed the highest calcium content (29.83 wt%).

4. Discussion

This in vitro study compared the remineralizing effects of F, F + HA, and HA toothpastes on artificial initial interproximal carious lesions adjacent to Class II resin composite and GIC restorations. Remineralization was assessed by micro-CT based on percentage changes in mineral density (%MD) and lesion depth (%LD). A 14-day pH cycling protocol simulating a high caries-risk oral environment (26) was adapted from Dechachart et al. (21) and Phyo et al. (15). The interproximal contact configuration was reproduced using acrylic resin premolar models based on the method described by Theerarath and Sriarj (14). The first null hypothesis was partially rejected because the effects of toothpaste type on MD and LD varied according to the restorative material and outcome assessed. The second null hypothesis was rejected because lesions adjacent to GIC and resin composite showed significantly different changes in MD and LD.

The restorative material adjacent to the lesion was an important determinant of the remineralization response. Across toothpaste conditions, lesions adjacent to GIC showed greater MD gains and less progression in LD than those adjacent to resin composite, consistent with previous studies demonstrating a protective effect of GIC on adjacent enamel (14, 15). This favorable response may be attributed to the fluoride-releasing and ion-exchange properties of GIC (27), which can create a local environment conducive to remineralization. The significant MD gain observed even in the untreated GIC control further supports an independent contribution of the restorative material. EDS findings were consistent with this interpretation: fluorine was detected in all enamel specimens adjacent to GIC, including the no-toothpaste control (0.58 wt%), whereas it was not detected in the resin composite control or composite/HA groups. In the resin composite groups, fluorine was detected only after application of fluoride-containing toothpastes. This pattern suggests that GIC provided an additional local source of fluoride independent of toothpaste exposure. However, because EDS primarily provides elemental information from the analyzed surface region, it cannot establish the chemical form or depth of fluoride incorporation. In contrast to the micro-CT findings, restorative material had no significant effect on surface roughness within any toothpaste condition ($p = 0.079-0.553$), and SEM showed broadly similar toothpaste-dependent surface patterns for both restorative materials. These findings suggest that restorative material primarily influenced lesion-level mineral changes, whereas toothpaste type had a greater effect on enamel surface characteristics.

Within the GIC groups, F and HA toothpastes produced significant MD gains and LD reductions following pH cycling, whereas F + HA toothpaste significantly reduced LD but did not significantly change MD. Although %MD change with F + HA was significantly lower than that with F and HA toothpastes, none of the toothpaste groups differed significantly from the untreated GIC control, suggesting that fluoride release from GIC itself contributed substantially to the remineralizing environment. Surface findings were consistent with this pattern. Ra decreased from 2494.66 ± 315.96 nm in the untreated GIC group to 1996.87 ± 326.34 nm with F, 1585.17 ± 255.25 nm with F + HA, and 1345.61 ± 229.74 nm with HA toothpaste. HA produced significantly lower Ra than the no-toothpaste and F groups, while F + HA showed an intermediate response. SEM similarly showed pronounced porosity in untreated specimens,

residual surface irregularities after F treatment, and comparatively smoother, more homogeneous surfaces with reduced apparent porosity after F + HA and HA treatment.

The favorable micro-CT response to HA toothpaste adjacent to GIC was accompanied by a distinct elemental profile. The GIC/HA group showed the highest calcium content (29.83 wt%) and Ca/P ratio (2.36), together with detectable fluorine (0.70 wt%). Taken together, the MD gain, LD reduction, lower surface roughness, smoother SEM morphology, and calcium-rich elemental composition are consistent with mineral deposition on the enamel surface in an environment containing HA-derived calcium and phosphorus and GIC-derived fluoride. Such conditions may favor mineral reprecipitation during pH cycling (2, 28). However, these findings do not constitute direct evidence of fluorohydroxyapatite or fluorapatite formation, because EDS cannot identify crystalline phases and a high Ca/P ratio alone does not establish the composition of the deposited mineral. Direct mineral-phase characterization would therefore be required to confirm this mechanism.

The F + HA group showed a different response. Despite lower surface roughness, reduced apparent porosity on SEM, and detectable fluorine (0.37 wt%), no significant MD gain was observed. This discrepancy suggests that improvement in surface characteristics does not necessarily correspond to mineral recovery throughout the lesion body. One possible explanation relates to the fluoride source in F + HA toothpaste. Sodium monofluorophosphate (MFP) requires enzymatic hydrolysis to release free fluoride ions (29), whereas phosphatase activity was absent from the present pH-cycling model. Consequently, the fluoride contribution of F + HA toothpaste may have been underestimated under the experimental conditions.

A different pattern was observed in lesions adjacent to resin composite. Overall, these groups showed net mineral loss after pH cycling, indicating that none of the tested formulations completely counteracted the demineralizing challenge. Fluoride toothpaste maintained relatively stable MD, whereas significant mineral loss occurred in the F + HA, HA, and control groups. The protective effect of fluoride toothpaste may be related to enhanced resistance of the enamel mineral phase to subsequent acidic challenge through fluoride-associated remineralization (2, 28). Despite the overall mineral loss, HA toothpaste had a favorable effect on lesion depth and was the only formulation to produce a significant LD reduction compared with the control ($p = 0.028$). Surface findings also demonstrated a clear toothpaste-dependent response. Mean Ra decreased from 2771.98 ± 265.69 nm in the no-toothpaste group to 1826.99 ± 159.63 nm with F, 1493.17 ± 289.44 nm with F + HA, and 1203.18 ± 251.99 nm with HA toothpaste. HA produced significantly lower roughness than the no-toothpaste and F groups, whereas F + HA showed intermediate values. SEM findings paralleled the roughness measurements: untreated specimens exhibited pronounced surface porosity, particularly at 5000 $\times$ magnification; F-treated specimens showed a comparatively smoother surface with residual localized irregularities; and F + HA- and HA-treated specimens exhibited more homogeneous surfaces with little apparent porosity, consistent with previous studies (6, 29, 30). These findings suggest that HA may preferentially act at or near the lesion surface and within enamel microporosities, thereby reducing lesion extension without necessarily producing a corresponding increase in bulk MD.

EDS further supports a fluoride-independent surface effect of HA in the composite group. No fluorine was detected in the composite/HA specimens, whereas fluorine was detected following treatment with F (0.55 wt%) and F + HA (0.28 wt%) toothpastes. Thus, the favorable surface morphology observed with HA toothpaste cannot be attributed to fluoride released from the restoration and is more consistent with an effect related to the HA formulation itself. However, the Ca/P ratio of 2.15 should be interpreted descriptively and does not identify the chemical nature of any deposited calcium-phosphate phase. Similarly, F + HA toothpaste reduced surface roughness and produced a relatively homogeneous SEM appearance despite the absence of a significant MD benefit, further indicating that surface modification and subsurface remineralization are related but distinct processes. The HA component may facilitate superficial mineral deposition, whereas limited enzymatic activation of MFP in the present model may have reduced the fluoride contribution to deeper lesion remineralization.

The present findings may also help explain inconsistencies among previous studies comparing fluoride- and HA-containing toothpastes. No significant differences in remineralization were observed between F + HA and F toothpastes in either restorative material group, consistent with Hornby et al. (12). In contrast, Körner et al. (31) reported greater remineralization with 1,400 ppm fluoride than with 10% HA toothpaste, whereas Tschoppe et al. (32) found no significant difference in MD between 20% HA and 1,400 ppm fluoride toothpastes but reported a significant reduction in LD with HA. These discrepancies may reflect methodological differences, including HA concentration, fluoride source, remineralization protocol, and the presence or absence of pH cycling. In addition, the present surface roughness and SEM findings suggest that HA-containing formulations may exert a pronounced surface effect through mineral deposition, adsorption, and occlusion of microporosities, whereas changes in overall lesion MD require mineral incorporation within the lesion body. Therefore, improved surface morphology does not necessarily correspond directly to increased MD.

To the authors' knowledge, this is the first in vitro study to examine HA-containing toothpastes in initial interproximal carious lesions adjacent to different Class II restorative materials. The findings suggest that both restorative material and toothpaste formulation influence lesion behavior. GIC provided a more favorable mineral environment, while HA-containing toothpastes improved enamel surface characteristics, with HA toothpaste showing particularly favorable outcomes adjacent to GIC. These findings indicate that interactions between restorative materials and daily oral-care products may be relevant to the management of early lesions adjacent to restorations. However, clinical recommendations cannot be made from this in vitro study, and clinical studies are needed to determine whether these effects translate into meaningful reductions in caries progression.

Several limitations should be acknowledged. The pH-cycling model cannot fully reproduce the complexity of the oral environment, including pellicle formation, biofilm dynamics, and phosphatase activity. The absence of phosphatase may have reduced fluoride release from the MFP-based F + HA toothpaste relative to clinical conditions (33), potentially contributing to the observed MD response. Although micro-CT, surface roughness, SEM, and EDS provided complementary information, SEM and EDS primarily characterize surface morphology and elemental composition and cannot determine the

chemical or crystalline nature of deposited mineral. Future studies incorporating direct mineral-phase characterization and clinically relevant models are warranted. In addition, HA possesses other biological properties, including dentinal tubule occlusion (34) and protection against acid erosion (6, 35), which were not assessed in the present study but may be clinically relevant for patients with dentinal hypersensitivity or erosion risk. Further clinical studies in these populations are needed.

Regarding the clinical implications, the findings suggest that the choice of restorative material may influence the remineralization potential of adjacent early carious lesions, with GIC providing a more favorable mineral environment than resin composite. However, the findings for 1500-ppm F toothpaste demonstrated a remineralizing effect, supporting its continued use as an effective option for caries prevention. HA-containing toothpastes may additionally improve enamel surface characteristics, particularly in lesions adjacent to GIC. Smoother enamel surfaces may offer potential clinical benefits by reducing plaque retention and bacterial adhesion (7, 8). In addition, HA-containing toothpaste maybe use in patients with xerostomia or reduced salivary flow, conditions that compromise the salivary reservoir of calcium, phosphate, and fluoride ions (36–38), calcium phosphate-based agents have demonstrated caries-preventive efficacy by directly supplementing ion availability at the tooth surface, independent of salivary buffering capacity (39) However, clinical studies are required before specific toothpaste–restoration combinations can be recommended.

6. Conclusion

The adjacent restorative material significantly influenced changes in MD and LD of initial interproximal carious lesions, with GIC providing a more favorable remineralizing environment than resin composite. Toothpaste effects varied according to the restorative material and outcome assessed. HA toothpaste showed favorable effects on LD and enamel surface characteristics, particularly adjacent to GIC, whereas F + HA did not demonstrate a consistent advantage over conventional F toothpaste. These findings suggest that both restorative material and toothpaste formulation may influence the behavior of early carious lesions adjacent to Class II restorations; however, clinical studies are required to confirm their clinical relevance.

Declarations

Ethics approval

This study was approved by the Human Ethics Committee (Reference number (HREC-DCU 2024-031)) and Institutional Biosafety Committee (DENT CU-IBC 019/2024), Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Informed consent

The present study did not involve human participants

Conflict of interest

The authors declare no conflicts of interest.

Author Contribution

Awanrut Muangthong: Methodology, validation, investigation, data collection and analysis, writing-original draft preparation. Dusit Nantanapiboon: Conceptualization, methodology, writing (review and editing), supervision. Wannakorn Sriarj: Conceptualization, methodology, writing (review and editing), supervision, project administration, funding acquisition. All authors read and approved the final manuscript.

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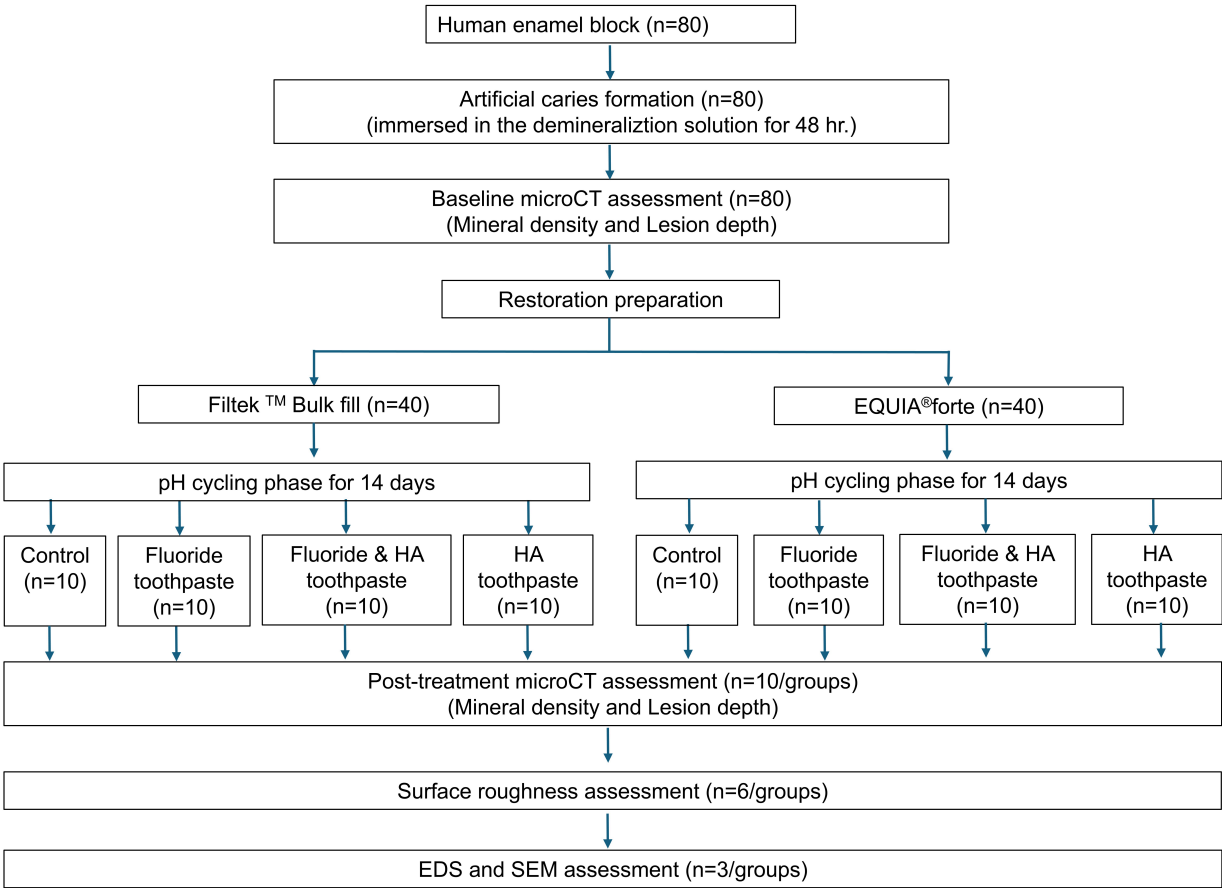
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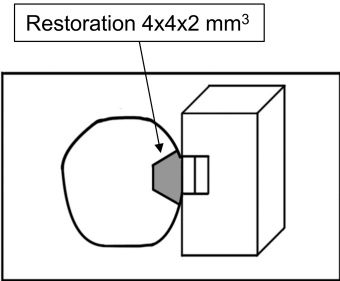
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Figures

A.



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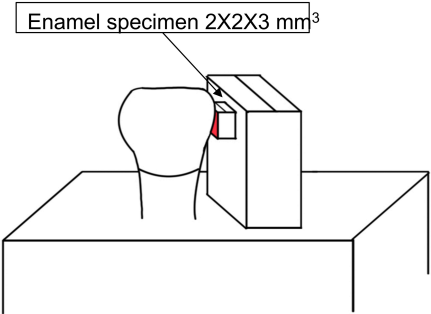


Figure 1

Experimental design and specimen preparation. (A) Study flow diagram. (B) Frontal view and (C) superior view of the experimental block setup designed to simulate proximal contact between the restorative material and the adjacent enamel specimen.

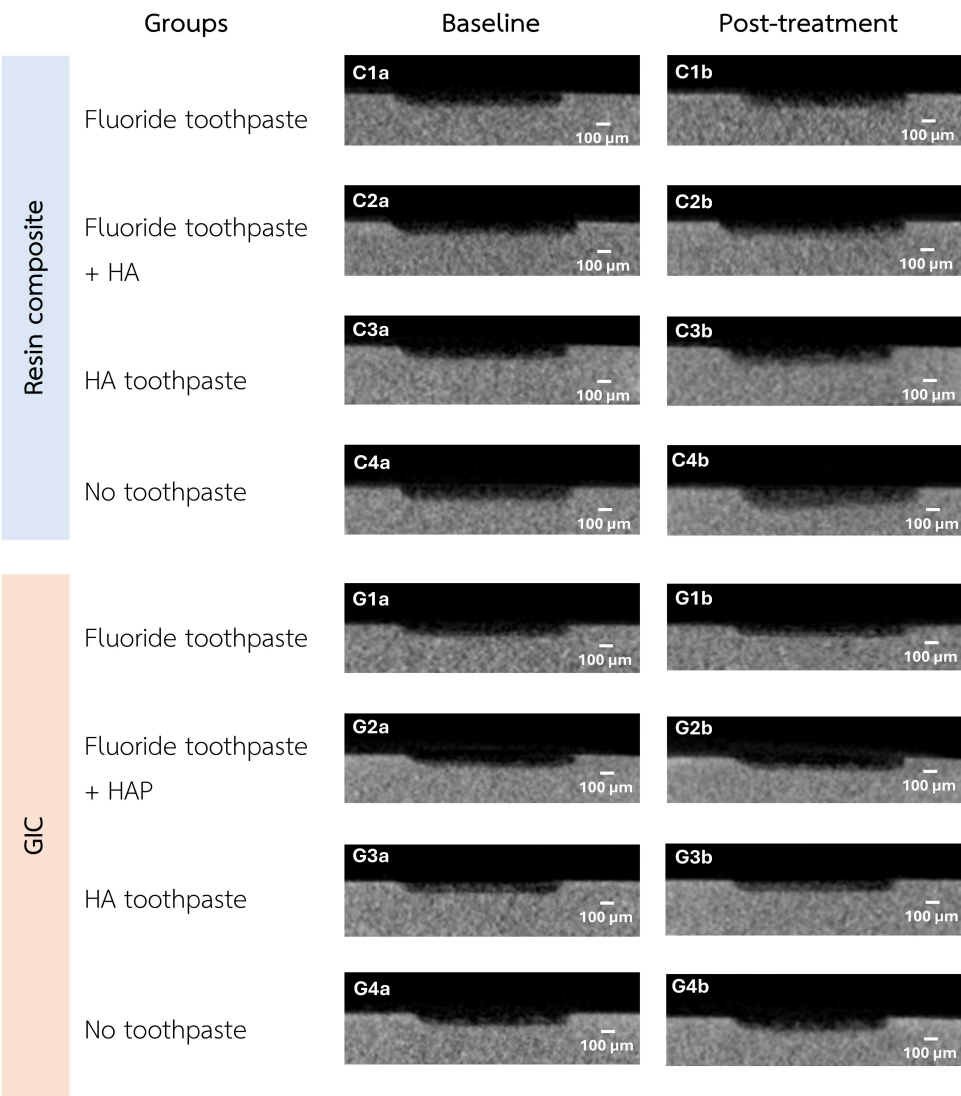


Figure 2

Representative micro-CT cross-sectional images of enamel specimens at baseline (C1a-C4a, G1a-G4a) and following post 14-day pH cycling (C1b-C4b, G1b-G4b). Rows 1–4: resin composite groups (C1–C4); rows 5–8: GIC groups (G1–G4). C1/G1 = fluoride toothpaste; C2/G2 = fluoride+HA toothpaste; C3/G3 = HA toothpaste; C4/G4 = no toothpaste control.

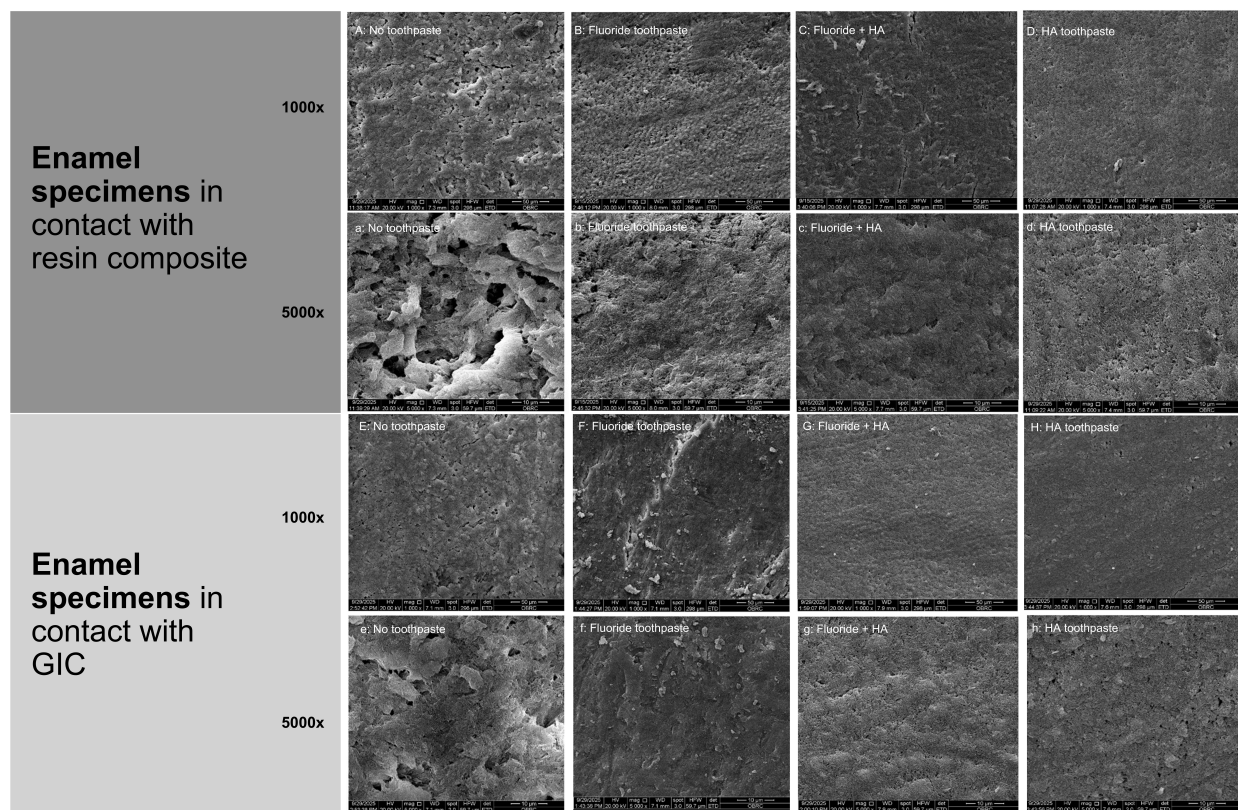


Figure 3

SEM photomicrographs of enamel specimens in contact with resin composite (A–D: 1000×; a–d: 5000×) and GIC (E–H: 1000×; e–h: 5000×) following exposure to different toothpaste types.

Supplementary Files

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