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Original Article

Remineralization potential of apacider mangosteen adhesive pastes on artificial carious lesions

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KEYWORDS
Apacider mangosteen adhesive paste;
Artificial carious lesion;
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Remineralization

Abstract *Background/purpose:* Attention to caries administration has altered toward an early finding of lesions and targeted to noninvasive management with a remineralizing agent. This study compared the remineralization potential of apacider mangosteen adhesive pastes (AMAP), 500-ppm fluoride toothpaste (FT500), and 1000-ppm fluoride toothpaste (FT1000) on artificial caries.

Materials and methods: Artificial caries were generated to enamel of eighty extracted human premolars. The specimens were allocated to four groups and subjected to pH-cycling with the application of testing agents (2 min each, for 10 days): (1) AMAP, dairy, (2) FT500, twice a day, (3) FT1000, twice a day, and (4) no treatment (NT). The surface microhardness was determined before demineralization, after demineralization, and after application of pH-cycling. The hardness, percent of hardness recovery (%HR), and percent of remineralization potential (%RP) were analyzed with ANOVA and Bonferroni's test ($\alpha = 0.05$). Polarized light microscopy (PLM) was assessed for lesion depth.

Results: Significant differences in remineralization were observed upon various agents compared to NT ($P < 0.05$). A significant difference in remineralization was found among AMAP, FT1000, and FT500 ($P < 0.05$). No significant difference in %HR and %RP was observed between AMAP and FT1000 ($P > 0.05$). PLM signified greater decrease in depth for AMAP, compared to FT1000 and FT500, but no depth reduction for NT.

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Conclusion: AMAP possesses comparable remineralization ability to FT1000. However, decreasing in depth of carious lesions was evinced with using AMAP more than FT1000 and FT500. AMAP was recommended as a potential remineralization material for handling initial caries.

Introduction

Dental caries is a common infectious disease that widely exaggerates the oral well-being of persons during this pandemic.[1] It can cause pain and discomfort, steering to limitations in terms of function and aesthetics.[2] A recent study reported that three-year-old children who have recently completed deciduous teeth had 51.7% of dental caries and decayed, missing, or filled teeth (DMFT) for 2.7 per person. While five-year-old children had 78.5% of dental caries and DMFT 4.4 per person.[3] Although the rate of dental caries is reduced, it is still high when compared with the previous reports.[3] Dental caries is an enthusiastic interchanging mechanism that naturally happens between the demineralized and remineralized process of the inorganic segment of the tooth structure.[4] The preponderance of the demineralized process can cause carious lesions and cavitation on the tooth surface. The carious lesion may progress or reverse based on the equilibrium between pathologic influences, comprising bacteria, fermentable carbohydrates, saliva dysfunction, and the protective mechanism of the remineralized and antibacterial products in adequate salivation. The initial carious lesions appear as white spots and locate at the shallow layer of the enamel surface.[4] The initial carious lesion represents the earliest signal of decay, in which the superficial surface of enamel continues undamaged whilst the underneath level is decalcified, thus running to the dental cavity if no sufficient treatment integrates. For the early enamel demineralization stage, the interprismatic inorganic contents are eradicated, which is consequently kept on with a sound superficial foundation of the initial carious lesion.[1] The process of demineralized enamel is slowly progressed, which allows for the feasible reverse process in case of the progression is initially identified and effectively treated. Initial carious lesions can be arrested and reversed by remineralization.[5] It can be restored to a healthy enamel as normal or stop spreading.[6] Lately, a traditional attitude in restorative treatment is primely concentrated on remineralized schemes to regenerate the tooth structure. Early detection of carious lesions, in common with the conventionally treated process, helps decrease significant expenses in dental care. Contemporary research has been penetrating for advanced processes to early detect caries and to render the non-invasive therapy feasible.

The enamel demineralization comprises the damage of inorganic compositions of hydroxyapatite (HA) crystalline configuration from the superficial and sub-surface area, steering to the attack of acid from bacterial plaque.[7] The mechanism of restoring the crystalline HA with inorganic particles was described as the process of remineralization. This naturally occurring process achieved the reestablishing of the loss of crystalline HA complex. The implemented procedure relates to the neutralized pH in physiological conditions in which the phosphate ions (PO_4^{3-}) and calcium ions (Ca^{2+}) from the saliva are reinstalled back to the caries lesion, serving as the foundation of the greater crystalline HA that is rather stronger to resist to acid attack.[8] Fundamentally, the demineralized–remineralized phenomenon happens simultaneously at the surface of enamel, and a substantial amount of inorganic particles are deficient from the crystalline HA complex with no damage to its foundation. Whilst the demineralized procedure is dominant, it destroys the HA crystalline integrity and eventually initiates cavitation. However, partially demineralized HA crystalline structure can be restored to its primitive stage if it is sufficiently exposed to the intraoral situations that encourage remineralization. Upon exposure to suitable oral circumstances, the remineralized process can increase dominance, leading to the restorable condition of the carious lesion. Once the escalation of protective aspects, the process can lean the equilibrium toward a remineralized process and initiate a reversible initial carious lesion.[9] To promote a remineralized process of the carious lesion, the increase in the quantity of Ca^{2+} or F^- in saliva appears to be essential. In this perspective view, remineralization has been advocated as a potential method for the contemporary management of dental caries.[9]

Numerous remineralization agents have been marketed in several forms, including sealants, chewing gums, mouth rinses, and toothpaste. Fluoride has been acknowledged as a substantial agent used for remineralization, and it has been utilized to stimulate the formation of fluorapatite, which is capable of increasing the acid resistance of dental enamel.[10] Fluoride is capable of inhibiting inorganic loss from the surface of dental enamel by encouraging adsorption to the partially demineralized crystalline lattices as well as attracting PO_4^{3-} and Ca^{2+} ions from the saliva fluid for developing the fluorapatite on the surface of the enamel, thus enhancing endurance for the enamel to confront the acid attack. Even though fluorapatite is greater stable and better resistant to acid than hydroxyapatite, a restricted remineralized process is achievable, due to the available quantity of PO_4^{3-} and Ca^{2+} ions in the oral circumstances.[11] Fluoride cannot steer the genesis of ordered and oriented mineral contents on the enamel surface in physiological conditions, which is essential for improving the mechanical properties of enamel. Fluoride simply endeavors to decrease the dissolution of hydroxyapatite instead of endorsing remineralization to counteract

the inorganic damage of the hydroxyapatite crystalline structure. It significantly affects flat enamel surface with relatively fewer effect on pit and fissure. However, an extreme rate of fluoride installation to the superficial shell of enamel probably obstructs the diffusion of the ions into the sub-surface layer of carious lesion, consequently, resulting in frustration to accomplish a mature remineralized process.[12] Furthermore, fluoride consumption for the period of dentitional development tends to enhance the possibility to develop fluorosis.[8] Therefore, the exploration of other materials that are capable of enhancing the remineralization process without the risks correlated with fluoride is a challenging paradigm.

Apacider mangosteen adhesive pastes (AMAP) were newly developed materials as a new alternate product for the management of initial carious lesions.[13] The AMAP is mainly comprised of apacider-AW® (Sangi, Tokyo, Japan) as a remineralizing agent and α-mangosteen as an antibacterial agent. The apacider-AW® is an inorganic antimicrobial agent, based on apatite containing silver and zinc metals.[14] An in vitro study of apacider on the enamel surface revealed a significantly increased enamel microhardness and increased remineralization of white spot lesions at a level similar to fluoride varnish and casein phosphor peptide amorphous calcium phosphate (CPP-ACP).[15] The AMAP is capable of enhancing remineralization activity from calcium-phosphate, while the α-mangosteen strongly promotes the antibacterial activity against cariogenic *Streptococcus mutans* from silver ions.[13] A study showed that AMAP application can provide acid resistance and enhance consistent mineral gain during the acid attack on artificial carious lesions.[13] The product is prepared in the form of an adhesive paste that can be applied onto the tooth surface. Since the adhesiveness of AMAP, it can stay on the tooth surface for a longer period compared to other remineralized products. Furthermore, the product possesses in white color, so it does not cause a yellowish staining on the surface of the tooth.[14,16]

Currently, the effective method to prevent dental caries is based on the use of fluoride. The children's teeth are recommended to brush as the eruption of primary dentition, at approximately 6 months, and firstly visited dental clinic approximately at the age of 1 year. Yet, excessive ingestion of fluoride during teeth development can cause noticeably detectable alterations in the structure of enamel such as dental fluorosis and staining. Hence, it is recommended by the centers for disease control and prevention (CDC) to start using toothpaste containing fluoride (FT) with 2 years old children. For children aged below 3 years, the amount of FT at a grain size of rice smearing on a toothbrush should be used. While children aged between 3 and 6 years, the amount of FT at the size of a pea (0.25 g) is suggested. While children aged above 6 years old, the appropriate development of reflex in swallowing is mature to avoid unintentional fluoride consumption. Still, some studies reported that children swallowed the FT while brushing their teeth, especially in young children, and when the toothpaste contains fluoride in large quantities.[17] Currently, toothpaste has been developed for young children and contains a low amount of fluoride.[18] The study has found that 250-ppm FT has less efficacy in dental caries prevention than 1000-ppm FT. However, 500- to 550-ppm FT may be effective in preventing dental caries equivalent to 1000-ppm FT.[18] Whereas, AMAP comprises an inorganic antimicrobial agent based on silver and zinc ions that are low toxic and enhances remineralizing activity from calcium-phosphate. The α-mangosteen is a natural extract from mangosteen peel that is effective in preventing dental caries and also not harmful.[19] As such, the objectives of this study were to assess the effects of AMAP in comparison to 500-ppm and 1000-ppm FT on remineralization of artificial enamel carious lesions. The results are considered a new alternative to prevent initial caries in the future. The null hypothesis for this study is no significant differences between AMAP and FT either 500- or 1000-ppm on remineralization of artificial enamel carious lesions.

Materials and methods

The experiment was approved by the KKU ethic committee for research in humans (Reference No: HE 612093) and followed the CRIS guidelines for in vitro study. The sample size was estimated according to Equation (1) using the Pi-face program version 1.76 (University of Iowa, Iowa, IA, USA) based on the former study[20] with the tests powers = 0.95, and α-error = 0.05.

$$\text{N per group} = \frac{\left(Z_{\alpha/2} + Z_{\beta}\right)^2 \left(s_1{}^2 + s_2{}^2\right)}{\left(\mu_1 - \mu_2\right)^2} \quad (1)$$

Which: Z_{α}=normal standard deviation = 1.96 (α = 0.05), Z_{β}=normal standard deviation = 1.28 (β = 0.1), $\mu_1 - \mu_2$ = difference of mean between groups = 7, and s = standard deviation (s_1 = 6.3, s_2 = 6.4).

The sample size of 20 samples per group was performed in the investigation.

Specimen preparation

Eighty human-extracted premolars through the indication of orthodontic treatment, with no developmental anomalies, white spot lesions, dental fluorosis, dental caries, abrasions, crazed lines, or fracture lines were chosen for the experiment. Each patient and guardian was informed and signed the informed consent before extraction. The extracted teeth were preserved in the thymol 0.1% solution (M-Dent, Bangkok, Thailand) till they were used for the experiment. The crown portion was separated from the root portion with a diamond blade under continuous cooling in a precise sectioning instrument (Mecatome-T180, Presi, Eybens, France) [Fig. 1a-(1)]. The crown portion was surrounded with the resin acrylic by leaving the buccal surface of enamel above the resin block [Fig. 1a-(2)]. The exposed enamel surface was coated with the nail varnish (Revlon, New York, NY, USA) except for the region of 4 × 4 mm in the middle portion was left uncoated [Fig. 1a-(3)], flattened with a 4000-grit abrasive grinding in a grinder-polisher machine (Ecomet, Buehler, Lake Buff, IL, USA) [Fig. 1b-(4)], cleansed and stored in 37°C deionized water (DW).

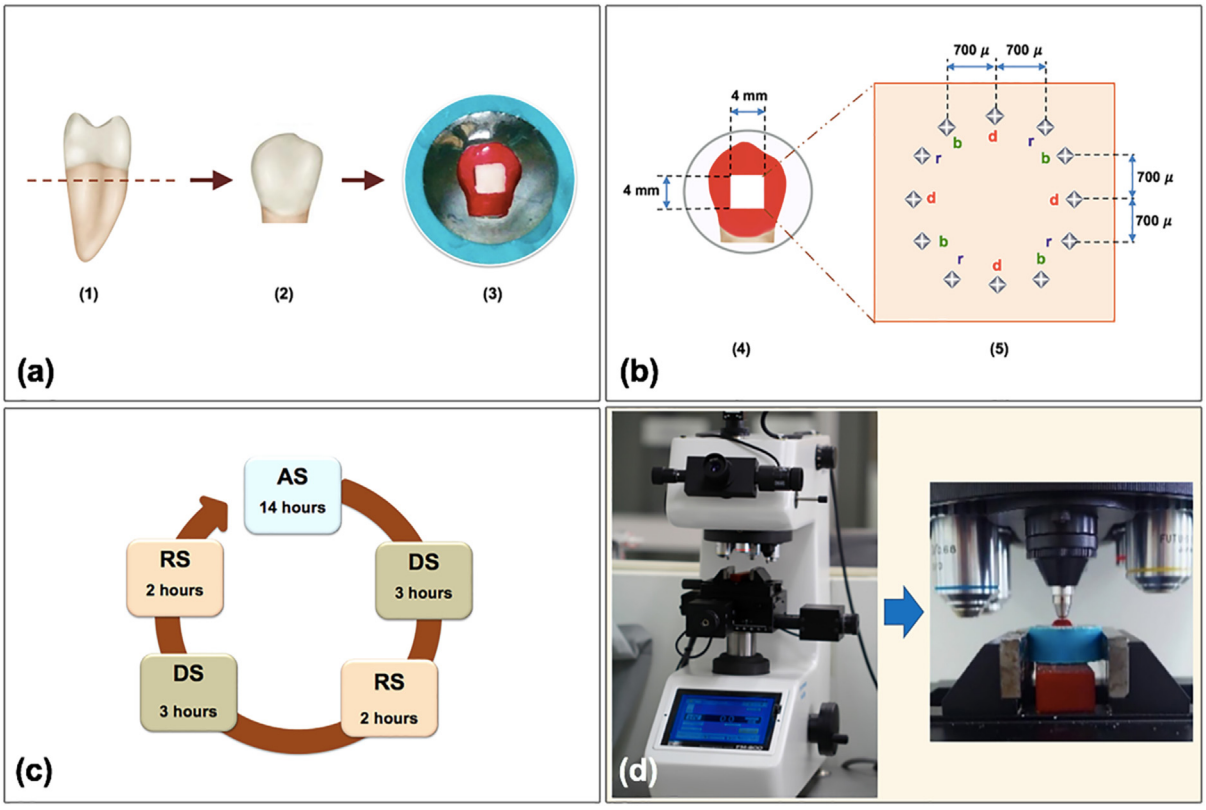

Figure 1 (a) Human bicuspid was horizontally sectioned at 1 mm below the cement enamel junction (1). The crown (2) was invested in the acrylic block (3) to create a flat surface area (b) of 4 × 4 mm^2 (4) for determination of microhardness at the specific location located 700 microns (μ) apart from each other (5). The specimens were treated in the cycling process comprising the demineralized solution (DS), remineralized solution (RS), and artificial saliva (AS) for 10 cycles (c) before determining Vicker hardness by diamond indenter (d).

Induction of artificial carious lesion

The artificial caries inducer (CI) was prepared in a gel form of demineralized solution (DS) that comprised 20 g/L of Carbopol-907 (BF-Goodrich, Cleveland, OH, USA), 500 mg/L of HA, 0.1% of lactic acid, and adjusted-pH to 5.0 by sodium-hydroxide.[20,21] The CI was used for inducing the artificial carious lesion. The tooth specimen was submerged in the DS and reserved in a humid atmosphere for 12 h and then cleansed with DW to generate a reliable artificial carious lesion at the enamel subsurface.[20,21]

Application of pH-cycling for investigated remineralized materials

The samples were randomly categorized into 4 groups (n = 20) according to different remineralization materials (Table 1) before being treated with an acid-challenged mode of pH-cycling condition.

Group AMAP: treated with the apacider mangosteen adhesive paste (AMAP, KKU, Khon Kaen, Thailand)
Group FT500: treated with 500 ppm fluoride toothpaste (Kodomo Grape, Lion, Bangkok, Thailand)
Group FT1000: treated with 1000 ppm fluoride toothpaste (Kodomo Ultra Shield, Lion, Bangkok, Thailand)
Group NT: preserved in DW to serve as a control.

The pH-cycling mode consists of a demineralized solution (DS) and remineralized solution (RS) together with artificial saliva (AS).[20–22] The DS, RS, and AS compositions were addressed in Table 1. Each solution was newly prepared for every cycle. The specimen was individually stored in the scientific vessel containing 10 ml of each solution and engaged in the pH-cycling and remineralized procedure for ten days in the 37 °C vibrating apparatus (Wise–Bath, Seoul, Korea). The pH-cycling embraced 3 h in DS, 2 h in RS twice a day, then submerged in AS for 14 h (Fig. 1c). The remineralized FT was prepared in a mash consistency by combining 15 g of FT with 45 ml of DW. The pH-cycling was performed for 10 days to treat specimens in a 5 ml solution of mash FT as per group – AMAP, FT500, FT1000, and NT. Group AMAP was applied with AMAP for 3 min once a day before the first immersion in the DS and rinsed with DW. Group FT500 and group FT1000 were soaked in their respective toothpaste slurry water for 2 min, before the first immersion in the DS and after the second immersion in the RS, and washed with DW after each application. Group NT was rinsed with DW twice a day.

Table 1 Materials, company, and compositions of toothpastes and solutions used in this study.

Materials	Company	Composition
Apacider mangosteen adhesive pastes (AMAP)	Patent No.1601000575, KKU, Khon Kaen, Thailand	Apacider® AW (Sangi, Tokyo, Japan), alpha-mangosteen, Fumed silica, Eudragit® (Evonik, Bitterfeld-Wolfen, Germany), Polyethylele glycol, 95% ethyl alcohol, Paraben
Fluoride toothpaste 500-ppm (FT500)	Kodomo Grape, Lion Bangkok, Thailand	Sodium fluoride 500-ppmF, Sorbitol, Water, Hydrated silica, Xylitol, Propylene, Glycol, CL77891, Cellulose gum, Flavor, Sodium lauryl sulfate, Sodium saccharin, Butylparaben, Methylparaben
Fluoride toothpaste 1000-ppm (FT1000)	Kodomo Ultra Shield, Lion, Bangkok, Thailand	Sodium fluoride 1000-ppmF, Sorbitol, Water, Hydrated silica, Xylitol, Propylene, Glycol, CL77891, Cellulose gum, Flavor, Sodium lauryl sulfate, Sodium saccharin, Butylparaben, Methylparaben
Artificial caries inducer (CI)	Biomaterial research, KKU Khon Kaen, Thailand	Lactic acid 0.1 mol/L, Hydroxyapatite 500 mg/L, Carbopol C907 20 g/L
Demineralizing solution (DS)	Biomaterial research, KKU Khon Kaen, Thailand	$CaCl_2$ 2.2 mM, KH_2PO_4 2.2 mM, CH_3COOH 0.05 M, pH adjusted to 4.4 with 1 M KOH
Remineralizing solution (RS)	Biomaterial research, KKU Khon Kaen, Thailand	$CaCl_2$ 1.5 mM, NaH_2PO_4 0.9 mM, KCL 0.15 M, pH 7.0.
Artificial saliva (AS)	Biomaterial research, KKU Khon Kaen, Thailand	$C_8H_{15}NaO_8$ 7.8 g/L, KCl 0.65 g/L, $MgCl_2$ 0.058 g/L, $CaCl_2$ 0.165 g/L, K_2HPO_4 0.804 g/L, KH_2PO_4 0.365 g/L, C_6H_5COONa 2.0 g/L, Adjusted pH 7.0

Abbreviations: AMAP: apacider mangosteen adhesive pastes, AS: artificial saliva, CI: artificial caries inducer, DS: demineralizing solution, FT500: fluoride toothpaste 500-ppm, FT1000: fluoride toothpaste 1000-ppm, KKU: Khon Kaen University, RS: remineralizing solution.

Evaluation of surface hardness

The surface hardness was assessed before applying the DS (Hb), after application in the DS (Hd), and after application in the pH-cycling and remineralized procedure (Hr). The hardness was unintentionally assessed at four locations, with 700 μ indentations away from each other [Fig. 1b-(5)]. The Vickers diamond indenter loaded for 100 g, with a lodging time of 15 s was introduced in a microhardness tester (Future-tech, Tokyo, Japan) (Fig. 1d). The indentation was diagonally measured (D1, D2) (Fig. 2a) and computed for Vickers hardness number (VHN).[10] Further calculations of hardness recovery (HR = Hr-Hd), percentage of surface hardness recovery (%HR), the potential of remineralization (RP), and percentage of remineralization potential (%RP) were performed according to Equation (2) and Equation (3).

$$\%HR = \frac{H_r - H_d}{H_b - H_d} x100 \quad (2)$$

$$\%RP = \frac{H_r - H_{r^*}}{H_d} x100 \quad (3)$$

Where: H_{r*} denotes hardness upon the applied pH-cycling & remineralization to the NT.

Microscopic determination

Each sample was sectioned in a longitudinal direction at the thickness of 200 μm, cleansed with DW, and then microscopically determined with a polarized light microscope (PLM, Eclipse-80i, Nikon, Kanagawa, Japan) at 10x intensification (10 per group). The PLM microscopy of the ordinary undamaged enamel and demineralized enamel were then accomplished at 10x intensification, which was utilized as references in contrast with other tested groups. The samples were gold-coated in a sputtering apparatus (Emitech-K500X, Quorum, Asford, UK) and examined for surface characters with the scanning electron microscope (SEM, S-3000N, Hitachi, Tokyo, Japan) (10 per group), compared with the SEM micrographs of the undamaged enamel and demineralized enamel at x2K intensification.

Statistical analysis

The data were analyzed using a statistical program (SPSS-version 20, IBM, Armonk, NY, USA). An analysis of variance (ANOVA) was implemented to verify significant differences in VHN at various phases of treatment including Hb, Hd, Hr, and HR, %HR, RP, and %RP of enamel. The Post-hoc Bonferroni's multiple comparisons were used to justify the statistically significant differences among groups at $\alpha = 0.05$. While SEM and PLM were evaluated qualitatively.

Results

The mean and standard deviation (sd) of Hb, Hd, Hr, HR, %HR, RP, and %RP for each group are displayed in Table 2 and Fig. 2b–d. ANOVA and post-hoc Tukey multiple comparisons exhibited no significant difference of Hb among the groups ($P > 0.05$), as shown in Tables 3a and 4a. The mean Hd for

Table 2 Mean, standard deviation of baseline hardness, hardness after artificial demineralization, hardness after application of pH-cycling & remineralization process, hardness recovery, percentage of hardness recovery, remineralization potential, and percentage of remineralization potential for groups of no treatment, apacider mangosteen adhesive pastes, fluoride toothpaste 500-ppm, and fluoride toothpaste 1000-ppm.

Group	Hb	Hd	Hr	HR	%HR	RP	%RP
	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd	Mean ± sd
NT	339.5 ± 18.4^{a}	163.9 ± 6.8^{b}	90.5 ± 3.1^{c}	-73.4 ± 7.8^{g}	-42.3 ± 6.4^{n}	–	–
AMAP	321.6 ± 34.6^{a}	169.9 ± 7.9^{b}	181.9 ± 4.2^{d}	11.9 ± 5.7^{h}	9.1 ± 7.9^{m}	91.3 ± 5.7^{p}	60.1 ± 10.5^{x}
FT500	320.8 ± 37.8^{a}	166.3 ± 4.6^{b}	161.3 ± 3.7^{e}	-5.0 ± 3.2^{k}	-4.5 ± 6.9^{o}	70.7 ± 5.1^{r}	46.2 ± 9.8^{y}
FT1000	328.9 ± 21.0^{a}	166.7 ± 9.3^{b}	173.0 ± 3.1^{f}	6.4 ± 8.1^{h}	3.6 ± 4.7^{m}	82.5 ± 4.2^{s}	52.3 ± 10.8^{z}

Different superscript letters in the same column denoted significant different between group ($P < 0.05$).
Abbreviations: AMAP: apacider mangosteen adhesive pastes, FT500: fluoride toothpaste 500-ppm, FT1000: fluoride toothpaste 1000-ppm, Hb: baseline hardness, Hd: hardness after artificial demineralization, Hr: hardness after application of pH-cycling & remineralization process, HR: hardness recovery, %HR: percentage of hardness recovery, NT: no treatment, RP: remineralization potential, %RP: percentage of remineralization potential, sd: standard deviation.

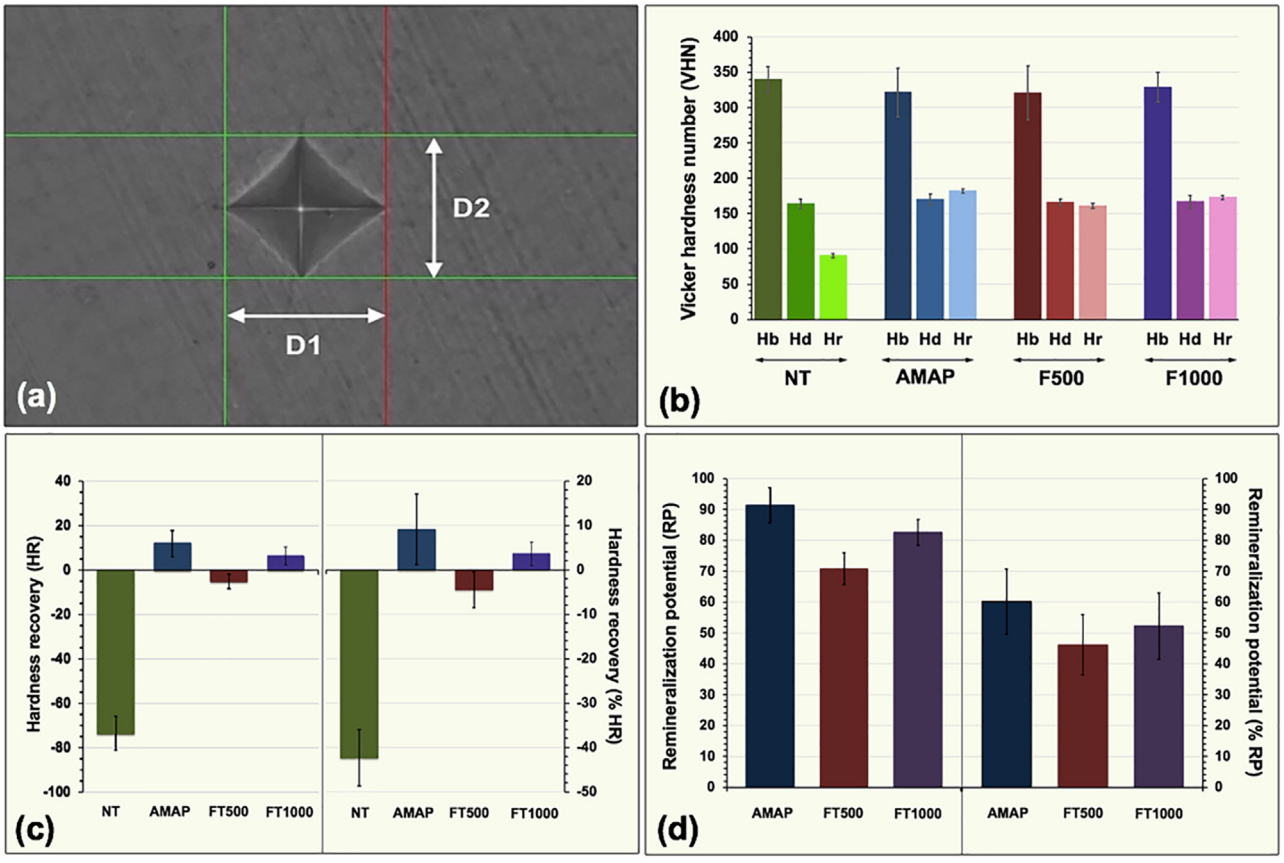

Figure 2 The indentation (a) was measured for diagonal length (D1, D2) and calculated for Vicker hardness number. (b) Mean, standard deviation of baseline hardness (Hb), hardness after the artificial formation of demineralization (Hd). hardness after application of pH-cycling (Hr), (c) hardness recovery determined between after application of pH-cycling and after the artificial formation of demineralization (HR= Hr-Hd), percentage of hardness recovery (%HR), (d) remineralization potential (RP), percentage of remineralization potential (%RP).

each group was reduced in comparison to the mean Hb (Fig. 2b). Nevertheless, no significant difference in Hd was shown midst the groups ($P > 0.05$), as presented in Tables 3(b) and 4(b). Once the pH-cycling & remineralization processes were introduced, the mean Hr for each group was significantly increased in comparison to Hd ($P < 0.05$), excepting the NT group ($P > 0.05$) (Fig. 2b). Significantly different mean Hr amid the tested groups were denoted, as presented in Table 3c. Bonferroni's multiple comparisons signified significant differences in the mean Hr amid the tested groups ($P < 0.05$), as displayed in Table 4c. ANOVA and Post-hoc multiple comparisons indicated significant

Table 3 An analysis of variance of baseline hardness, hardness after artificial demineralization, hardness after application of pH-cycling & remineralization process, hardness recovery, percentage of hardness recovery, remineralization potential, and percentage of remineralization potential.

a. One-way ANOVA of Hb					
Source	SS	df	MS	F	*P*
Between Group	4533.021	3	1511.007	1.774	0.159
Within Group	64,742.526	76	851.875		
Total	8,662,082.167	80			
b. One-way ANOVA of Hd					
Source	SS	df	MS	F	*P*
Between Group	359.362	3	119.787	2.217	0.093
Within Group	4106.602	76	54.034		
Total	2,228,008.938	80			
c. One-way ANOVA of Hr					
Source	SS	df	MS	F	*P*
Between Group	103,955.505	3	34,651.835	2738.627	<0.001
Within Group	961.628	76	12.653		
Total	1,945,596.709	80			
d. One-way ANOVA of HR					
Source	SS	df	MS	F	*P*
Between Group	93,937.552	3	31,312.517	741.540	<0.001
Within Group	3209.201	76	42.226		
Total	115,220.629	80			
e. One-way ANOVA of %HR					
Source	SS	df	MS	F	*P*
Between Group	32,251.418	3	10,750.473	247.250	<0.001
Within Group	3304.490	76	43.480		
Total	41,369.787	80			
f. One-way ANOVA of RP					
Source	SS	df	MS	F	*P*
Between Group	4267.100	2	2133.550	83.897	<0.001
Within Group	1449.535	57	25.430		
Total	404,479.868	60			
g. One-way ANOVA of %RP					
Source	SS	df	MS	F	*P*
Between Group	1961.632	2	980.816	9.138	<0.001
Within Group	6117.940	57	107.332		
Total	175,749.378	60			

Abbreviations: ANOVA: analysis of variance, df: degree of freedom, F: F-ratio, Hb: baseline hardness, Hd: hardness after artificial demineralization, Hr: hardness after application of pH-cycling & remineralization process, HR: hardness recovery, %HR: percentage of hardness recovery, MS: mean square, *P*: *P*-value, RP: remineralization potential, %RP: percentage of remineralization potential, SS: sum of squares.

differences in the mean HR among the tested groups ($P < 0.05$) (Fig. 2c, Tables 3d and 4d). Significant differences in the mean %HR among the tested groups ($P < 0.05$) were noticed, except for AMAP-FT100 (Fig. 2c, Tables 3e and 4e). The highest %HR was discovered for the group that was treated with AMAP, followed by the FT1000 group and the FT500 group, respectively. ANOVA suggested significant differences in the mean RP among the tested groups ($P < 0.05$) (Fig. 2d, Tables 3f and 4f). Significant differences in the mean %RP amid groups ($P < 0.05$) were denoted, except for AMAP-FT1000 (Fig. 2d, Tables 3g and 4g). The %RP of AMAP and FT1000 were equivalent. The administration of AMAP, FT500, and FT1000 signified significantly competent remineralization potential to the demineralized enamel ($P < 0.05$) compared to the NT group, but their capability of remineralization of AMAP was higher than FT1000 and FT500 respectively. Nevertheless, the degree of remineralization potential of AMAP and FT1000 was comparable.

The manifestation of a caries lesion as well as the advancement of the remineralized procedure was described by the PLM microscopy (Fig. 3) in contrast to the PLM of undamaged enamel with no substantiation of the caries lesion (Fig. 3a). A noticeable shadowy zone and increasing depth of lesion were detected on the PLM of the synthetic carious sample (Fig. 3b). Once the pH-cycling & remineralization procedure was applied, the decreasing depth of lesion for each experimentally tested toothpaste is indicated (Fig. 3d–f), except for the control (NT) group indicated an increase in lesion depth (Fig. 3c) in comparison

Table 4 Post hoc Bonferroni multiple comparisons of baseline hardness, hardness after artificial demineralization, hardness after application of pH-cycling & remineralization process, hardness recovery, percentage of hardness recovery, remineralization potential, percentage of remineralization potential among groups of no treatment, apacider mangosteen adhesive pastes, fluoride toothpaste 500-ppm, and fluoride toothpaste 1000-ppm.

a. Bonferroni multiple comparisons of Hb

Group	NT	AMAP	FT500	FT1000
NT	1.000	0.336	0.273	0.99
AMAP		1.000	0.99	0.99
FT500			1.000	0.99
FT1000				1.000

b. Bonferroni multiple comparisons of Hd

Group	NT	AMAP	FT500	FT1000
NT	1.000	0.075	0.99	0.99
AMAP		1.000	0.731	0.99
FT500			1.000	0.99
FT1000				1.000

c. Bonferroni multiple comparisons of Hr

Group	NT	AMAP	FT500	FT1000
NT	1.000	0.001	0.001	0.001
AMAP		1.000	0.001	0.001
FT500			1.000	0.001
FT1000				1.000

d. Bonferroni multiple comparisons of HR

Group	NT	AMAP	FT500	FT1000
NT	1.000	0.001	0.001	0.001
AMAP		1.000	0.001	0.048
FT500			1.000	0.001
FT1000				1.000

e. Bonferroni multiple comparisons of %HR

Group	NT	AMAP	FT500	FT1000
NT	1.000	0.001	0.001	0.001
AMAP		1.000	0.001	0.059
FT500			1.000	0.001
FT1000				1.000

f. Bonferroni multiple comparisons of RP

Group	AMAP	FT500	FT1000
AMAP	1.000	0.001	0.001
FT500		1.000	0.001
FT1000			1.000

g. Bonferroni multiple comparisons of %RP

Group	AMAP	FT500	FT1000
AMAP	1.000	0.010	0.99
FT500		1.000	0.060
FT1000			1.000

Abbreviations: AMAP: apacider mangosteen adhesive pastes, FT500: fluoride toothpaste 500-ppm, FT1000: fluoride toothpaste 1000-ppm, Hb: baseline hardness, Hd: hardness after artificial demineralization, Hr: hardness after application of pH-cycling & remineralization process, HR: hardness recovery, %HR: percentage of hardness recovery, NT: no treatment, RP: remineralization potential, %RP: percentage of remineralization potential.

to the lesion depth of the artificially demineralized enamel (Fig. 3b). This suggested that all tested kinds of toothpaste were able to generate remineralization of the synthetic caries lesion. The reduction in caries lesions for AMAP treated group was superior to the FT1000 and FT500 treated groups, respectively. Yet, the reduction in the depth of carious lesion for the AMAP group probably indicated a slightly better than that of the FT1000 treated group. Enhancing in the depth of carious lesion in the NT group was noted, and depth, signifying no remineralized establishment was offered upon the pH-cycling procedure.

The scanning electron microscopy for each treated group was detected and compared with the SEM micrograph of undamaged enamel at ×2K magnification (Fig. 4a). The artificially produced carious specimen revealed an asymmetrical configuration of slashes and damaged zone with extreme signs of porous (Fig. 4b) in comparison to the even and undamaged typical enamel structure (Fig. 4a). The SEM micrograph of the NT group exhibited several porosities and unevenness apparent, typically related to no establishment of remineralization upon pH-cycling procedure (Fig. 3c). The SEM micrograph for the AMAP group exhibited an even and consistent appearance, representing the establishment of a new HA layer and remineralized process of the carious area (Fig. 4d). The SEM micrograph for the FT500 group revealed a minor rough with micropore areas, that indicated approximately remineralized establishments of fluorapatite to demineralized zone, without absolute remineralized process (Fig. 4e). The SEM micrograph for the FT1000 specimen denoted a thin mineral deposition with an imperfect apposition of the craters and some remaining porous produced from the formerly stimulated caries lesion (Fig. 4f), that implies imperfection of remineralized capacity of FT1000 throughout the whole carious area. The SEM micrographs denoted a development of a new HA deposit and remineralized process established on the produced caries lesion treated with AMAP, FT500, and FT1000 at different quantities. Contrarily, hidden porosities and more noticeable asymmetrical destructive configuration of destruction-induced caries lesions were detected in the SEM micrograph of the NT group (Fig. 4c).

Discussion

Remineralization of incipient caries was recently considered a prophylactic approach in contemporary restorative dentistry. This experiment assessed the capabilities of AMAP, FT500, and FT1000 to remineralize initial caries lesions of enamel. The study signified that AMAP, FT500, and FT1000 possessed significant remineralized ability in retrieving the demineralized enamel compared with the untreated demineralized enamel. This implies that the experimental toothpaste was capable of generating a remineralized process for the artificially carious enamel. The experimental study indicated a significantly different efficacy in remineralization among groups of toothpaste, as indicated by the rejection of the null hypothesis in the remineralization capability of the test products. However, the remineralization potential between AMAP and FT1000 group seem to be comparable. The remineralization effects on artificially demineralized enamel were evidenced by SEM. Particles were found to be deposited on the

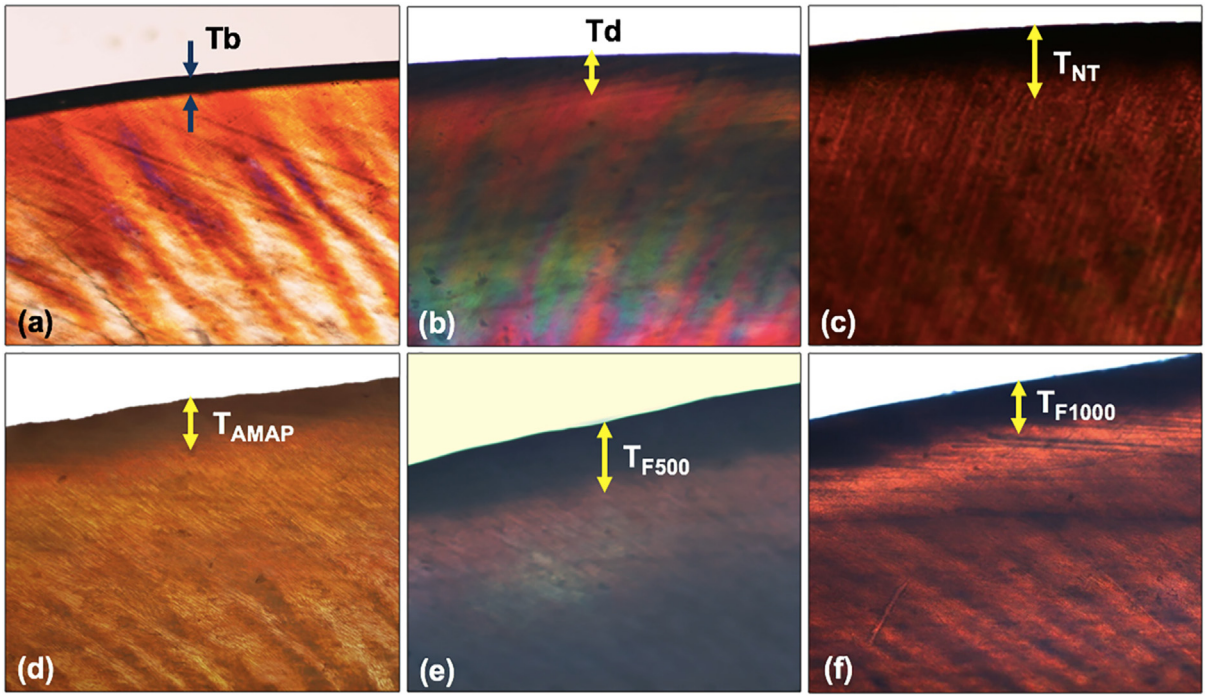

Figure 3 Polarized light microscope (PLM) at ×10 of enamel (a), enamel after the artificial formation of demineralization (b), demineralized enamel after application of pH-cycling without remineralization agent (c) and with remineralization agent either apacider mangosteen adhesive pastes (AMAP) (d) or fluoride toothpaste 500-ppm (FT500), (e). or fluoride toothpaste 1000-ppm (FT1000) (f) was used to verify mechanism and thickness (T) of the demineralization and remineralization process upon receiving specific tested materials.

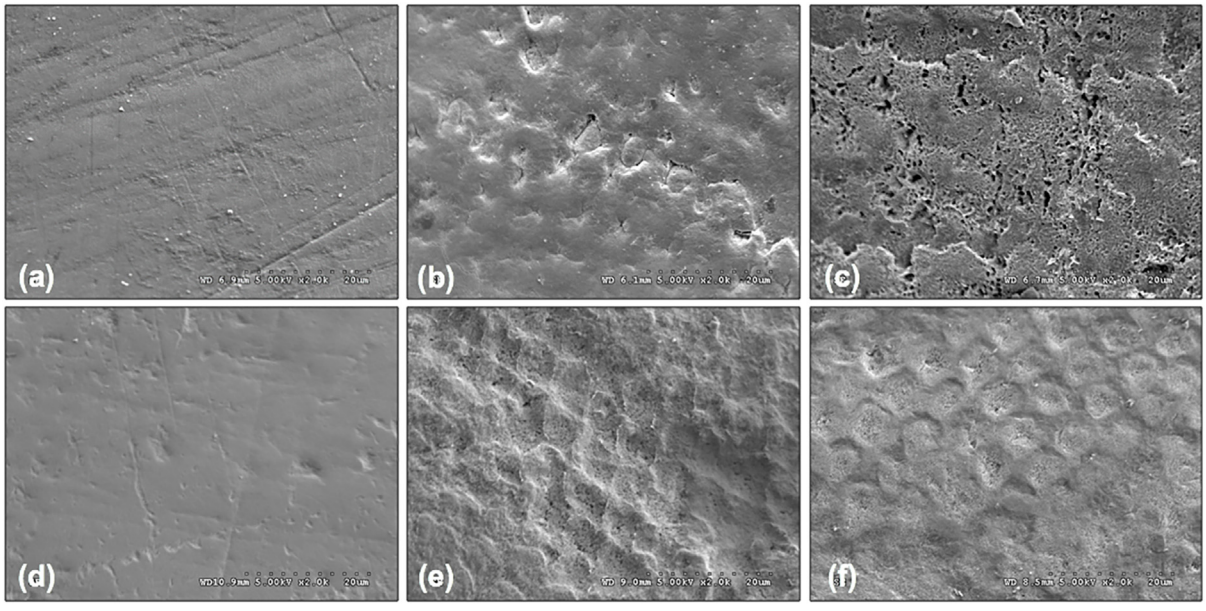

Figure 4 Scanning electron microscopy (SEM) at x2K of enamel (a), enamel after the artificial formation of demineralization (b), demineralized enamel after application of pH-cycling without remineralization agent (c) and with remineralization agent either apacider mangosteen adhesive pastes (AMAP) (d) or fluoride toothpaste 500-ppm (FT500), (e). or fluoride toothpaste 1000-ppm (FT1000) (f).

demineralized enamel surface of the AMAP, FT500, and FT1000 groups, compared with the NT. Specifically, the PLM clarified a decrease in the depth of carious lesions once the demineralized enamel was treated with each type of toothpaste, compared with the NT group. Furthermore, the AMAP signified more superior capability in remineralization than both FT1000 and FT500 in comparison with NT, as supported by SEM.

Artificial enamel carious lesions can be prepared by various techniques for the remineralization assessment of therapeutic agents. In other studies, the lesions were prepared by lactate or acetate gel at pH 4.4–5.0, to simulate organic acid produced by cariogenic bacteria. However, to mimic in vivo carious lesions, there should be subsurface lesions with less demineralized surface layers.[22] Therefore, we used synthetic polymer gels as an artificial caries inducer (CI), comprised of polyacrylic acid (Carbopol™C907), lactic acid, and hydroxyapatite (Table 1), for artificial caries formation.[23] Polyacrylic acid was supplemented as the main factor to maintain the surface layer and generate in vitro subsurface caries foundation.[6] In the pH cycling model to simulate pH conditions in an oral environment with dynamic mineral loss and gain, remineralizing agents were applied in the model for 10 days.[22] The application of fluoride toothpaste was designed to mimic clinical situations under the manufacturer's recommendation; however, the AMAP paste was applied on a one-time diary basis until the end of the ten days of the experimental cycle. The study indicated that the ultimate amount of demineralization of enamel happened in the NT group, which was solely exposed to the demineralizing solution without any agent application. The demineralized solution of pH 4.4 was selected to control the demineralized process to generate incipient caries, which were then accurately measured.[7,24] Mild organic acids and acid buffers, such as acetate acid, was used to generate carious lesions that imitate natural caries.[4,25]

This study showed partial remineralization on artificial enamel carious lesions which were possibly due to calcium and phosphate ions deficiency.[1] The deficiency of ions was attributed to the dissolution of hydroxyapatite crystal during artificial carious lesions formation, therefore, fluorapatite and calcium fluoride (CaF_2) synthesis were interrupted.[9] The AMAP paste was able to provide calcium and phosphate ions[13] that simultaneously diffuse into subsurface lesions for molecular structure restoration. Similarly, Sodata's study also observed homogeneous remineralization of artificial carious lesions after AMAP application.[13] The porosity and depth of artificial carious lesions play a critical role in mineral diffusion. Larger porosities can induce higher mineral deposition, however, deeper lesions with longer distances can create difficulty for mineral ion absorption.[22] The results of the AMAP group can be explained that small molecules of phosphate and calcium ions could be absorbed into the deeper porosity of enamel and deposited significantly more amounts of the inorganic than fluoride as reflected by the higher surface hardness.[5,26]

The AMAP-treated tooth exhibited harmonized smooth enamel with no porosity under magnification of x2K. The results were consistent with other studies that described the effects of apacider on enamel remineralization. The studies showed that the surface of enamel treated with apacider exhibited more harmonized and smooth surfaces.[13,15,20] In addition, the study revealed that the surface morphology of healthy tooth samples after acid attacks with AMAP revealed uniform smooth enamel compared with the untreated tooth samples.[13] Furthermore, the study stated that AMAP-treated tooth exhibited a more uniform and smoother enamel surface than the fluoride-treated tooth.[20] This could be described as the partially demineralized crystalline surface inside the lesion acting as a "nucleator", and new surfaces grew on the crystal structure. This process constitutes remineralization, which is the substitution of minerals in the partially demineralized areas of the carious lesion of enamel.[5] The AMAP is prepared in the form of an adhesive paste that can be smeared on the tooth surface. The anticariogenic process of AMAP relates to the integration of nano complexes upon the tooth surface and acts as a reservoir of phosphate and calcium that could be absorbed into the inner portion of enamel porosities, and drastically greater amounts of the mineral were accumulated at the subsurface lesions. Higher remineralization potential for AMAP, compared to both FT500 and FT1000, was found in our study as evidence from the SEM analysis that AMAP treated group showed a harmonized smooth surface with a minute amount of porosities, whilst the FT-treated groups displayed a rough appearance and minor porous. This is probably related to the similar characteristics of AMAP to enamel configuration which possibly generated the stimulation of the remineralized process as well as engendering a uniform complex HA configuration for the demineralized enamel after the paste was introduced[14] On the contrary, the fluoride encouraged the establishment of intraoral fluoride reservoirs as a result of the calcium fluoride (CaF_2) formation. This intricate structure encompasses the synthetic HA adhering directly to the natural crystal structure of enamel. Accordingly, the remineralized enamel is comparable in hardness to regular enamel.[10] Vice versa, the remineralized enamel upon being treated with FT demands phosphate and calcium ions to formulate fluorapatite on the carious enamel, which limitedly presented these ions in saliva, thus the remineralized procedure could not be fully accomplished.[27] Additionally, the SEM confirms that the AMAP displayed more capability in remineralization potential than FT. Furthermore, the remineralization potential of artificial saliva was also restricted by phosphate and calcium, which caused the imperfect establishment of fluorapatite and remineralization.[13,16]

The PLM evaluation was considered in this study for evaluating the initial lesion and differentiation of enamel structure. The PLM photomicrograph allowed the precise and direct measuring of the depth of demineralization to compare the effect of different tested materials. This accuracy is essential for the truthful evaluation of any therapeutic involvement since the material might not preclude demineralization, nevertheless, it could lessen the area of the affected tooth surface in addition to the quantity of inorganic loss and the lesion depth.[28,29] The photomicrograph after lesion formation and before agent application showed a thickening band underneath the outer surface of the enamel. In the sample that received AMAP, there was a smaller band in terms of color intensity and the bandwidth beneath the outer surface of enamel, as compared to the FT500, FT1000 group, and NT group, indicating the complete remineralization of AMAP. The investigation of the PLM micrographs proved that the acid endurance in the group treated with AMAP offered the highest reduction in the depth of demineralized enamel in comparison with another group. This is probably due to the main composition of AMAP being calcium-phosphate which is composed of calcium ions and phosphate ions. The calcium ion has a radius of 0.99 Å and the phosphate ion has a radius of 1.15 Å. These particles smaller than fluoride with a radius

of 1.32 Å offered AMAP a superior remineralization. The nano-sized crystal structure can be accumulated in the bare bones of caries lesions, finally decreasing the depth of the lesion. The results are inconsistent with other studies, in which PLMs displayed that the apacider and AMAP exhibited the depths of demineralized enamel fewer than other remineralizing agents.[14,15,20]

This study indicated that the %HR and the %RP of AMAP were comparable to that of FT1000. Nevertheless, PLM and SEM explorations discovered that the AMAP possessed more remineralization potential than FT1000, which was also inconsistent with another study.[30] The AMAP group exhibited better remineralization potential than the FT1000, FT500, and the control group as shown in the SEM and PLM. The smaller particle size and the better activity of AMAP possibly help the particle penetrate through the enamel surfaces and constantly fulfill the porosity of the carious lesion better than fluoride.[30] The SEM micrographs confirmed that AMAP was capable of forming a homogenous apatite layer, which corresponded with the previous studies.[30] In addition, the study suggested that AMAP is similar to FT1000 as increasing in surface hardness of the caries lesion. Another serious issue that could increase the capability for remineralization of the AMAP relates to the acid atmosphere upon pH-cycling because acid conditions escalate the solubility of AMAP, thus increasing the deposition of AMAP on the enamel surface.[13,30] The pH-cycle process was intended to imitate the acid-challenging situation in the mouth, therefore this might potentiate the remineralization effect of AMAP above FT1000 and FT500, as confirmed by PLM and SEM. The tested groups, except the NT group, revealed a diminution in the depth of caries, as designated by PLM, nonetheless, the absolute remineralized process was not succeeded. The FT group assumed that the hydroxyl ions were replaced by fluoride ions and exhibited an exothermic reaction on the external surface, whilst the inner portion gradually produced fewer exothermic conditions, thus steering the superficial hydroxyl ions to be effortlessly substituted by fluoride ions.[11] Thus, the external surface developed an extremely mineralized layer or hypermineralization, and this reduced the ability of minerals to infiltrate into the inner portion, as indicated by the PLM micrograph of the FT group.

The pH-cycle process was intended to imitate an acid-challenging situation and mimic active mineral permeation related to naturally occurring carious behavior.[21] The pH-cycling process is efficiently considered to estimate carious advancement established on crystalline alterations and the hardness of enamel.[22] Synthetic caries were formulated by the synthetic gel, comprising polyacrylic acid to maintain the superficial surface as well as to generate the subsurface carious lesion, similar to the naturally occurring initial caries.[21] The restrictions of the study related to an experimental design were the limited ability to mimic dental biofilm and salivary oral pellicles as presented in the mouth. Furthermore, every specimen might be different, based on the age of donors and the exposure to the oral environment. These lead to deviation in responses to acid-challenged situations. Moreover, the intervals of the demineralized-remineralized process were shorter than the natural interval of the in-vivo condition.[22] Further scientific experiments are suggested for *in-situ* clinical investigations. Yet, the clinical implication of this research was crucial by way of leading the investigation for novel remineralization materials to deliver worthy data for dental clinicians to pursue efficient clinical practice.

In summary, this in vitro experiment allocated the remineralization potential of AMAP to the initial caries lesion equivalent to FT1000. Both AMAP and FT1000 demonstrated more remineralized capability than FT500 for carious enamel remineralization. Thus, AMAP and FT1000 should be considered therapeutic and preventive materials for initial carious lesions and may be recommended for high-risk groups of dental caries in children as well as patients with salivary function problems. Additional clinical trials are desired to endorse the remineralization potential of AMAP. In addition, AMAP is a novel product that probably offers a more cost-effective therapeutic procedure for dental caries in a medically compromised patient as well as the uncooperative child who may otherwise has difficulty with oral hygiene care. The application of once daily-AMAP or the use of twice-daily FT1000 has significant remineralization potential on artificial demineralized enamel lesions. On the contrary, the use of twice-daily FT500 continuously indicated a less significant remineralization effect on artificial demineralized enamel lesions.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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