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Research Report

Efficacy of various topical agents to prevent enamel demineralization

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ABSTRACT

***Background**: Enamel demineralization is a common and undesirable side effect of fixed appliance orthodontic treatment. Many sudies showed that the prevalence varied between 2–96%. There are many ways to prevent demineralization and increased remineralization such as oral hygiene instruction and by topical application such as acidulated phosphate fluor (APF) casein phospo peptide-amorphous calcium phosphate (CPP-ACP), casein phospo peptide-amorphous calcium phosphate plus (CPP-ACPF). **Purpose**: The purpose of this in-vitro study was to evaluate the efficacy of various topical agents to prevent enamel demineralization. **Methods**: Fourty extracted human premolars were allocated to 1 of 4 groups: 1.23% APF gel; 10% CPP-ACP paste; 10% CPP-ACPF paste; and untreated control. All samples were subjected to pH cycling treatment for 12 days through a daily procedure of demineralization solution with pH 4 for 6 hours and remineralization solution with pH 7 for 18 hours. Microhardness testing were done before and after pH cycling and the delta hardness values were determined. **Results**: APF, CPP-ACP and CPP-ACPF application significantly prevent lowering of enamel microhardness value compared with untreated control group. Kruskal-Wallis, ANOVA, Mann-Whitney U, Tukey and Bonferroni Post-Hoc multiple comparison test showed significant difference between mean delta microhardness value of CPP-ACPF and CPP-ACP group with APF group, but there is no significant difference between mean delta microhardness value of CPP-ACPF and CPP-ACP group. **Conclusion**: APF, CPP-ACP and CPP-ACPF prevent enamel demineralization. CPP-ACP and CPP-ACPF prevent demineralization more than APF.*

***Key words**: Enamel demineralzation, topical agents, enamel microhardness testing*

ABSTRAK

***Latar belakang**: Demineralisasi email merupakan efek samping negatif yang sering dijumpai pada perawatan ortodontik cekat. Beberapa penelitian menyatakan bahwa prevalensinya bervariasi 2–96 persen. Ada beberapa cara untuk mencegah demineralisasi dan meningkatkan remineralisasi, misalnya dengan instruksi kebersihan mulut dan menggunakan bahan topical aplikasi seperti acidulated phosphate fluor (APF) casein phospo peptide-amorphous calcium phosphate (CPP-ACP), casein phospo peptide-amorphous calcium phosphate plus (CPP-ACPF). **Tujuan**: Tujuan penelitian in vitro ini adalah untuk mengevaluasi efektivitas berbagai agen topikal untuk mencegah demineralisi email yang dilihat dengan uji kekerasan mikro permukaan email. **Metode**: emapat puluh gigi premolar yang sudah di ekstraksi dibagi dalam 4 kelompok: aplikasi gel 1,23% APF; aplikasi psta 10% CPP-ACP; aplikasi pasta 10% CPP-ACPF dan kelompok Kontrol. Semua sampel diberikan perlakuan siklus pH selama 12 hari yang terdiri dari perendaman dalam larutan demineralisasi dengan pH 4 selama 6 jan dilanjutkan dengan perendaman dalam larutan remineralisasi dengan pH 7 selama 18 jam. Uji kekerasan dilaku kan sebelum dan sesudah perlakuan serta diperoleh juga data delta kekerasan. **Hasil**: Semua aplikasi agen topikal tersebut dapat mencegah demineralisasi email secara signifikan dibandingkan kelompok kontrol. **Kesimpulan**: APF, CPP, ACP, dan CPD-ACPF mencegah demineralisasi enamel. CPP-ACP dan CPD-ACPF mencegah demineralisasi lebih baik dibanding APF.*

***Kata kunci**: Demineralisasi email, agen topikal, uji kekerasan permukaan email*

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INTRODUCTION

Enamel is the hardest tissue of the human body consist of 96% hydroxyapatite, 4% organic content and water. Enamel hardness is influenced by the total mineral content and structural characteristics of the prisms.[1] Microhardness value for sound enamel varied between 292–390 knoop hardness number (KHN).[2,3]

Enamel demineralization around orthodontic brackets is a common and undesirable side effect of fixed appliance orthodontic treatment, with incidence and prevalence varied between 2–96%.[4,5] Clinically, early enamel demineralization lesion appear as white spot, which is a white opaque area and is softer than the surrounding enamel. Carbohydrate consumption will cause the saliva and plaque fluid pH to fall to critical pH (between 4.5–5.5) as acids are produced from fermentation of the carbohydrate by bacteries such as *Streptococcus mutans* (*S. mutans*) and *Lactobacillus* which then will cause hydroxyapatit to dissolve. *S. mutans and lactobacillus* levels were found to be significantly elevated during active orthodontic treatment. Saliva is the most important defense mechanism against formation of demineralization lesion. Saliva parameters such as flow rate, pH, and buffer capacity influence the dynamics between demineralization and remineralization.[6]

Bracket placement increases plaque retention sites and limiting salivary flow access, especially on enamel area between brackets and gingival margins and below orthodontic wire. Bracket placement also complicates tooth cleansing by the patient.[5] Preventive measures that can be done to reduce the risk of demineralization during orthodontic treatment are patient education and oral hygiene instruction, routine professional cleaning, and giving additional topical agents that contain fluoride and casein phospho peptide–amorphous calcium phosphate (CPP-ACP).[7,8]

The efficacy of fluoride topical agents to prevent enamel demineralization during orthodontic treatment has been proven as a fact. There are several methods to deliver fluoride to teeth during orthodontic treatment which include toothpaste with a higher fluoride content, fluoride rinse, gel/foam and varnish application.[8] Previous researches have shown that CPP-ACP also can reduce demineralization risk because of its ability to stabilize free calcium and phosphate ions in plaque surrounding the enamel thereby helping to maintain a state of supersaturation with respect to enamel and its ability to buffer plaque pH, thus promoting remineralization and depressing demineralization. CPP-ACP at 0.5–1.0% w/v produced a reduction in caries activity similar to that of the 500 ppm fluoride containing solution.[9–11] Products that contain CPP-ACP that are available in the Indonesian market is GC Tooth Mousse™ (GC Corporation). CPP-ACP and fluoride have been shown to have a synergistic effect enhancing the potential to depress demineralization and promote remineralization.[9,12,13] Recently, a new variant of the GC Tooth Mousse which is the GC Tooth Mousse Plus™ is introduced to the Indonesian market, it contained additional 0.2% NaF (900 ppm). This product is expected to have added benefit of the synergistic effect between CPP-ACP and fluoride. Until this research is done, the writer has not encountered any research that tested the efficacy of the casein phospho peptide–amorphous calcium phosphate paste (CPP-ACPF) in reducing demineralization risk on enamel surrounding orthodontic brackets.

Microhardness testing is a quick, simple, and non-destructive test. Knoop microhardness test is the most common test to determine the microhardness value of surface enamel, with 1–1000 g indenter load. There is a linear relationship between the square root of KHN and the mineral content of dental tissues; therefore the demineralization process of enamel could be detected by the reduction of enamel microhardness. Loss of enamel mineral will increase its porosity thereby reducing its ability to resist the load of the indenter causing deeper and wider indentation mark.[2,14] The purpose of this research is to analyze the efficacy of topical fluor application such as APF, CPP-ACP and CPP-ACPF to prevent enamel demineralization using microhardness testing and to know which topical agent is the most effective.

MATERIALS AND METHODS

Forty extracted human premolars that never been bonded with orthodontic bracket, free of filling, stain, white spot and carious lesion, and without structural enamel defects are cleaned and stored in saline solution until it is time to be used in the research. Roots were sectioned at the cement-enamel junction using low speed hand piece and diamond disc. The crowns were then fixated on a decorative self-curing resin using 20 mm in diameter and 10 mm thick pipe as mold with the buccal/lingual side facing upward.

Each specimen is polished with silicone carbide paper no. 1500 and 2000 continued with 1µm alumina polishing solution and polishing cloth on top of a polishing machine. Each specimen is polished until maximum of 200 µm of enamel thickness is taken. After polishing, microhardness testing is done with knoop indentation to determine inital surface microhardness value of each specimen. Forty specimens were allocated to 1 of 4 groups: 1.23% APF gel; 10% CPP-ACPF (GC Tooth Mouse); 10% CPP-ACP (GC Tooth Mouse Plus); and untreated control.

Specimens from application group were applied with 0.5 ml of corresponding topical agents for 5 minutes. Specimens from untreated group receive no topical agent application. All specimens were then placed in 20 ml 37° C, pH 4 demineralizing solution (containing 2.2 mM/L $CaCl_2$, 2.2 mM/L KH_2PO_4 dan 50 mM/L acetic acid)[16] for 6 hours. After that the specimens were removed and placed in 20ml 37° C, pH 7 remineralizing solution (containing 1.5 mM/L $CaCl_2$, 0.9 mM/L KH_2PO_4, dan 130 mM/L KCl)[16] for 18 hours. After that, all specimens were removed and rinsed with aquadest and dried using paper towel. This cycle was

repeated 12 times. Specimens from CPP-ACP and CPP-ACPF group were reapplied with 0.5 ml of corresponding topical agents for 5 minutes after each cycle, whereas specimens from APF group were not. After 12 cycles, microhardness testing was done with Knoop indentation to determine final surface microhardness value of each specimen. Initial and final microhardness value of each specimen is a mean value from 3 indentations. Delta microhardness value is the difference between initial and final microhardness value. The data of these result was analyzed using ANOVA and Tukey test.

RESULTS

Table 1. Enamel microhardness of control and treated groups

Group	n	$\overline{X}$ Initial Hardness	$\overline{X}$ Final Hardness	$\overline{X}$ Δ (Delta)
Control	10	359.9 ± 9.63	73.3 ± 18.07	286.6 ± 20.75
APF	10	359.9 ± 15.1	241.4 ± 25.27	118.5 ± 23.78
CPP-ACPF	10	359.9 ± 11.32	332 ± 15.08	27.9 ± 12.77
CPP-ACP	10	359.9 ± 13.51	329 ± 11.78	30.9 ± 12.77

* Showed significant differences with control group ($p < 0.05$).

APF, CPP-ACP and CPP-ACPF application significantly prevent lowering of enamel microhardness value compared with untreated control group (Table 1). This means that all topical application agents is effective in preventing enamel demineralization compared to untreated control group.

ANOVA and Tukey test showed significant difference between mean delta microhardness value of CPP-ACPF and CPP-ACP group with APF group, but there is no significant difference between mean delta microhardness value of CPP-ACPF and CPP-ACP group (Table 2 and 3).

Table 2. ANOVA test of delta microhardness value

Group	n	$\overline{X}$ ð (KHN)	ANOVA	
			F	p
Control	10	286.6 ± 20.75	444.973	0.001
APF	10	118.5 ± 23.78		
CPP-ACPF	10	27.9 ± 12.77		
CPP-ACP*	10	30.9 ± 12.77*		

* Showed no statistical difference, significant at $p < 0.05$

Table 3. Tukey test of delta microhardness value

Group	Mean ð (KHN)	Group	Mean ð (KHN)	Tukey (p)
Control	286.6	APF	118.5	$p < 0.05$
		CPP-ACPF	27.9	$p < 0.05$
APF	118.5	Control	286.6	$p < 0.05$
		CPP-ACPF	27.9	$p < 0.05$
CPP-ACPF	27.9	Control	286.6	$p < 0.05$
		APF	118.5	$p < 0.05$

DISCUSSION

There are a linear relationship between the square root of KHN and the mineral content of enamel and dentin.[14] Fujimaru *et al*.[2] and White *et al*.,[17] compared contact microradiography (CMR), which is the gold standard to measure mineral content of dental tissues, with microhardness testing and found the result from both methods showed insignificant differences and strong correlation ($p < 0.01$, $r^2 = 0.94$) thus enamel microhardness testing can be used as a simpler alternative to evaluate enamel remineralization and demineralization process.[16] It is more valuable to a clinician to know enamel surface microhardness than to know its mineral content. Enamel is not a homogen material, it consists of inorganic and organic materials, therefore the mean of 3 indentations was calculated for each specimen to increase the accuracy of the hardness value.

The best way to simulate the process of demineralization and remineralization in vivo, in which demineralization occurs in an neutral pH.[18–20] The in vitro pH cycling model can be varied in the time intervals of the demineralization and remineralization phase and the number of cycles.[16–18] In this research, 12 cycles were done with each cycle consisting of 6 hours demieralization followed by 18 hours remeralization. This model was showned as the best model to simulate the mineral loss that occurs in vivo around orthodontic brackets.[19] The 5 minutes topical agent application is in accordance with recommendation from American Dental Association (ADA).[21] APF were applied only once before the 12 cycles to simulate professional application which was done every 3 to 6 months, whereas CPP-ACP and CPP-ACPF pastes were re-applied at the beginning of each cycle to simulate daily use by the patient. Therefore, the in vitro method used in this research may represent the in vivo conditions of the demineralization challenge experienced by a patient during fixed orthodontic treatment.

Delta microhardness value of CPP-ACP group is not distributed normally caused by one speciment with a KHN way above mean. The probable explanation for this is a possible nonuniform time of topical agent application or length of demineralization/remineralization cycle. Eventhough the time had been strictly scheduled and monitored, this can still happen considering the number of specimens.

The efficacy of topical fluoride to prevent enamel demineralization.[17] The efficacy of CPP-ACP paste to prevent enamel demineralization is in accordance with studies done by Oshiro *et al.*,[10] Hodnett and Sato *et al*.,[22] Reynolds *et al*.,[13] and Kumar *et al*.,[9] found a synergistic effect between CPP-ACP and topical fluoride agent which decreases enamel demineralization and enhances enamel repair with remineralization process and furthermore the combination effect of the two is better than just using either one. Recently, a new variant of GC Tooth Mousse™ (contatining 10% w/v CPP-ACP), the GC Tooth Mousse Plus™ (containing additional 0.2% 900 ppm NaF), is introduced to the Indonesian market. This product is expected to have the combined effect of topical fluoride and CPP-ACP within one product. Until this research is done, the writer has not encountered any research that tested the efficacy of the GC Tooth Mousse Plus™ (CPP-ACPF) in reducing demineralization risk on enamel surrounding orthodontic brackets. The result of this research showed CPP-ACPF and CPP-ACP application prevent demineralization more significantly than 1.23% APF, which is in accordance with the result of a study done by Sato *et al*.,[22] Statistical analysis showed no significant difference between CPP-ACPF and CPP-ACP even though delta microhardness value of CPP-ACP group is less than CPP-ACPF group (Table 1). Further in vitro and in vivo research is needed to compare the efficacy of CPP-ACPF and CPP-ACP paste in preventing enamel demineralization especially in patients undergoing fixed orthodontic treatment.

In conclusion topical agents such as APF gel, CPP-ACP paste and CPP-ACPF could prevent demineralization. CPP-ACP paste and CPP-ACPF application to prevent demineralization were more effective then APF.

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