# Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP-ACPF) as an Enamel Remineralization

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Abstract: Dental erosion is an oral problem which often occurs in children and adolescents. The most common cause of dental erosion is excessive consumption of soft drinks. One of the methods to prevent dental erosion caused by soft drinks is using products that are able to increase the remineralization of dental structure. A remineralization agent that can be used is CPP-ACPF which contains calcium, phosphate, and fluoride. Purpose: To examine the microporosity changes on the enamel surface of primary teeth exposed to soft drinks followed by application of CPP-ACPF. Methods: Five primary lower incisor crowns were vertically separated in half. Two samples (4 × 4 × 2 mm) were made by dividing one tooth into each group sample. Group A was immersed in 50 ml of soft drink (Coca Cola®) for 15 minutes for 7 days, while in group B, this was followed by CPP-ACPF application for 4 minutes twice a day for 7 days. Samples were scanned with a Scanning Electron Microscope (SEM) with 1000× magnification and the enamel surface microporosity was scored using Dudea criteria. Results: The average number of micropores on the enamel surface of primary tooth group A was 3,7500±0,43301, while in group B it was 1,6000±0,45415. Conclusion: There was a significant decrease in microporosity on the enamel surface after CPP-ACPF application in primary teeth exposed to soft drinks.



## 1 INTRODUCTION

Dental erosions, along with dental caries, are the two most common oral diseases in children and adolescents (Amaechi 2015). Based on an epidemiological systematic review of 22 studies, it was estimated that the prevalence of dental erosion is30.4% worldwide (Salas et al. 2014).

Dental erosion has a continuous impact on the deeper dental tissue layer which leads to exposure of the dentine, and consequently the pulp. The exposure of dentine tubules is a cause of dental hypersensitivity, and the exposure of the pulp, as a result of considerable loss of the dentine covering the pulp chamber, leads to its inflammation and vitality loss, and consequently, makes endodontic treatment necessary. The loss of dental tissues on the occlusal surfaces of the posterior teeth leads to an occlusal height decrease. Occlusal abnormalities, face and neck muscle pains, as well as temporomandibular joint disorders may also occur (Ewa & Szymanska 2014).

Dental erosions can be caused by intrinsic and extrinsic factors. The intrinsic cause is when the tooth surface comes into contact with stomach acid during vomiting or reflux, while the most common extrinsic cause is the excessive consumption of soft drinks (Amaechi 2015). Literature states that in young individuals a global rise in the consumption of soft drinks is the most significant factor for the occurrence of dental erosion (Koch et al. 2016).

In recent decades, the consumption of soft drinks has increased considerably. Globally this beverage consumption increased from 9.5 gallons per person per year in 1997 to 11.4 gallons in 2010 (Basu et al. 2013). In the Asia-Pacific region, Indonesia is one of the fastest growing soft drinks markets, third after China and India in sales volume from 2008 – 2013 (Taylor & Jacobson 2016). In Indonesia, soft drink sales increased by 10% from 40.2 trillion rupiah in 2008 to 48.2 trillion in 2010. In 2011, Business Monitor International predicted this figure to increase by 13% to 86.9 trillion rupiah by 2015.

One way to prevent dental erosion caused by soft drinks is by application of products that can improve



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the remineralization of tooth structure. The most commonly used remineralization materials are sodium fluoride (NaF) contained in toothpaste, mouthwash, gel and varnish. Other than fluoride, calcium and phosphate ions are required in the process. Both of these ions are present in milk and its derivative products, for example in products containing nanocomplex milk protein (casein phosphopeptide, CPP) that stabilize amorphous calcium phosphate (ACP) and form the CPP-ACP complex (Carvalho et al. 2014). CPP-ACP is formulated with fluoride with a composition of 10% CPP-ACP and 0.2% (900 ppm) NaF to increase its effectiveness (Banava et al. 2015). The study by Ashwini et al. showed that CPP-ACP and CPP-ACPF can increase surface hardness in bleached enamel. In that study, surface hardness on enamel treated with CPP-ACPF was higher than that given CPP-ACP, although the difference was not significant (Ashwini et al. 2012).

The use of CPP-ACPF is not as common and has not been as widely used as fluoride in preventing dental erosion. Thus, studies of CPP-ACPF and its relation to dental erosion are much fewer than that of fluoride. CPP-ACPF is a specific product used to remineralize dental enamel, so researchon CPP-ACPF as a remineralization material for dental erosion caused by soft drinks is needed. This study aimed to examine the microporosity changes on the enamel surface of primary teeth exposed to soft drinks followed by application of CPP-ACPF.

## 2 MATERIALS AND METHODS

This study was an experimental laboratory research with a post-test only group design. This study received ethical clearance from the Ethics Committee on Health Research of the Faculty of Dental Medicine Universitas Airlangga (292/KKEPK.FKG/XII/2016).

The sample for the study was prepared from five extracted primary lower incisor teeth that met the sample criteria. The sample criteria were retained primary teeth the crown of which was free of caries, abrasion, enamel damage, or structural and anatomical abnormalities, no restoration on the crown, and the teeth had never been given topical fluoride applications.

After extraction, the teeth were cleansed of soft tissue debris with a sterile gauze. The teeth were disinfected in 5% sodium hypochlorite solution for 1 hour. The teeth were stored in 0.1% thymol at 4°C and used within 1 month after extraction (Ferrazzano et al. 2012; Carvalho et al. 2013; Poggio et al. 2013).

The crowns were cut 2 mm from the cemento enamel junction (CEJ) to be separated from the root part with a double-faced diamond disc (Diamond Discs, Meisinger USA LLC, Centennial, USA) mounted on a contra angle handpiece. The crowns were polished with pumice dust using a brush.(Ashwini et al. 2012; Ferrazzano et al. 2012) The crowns were cut and divided into two vertical halves to obtain two enamel samples(4 × 4 × 2) (Carvalho et al. 2013; Shetty et al. 2014).

The samples were embedded in acrylic resin (Hillon Self Cured Acrylic, S. Court Limited, United Kingdom) and divided into two groups, group A which was the control group and group B which was the treatment group. Each sample in group A was completely immersed in 50 ml of Coca Cola (Coca Cola, Coca Cola Amatil Indonesia, Bekasi, Indonesia) for 15 minutes at room temperature 7 days in a row without being followed by any treatment (Carvalho et al. 2013; Bertoldi et al. 2015).

Each sample in group B was also immersed in 50 ml of Coca Cola for 15 minutes at room temperature 7 days continuously followed by application of CPP-ACPF paste (GC Tooth Mousse Plus™, Recaldent, Tokyo, Japan) for 4 minutes twice a day for 7 days. Application was performed by using a microbrush (Microbrush, Dochem, Shanghai, China).

After each immersion and application, the sample was rinsed with aquades (Onelab Waterone™, PT Jayamas Medica Industri, Sidoarjo, Indonesia) and stored in artificial saliva (Bertoldi et al. 2015). All samples were then immersed in artificial saliva solution and incubated at 37°C for 8 hours (Rallan et al. 2013). Samples were dried and analyzed using SEM (ZEISS EVO MA 10, ZEISS, Cambridge, United Kingdom) with 1000× magnification (Poggio et al. 2013).

The analysis was conducted using Dudea criteria by dividing the images of SEM results into four quadrants and each quadrant was assessed and scored as follows: (1) smooth, normal enamel; (2) fissures on the enamel surface; (3) images of mildly increased porosity; (4) images of exposed enamel prisms and dissolution; then the average score was calculated (Dudea et al. 2009; Wahluyo 2013). The data was analyzed using the statistical package for social sciences (SPSS) version 20.0 (IBM SPSS Statistics 20, IBM Corp, New York, United States) and the Kolmogorov-Smirnov test and paired sample t test were performed. The confidence level of the

study was kept at 95%; hence a p value < 0.05 indicated a statistically significant difference.

## 3 RESULTS

The representative image of SEM results of enamel surface of primary tooth exposed to soft drinks without CPP-ACPF application (group A) are shown in Figure 1, whereas the representative image of SEM results of enamel surfaces of primary tooth exposed to soft drinks with CPP-ACPF application (group B) are shown in Figure 2.

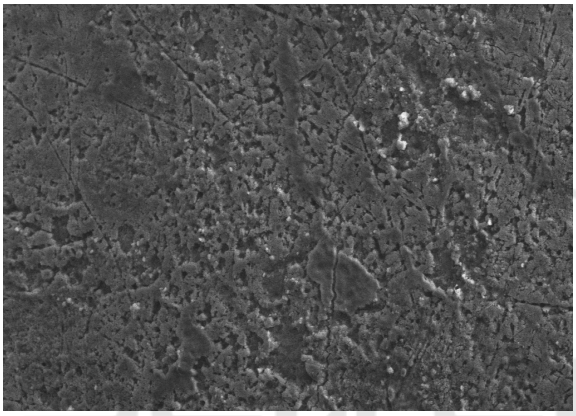

Figure 1: Image of SEM result group A

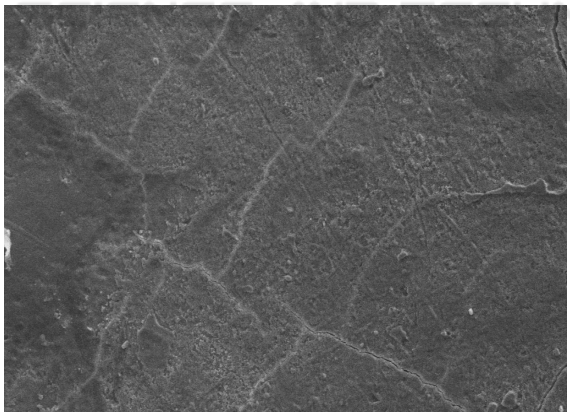

Figure 2: Image of SEM result of group B

The data obtained was tested to determine whether the sample data was normally distributed using the Kolmogorov-Smirnov test in each treatment group.

Table 1: The result of Kolmogorov-Smirnov test

| Group | N | X ± SD | Sig. (2-tailed) |
|---|---|---|---|
| Group A | 5 | 3,7500 ± 0,43301 | 0,692 |
| Group B | 5 | 1,6000 ± 0,45415 | 0,955 |

The results of the Kolmogorov-Smirnov test on group A were 3.7500on average with a standard deviationof 0.43301 and Asymp. Sig. (2-tailed) 0.692, while the results of the Kolmogorov-Smirnov test on group B were 1.6000 on average with a standard deviation of 0.45415 and Asymp. Sig. (2-tailed) 0.955. An Asymp. Sig. (2-tailed) value greater than 0.05 indicates that the research data was normally distributed.

After the normality test was performed and gave normal results, it was continued by paired sample t test, which was performed to test two paired samples and compare their mean. Based on the test, the Sig. (2-tailed) value was 0,000 which met the requirements of p <0.05. This shows that there were significant differences between the two sample groups.

## 4 DISCUSSION

The results of this study showed that there was a significant difference in the microporosity on the enamel surface of primary teeth exposed to a soft drink with CPP-ACPF application compared to the group without CPP-ACPF application with significantly lower values in the group with CPP-ACPF application. This suggests that there was a change after CPP-ACPF application in the microporosity on the enamel surface of primary teeth exposed to the soft drink characterized by decreased microporosity.

To simulate the clinical state, primary teeth were used that met the sample criteria and stored in artificial saliva. Each used primary tooth was cut into two vertical halves to become two samples and treated differently. This was to create research standards and minimize the number of confounding variables.

Dental erosion is a cyclic process influenced by demineralization, remineralization and abrasion, where typically the balance is shifted towards mineral loss due to the consumption of dietary acids. It usually takes a period of months or even years for the lesion to develop (Rallan et al. 2013).

Soft drinks contain high amounts of sugar, calories and caffeine, and provide no valuable nutrition. A carbonated beverage is a mixture of phosphoric acid, sugar, caffeine, coloring and flavoring agents. The active ingredient in these beverages is phosphoric acid, with an acidic pH generally less than three, which is the same as acetic acid. However, it doesn't taste like acid as manufacturers add large quantities of sugar. A high amount of phosphoric acid is added to keep the water sterile as no bacteria can live in such acidic conditions. The solution of phosphoric acid in cola drinks is strong enough to cause human teeth to become soft within 2 days (Damle et al. 2011).

Soft drinks contain a high proportion of H+ ions which can lower the pH of the oral cavity to below the critical pH threshold 5.5, and return to neutral pH within 20 to 50 minutes. A decrease in pH in the oral cavity causes the displacement of calcium and phosphate ions which are the main components of the tooth structure (Banava et al. 2015; Bertoldi et al. 2015).

Saliva in the oral cavity serves as the first protector against the acidic conditions. Another feature of saliva, with its buffer capacity, is to keep calcium and phosphate ions in the oral cavity at saturation point, protecting the hydroxyapatite crystals and increasing enamel remineralization. But the equilibrium can shift toward demineralization of tooth structure. This may be due to active caries or bacterial plaque. Thus, the application of remineralization agents is important to improve remineralization (Heshmat et al. 2014).

CPP-ACPF paste is one of the remineralization agents that has the potential to cope with dental erosion better than the more commonly used remineralization agents, fluoride and CPP-ACP. It is proven ina study by Shetty that CPP-ACPF remineralizes enamel subsurface lesions better than NaF and CPP-ACP. NaF showed better results than CPP-ACP in enamel remineralization although it was not statistically significant (Shetty et al. 2014).

The CPP-ACPF paste contains casein phosphopeptide-amorphous calcium phosphate complex with fluoride. Casein can work in acidic and alkaline environments. When the CPP-ACPF paste is applied, the paste is in contact with saliva, pellicle, and plaque, and acts as a storage of calcium which increases the remineralization potential. In an acid environment, the ACP will be separated from CPP, increasing the amount of calcium and phosphate ions in the saliva and stabilizing the amount of both ions on the tooth surface. So, if there is a white spot lesion, which is the first sign of caries, the active ions will enter the lesion and increase remineralization. Increased remineralization can also be due to the pH being neutral due to calcium and phosphate in plaque (Rallan et al. 2013; Heshmat et al. 2014).

The fluoride content of CPP-ACPF is 0.2% or equivalent to 900 ppm NaF. Fluoride is also important for the treatment of white spot lesions and preventing caries. Fluoride can work synergistically with a variety of caries prevention materials and methods. The fluoride content of CPP-ACPF causes localized calcium, phosphate, and fluoride ion activities, it keeps the amount of calcium and phosphate ions in saturation and suppresses demineralization. If calcium and phosphate ions are needed to improve remineralization, along with those two ions, fluoride is required for the formation of fluorapatite crystals and fluorhydroxyapatite which are relatively more resistant to acids (Rallan et al. 2013; Heshmat et al. 2014). This leads to a significant decrease in microporosity on the enamel surface after CPP-ACPF application in primary teeth exposed to soft drinks.

# 4 CONCLUSIONS

There was a significant decrease in microporosity on the enamel surface after CPP-ACPF application in primary teeth exposed to soft drinks.

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