EFFECT OF VARIOUS FORMS OF CALCIUM IN DENTAL PRODUCTS ON HUMAN ENAMEL MICROHARDNESS *IN VITRO*

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Abstract. The aim of this *in vitro* study was to compare the remineralization potential of dental products containing calcium on human enamel softened by soft drinks. Fifty sound human premolar teeth were randomly divided into 5 treatment groups (n=10): artificial saliva, 1,000 ppm fluoride toothpaste, CPP-ACP paste, CPP-ACP with 900 ppm fluoride paste and tricalcium phosphate with 950 ppm fluoride paste. All specimens were immersed in cola soft drink and artificial saliva for 10 cycles of 5 seconds each; this procedure was repeated twice at six-hour intervals. All specimens were remineralized by treatment with the dental products mentioned above for 5 minutes and kept in artificial saliva at 37ºC for 6 hours. The surface microhardness of the enamel was measured with a Vickers hardness tester (100 grams, 15 seconds) at baseline, after erosion and after remineralization. Comparison of the mean microhardness numbers within groups was made by one-way repeated measures ANOVA and between groups with the one-way ANOVA with a level of significance of $p<0.05$. The mean surface microhardness in all groups decreased significantly after being eroded by the soft drink and increased after treatment. After remineralization treament, the mean surface microhardness of the artificial saliva group was significantly less than the other groups. The CPP-ACP paste, CPP-ACP with 900 ppm fluoride paste and tricalcium phosphate with 950 ppm fluoride paste treatments all increased the hardness of the teeth *in vitro*.

Keywords: CPP-ACP, erosion, fluoride, microhardness, soft drink, tricalcium phosphate

INTRODUCTION

Dental erosions are areas of localized loss of dental hard tissue chemically etched away from the tooth surface by acid dissolution without bacterial involvement (Ten Cate and Imfeld, 1996). This process may be caused by extrinsic or instrinsic agents. Extrinsic agents include acidic foodstuff, beverages or snacks or may occur following environment exposure to acidic agents (Eccles and Jenkins, 1974; Asher and Read, 1987). Hemingway *et al* (2006) reported dietary acids are the

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most important extrinsic factor.

As lifestyles have changed, there has been a 50% increase in consumption of soft drinks over the past few decades, especially among children and adolescents (Tehmassebi *et al*, 2006). Carbonated soft drinks contain carbonic acid and organic acids (commonly citric acid) are frequently added to improve taste. Citrate anions chelate calcium ions, decreasing the amount of free ionic calcium available in both the saliva and at the enamel surface, thereby enhancing demineralization and limiting the potential for remineralization (Cai *et al*, 2007).

Dental erosions are becoming increasingly important to long-term dental health. Therefore, it seems reasonable to search for effective agents for prevention or repair of these erosions. Fluoride has been used as an anti-erosive agent (Lussi *et al*, 2008); however, some studies have shown fluoride has insufficient efficacy (Larsen and Richards, 2002; Wang *et al*, 2008). Inclusion of minerals, such as calcium and phosphate, may enhance its anti-erosion benefits. Rirattanapong *et al* (2011) reported Tooth mousse [Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)], Tooth mousse Plus (same as Tooth mousse with the addition of fluoride at 900 ppm)(CPP-ACFP) and Clinpro Tooth crème [Tricalcium phosphate (TCP) with 950 ppm of fluoride (TCPF)] have anti-erosive properties.

The objective of the present study was to evaluate the efficiency of calcium in various dental products against erosions caused by soft drinks.

MATERIALS AND METHODS

Specimen preparation

This study was approved by the Ethics Committee of Mahidol University. Fifty fresh sound premolars extracted for orthodontic purposes, were collected and kept in 0.9% normal saline and the radicular part of each tooth was removed. The specimens were embedded in self-cured acrylic resin. The labial surfaces were wet ground using 400, 600, 1,200, 2,000 and 2,500 grit silicon carbide paper to obtain a smooth flat surface. A 3x4 mm test window was demarcated by scalpel cuts to assist in specimen orientation during the surface microhardness test. Baseline surface microhardness of sound enamel was measured on the labial surface by means of a Vickers indenter (FM-700e Type D, Future-Tech, Tokyo, Japan) with 100 grams of force for 15 seconds (Moupomé *et al*, 1999). Four indentations per test were performed on each specimen during each experimental stage.

Erosion procedure

The pH of a Cola soft drink (Coca-Cola, ThaiNamthip, Bangkok, Thailand) and artificial saliva [containing 0.65 grams per liter potassium chloride British Pharmacopoeia (BP), 0.058 g/l magnesium chloride BP, 0.165 g/l calcium chloride BP, 0.804 g/l dipotassium hydrogen phosphate US pharmacopeia, 0.365 g/l potassium dihydrogen phosphate, 2 g/l sodium carboxymethyl cellulose BP and deionized water to make 1 liter (modified from Amaechi *et al*, 1999)] were measured by a pH meter (Thremo Scientific Orion 3 star RDO® portable pH meter, MA, USA). All specimens were immersed in cola for five seconds, then in artificial saliva for another five seconds. Ten cycles of the immersion process were conducted at room temperature. The process was repeated two times at six-hour intervals. Between each test, the specimens were stored in artificial saliva at room temperature. After the erosion process was completed, all the specimens were rinsed in deionized water

Table 1
Materials used in this study.

Material	Manufacturer
Sodium fluoride 1,000 ppm (Colgate total® professional clean) lot no.00132J1	Colgate-Palmolive Company, Thailand
Casein phosphopeptide- amorphous calcium phosphate (Tooth Mousse) lot no.100722s	GC, Tokyo
Casein phosphopeptide- amorphous calcium phosphate with sodium fluoride 900 ppm (Tooth Mousse plus) lot no.100416s	GC, Tokyo
Tricalcium phosphate with sodium fluoride 950 ppm (Clinpro™ Tooth Crème) lot no.90413	3M ESPE, USA

and blotted dry. The Vickers indenter test was performed, by making 4 indentitions at least 120 m apart to determine the microhardness value.

Remineralization procedure

The materials used in this study are shown in Table 1. The specimens were randomly divided into five groups of 10 teeth each: the teeth in the groups were treated as follows: Group 1 (control group) received no treatment. Group 2 was treated with a 0.5 mm layer of 1,000 ppm of fluoride (1,000 ppmF), Group 3 was treated with a 0.5 mm layer of CPP-ACP, Group 4 was treated with a 0.5 mm layer of CPP-ACPF and Group 5 was treated with TCPF for 5 minutes each. All specimens were stored at 37ºC for 6 hours in artificial saliva after the remineralization process was completed. The specimens were washed with deionized water and blotted dry. The Vickers indenter test was performed with 4 indentations at least 120 m apart to detect the microhardness value.

Statistical analysis

One-way repeated measure analysis of variance (ANOVA) was used to compare the surface microhardness values at baseline, after erosion and after remineralization, with the level of significance set at $p<0.05$. One-way ANOVA and Student-Newman-Keuls (SNK) test were used to compare the mean surface microhardness values between groups with a level of significance set at $p<0.05$.

RESULTS

The mean surface microhardness values at baseline, after erosion and after remineralization are shown in Table 2. The mean baseline microhardness value (±SD) was 342.1 ± 21.9 Vickers Hardness Number (VHN). The mean baseline microhardness values were not significantly different among the study groups.

After erosion with the cola (pH=2.8), The microhardness values for each group were not significantly different from each other. The mean surface microhardness value (±SD) was 293.6 ± 21.1 VHN, with a 14.2% reduction from baseline, a significant decrease in microhardness values from baseline.

After remineralization, the mean surface microhardness values increased significantly compared to the values after erosion. The microhardness value of the

Table 2
Enamel microhardness at baseline, after erosion and after remineralization (N=50).

Group	Condition (Mean VHN±SD)		
	Baseline	After erosion	After remineralization
Artificial saliva	339.95±22.16	291.45±16.99	292.41±19.81
1,000ppmF-toothpaste	347.26±31.23	306.26±29.19	336.33±29.95
CPP-ACP	345.93±20.62	293.57±13.55	326.77±15.62
CPP-ACP +900ppmF	343.94±14.58	293.15±22.06	323.77±37.52
TCP +950ppm F	333.37±19.41	283.82±17.88	322.65±22.43

Within columns, all groups were significantly different from the control (One-way ANOVA, $p<0.05$)
Among columns, all groups were significantly different from the other columns (One-way repeated measures ANOVA, $p<0.05$)
VHN, Vickers hardness number

teeth treated only with artificial saliva was the lowest compared to those treated with the other dental products.

DISCUSSION

The baseline surface microhardness values in this study ranged from 276.4-347.3 VHN. This study required a flat area to measure microhardness, thus the area was not the original surface, which might explain the range in baseline values. These values were similar to those found by Panich and Poolthong (2009) and Maupomé *et al* (1999) but higher than those found by Seow and Thong (2005) and Wongkhantee *et al* (2006). The microhardness values were different because of specimen preparation, differences in the part of the tooth used and the types of teeth used (Cuy *et al*, 2002).

In this study, after specimens were eroded, the mean microhardness percent reduction from baseline was 14.16%. This result is similar to that of Panich and Poolthong's study (2009), but different from the study by Srinivasan *et al* (2010), in which the mean microhardness reduction was 24.5%. This difference might have been due to the timing of the erosion process. This study followed the method of Wongkhantee *et al* (2006) of which the erosion process was performed three times at six-hour intervals to represent three mealtimes.

In this study, remineralization occurred in the artificial saliva group. Many previous studies have pointed to the resistance to erosion of enamel surfaces by saliva. Enamel demineralizied caused by acidic beverages has a tendency to reharden following exposure to saliva in the mouth (Amaechi and Higham, 2001) or to remineralizing solution (Shannon *et al*, 1977; Kim *et al*, 2001). However, the recovery of microhardness was the lowest in the artificial saliva treatment group, compared to the other treatment groups.

After remineralization the various treatment groups, except for artificial saliva alone, were not significantly different from each other.

There is convincing evidence for the effectiveness of fluoride in caries prevention. Many studies have described the preventive effect of different fluoride formulations on dental erosions or their effects on abrasion resistance after an erosive attack. The results of this study are similar to that of Rirattanapong *et al* (2011) who found fluoride at 1,000 ppm could reharden enamel erosions caused by chlorinated water.

CPP-ACP is based on a nano-complex of milk protein casein phosphopeptide with amorphous calcium phosphate (ACP). The mineralization mechanism of CPP-ACP involves localization of ACP at the tooth surface, which buffers free calcium and phosphate ions; these ions depress demineralization and promote remineralization (Reynold and Black, 1987). Our findings are consistent with the findings of many studies demonstrating the remineralization effects of CPP-ACP (Cai *et al*, 2007; Tantbirojn *et al*, 2008).

Our findings are similar to those of Rirattanpong *et al* (2011) who found CPP-ACP with fluoride at 900 ppm can remineralize eroded enamel. It is likely the combination of CPP-ACP and fluoride resulted in co-localization of calcium and phosphate ions with fluoride ions at the enamel surface, presumably as CPP-ACPF nanocomplexes (Cross *et al*, 2004). Srinivasan *et al* (2010) reported an additive effect between CPP-ACP and fluoride to prevent caries. Our study did not find an additional benefit by adding fluoride to CPP-ACP compared with using CPP-ACP alone. This may be due to differences in study design.

Tricalcium phosphate (TCP) is a hybrid material created with a milling technique that fuses beta tricalcium phosphate and sodium lauryl sulfate or fumaric acid. This blending results in a functionalized calcium and a free phosphate, designed to increase the efficacy of fluoride remineralization (Karlinsey and Markey, 2009). When TCP comes into contact with the tooth surface and is moistened by saliva, the protective barrier breaks down, making calcium, phosphate and fluoride ions available to the teeth. Our results are in agreement with those of *in vitro* studies (Karlinsey *et al*, 2009; Rirattanapong *et al*, 2011) showing TCP with fluoride can promote remineralization of eroded enamel.

In conclusion, the various forms of calcium in dental products were all equally effective in increasing the surface microhardness of eroded enamel caused by soft drinks *in vitro*.

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