EFFECT OF FIVE DIFFERENT DENTAL PRODUCTS ON SURFACE HARDNESS OF ENAMEL EXPOSED TO CHLORINATED WATER *IN VITRO*

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Abstract. The objective of this study was to assess the effect of five different dental products on surface microhardness of enamel exposed to chlorinated water *in vitro*. Sixty human sound premolar teeth, extracted for orthodontic reasons, were used and randomly divided into 6 groups (10 specimens each group: artificial saliva, 1,000 ppm fluoride toothpaste, CPP-ACP paste, CPP-ACP with 900 ppm fluoride paste, CPP toothpaste and tricalcium phosphate with 950 ppm fluoride paste. All specimens were immersed in chlorinated water at pH 5 for 24 hours and then remineralized by coating with a dental product from each group for 5 minutes and kept in artificial saliva at 37°C for 6 hours. The surface microhardness of the enamel was measured with a Vickers hardness tester at baseline, after erosion by chlorinated water and after remineralization. Comparisons of the mean microhardness within each group were made a one-way repeated measures ANOVA and between groups using a one-way ANOVA and an LSD test at $p < 0.05$. The mean surface microhardness in all groups decreased significantly after eroding with chlorinated water and increased after remineralization. After remineralization, the mean surface microhardness of the artificial saliva group was significantly less than the other groups. Five different dental products (1,000 ppm fluoride toothpaste, CPP-ACP paste, CPP-ACP with 900 ppm fluoride paste, CPP toothpaste and tricalcium phosphate with 950 ppm fluoride paste) increased the hardness *in vitro* of eroded enamel caused by chlorinated water.

Keywords: chlorinated water, CPP-ACP, erosion, fluoride, microhardness, tricalcium phosphate

INTRODUCTION

Dental erosion is defined as a loss of tooth surfaces caused by intrinsic and extrinsic factors (Wiegand *et al*, 2004). Intrinsic factors include vomiting and gastroesophageal reflux. Extrinsic factors include exposure to acidic foods, drinks, medications or other lifestyle factors (Caglar *et al*, 2005).

There have been several case reports of competitive swimmers suffering dental erosion from swimming in gas-chlorinated pools (Centerwall *et al*, 1986). A pH of 5.5 is considered to be the critical pH for enamel dissolution. Chlorine is the chemical most often used to keep swimming pools free of bacteria. When chlorine is

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added into water, it produces hypochlorous acid (HOCl) and hypochloride ion (OCl). Extremely high levels of chlorine in the water cause a decrease in the pH of the swimming pool water. In 2003, 139 swimming pools in 15 provinces of Thailand were examined by the Dental Public Health Division, Department of Health, to assess the risk of dental erosions among swimmers; their results showed 31.2% of swimming pools had a pH < 5.5, increasing the risk to swimmers (Chanduaykit *et al*, 2005).

Dental erosions are important in the long-term management of the health of teeth. It is important to search for agents to prevent or repair of dental erosions. Although there is convincing evidence of the preventive effects of fluoride against dental erosions and abrasion resistance after an erosive attack (Lussi *et al*, 2004), the following newer dental products claim to prevent erosions: Tooth Mousse (CPP-ACP), Tooth Mousse Plus (CPP-ACFP), Hi Herb toothpaste (CPP) and Clinpro Tooth créme (tricalcium phosphate).

The objective of the present study was to evaluate the effect of five dental products on the surface microhardness of enamel exposed to chlorinated water *in vitro*.

MATERIALS AND METHODS

Specimen preparation

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

Erosion procedure

All specimens were soaked in chlorinated water at room temperature for 24 hours. The chlorinated water was prepared from $Ca(ClO)_2$ 3 mg dissolved in 3,000 ml tap water and adjusted to a pH of 5.0 with 0.1 M HCl. All the specimens were then rinsed in deionized water and blotted dry. The Vickers Indenter Test was performed, with indentations at least 120 m apart, and the average of the 4 indentations was used as the microhardness value.

Remineralization procedure

The specimens were randomly divided into six groups of 10 teeth each: Group 1 (control group) received no treatment, groups 2,3,4,5 and 6, were treated with a 0.5 mm layer of 1,000 ppmF, CPP-ACP, CPP-ACP plus 900 ppmF, CPP, and tricalcium phosphate plus 950 ppmF, respectively, for 5 minutes (Table 1). All specimens were stored at 37ºC for 6 hours in 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 as modified from Amaechi *et al* (1999). After the

Table 1
Studied materials.

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
Casein phosphopeptide (Hi Herb®) lot no. 6829092009	Sahaphattanaphiboon Company, Thailand
Tricalcium phosphate with sodium fluoride 950 ppm (Clinpro™ Tooth Créme) lot no.90413	3M ESPE, USA

Table 2
Enamel microhardness at baseline, after erosion and after remineralization (N=60).

Group	Condition (Mean VHN±SD)		
	Baseline	After erosion	After remineralization
Artificial saliva	344.42 ± 14.40	305.05 ± 11.58	313.29 ± 10.13
1,000 ppmF-toothpaste	341.36 ± 15.02	311.74 ± 14.01	328.66 ± 14.22
CPP-ACP	346.75 ± 10.29	314.67 ± 12.62	333.64 ± 14.00
CPP-ACP +900ppmF	340.51 ± 14.67	301.95 ± 11.81	326.12 ± 9.17
CPP toothpaste	345.45 ± 13.45	313.56 ± 12.64	329.48 ± 13.95
TCP +950ppm F	344.76 ± 18.51	314.93 ± 15.72	332.15 ± 16.13

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

remineralization process was completed, the specimens were washed with deionized water and blotted dry. The Vickers Indenter Test was performed, with indentations at least 120 m apart, and the average of the 4 indentations was used as the microhardness value.

Statistical analysis

A one-way repeated measure analysis of variance (ANOVA) was used to compare the surface microhardness values at baseline, after erosion and after remineralization with significance set at $p<0.05$. The one-way ANOVA and least significant difference (LSD) were used to compare the mean surface microhardness values among the groups with a significance level 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 was 343.82±13.92 VHN. One-way ANOVA revealed no statistically significant difference among the groups at baseline.

After erosion, one-way ANOVA results showed no statistically significant difference among groups. The average enamel microhardness was 310.31 VHN, a 9.76% reduction from baseline.

After remineralization, the mean microhardness values increased siginificantly compared to the mean microhardness values after erosion. The one-way ANOVA results show the microhardness value of the artificial saliva control was lower than the other dental products.

DISCUSSION

The baseline enamel microhardness value in this study was 343.87±14.14 VHN. This is similar to studies by Panich and Poolthong (2009) and Maupomé *et al* (1999) but higher than the studies by Seow and Thong (2005) and Wongkhantee *et al* (2006). The microhardness values may have been different due to differences in specimen preparation, area of the tooth and type of tooth used (Cuy *et al*, 2002).

After erosion with chlorinated water, the mean microhardness reduction was 9.76% below baseline. This reduction is different from a study by Chuenarrom *et al* (2010), in which the mean microhardness reduction was 23.2%. This difference may have been due to different pH levels or timing of the erosion process.

In this study, remineralization occurred in the artificial saliva group. The artificial saliva was produced using the formulation of Amaechi *et al* (1999). The artificial saliva contained potassium chloride, magnesium chloride, calcium chloride, dipotassium hydrogen phosphate and potassium dihydrogen phosphate, which may help remineralize enamel. Results from several studies have shown artificial saliva can reharden eroded enamel (Devlin *et al*, 2006; Lussi *et al*, 2008). However, the rehardening of the eroded enamel with artificial saliva was less effective than with the other dental products.

Remineralization by 1,000 ppm fluoride toothpaste, CPP-ACP, CPP-ACP plus 900 ppm fluoride, CPP toothpaste, tricalcium phosphate, resulted in equal levels of microhardness that were not siginificantly different among the groups. Application of fluoride to the enamel surface can cause remineralization. The results of this study are similar to studies by Magalhães *et al* (2008) and Moretto *et al* (2010) who found fluoride 1,000 ppm can reharden enamel.

The CPP-ACP complex acts as a vehicle for calcium and phosphate, transporting it to the tooth surface and localizing it in plaque and salivary pellicles (Reynolds *et al*, 2009). A reduction in pH results in a release of calcium and phosphate ions, which act to promote remineralization. Most studies of CPP-ACP have focused on caries; a few studies have shown that CPP-ACP can reduce erosion from chlorinated water (Vongsawan *et al*, 2010), reduce tooth wear from abrasion (Ranjikar *et al*, 2009) and can increase enamel hardness following erosive by soft drinks (Panich and Poolthong, 2009).

A study by Srinivasan *et al* (2010) showed both CPP-ACP and CPP-ACP with 900 ppm fluoride significantly remineralized softened enamel, and the CPP-ACP and fluoride combination had greater remineralization potential than CPP-ACP, but the result of our study showed no significant difference between CPP-ACP and CPP-ACP plus fluoride. This may be due to a different study design.

There has been much interest in CPP as an anticariogenic and remineralizing agent. CPP is produced by enzymatic digestion of casein, generally by trypsin (FitzGerald, 1998). Recently, CPP was found to reduce caries incidence during a clinical trial (Rao *et al*, 2009) that found no difference between CPP and fluoride 1,190 ppm, similar to our study.

Tricalcium phosphate (TCP) is a new hybrid material created with a milling technique that fuses beta tricalcium phosphate and sodium lauryl sulfate or fumaric acid. 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 tooth. A study by Karlinsey *et al* (2009) showed an innovative form of tricalcium phosphate plus fluoride 225 ppm can remineralize softened enamel but there are no published studies of TCP with 900 ppmF.

The five different dental products (1,000 ppm fluoride toothpaste, CPP-ACP paste, CPP-ACP with 900 ppm fluoride paste, CPP toothpaste and tricalcium phosphate with 950 ppm fluoride paste) increased the surface microhardness of eroded enamel by chlorinated water *in vitro*.

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