EFFECT OF ADDING TRICALCIUM PHOSPHATE TO FLUORIDE MOUTHRINSE ON MICROHARDNESS OF DEMINERALIZED PRIMARY HUMAN TOOTH

Praphasri Rirattanapong[1], Kadkao Vongsavan[1], Chavengkiat Saengsirinavin[2] and Pimonchat Phuekcharoen[3]

[1]Department of Pediatric Dentistry, Faculty of Dentistry; [2]Research Unit, Faculty of Dentistry, Mahidol University, Bangkok; [3]Dental Department, Khao Khitchakut Hospital, Chantaburi, Thailand

Abstract. The purpose of the present study was to evaluate the effect of fluoride mouthrinse containing tricalcium phosphate on microhardness of demineralized primary enamel. Thirty-six sound primary incisors were immersed in a demineralizing solution (pH 4.4) for 96 hours at 37°C to create artificial caries-like lesions. After artificial caries formation, the specimens were randomly divided into 3 groups (with 12 specimens in each group): Group A: deionized water; Group B: 0.05% NaF plus 20 ppm tricalcium phosphate mouthrinse and Group C: 0.05% NaF mouthrinse. All the specimens were immersed for 1 minute at 37°C three times per day for 7 days in the respective mouthrinse among pH cycling. The surface microhardness was examined using a Vickers hardness tester (100 grams for 15 seconds) at baseline, before and after the pH-cycling procedure. Data were analyzed using one-way ANOVA and Tukey's multiple comparison tests with a significance level of 0.05. After treatment, Group A had a significantly lower surface microhardness value than the other two groups (p=0.000); however, there was no significant difference between Groups B and C (p=0.728). We concluded fluoride mouthrinse containing tricalcium phosphate and fluoride mouthrinse have similar remineralizing effects on microhardness of demineralized primary teeth.

Keywords: fluoride mouthrinse, microhardness, primary enamel, remineralization, tricalcium phosphate

INTRODUCTION

Dental caries are common among children in Thailand (Narksawat *et al*, 2011). The prevention of dental caries in children and adolescents is a priority for dental practices and is more cost-effective than treatment. Fluoride is an important popular agent for promoting remineralization and is available in a variety of forms, such as toothpaste, mouthrinse, gel and solution (Marinho *et al*, 2003).

Many studies have confirmed the benefits of fluoride mouthrinses against caries over the past 30 years, especially among children (Marinho *et al*, 2003, 2004; Divaris *et al*, 2012). School based programs for dental health have been conducted world-wide as a public health preventive measure (Reich *et al*, 2002). Fluoride is not

Correspondence: Praphasri Rirattanapong, Department of Pediatric Dentistry, Faculty of Dentistry, Mahidol University, 6 Yothi Street, Bangkok 10400, Thailand.
Tel: 66 (0) 2200 7821 ext 30
E-mail: praphasri.rir @mahidol.ac.th

the only agent used for remineralization; calcium and phosphate have also been used (Featherstone, 2008). Calcium and phosphate ions are the primary constituents of tooth enamel (Gurunathan *et al*, 2012). Adequate quantities of these ions must be present for remineralization to occur (Cury and Tenuta, 2009). Schemehorn and colleagues (1999) evaluated the addition of calcium and phosphate to fluoride toothpaste or mouthrinse can improve remineralization and increase fluoride uptake.

Tricalcium phosphate has been developed by fusing beta-tricalcium phosphate (β-TCP) with sodium lauryl sulfate or fumaric acid (Karlinsey and Pfarrer; 2012). This combination is designed to assist with the fluoride in remineralization (Twetman *et al*, 2004; Shen *et al*, 2011). Tricalcium phosphate provides catalytic amounts of calcium to boost fluoride efficacy and can be mixed with fluoride in mouthrinse or dentifrice, because it does not react before reaching the tooth surface (Karlinsey *et al*, 2010a). When the tricalcium phosphate comes into contact with the tooth surface and is moistened by saliva, it breaks down, making the calcium, phosphate and fluoride ions available for the tooth. The fluoride and calcium then react with the weakened enamel to provide a seed for enhanced mineral growth (Karlinsey *et al*, 2010b; Shen *et al*, 2011).

Many studies have evaluated remineralization of enamel lesions on permanent tooth by various fluoride and calcium phosphate containing mouthrinses (Kumar *et al*, 2008; Puig-Silla *et al*, 2009; Moezizadeh and Alimi, 2014). However, there have been no studies comparing the efficacy of fluoride only mouthrinse with fluoride combined with tricalcium phosphate mouthrinse on microhardness of demineralized enamel in primary human teeth.

The objectives of this *in vitro* study were to evaluate and compare the effects of fluoride mouthrinse with fluoride containing tricalcium phosphate mouthrinse on the microhardness of demineralized enamel in primary human teeth.

MATERIALS AND METHODS

Specimen preparation

Thirty-six human primary incisors were used for this study and stored in normal saline solution at room temperature until use. The radicular part of each tooth was removed. The specimens were embedded in self-cured acrylic resin, with the labial surface leveled on top and lying flat and parallel to the horizontal plane. Specimens were polished using silicon carbide sandpaper using progressively fines grit (400, 600, 1,200, 2,000 and 2,500) to obtain a flat smooth surface and then stored in deionized water at room temperature until use. The baseline microhardness of the enamel was measured on the labial surface using a Vickers indenter (FM-700e Type D, Future-tech,Tokyo, Japan) with 100 grams of force for 15 seconds (Rirattanapong *et al*, 2012). Four indentations per test were performed and the microhardness value was an average of the four readings.

This study was approved by The Ethics Committee of Mahidol University. (MU-DT/PY-1RB 2013/049.0509).

Demineralizing and remineralizing solutions preparation

Demineralizing solution 1 (D1) consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid with the pH being adjusted to 4.4 using 1M KOH. Demineralizing solution 2 (D2) contained the same components as D1, but the pH was adjusted to 4.7 by the addition of 1M KOH. The remineralizing solution

(R) was comprised of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl with the pH being adjusted using 1 M KOH to 7.0 (Thaveesangpanich *et al*, 2005). The demineralizing and remineralizing solutions were freshly prepared for each pH-cycling period and the pH was checked each time prior to use.

Artificial caries lesion formation

Each specimen was immersed in 3 ml of D1 and incubated at 37°C (Sheldon manufacturing, model 1545, Cornelius, OR) for 4 days (Thaveesangpanich *et al*, 2005). The specimens were then immersed in artificial saliva. The artificial saliva used consisted of 0.65 grams per liter KCl (British Pharmacopoeia, Norwrick, UK), 0.058 g/l $MgCl_2$ (British Pharmacopoeia), 0.165 g/l $CaCl_2$ (British Pharmacopoeia), $K_2(HPO_4)_2$ (Pharmacopoeia, Rockville, MA), $KH_2(PO_4)_3$ (British Pharmacopoeia), 2 g/l $NaCO_2CH_3$ cellulose (Pharmacopoeia, Rockville, MA) and deionized water was added to make 1 liter (modified from Amaechi *et al*,1999). Microhardness was measured by a Vickers indenter test.

Grouping

After artificial carious lesion formation, the specimens were pooled and randomly assigned to one of three groups (n=12): Group A (control) - deionized water, Group B - 0.05% sodium fluoride and 20 ppm tricalcium phosphate mouthrinse, Group C - 0.05% sodium fluoride mouthrinse.

pH-cycling process

An experimental process intended to imitate the changes in pH in the oral environment for seven days was employed in the present study (Yimcharoen *et al*, 2011). Each cycle involved placing in D2 for 3 hours twice daily and placing in R for 2 hours in between. All the specimens were immersed and agitated for 1 minute, three times a day in one of the treatment rinses at room temperature (Moi *et al*, 2008). All of the specimens were placed in remineralizing solution overnight at 37°C in a controlled environment incubator shaker at 150 rpm (Series 25 incubator shaker, Ramsey, MN). At the end of the pH-cycling period, the microhardness for each tooth was measured by the Vickers indenter test.

Statistic analysis

The one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare microhardness values at baseline, before and after pH-cycling and compare the mean microhardness values among the groups. Significance was set at $p< 0.05$.

RESULTS

The mean microhardness values at baseline, before and after the pH-cycling are shown in Table 1. At baseline, the mean microhardness value (±SD) was 328.453±26.566 Vicker Hardness Number (VHN). The mean baseline microhardness values were not significantly different among the groups (p=0.271).

Before pH-cycling period, the microhardness values were not significantly different among the groups (p=0.526) and were at significantly lower values than baseline (p=0.000) having a 61.19±9.8% reduction.

After pH-cycling, groups B and C had a significant increase in mean microhardness compared to before pH-cycling. Group A had a significantly lower microhardness than Groups B and C (p=0.000). There was no significant difference in microhardness values between Groups B and C after pH-cycling (p=0.728).

Table 1
Microhardness values at baseline, before and after 7 days of pH-cycling.

Group	Treatment	Condition (Mean±VHN)		
		Baseline	Before pH-cycling	After pH-cycling
A	Deionized water	332.332±23.218[a]	136.171±31.750[b]	179.318±25.593[b]
B	0.05%NaF plus TCP	318.320±27.499[a]	122.106±32.051[b]	231.848±18.729[c]
C	0.05%NaF	334.705±27.913[a]	124.338±31.199[b]	225.101±19.986[c]

Same letters indicate no statistically significant difference ($p \geq 0.05$).

DISCUSSION

The mean baseline microhardness value was 328.45±26.57 Vicker Hardness Number (VHN) and ranged from 264.85-367.61 VHN. Our study required a flat area to measure surface microhardness, so we used an area that was not the original surface after preparation; it could explain the range in the baseline values. Our mean baseline microhardness value was similar to those found by Rirattanapong *et al* (2012) (342.1±21.9 VHN) and Maupomé *et al* (1999) (344.2±32.4 VHN), but higher than that found by Vongsawan *et al* (2014) (295.75±15.79 VHN). This difference could be due to differences in tooth preparation as described above. Prior to pH-cycling, the mean microhardness reduced by 61.19% from baseline. This reduction is similar to a study by González-Cabezas *et al* (2012), which had a 66.67% reduction in microhardness. The small difference between our study and their studies could be due to different pH levels, solution compositions or timing of demineralization (Cuy *et al*, 2002).

The American Academy of Pediatric Dentistry (2012) guidelines on fluoride therapy recommended fluoride mouthrinses as an adjunct to fluoride toothpaste for individuals who are at high risk of developing dental caries. Many studies have shown that fluoride mouthrinse can promote enamel remineralization (Marinho *et al*, 2003, 2004; Divaris *et al*, 2012).

Our study confirmed the ability of fluoride mouthrinse to improve microhardness of primary enamel lesion. This confirms the protective effect of fluoride mouthrinse on primary enamel, similar to previous studies (Divaris *et al*, 2012; Rirattanapong *et al*, 2015).

Calcium and phosphate have also been used for remineralization. A new prospective calcium phosphate system can be prepared by modifying calcium phosphate to form a more functional calcium phosphate that better interacts with demineralized enamel to boost remineralization (Karlinsey *et al*, 2012). Previous studies found a synergistic effect between fluoride and calcium phosphate (Karlinsey *et al*, 2009; 2010c; 2012). Karlinsey and colleagues (2009) found fluoride with tricalcium phosphate provided significantly better surface strengthening than fluoride alone for bovine enamel.

Our findings showed the addition of tricalcium phosphate provided no additional benefit over the fluoride only preparation. This differs from previous studies that showed a synergistic effect between tricalcium phosphate and fluoride (Karlinsey *et al*, 2009; 2012). Differences in study

design and measures of remineralization could explain why our results disagreed with other studies (Rirattanapong *et al*, 2011). Some studies used bovine enamel or permanent human teeth. In our study we used primary human teeth, which have less structure, making the transfer of fluoride, calcium and phosphate ions across the crystals less pronounced than in permanent teeth (Alamoudi *et al*, 2013). Differences in pH, solution composition, pH-cycling and tricalcium phosphate concentration used in mouthrinse preparation could result in dissimilar results among studies (Maupomé *et al*, 1999; Hayashi-Sakai *et al*, 2012).

Studies of remineralization have employed various methods for assessing remineralization of carious lesions, such as microradiography (Epasinghe *et al*, 2014), polarized light microscopy (Rirattanapong *et al*, 2015), surface microhardness (Rirattanapong *et al*, 2012), mineral analysis of calcium phosphate and fluoride phases (Hirata *et al*, 2013) and scanning microscopy (Epasinghe *et al*, 2014). Assessing, surface microhardness is a relatively simple, non-destructive, rapid comparative measure of hardness. However, this measurement can be affected by sample preparation, indenter condition, reading error and area selection (Guitierrez-Salazar and Reyes-Gasga, 2001). The different methods used in determining demineralization and remineralization of primary enamel may also have contributed to the different results (Guitierrez-Salazar and Reyes-Gasga, 2001). Further studies, both *in vitro* and *in vivo,* are needed.

In conclusion, fluoride mouthrinse improved remineralization of primary teeth but the addition of tricalcium phosphate to the fluoride provided no additional benefit and its addition cost makes it unwarranted for use in primary teeth.

REFERENCES

Alamoudi SA, Pani SC, Alomari M. The effect of the addition of tricalcium phosphate to 5% sodium fluoride varnishes on the microhardness of enamel of primary teeth. *Int J Dent* 2013; 2013: 486358.

Amaechi BT, Higham SM, Edgar WM. Factors influencing the development of dental erosion *in vitro*: enamel type, temperature and exposure time. *J Oral Rehabil* 1999; 26: 624-30.

American Academy of Pediatric Dentistry. Guideline on fluoride therapy (revised 2012). *Clin Guide Ref Manual* 2012; 34: 16: 2-5.

Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? *Braz Oral Res* 2009; 23 (suppl 1): 23-30.

Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. *Arch Oral Biol* 2002; 47: 281-91.

Divaris K, Rozier RG, King RS. Effectiveness of a school-based fluoride mouthrinse program. *J Dent Res* 2012; 91: 282-7.

Epasinghe DJ, Yiu CK, Burrow MF. Synergistic effect of Proanthocyanidin and CPP-ACFP on remineralization of artificial root caries. *Aust Dent J* 2014 Nov 1. doi.10.1111/adj.12249. [Epub ahead of print].

Featherstone JD. Dental caries: a dynamic disease process. *Aust Dent J* 2008; 53: 286-91.

Gonzáles-Cabezas C, Jiang H, Fontana M, Eckert G. Effect of low pH on surface rehardening efficacy of high concentration fluoride treatments on non-cavitate lesions. *J Dent* 2012; 40: 522-26.

Gurunathan D, Somasundaram S, Kumar SA. Casein phosphopeptide-amorphous calcium phosphate: a remineralizing agent of enamel. *Aust Dent J* 2012; 57: 404-8.

Gutierrez-Salazar MP, Reyes-Gasga J. Enamel hardness and caries susceptibility in human teeth. *Rev Latin Am Met Mat* 2001; 21: 36-40.

Hirata E, Danelon M, Freire IR, Delbem AC. In vitro enamel remineralization by low-fluoride toothpaste with calcium citrate and sodium trimetaphosphate. *Braz Dent J* 2013; 24: 253-7.

Hayashi-Sakai S, Sakai J, Sakamoto M, Endo H. Determination of fracture toughness of human permanent and primary enamel using an indentation microfracture method. *J Mater Sci Mater Med* 2012; 23: 2047-54.

Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Surfactant-modified β-TCP: structure, properties, and *in vitro* remineralization of subsurface enamel lesions. *J Mater Sci Mater Mad* 2010a; 21: 2009-20.

Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Preparation, characterization and *in vitro* efficacy of an acid-modified beta-TCP material for dental hard-tissue remineralization. *Acta Biomater* 2010b; 6: 969-78.

Karlinsey RL, Mackey AC, Walker ER, Frederick KE, Fowler CX. *In vitro* evaluation of eroded enamel treated with fluoride and a prospective tricalcium phosphate agent *J Dent Oral Hygien*e 2009; 1: 52-8.

Karlinsey RL, Mackey AC, Walker ER, Walker TJ, Fowler CX. Mineralization of eroded dental enamel seeded with fluoride and a tricalcium phosphate ternary biomaterial. *Adv Sci Technol* 2010c; 76: 42-7.

Karlinsey RL, Pfarrer AM. Fluoride plus functionalized β-TCP. A promising combination for robust remineralization. *Adv Dent Res* 2012; 24: 48-52.

Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an *in vitro* study. *Aust Dent J* 2008; 53: 34-40.

Marinho VC, Higgins JP, Logan S, Sheiham A. Fluoride mouthrinses for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2003; (3): CD002284.

Marinho VC, Higgins JP, Sheiham A, Logan S. One topical fluoride (toothpastes, or mouthrinses, or gels, or varnishes) versus another for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2004; (1): CD00278.

Moezizadeh M, Alimi A. The effect of casein phosphopeptide-amorphous calcium phosphate paste and sodium fluoride mouthwash on the prevention of dentine erosion: An *in vitro* study. *J Conserv Dent* 2014; 17: 244-9.

Maupomé G, Aquilar-Avila M, Medrano-Ugalde H, Borges-Yáñez A. *In vitro* quantitative microhardness assessment of enamel with early salivary pellicles after exposure to an eroding cola drink. *Caries Res* 1999; 33: 140-7.

Moi GP, Tenuta LMA, Cury JA. Anticaries potential of a fluoride mouthrinse evaluated by *in vitro* by validated protocols. *Braz Dent J* 2008; 19: 91-6.

Narksawat K, Boonthum A, Tonmukayakul U. Roles of parents in preventing dental caries in the primary dentition among preschool children in Thailand. *Asia Pac J Public Health* 2011; 23: 209-16.

Puig-Silla M, Montiel-Company JM, Almerich-Silla JM. Comparison of the remineralizing effect of a sodium fluoride mouthrinse versus a sodium monofluorophosphate and calcium mouthrinse: an in vitro study. *Med Oral Patol Oral Cir Bucal* 2009; 14: 257-62.

Reich E, Petersson LG, Netuschil L. FDI Commission: mouthrinses and dental caries. *Int Dent J* 2002; 52: 337-45.

Rirattanapong P, Vongsawan K, Saengsirinavin C, Phuekcharoen P. Efficacy of fluoride mouthrinse containing tricalcium phosphate on primary enamel lesions: a polarized light microscopic study. *Southeast Asian J Trop Med Public Health* 2015; 46: 168-74.

Rirattanapong P, Vongsavan K, Surarit R, Tanaiutchawoot N, Charoenchokdilok V, Jeansuwannagorn S. Effect of various forms of calcium in dental products on human enamel microhardness *in vitro*. *Southeast Asian J Trop Med Public Health*

2012; 43: 1053-8.

Rirattanapong P, Vongsawan K, Tepvichaisillapakul M. Effect of five different dental products on surface hardness of enamel exposed to chlorinated water *in vitro*. *Southeast Asian J Trop Med Public Health* 2011; 42: 1293-8.

Schemehorn BR, Orban JC, Wood GD, Fischer GM, Winston AE. Remineralization by fluoride enhanced with calcium and phosphate ingredients. *J Clin Dent* 1999; 10 (1 Spec No): 13-6.

Shen P, Manton DJ, Cochrane NJ, *et al*. Effect of added calcium phosphate on enamel remineralization by fluoride in a randomized controlled *in situ* trial. *J Dent* 2011; 39: 518-25.

Thaveesangpanich P, Itthagarun A, King NM, Wefel JS, Tay FR. *In vitro* model for evaluating the effect of child formula toothpastes on artificial caries in primary dentition enamel. *Am J Dent* 2005; 18: 212-6.

Twetman S, Petersson L, Axelsson S, *et al*. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. *Acta Odontol Scand* 2004; 62: 223-30.

Vongsawan K, Surarit R, Rirattanapong P. Effect of xylitol and fluoride in varnish on bovine teeth surface microhardness. *Southeast Asian J Trop Med Public Health* 2014; 45: 505-10.

Yimcharoen V, Rirattanapong P, Kiatchallermwong W. Effect of casein phosphor-peptide toothpaste versus fluoride toothpaste on remineralzation of primary teeth enamel. *Southeast Asian J Trop Med Public Health* 2011; 42: 1032-40.