THE REMINERALIZATION EFFECT OF BIOACTIVE GLASS ON ENAMEL CARIES-LIKE LESIONS IN PRIMARY TEETH

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Abstract. Fluoride toothpastes can prevent caries but excessive of fluoride during early childhood can cause dental fluorosis. Newer products have been developed in an attempt to promote remineraliztion without causing fluorosis, such as bioactive glass. The aim of this study was to evaluate the remineralization effect of bioactive glass on enamel caries-like lesions compared with fluoride based products in primary teeth using micro-computed tomography to be an alternative remineralizing agent to avoid risk of fluorosis especially in young children. Fifty sound primary incisors were coated with nail varnish, leaving a 1x1 mm window and then immersed in a demineralizing solution for 96 hours to produce artificial enamel caries-like lesions. These teeth were then randomly divided into five groups of 10 teeth each group. Group A: control (artificial saliva), Group B: bioactive glass (NovaMin)(Dr.Collins Restore® toothpaste), Group C: 0.11% NaF (500 ppmF) (Colgate® Babies toothpaste), Group D: 0.22% NaF (1,000 ppmF) (Colgate® great regular toothpaste), and Group E: CPP-ACP (GC Tooth Mousse®). Each tooth was treated with its respective slurry twice daily for 2 minutes during the pH-cycling period. The pH-cycling method was carried out for 7 days. Each tooth was examined to determine mineral density using a micro-CT (Skyscan1173) three times: baseline, post-lesion formation, and post-treatment. The percent change in mineral density for each group and comparison among groups was conducted using the one-way ANOVA and LSD tests at a 95% level of confidence. Results showed all tested toothpaste dentifrices were effective for remineralization of caries-like lesions in primary teeth. Statistical analysis indicated that bioactive glass has no significant remineralization effect to 500 ppm F, and CPP-ACP but significantly less than 1,000 ppm F containing toothpaste. In conclusion, bioactive glass may be used in place of 500 ppm fluoride or CPP-ACP for remineralization of caries without the risk of fluorosis especially in young children,

Keywords: bioactive glass, micro-computed tomography, NovaMin, primary teeth, remineralization

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INTRODUCTION

Dental caries are caused by acids from bacterial metabolism diffusing into tooth enamel and dentine and dissolving the minerals (Featherstone, 2008). Treat-

ment of dental caries can be expenisve. The agents used to treat dental caries should be safe, convenient and effective. Recently dental research has focused on agents effective for remineralization and prevention of demineralization (Pitts and Wefel, 2009).

Fluoride is effective in promoting remineralization of early caries lesions in tooth enamel through formation of fluorapatite (Cury and Tenuta, 2009). In children, the major source of topical fluoride is toothpaste (Kanduti *et al*, 2016). However, studies (Do *et al*, 2014; Abanto *et al*, 2009) have found overuse of fluoride toothpaste can cause dental fluorosis, especially in young children. New materials have been developed to decrease the risk of fluorosis yet promote remineralization, such as bioactive glass and casien phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Mehta *et al*, 2014).

CPP-ACP assists in localization of ACP on the tooth surface (Zhou *et al*, 2014), it has buffering activities of free calcium and phosphate ions, helping to maintain a state of supersaturation, thereby lessening enamel demineralization and enhancing remineralization (Hassanein and El-Brolossy, 2006). The remineralizing potential of CPP-ACP has been shown in several studies (Walker *et al*, 2009; Yimcharoen *et al*, 2011).

Bioactive glass was originally developed as a bone regenerative material and recently has been used for oral care applications such as toothpaste, mouthrinse (Burwell *et al*, 2009). Bioactive glass is composed of synthetic mineral containing sodium, calcium, phosphorous and silica, all naturally occurring in the body. It has been shown *in vitro* and *in situ* to reduce hypersensitivity by occluding exposed dentinal tubules, and decrease gingivitis and bleeding (Tai *et al*, 2006). It is hypothesized to form a mechanically strong hydroxyapatite-like layer on the dentine surface which is resistant to acid challenges (Earl *et al*, 2011). However, it is unclear if it can inhibit demineralization or enhance remineralization (Madan *et al*, 2011). Most studies of bioactive glass have been on permanent teeth rather than primary teeth. Therefore, we aimed to determine the remineralization effect of one formulation of bioactive glass (NovaMin) compared with fluoride toothpaste containing either 500 ppm or 1,000 ppm and CPP-ACP on artificially created carious lesions on primary teeth. This evaluation would be determined by using a micro-CT.

MATERIALS AND METHODS

Specimen preparation

We conducted this study on 50 sound primary incisor teeth. On each tooth, the radicular part was cut off using a high speed fissure carbide bur. Each tooth was the coated with two layers of nail varnish (Revlon, New York, NY) leaving a 1x1 mm window in the middle 1/3 of the crown on the labial surface. Each tooth was fixed with utility wax and putty silicone to kinetic mount, which could remove and repeatedly be repositioned on a micro-CT rotation stage.

Demineralizing and remineralizaing solutions preparation

In our study, we used 2 demineralizing solutions: demineralizing solution 1 (D1) was used to create caries lesions and demineralizing solution 2 (D2) was used for pH-cycling to imitate oral conditions. These solutions were prepared according to Rirattanapong *et al* (2010). D1 consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid at a pH adjusted to 4.4

using 1M KOH. D2 was comprised of the same components as D1, but the pH adjusted to 4.7 using 1M KOH. We also created a remineralizing solutions (R) which consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 M KCl at a pH adjusted to 7.0 using 1M KOH.

Artificial caries formation

After creating the 1x1 mm window using nail varnish, each tooth was then immersed in 3 ml D1 and incubated at 37C° (Sheldon Manufacturing, model 1545, Corneline, OR) for 96 hours to form carious lesions (Rirattanapong *et al*, 2010). Each tooth was then ultrasonically cleaned in deionized water, wiped dry with tissue paper and then immersed in artificial saliva modified from Amaechi *et al* (1999), until used.

Grouping

After artificial carious formation, the 50 study teeth were randomly divided into five groups of 10 teeth each. Group A; artificial saliva; Group B; bioactive glass (NovaMin) (Dr.Collins Restore® toothpaste); Group C; 0.11% NaF (500 ppmF) (Colgate® Babies toothpaste); Group D; 0.22% NaF (1,000 ppmF)(Colgate® great regular toothpaste); Group E; CPP-ACP (GC Tooth Mousse®).

Preparation of treatment slurry

The treatment slurry was prepared in a 3:1 ratio of deionized water and the studied toothpaste (Vahid *et al*, 2012). Each tooth was placed in the respective slurry for that group and stirred with a magnetic stirrer for 20 minutes (Mehta *et al*, 2014).

pH-cycling process

Each specimen was pH cycled for 7 days using D2 and R (Rirattanapong *et al*, 2016). Each tooth was placed in D2 for 3 hours twice daily and R for 2 hours between these 2 times and overnight. Each tooth was treated with its respective slurry twice daily for 2 minutes each time: the first time before the first demineralization of the day and the second time after the second demineralization of the day. After the 7 days of pH-cycling, the specimens were then placed in artificial saliva until examined. The solutions and slurry were changed daily. The pH level of the demineralizing and remineralizing solutions was measured with pH meter before each cycle. Separate containers were used for each tooth in the study.

Evaluation technique

The tooth was examined 3 times during the study; at baseline, post-lesion formation and post-treatment with slurry. The examination was made with a micro-CT (Skyscan 1173; Bruker, Kontich, Belgium). Each specimen was fixed temporarily with wax to a silicone block with the window horizontal. The micro-CT tube voltage was set at 70 kV and a current of 114 μA was used (Mulder *et al*, 2004). The resolution was set at 6.8 μm. Hydroxyapatite phantom, consisting of two concentrations of hydroxyapatite crystal (0.25 and 0.75 gHAp cm^{-3}) was used to calibrate the mineral density (MD) (Wang *et al*, 2016).

The scan results were reconstructed using NRecon software (version 1.6.8.0, Skyscan; Bruker, Belgium). The data were then imported into DataViewer (version 1.5.0, Skyscan; Bruker, Belgium) and then analyzed using CTAn software (version 1.16.1.0, Skyscan; Bruker, Belgium). Greyscale values was converted into MD values (gHAp cm^{-3}) using a linear attenuation curve based on the greyscale values obtained from the mineral phantoms. Each cross-sectional image that included a caries lesion was used to calculate the mineral density.

Table 1
Mineral density at baseline, post-lesion formation, post-treatment by treatment group.

Group	Treatment	Mean(± SD) mineral density (g/cm^3)			
		Baseline	Post-lesion formation	Post-treatment	Percentage change
A	Artificial saliva	1.069(±0.088)^a	0.721(±0.079)^b	0.701(±0.082)^c	-1.486(±12.383)^f
B	Bioactive glass (Dr.Collins Restore®)	1.010(±0.101)^a	0.737(±0.036)^b	0.858(±0.073)^d	19.673(±8.863)^g
C	0.11%NaF (500 ppmF) (Colgate® Barbies)	1.083(±0.066)^a	0.782(±0.049)^b	0.893(±0.084)^d	11.749(±7.616)^g
D	0.22%NaF (1,000 ppmF) (Colgate® great regular)	1.093(±0.119)^a	0.795(±0.089)^b	0.994(±0.102)^e	32.532(±14.135)^h
E	CPP-ACP (GCTooth Mousse®)	0.993(±0.071)^a	0.744(±0.115)^b	0.835(±0.095)^d	17.605(±6.610)^g

The same letters indicate $p \geq 0.05$.

Intra-examination reliability

Ten teeth (20% of the studied samples) were randomly chosen and re-examined by the same examiner under the same conditions and the intra-examiner reliability was calculated using the Intraclass Correlation Coefficient.

Statistical analysis

Means and standard deviations for tooth mineral density were calculated for each group. The one-way analysis of variance (ANOVA) and least significant difference (LSD) method were used to compare the mean mineral density values at baseline, post-lesion formation, post-treatment and the percent changes in lesion depth. These were used to determine the difference in mineral density among the study groups. Statistical analysis was performed using SPSS software, version 22.0 (IBM, Armonk, NY). Significance was set at $p<0.05$.

Ethical considerations

This study was approved by the Ethics Committee of Mahidol University (MU-DT/PY-IRB 2016/011.2406).

RESULTS

Intra-examiner reliability was good with an Intraclass Correlation Coefficient of 0.993.

The means and standard deviations for mineral density measured at baseline, post-lesion formation and post-treatment are shown in Table 1. The mean of mineral density at baseline for all the groups was ranged from 0.993 g/cm^3 to 1.093 g/cm^3. After D1 caries lesion formation, the mean mineral density ranged from 0.721 g/cm^3 to 0.795 g/cm^3. No significant differences were seen among study groups at baseline or post-lesion formation.

Table 1 shows the percent change in mineral density for each group after slurry treatment. There were increases in mineral density (±SD) in group B,C,D and E after slurry treatment of 19.673(±8.863)%, 11.749(±7.616)%, 32.532(±14.135)% and 17.605(±6.610)%, respectively. Group A had a decrease in mineral density at the third check of -1.486(±12.383)%.

Statistical analysis indicated that bioactive glass has no significant difference

in remineralization of caries in primary teeth to 500 ppm fluoride toothpaste or CPP-ACP but has significantly lower remineralization than 1,000 ppm fluoride toothpaste.

DISCUSSION

In this study, the mineral density of all teeth were measured at baseline from the surface of the enamel to 100 μm deep, the results ranged from 0.993 g/cm^3 to 1.093 g/cm^3. In contrast to the study by Hayashi-Sakai *et al* (2016) using micro-CT to analyze enamel mineral density of primary central incisor found a range of 1.19 g/cm^3 to 1.84 g/cm^3. These differences might be the variations between individuals, ethnic groups, or geographic regions where teeth were collected (Wong *et al*, 2004). However, in this study although all specimens were from different teeth but the variation among them did not yield any major effect on the results as confirmed by the $p > 0.05$ for all the mineral density measurements at baseline and post-lesion formation.

Ours is the first study to compare the efficacy of bioactive glass, 500 and 1,000 ppmF and CPP-ACP for remineralization of caries-like lesions in primary teeth using a micro-CT. A previous study of remineralization effect of bioactive glass on primary teeth (Prabhakar and Arali, 2009) found it could be used as an alternative to fluoride as a remineralizing agent; however, the concentrations of fluoride and bioactive glass in that study were not reported.

In our study, we found no significant difference in the remneralization effect of bioactive glass compared to CPP-ACP, similar to a previous study (Preethee *et al*, 2011) but in contrast to another study (Mehta *et al*, 2014) that found bioactive glass had better remineralizing potential than CPP-ACP.

In our study bioactive glass gave similar remineralization of caries in primary teeth to 500 ppm fluoride toothpaste and CPP-ACP but lower remineralization than 1,000 ppm fluoride toothpaste. In conclusion, bioactive glass may be used in place of 500 ppm fluoride or CPP-ACP for remineralization of caries without the risk of fluorosis especially in young children.

CONFLICTS OF INTEREST

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

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