Original Article

Comparison of the effect of fluoride gel and two toothpastes with different materials on remineralization of initial carious lesions in primary teeth

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Abstract

Introduction: Various types of toothpastes are claimed to be able to improve initial enamel caries. This study compared the effect of fluoride gel and two toothpastes on remineralization of initial caries lesions in primary teeth. **Materials and Methods:** A total of sixty-four sound extracted primary canine were immersed in demineralizing solution at 37°C for 96 h to produce artificial caries *in vitro*. Enamel pieces (3 × 3 × 5 mm) were prepared from each tooth and mounted in self cure acrylic blocks. The specimens were randomly assigned to four groups ($n = 16$) based on treatment agent (fluoride gel, Chitodent toothpaste, ReminPro toothpaste, no treatment as control) and underwent a pH cycling model for 10 days. Vickers microhardness (VH) was measured before and after treatment. Data was analyzed using analysis of variance and paired *t*-test by SPSS 18 ($P < 0.05$). **Results:** No significant difference was found in VH between groups at baseline (fluoride group: 265.9 ± 44.8, Chitodent group: 282.6 ± 34.6, ReminPro group: 266.5 ± 26.6, control: 272.7 ± 32.5; $P = 0.516$). Microhardness significantly increased after exposure to ReminPro toothpaste (VH change: 24.1%, $P < 0.001$) and fluoride gel (VH change: 10.9%, $P = 0.046$), but no significant changes were observed in Chitodent (VH change: 2.8%, $P = 0.635$) and control (VH change: - 2.2%, $P = 0.181$) groups. **Conclusion:** ReminPro toothpaste might be effective in remineralizing initial carious lesions of primary teeth.

Keywords: Chitosan, dental caries, fluorides, toothpastes

Introduction

Tooth decay, despite its declining prevalence in most developed countries, remains a major health problem, especially among children.[1] Tooth decay is pathophysiologically associated with imbalanced remineralization (Ca^{2+} and $PO4^{3-}$ deposition) and demineralization of dental structures.[2-4] Carious lesions in deciduous teeth usually develop more rapidly than permanent teeth because of their thinner enamel, lower microhardness, lower mineral content, and irregular crystal structure, as well as some children's eating habits, including higher acidic beverages and sugar intake.[2]

The goal of tooth decay prevention strategies in children and adolescents is to stop the caries process in early stages and remineralize the damaged tooth surface.[5] This treatment provides sufficient amounts of calcium, phosphate, and fluoride ions in the oral environment. Several non-invasive methods using remineralizing agents have been proposed to provide these ions in the oral cavity.[6]

Remineralizing agents include fluorides, compounds containing calcium and phosphate, xylitol, polypopamine, oligopeptides,

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theobromine, arginine, and self-assembling peptides, as explained below[7]:

- Fluoride products facilitate the transfer of calcium and phosphate ions into crystalline lattices, resulting in formation of fluoroapatite, and increase tooth resistance to acid. The main method for stabilizing primary decay lesions is through remineralization. However, fluorosis-related dental fluorosis is a major concern, especially in children.[4,8]
- ReminPro is a new remineralizing agent containing hydroxyapatite, fluoride, and xylitol; it is recommended for controlling dentin sensitivity, preventing demineralization and enamel erosion, and improving the remineralization of carious lesions. The content of fluoride and hydroxyapatite enhances remineralization and strengthens the enamel surface, and xylitol acts as an antibacterial agent.[9,10]
- Chitosan is a biocompatible and non-toxic natural polymer used to control decay in some toothpastes, mouthwashes, and chewing gums and can inhibit the growth and adhesion of cariogenic bacteria and improve the enamel demineralization process.[11-13]

It is very important to find low-cost and easy-handling methods for prevention and treatment of carious lesions in children, particularly those who live in undeveloped/developing countries. Therefore, this study tends to compare the effect of fluoride gel and two types of toothpastes, Chitodent and ReminePro, on remineralization of initial carious lesions of deciduous teeth.

Materials and Methods

Sampling

This study was conducted *in vitro* on 64 primary canines without caries, cracking, restoration, erosion, or hypoplasia, extracted for orthodontic reasons. The teeth were immediately stored in 0.1% thymol solution after extraction. The teeth were cleaned with a scalpel blade No. 15 and a brush in a low-speed handpiece, and polished with 4000 size silicon carbide paper. The adicular section of each tooth was removed by making cuts at cementum-enamel junction (CEJ) using a diamond disk. Then, enamel blocks (3 × 3 × 3 mm) were made from buccal surface of the samples and each block was mounted in self-polymerizing acrylic.

Products

1. 2% sodium fluoride gel (Master Dent; USA)
2. Chitodent toothpaste (Emsland, Germany) containing fluoride-free chitosan
3. Remin-Pro toothpaste (VOCO GmbH, Cuxhaven, Germany) containing fluoride, hydroxyapatite, and xylitol

An aqueous solution was made from each product by adding deionized water by weight ratio of 1 to 3 (17 g product with 51 ml deionized water) and mixing by a vibrator (Votech, Korea).

Demineralizing and remineralizing[14-16]

1. Demineralizing solution containing $CaCl_2$ (2.2 mM), NaH_2PO_4 (2.2 mM), acetic acid (0.05 M); pH was set at 4.5 using KOH (1 M).
2. Remineralizing solution containing $CaCl_2$ (1.5 mM), NaH_2PO_4 (0.9 mM), KCl (0.15 M); pH was set at 7.

Producing artificial initial carious lesions

Artificial initial carious lesions were formed by demineralization. The samples were immersed in 1 L of demineralization solution in an incubator (Innova, USA) at 37°C for 96 h. Then, the samples were rinsed with deionized water and stored in normal saline until the first microhardness was measured.[14]

Microhardness measurement

Microhardness of each sample was measured by a Vickers microhardness device (Buhler, Germany) under a load of 50 g for 5 s by an indentor at four different points 1 mm apart using the formula HV (Vickers Hardness) =1.854 (F/D2), where F is the amount of load applied (kg force) and D is diameter of the indent area (mm) achieved by the indentor.[15] The average microhardness of four points was recorded as sample microhardness. Samples with microhardness ranging from 373.50 to 217.76 were selected for the study.[15]

Sample grouping

After measuring the microhardness, the samples were randomly assigned to four groups (*n* = 16):

1. Group fluoride: Treatment group with 2% sodium fluoride gel
2. Group Chitodent: Chitodent toothpaste treatment
3. Group ReminPro: ReminPro toothpaste treatment
4. Group control: without treatment

All samples were exposed to a pH-cycling model for 10 days.[14] In this model, each sample was placed separately in demineralization solution (10 ml) twice a day for 3 h and in remineralization solution (10 ml) once a day for 2 h between two demineralization steps. The samples of each group except the control group were placed in the solution of the studied products (4 ml of aqueous solution for each sample) for 60 s before the first demineralization and before and after the second demineralization. Then, all samples were kept in the remineralization solution in an incubator at 37°C overnight.

After each pH-cycling, the samples were rinsed with deionized water for 30 s to prevent the solutions from interacting.

An aqueous solution of each freshly made product was used for each demineralization and remineralization cycle; pH of the solutions was measured daily by a pH meter (Jenway 3310, UK). Then, microhardness of each sample was measured and recorded as described previously.

Statistic analysis

Data were analyzed by SPSS 18 software. The groups were compared by analysis of variance (ANOVA) and paired t-test ($P < 0.05$).

Results

Analysis of variance (ANOVA) showed no significant difference in pre-treatment microhardness between four groups ($P = 0.516$). Descriptive statistics of pre- and post-treatment microhardness are displayed in Table 1. After treatment, microhardness was most likely to occur in ReminPro group (24.1%), followed by fluoride (10.9%), chitodent (2.8%) and control (-2.2%) groups [Table 1, Figure 1].

Paired t-test showed that post-treatment microhardness significantly increased in fluoride ($P = 0.046$) and ReminPro ($P < 0.001$) groups; however, no significant change was observed in chitodent ($P = 0.635$) and control ($P = 0.181$) groups [Table 2].

Discussion

Prevention and treatment of primary carious lesions remains one of the most important dental challenges. Non-invasive methods including remineralization are essential treatments for these lesions, especially in children. Today, remineralization is growing as a biological approach.[3]

Mineral content measurement techniques,[10] surface microhardness assessment,[16] scanning electron microscope, and transverse microradiography are used to evaluate the effect of various agents on tooth enamel remineralization.[13] Microhardness assessment is a simple, rapid, non-destructive method capable of repeated measurements that shows mineral changes resulting from therapeutic interventions.[16] Enamel hardness can be measured by many techniques, including Vickers hardness test. This test is suitable for determining the hardness of highly brittle materials such as tooth structure.[17] Therefore, the present study used the Vickers microhardness technique.

This study designed pH cycling protocol to simulate dynamics of remineralization–demineralization in the oral cavity. The pH cycling protocol used for this study is based on a model described by Malekafzali *et al.*[14] During the brushing process, the toothpaste is mixed with saliva in the mouth and thus diluted. In order to simulate oral conditions, the tested agents (fluoride gel and two toothpastes) were diluted with water in a ratio of 1: 3 by volume and the resulting aqueous solution was used.

Samples with initial microhardness ranging from 217.76 to 373.50 were selected for this study; the microhardness that is similar to hardness of healthy enamel.[15] Analysis of variance showed no significant difference between the groups in terms of microhardness at baseline, which indicates that the groups were similar in terms of microhardness.

In this study, Reminpro group had the highest microhardness followed by Foraide, Chitodent, and control groups. Microhardness significantly increased after treatment in Reminepro group and fluoride group. In chitodent group, a tendency to increase was observed but it was not statistically significant. In the control group, microhardness decreased, although this reduction was not significant.

Microhardness increased significantly after exposure to fluoride gel. Consistent with this finding, Hamba *et al.* reported an increase in surface and subsurface mineral density, which reflected the remineralization of carious lesions.[17,18] Numerous studies have indicated the positive effect of fluoride on enamel microhardness.[19-21] Fluoride is the golden standard in remineralization of tooth structure. Fluoride prevents demineralization by forming fluoropatite crystals. These crystals are more resistant to acidic agents than hydroxyapatite crystals. In addition, fluoride enhances the growth of new fluoropatite crystals and inhibits acid production by cariogenic bacteria.[22] In contrast to the present study, clinical trials by Huang *et al.*[23] showed that fluoride varnish was not better than control group (conventional home care technique) for treating white matter lesions (WSLs).

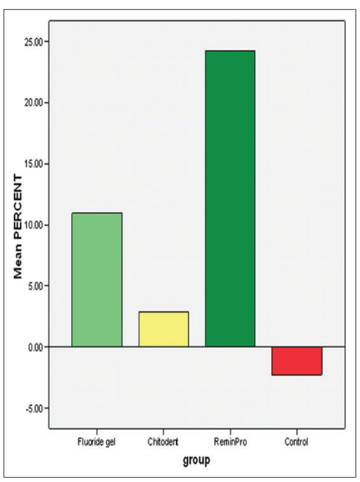

Figure 1: Exchange % of microhardenss in the groups

Table 1: Descriptive statistics of microhardness in groups

Group	*n*	Microhardness				
		Min-Max (pre-treatment)	Min-Max (post-treatment)	Mean±SD (pre-treatment)	Mean±SD (post-treatment)	Change (%)
Flouride	16	224.1-363.2	177.9-403.7	265.9±44.8	293.7±64.3	27.7 (10.9%)
Chitonent	16	225.1-322.2	184.5-390.2	282.6±34.6	288.7±52.3	6.1 (2.8%)
Remin-Pro	16	221.1-315.3	256.9-391.5	266.5±26.6	330.1±40.2	63.6 (24.1%)
Control	16	219.6-334.6	194.8-298.8	272.7±32.5	265.4±26.8	-7.3 (-2.2%)

Table 2: Intra-group comparison of pre- and post-treatment microhardness in the groups

Group	Microhardness (mean±SD)		P*
	Pre-treatment	Post-treatment	
Flouride	265.9±44.8	293.7±64.3	0.046
Chitonent	282.6±34.6	288.7±52.3	0.635
Remin-Pro	266.5±26.6	330.1±40.2	<0.001
Control	272.7±32.5	265.4±26.8	0.181

*Paired *t*-test

They found no significant difference between fluoride varnish group and control group and attributed this to high adsorption of fluoride by surface layer of WSLs, which may reduce the adsorption of calcium and phosphate and thus lead to inadequate remineralization of deeper layers. In addition, Huang *et al.*[23] evaluated WSLs by visual examination 2 months after application of fluoride varnish. However, clinical evaluation of WSPs treated with remineralization techniques showed that clinical changes could be detected after 3 months.[24]

Microhardness increased significantly in Reminpro group. Similarly, clinical trials conducted by Heravi *et al.*[25] and Ebrahimi *et al.*[10] showed that Reminpro significantly increased mineral content and decreased the area of white spots (WSLs) compared to the control group. Rafiei *et al.*[26] reported that CO_2 laser used simultaneously with Reminpro toothpaste significantly increased the microhardness of WSLs in deep enamel layers compared to laser (no laser) or control (no laser or reminepro) groups. Consistent with the present study, several studies also reported that Reminpro toothpaste had a positive effect on remineralization of bleached enamel.[17,27,28] These findings indicate the ability of Reminepro to remineralize and fill the porous areas. Reminpro contains fluoride as well as calcium and phosphate in the form of hydroxyapatite and xylitol. It seems that hydroxyapatite can fill the pores of primary decay lesions, seal the fluoride in the rhyming compound; xylitol has antibacterial effects that can stop demineralization and increase remineralization of primary enamel lesions.[29] However, the present study was conducted *in vitro*; it appears that the increase in microhardness is independent of antibacterial effects of xylitol. Considering the antibacterial effect of xylitol and thus inhibiting demineralization, therefore, Reminpro may show better efficacy in clinical settings and clinical studies should be performed to confirm it.

According to findings of the present study, the rate of microhardness changes in the reminepro group (24.1%) was higher than the fluoride group (10.9%). Consistent with this finding, Mohammadipour *et al.*[11] found that remineralization of primary decay by Reminpro is higher than 2% sodium fluoride and Reminpro increases the hardness of tooth enamel. Bilgin *et al.*[12] reported that Reminpro had stronger remineralizing effects on enamel WSLs than sodium fluoride and fluoride varnishes. The superiority of Reminpro toothpaste over fluoride gel can be attributed to the presence of calcium and phosphate in Reminpro toothpaste. Fluoride ions in the presence of calcium and phosphate ions can replace minerals lost in primary caries lesions through remineralization, while fluoride alone cannot lead to remineralization.[27] Fluoride ions in the presence of calcium ions and phosphorus ions lead to remineralization of enamel lesions through formation of fluoropatite crystals. Therefore, fluoride-containing products used alone limit the insufficient amount of calcium ion and phosphate remineralization.[14] Consistent with this, Kamal *et al.*[30] reported that fluoride, along with a self-assembling peptide that leads to adsorption of calcium ions by forming fibrillar 3D scaffolds, further increased remineralization than fluoride alone.

Microhardness did not significantly changed in Chitodent group. The main ingredient in this toothpaste is chitosan. Therefore, it seems that chitosan is not able to remineralize primary carious lesions according to the protocol used in the present study. Two studies evaluating the effect of chitosan-containing compounds (chitosan hydrogel containing amelogenin-derived peptide) showed that microhardness increased and remineralization of enamel was improved.[13,31] Ruan *et al.*[14] also showed that amlogenin-chitosan was effective in repairing primary carious lesions by increasing the growth of apatite crystals and reducing the depth of lesions. The increase in microhardness and improvement of tooth mineral structure reported in these studies can be attributed to amelogenin-derived peptide (QP5). In animal models, QP5 has been shown to enhance remineralization of primary enamel caries.[32,33] Chitosan, on the contrary, acts as a carrier of QP5 and has antibacterial effects exerted by acting on bacterial cell wall. Chitosan inhibits the growth of *Streptococcus mutans*, lactic acid production, and metabolic activity.[13] Therefore, it seems that chitosan alone does not directly affect the enhancement of enamel remineralization *in vitro*. However, due to its effectiveness mechanisms (inhibition of bacteria and reduction of acid production), chitodent toothpaste may improve primary carious lesions in a clinical setting, which requires further clinical studies to confirm.

One of the limitations of this study was that it was performed *in vitro*. Although attempts have been made to simulate the clinical condition, it is not possible to provide complex oral cavity conditions.

Conclusion

Reminpro showed the highest increase in microhardness compared to other agents. Therefore, this toothpaste can be used successfully for remineralization of primary carious lesions.

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Conflicts of interest

There are no conflicts of interest.

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