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The effect of Remin Pro on the microhardness of initial enamel lesions in primary teeth: An *in vitro* study

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ABSTRACT

Background: Remineralization is currently considered a treatment option for many clinicians. The present study was conducted to compare the effect of three remineralizing agents on microhardness of initial caries lesions in primary teeth.

Materials and Methods: In this *in vitro* study, 96 enamel samples were prepared. Microhardness was first measured for all the samples using the Vickers microhardness test. After developing the initial caries lesions, the microhardness of all the demineralized samples was measured, and the samples were then divided into four groups (n = 24). Casein phosphopeptide–amorphous calcium phosphate in Group 1, Remin Pro in Group 2, and acidulated phosphate fluoride gel in Group 3 were placed on the samples for 4 min. The control group received no treatments. The microhardness of the samples was measured again following a pH cycle of 5 days. The data were analyzed by ANOVA and the *post hoc* test at the significance level of $P < 0.05$.

Results: The mean microhardness reduced significantly in all the groups following the development of initial caries lesions and after the pH cycle of 3 days ($P < 0.001$). After the remineralization and pH cycle of 5 days, the mean microhardness was significantly lower in the control group compared to the other three groups ($P < 0.001$) and had increased in the three treated groups. The microhardness recovery rate also increased in the treated groups compared to the control group, but no significant differences were observed between the three groups themselves ($P > 0.05$).

Conclusion: Remin Pro can be used as an effective substance for preventing tooth caries in children.

Key Words: Acidulated phosphate fluoride, casein phosphopeptide–amorphous calcium phosphate, dental caries, Remin Pro, tooth remineralization

INTRODUCTION

Dental caries is the result of microbial metabolism on the tooth surface and causes a significant loss of tooth minerals. The first clinical sign of dental caries is an appearance of white spot lesion on the tooth surface, which is due to the demineralization of enamel subsurface. The initial lesion or white spot can be treated using noninvasive techniques by remineralization of enamel.[1]

Fluoride is the best-known substance for boosting the mineralization of enamel and dentin, and its topical application creates fluoroapatite crystals, reduces caries, and stops the initial caries lesions.[2]

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Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is well capable of stabilizing calcium and phosphate in a solution and it can thus increase the calcium–phosphate level in dental plaque and act as a reservoir of calcium and phosphate ions and result in remineralization.[3,4]

Remin Pro is composed of three effective compounds, including hydroxyapatite, fluoride, and xylitol, to protect against demineralization and enamel abrasion. The hydroxyapatite contained in this composition fills the surface enamel lesions and thus creates a smooth surface. Its fluoride content is 1450 ppm of sodium fluoride, and its xylitol content reduces the harmful effects of bacteria and lactic acid metabolic products, which are all effective in remineralization and strengthening the tooth enamel.[5,6]

Zhou *et al*. studied the remineralization of initial enamel lesions in primary teeth using CPP-ACP. They found that, compared to sodium fluoride (NaF), CPP-ACP paste significantly increases the size of the hydroxyapatite crystals and more effectively increases enamel calcium and phosphate levels.[7] Another study examined the effect of Remin Pro on enamel surface roughness after bleaching and found no significant differences between the effects of CPP-ACP and Remin Pro and therefore concluded that both substances are equally effective in reducing enamel surface roughness after bleaching.[8]

Currently, preventive and minimally invasive approaches are considered for the treatment of white spot lesion, so selecting appropriate methods is an important factor in avoiding the need for invasive treatment options. Therefore, the purpose of the present *in vitro* study was to compare the remineralization capacity of acidulated phosphate fluoride (APF), CPP-ACP, and Remin Pro on initial caries lesions in primary teeth by assessing enamel microhardness.

MATERIALS AND METHODS

Experimental design

The present *in vitro* study was conducted at the School of Dentistry of Mashhad University of Medical Sciences. The sample size was determined as 24 per group with a 95% confidence interval and an 80% statistical power. A total of 48 human primary molar teeth with intact enamel surface and devoid of any hypoplasia, hypocalcification, enamel cracks, abrasion, erosion, and dental caries on lingual and buccal surfaces were used for the study.

Specimens' preparation

Following extraction, the teeth were cleaned of plaque and soft tissue debris. They were disinfected in 0.2% thymol at room temperature for a month and then stored in saline until the tests began.

Forty-eight tooth crowns were separated from the roots at a cementoenamel junction. Each crown was sectioned mesiodistally into labial and lingual fragments using a water-cooled diamond disc (D&Z. Engelskirchen, Germany). Each enamel section was then embedded in self-polymerizing resin (Acropars 100, Tehran, Iran) The enamel surface was then serially ground wet using 400, 600, and 1200 grit silicon carbide abrasive papers (CB-C, SAIT ABRASIVI, Italy) and polished with 1 µm and 0.05 µm colloidal silica and alumina polishing suspension (Kemet, United Kingdom). Specimens were inspected for any defects under a stereomicroscope (Blue Light, USA) at ×40 magnification and discarded if cracked or pitted. A 3 mm × 3 mm window was designed at the center of each enamel specimen and was then divided into three imaginary parts of equal sizes in preparation for the test. The other parts of the enamel specimen were covered with three layers of nail varnish (Zoya, Cleveland, USA).

Microhardness analysis step one

Ninety-six specimens were tested for surface microhardness (SMH) using a Vickers microhardness tester (Haresh, Iran). A 25-g vertical load was exerted on the surface of the samples for 10 s with a Vickers diamond indenter. The enamel microhardness of each sample was measured at three points <0.5 mm apart, and the mean of these three values was recorded for each sample.

Cariogenic step

The pH cycle: To create artificial initial caries, each sample remained in contact with solutions. The pH cycling protocol consisted of first immersion of specimens in 1.5 liters of demineralizing solution for 3 h and then in 1.5 liters of remineralizing solution for 21 h.

The demineralizing solution was composed of 0.9 mM NaHPo4, 1.4 mM CaCl2, 0.05 M acetate buffer, and 0.03 ppm F, which was set with NaOH 50% until a pH of 5.5 was reached.[9]

The remineralizing solution was composed of 0.9 mM NaHPo4, 1.5 mM CaCl2, 0.1 M Tris buffer, and 0.05 ppm F, which was set with NaOH 50% until a pH of 7 was reached.[9]

After this step, the samples were placed in artificial saliva with a pH of 7 for 4–8 h, and the pH cycles were repeated three times.

Microhardness analysis step two

After the development of the initial enamel lesion, all the samples underwent a Vickers microhardness test with the same static load and time for their microhardness to be assessed.

Treatment step

In this step, experimental samples were randomly divided into four groups (n = 24), and the mineralization process was performed on them with different substances [Table 1].

Group 1 (casein phosphopeptide–amorphous calcium phosphate)

A layer of GC tooth mousse (MI Paste, GC Corporation, Tokyo, Japan) was applied on the enamel surface for 4 min and was then removed.

Group 2 (Remin Pro)

A layer of Remin Pro cream (VOCO, Cuxhaven, Germany) was applied on the surface of the samples for 4 min and was then removed.

Group 3 (acidulated phosphate fluoride)

The enamel samples were entirely covered with a layer of APF 1.23% gel (Ionite, Dharma Research, Inc., USA) for 4 min and were then cleared off the gel.

Group 4 (controls)

The samples in the control group were not treated with any substances.

To assess enamel resistance against acid, all the samples underwent a 5-day pH cycle by being placed in a demineralization solution for 3 h each day and then in remineralization solution for 21 h.

Microhardness analysis step three (posttreatment stage)

The same hardness test was used once again under the same conditions in the enamel samples after treatment for microhardness measurements.

Statistical analysis

The collected data were analyzed using the repeated measures ANOVA, the one-way ANOVA, and the mixed ANOVA. The pairwise comparison of the groups was performed using Bonferroni's *post hoc* test and the Dunnett's T3 test. The level of statistical significance was set at $P < 0.05$ for all the tests. The percentage of microhardness recovery was calculated using the following formula ([posttreatment microhardness − initial sample microhardness]/initial microhardness)/100.

RESULTS

Enamel surface microhardness was measured in the study groups in three steps: (1) at baseline, (2) following the induction of initial enamel lesions, and (3) after remineralization.

The normal distribution of microhardness data at different steps of study in each of four experimental groups was confirmed by the Kolmogorov–Smirnov test ($P > 0.05$).

Mixed between-within subjects ANOVA revealed that experimental steps ($P < 0.001$ $F = 81.143$), type of remineralizing agents ($P = 0.010$ $F = 3.994$), and their interaction ($P < 0.001$ $F = 24.673$) had significant effects on the microhardness of enamel.

The mean surface microhardness reduced in all the groups from step one to step two, but then increased in step three in all the groups except for the control group. Significant changes were observed in the mean microhardness of all the four groups from step one to step three, and Bonferroni's pairwise comparison of the CPP-ACP and control groups showed significant differences in the mean values obtained in the three steps ($P < 0.001$). In the Remin Pro and fluoride groups, however, the mean values reduced significantly from step one to step two ($P < 0.001$) and increased significantly from step two to step three ($P < 0.001$), but no significant differences were observed between step one and step three [Table 2].

Table 1: Materials used in this study

Materials	Chemical composition	Manufacturer
Tooth mousse	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propyleneglycol, silicondioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zincoxide, sodiumsaccharin, ethyl p-hydroxybenzoate, magnesiumoxide, guargum, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate	GC Corporation, Tokyo, Japan
Remin Pro	Sodiumfluoride, ethanoliccolophony, Hydroxyl apatite Xylitol	VOCO GmbH, Cuxhaven, Germany
Ionite APF gel	Acidulated Phosphate Fluoride (1.23% Fluoride Ion), Xylitol, Vitamin E	Dharma Research, Inc., USA

CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; APF: Acidulated phosphate fluoride, CMC-Na: Sodium carboxyl methyl cellulose

The groups were compared to one another in each step, and the one-way ANOVA showed no significant differences between the four groups in the mean microhardness obtained in steps one and two, but in step three, the groups were significantly different from one another in terms of their mean microhardness ($P < 0.001$). The Dunnett's test showed a significantly lower mean micro hardness in the control group compared to the other three groups in step three, but no significant differences were observed between the other three groups [Table 3].

The assessment of changes in the microhardness of the samples compared to their initial state (enamel surface microhardness recovery) showed significant differences between the groups ($P < 0.001$), and the control group showed a significantly lower mean compared to all the other groups; however, no significant differences were observed between the three other groups themselves. Overall, microhardness recovery was observed in the demineralized enamel in all experimental groups treated with the three different substances compared to the control group [Figure 1].

Table 2: The mean and standard deviation of microhardness by group and step

Substance type	Step	*n*	Mean	SD	Test result (*P*)
CPP-ACP	One	24	333.87[a]	75.39	<0.001
	Two	24	276.18[b]	61.33	
	Three	24	379.43[c]	60.29	
Remin Pro	One	24	339.76[a]	85.50	<0.001
	Two	24	276.63[b]	67.12	
	Three	24	354.32[a]	100.12	
Fluoride	One	24	340.04[a]	54.30	<0.001
	Two	24	280.75[b]	58.43	
	Three	24	340.95[a]	60.73	
Control	One	24	329.37[a]	51.42	<0.001
	Two	24	270.68[b]	34.71	
	Three	24	232.87[c]	43.91	

*In each group, different characters indicate significant differences between the steps. CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate; SD: Standard deviation

DISCUSSION

Given the importance of the enamel surface layer in the progress of dental caries, the assessment of changes in this area is crucial. Since surface enamel has higher acid resistance, reducing the levels of calcium and phosphate ions leads to the initial enamel lesion with an almost healthy surface.[10] In the present study, the remineralization effect of Remin Pro was compared with the effect of CPP-ACP and APF on initial enamel lesions in primary teeth.

The process of enamel remineralization is assessed using relatively simple, quick, and nondestructive

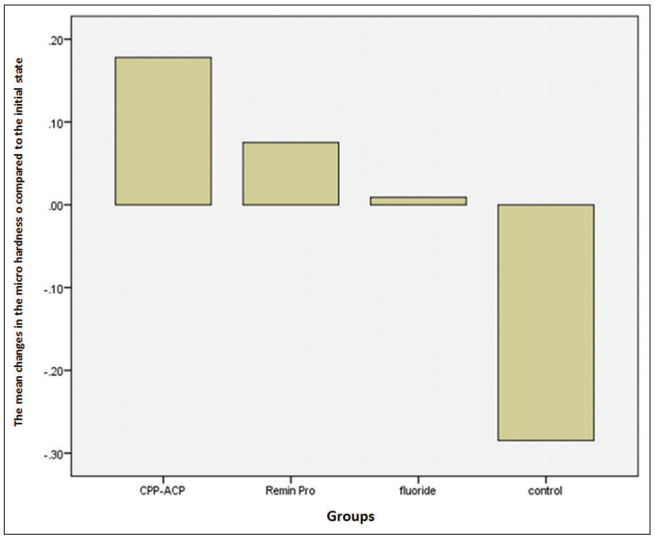

Figure 1: The mean changes in the microhardness of the samples compared to the initial state in the four groups.

Table 3: The mean and standard deviation of microhardness in the different groups by step

Step	Group	*n*	Mean	SD	Minimum	Maximum	Test result
One	CPP-ACP	24	333.87	75.39	193.3	486.0	*F*=0.135
	Remin Pro	24	339.76	85.50	162.8	438.0	*P*=0.939
	Fluoride	24	340.04	54.30	241.3	443.0	
	Control	24	329.37	51.42	227.6	446.0	
Two	CPP-ACP	24	276.63	67.12	143.3	372.0	*F*=0.127
	Remin Pro	24	276.63	67.12	143.3	372.0	*P*=0.944
	Fluoride	24	280.75	58.43	190.0	423.6	
	Control	24	270.68	34.71	200.3	360.3	
Three	CPP-ACP	24	379.43[a]	60.29	264.6	471.6	*F*=20.84
	Remin Pro	24	354.32[a]	100.12	171.3	539.3	*P*<0.001
	Fluoride	24	340.95[a]	60.73	261.0	477.0	
	Control	24	232.87[b]	43.91	144.3	335.0	

*In step three, different characters indicate significant differences between the substance types. SD: Standard deviation; CPP-ACP: Casein phosphopeptide-amorphous calcium phosphate

hardness tests.[11-13] This study used Vickers test for measuring hardness in three steps, including (1) baseline, (2) after the induction of the initial enamel lesion, and (3) after the application of the remineralizing substances and the pH cycles.[13] Vickers test is suitable for determining the hardness of brittle materials such as enamel.[14] The average Vickers microhardness value for enamel is in the range from 250 to 360.[14] In this study, the microhardness values were in the range from 329 to 340, which was within the standard range.

Sample preparation is a critical step which may affect Vickers microhardness data, because any tilt or uneven surface may yield a too large indentation and thus smaller Vickers microhardness measurement, so the enamel microhardness of each sample was measured at three points <0.5 mm apart to avoid any operational bias and the mean of these three values was recorded for each sample.

The identical enamel surface microhardness values obtained in the first step showed that the samples were similar before surface treatment and that they were in similar conditions and were all suitable for the test.

A variety of demineralizing solutions are available with different compound concentrations affecting the final pH of the solution and the sample's shelf life. These solutions are reported to have a pH between 3.5 and 5.5 and an application duration of 2 h to 21 days.[15] In the present study, the samples were immersed in a solution with a pH of 5.5 for 3 days to develop a demineralized subsurface layer. The concentration of both calcium and phosphate ions in the demineralized solution was 50% of the saturation level, so that dissolving would occur only in the enamel subsurface layer. In addition, the fluoride contained in the solution prevents enamel demineralization by forming fluoroapatite on the enamel surface and thus produces the initial enamel lesion with a healthy surface layer.[16] The results of the present study showed that the surface microhardness was significantly lower in all the four groups after the demineralization step compared to baseline; this finding is consistent with the results of previous studies.[11,13]

The role of calcium and phosphate ions in the demineralization and remineralization process is determined, but the effect of organic content of oral saliva should also be considered. In an attempt to mimic the tooth environmental condition, artificial saliva containing organic and inorganic compounds was prepared to be used as a thin layer applied over the enamel samples before the remineralization step.[17]

In the third step, the mean surface microhardness was significantly higher in the three treated groups compared to the control group, as consistent with the results of some other studies.[5,18,19] These positive changes in enamel microhardness after treatment steps are indicative of proper remineralization capacity of CPP-ACP, Remin-Pro, and APF.

In the APF group, the enamel surface microhardness increased significantly in the third step compared to in the second step. It means that fluoride ion in APF replaces the hydroxyl ions in the hydroxyapatite structure and creates fluorohydroxyapatite, which is more resistant to demineralization. The caries-preventive action of fluoride is mainly topical, and the application of compositions with high concentrations of fluoride in the form of varnish or gel leads to the formation of surface globules of calcium fluoride in dental enamel, which acts as a physical barrier, preventing contact between the acid and enamel or as a source of fluoride and continuously releases this substance and thus inhibits demineralization.[20]

The mean microhardness was 379.42 ± 60 after the application of CPP-ACP paste, which is higher than the second step. This finding is due to casein phosphopeptide presence in CPP-ACP, which stabilizes calcium and phosphate and also facilitates the formation of calcium phosphate nanocomplexes on the tooth surface. These compounds will, in turn, act as a source of minerals for the remineralization process. In fact, the insoluble calcium phosphate becomes soluble in the presence of casein phospholipids. Subsequently, amorphous calcium phosphate is formed, localized on the tooth surface, and acts as a source of calcium and phosphate ions. It helps calcium and phosphate ions to travel deep into lesions through the porous layer on the white lesion and to encourage the remineralization of enamel crystals. This material is also capable of rapidly turning into hydroxyapatite, which is then deposited on the tooth surface.[21]

The role of Remin Pro in remineralization of incipient enamel lesions has been approved previously.[6] Several studies indicated that Remin Pro increases

microhardness of bleached enamel.[8,14] In agreement with the finding of our study, Tabatabaaei has also confirmed the efficacy of Remin Pro for increasing the microhardness of incipient enamel lesion.[5] Remin Pro contains calcium–phosphate as hydroxyapatite along with xylitol and fluoride.[21] Previous studies have shown that when remineralization occurs in the presence of calcium and phosphate ions, fluoride ions can replace the materials lost in the initial caries lesions. Together, the fluoride ions and calcium–phosphate ions in the composition of Remin Pro affect the enamel subsurface.[5,21] According to the manufacturers, Remin Pro is capable of filling porosities in the enamel and forms a protective layer on the tooth surface that prevents the adhesion of bacterial plaque to the tooth surface.[5] Moreover, the xylitol contained in Remin Pro prevents caries by interfering with the metabolism action and the fermentation of sucrose and reducing acid production by microorganisms.[22] The use of Remin Pro in the biological environment of the oral cavity appears to be more effective than its *in vitro* use due to the antimicrobial properties of its xylitol content.[5]

Contrary to the results of our study, Valian demonstrated that neutral sodium fluoride had a more significant impact on increasing the microhardness compared to Remin Pro.[22] It seems that this controversy is due to different materials (NaF) and experimental setup.

In our study, the mean enamel microhardness was higher in the CPP-ACP group than in the Remin Pro and APF groups, although the difference was not statistically significant; this finding is consistent with the results obtained by Esfahani[21] and Poggio.[23] Furthermore, the recovery of enamel surface microhardness was higher after remineralization compared to the initial state in the CPP-ACP group compared to the Remin Pro and fluoride groups.

These findings show the more exceptional ability of CPP-ACP to remineralize incipient enamel lesion compared to the other two substances. CPP-ACP inserts amorphous calcium phosphate into dental plaque and neutralizes the action of free calcium phosphate ions, and, by creating a supersaturated enamel, restricts the loss of minerals and boosts enamel remineralization.[3,17]

Although our study could not wholly simulate oral cavity conditions, it presented the potential of Remin Pro and CPP-ACP pastes for remineralizing initial enamel lesions. It is assumed that the effect of these pastes will be improved under oral conditions.[8,24] Furthermore, the application of Remin Pro and CPP-ACP may have some advantages over APF as they are good tasting and are not toxic in higher dosage, so that they could be applied safely by either patients or clinicians.[6]

CONCLUSION

The current results revealed that APF, CPP-ACP, and Remin Pro have the same remineralization capacity for increasing demineralized enamel hardness. Hence, Remin Pro can be used as an effective substance for preventing tooth caries in children.

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Conflicts of interest

The authors of this manuscript declared that they have no conflicts of interest, real or perceived, and financial or non-financial in this article.

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