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Original Article

The effects of sodium hexametaphosphate combined with other remineralizing agents on the staining and microhardness of early enamel caries: An *in vitro* modified pH-cycling model

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ABSTRACT

Background: This study was conducted to determine the effects of sodium hexametaphosphate (SHMP) combined with other remineralizing agents on the staining and microhardness of early enamel caries.
Materials and Methods: in This *in vitro* study The enamel buccal surfaces of 70 bovine incisors were classified into seven study groups (n = 10). Remineralizing agents were employed alone and in combination with SHMP in different groups, including: (1) 8% SHMP, (2) 2% sodium fluoride, (3) 2% sodium fluoride + SHMP, (4) Remin Pro®, (5) Remin Pro®+SHMP, (6) MI Paste Plus, and (7) MI Paste Plus + SHMP. A modified pH-cycling technique was used to reconstruct the dynamics of caries. Colorimetric and microhardness analyses were conducted before demineralization (T_1), after caries formation (T_2), and after the remineralizing treatment (T_3). The data were analyzed by the one-way analysis of variance and the repeated measurement analysis ($P > 0.05$).
Results: After remineralizing cycles, the experimental groups treated with either SHMP alone or in combination with other materials showed less significant changes in the three variables of color (Δa, Δb, and ΔL) and the overall color change (ΔE). The enamel caries treated with Remin Pro® presented the highest color change, while Remin Pro®+ SHMP resulted in the least changes. The mean value of microhardness after remineralization improved significantly in all groups, except in the MI Paste Plus + SHMP group that showed the lowest value. In contrast, the highest microhardness value was recorded for Remin Pro®, being comparable to that of the sound teeth ($P > 0.05$).
Conclusion: SHMP, either alone or combined with remineralizing agents, created the least staining. Remineralizing materials alone showed higher surface hardness, while sodium fluoride alone showed higher surface hardness when combined with SHMP.

Key Words: Casein phosphopeptide-amorphous calcium phosphate, fluoride , remineralization, sodium hexametaphosphate

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INTRODUCTION

Dental caries is a chronic, infectious, and multifactorial disease induced by the metabolic activities of cariogenic bacteria.[1] In the presence

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of carbohydrates, cariogenic bacteria attached to the dental surface called the biofilm produce acidic byproducts and decrease the biofilm pH. In this acidic environment, inorganic dental structures get dissolved and the demineralization process begins. At initial stages, enamel and dentin are broken down, with dental cavities formed.[2,3] The term "white spot lesion (WSL)" was first introduced by Fejerskov *et al*.[4] to describe the initial visible sign of caries on enamel surfaces. WSLs are noncavitated lesions with chalky and matte surfaces, which are detectable only in dry conditions.[1,4] This incipient caries is localized in enamel and can be remineralized, since its crystalline rod structure remains intact, making these etched crystals act as remineralization cores.[1,3] Not only do WSLs produce an unesthetic and disturbing appearance, but also they may progress into caries. In the remineralization procedure, dissolved calcium (Ca) and phosphate (P) ions are precipitated once more and formed by remineralizing agents.[2,3] Therefore, by the early and proper detection of white spots and replacement of remineralization procedures with aggressive treatments, caries activities stop without damaging healthy teeth.[2]

Fluoride-containing compounds, including sodium fluoride and stannous fluoride, have proven to exert some effects on the remineralization mechanism.[5] The trace amount of fluoride increases the precipitation of calcium and phosphate and strengthens enamel against acidic attacks by producing fluorapatite, that is, a material with more resistance against caries attacks than hydroxyapatite.[6]

Several fluoride substitutions, such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and hydroxyapatite, which are the main elements of dental structures have also been reported to have promising results for caries management.[7,8]

Casein phosphoprotein-ACP (CPP-ACP) is composed of ACP with casein phosphoprotein and keeps Ca and P saturated around the teeth, thereby enhancing their precipitation.[9,10] MI Paste Plus (GC Corporation, Tokyo, Japan) is a commercial product that contains CPP-ACP and 900-ppm fluoride (CPP-ACPF). Remin Pro® (VOCO GmbH, Cuxhaven, Germany) is a remineralizing cream formulated with hydroxyapatite, fluoride, and xylitol. It is believed that hydroxyapatite can produce precipitates on enamel surfaces and fill all porosities, thereby increasing enamel hardness and improving its mechanical properties.[11,12]

Sodium hexametaphosphate ($Na_6P_6O_{18}$) (SHMP) was another remineralizing material that was introduced in 2000 as an effective anti-tartar component in toothpastes.[13] This agent that is also called polypyrophosphate offers a wide range of therapeutic and cosmetic properties. SHMP is a longer-chain variant of pyrophosphate; a traditional ingredient used to inhibit surface stains and calculus. It is composed of 10–12 repeating pyrophosphate subunits, so it provides better coverage and retention on the tooth surface than pyrophosphate. SHMP prevents staining and eliminates remaining stains, due to its strong capacity for the adsorption of tooth minerals and desorption of pellicle proteins containing stains.[14] In addition, it exerts anti-inflammatory effects on gingivitis and is useful in the treatment of dental hypersensitivity.[13] Numerous clinical and technical studies have demonstrated the stain removal and prevention properties of SHMP in the forms of dentifrices, mouth rinses, and chewing gums.[14-17]

In spite of its anti-stain properties, SHMP demonstrates anticaries effects. SHMP with a negative charge can be absorbed at the positive sites of enamel surfaces, thereby forming a protective layer on enamel against erosive and acidic factors. In addition, it has three phosphate groups that provide binding points for the retention of ions, including Ca^{++} and CaF^{+}.[15] During the remineralization phase in the oral cavity, pigments soluble in saliva can enter into the newly formed mineral phase as the surface of the caries is mineralized. This event turns the unesthetic appearance of the hardened initial enamel caries into something called the "brown spot." Some products, such as stannous fluoride with a remineralizing capacity, still have limitations, including the potential for the extrinsic tooth stain. In addition, the combination of the favorable properties of SHMP with other remineralizing products may help prevent caries extension and surface staining.

It seems that remineralizing agents exert synergic effects when used together. Recent research revealed that 250-ppm fluoride in combination with 0.5%–1% SHMP have the same effect as 1100-ppm fluoride in enamel remineralization.[18,19] Besides, the supplementation of a 1100-ppm F dentifrice with 1% HMP exerted a higher inhibitory effect on enamel demineralization than a dentifrice containing the same amount of fluoride *in vitro*.[20,21] A study performed by Pfarrer *et al*.[16] showed the enhanced anticaries performance of the stabilized stannous fluoride/SHMP

dentifrice compared to a sodium fluoride control dentifrice.

Therefore, this *in vitro* study was conducted to assess the effects of SHMP combined with mineral pastes containing 2% sodium fluoride, MI paste Plus, and Remin Pro® on the microhardness and staining of early enamel caries.

MATERIALS AND METHODS

This *in vitro* study was performed on 70 extracted sound bovine incisors. In the beginning, the teeth were cleaned with rubber caps and a slurry of pumice, and then they were examined under a ×20 stereomicroscope (Dino lite Pro, Anmo Electronics Corp, Taiwan) to discard the ones with cracks, developmental and structural defects, and carious lesions. The teeth were immersed in a 0.1% thymol solution at room temperature for a week and then in a normal saline solution until the commencement of the study.

Their anatomic crowns were cut at a cementoenamel junction level by a slow-speed water-cooled diamond saw (CNC Machine, Nemo, Karaj, Iran) and embedded in a self-cure acrylic resin (Acropars, Marlic Co., Tehran, Iran) to obtain approximately a 6 mm × 6 mm enamel window at the center of the buccal surface of each tooth. To make the flat surfaces suitable for the microhardness test, the enamel surfaces were polished with 100- and 600-grit silicon carbide papers (Starcke, Germany) under a cooling water flow. Next, their surface microhardness and initial color were assessed by the Vickers test (Micro Hardness Tester, Koopa Pazhoohesh, Iran, Model: MH3) and a colorimeter (Chroma Meter, Konika Minolt, Japan) for the first time (T1).

After the assessment, each specimen was stored in a container containing a demineralization solution for 4 weeks until WSLs appeared. The ingredients of the demineralization and remineralization solutions employed in this study are presented in Table 1. Later on, the Vickers and colorimetry analyses were done for the second time (T2). To reproduce the oral environment, the pH-cycling technique recommended by Ten Cate as well as Duijsters[22] and modified by Featherstone[23] was employed. To simulate the demineralizing and remineralizing cycles as well as the staining potential, the test regimen in each 24-h time period included a 6-h demineralization period, a 16-h remineralization period, as well as twice the 1-h staining immersion, and twice the 5-min surface treatments for each phase. The schematic duration of the phases is presented in Figure 1.

According to the modified pH-cycling method used, all specimens were stored in a demineralization solution for 6 h at 37°C. Each tooth crown was immersed individually in 40 ml of the demineralizing solution. The specimens were then removed from the solution and rinsed in the deionized water. Next, the specimens were subjected individually to the different remineralizing agents for about 5 min. Due to the lack of similar previous research, at first, a pilot study was carried to find out the appropriate sample size. Then, a formula was used to calculate the sample size based on the mean value, standard deviations, standard errors, and so on. A total of eight samples in each group was obtained which was increased to ten for more accuracy. The enamel samples were assigned randomly into seven groups (n = 10), according to the treatment applied to the demineralized enamel.

- Group 1: SHMP 8%
- Group 2: 2% sodium fluoride (MasterDent, USA)
- Group 3: A combination of 2% sodium fluoride and 8% SHMP
- Group 4: A commercial cream containing hydroxyapatite (Remin Pro®, VOCO, Germany)
- Group 5: Remin Pro® combined with 8% SHMP
- Group 6: A commercial paste containing CCP-ACPF (MI Paste Plus, GC, Japan)
- Group 7: The combination of MI Paste Plus and 8% SHMP.

After the remineralization treatments, all specimens were rinsed and immersed in a remineralization solution at 37°C overnight for 16 h to simulate the remineralization stage of the caries process [Figure 1]. Before the start of each demineralization and

Table 1: Demineralization and remineralization solutions contents (mmol/L)

Solutions	Contents						
	Calcium chloride	Sodium phosphate	Acetic acid	Potassium chloride	Potassium hydroxide	Cacodylate buffer	pH
Demineralization solution	2.2	2.2	0.05	-	1	-	4.4
Remineralization solution	1.5	0.9	-	150	-	20	7

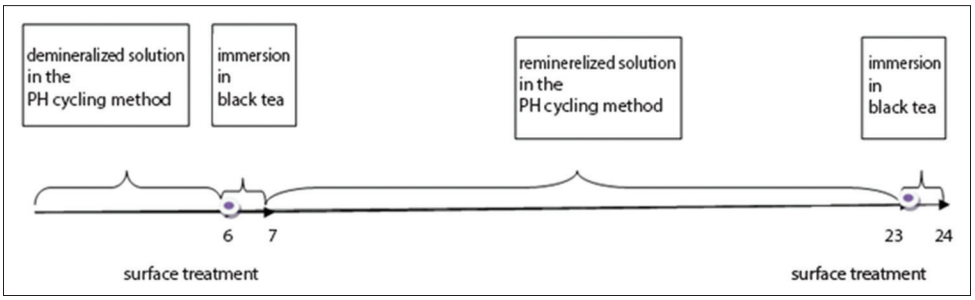

Figure 1: A schematic image of the pH-cycle model which was used in this study. Each cycle consisted of 6 h immersion in demineralizing solution and 16 h immersion in remineralizing solution. Before each demineralization and remineralization cycle, different treatments were applied to the enamel surfaces and then specimens were immersed in the black tea solution for 55 min. These cycles were repeated for 14 consecutive days.

remineralization cycle, the specimens were immersed in a black tea solution for about 1 h. The cycles were repeated for 14 consecutive days. At the end of day 14, all specimens were rinsed with ionized water, with their surface hardness and color change measured for the third time (T3). The total color change was calculated using the formula below, based on the three parameters of color, including a, b, and L for each tooth.

Statistical analysis

The normal distribution of the data was confirmed by the Shapiro–Wilk normality test. The one-way analysis of variance (ANOVA) was used to detect any significant differences in the microhardness and color change values among the study groups in each of the three evaluation stages. A repeated measurement analysis was performed to determine any significant alterations in the variables before and after the treatments in each of the study groups. Due to the lack of normality for the data, the Friedman analysis was performed in some measurements (ΔE_{12} in Sodium fluoride + SHMP and ΔE_{23} in Remin Pro + SHMP). The data were analyzed using SPSS Statistics (Statistical Package for the Social Sciences, Version 16.0, SPSS Inc., Chicago, IL, USA), with the significance level determined at $P < 0.05$.

RESULTS

Color changes

In this *in vitro* study, the differences in each color parameter, that is, L, a, and b, as well as ΔE, were analyzed separately by the one-way ANOVA for each experimental group. The measurements were carried out in three stages, including Δ_{1-2} (the comparison between the intact tooth and the state after demineralization), Δ_{2-3} (the comparison between the state after demineralization and surface treatments), and Δ_{1-3} (the comparison between the intact tooth and the state after surface treatments). Regarding the color change parameters, significant differences were observed among the study groups in terms of Δ_{1-3} and Δ_{2-3} ($P < 0.001$).

The results obtained from the ANOVA test and the Friedman test concerning the three parameters of color, that is, L, a, and b, as well as the overall color change (ΔE) implied that the experimental groups treated with SHMP, either alone or combined with other materials, showed the least difference in terms of Δ_{1-3} and Δ_{2-3}. Regarding the color parameters, the greatest and the least alterations between Stages 2 and 3 among experimental groups were observed in the Remin Pro® and Remin Pro® + SHMP groups, respectively.

The Remin Pro®-treated group showed a significantly higher color change than other experimental groups in terms of ΔE_{1-3} and ΔE_{2-3}, indicating no significant difference with the sodium fluoride group [Table 2].

Microhardness

The data obtained from the microhardness evaluation showed a significant difference among the study groups only after surface treatments ($P < 0.001$). After demineralization, the microhardness value decreased significantly in all the study groups. The highest and lowest microhardness values after surface treatments were observed in the Remin Pro® group and the MI Paste Plus + SHMP group, respectively. The mean value of microhardness after the remineralization process improved significantly in all the study groups, except in the MI Paste Plus + SHMP group. In all study groups, after remineralization treatments, despite an improvement in the surface hardness, the final microhardness value was significantly lower than that of the intact tooth, except for the Remin Pro® group that showed no significant difference with the sound enamel [Table 3].

The correlation coefficient

The correlation coefficient between ΔE and surface hardness is shown in Table 4. There was a significant positive correlation between stages 2 and 3 of the evaluation in the Remin Pro®-treated group.

DISCUSSION

The present study was performed to evaluate whether the addition of SHMP to different remineralizing factors would improve their protective effects on WSLs or not.

The surface microhardness test is generally proposed for the assessment of the remineralization efficacy of mineral agents through the measurement of the surface strength.[24] In the current study, microhardness and colorimetry assessments were made in three stages, with the first stage related to sound enamel samples, the second stage associated with tooth immersion in a demineralizing solution, and the final stage connected with the application of different remineralizing regimens. The lack of a significant difference among the groups after the initial measurement ($P = 0.052$) confirmed the presence of the homogeneous enamel in all groups; in addition, the second microhardness measurement ($P = 0.98$) determined the presence of a comparable enamel demineralization pattern in the study groups. However, a significant difference was observed after treatments among the study groups ($P < 0.001$). The further analysis indicated that among study groups, the average value of the measured microhardness was highest for Remin Pro®, being the same as the value for the sound teeth, while there was no significant difference between other test groups. It is worth noting that Remin Pro® contains xylitol, hydroxyapatite, and fluoride.[25] According to various studies, hydroxyapatite particles can precipitate on enamel surfaces, fill all porosities, and increase their surface hardness and strength against acidic attacks.[12] In agreement with the current study, Swarup and Rao[26] reported that 10% hydroxyapatite could remineralize incipient caries and improve enamel hardness significantly in comparison with 2% sodium fluoride. This finding is in agreement with the study by Bilgin *et al.*,[7] which reported that Remin Pro® produced stronger remineralization effects on enamel WSLs than CPP-ACPF, sodium fluoride, and fluoride varnishes. In contrast, Salehzadeh Esfahani *et al.*[25] reported that the remineralizing effects of Remin Pro® on enamel microhardness were weaker than those of CPP-ACP. According to the current study results, although the Remin Pro® group showed the highest measured microhardness, it showed the highest staining capacity as well. Due to the lack of similar research on staining during remineralization cycles, this part of the study cannot be compared with other studies.

Based on the current study results, MI Paste Plus could not remineralize early enamel caries

Table 2: The mean value and standard deviation of ΔE in different treatment groups in different stages of the study

Study groups	*n*	Mean±SD		
		ΔE1-2	ΔE1-3	ΔE2-3
SHMP	10	7.2±2.2	13.8±5.8	10.7±4.4
Sodium fluoride	10	7±2.1	24.7±5.6	20.9±3.7
Sodium fluoride + SHMP	10	7.9±2.3	15.3±5.3	14.4±4.4
Remin Pro®	10	6.7±1.7	25±4.3	24.5±6.1
Remin Pro® + SHMP	10	9.9±3.7	14±6.4	8.6±5.5
MI Paste Plus	10	10.2±3.3	19.9±4.2	16.2±2.9
MI Paste Plus + SHMP	10	9.6±2.2	13.2±7	10.9±5.5
Result of analysis of variance (*F*, *P*)		3.84, 0.005	8.60, <0.001	15.10, <0.001

SHMP: Sodium hexametaphosphate, SD: Standard deviation

Table 3: The mean value and standard deviation of testing groups in different stages of the study

Study groups	*n*	Mean±SD			Test result analysis of variance with repeated measures (*F*, *P*)
		T_1	T_2	T_3	
SHMP	10	225.2±14.8	99.0±34.4	124.9±44.7	76.0, <0.001
Sodium fluoride	10	211.3±11.6	100.0±28.3	138.0±19.5	72, <0.001
Sodium fluoride + SHMP	10	227.9±15.1	105.4±27.5	138.2±32.4	87.2, <0.003
Remin Pro®	10	228.4±12.6	107.6±13.1	212.4±24.5	174.7, <0.001
Remin Pro® + SHMP	10	208.5±25.2	105.6±20.0	136.8±28.3	115.6, <0.001
MI Paste Plus	10	225.1±15.9	101.9±17.7	127.6±17.6	275.1, <0.001
MI Paste Plus + SHMP	10	228.7±25.6	101.8±27.8	121.7±42.0	55.7, <0.001
Result of analysis variance test (*F*, *P*)		2.22, 0.052	0.16, 0.985	10.02, <0.001	Interaction 7.44, <0.001

SHMP: Sodium hexametaphosphate, SD: Standard deviation

Table 4: the correlation coefficient of ΔE and ΔH in different treatment groups and different stages of the study

Study groups	ΔE_{1-2}-ΔH_{1-2}	ΔE_{2-3}-ΔH_{2-3}	ΔE_{1-3}-ΔH_{1-3}
SHMP	Rp=0.207	Rp=0.533	Rp=0.090
	P=0.566	*P*=0.112	*P*=0.804
Sodium fluoride	Rp=0.120	Rp=0.114	Rp=0.355
	P=0.741	*P*=0.755	*P*=0.314
Sodium fluoride + SHMP	Rp=0.818	Rp=0.381	Rp=0.421
	P=0.004	*P*=0.277	*P*=0.225
Remin Pro®	Rp=0.016	Rp=0.638	Rp=0.510
	P=0.965	*P*=0.047	*P*=0.132
Remin Pro® + SHMP	Rp=0.038	Rp=0.188	Rp=0.516
	P=0.917	*P*=0.603	*P*=0.127
MI Paste Plus	Rp=0.231	Rp=0.118	Rp=0.210
	P=0.521	*P*=0.745	*P*=0.560
MI Paste Plus + SHMP	Rp=0.256	Rp=0.406	Rp=0.247
	P=0.459	*P*=0.245	*P*=0.491

SHMP: Sodium hexametaphosphate

effectively, either alone or combined with SHMP. In agreement a recent study revealed the CPP-ACP was unable to remineralize enamel subjected to extrinsic erosion, being ineffective in preventing the erosion of enamel.[27] In the literature, there are some controversies observed concerning the effectiveness of CPP-ACP. While some studies have not indicated any favorable effects for this agent on enamel caries,[27-29] others have reported an increase in enamel microhardness after the use of CPP-ACP, with a protective effect on the demineralization of human tooth enamel against acidic conditions.[30,31]

In the current study, the colorimetry test and the CIE Lab parametric system were employed to evaluate color changes. If the ΔE value exceeded 3.3, the color change would be clinically obvious. In this study, after the demineralization phase and pH-cycling, the measured ΔE value exceeded 3.3 in all test groups. In addition, the difference in the ΔE value among different stages of the study in SHMP-treated groups was lower than others. This finding implies that the addition of SHMP to remineralizing agents reduces staining and pigment adsorption during the remineralization process. During the brushing process, SHMP molecules dissolved rapidly due to their high solubility in water. In addition, they reacted chemically to remove the stains, leaving a protective layer on the tooth surface to prevent new stain adsorptions.[14] It should be considered that when a mineral agent, such as sodium fluoride remineralizes the surface of the initial enamel caries in the oral cavity, the pigments present in the saliva, and dental plaque along with mineral ions precipitated in caries affect the surfaces. As a result, the new hardened surface, against its high resistance against the offense of future caries, will have an unesthetic appearance. As a result, some patients are not satisfied with the appearance and would like to restore the lesions, not for caries but for esthetic reasons. In line with the current study, Bartizek *et al.*[32] reported that 3.2% SHMP containing local materials inhibited staining; in the same vein, Koyasu *et al.*[33] stated that SHMP was effective in stain removal due to its high binding affinity to calcium, and considering its negative charge. The extrinsic stain removal efficacy of an experimental stannous fluoride + SHMP dentifrice was also confirmed in a 6-week clinical trial study by He *et al*.[14] In contrast to Gerlach *et al.*[34] who reported that SHMP-containing toothpastes were comparable with high-abrasive toothpastes in extrinsic stain removal effects, SHMP presented the same abrasiveness with stannous fluoride.[35] Hence, the lower color change in SHMP-treated groups cannot be the result of the abrasive property.

In the present study, the microhardness value of the sodium fluoride group was greater than that of the SHMP group; however, the difference was not significant. This outcome is in contrast to the findings of the study by do Amaral *et al.*[15] that indicated a stronger inhibitory effect for SHMP than 1100-ppm fluoride in erosive conditions. The difference between these two studies seems to have been due to the different pH-cycling method used.

According to the present study, SHMP-containing groups demonstrated lower microhardness values, except the sodium fluoride + SHMP group that showed a higher microhardness value than sodium fluoride alone. A study by da Camara *et al.*[20] revealed that SHMP combined with fluoride enhanced the fluoride concentration on the biofilm by the binding of Ca^{2+} to the SHMP molecule, thereby reducing the loss of minerals in demineralization conditions. SHMP produce a more electron-donor site on enamel surface which can facilitate greater adsorption of Ca and PO_4 ions.[36] In agreement with these findings, Dalpasquale *et al.*[37] reported that 1100-ppm fluoride-SHMP-containing toothpastes strengthened enamel more than usual toothpastes under demineralization conditions. Furthermore, a recent study revealed the addition of 9% SHMP to a gel containing 4500-ppm fluoride concentration was able to significantly enhance the remineralization

of artificial carious lesions when compared to other groups containing 900-ppm or 12,300-ppm fluoride.[38]

Therefore, given the past study results and the present study findings, it can be concluded that SHMP has a synergistic effect only with fluoride supplements, with no complementary effect in combination with CPP-ACP or hydroxyapatite. Thus, the combination of SHMP and sodium fluoride can be effective in restoring enamel lesions, while maintaining esthetics; in addition, the combination could be considered as a safe and a more efficacious alternative for sodium fluoride supplements. It is worth noting that the SHMP's ability to form a strong complex with metal ions[39] might reduce the presence of Ca^{2+} released from CPP-ACP and decrease Ca^{2+} deposition in caries lesions, thereby decreasing the effects of these agents on remineralization. Another factor that might have affected the microhardness of SHMP-containing groups in this study was the lack of information about the proper amount and percentage of SHMP in combination with CPP-ACP and hydroxyapatite-containing materials. da Camara *et al.*[20] stated that the exact antimicrobial concentration of SHMP was unknown, yet in a pilot study, they reported some inhibitory effects for 6% SHMP. However, they stated that with an increase in the SHMP concentration, its synergistic effect with fluoride decreased. Therefore, the lower microhardness value of the SHMP-containing groups in the current study might have been associated with its 8% concentration. do Amaral *et al.*[15] demonstrated a synergistic effect only between fluoride and 1% HMP. However, the duration of the inhibitory effect was longer when 1% or 8% SHMP were combined with fluoride. A recent study that used the same percent of SHMP (9%) with sodium fluoride revealed the greater remineralization potential of the toothpaste containing SHMP + F compared with F alone. However, the recent studies that evaluated the remineralizing affect of the toothpastes containing nano-sized SHMP and fluoride, the addition of 0.5% SHMP nanoparticles to a conventional 1100-ppm fluoride toothpaste promoted remineralization of artificial caries lesions and significantly affected the composition of biofilm formed with higher values of fluoride and calcium.[40-42]

The results of the current study revealed that the highest measured microhardness was obtained in Remin pro®-treated groups after pH-cycling duration among the study groups. It should be noted that although Remin Pro® testing group showed the same microhardness value as intact tooth, the highest color change among experimental groups during remineralization might have necessitated the esthetic treatments for these strengthened stained areas. The strong and positive correlation between microhardness and overall color change observed only in the Remin Pro® group among experimental groups confirmed this outcome. It should be noted that enamel WSLs treated with the combination of Remin Pro® and SHMP had the lowest alteration in color parameters after remineralization; however, the hardness value decreased in this group.

Given its laboratory nature, this study had certain limitations that might have affected the results obtained. The oral environment, remineralization and demineralization cycles, the salivary flow rate, as well as oral protective factors, including salivary proteins and bacteria could not be considered *in vitro*. Furthermore, the present study was used the bovine teeth as a substitute for human teeth in evaluating the different remineralizing protocols. However, specimens generated from human teeth are preferred for dental researches, but some disadvantages such as inadequate quantity and quality, relatively small and curved surface area, infection hazard, and ethical issues limited their application. However, bovine teeth not only easily obtain, but also they have a relatively large flat surface and do not have caries lesions and other defects which might affect outcomes. According to Yassen *et al.*[43] literature review, although there are several differences between the bovine and human teeth in both dentin and enamel structure, the bovine teeth can be used as an alternative to human substrate in both dental erosion/abrasion and dental caries studies.

The evaluation of the synergistic effects of remineralizing agents could be an appealing subject in the future studies. Further studies should be conducted *in vivo* to evaluate the effects of protective factors on the oral cavity, over a long period of time.

CONCLUSION

Based on the results obtained, it could be concluded that the addition of 8% SHMP to 2% sodium fluoride, Remin Pro®, and MI Paste Plus reduced the staining of early enamel caries during the remineralization phase, while it could improve remineralization only in the sodium fluoride-treated group. In addition, it

should be noted that Remin Pro® can improve the hardness of enamel caries up to the level of sound teeth, yet it can be accomplished by the highest pigment adsorption.

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Conflicts of interest

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

REFERENCES

1. Heymann HO, Swift EJ Jr., Ritter AV. Sturdevant's Art and Science of Operative Dentistry. 6th ed. St. Louise: Mosby; 2014.
2. Summitt JB, Schwartz RS, Hilton TJ, Robbins JW,. Fundamentals of Operative Dentistry: A Contemporary Approach. 3rd ed. Hanover Park, Illinois: Quintessence Pub; 2006.
3. Theodore M, Heymann HO, Edward J. Sturdevant's Art & Science of Operative Dentistry. 5th ed. St. Louise: Mosby; 2006.
4. Fejerskov O, Nyvad B, Kidd E. Clinical and histological manifestations of dental caries. In: Fejerskov O, Kidd E, editors. Dental Caries: Disease and Its Clinical Management. New Jersey, Hoboken: Blackwell Pub; 2003. p. 71-97.
5. Ten Cate JM. *In vitro* studies on the effects of fluoride on de- and remineralization. J Dent Res 1990;69:614-9.
6. Gessner BD, Beller M, Middaugh JP, Whitford GM. Acute fluoride poisoning from a public water system. N Engl J Med 1994;330:95-9.
7. Bilgin G, Yanikoglu F, Tagtekin D, Stookey G, Schemeron B. Remineralization potential of different agents and assessment by a new caries detection device. Gavin J Dent Sci 2016;2016:39-43.
8. Gavrila L, Maxim A, Balan A, Stoleriu S, Sandu AV, Serban V, *et al*. Comparative study regarding the effect of different remineralizing products on primary and permanent teeth enamel caries lesions. Rev Chim 2015;66:1159-61.
9. Cross KJ, Huq NL, Palamara JE, Perich JW, Reynolds EC. Physicochemical characterization of casein phosphopeptide -amorphous calcium phosphate nanocomplexes. J Biol Chem 2005;280:15362-9.
10. Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate: The scientific evidence. Adv Dent Res 2009;21:25-9.
11. Li X, Pan D, Lin S, Zhuang Z, Lin Z. Facile *in vitro* hydroxyapatite remineralization of human enamel with remarkable hardness. CrystEngComm 2013;15:4351-6.
12. Tschoppe P, Zandim DL, Martus P, Kielbassa AM. Enamel and dentine remineralization by nano-hydroxyapatite toothpastes. J Dent 2011;39:430-7.
13. Sensabaugh C, Sagel ME. Stannous fluoride dentifrice with sodium hexametaphosphate: Review of laboratory, clinical and practice-based data. J Dent Hyg 2009;83:70-8.
14. He T, Baker R, Bartizek RD, Biesbrock AR, Chaves E, Terézhalmy G, *et al.* Extrinsic stain removal efficacy of a stannous fluoride dentifrice with sodium hexametaphosphate. J Clin Dent 2007;18:7-11.
15. do Amaral JG, Delbem AC, Pessan JP, Manarelli MM, Barbour ME. Effects of polyphosphates and fluoride on hydroxyapatite dissolution: A pH-stat investigation. Arch Oral Biol 2016;63:40-6.
16. Pfarrer A, White D, Rapozo-Hilo M, Featherstone J. Anticaries and hard tissue abrasion effects of a "dual-action" whitening, sodium hexametaphosphate tartar control dentifrice. J Clin Dent 2002;13:50-4.
17. Schiff T, Saletta L, Baker RA, He T, Winston JL. Anticalculus efficacy and safety of a stabilized stannous fluoride/sodium hexametaphosphate dentifrice. Compend Contin Educ Dent 2005;26:29-34.
18. da Camara DM, Miyasaki ML, Danelon M, Sassaki KT, Delbem AC. Effect of low-fluoride toothpastes combined with hexametaphosphate on *in vitro* enamel demineralization. J Dent 2014;42:256-62.
19. Pfarrer AM, McQueen CM, Lawless MA, Rapozo-Hilo M, Featherstone JD. Anticaries potential of a stabilized stannous fluoride/sodium hexametaphosphate dentifrice. Compend Contin Educ Dent 2005;26:41-6.
20. da Camara DM, Pessan JP, Francati TM, Santos Souza JA, Danelon M, Delbem AC, *et al.* Synergistic effect of fluoride and sodium hexametaphosphate in toothpaste on enamel demineralization *in situ*. J Dent 2015;43:1249-54.
21. da Camara DM, Pessan JP, Francati TM, Souza JA, Danelon M, Delbem AC, *et al.* Fluoride toothpaste supplemented with sodium hexametaphosphate reduces enamel demineralization *in vitro*. Clin Oral Investig 2016;20:1981-5.
22. ten Cate JM, Duijsters PP. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res 1982;16:201-10.
23. Featherstone JDB, O'Reilly MM, Shariati M, Brugler S. Enhancement of remineralization *in vitro* and *in vivo*. In: Leach SA. Factors affecting de- and remineralization of the teeth. Oxford: IRL Press; 1986. p. 23-34.
24. Uysal T, Amasyali M, Koyuturk AE, Ozcan S. Effects of different topical agents on enamel demineralization around orthodontic brackets: An *in vivo* and *in vitro* study. Aust Dent J 2010;55:268-74.
25. Salehzadeh Esfahani K, Mazaheri R, Pishevar L. Effects of treatment with various remineralizing agents on the microhardness of demineralized enamel surface. J Dent Res Dent Clin Dent Prospects 2015;9:239-45.
26. Swarup JS, Rao A. Enamel surface remineralization: Using synthetic nanohydroxyapatite. Contemp Clin Dent 2012;3:433-6.

27. Scardini ID, Dias MK, Castro MC, Guedes AP, Peixoto LM. Effect of CPP-ACP on enamel eroded extrinsically: *In vitro* study. Rev Gaúcha Odontol 2018;66:21-8.
28. Carvalho FG, Brasil VL, Silva Filho TJ, Carlo HL, Santos RL, Lima BA, *et al.* Protective effect of calcium nanophosphate and CPP-ACP agents on enamel erosion. Braz Oral Res 2013;27:463-70.
29. Rallan M, Chaudhary S, Goswami M, Sinha A, Arora R, Kishor A, *et al.* Effect of various remineralising agents on human eroded enamel of primary teeth. Eur Arch Paediatr Dent 2013;14:313-8.
30. de Alencar CR, Magalhães AC, de Andrade Moreira Machado MA, de Oliveira TM, Honório HM, Rios D, *et al. In situ* effect of a commercial CPP-ACP chewing gum on the human enamel initial erosion. J Dent 2014;42:1502-7.
31. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC, *et al.* Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res 2004;38:551-6.
32. Bartizek RD, Walters P, Biesbrock AR. The prevention of induced stain using two levels of sodium hexametaphosphate in chewing gum. J Clin Dent 2003;14:77-81.
33. Koyasu M, Shiba T, Kawazoe Y, Manabe A, Miyazaki T. Ultraphosphate, a potent stain control agent that is effective for both stain removal and prevention of stain deposition. Dent Mater J 2014;33:252-60.
34. Gerlach R, Ramsey LL, White DJ. Extrinsic stain removal with a sodium hexametaphosphate-containing dentifrice: Comparisons to marketed controls. J Clin Dent 2002;13:10-4.
35. de Oliveira TA, Scaramucci T, Nogueira FN, Simões A, Sobral MA. Effect of mouthrinses with different active agents in the prevention of initial dental erosion. Indian J Dent Res 2015;26:508-13.
36. Neves JG, Danelon M, Pessan JP, Figueiredo LR, Camargo ER, Delbem AC, *et al.* Surface free energy of enamel treated with sodium hexametaphosphate, calcium and phosphate. Arch Oral Biol 2018;90:108-12.
37. Dalpasquale G, Delbem AC, Pessan JP, Nunes GP, Gorup LF, Neto FN, *et al.* Effect of the addition of nano-sized sodium hexametaphosphate to fluoride toothpastes on tooth demineralization: An *in vitro* study. Clin Oral Investig 2017;21:1821-7.
38. Gonçalves FM, Delbem AC, Pessan JP, Nunes GP, Emerenciano NG, Garcia LS, *et al.* Remineralizing effect of a fluoridated gel containing sodium hexametaphosphate: An *in vitro* study. Arch Oral Biol 2018;90:40-4.
39. Genichiro Kura SO, Kura S. Complex formation of cyclic phosphate anions with bivalent cations. J Inorg Nucl Chem 1974;36:1605-9.
40. Danelon M, Garcia LG, Pessan JP, Passarinho A, Camargo ER, Delbem AC, *et al.* Effect of fluoride toothpaste containing nano-sized sodium hexametaphosphate on enamel remineralization: An *in situ* study. Caries Res 2018;53:260-7.
41. Garcia LS, Delbem AC, Pessan JP, Dos Passos Silva M, Neto FN, Gorup LF, *et al.* Anticaries effect of toothpaste with nano-sized sodium hexametaphosphate. Clin Oral Investig 2018. p 1-8. doi: 10.1007/s00784-018-2773-7. [Epub ahead of print]
42. Emerenciano NG, Botazzo Delbem AC, Pessan JP, Nunes GP, Souza Neto FN, de Camargo ER, *et al. In situ* effect of fluoride toothpaste supplemented with nano-sized sodium trimetaphosphate on enamel demineralization prevention and biofilm composition. Arch Oral Biol 2018;96:223-9.
43. Yassen GH, Platt JA, Hara AT. Bovine teeth as substitute for human teeth in dental research: A review of literature. J Oral Sci 2011;53:273-82.