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The Effect of Different Percentages of Nano-bioactive Glass in the Synthesized CPP/ACP Paste on the Remineralization of Demineralized Enamel

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Abstract

Background: The preventive treatments of primary caries lesions are essential for preventing destructive damage to the tooth structure. One of the common treatments is the application of casein phosphopeptide-amorphous calcium phosphate (CCP/ACP) paste on the enamel surface. The aim of this study was to investigate the effect of different percentages of nano-bioactive glass (nBG) incorporation into synthesized CPP/ACP paste on the remineralization of demineralized enamel.

Methods: In general, 24 extracted human intact premolar teeth were selected, and their crowns were removed for this purpose. Each crown was cut into two halves, and each half was considered as a sample. The samples were placed in a demineralizing solution at a pH rate of 4.6 for 8 hours, in artificial saliva for 1 hour, and again in a remineralizing solution at a pH rate of 7 for 15 hours. The pH cycling was performed for 14 days to demineralize the enamel surface. The samples were randomly divided into 3 groups (n=16), including G1 (without treatment), G2 (treated with synthesized CPP/ACP paste containing 5% nanobioglass), and G3 (treated with synthesized CPP/ACP paste containing 10% nanobioglass). The paste was then placed directly on the surface of the demineralized enamel for 4 minutes (twice a day for 28 days). The samples were subjected to the Vickers microhardness test. Finally, data were analyzed using SPSS (version 19) and the analysis of variance and Tukey's tests ($\alpha=0.05$).

Results: There was a significant difference between microhardness values in G1 and G2, as well as G1 and G3 ($P<0.05$). However, no statistically significant difference was observed between G2 and G3 ($P>0.05$).

Conclusions: The results showed adding bioactive glass into synthetic CPP/ACP paste increases enamel remineralization in spite of the percentage of bioactive glass incorporation.

Keywords: Bioactive glass, Enamel remineralization, Microhardness, CPP-ACP

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Background

Dental health plays an important role in individual health. In the past, dental caries were mostly treated by filling with restorative materials by dentists. However, preventive treatments (e.g., fissure sealants and fluoride therapy) are now performed instead (1). Enamel is composed of densely packed hydroxyapatite (HA) crystals, which together form enamel prisms that have a key-shaped cross-section and are made by ameloblasts. Each prism starts at the dentinoenamel junction and reaches the tooth surface as a rod (2,3). Primary demineralized areas of the enamel are milky white spots in contrast to intact enamel and rapidly progress in cariogenic conditions, destroying the enamel structure. According to Gorelick et al, caries are found in 24% of people (4). Before the start of the treatment, it is important to diagnose patients at high risk of decalcification. Patients with a plaque above 3, or decayed, missing, and filled teeth (DMFT index) above 8, or early lesions above 4 are among those who undergo professional preventive health measures or chemical treatments with anticariogenic agents (5).

In addition to the need for mechanical oral hygiene,

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various chemical methods such as the application of various forms of fluoride, including fluoride-releasing varnishes and adhesives, casein phosphopeptide-amorphous calcium phosphate (CCP/ACP), and newer methods (e.g., laser radiation) have been proposed for the treatment of these lesions.

The most common chemical method is the use of fluoride-containing agents in various forms as a proven method for the enhancement of remineralization. Fluoride ions can enter the tooth structure by replacing hydroxyl groups or fluorapatite deposits (6).

Damage to the enamel can be reversed using mineralizing agents. Previous studies showed the positive effect of CPP/ACP paste on enamel remineralization (7,8). On the other hand, another group of these mineralizing substances is bioactive glasses. Recent studies have reported that bioactive glass can inhibit and reverse the progression of primary caries in the enamel. In addition, it can form a protective layer of hydroxycarbonate apatite on the surface of the demineralized enamel or enhance remineralization in the enamel. Bioglass 45S5 (made by Hench) has high biocompatibility, can form hydroxycarbonate apatite (a biological mineral) in body fluids, and is widely used in bone regeneration and tissue engineering (9,10).

Considering the lack of sufficient information regarding adding and optimizing the nano-bioactive glass (nBG) percentage into CPP/ACP paste in order to induce enamel remineralization, the present study was designed to evaluate the effect of the incorporated percentages of nBG in a synthesized CPP/ACP paste on the remineralization of the demineralized enamel.

In a recent study by Yann et al, calcium replacement with strontium (due to its larger ionic radius than calcium) led to further expansion of the glass lattice and increased the decomposition and formation of apatite. Moreover, the inclusion of strontium into the bioglasses structure enhanced the remineralization of the enamel (11).

Materials and Methods

Bioglass Synthesis

Nanobioglass was synthesized using the sol-gel method (12). Water/ethanol solution (2:1), namely, tetraethyl orthosilicate was mixed with calcium nitrate. By adding 1 M of citric acid, the pH of the solution was adjusted to 2. This system was stirred until obtaining a clear solution was (solution A). The solution of 2% PEG (MW: 2000) and diammonium hydrogen orthophosphate was prepared, and its pH was adjusted to 10 (solution B) by adding ammonia. Both solutions A and B were mixed under a stirrer for 10 hours to obtain a homogeneous gel. After rinsing with deionized water and filter, the resulting white gel was dried and lyophilized, and finally, calcined for 10 hours at 650˚C.

CPP/ACP Synthesis

A 10% by weight/volume solution of casein was prepared (pH = 8). The pH was adjusted by adding NaOH, and then trypsin was added by 0.2% w/w and dissolved for 2 hours at 50˚C. After dissolving by adding HCl, the pH was adjusted to 4.6. The formed chemical deposition was separated using a centrifuge. At this stage, the pH of the formed deposition was adjusted to 9 using NaOH. Then, $CaCl_2$ (one mole), Na_2HPO_4 (one mole), and NaF (200 mmol) were slowly added, along with continuous shaking. Certain concentrations of $CaCl_2$, Na_2HPO_4, and NaF were added as well. The final solution was filtered by a microfilter (0.1-0.2 µm). The remaining depositions, after filtration, were brought up to the volume by distilled water with a volume equal to or 5 times its volume. The final depositions were dried by the spray. The resulting white powder contained 50% CPP and 40% ACP (13).

CPP/ACP paste containing bioactive glass was obtained from a physical mixture of CPP/ACP powder and bioglass with a weight ratio of 5% and 10% in double-distilled water (14).

Preparation of Specimens

A total of 24 extracted intact human premolars were selected based on the study purpose. These teeth were obtained from patients with orthodontic treatment needs referring to the orthodontic department of Hamadan Dental School. Teeth were stored in a 10% formalin solution. Contamination and excess tissues on the surface of individual teeth were removed by a surgical scalpel blade No. 15. The plaques were cleaned and removed at low speed by a brush placed on a handpiece and placed in de-ionized water at room temperature 24 hours before the experiment. Then, the extracted teeth had intact crowns, were free of caries and fillings, and had no hypoplasia, decalcification lesions, fractures, or enamel cracks.

Next, the teeth were mounted in amalgam capsules using a capstone for sectioning and placing in a disc inserted on the cutting machine. The samples were mesiodistally cut by a 0.3 mm diamond disc (Ham Co. Machines, Inc., Rochester/USA) using continuous water cooling and then separated from the cemento-enamel junction via the cutting disc. The teeth were mesiodistally divided into two parts and then mounted in orthodontic resin. The outer surfaces of samples were polished using 600, 1000, and 2000 grit silicon carbide abrasive paper and a diamond paste-impregnated brush (1-5 µm). The samples were cleaned with distilled water and assessed under a microscope to evaluate the presence of cracks or defects (10). The samples with cracks or defects were excluded from the study. For pH cycling, samples were immersed in a demineralizing solution (pH = 4.6) for 8 hours, in artificial saliva for 1 hour, and then again in a remineralizing solution (pH = 7) for 15 hours. This cycle was continued for 14 days to demineralize the enamel surface. Demineralizing and remineralizing materials

Table 1. Descriptive Distribution of the Studied Groups

	N	Mean	Standard Deviation	Standard Error	95% CI for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	16	269.5100	25.70124	6.42531	255.8148	283.2052	214.66	313.66
5%	16	322.6825	37.01026	9.25257	302.9611	342.4039	249.33	400.00
10%	16	334.5162	28.03772	7.00943	319.5760	349.4565	289.90	384.00
Total	48	308.9029	41.41383	5.97757	296.8776	320.9282	214.66	400.00

were changed every 2 days (7).

All samples were randomly assigned to three equal groups (n = 16) according to surface treatment G1 (Control, synthetic CPP/ACP without nBG), G2 (synthetic CPP/ACP containing 5% bioglass), and G3 (synthetic CPP/ACP containing 10% bioglass). The samples were dried for 5 seconds until the moisture disappeared, and CPP/ACP was placed directly on the surface of the demineralized enamel for 4 minutes twice a day for 28 days (8).

The microhardness of the samples was measured by the Vickers test using a microhardness tester (Micrometer 1, Buehler, Lake Bluff, IL, USA) microscope to measure the amount of indentation generated by the force of 500 grams at three points for 5 seconds, and then the average microhardness was calculated for the three points. Data were analyzed by SPSS (version 19) using the analysis of variance (ANOVA) and Tukey's tests ($\alpha = 0.05$).

Results

The mean, standard deviation, minimum, and maximum values of surface hardness in each of the study groups are presented in Table 1.

The mean microhardness strength was 10% higher in the bioglass group (334.51 ± 28.03) compared to the other groups, and the mean microhardness strength was lower in the control group in comparison with the other groups (269.51 ± 25.70).

The results of the Kolmogorov-Smirnov test indicated the normal distribution of microhardness in all groups ($P > 0.05$). One-way ANOVA revealed statistically significant differences in the mean microhardness between the groups ($P < 0.001$, Table 2).

According to the results of Tukey's post hoc test for the pairwise comparison of the studied groups, the control group (G1) was significantly different from 5% (G2) and 10% bioglass (G3) groups ($P < 0.001$), but no statistically significant difference was observed between the 5% (G2) and 10% bioglass (G3) groups ($P = 0.524$, Table 3).

Table 2. Results of the ANOVA Test of the Mean Microhardness of the Studied Groups

	Sum of Squares	df	Mean Square	F	*P* Value
Between groups	38363.546	2	19181.773	20.432	0.000
Within groups	42246.409	45	938.809		
Total	80609.956	47			

Note. ANOVA: Analysis of variance.

Discussion

Nowadays, dental sciences increasingly emphasize prevention and health education instead of restorative and neurosurgery treatments. Moreover, caries prevention has manifested the need to produce anti-caries and enamel restorative materials more than before, which is of high interest to many researchers who are attempting to make more effective products for enamel repair (15-18).

CPP/ACP is a commonly used material in tooth remineralization that promotes remineralization and prevents the colonization of cariogenic bacteria on dental surfaces by maintaining a saturation state of essential minerals. Most studies have proven the effect of CPP/ACP on enamel remineralization and its effectiveness in the reduction of caries.

The present study sought to investigate the effect of different inclusion percentages of nBG in the synthesized CPP/ACP paste on the remineralization of demineralized enamel. Unlike fluoride, CPP/ACP is safe to swallow, has a better taste, and adequately provides the required calcium and phosphate of the enamel compared with other remineralizing materials (19). Soltanimehr et al studied the effect of CPP/ACP on enamel microhardness and concluded that this paste regenerated enamel and increased the surface microhardness of the enamel (8).

Likewise, Khamverdi et al evaluated the effects of CO_2 laser and CPP/ACP on the reinforcement of demineralized enamel and found that enamel microhardness effectively increased using CO_2 laser and CPP/ACP after demineralization (7). Moreover, Soltanimehr et al reported that CPP/ACP enhanced the remineralization ability of the enamel (8). Similarly, Zhang et al confirmed the effect of CPP/ACP paste on tooth enamel (20).

According to the results of previous investigations regarding the effect of bioactive glass on enamel remineralization, it seems that using this material will be effective by the efficient use of bioactive glass on the teeth, particularly during teeth whitening with bleaching, the time, and the number of its appropriate uses, but it requires further studies (10).

Based on the obtained results, the surface microhardness mean of the enamel increased significantly in groups

Table 3. Pairwise Comparison of the Mean Microhardness of the Studied Groups

Multiple Comparisons						
Dependent Variable: Microhardness						
Tukey HSD						
(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	*P* Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	5%	-53.17250*	10.83287	0.000	-79.4272	-26.9178
	10%	-65.00625*	10.83287	0.000	-91.2609	-38.7516
5%	Control	53.17250*	10.83287	0.000	26.9178	79.4272
	10%	-11.83375	10.83287	0.524	-38.0884	14.4209

Note. * The mean difference is significant at the 0.05 level.

containing 5% and 10% bioactive glass compared to the control group. This can be attributed to its material nature. Bioactive glass is composed of amorphous sodium-calcium-phosphate, the contact of which with the saliva causes the release of calcium and phosphate from the glass. A transient increase in pH results in the deposition of calcium and phosphate on the tooth surface (21).

Additionally, Bächli et al evaluated the demineralization potential of demineralized dentin and observed high remineralizing ability in the demineralized dentin, particularly after the use of dentin matrix proteins (22).

Regarding incorporating remineralizing agents such as fluoride to CPP/ACP, Khamverdi et al concluded that a combination of anti-caries agents could have a synergistic effect on the repair of demineralized enamel (23), which is in line with the results of the current study.

In a study by Alhussain et al on the remineralization of artificial caries lesions by fluoride and bioactive glass toothpaste, toothpaste containing bioactive glass and fluoride had a better effect than using each substance alone, indicating the synergistic effect of these substances on the remineralization of the enamel (24).

Bioactive glass is a material that can bind to HA crystals, which is widely employed in bone regeneration. Accordingly, this substance was used by many researchers to restore enamel caries, yielding noteworthy results (25,26).

In another study, Narayana et al applied a bioactive glass, fluoride, and CPP/ACP to evaluate the remineralization ability of bioactive glass on artificial caries and introduced bioactive glass as an effective substance in remineralization (27). In line with our results, Mehta et al investigated the effect of CPP/ACP and bioactive glass on dental remineralization and reported that both had an acceptable effect on dental remineralization, but bioactive glass led to better results (28).

Another finding of this study demonstrated no statistically significant differences between 5% and 10% groups probably due to the limited ability of HA crystals to absorb this substance, its placement in HA crystals, and the limited involved surface area.

Likewise, Palaniswamy et al examined different effects of bioactive glass and CCP/ACP paste on the remineralization of early enamel lesions. Their results revealed that microhardness significantly increased in samples treated with bioactive glass after 10 days, but the other group represented better results after 15 days (26). The results of our study indicated that the effect of bioglass and CCP/ACP paste was higher compared to the paste alone after 28 days, suggesting the higher synergistic effect of the two substances when used together.

Furthermore, Wu et al studied the remineralizing effects of bioactive glass on dentin in comparison to ACP/CPP sodium fluoride paste and found the highest depth of remineralization in the BAG group, demonstrating the better effect of bioactive glass on dentin remineralization (29).

Körner also compared the remineralization ability of fluoride and two different bioglasses and reported that the fluoride group alone showed more remineralization compared to the combined use of fluoride and bioglass (30). They attributed this issue to the lack of deep penetration of minerals due to the accumulation of calcium, phosphate, and fluoride on the surface of the enamel lesion (31).

This was an in vitro study for stimulating oral conditions, designing future studies is recommended in this regard. Finally, it is suggested that nBG particles be characterized and the degree of cytotoxicity be evaluated as well.

Conclusions

Under the limitations of the present study, the results demonstrated that the incorporation of nBG particles into CPP/ACP paste increased the microhardness for the treatment of demineralized enamel, and this increase is not dependent on the percentage of nBG.

Authors' Contribution

Conceptualization: Zahra Khamverdi.
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Writing – original draft: Alireza Mazaheri.
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Competing Interests

The authors declare that they have no conflict of interests.

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