AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 9, No. 1, January (2022) — PP. 61:72

Remineralization Efficiency of Different Toothpastes on Human Enamel Subjected to Acid Challenge: in Vitro Study

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Codex : 08/22.01

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http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2021.66174.1346

Restorative Dentistry
(Removable Prosthodontics, Fixed Prosthodontics, Endodontics, Dental Biomaterials, Operative Dentistry)

ABSTRACT

Purpose: The objective of this in vitro study was to evaluate and compare the remineralizing potential of dentifrices containing nanohydroxyapatite, fluoride, and bioactive glass with and without fluoride on enamel by assessing the enamel surface microhardness the enamel structural and elemental analysis through Energy Dispersive X-ray Analysis (EDX). **Methods:** Sound extracted third molars were divided into 5 groups. Group A (n=15): Hydroxyapatite toothpaste (Karex) was used to treat the teeth; Group B (n=15): teeth were treated with Hydroxyapatite and Fluoride containing toothpaste (Apacare); Group C (n=15): teeth were treated with ChloroCalcium Phosphosilicate containing toothpaste (Biomin C); Group D (n=15): teeth were treated with FluoroCalcium Phosphosilicate containing toothpaste (Biomin F); negative control group (n=15): teeth not subjected to any treatment. All teeth (experimental and negative control groups). After 2 weeks of the dynamic pH-cycling; 10 teeth of each group were subjected to microhardness assessment, while 5 teeth of each group were subjected to EDX Analysis. **Results:** After 2 weeks of pH-cycling, all experimental groups (A, B, C, and D) showed a percent increase of enamel surface microhardness. Group D reported the highest percent increase (15.07%) while teeth that were not subjected to any treatment (negative control group) showed a percent decrease (-15.7%). Fluoride and calcium ions recorded a significantly higher percent increase in group D, while a significantly lower value was recorded in the control group. **Conclusion:** All the experimental toothpaste had the potential to remineralize enamel surface subjected to dynamic pH-cycling, but the incorporation of fluoride with the bioactive glass technology as in Biomin F toothpaste had the maximum effect on the demineralized enamel surface.

KEYWORDS

Bioactive glass, hydroxyapatite, remineralization, enamel microhardness.

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INTRODUCTION

Recently there has been a shift in caries treatment, from the traditional surgical approach to the minimally invasive approach. Minimally invasive dentistry (MID), is a modern evidence-based approach[1] that combines prevention, remineralization, and minimal intervention for the placement and replacement of restorations[2]. MID attempts to remineralize or arrest lesions to the fullest degree possible as there is no replacement for the normal tooth structure. Remineralization is considered an ideal way for the treatment of carious lesions that do not need surgical intervention. This is because of fact that remineralization restores the lost minerals within a demineralized matrix or tooth[1]. Therefore, improving the process of remineralization with the aid of different remineralizing products is considered the best method for caries control[3].

Currently, it is well established that fluoride has both preventive and remineralizing effects. Featherstone explained the anticaries effect of fluoride through three main mechanisms. Firstly, fluoride acts as an inhibitor for both, the metabolic and physiological pathways in the cariogenic biofilm that produces organic acids that demineralize dental hard tissues. Secondly, fluoride inhibits demineralization by replacing ions in hydroxyapatite (HAP), resulting in the formation of fluorapatite (FAP). When compared to HAP, FAP is more resistant to acid dissolution. Finally, fluoride can remineralize the dental hard tissue through its attachment onto the surface of the crystal and followed by attracting calcium and phosphate ions to nucleate for new mineral formation and growth[1].

Even though fluoride interventions appear to have the most consistent benefit in preventing caries development and remineralizing initial lesions with the highest level of supporting evidence, there are limitations to what application of fluoride alone can achieve in terms of caries prevention and remineralization. These limitations could also be associated with the very fact that at a pH level below 4.5, fluoride encompasses a lower effect; fluoride still requires calcium and phosphate ions during a bioavailable form in saliva and other sources to be effective. Furthermore, while the effect of fluoride is dose-dependent and increases with increasing the dose, there is a limit to how high the fluoride dose can be increased to achieve the desired effect without the risk of side effects as dental fluorosis and toxicity. The aforementioned limitations explain the need for new strategies that could work better than or as well as fluoride but allow increasing dosage for increased effectiveness without safety concerns[4]. Therefore, many non-fluoride remineralizing materials have been introduced and incorporated into toothpaste and oral care products.

One of these non-fluoride materials is bioactive glass (BAG). This substance has several unique characteristics; one of which is having the ability to biomimetically mineralize the tissues in a way like the body's mineralization characteristics. BAG consists of minerals naturally present in the fluids of the body. Upon contacting water, saliva, or other body fluids, it releases calcium, phosphorus, sodium, and silicon ions leading to hydroxyapatite crystals formation. The formed hydroxyapatite crystals are equivalent both chemically and structurally to naturally occurring biological apatite which is thought to help in the remineralization process[3].

Since the traditional bioactive glass composition is deficient in fluoride, which is essential for the remineralizing process and caries resistance. A new toothpaste was introduced into the market which is formed of fluoride incorporated bioactive glass[5].

Another material that has been introduced and widely investigated is nano-HA. It is considered to be very promising because it is biocompatible, bioactive and similar to the bone and mineral structure of teeth[6]. HAP nanoparticles have been added into toothpaste or mouthwashes to promote remineralization of demineralized enamel or dentin through the deposition of HAP nanoparticles in the lesions[1].

Hence, the goal of this in vitro study is to assess and compare the remineralizing potential of toothpastes containing nanohydroxyapatite, fluoride, and bioactive glass with and without fluoride on enamel microhardness. We hypothesized that when used daily as instructed by the manufacturer each of these toothpaste formulations promotes remineralization.

MATERIAL AND METHODS

Remineralizing Agents

Four commercially remineralizing toothpaste were used in this study.

Hydroxyapatite without fluoride (Karex) (Dr. Kurt Wolff GmbH & CO. KG, Bielefeld, Germany)

Hydroxyapatite with Sodium Fluoride (1450 ppm) (Apacare) (Cumdente GmbH, Tübingen, Germany

ChloroCalcium Phosphosilicate (Biomin C) (BioMin Technologies Limited, London, England).

FluoroCalcium Phosphosilicate (Biomin F) (600 ppm) (BioMin Technologies Limited, London, England).

Demineralizing Solution

Pepsi® Soft drink with 1.28 pH (Carbonated water, 42.5 g/35 cl sucrose, phosphoric acid [7].

Study Design

Enamel surface microhardness was evaluated before and after treatment of extracted sound human molars with different toothpaste to compare its enamel remineralizing effect. Seventy-five extracted molars were used in this in vitro study. Molars were randomly assigned to one of five groups of 15 teeth each, four experimental groups according to the remineralizing agent used, and one control group with no treatment (Negative Control Group). Group A (n=15): teeth were treated with Hydroxyapatite containing toothpaste (Karex); Group B (n=15): teeth were treated with Hydroxyapatite and Fluoride containing toothpaste (Apacare); Group C (n=15): teeth were treated with ChloroCalcium Phosphosilicate containing toothpaste (Biomin C); Group D (n=15): teeth were treated with FluoroCalcium Phosphosilicate containing toothpaste (Biomin F); negative control group (n=15): teeth not subjected to any treatment. All teeth (experimental and negative control groups) were subjected to dynamic pH cycling to simulate the demineralization-remineralization cycle that happens in the oral cavity. 10 teeth of each group were subjected to microhardness assessment at baseline and after the two weeks of the dynamic pH cycling, while 5 teeth of each group were subjected to energy dispersive X-ray analysis (EDX Analysis) at baseline and after the two weeks.

Sample Preparation

Seventy-five sound extracted third molars for orthodontic and surgical reasons were used in this study. Teeth were collected from patients (ranged from 18 to 25 years old) visited the Surgery Clinic, Faculty of Dental Medicine, Al Ahram Canadian University. Teeth with restorations, enamel cracks, caries, erosion, developmental defects, or white spot lesions were not included[8]. Disinfection of the selected molars was done using a solution of 5.25% sodium hypochlorite solution for 1 hour. Decoronation of all selected molars was carried out by sectioning the roots 2 mm cervical to the cementoenamel junction using a water-cooled diamond saw (Isomet® 5000 Linear Precision Saw; Buehler Ltd., Lake Bluff, USA). All the crowns were embedded vertically in an auto-polymerizing acrylic resin block (3.0 cm × 3.0 cm × 3.0 cm). The crowns were scraped with a hand scaler and washed under running tap water to remove any residual tissues and debris; then polished with fluoride free pumice paste[10]. An acid-resistant varnish coating was applied on all teeth surfaces except a window of 2 mm X 2 mm on the middle third of the buccal surfaces[11].

Using a water-cooled diamond saw each tooth was sectioned into 2 halves mesiodistally. Custom-made plastic molds were prepared with the dimension of 3 mm height and 20 mm diameter poured with cold cure acrylic resin (Acrostone dental factory, Egypt). The buccal half [12]of each tooth was fixed using superglue on the custom-made acrylic resin block; so that the buccal surfaces were available for treatment to be treated. For easy identification, each acrylic disc with the glued sample was numerically coded at its base using a waterproof permanent marker. Each group of samples was put in a separate glass container containing 10 ml of artificial saliva at 37 °C in the incubator.

In the laboratory artificial saliva was made by dissolving [0.4gm sodium chloride (NaCl), 1.21 gm potassium chloride (KCl), 0.78 gm sodium dihydrogen dehydrate ($NaH_2PO_4.2H_2O$), 0.005 gm hydrated sodium sulfide ($Na_2S.9H_2O$), 1gm urea $CO(NH_2)_2$] in 1000 ml of deionized water. This mixture was treated by 10N sodium hydroxide until the pH value was measured using a pH meter to be 6.75±0.15[13].

Toothpaste Application

The samples in the experimental groups (A, B, C, D) were treated with the corresponding tooth-paste: Hydroxyapatite without Fluoride (karex); Hydroxyapatite and Fluoride (Apacare); Chloro-Calcium Phosphosilicate (Biomin C); Fluorocal-cium phosphosilicate (Biomin F) twice daily for 2 weeks. For 2 minutes the toothpaste was rubbed in a circular motion on the window of 2 mm X 2 mm on the middle third of the buccal surface using a micro brush for 2 minutes. Then the paste was kept on the surface for 30 seconds. Finally, the samples were rinsed carefully under running tap water to remove any excess paste. After drying the samples with clean absorbent, each group was returned to its container containing artificial saliva (10ml) in the incubator.

Dynamic pH-Cycling

All samples (experimental groups as well as the negative control group) were subjected to the pH-cycling regimen (dynamic model) for 2 weeks to simulate the drop of pH that occurs in the oral cavity every day. A completed two cycles were done in 24 hours. Each group was immersed separately in 10 ml artificial saliva for 11.5 hours before being removed and immersed in 10 ml of the demineralizing solution for 30 minutes[14]. The demineralizing solution used in this study was Pepsi®[15]. After 30 min., the specimens were removed from Pepsi® then each group was washed separately in 10 ml distilled water for 5 minutes. The negative control group was returned to the artificial saliva immediately with no treatment. The remineralizing toothpaste was applied on the buccal surfaces of the experimental samples (Group A, B, C, D) as previously described then the samples were returned to the artificial saliva (10 ml). During the two weeks of pH-cycling the artificial saliva was changed every 24 hours.

Surface Microhardness Assessment (SMH)

The surface microhardness of sound untreated enamel was measured at baseline and after 2 weeks of the dynamic pH-cycling. The surface microhardness of the enamel specimens was measured with Digital Display Vickers Microhardness Tester equipped with a Vickers diamond indenter and a 20X objective lens. For 20 seconds a load of 300g was applied to the surface of the specimens. Three indentations were evenly placed on the surface of each specimen and not closer than 0.5 mm to the adjacent indentations. The diagonals lengths of the indentations were measured by a built-in scaled microscope and Vickers values were converted into microhardness values.

Microhardness calculation

The following equation was accustomed to calculate microhardness:

HV=1.854 P/d2

Where HV refers to Vickers hardness in Kgf/mm2, P refers to the load in Kgf and d refers to the length of the diagonals in mm[16].

Energy Dispersive X-Ray Analysis (SEM-EDX Analysis)

Elemental analysis was done at baseline and after 2 weeks of pH cycling[17] to observe and compare the structural analysis before and after treatment with different toothpaste.

Speciemen Preparation for SEM- EDX

The enamel specimens were sputtered with a thin film of gold foil. The specimens were then assessed for mineral contents (mass/atomic percentage) using Energy-dispersive X-ray analysis (EDX). The digital outputs of the EDX values were numerically interpreted at baseline and after 2 weeks of pH-cycling.

Statistical Analysis

The data was presented as mean, standard deviation (SD) values. Data were explored for normality using the Kolmogorov-Smirnov test of normality. The results of the Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data), therefore, independent one-way analysis of variance, followed by Tukey's post hoc test was used to compare between groups.

Percent change was calculated by the formula:

$$\frac{\text{Value after-value before}}{\text{Value before}} \quad \text{X } 100$$

The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

RESULTS

Surface Microhardness Assessment (SMH) Results:

SMH assessment results of all experimental and control groups at baseline and after treatment with the experimental toothpaste are shown in (Table 1 and Fig.1). When comparing the percent change of microhardness of enamel surfaces before and after dynamic pH-cycle and remineralization, the highest mean percent increase was recorded in group D (15.07), followed by group B (14.92), then group C (10.56) and group A (10.38), with a percent decrease in the control group (-15.7). ANOVA test revealed that there was a statistically significant difference between groups was statistically significant (p=0.00). Tukey's post hoc test revealed that group D (Biomin F), group B (Apacare) were not significantly different. Moreover, group C (Biomin C) and group A (Karex) were not significantly different (Table 1, Fig. 2).

SEM-EDX Analysis Results:

SEM Analysis:

The SEM photographs of the enamel surfaces are shown in Fig. 3,4,5,6 and 7. The surface of enamel in the control group, is smooth before demineralization with some pits and scratches as shown in Fig.3 (a). When the acid erodes the enamel specimen, many micro-pores appear on the surface, as illustrated in Fig.3 (b).

The enamel surfaces of all experimental groups before pH-cycling and remineralization were smooth with some pits and scratches as shown in Fig. 4(a), 5(a), 6(a), and 7(a)

After using Biomin F toothpaste (Group D), well-organized enamel rods; with thickening of interprismatic substance were observed denoting highly mineralized enamel surface as shown in Fig.4 (b). Group B (Apacare) had some rod cores partially occluded with clearly thickened interprismatic substance (figure 5(b)). Group C (Biomin C) showed prismatic enamel configurations that had been hidden by mineral depositions with some empty rods as shown in figure 6(b). Group A (Karex) had a relatively smooth surface with less clearly seen enamel rod ends. Some rods revealed complete remineralization while most of them were still empty (Figure 7(b)).

Table (1) *Descriptive statistics and comparison between enamel surface microhardness before and after treatment with different remineralizing toothpaste after dynamic pH-cycling; and percent change after treatment with toothpaste. (ANOVA test)*

		Mean	Std. Dev	Std. Error	95% Confidence Interval for Mean		Min.	Max	F value	P value
					Lower Bound	Upper Bound				
Baseline	Group A	300.43[a]	1.48	.47	299.4	301.49	298.8	302.1		
	Group B	283.61[a,b]	30.43	9.62	261.8	305.38	253.7	313.0		
	Group C	291.54[a,b]	28.52	9.02	271.1	311.95	263.8	318.8	12.17	0.00*
	Group D	244.39[c]	9.33	2.95	237.7	251.06	235.4	253.4		
	Control	274.83[b]	8.66	2.74	268.6	281.03	269.0	290.1		
After Remineralization	Group A	331.63[a]	1.12	.35	330.8	332.43	330.5	332.8	54.88	0.00*
	Group B	325.02[a]	25.45	8.05	306.8	343.22	299.6	349.2		
	Group C	322.08[a]	28.81	9.11	301.5	342.69	294.5	349. 7		
	Group D	281.23[b]	11.25	3.56	273.2	289.28	270.4	292.1		
	Control	231.59[c]	4.33	1.37	228.5	234.68	227.3	236.8		
Percent change after treatment with toothpaste	Group A	10.38[b]	.18	.06	10.25	10.52	10.17	10.67		
	Group B	14.92[a]	3.38	1.07	12.51	17.34	11.52	18.88		
	Group C	10.56[b]	.94	.30	9.88	11.23	9.63	11.62	576.9	0.00*
	Group D	15.07[a]	.21	.07	14.92	15.22	14.87	15.28		
	Control	-15.70[c]	1.45	.46	-16.73	-14.67	-18.38	-14.62		

*Significance level p≤0.05, *significant*

Tukey's post hoc test means sharing the same superscript letter are not significantly different.

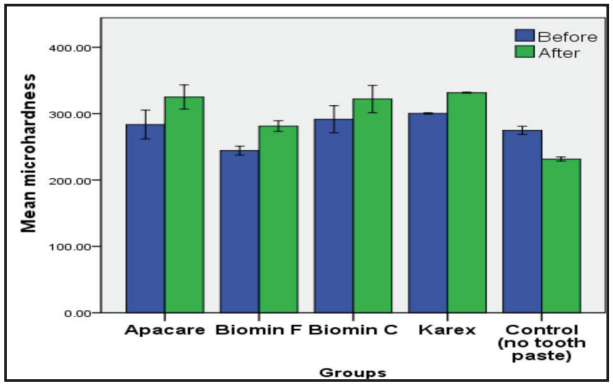

Figure (1) Bar chart illustrating mean enamel microhardness before and after treatment with each toothpaste.

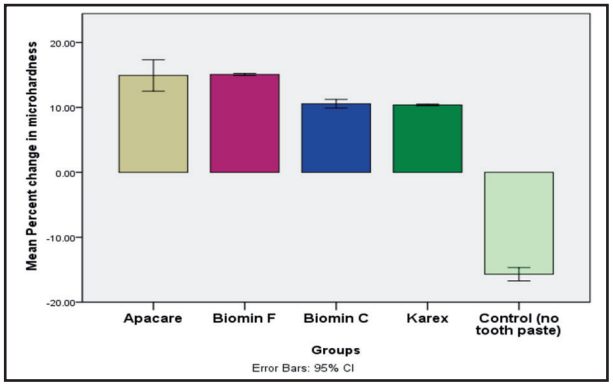

Figure (2) Bar chart illustrating percent change of enamel microhardness after treatment with each toothpaste, showing percent increase of all experimental groups while the control group recorded percent decrease.

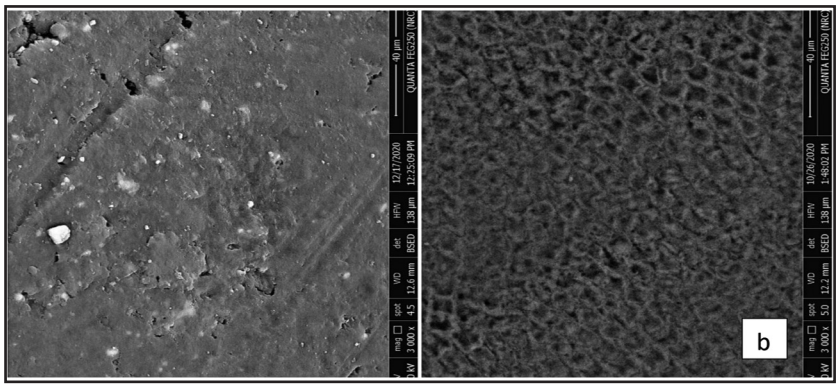

Figure (3) The SEM photographs of the enamel surface of the control group under the different conditions: (a) before demineralization, (b) after demineralization (both under 3000x magnification)

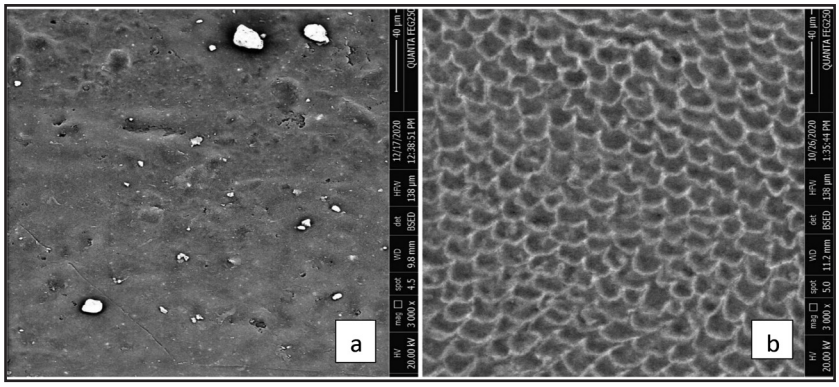

Figure (4) The SEM photographs of the enamel surface of group D under the different conditions: (a) normal state, (b) after treating with Biomin F toothpaste (both under 3000x magnification)

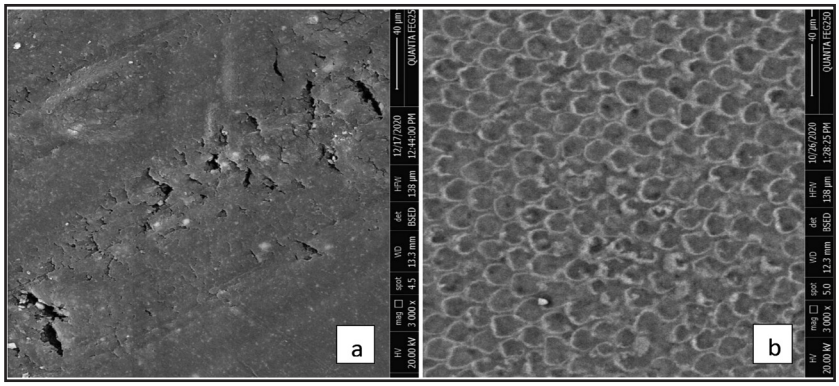

Figure (5) The SEM photographs of the enamel surface of group B under the different conditions: (a) normal state, (b) after treating with Apacare toothpaste (both under 3000x magnification)

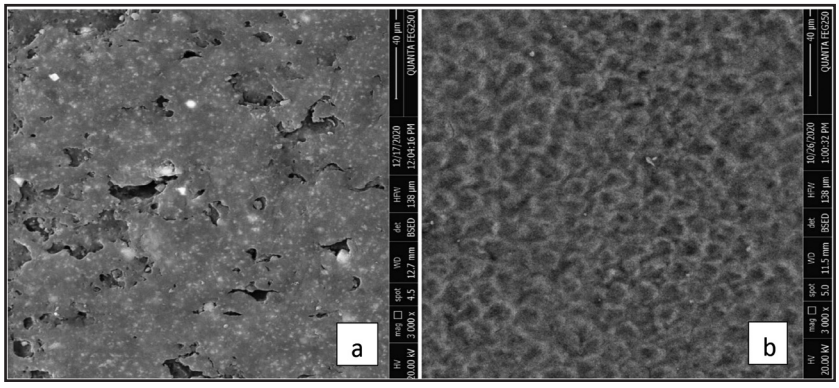

Figure (6) The SEM photographs of the enamel surface of group C under the different conditions: (a) normal state, (b) after treating with Biomin C toothpaste (both under 3000x magnification)

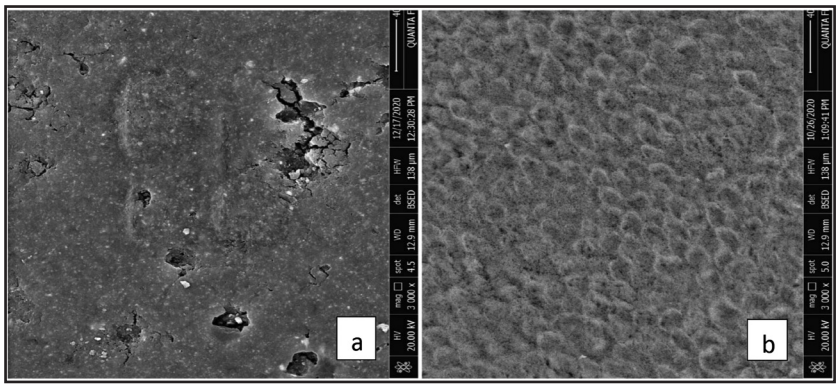

Figure (7) The SEM photographs of the enamel surface of group A under the different conditions: (a) normal state, (b) after treating with Karex toothpaste (both under 3000x magnification)

EDX Analysis:

Elemental analysis of all enamel specimens before pH-cycling and remineralization is shown in table 2 and figures (8-9). Carbon (C) ions recorded a significantly higher value in the negative control (p=0.00). All experimental groups showed a percent decrease, which was significantly different from the percent increase noted in the negative control (p=0.00). Fluoride (F) ions recorded a significantly higher percent increase in group D (Biomin F), with a significantly greater decrease in all other groups (p=0.00). Phosphorous (P) ions recorded a significantly higher percent increase in group C (Biomin C), followed by group A (Karex) and group B (Apacare), with a significantly greater decrease in the negative control (p=0.00). Calcium (Ca) ions recorded **a** significantly higher percent increase in group D (Biomin F), with a significantly greater decrease in the negative control (p=0.00).

Table (2) *Descriptive statistics and comparison between elemental analysis of enamel before and after treatment with different remineralizing toothpaste after dynamic pH-cycling (ANOVA test)*

		Group A	Group B	Group C	Group D	Negative control	F value	P value
C	Before	47.94^{a} ±6.3	46.77^{a}±5.5	48.78^{a} ±4.6	48.11^{a} ±8.3	3.86^{b}±0.6	120.9	0.00*
	After	5.55^{b}±0.14	3.49^{c}±0.4	5.24^{b}±0.1	4.01^{c}±0.6	49.57^{a}±6.2	128.6	0.00*
	% b change	-88.42^{b}±7.8	-92.54^{b}±8.65	-89.26^{b}±8.4	-91.66^{b}±7.6	1184.2^{a}±97.5	1115.2	0.00*
F	Before	3.19^{a,b}±0.82	3.35^{b}±0.8	2.56^{b}±0.7	1.36^{c}±0.3	4.26^{a}±0.94	20.77	0.00*
	After	1.60^{b}±0.36	1.32^{b}±0.3	1.75^{b}±0.31	6.05^{a}±1.5	1.70^{b}±0.30	73.67	0.00*
	% change	-49.84^{c}±11.76	-60.6^{b}±16.2	-31.64^{c}±7.9	344.85^{a}±50.2	-60.09^{b}±17.6	477.72	0.00*
P	Before	1.81^{b}±0.24	2.01^{b}±0.4	1.45^{b}±0.18	3.14^{b}±0.21	16.89^{a}±2.9	13.24	0.00*
	After	16.67^{a}±3.8	18.51^{a}±3.5	16.8^{a}±3.9	17.58^{a}±4.2	7.51^{b}±1.3	17.72	0.00*
	% change	820.99^{a}±102.5	820.9^{a}±98.2	1058.62^{a} ±127.6	459.87^{b}±43.9	-55.54^{c}±8.76	247.5	0.00*
Ca	Before	1.54^{b}±0.29	1.72^{b} ±0.4	1.50^{b}±0.41	0.92^{b} ±0.32	32.47^{a}±5.64	303.5	0.00*
	After	35.37^{a} ±6.9	41.76^{a}±8.92	33.94^{a} ±7.03	37.19^{a}±6.75	5.09^{b}±1.1	46.85	0.00*
	% change	2196.75^{b}±103.2	2327.91^{b}±167.3	2162.67^{b}±110.6	3942.39^{a}±207.6	-84.32^{c}±8.5	1096.5	0.00*

*Significance level p≤0.05, *significant, ns=non-significant*

Tukey's post hoc test: within the same comparison (each row): means sharing the same superscript letter are not significantly different.

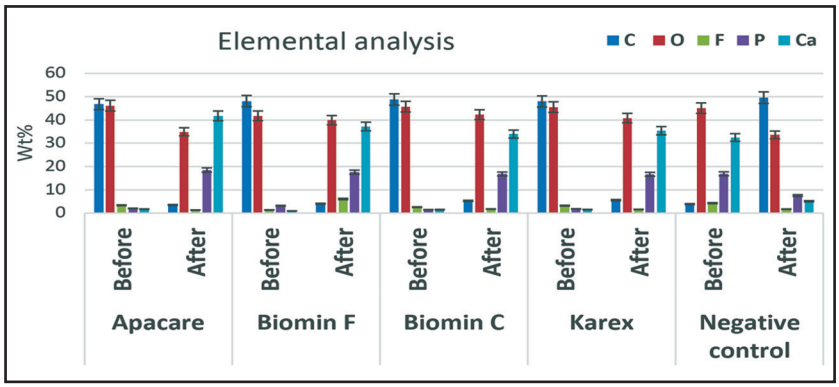

Figure (8) Bar chart illustrating the mean value of elemental analysis before and after toothpaste application and pH-cycling.

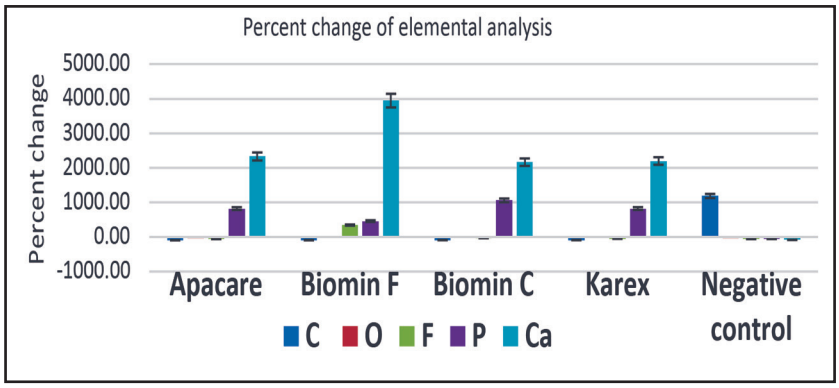

Figure (9) Bar chart illustrating percent change of elemental analysis before and after toothpaste application and pH-cycling.

DISCUSSION

The minimally invasive dentistry (MID) concept dictates reducing cariogenic bacteria count, decreasing demineralization together with effective remineralization of early enamel caries lesions[1]. Toothpaste containing remineralizing agents is one of the caries preventive methods that ensure to some extent effective remineralization with subsequent caries arrest. Many toothpastes of different active ingredients are available in the market nowadays. Toothpaste containing fluoride are the most widely used at home and were proven to reduce dental caries prevalence. Fluoride not only prevents demineralization but also aids in the remineralization of the enamel surface, resulting in the formation of fluorapatite layer[18]. Although fluoride is the cornerstone of non-invasive caries management, its use primarily results in surface-only remineralization without mineralizing deeper lesions. A remineralization system should ideally provide stabilized bioavailable calcium, phosphate, and fluoride ions that promote subsurface mineral gain instead of surface mineral deposition[19].

Therefore, many emerging non-fluoride remineralizing materials have been incorporated into toothpaste such as bioactive glass, HAP. Because of its exceptional mineral composition, which is very close to that of bone and teeth, Bioactive Glass (BAG) is biocompatible and has been used as a remineralizing agent over the last decade [20].

The objective of this study was to evaluate and compare the enamel remineralizing potential of different commercially available toothpaste containing various active ingredients using surface microhardness assessment. The microhardness of enamel surface was evaluated before and after treatment of extracted sound human molars with toothpaste containing Hydroxyapatite (Karex), toothpaste containing Hydroxyapatite, and Sodium fluoride (Apacare), toothpaste containing Chlorocalcium

phosphosilicate (Biomin C), and toothpaste containing Fluorocalcium phosphosilicate (Biomin F). All enamel specimens (experimental and negative control groups) were subjected to dynamic pH-cycling. The demineralizing solution used in this study was Pepsi® to simulate the pH drop that happens in the oral cavity after soft drink intake; as it can cause enamel dissolution and demineralization[15]. To simulate the dynamic process of demineralization and remineralization that occurs in the oral cavity, the dynamic pH-cycling model was used[17]. Artificial saliva was used as a storage medium for all experimental and control teeth to simulate the remineralizing capacity of human saliva[21].

The purpose of this study was to evaluate the effects of different commercially available toothpaste on enamel remineralization using the Vickers Microhardness test and SEM-EDX analysis[22].

The objective of baseline SMH determination is to compare and calculate the percent changes that occur before and after pH-cycling[23]. Microhardness results showed a clear difference between baseline (sound teeth) and remineralization values. The microhardness values for the control groups were decreased after the pH-cycling and stored in artificial saliva, this confirms the demineralizing and erosive effects of the acidic soft drink Pepsi®[15]. At the end of the study, all experimental groups treated with the tested toothpaste reported a significant increase in microhardness values when compared to the control group that didn't receive any treatment. Microhardness results were confirmed by the EDX analysis results which revealed that toothpaste treated groups had a significantly greater increase in mineral content than that of the control group, indicating that the products have a remineralizing capability.

The highest mean percent increase was recorded in Biomin F group (fluorocalcium phosphosilicate), followed by Apacare group (HAP+NaF) then Biomin C group (Chlorocalcium phosphosilicate), and Karex group (HAP), while control group recorded a percent decrease. The results were consistent with previous studies that reported that the components within the 5% fluorocalcium phosphosilicate dentifrice slowly dissolved to release calcium, phosphate, and fluoride ions which increased their bioavailability. FAP is formed when these ions precipitate and crystallize(24). The significant increase of enamel microhardness in Biomin F (fluorocalcium phosphosilicate) is explained and confirmed by the significantly high value of Fluoride ions recorded by EDX analysis results. Biomin F can supply a low level of fluoride for up to 12 hours after application. The slow and controlled supply of fluoride is far more efficient in its use of fluoride. Demineralized crystals serve as nuclei for the accumulation of new minerals and the result is a fluoride-rich, carbonate-poor, acid-resistant surface mineral layer, the FAP(25).

Biomin F group (fluorocalcium phosphosilicate) also showed an increase in Ca and P percentage. According to Mony *et al*(25) and Eggerath(26), this increase in Ca and P ions could be due to the presence of fluoride ions in the remineralizing agents used. This result is inconsistent with Gjorgievska *et al*.(27) who conducted that the addition of fluoride had no synergistic effect on remineralization. It was reported that when FBAG was used, HAP was formed after 24h. the HAP formation was reinforced by the F ions. FBAG releases more ions in an acidic environment and forms HAP(28).

EDX analysis results showed a significant increase of Ca and P ions concentration in Apacare group (HAP+NaF); when compared to Karex group (which is based on HAP only), resulting in repair of the demineralized enamel surface. This is explained by Heravi *et al*.(29) who suggested that the presence of hydroxyapatite particles with fluoride lead to an increase in the concentration of Ca and P ions. Despite it was previously suggested that hydroxyapatite causes more efficient delivery of calcium and phosphate ions (29), this study results found that the higher minerals levels were recorded for the hydroxyapatite free Biomin F group.

Both Biomin C (Chlorocalcium phosphosilicate) and Karex (HAP only) do not contain fluoride in their compositions, this explains the significant decrease of F ions in enamel surfaces reported by the EDX analysis. The EDX analysis results revealed a significant higher value of Carbon (C) ions in the control group when compared to the experimental groups. This remarkable increase could be the major cause of disturbances to enamel HAP crystals. The carbon ions could substitute the phosphate ions or hydroxyl groups leading to increase apatite solubility causing enamel demineralization(30).

The SEM images of enamel surfaces before and after treatment with the experimental toothpaste confirmed the dynamic remineralization process with mineral deposition and with decreasing pore volume. This is further supported by the significant increase in fluoride, calcium, and phosphorus levels reported by EDX analysis.

CONCLUSION

All the experimental toothpaste had the potential to remineralize enamel surface subjected to dynamic pH-cycling, but the incorporation of fluoride with the bioactive glass technology as in Biomin F toothpaste had the maximum effect on the demineralized enamel surface. Adding HAP and fluoride in one toothpaste as in Apacare toothpaste was better than the effect of toothpaste based on HAP only as in Karex toothpaste. Bioactive glass technology as in Biomin C toothpaste showed higher remineralization potential than Karex toothpaste. All toothpaste supporting the noninvasive means of managing early enamel carious lesions.

CONFLICT OF INTEREST

None declared.

FUNDING

No funding was received for this study.

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