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Assessment of Remineralizing Effect of Bioactive Glass Based Toothpastes: an In-Vitro Comparative Study

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ABSTRACT

Purpose: The purpose of the study was to assess the enamel remineralization potential of two bioactive glass- based toothpastes: Biomin® based toothpaste in comparison with Novamin® based toothpaste with sodium fluoride. **Material and methods:** Forty premolars were selected and divided equally into two groups named Novamin and Biomin groups after discarding their roots. First, the samples were soaked in a demineralizing solution for three days to get white spot lesion. Then, after rinsing them with deionized water, toothpastes were applied in the form of slurry twice daily, for two minutes each. The remineralization process lasted for two weeks. Environmental Scanning Electron Microscope (ESEM) was used to assess the remineralization degree. It was attached to Energy-dispersive X-ray spectroscopy (EDX) unit to measure the Ca, P weight percent and also the Ca: P for each sample. Three EDX readings were taken for each sample; baseline, after demineralization and after remineralization. **Results:** The results showed that both toothpastes have remineralization potential without statistically significant difference between them except in phosphate weight percent which was higher in the Biomin group. **Conclusions:** Within the limitations of the present study we concluded that the mineral content of the demineralized enamel significantly increase with using both Biomin and Novamin containing toothpastes twice daily for two weeks. Biomin may be more effective than Novamin in remineralization of demineralized enamel.

KEYWORDS

Bioactive glass, Biomin, Demineralized enamel, Novamin, White spot lesion.

INTRODUCTION

Dental caries is considered one of the most ancient, and the most frequent lesions occurred in humans. It comes in the second place among the most common causes of tooth loss, after periodontal disease.

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Recently, there is a growing attention to the medical model of disease management, newer strategies highlight disease prevention and preservation of tooth structure, instead of the surgical model, which focused attention on restorative treatment. Fluoridated water supplies and habitual factors, such as diet, surely effect caries process. If people can effectively fight caries with daily brushing, the need for restorative treatment can be averted [(1)].

The everyday use of fluoride containing toothpaste is yet recognized as the most efficient and simplest method of caries prevention and tooth remineralization for large populations. However, in order to form fluorapatite $[Ca_{10} (PO_4)_6 (F)_2]$ a high concentration of bioavailable calcium and phosphate ions in relation to the fluoride ions are required [(2)]. Bioavailable calcium and phosphate ions can be supplied by saliva, but even in healthy individuals, style of living, nutrition and other considerations can affect 9bioavailability of salivary calcium [(3)].

The invention of bioactive glasses (Bioglass®) by Dr. Larry Hench in the late 1960s caused a revolution in regenerative medicine. It boosts the formation of new bone by enhancing the body's own mechanism. This provided a whole new approach to biomaterials. Bioactive glass can provide the oral environment with the fundamental elements (calcium and phosphate) to remineralize teeth. This could be useful for many individuals with reduced calcium, phosphate and fluoride ions caused by reduced salivary flow due to old age, some medical drugs, Sjögren's Syndrome, diabetes and radiation therapy. Also, females have high caries risk due to insufficient salivary calcium levels at different periods in their lives including ovulation, gestation and post-menopause, leading to the same final outcome as decreased saliva fluoride efficacy [(4)].

Toothpastes that effectively produce ideal remineralization are being researched globally, as they can minimize the need for unwanted removal of healthy tooth structures, which occurs during routine restorative dental treatments. Therefore, this study was performed to evaluate the enamel remineralization ability of two bioactive glass-based toothpastes: the first one is Biomin®-based toothpaste and the second one is Novamin® based toothpaste with sodium fluoride.

MATERIALS AND METHODS

The materials used, their specifications, compositions and manufacturers are present in (Table 1) and the laboratory prepared solutions are present in (Table 2).

1. Collection of teeth:

Forty human premolars freshly extracted for orthodontic purposes were selected for this study. Teeth were rinsed thoroughly with running water and cleaned with soft brush after extraction to remove the soft debris from their surfaces. Teeth then were stored in 0.1% thymol [(5)].

2. Grouping of the teeth:

The teeth were equally divided randomly into 2 groups as follows:

Group I: 20 teeth were treated with Novamin toothpaste (Sensodyne Repair and protect, GlaxoSmith-Kline (GSK) Company).

Group Π: 20 teeth were treated with Biomin toothpaste (BioMinF, BioMin Technologies limited).

3. Preparation of teeth:

The teeth were cut 1 mm below the cemento-enamel junction using the low-speed diamond disk to discard the root part. Then the samples had been rinsed with deionized water [(6)]. All crown surfaces were covered with acid resistant nail polish (LUNA) leaving the 3 mm × 3 mm of window on buccal surface [(7)]. Baseline assessment had been done using Environmental Scanning Electron Microscope (ESEM) and Energy-dispersive X-ray spectroscopy (EDX).

4. Demineralization:

Each sample had been immersed in the demineralizing solution for 72 hours, until white spot lesions were obtained. Then the samples had been taken away from the solution and rinsed with deionized water to stop the demineralization process and to remove any residuals of the solution. ESEM assessment and EDX analysis were done again.

5. Remineralization:

Toothpaste slurries (the remineralizing solution) were prepared by mixing toothpaste and artificial saliva (ratio 1:3 w/v). This ratio is in conformity with the European standards for preparing artificial saliva/toothpaste slurries (EN ISO 11609) [9, 10]. Each sample was soaked in the remineralizing solution for 2 minutes [11], then, the samples were rinsed with the deionized water and stored separately in the artificial saliva [12]. This step was repeated twice daily (every 12 hours) for 2 weeks, during this period the artificial saliva were replenished every 48 hours [8]. The ESEM assessment and EDX analysis were done again.

The amount of remineralization was measured qualitatively by comparing the scanning electron microscope pictures and the Energy dispersive X-ray analysis values at different stages of study (before demineralization, after demineralization and after remineralization). The measurement were done at Egyptian Mineral Resources Authority (Central Laboratories) using SEM Model Quanta 250 FEG (Field Emission Gun) attached with EDX Unit, with accelerating voltage 30 K.V., magnification 14x up to 100000 and resolution for Gun. 1n (FEI company, Netherlands).

Table (1): *The materials used, their specifications, compositions and manufacturers.*

Material	Specifications	Composition	Manufacturer	Batch no.
Sensodyne Repair and Protect Powered by Novamin toothpaste	Remineralizing agent.	• Calcium Sodium Phosphosilicate (Novamin) • Sodium fluoride (1450ppm Fluoride) • Glycerin • PEG-8 • Hydrated Silica • Cocamidopropyl Betaine. • Sodium Methyl Cocoyl Taurate • Aroma • Titanium Dioxide • Carbomer • Sodium Saccharin	GlaxoSmith-Kline (GSK) Company.	B8002703
BioMinF Armour for teeth toothpaste	Remineralizing agent	• Fluoro Calcium-PhosphoSilicate (Biomin) • Glycerin • Silica • PEG 400 • Sodium-Lauryl Sulphate • Titanium Dioxide • Aroma • Carbomer • Potassium Acesulfame • Contains maximum 530ppm of available fluoride when packed.	BioMin Technologies limited.	PMCB0022

Table (2): *The laboratory prepared solutions.*

Solution	Composition	Used for	PH
Artificial saliva	• 2.20 g/L gastric mucin • 1.45 mmol/L $CaCl_2$ $2H_2O$ • 5.42 mmol/L KH_2PO_4 • 6.50 mmol/L NaCl • 14.94 mmol/L KCl. PH was adjusted to 7.0 using KOH.	• Storage medium for the samples. • Toothpaste slurry formation.	7.0
Demineralizing solution	• 2.2 mM $CaCl_2$ • 2.2 mM NaH_2PO_4 • 0.05 M lactic acid • 0.2 ppm fluoride. The pH was adjusted to 4.5 with 50% NaOH.	Artificial sub-surface lesion formation.	5.0
Thymol 0.1%	Thymol powder, deionized water.	Preservative media for the teeth.	

RESULTS

1. Environmental scanning electron microscope examination (Fig. 1 & 2):

The base line:

The sound enamel showed the same characters and features which are smooth surface with relatively variable appearance such as aprismatic enamel, preikymata (perikymata grooves separated from each other by perikymata ridge), prism end markings, cracks, pits, elevations and pores.

After demineralization:

In both groups the demineralized pattern was the same. The demineralization significantly altered the prismatic structure of sound enamel. All samples showed alteration in the prismatic structure of sound enamel with varying degrees of areas of dissolution indicating demineralization, this demineralization represented by pores, which characterized by typical honey comb or fish scales appearance.

After remineralization:

Enamel surface treated with both toothpastes showed partial restoration of the surface structure of the enamel. It can be observed by considerable disappearance of dissolution areas with minimizing the porosity size indicating remineralization. In both groups, the enamel surface showed areas of uniform and smooth enamel surface and some prism cores had been completely obliterated and not remarkably visualized.

2. Energy- dispersive X-ray spectroscopy statistical analysis (Table 3):

Analysis of Variance (ANOVA) was used to study the effect of material, De/Re-mineralization process and their interaction on mean Ca, P weight% and Ca: P ratio. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

2.1. Calcium (Ca) weight %:

a. Comparison between materials:

Whether at base line, after de-mineralization or after re-mineralization; there was no statistically significant difference between mean Ca weight % of Novamin and Biomin.

b. Comparison between De/Re-mineralization processes:

Whether with Novamin or Biomin; there was a statistically significant decrease in mean Ca weight % after demineralization followed by a statistically significant increase after re-mineralization. However, the mean Ca weight % after re-mineralization showed statistically significantly lower mean value compared with baseline Ca weight %.

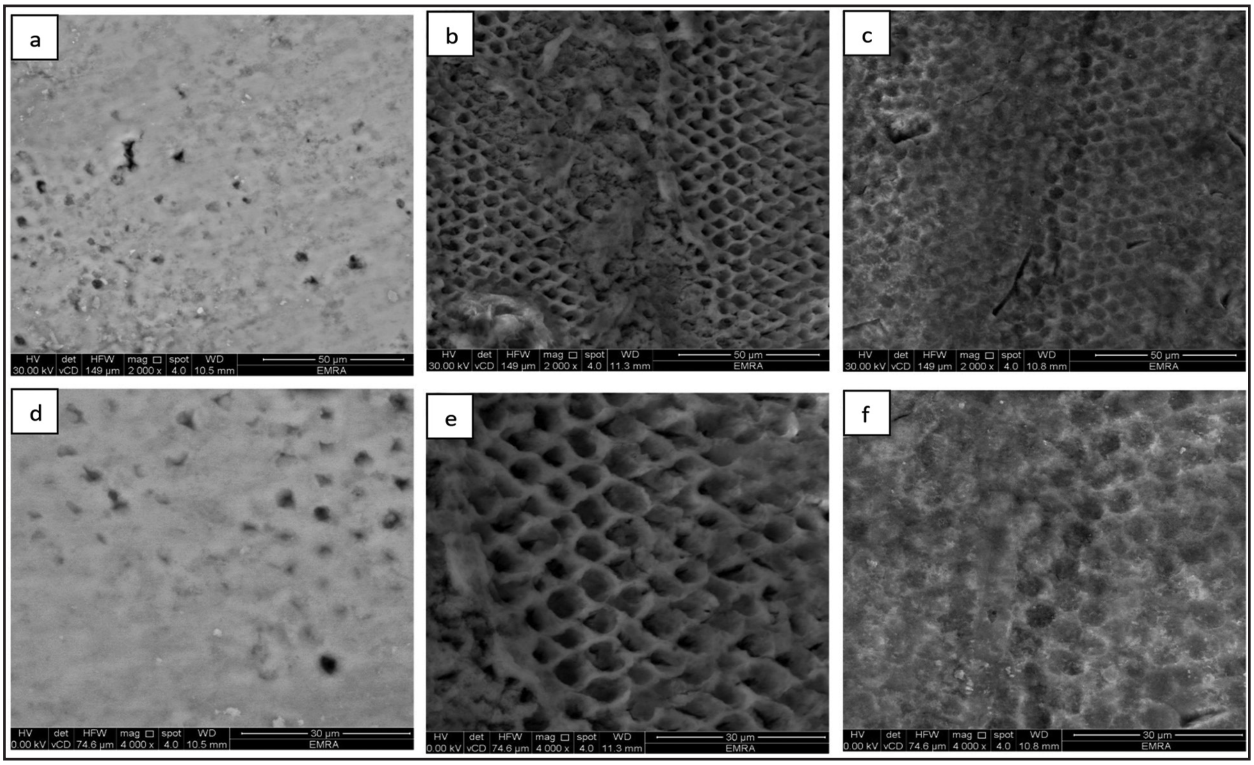

Figure (1): ESEM of the same enamel surface in 3 stages: at base line (a & d), after demineralization (b & e) & after remineralization with Novamin (c & f) showing considerable restoration of the enamel surface and closure of the pores. (Orig. Mag. 2000x and 4000x)

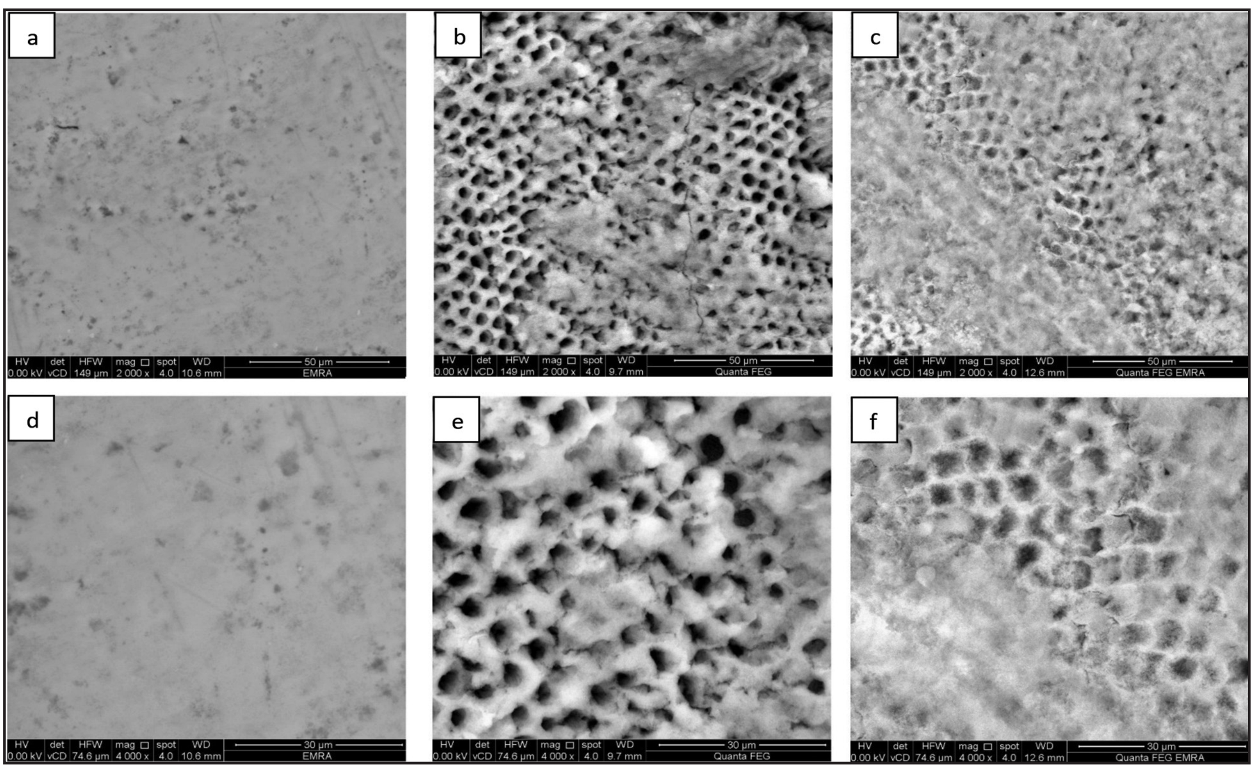

Figure (2): ESEM of the same enamel surface in 3 stages: at base line (a & d), after demineralization (b & e) & after remineralization with Biomin (c & f) showing considerable restoration of the enamel surface and closure of the pores. (Orig. Mag. 2000x and 4000x)

2.2. Phosphorus (P) weight %:

a. Comparison between materials:

Whether at base line, after de-mineralization or after re-mineralization; there was no statistically significant difference between mean P weight % of Novamin and Biomin.

b. Comparison between De/Re-mineralization processes:

With Novamin; there was a statistically significant decrease in mean P weight % after demineralization followed by a statistically significant increase after re-mineralization. However, the mean P weight % after re-mineralization showed statistically significantly lower mean value compared with baseline P weight %.

While with Biomin; there was a statistically significant decrease in mean P weight % after demineralization followed by a statistically significant increase after re-mineralization. The mean P weight % after re-mineralization showed non-statistically significant difference from baseline P weight %.

2.3. Calcium: Phosphorus ratio (Ca: P ratio):

a. Comparison between materials:

Whether at base line, after de-mineralization or after re-mineralization; there was no statistically significant difference between mean Ca:P ratio of Novamin and Biomin.

b. Comparison between De/Re-mineralization processes:

Whether with Novamin or Biomin; there was no statistically significant change in mean Ca: P ratio after de-mineralization/re-mineralization process.

Table (3) *The comparison between Ca weight %, P weight % and Ca:P ratio mean values with different interactions of variables*

De/Re-mineralization process	Novamin			Biomin		
	Mean of Ca weight %	Mean of P weight %	Mean of Ca:P ratio	Mean of Ca weight %	Mean of P weight %	Mean of Ca:P ratio
Base line	42	20.3	2.07	43.3	20.1	2.16
De-mineralization	36.8	18	2.07	37.8	18.1	2.11
Re-mineralization	40.5	19.4	2.09	40.9	19.7	2.08

DISCUSSION

By using scanning electron microscope, all sound specimens in both groups showed relatively intact smooth enamel surface with adequately variable appearance showing features like aprismatic enamel, perikymata, prism ends markings, cracks, pits, elevations and pores. This findings are almost constant and indisputable features of sound enamel which were in accordance with most of the studies [13,14]. The demineralized specimens in both groups showed a prismatic pattern of destruction with a honeycomb appearance. In some specimens, the dissolved prism ends gave the enamel surface an appearance of fish scales. These findings are obvious in most other studies of incipient caries [15,16].

Regarding the results of SEM after remineralization, both groups showed big difference from the demineralized images with obvious restoration of the demineralized enamel surface represented by areas of complete uniform and smooth enamel surface and some prism cores had been completely obliterated and not remarkably visualized. There was no clear difference between the two groups. This result was similar to earlier studies where the SEM images showed remineralization potential of the Novamin toothpaste [17,18]. The result was also close to a previous study was done on Biomin where it showed remineralization potential, but the SEM images showed formation of a newly formed layer having crystal like structures and better restoration of the enamel surface. That may be due to the longer duration of the Biomin application in that study as the paste was put on the white spot lesion for complete 24 hours [19].

As to Energy dispersive X-ray spectroscopy (EDX) result, it supported the SEM images and showed that the toothpastes used in this study have remineralizing potential. EDX test was used for surface mineral content analysis during different stages of testing (baseline, after demineralization and after remineralization). It was an important issue to measure the samples before demineralization to estimate the amount of mineral loss after demineralization and the amount of mineral restoration after remineralization in relation to the baseline mineral. The calcium and phosphate weight percent were measured in all samples. Fluoride peaks couldn't be detected at any sample, which may be ascribed to the low photon energy of the fluoride, which may make it hard to be detected by EDX.

The mean values of weight percentage of calcium and phosphate either before demineralization (baseline) or after demineralization showed no statistically significant difference between the two groups. Both groups showed significant decrease in mineral content after demineralization with approximated values. This finding was logical outcome because they all treated with the same demineralizing solution. After remineralization, both groups showed significant increase in their mineral content. These findings had been in accordance with several studies that used EDX test, in which application of Novamin significantly increase the Calcium content of the enamel layer [20,21]. They had also been similar to another study investigated the enamel remineralization potential of Novamin where they found that both calcium and phosphate content had significantly increased [22].

Most of the studies conducted on Biomin investigated its ability to treat hypersensitivity and to occlude the dentinal tubules. The studies investigated its enamel remineralization potential are very scarce. The results of the present study had been similar to a previous study investigated the remineralizaing effect of Biomin using EDX test where the calcium and phosphate contents in the group treated with Biomin showed significant increase comparing to the control group which had no treatment after demineralization [19]. The result had also been similar to another Biomin study which put Biomin toothpaste in comparison with other two toothpastes which are Medicinal Nanohydroxy apatite containing calcium hydrogen phosphate and Anticay 5% (calcium sucrose phosphate with inorganic amorphous calcium phosphate). In that previous study the Biomin group showed significant increase in calcium and phosphate content compared to the other two groups [23].

Unfortunately we couldn't find many studies comparing Novamin to Biomin toothpastes regarding to their enamel remineralization potential. Most of them compared the dentinal tubules occluding capacity where the Biomin showed higher efficiency. This may be due to its smaller particles than Novamin, which may help the bioglass better infiltration into dentinal tubules [24]. There is a study conducted to compare the effect of Novamin and Biomin in remineralizing the demineralized enamel using Micro-computed tomography, which was used to investigate mean enamel volume changes, and Microscopes profilometer, which was used to investigate mean surface loss or gain. Both toothpastes were applied to the demineralized enamel surface for five minutes and 24 hours. The results showed that there was no significant difference in the remineralization capacity between the two agents [(25)].

By comparing the mean calcium and phosphate weight percentage of the remineralized enamel to the baseline values, the calcium values after remineralization didn't reach the baseline in both groups. The phosphate values on the other hand were different. In the Novamin group, they didn't reach the base line, while in the Biomin group they did. That may be due to high phosphate content of Biomin, as the company claimed that it is three times that of Novamin. High phosphate content glasses form apatite considerably faster (within 6 h) than low phosphate content glasses. Also, an increase in phosphate content favors apatite formation instead of fluorite (CaF_2) as the release of phosphate would affect the supersaturation and thereby favour apatite deposition [(26)]. As we know the main inclusion that makes tooth mineral much less acid resistant than hydroxyapatite or fluorapatite is the carbonate ion that substitutes for the phosphate ion in the crystal lattice, creating defects. That is because the presence of carbon in apatite leads to lattice distortion which in turn causes crystalline defects and micro-stresses in the network. These stresses and defects have a critical effect on the apatite solubility [(27)]. So, the Biomin group which has higher phosphate weight percent may also has less carbon impurities, which makes it less acid soluble

However, one of this study limitations we must take into consideration that remineralization in vitro may be entirely different in comparison to dynamic complex biological system which is the case in oral environment. In addition the difference could be related to the substrate; in this study we used human teeth only, while there is other studies used bovine teeth. Artificial carious lesions formed in bovine tooth enamel are varied from the ones formed in human teeth. Also we used permanent teeth while other studies used primary teeth. We chose to use permanent premolar teeth because white spot lesions is more common in permanent teeth especially after orthodontic appliances. Also, the premolars are the most easily found sound teeth. Primary and permanent enamel substrates reacted differently to remineralization agents [(28)].

CONCLUSIONS

Within the limitations of the present study, we concluded that:

- The mineral content of the demineralized enamel significantly increase with using both Novamin and Biomin containing toothpastes twice daily for two weeks.
- Biomin may be more effective than Novamin in remineralization of demineralized enamel.
- The use of bioactive glass toothpastes as a remineralizing agents are promising in repairing early carious lesions.

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