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An In-Vitro Evaluation of Enamel Remineralization potential of BioMin toothpaste in primary teeth

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ABSTRACT

This study aimed to evaluate the remineralization potential BioMin-F compared to a commonly used standard toothpaste (Signal®) in primary teeth. Thirteen enamel blocks of primary canines were divided randomly into two groups: Group 1 (n = 15; Experimental), BioMin-F toothpaste group, and Group 2: (n = 15; Control), Signal toothpaste. The samples were initially evaluated for baseline surface microhardness; later on, these samples were placed in the demineralizing solution for 69 h, and post-demineralization surface microhardness was measured. Thereafter, the samples were stored in the toothpaste for 3 minutes twice daily for 15 days, and surface microhardness was recorded. The surface microhardness was evaluated using a Vickers microhardness tester. Statistical analysis was done using dependent and independent sample tests. The results showed that there is a statistically significant difference in the mean enamel microhardness after remineralization (p=0.000) between the BioMin-F (353.62) and Signal (300.62) groups in favor of the BioMin-F group. The BioMin-F toothpaste showed promising potential to promote remineralization of demineralized primary human enamel.

1. Introduction

The remineralization potential of damaged tooth surfaces is appreciable, especially in children. As it is well documented that white spot lesions are the earliest macroscopic evidence of enamel caries, the enamel surface layer stays intact during subsurface demineralization, but without any intervention, it will eventually collapse into cavitation [1], [2]. Standard procedures for protection of these teeth are fissure sealing and topical fluoride application. So far, none of these procedures are completely efficient. Therefore, attempts have been made to find an effective anticariogenic and remineralizing agent to have ions directly delivered to when and where they are needed most. This leads to the development of aided remineralization [3].

Till date, the researches in the literature support that fluoride treatment remains the best remineralizing method for early enamel caries [4], [5]. However, it is difficult for fluoride to result in oriented and ordered mineral crystals on the surface of enamel under physiological conditions due to the lack of ability to guide the formation of mineral crystals. The ordered orientation is essential for the mechanical properties of

enamel. Thus, the aim of an ideal mineralizing agent should be to achieve the organization and microarchitecture of mineral crystal as close the natural ones as possible [6].

Bioactive glass (BG) is a biocompatible material, traditionally used because of its osteogenic properties [7], but its use in dentistry has been encouraged recently due to its compositional resemblance to bone and dental enamel [8]. It is composed of 45% silica, 26.9% calcium oxide, 24.4% sodium oxide, and 6% phosphorus pentoxide in weight percentage and lacks fluoride [9].

Since the introduction of BAG in the late 1960's by Prof. Larry Hench, such materials have been modified and various compositions have been synthesized [10]. In general, because of a controlled degradation over time, the ions released by such bioactive glasses can interact with the body tissues and offer various therapeutic effects such as repair and regeneration of bone and dental tissues [11]. In dentistry, they are currently being successfully used for enamel remineralization and dentin tubule occlusion [8].

BioMin contains calcium and phosphate but also has fluoride (600 ppm) within the glass rather than as soluble addition; this allows longer term delivery of fluoride [12]. The ration of these three ions reflects the molecular stoichiometry of Fluoroapatite and also the phosphate concentration is three times that of Nova Min. The particle size of BioMin is also smaller than NovaMin which allows greater physical occlusion of open dentin tubules [12].

With this background, this study was undertaken to evaluate the ability of topically applied BioMin-F in remineralization of enamel surface that has been exposed to an artificial caries challenge in a simulated oral environment.

2. Materials and Methods

2.1 Collection and preservation of teeth

A comparative in vitro study was proposed to evaluate the effectiveness of BioMin-F toothpaste on the surface microhardness of enamel carious-like lesions. Thirty caries-free primary canines extracted for orthodontic reasons without any visible caries, hypoplastic lesions, and white spots on any surface of the tooth were collected, cleaned of soft tissue debris, and stored in 0.5% chloramine T solution for one week. Then the teeth were transferred to other plastic containers containing distilled water.

Each of the studied teeth was given a number between 1 and 30. Afterward, the teeth were divided randomly through randomization.com into two groups: Group 1 (n=15): BioMin-F toothpaste, and Group 2 (n=15) Signal toothpaste. Single-blinded trials were adopted in this study so that the assessors would not know which toothpaste was used.

2.2 Specimen preparation and Enamel window formation

The roots of the teeth were cut using a water-cooled diamond bur. Then enamel blocks of 4mm (length) x 2mm (breadth) x 4mm (width) were cut from each tooth. All 30 enamel blocks were subjected to enamel window formation. This was done to limit the area of demineralization followed by remineralization only in the window area.

Each enamel block was mounted with an acrylic mold. Each enamel block was trimmed using graded-roughness sandpaper under water cooling, as a maximum of 50-100 microns were removed to obtain a flat surface (Figure 1-A). The mounted 30 enamel blocks were analyzed using a Vickers hardness testing

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machine to record the surface microhardness of the sound enamel surface.

The surface microhardness was carried out using a Galileo LTF-Isoscan CN03 microhardness tester with a Vickers hardness pyramidal indenter and an applied load of 300 g for 15 seconds (Figure 1-B). Microhardness was measured according to the following equation:

$$HV = \frac{1854 \times P}{D^2}$$

As: P (Applied load in grams), and D (The mean diameter of the footprint left by the pyramid in microns).

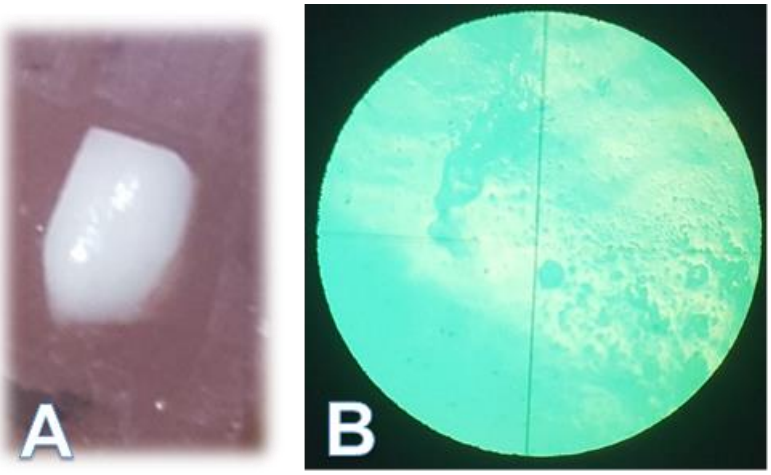

Figure 1. (A) The mounted enamel blocks, (B) Microhardness measurement

2.3 Demineralization Process

Each sample was then subjected to a demineralizing solution for a period of 69 hours. This was done to create artificial carious lesions of approximately 100–120 μm deep among the selected teeth. All samples were immersed in the first demineralization solution for an hour (the first solution was prepared using the following chemicals: 2mM calcium chloride, 2mM sodium phosphate and 0.05M acetic acid and 0.1 M calcium hydroxide to adjust the pH to 4.5), then each sample was washed with 5 ml of distilled water and immersed in the second demineralization solution (the second solution was prepared using following chemicals: 2mM calcium chloride, 2mM sodium phosphate and 0.1 M calcium hydroxide to adjust the pH to 6.8) for 22 hours to alternate remineralization and demineralization to simulate the changes occurring in the oral cavity.

This process was repeated three times until the initial caries lesion visible on the buccal surface was obtained. The enamel blocks were also again analyzed using the Vickers hardness testing machine to record the surface microhardness of the enamel surface

2.4 Treatment procedure:

The mounted enamel blocks were stored in distilled water. Teeth specimens were cleaned and dried and then subjected to the application of the two toothpastes. The toothpastes were applied for 3 minutes twice daily for 15 days using a toothbrush and then rinsed with water. The enamel blocks were also again analyzed using a Vickers hardness testing machine to record the surface microhardness of the enamel surface.

3. Results

The study sample consisted of 30 extracted primary canines, divided into two equal groups (50%). To study the differences in the effect of the toothpaste used on the mean of enamel microhardness between the studied groups (Signal/ BioMin) according to the studied period (Baseline, after demineralization and After toothpaste application), an independent sample t-test was applied (Table 1).

Table 1. Means and standard deviations of enamel microhardness in each studied group and independent sample t-test results (*Statistically differences)

Studied period	Studied groups	N	Mean	SD	Differences between Means	t-value	P-value
Baseline	BioMin toothpaste	15	405.27	45.458	2.14	1.126	0.334
	Signal toothpaste	15	403.13	36.353			
After demineralization	BioMin toothpaste	15	147.62	28.70	0.4	0.002	0.998
	Signal toothpaste	15	147.22	26.099			
After toothpaste application	BioMin toothpaste	15	353.62	42.886	53	20.747	**0.000**
	Signal toothpaste	15	300.62	46.935			

Table 1 shows that there is a statistically significant difference in the mean enamel microhardness after remineralization (p=0.000) between the BioMin and Signal groups in favor of the BioMin group. The mean enamel microhardness after remineralization using BioMin toothpaste /353.62/ was better than the mean enamel microhardness using Signal toothpaste /300.62/.

To study the differences in the effect of the toothpaste used on the mean of enamel microhardness during the studied periods (Baseline, after demineralization, and After toothpaste application), a dependent sample t-test was applied (Table 2).

Table 2. Means and standard deviations of enamel microhardness in each studied period and dependent sample t-test results (*Statistically differences)

Studied groups	Studied periods	Mean	SD	Pairwise comparison	Differences between Means	t-value	P-value
BioMin toothpaste	Baseline	405.27	45.458	Baseline - After demineralization	257.65	43.603	**0.000**
	After demineralization	147.62	28.70	Baseline - After toothpaste application	51.65	11.621	**0.000**
	After toothpaste application	353.62	42.886	After demineralization - After toothpaste application	-206	-35.069	**0.000**
Signal toothpaste	Baseline	403.13	36.353	Baseline - After demineralization	255.91	53.655	**0.000**
	After demineralization	147.22	26.099	Baseline - After toothpaste application	102.51	29.170	**0.000**
	After toothpaste application	300.62	46.935	After demineralization - After toothpaste application	- .1534	23	**0.000**

In the BioMin group: the mean of enamel microhardness in the baseline was/405.27/ greater than the after demineralization /147.62/ (p= 0.000), and the mean of enamel microhardness in the baseline was also greater than after toothpaste application /353.62/ (p= 0.000). Finally, the mean of enamel microhardness after toothpaste application was greater than the mean of enamel microhardness after demineralization (p= 0.000).

In the Signal group: the mean of enamel microhardness in the baseline was/403.13/ greater than the after demineralization /147.22/ (p= 0.000), and the mean of enamel microhardness in the baseline was also greater than after toothpaste application /300.62/ (p= 0.000). Finally, the mean of enamel microhardness after toothpaste application was greater than the mean of enamel microhardness after demineralization (p= 0.000).

4. Discussion

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Clinical evidence suggests that fluoride is effective in preventing caries onset and arresting or possibly reversing the process of demineralization. Although the demineralizing efficacy of fluoride is substantially justified, the material is not able to surpass the high caries challenge posed in few individuals, and this highlights the need to find newer method to enhance remineralization process. The key requisite in the remineralization of enamel and dentin is the availability of calcium in the oral environment. This has led to the development of various calcium-based systems that enhance the availability of calcium and phosphate [13].

The current in vitro study aimed to evaluate the ability of BioMin toothpastes to remineralize enamel of primary teeth compared to a commonly used fluoride-containing toothpaste such as Signal. BioMin contains calcium and phosphate but also has fluoride within the glass rather than as soluble addition; this allows longer term delivery of fluoride. It is considered as a breakthrough in remineralization because current systems are dependent on adequate saliva as a source of calcium and phosphate, but bioactive glasses are enriched with these ions [14].

The buccal surfaces of primary maxillary or mandibular canines were chosen because of their wide surface which allows doing mechanical tests better [15]. The roots of the teeth were scaled to remove attached soft tissue remnants, and the teeth were preserved in chloramine solution for a week for disinfection [16]. Teeth were then preserved with distilled water until a microhardness test was conducted to avoid drying out of its structure, which makes it brittle and breakable [17].

demineralization and remineralization solutions containing calcium and phosphate were used at intermittent intervals to stimulate the formation of initial caries lesions to simulate the clinical situation [18]. The three toothpastes were applied in the same quantity, time of application, and number of application times to standardize and control the procedures [15], [19].

Vickers method was chosen in the present study because the pyramid shaped indent obtained is accurate to measure and detect visually and digitally [20]. In this study, the null hypothesis was rejected, as the BioMin-F toothpastes has a better remineralization ability than the Signal toothpastes, this can be attributed to the BioMin-F particles adhering to the enamel surfaces and delivering essential remineralizing ions (calcium, phosphate, and fluoride) immediately [21].

The results of this study agreed with [22] study, which evaluated the microhardness of the demineralized enamel after applying the BioMin-F or fluoridated toothpaste. The statistical results indicated superior fracture resistance for the enamel group treated with BioMin-F toothpaste.

The results of the current study agreed with many similar in-vitro studies, as the enamel microhardness decreased after the demineralization process and increased after applying BioMin-F toothpaste [23], [24]. The results of the current study also agreed with [25] study, as the rate of enamel remineralization in samples treated with BioMin-F was higher compared to the control group.

There were a few limitations to this study. The sample size was small. The lesions were artificially induced and were not natural carious lesions. Similar studies can be conducted by storing the tooth samples in artificial saliva which would mimic the oral environment and improve the validity of the study.

5. Conclusion

Within the limitations of this in vitro study, it may be concluded that BioMin-F toothpaste showed a

substantial potential to promote remineralization of demineralized primary human enamel. Future in-vivo studies are recommended to assess its clinical effectiveness.

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