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REMINERALIZATION OF DECALCIFIED ENAMEL IN PRIMARY TEETH VIA A NEW BIOACTIVE GLASS PASTE: AN IN VITRO STUDY

This manuscript was presented as a poster at the Alexandria International Dental Congress 2024

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ABSTRACT

BACKGROUND: Fluoride toothpaste is widely used to prevent and limit caries. Exploring alternative formulations that offer additional benefits could be valuable. This study aimed to compare the effects of experimental bioactive glass (BioMin) with commercial fluoridated toothpaste on the sound primary teeth demineralized in-vitro.

METHODS: Sixty exfoliated anterior primary teeth were split into three equal groups All samples were exposed to a demineralization solution for a period of 96 hours., followed by separate remineralization for each group: Group I (artificial saliva), Group II (fluoridated toothpaste), and Group III (BioMin toothpaste) for 15 days. The outermost microhardness of each sample was evaluated via a Vickers microhardness apparatus, and the lesion depth was determined via a polarised light microscope and ImageJ 1.46r software. Data was evaluated using one-way ANOVA and Tukey's HSD Test (honestly significant difference)

RESULTS: compared with the controls ($P < 0.001$) and fluoridated toothpaste ($P < 0.05$), the Biomin toothpaste demonstrated significantly superior results in terms of microhardness and lesion depth. In contrast, the fluoridated toothpaste led to a statistically insignificant rise in microhardness and a reduction in lesion depth ($P = 0.52$ and 0.78 respectively). Qualitative assessment showed that both agents contributed to reducing lesion depth.

CONCLUSION: The new bioactive glass paste (BioMin® F) had a more significant effect than fluoridated toothpaste in treating artificial caries caused by enamel demineralization.

KEYWORDS: Artificial caries, Enamel demineralization, Fluoridated toothpaste, Bioactive glass (BioMin) toothpaste, Primary teeth

ABBREVIATIONS: F; fluoride, HSD; honestly significant difference, BAG: bioactive glass, SD:standard deviation: Vickers hardness number ,SEM: scan electron microscope, CPP-ACP: casein phosphopeptide amorphous calcium phosphate.

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BACKGROUND

Dental caries remains the most rampant long-lasting disease worldwide, affecting 60–90% of school-aged children and the overwhelming majority of adults. It is a disease caused by biofilms, influenced by nutrition, and is not transmissible. Its dynamic etiology involves a mineral loss in dental hard tissues, resulting in demineralization and remineralization phases. Biological, behavioural, psychological, and environmental variables all contribute to the development of the disease. (1,2).

In recent years, two factors have dramatically changed the management of dental caries: the noticeably slow rate of progression of active initial carious lesions (3) and the acknowledgment that early phases can be avoided, switched, or halted primarily by managing the etiological elements (4). These two factors have paved the way for the implementation of preventive measures when the lesions are most likely to be halted.

Rather than removing part of the tooth structure and filling, management of initial caries lesions should be performed conservatively via safe methods including remineralization therapy, behavioral modifications, and the use of fluoride-containing medications. Remineralization aims to arrest the course of the lesion or, ideally, repair it. (2).

Fluoride has long been the standard for avoiding early enamel caries (5). Fluoride improves caries resistance through a variety of mechanisms, including increased enamel resistance, faster maturation, remineralization of incipient caries,

interference with microorganisms, and enhancement of tooth morphology.

However, these cariostatic treatments are insufficient to address those with elevated risk (5), and the major shortcoming is the fact that the small level of calcium and phosphate ions in saliva limits the ability to remineralize enamel (6). This has resulted in the development of numerous novel materials that may offer critical elements for remineralization. Some of them are bioactive glasses (BAG).

Bioactive glass (BAG) is a ceramic substance made of amorphous sodium-calcium-phosphosilicate that is extremely reactive in water and when finely powdered, can physically block dentinal tubules. It is regarded as a unique substance with various novel characteristics; the most essential aspect is its potential to act as a synthetic mineralizer, mirroring the body's mineralizing features (7).

The remineralizing capabilities of bioactive glass (BAG) have been evaluated in several studies, which demonstrate its effectiveness in alleviating dentine hypersensitivity by occluding the dentinal tubules.(8,9) Other research indicates its role in preventing enamel erosion from beverages, (10)while another study highlights its ability to treat white spots resulting from orthodontic brackets.(11) Additionally, BAG has been found to act as a reservoir for ions that can be released in areas susceptible to demineralization.

Previous research focused on the effects of Biomin F on permanent teeth, evaluating factors like microhardness,mineral content through techniques like EDX analysis , Raman spectroscopy, and X-ray diffraction (XRD) (9,10,11). However, there hasn't been any research measuring the reduction in lesion depth by this paste, nor have there been studies on the use of Biomin for children with primary teeth as primary teeth are different from permanent teeth as they have thinner enamel and lower mineral content. The null hypothesis suggests that there is no difference between the two remineralizing agents, So the question is, Is there any differences in the effectiveness of the first remineralizing toothpaste (group II) compared to the second toothpaste (group III) in demineralized primary teeth?

Therefore the goal of this study was to evaluate and compare the remineralization effects of Biomin toothpaste for kids (580ppm) and conventional toothpaste (1450ppm) on experimentally created carious lesions in deciduous front teeth. The surface microhardness was assessed with microhardness equipment, and the lesion extent was measured with a polarized light microscope.

MATERIALS AND METHODS

This experimental research received approval from the ethics committee with a code 429/2024 at the AASTMT Alamein campus Faculty of Dentistry. The minimum sample size was determined on the basis of a prior study (12). A sample size of 20 teeth per group (totalling 60 teeth across 3 groups) was considered sufficient (13), meeting statistical significance with 80% power (β=20%) and a significance level of 95% (α=0.05) (14). G Power version 3.1.9.2 was employed to calculate the sample power size (15).

The sample size was estimated using this formula:

$$\text{Sample size} = \frac{\frac{z^2 \times p\,(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p\,(1-p)}{e^2 N}\right)}$$

Sixty sound deciduous anterior teeth were procured from the Department of Pediatric Dentistry and the Dental Public Health Clinic at the AASTMT Faculty of Dentistry. Visual examination ensured compliance with the inclusion criteria, confirming the absence of caries, previous fillings, developmental anomalies, and cracks through magnification. Samples were kept in saline at room temperature (16). Random allocation was performed via a computer-generated set of number sequences.

Teeth Setting

The enamel of the teeth was brushed with fluoride-free pumice, flushed with purified water, and then dried in the air. A 3×4 mm piece of self-adhesive tape was placed above the cementoenamel junction on the facial surface of each tooth. Acid-resistant nail polish was painted on all dental surfaces. (16). After the nail coating was desiccated, the strips were removed revealing a 3×4 mm enamel window on the facial surface of the samples (17). Each tooth was then immersed in a self-curing acrylic material and placed inside a mold, with the facial surface facing upward. (18).

Grouping and methods

In Group I, control group with 20 deciduous teeth was labelled from (1-20) and was kept in artificial saliva. In Group II, conventional toothpaste with 20 deciduous teeth labelled from(21-40) treated with a standard fluoride toothpaste (Signal with1450 ppm Fluoride), Group III: Biomin Toothpaste: This group consisted of 20 primary teeth labelled 41-60 and treated with Biomin toothpaste (Glycerin,silica, PEG 400, fluoro, calcium, phosphosilicate and fluoride 580 ppm).

Artificial carious lesion formation

Following the measurement of the baseline microhardness in groups I, II, and III, all the samples from these groups were immersed in a demineralizing solution. The demineralizing solution, composed of 50 mM (CH_3COOH), 2.2 mM ($Ca(NO_3)_2 \cdot 2H_2O$), 2.2 mM (KH_2PO_4), and NaOH to adjust the pH to 4.2, was used at a

volume of 10 ml per tooth. The immersion occurred at thirty-seven degrees for ninety-six hours without vibration (19). The demineralization solution was produced in a laboratory lab of the College of Pharmacy at the AASTMT. (19)..

Preparation of artificial saliva (19)

The remineralizing solution (1.5 mM CaCl2, 0.9 mM NaH2PO4, and 0.15 M KCL, pH 7.0)

Application of toothpastes

Every tooth underwent manual brushing via a soft micro toothbrush, and the paste was left on the tooth surface thirty times, with only a small amount used each time. Every washing cycle lasted for 15 seconds, and all teeth were placed in colloid water with the corresponding toothpaste. (16) The liquid of the paste, made daily before usage, was generated by mixing toothpaste and purified water. at a 1:3 ratio and left for an hour on a sonicator to agitate the paste in the water (20). The samples were brushed twice daily and then kept in manufactured saliva till the next day, lasting 15 days(16).

Microhardness assessment.

The enamel of each tooth in groups (I, II, and III) was tested using a Vickers microhardness instrument (Wilson microhardness tester, Japan) with a 25-gm force for 5 seconds. Three points were made on the surface of each sample, and the average of these points was determined. (21). Microhardness assessment occurred at two key points: after the initial formation of caries (first assessment) and after the study (second assessment).

Polarized light microscopic assessment.

Longitudinal ground sections of the tooth with a thickness of approximately 15 µm were placed with Canada balsam and over the glass slide. Depth of the lesion was conducted via ImageJ software (version 4.6) (22). The average depth in each sample was calculated by taking measurements from three lines: one on both sides and the other in the middle of the defect. These lines were perpendicular to the surface and reached to the sound zone.

Static evaluation

Data was processed using SPSS by IBM for Windows operating system 23.0. A thorough data review was conducted to identify and rectify any errors during the data entry process. Normality checks were performed via the Sharpino-Wilk test,and all variables exhibited a normal distribution. Consequently, the means and standard deviations (SDs) were calculated. (23) Differences in surface microhardness and lesion depth among the three groups were calculated via one-way ANOVA and Tukey's HSD (honestly significant difference) test. Differences in lesion depth among the three groups were calculated using a significance set at $P \leq 0.05$. To quantify the percent change in the microhardness, the following equation was employed:

$$\frac{Microhardness\ after\ tooth\ paste\ use - Microhardness\ after\ demineralization}{Microhardness\ after\ demineralization} \times 100$$

The percentage of the variation in lesion depth for every group was computed via the following method: (23)

$$\frac{Depth\ in\ treated\ tooth - Depth\ in\ untreated\ tooth}{lesion\ depth\ in\ untreated\ tooth} \times 100$$

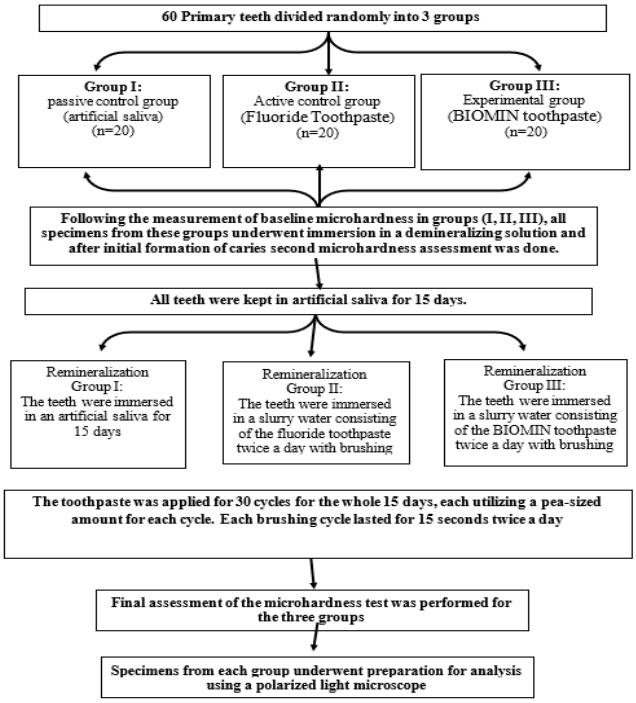

Figure (1): Diagram of Planning

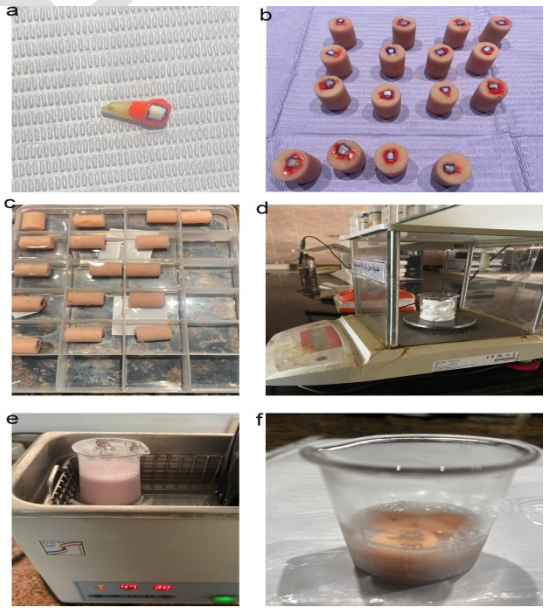

Figure (2): Showing steps of preparation for the remineralization challenge: a) A 3×4 mm square window was made above the CEJ (cementoenamel junction). b) Each tooth was then placed in a self-curing acrylic material, with its outer surface facing

upwards. c) Teeth underwent immersion in a demineralizing solution. d) Weighting the toothpaste for the slurry preparation. e) A Mix of toothpaste and deionized water which was left for an hour on a sonicator to agitate the paste in the water. f) Teeth were soaked in colloid water with the appropriate toothpaste two times per day for fifteen consecutive days.

RESULTS

The surface microhardness (mean ± standard deviation (SD) (Table 1, and Figure 5).

The descriptive statistics for the VHN data of the three test groups used are the "mean ± SD" (mean and standard deviation) —Group I (control), Group II , and Group III (Biomin paste)—were (146.095±440, (169.975±82), and (284.045±33), respectively. One-way ANOVA yielded a P-value significantly less than 0.05 and equal to 2.35×10^{-10} , indicating statistically significant differences in VHN among the three groups. Tukey's HSD test results revealed that the mean VHN of Group III (Biomin group) was significantly greater than that of both Group I (146.095) and Group II(169.975) , with p-values ≤0.001. There was no significant difference in the mean VHN between Group I and Group II , with a p-value of 0.317. These findings suggest that compared with artificial saliva and Signal 2, Group III(284.045) treated with Biomin is more effective at increasing the hardness of demineralized enamel but there was no significant difference between the artificial saliva and Signal 2 treatments. Compared with untreated teeth Group I had a 93.01% increase in surface microhardness, whereas Group II and Group III had increases in surface microhardness of 127.23% and 275.16%, respectively. These results revealed significant differences among the groups, with Biomin having the greatest increase in surface microhardness. The Saliva and Signal groups had intermediate surface microhardness, values, which were not significantly different from each other.

The depth in the demineralized areas (mean ± standard deviation (SD) (Table 2, and Figure 4). The average depth was (mean ±SD) 133 ± 37.4 µm for Group I (passive control), 121.75 ± 55.05 µm for Group II (active control), and 50.45 ± 24.3 µm for Group III .One-way ANOVA revealed that the p-value was significantly less than 0.05 (1.41×10^{-17}), indicating statistically significant differences in lesion depth among the three groups. Tukey's HSD test revealed a reduction in lesion depth for Group II compared with Group I, although this difference was not statistically significant (P=0.780). However, there was a statistically significant reduction in lesion depth between Group I and

^{17}), indicating statistically significant differences in lesion depth among the three groups. Tukey's HSD test revealed a reduction in lesion depth for Group II compared with Group I, although this difference was not statistically significant (P=0.780). However, there was a statistically significant reduction in lesion depth between Group I and Group III (P<0.001) and between Group II and Group III (P<0.001). compared with untreated teeth, Group I experienced a 35.75% decrease in lesion depth, Group II and Group III showed reductions of 41.20% and 74.42%, respectively. These results indicated significant differences among the groups, with Biomin paste resulting in the lowest lesion depth. The Saliva and Signal groups presented intermediate lesion depths, which were not significantly different. (Table 2)

c) The polarized micrograph revealed that the sound enamel sample displayed the typical arrangement of enamel rods with alternating Hunter–Shreger bands (HSBs). It also demonstrated a structureless area of the enamel surface that appeared as a continuous band (Fig 3a). The protective effect of fluoridated toothpaste was evident by the reduced lesion depth in Group II (Fig. 3d) compared with Group I (Fig. 3c). The negative birefringence highlighted the impact of this paste. The majority of samples in Group I (Fig 3c) showed obvious black bands extending from the enamel surface, the disappearance of HSBs within the affected region results in a significant level of positive birefringence (magnification: 40×). The effect of bioactive glass paste was demonstrated by the significant reduction in lesion depth in Group III (Fig. 3e) compared with Group I. Most samples had a heavily mineralized surface layer. (Fig. 3e).

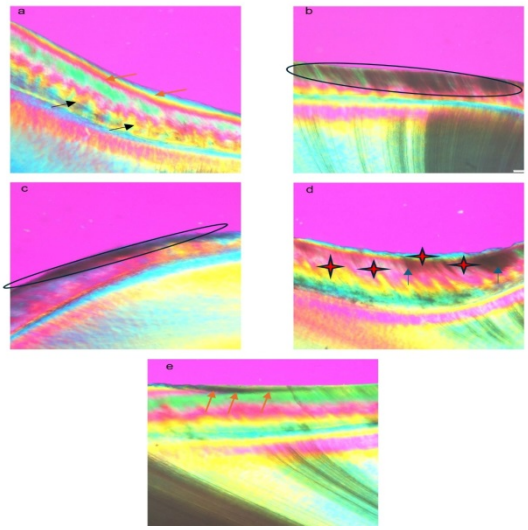

Figure (3): A polarized light microscopy image of a longitudinally ground section: a) Typical enamel with HSBs (black arrows) and a prism-free surface layer (red arrows). The untreated sample (b) has a distinct dark and deep demineralized enamel band that represents about half of the enamel thickness

and exhibits a high degree of positive birefringence. Saliva treatment of enamel (c) results in a significant reduction in lesion depth and evident negative birefringence (circle). Specimens treated with fluoridated toothpaste show areas of homogenous remineralization of lesions (stars) while other areas show a broad, deep dark demineralization band (blue arrows) (d). Enamel treated with bioactive glass paste (e) showing apparent lesion limitation (red arrows) and whole enamel thickness remineralization. Magnification ×4

Table 1 : Vickers the microhardness (VHN) readings for all groups

	Saliva (Group I)	Fluoridated toothpaste (Group II)	Bio-Min toothpaste (Group III)	One-way ANOVA test (P value)	Tukey's HSD Test (P value)
Mean±SD	146.095±44	169.975±82	284.045±33	p<0.001*	P < 0.001* Except between Group I and II =0.317
Median	151.75	152.50	282.55		
Min–Max	69.6-221.7	22.9-283.3	196.8-360.0		

HSD= **Honestly significant difference** * significant (p <0.05)

Table 2 The lesion values for depth in micrometers for all groups

	Saliva (Group I)	Fluoridated toothpaste (Group II)	Bio-Min toothpaste (Group III)	One-way ANOVA test (P value)	Tukey's HSD Test (P value)
Mean±SD	133±37.4	121.75±55	52.95±24	**p<0.001***	**P<0.001* Except between Group I and II=0.780**
Median	124.5	131.5	51		
Min–Max	77-179	50-228	0-118		

HSD= **Honestly significant difference** * significant (p <0.05)

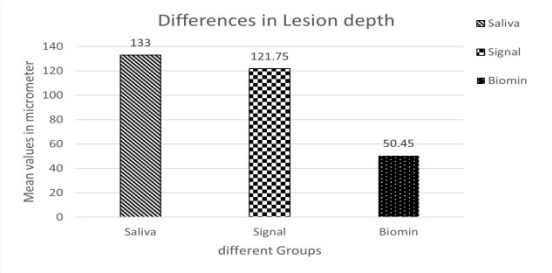

Figure (4): Difference in the lesion depth between the groups (the vertical axis shows the mean values in micrometers)

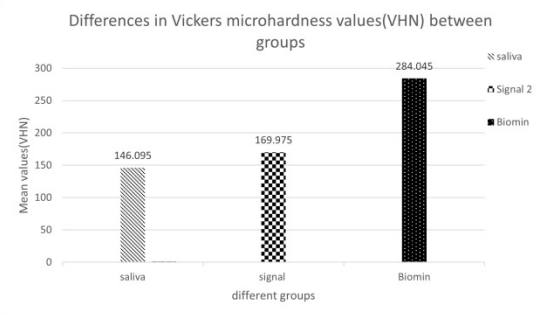

Figure (5): Shows the difference in the microhardness values between the groups after remineralization (the vertical axis shows the mean values in VHN).

DISCUSSION

This study evaluated the effectiveness of an experimental new bioactive glass paste(580ppm) formulation versus commercial fluoride toothpaste(1450ppm) in treating enamel demineralization in primary teeth caused by acid exposure. The findings indicated that the two agents had different levels of effectiveness in addressing carious lesions, leading to rejection of the null hypothesis.

The study lasted 15 days because the ions released from the Bioactive Glass (BAG) take at least two weeks to form Hydroxyl Carbonate Apatite (HCA), which closely resembles natural tooth mineral. These particles adhere to the tooth surface and continuously release ions, forming strong, removable-resistant bonds. (24)

Biomin Kid was used in this study for primary teeth because it has a lower fluoride content than Biomin for adults, which is 580 ppm. This lower fluoride level makes it safer for children and helps protect them from dental fluorosis.

Biomin showed a significant increase in microhardness and decrease in lesion depth in comparison to the other two groups. In support of these results, Abbassy MA et al. (25) demonstrated that, compared with control glass paste, bioactive glass pastes significantly enhanced the acid resistance of demineralized enamel and dentin.

As in group I, saliva contains small amount of calcium, phosphate and fluoride,the existence of calcium and phosphate ions in saliva may help with enamel remineralization. However, if acid challenges pass this physiological remineralization mechanism, different treatment strategies are required to promote remineralization. The natural physiological remineralization-demineralization of the tooth structure may be disrupted by several factors, such as salivary gland dysfunction, fermentable carbohydrates, and cariogenic factors. (26)

Fluoride helps prevent tooth decay by preventing demineralization and encouraging the remineralization of early cavities. It is the most frequently used agent for this purpose. When the pH increases, fluoride promotes the formation of new and larger crystals containing more fluoride, reducing enamel demineralization by forming fluorhydroxyapatite crystals and increasing remineralization. (27)

Fluoride toothpaste is among the most thoroughly researched products, with established safety and effectiveness for caries control in children under six (28). In this study, the use of fluoridated toothpaste confirmed its ability to reduce demineralization. The treated samples with fluoridated toothpaste presented a non-significant increase in microhardness values compared to the untreated samples (29), and the lesion depth in the fluoridated samples was lesser than that in the untreated samples with no statistically significant difference (30).

On the other hand, topical fluoride paste cannot infiltrate deep lesions or eliminate them. Consequently, Fluoride's cariostatic properties alone are insufficient for managing patients with a high caries risk, and incorrect fluoride usage can result in undesirable effects such as dental fluorosis (25).

This limitation led to the development of Biomin, which is rich in calcium and phosphate. Biomin penetrates the porous enamel subsurface, promoting the remineralization of deep demineralized enamel lesions other than just remineralizing the outer enamel surface, as seen with fluoride alone and this was in the same line with the results of this study.(31)

Biomin can provide low levels of fluoride for up to 12 hours after brushing because of its slow and controlled release of fluoride ions; therefore, even if the fluoride level in biomin does not exceed 580 ppm it gives the sufficient amount of fluoride needed. Previous research has shown that the amount of fluoride in Biomin is enough to facilitate apatite formation as fluorapatite occurs at a pH about one unit lower than that of hydroxyapatite. Fluoride in Biomin has been shown to convert the brushite, octacalcium phosphate, and amorphous calcium phosphate into apatite it also enhances the formation of acidic calcium-phosphate salts on the defected lesion (14).

The qualitative evaluation was done by the polarized light microscope, which is recognized as the most perceptive and descriptive-analytical. approach for identifying histological changes in areas of enamel defect. In this study, the histological evaluation results correlated with changes in microhardness. Compared with the control samples, all the treated samples presented a reduction in lesion extent, accompanied by a decrease in the positive birefringence of the lesion body.

Moreover, the present investigation revealed that the extent of the lesions differed among the samples treated with Biomin paste, with many exhibiting a strongly mineralized outermost layer (which is negatively birefringent) not observed in the other samples. This difference is largely attributed to the high calcium and phosphate contents with a small sized particles which enhance the penetration the porous enamel subsurface.

Many studies on Biomin have focused on its ability to seal dentinal tubules and address hypersensitivity. Systematic reviews revealed that, in the basis of in vitro evidence alone, bioactive glasses could improve enamel remineralization compared with other remineralizing agents, such as fluoride and CPP-ACP (32,33).

Aidaros et al. (34) conducted an in-vitro study on the surface of the permanent third molars. The agents in their study included Biomin for adults (1450 ppm) but in this study, we used Biomin for kids(580 ppm). SEM and elemental analysis were used in their study to evaluate the surface topography and the percentage of minerals, in contrast to this study which focused on measuring the depth of the lesion in primary teeth. The application regimen was : Two minutes, two times a day, lasting 15 days They found that all experimental kinds of toothpaste could remineralize enamel surfaces, but Biomin toothpaste, which combined fluoride with bioactive glass technology, had the best outcomes.

Importantly, an in vitro study may not fully replicate the results of an in vivo study, as it may not capture several stages of the caries progression. The study's limitations include the inability to account for oral characteristics such as biofilms, oral flora, various salivary elements, individuals' food patterns, and dental hygiene habits.

The hypothesis regarding the action of the Biomin paste mentioned above was formulated on the basis of observations from the current study and previous research. Hence, future studies could be enriched by crafting pH-cycling models that closely resemble in-vivo conditions. These models could involve solutions that mimic the ionic

concentration and pH of plaque fluid and are tailored to individuals with varying levels of caries risk. Furthermore, incorporating organic salivary components could enhance the fidelity of these models, offering deeper insights into the effects of treatments on dental health.

CONCLUSION

The new bioactive glass paste (Biomin toothpaste for kids (580 ppm) demonstrated a more significant effect than fluoridated toothpaste (1450 ppm) in treating enamel demineralization induced by artificial cariogenic trials. Consequently, it could be considered a potential alternative treatment option for managing carious lesion in primary teeth.

Competing interests

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Ghada Mahmoud was responsible for conceptualizing the study, overseeing the data analysis, and revising the manuscript draft. Heba Yousif handled the microhardness test measurements and their analysis. Maha Montaser prepared, examined, and analyzed the teeth under a polarized microscope and calculated the lesion depth. All the authors participated in designing the study, providing feedback, and approving all the versions of the manuscript.

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DECLARATIONS

Human Ethics and Consent to Participate Declarations: The samples used in this study were exfoliated primary teeth from children. Since the children's guardians were illiterate and the study posed minimal risk, verbal informed consent was obtained from the parents, agreeing to the use of the teeth for research purposes instead of discarding them as waste. This process was conducted in compliance with the institution's regulations and the ethical principles endorsed by the Research Ethics Committee.

Ethics Approval declaration:

Before the study began, ethical approval was secured from the Ethics Approval Committee at the Arab Academy for Science, Technology and Maritime Transport (AASTMT) , College of Dentistry, Egypt (429/2024). All techniques followed the Helsinki Declaration

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