Original Paper

Dentin Tubule Occlusion Potential of Novel Dentifrices Having Fluoride Containing Bioactive Glass and Zinc Oxide Nanoparticles

Abdul Samad Khan[a]   Imran Farooq[b]   Kawther Moosa Alakrawi[c]   Hina Khalid[d]
Omar Waqas Saadi[e]   Abbas Saeed Hakeem[f]

[a]Department of Restorative Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [b]Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [c]College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia; [d]Interdisciplinary Research Centre in Biomedical Materials, COMSATS University Islamabad, Lahore Campus, Lahore, Pakistan; [e]Department of Mechanical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates; [f]Center of Excellence in Nanotechnology, King Fahd University of Petroleum and Minerals, Dhahran, Saudi Arabia

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Highlights

- We analyzed the potential of dentin tubule occlusion of two novel experimental dentifrices, having fluoride containing bioactive glass and zinc oxide nanoparticles.
- Experimental dentifrices were found capable of occluding more tubules than fluoride dentifrice, and their results are comparable to commercially available bioactive glass dentifrices.
- The results can aid in offering appropriate recommendations for dentifrice for patients, especially those suffering from dentin sensitivity.

Abstract

Objective: To compare the in vitro potential of dentin tubule occlusion of two novel experimental dentifrices consisting of fluoride containing bioactive glass (BG) with zinc oxide nanoparticles. ***Materials and Methods:*** Forty-eight dentin discs ($n = 48$) were divided into 6 groups ($n = 8$), based on their brushing dentifrices: Group 1 = artificial saliva (AS; control); Group 2 = fluoride dentifrice (Colgate Palmolive©, UK); Group 3 = experimental nonactive agent dentifrice; Group 4 = experimental dentifrice with 1.5% BG; Group 5 = experimental dentifrice with 4% BG; and Group 6 = BioMinF© dentifrice. Postbrushing, the discs were subjected to acidic challenge with 6% wt citric acid (pH = 4.0) for 1 min. Scanning electron microscope (SEM) and energy-dispersive X-ray (EDX) spectroscopy were performed pre- and post-citric acid challenges, and percentages of tubule occlusion assessed. ***Results:*** SEM micrographs of group 1 (AS) show no tubule occlusion (0%), whereas those of groups 2 and 3 show partial tubule occlusion (25 to <50% of tubules occluded). The SEM micrographs of dentifrices containing fluoride-BG (groups 4, 5, and 6) show that most of the tubules (>50 and <100%) were occluded. For all the groups (excluding group 1), pre- and post-citric acid challenge values are statistically significant ($p < 0.05$). EDX analysis reveals the presence of zinc in experimental dentifrices only. ***Conclusion:*** The results of

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novel experimental dentifrices are comparable to those of the BioMinF©, in terms of tubule occlusion. Dentifrices containing BG could serve as an alternative in dentin sensitivity management.

Introduction

Dentin is a mineral-bearing tissue, composed of tiny dentinal tubules, whose exposure due to enamel loss and gingival recession results in dentin hypersensitivity (DH) [1]. DH is a common problem, and its prevalence has been reported to be as high as 74% [1]. The most widely accepted theory is the hydrodynamic theory, which explains that DH is caused by the exposure of dentinal tubules in which the movement of fluids, in response to stimuli, activates nerve endings in the pulp and causes sharp pain for a short duration [2]. The extrinsic factors that contribute to dental erosion leading to the exposure of tubules are related to acids of dietary or medicinal origin; besides, they are also associated with certain behavioral factors. The causes of DH include caries, gingival recession, tooth wear, and fractures [3].

One solution to minimize DH is to block the open dentinal tubules [1]. With aging, dentin can change to sclerotic dentin, as the tubules can get occluded by minerals [4]. However, this process is slow, and the patient may stay in pain for a long time. It has been reported earlier that the use of desensitizing dentifrices could reduce DH immediately and effectively [5]. It is also possible to reverse demineralization by deploying mineralizing agents on the tooth surface. Bioactive glass (BG) is one such type of mineralizing agent, which is capable of bonding chemically to the hard dental tissues [6]. The components of BG are oxides of calcium, sodium, phosphorus, and silica, in ratios that impart bioactivity [7]. These BGs were introduced in the late 1960s primarily for osteogenesis; however, since then, their inclusion in various dental products, especially dentifrices, has been increasing [8]. As the bone and dentin are similar in structure, the use of BGs on dentin could prove useful in attaining clinical benefits [9].

The reaction of BG in aqueous solution brings about a change in its structure and chemical composition, as it dissolves and leads to the formation of hydroxycarbonated apatite (HAP) [8]. The fluoride of traditional BGs is not inside their glass structure, although that would be more beneficial in ensuring the formation of strong fluorapatite (FAP) crystals [10]. One such commercially available toothpaste is BioMinF©, which has shown good tubule occlusion properties in a previous in vitro study [11]. Based on a recent in vitro study, Kanwal et al. [12] conclude that incorporation of fluoride in a BG toothpaste helps it in forming fluoridated apatite in Tris buffer solution, which has better clinical durability. In addition, fluoride in combination with other desensitizing agents in toothpaste shows increased efficacy in caries prevention and desensitization [13].

This study was conducted out to develop BGs of new compositions and test them for their dentin tubule occlusion capability. Accordingly, two novel compositions of dentifrices, having fluoride-containing BG and nano-zinc oxide (ZnO) powders as active ingredients, were evaluated for their potential in dentin tubule occlusion and the results compared with that of a standard fluoride dentifrice and BioMinF©. The null hypothesis (H_0) was that all tested dentifrices will have similar dentin tubule occlusion competence.

Materials and Methods

Ethical clearance was obtained from the Scientific Research Unit of the institute, and all the ethical protocols were strictly followed.

Composition of Dentifrices
A standard fluoride dentifrice (Colgate-Palmolive©, UK) and BioMinF© were used in this study for the purpose of comparison with the new experimental dentifrices. A nonactive agent of dentifrice composition and 2 experimental dentifrice compositions with active agents were developed and used in this study. All the chemicals used were of analytical grade. Initially, the basic ingredients were mixed in optimum ratio and allowed to form a homogeneous solution. Then 1.5% wt. of titanium dioxide (Sigma Aldrich, St. Louis, MO, USA) and 3% wt. of ZnO nanoparticles [14] were added in increments to avoid agglomeration. Fluoride-BG (7.5% mol) was synthesized following the sol–gel precipitation method of Gul et al. [15]. The fluoride-BG thus obtained was incorporated into the experimental toothpastes in different concentrations, that is, 1.5 and 4% wt/wt. The composition of each dentifrice (with its active and basic ingredients) is given in Table 1.

Preparation of Dentin Discs and Grouping of Specimens
For this study, extracted noncarious teeth were collected from Oral and Maxillofacial Surgery Clinics of the institute. Forty-eight dentin discs of 2.0 mm (± 0.2 mm) size were prepared by cutting each tooth horizontally (mesio-distally) over cemento-enamel junction, utilizing cooled diamond saw (Isomet® 5000 Linear Precision Saw, Buehler Ltd., Lake Bluff, IL, USA). The occlusal enamel, 2.5 mm down the cusp tip, was also removed and the teeth were cut mesio-distally. The upper surfaces of the dentin discs were marked, and the unmarked surfaces were etched with 37% orthophosphoric acid for 20 s to open the tubules and to free them from any organic material. Post-etching, the discs were washed with dis-

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Table 1. Showing composition of all the dentifrices used in this study

Dentifrice	Basic ingredient (wt%)	Active ingredients (wt%)
Colgate®	CaCO$_3$, Aqua, sorbitol, sodium lauryl sulfate, aroma, cellulose gum, sodium bicarbonate, tetrasodium pyrophosphate, benzyl alcohol, sodium saccharin, sodium hydroxide, limonene	Sodium monofluorophosphate 1.1% w/w, 1,450 ppm F
Non-BG dentifrice	Glycerol ~32%	–
1.5% BG dentifrice	Sodium benzoate ~1% Methyelcellulose ~1% Peppermint (flavoring agent) ~ 1% Sodium lauryl sulfate ~ 2% CaCO$_3$	TiO$_2$ 1.5% wt. ZnO nanoparticles 3% wt. F-BG 1.5% wt. Herbal product 3% wt
4% BG dentifrice	~27% wt. Water ~ 36%	TiO$_2$ 1.5% wt. ZnO nanoparticles 3% wt. F-BG 4% wt. Herbal product 3% wt.
BiominF®	Glycerin, silica, PEG 400, fluoro calcium silicate, sodium lauryl sulfate, titanium dioxide, aroma, carbomer, potassium acesulfame, fluoride up to 530 ppm	BioMin® (fluoro calcium phosphosilicate)

BG, bioactive glass; CaCO$_3$, calcium carbonate ; TiO$_2$, titanium dioxide; ZnO, zinc oxide; F-BG, fluoride-bioactive glass;

tilled water for 1 min and randomly divided into the following 6 groups, each group receiving 8 discs ($n = 8$), based on their treatment with different dentifrices: Group 1 = discs brushed with artificial saliva (AS; control); Group 2 = discs brushed with fluoride dentifrice (Colgate Palmolive©, UK); Group 3 = discs treated with experimental non-active agent dentifrice; Group 4 = discs treated with experimental dentifrice, having 1.5% BG; Group 5 = discs brushed with experimental dentifrice, containing 4% BG; and Group 6 = discs treated with Biomin-F dentifrice.

The AS was prepared by mixing the following chemicals in 1,000 mL of deionized water, as proposed by Fusayama et al. [16]: NaCl: 0.400 g, KCl: 0.400 g, NaH$_2$PO$_4$. H$_2$O: 0.69 g; CaCl$_2$. H$_2$O: 0.795 g; Na$_2$S. 9H$_2$O: 0.005 g. The pH of the freshly prepared AS was 5.5, which was adjusted to the neutral pH of 7.0 by adding aliquots of NaOH [10].

Tooth Brushing Protocol for Dentin Discs

Discs of group 1 were brushed with 1 mL AS and those of other groups with 1 g of their respective dentifrices for 2 min, twice a day (once in the morning and once in the afternoon) for 7 days. The dentifrice was mixed with deionized water in the ratio of 1:2, and a load of 200 g was applied to the toothbrush (Medium bristled, Oral B®, Pro-Flex™, USA) head to ensure that it contacted the samples. Each sample was treated for 150 cycles/min inside a toothbrush simulation machine (Toothbrush simulator; model ZM-3.8, Germany). Each day, post-brushing, the discs were washed with distilled water for 1 min and then returned to their respective containers containing AS at 37°C.

Citric Acid Challenge

Post-brushing, the discs were subjected to citric acid (6% wt.) challenge for 1 min under dynamic conditions. The citric acid was freshly prepared by adding 6 g citric acid powder (FunFresh Foods©, San Clemente, CA, USA) to 100 mL of deionized water, and the pH was maintained at 4. After citric acid exposure, the discs were washed with distilled water and left to air dry for 24 h before analyzing them by scanning electron microscope (SEM).

Analysis of Tubule Occlusion by SEM Energy-Dispersive X-Ray Analysis

The dentin discs were mounted on stubs, given sputter coating with gold and then analyzed, using an SEM (FEI, Inspect F50, The Netherlands) with energy-dispersive spectroscopy (EDS, Oxford Instruments, UK). The discs were evaluated pre- and post-citric acid challenges. Micrographs were taken from the central areas marked on the discs, utilizing 5 and 20 kV electron modes, at 10 and 15 Kx magnifications. The percentage of the blocked dentinal tubules was calculated by dividing the number of occluded tubules in a single micrograph with the total number of tubules present in that image, and then multiplying it with 100. Three independent blinded reviewers (who were not part of the study group) were requested to analyze the percentages of tubule occlusion, following the criteria given by Pathan et al. [17]: No occlusion = 0%, mostly unoccluded (<25% of tubules occluded), partially occluded (25 to <50% of tubules occluded), mostly occluded (50 to <100% of tubules occluded), and occluded (100% of tubules occluded). The reviewers performed the analysis three times and then submitted the mean of their analytical results. The data of the three reviewers were pooled to arrive at the final occlusion percentage. The inter-reviewer agreement between the three reviewers' results, determined by using Kappa statistics, was found to be high (Kappa index: 89.9%).

Fig. 1. SEM micrograph of a dentin disc that was brushed with fluoride dentifrice (**a**) pre-citric acid challenge and (**b**) post-citric acid challenge.
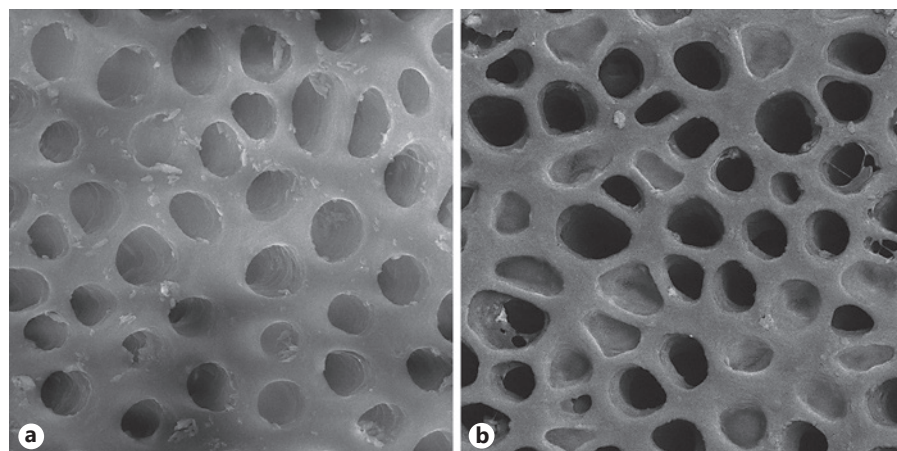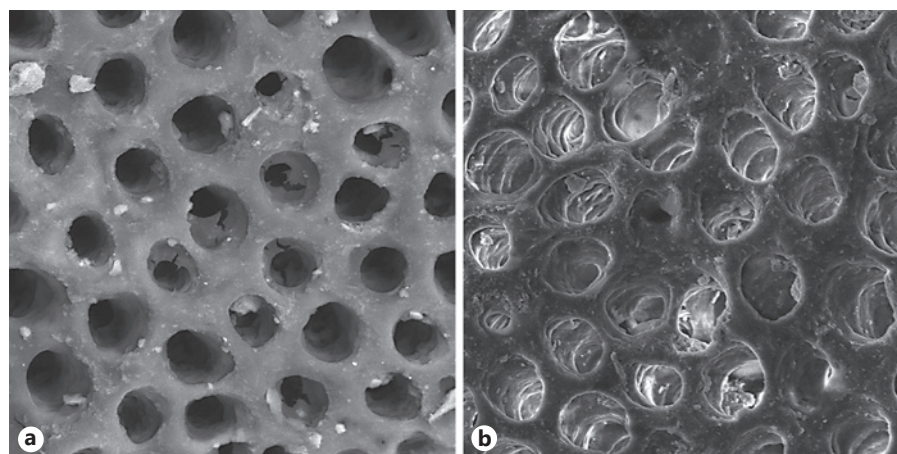

Fig. 2. SEM micrograph of a dentin disc that was brushed with non-BG experimental dentifrice (**a**) pre-citric acid challenge and (**b**) post-citric acid challenge.

Statistical Analysis

Data analysis was performed using SPSS-20.0 (IBM product, Chicago, IL, USA). Paired sample t test was applied to compare pre- and post-citric acid challenge tubule occlusion values, within each group. Tukey's HSD post hoc test was used for comparison of post-citric acid challenge results among the groups. p value ≤0.05 was considered a statistically significant difference between means.

Results

The SEM micrographs, belonging to group 1 (AS), showed no tubule occlusion (0%), both pre-and post-citric acid challenges (not shown). The SEM micrographs of group 2 (Colgate©) showed only partial tubule occlusion (25 to <50% of tubules occluded), pre- (Fig. 1a) and post-citric acid challenges (Fig. 1b). A similar finding was observed in group 3 (non-BG experimental dentifrice), where again the tubules were partially occluded (25 to <50% of tubules occluded), pre-and post-citric acid chal-

lenges (Fig. 2a, b). Compared with groups 1, 2, and 3, group 4 (containing 1.5% BG) showed superior tubule occlusion, pre- and post-citric acid challenges, and most of the tubules (>50 and <100%) remained occluded (Fig. 3a, b). The findings for group 5 (containing 4% BG), where most of the tubules (>50 and <100%) remained occluded, pre- and post-citric acid challenges (Fig. 4a, b), are similar to those of group 4. The findings for Group 6 are similar to those for groups 4 and 5, where again most of the tubules (>50 and <100%) were blocked both pre-and post-citric acid challenges (Fig. 5a, b).

Energy-dispersive X-ray results (Table 2) show elemental composition of dentin surface post-citric acid challenge. The experimental dentifrices show that Ca/P is approximately 2.0, whereas fluoride is rare in all the groups. It is noteworthy that the percentage of zinc is higher in the experimental dentifrices (i.e., 1.5 and 4% BG) than in other dentifrice groups. Similarly, Ti (due to TiO_2) was also visible in experimental dentifrices (Fig. 6a–d). For all the groups (excluding group 1), paired sample

Fig. 3. SEM micrograph of a dentin disc that was brushed with experimental dentifrice containing 1.5% BG (**a**) pre-citric acid challenge and (**b**) post-citric acid challenge.
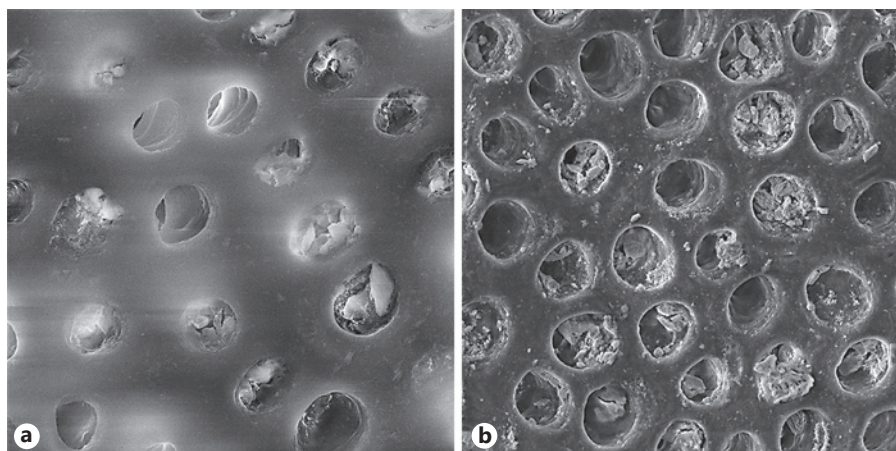

Fig. 4. SEM micrograph of a dentin disc that was brushed with experimental dentifrice containing 4% BG (**a**) pre-citric acid challenge and (**b**) post-citric acid challenge.
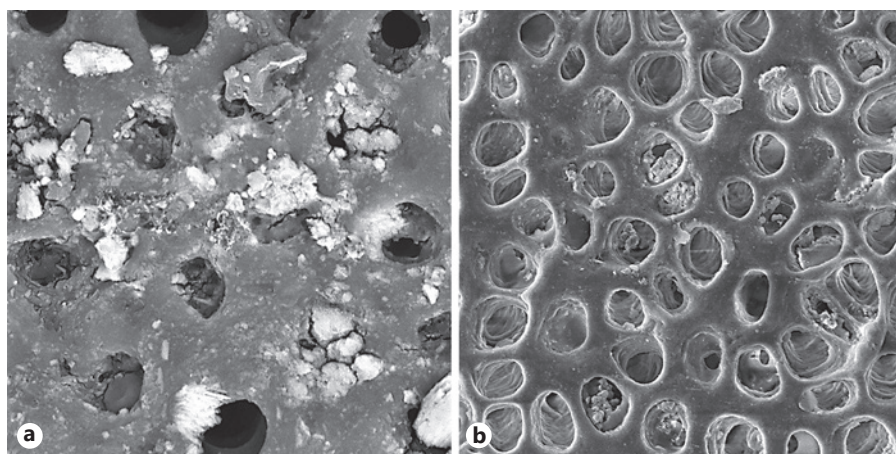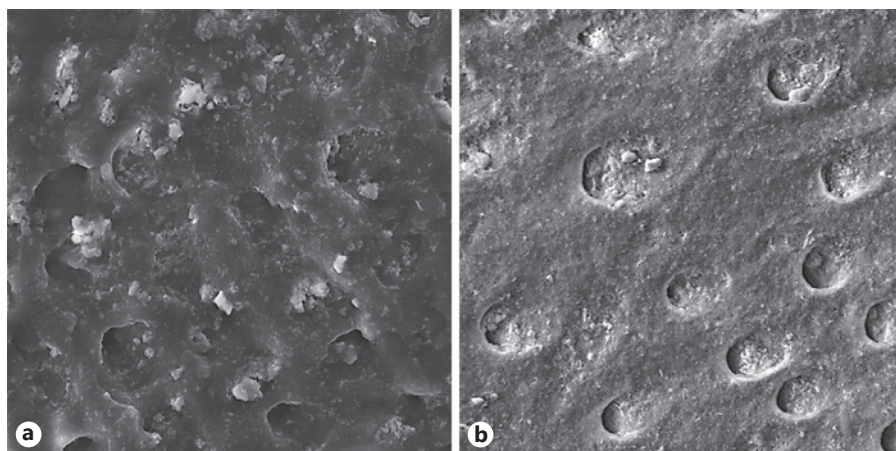

Fig. 5. SEM micrograph of a dentin disc that was brushed with BioMinF© (**a**) pre-citric acid challenge and (**b**) post-citric acid challenge.

t test reveals statistically significant differences ($p < 0.05$), within the group, between pre- and post-citric acid challenge values (Table 3). Intergroup comparisons reveal statistically significant differences ($p < 0.05$) in post-citric acid challenge tubule occlusion values (except between groups 2 and 3, groups 4–6, and groups 5 and 6; Table 4).

Discussion

This study investigated the tubule occlusion competence of two novel fluoridated-BG experimental dentifrices and compared their competences with those of standard fluoride, nonactive BG dentifrice and BioMinF©.
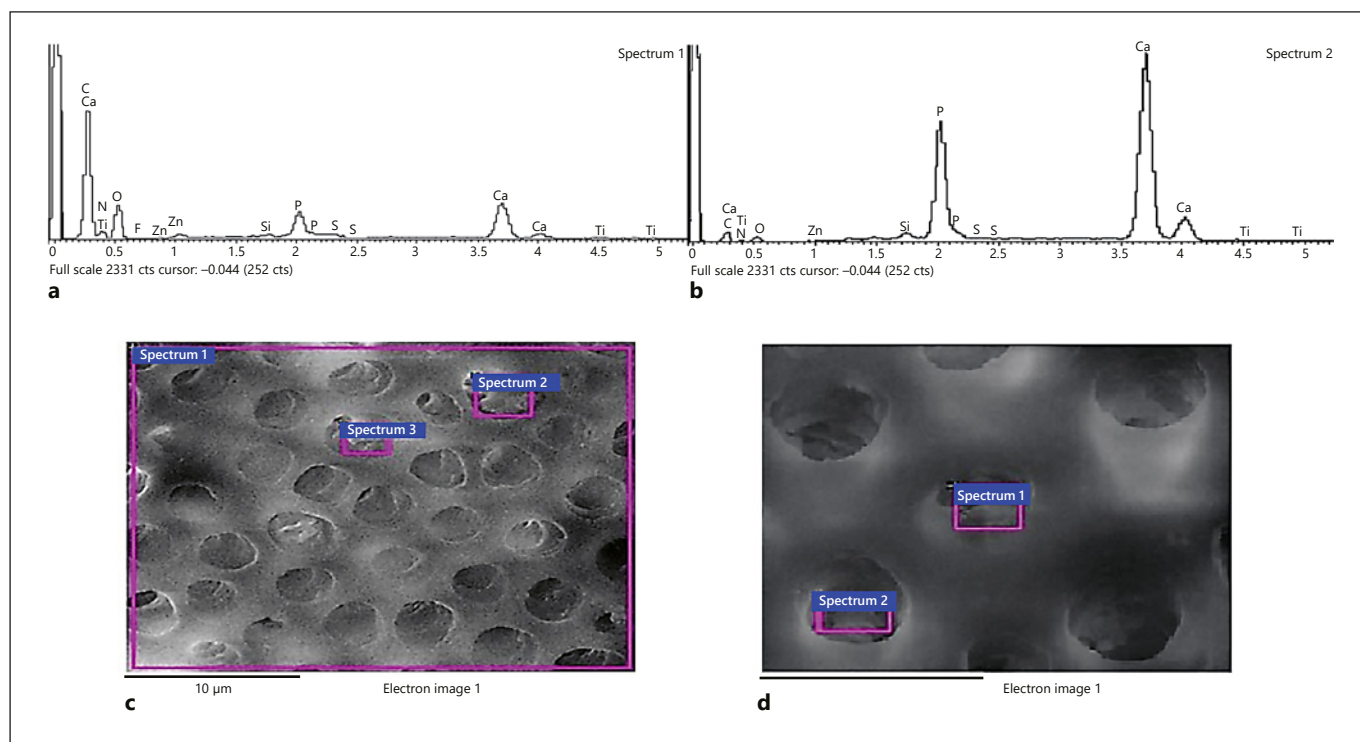
Fig. 6. Showing EDX spectra of (**a**) dentin discs brushed with 1.5% BG dentifrice post citric acid challenge, (**b**) dentin discs brushed with 4% BG dentifrice post citric acid challenge, (**c**) SEM micrograph showing EDX analysis area of dentin disc which was brushed with 1.5% BG, and (**d**) SEM micrograph showing EDX analysis area of dentin disc which was brushed with 4% BG.

Table 2. EDX results showing elemental composition of different toothpaste groups

Groups	Wt%										
	C	N	O	F	Si	P	S	Ca	Ti	Zn	total
Group 2	46.60	21.83	32.15	−1.06	−0.03	−0.06	0.06	0.35	−0.01	0.00	100
Group 3	45.80	20.62	31.90	0.09	0.12	0.44	0.17	0.90	−0.01	0.03	100
Group 4	38.78	−0.47	39.25	−0.13	0.12	1.37	0.08	2.63	17.41	0.94	100
Group 5	17.46	9.88	13.09	−0.11	0.66	18.08	−0.01	39.97	0.04	0.94	100
Group 6	13.63	0.85	41.55	0.01	15.18	0.04	0.49	30.12	−0.05	−0.18	100

EDX, energy-dispersive X-ray.

The results indicate that pre- and post-citric acid challenge BioMinF© dentifrices, containing 1.5% BG, and 4% wt/wt BG were able to block most of the tubules (>50 and <100%). For the control group containing AS, no tubule occlusion was seen. Human saliva plays an important role in transporting essential ions such as calcium and phosphate to the dentin surface; however, this process by itself cannot plug these ions inside the tubules to the extent necessary to occlude them and thus decrease DH [11]. For dentifrice groups containing fluoride, and those containing no BG, tubule occlusion was only partial (<25 and <50%). Incorporation of fluoride in dentifrices is beneficial to tooth structure because of the strong association between the use of fluoride and prevention of dental caries [18]. Fluoride helps in the formation of FAP from HAP through substitution of hydroxyl ion for fluoride

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ion [19] in the apatite structure of enamel. The FAP is known to have better chemical stability and lesser bioresorption rate than HAP [20]. Fluoride is quite effective in enamel remineralization, but its effect on DH is rather limited [21]. This finding can be attributed to the fact that fluoride from a regular dentifrice can be washed away quickly by salivary flow, and the amount of FAP that can thus form is debatable [22].

Calcium and phosphate ions are integral parts of hard tissues of the human body, such as bones and teeth [23], and all the BG compositions that are available till date contain these two important ions [7]. The surface reaction that occurs when the BG comes in contact with a bone comprises the following main steps: formation of silanol (Si-OH) groups on the surface of BG, silica dissolution, and the development of an amorphous calcium phosphate layer, which in turn crystallizes as HAP [8]. As both human bone and dentin are similar in their chemical compositions, it can be predicted that the material, which forms a strong bond with the former, will also form a similar bond with the latter [24]. Bakri et al. [25], based on their in vitro analysis of dentin tubule occlusion competence of a BG dentifrice and comparison of that result with that of an arginine and fluoride containing dentifrice, conclude that after acidic challenge, BG toothpaste shows better tubule occlusion than other groups. Previously, Shaikh et al. [11], report that BioMinF© demonstrates better blockage of patent tubules of dentin discs as compared to Novamin© dentifrice. In the present study, we observed similar tubule occlusion competence for BioMinF©. Our novel BG-containing dentifrices also showed similar results in respect of BioMinF©. The presence of BG (1.5 and 4% wt/wt.) BG in those dentifrices could have contributed to better tubule occlusion by virtue of its surface reaction with dentin, which ensures enhanced plugging of open tubules. Moreover, nano-ZnO powders in experimental dentifrices offer an additional advantage. ZnO powder (3% wt.) is added as a preservative to toothpastes, which in aqueous suspension inhibits not only dentin demineralization but also induces antimicrobial action by yielding zinc ions (Zn^{2+}) and reactive oxygen species [25]. The partial dissolution of ZnO particles releases Zn^{2+} ions in aqueous suspension, which display antimicrobial action against many bacterial and fungal strains through direct contact with cell wall [26].

The ideal dentifrice for treating DH should not only block the tubules but also preserve the occlusion of tubules when faced with acidic challenge [10]. The experimental dentifrices of this study blocked a majority of the tubules and also retained most of the tubule occlusion,

Table 3. Statistical values when pre- versus post-citric acid challenge tubule occlusion values were compared within each group

Groups			Paired samples test, paired differences			t	df	p value
			mean	SD	SEM			
2				0.75			7	0.001
3	Pair 1	Pre – post	2.00	0.92			7	0.000
4	Pair 1	Pre – post	2.50	1.51			7	0.002
5	Pair 1	Pre – post	2.87	3.09	1.09279	2.631	7	0.034
6	Pair 1	Pre – post	2.62	1.76	0.62500	4.200	7	0.004

Paired sample t test, group 1 values are not shown as they resulted in no tubule occlusion.

Table 4. Showing Tukey HSD – post hoc test results when post-citric acid challenge tubule occlusion values were compared among the groups

Group	Group(s)	Mean difference	SE	p value
2	3	1.12	2.36	0.999
	4	−60.37*	2.36	0.000
	5	−67.12*	2.36	0.000
	6	−64.50*	2.36	0.000
3	4	−61.50*	2.36	0.000
	5	−68.25*	2.36	0.000
	6	−65.62*	2.36	0.000
4	5	−6.75	2.36	0.084
	6	−4.12	2.36	0.588
5	6	2.62	2.36	0.921

* The mean difference is significant at the 0.05 level.

post-acidic challenge. The traditional BG composition (45S5, Bioglass©) is devoid of fluoride and contains only calcium sodium phosphosilicate [27]. The dentifrice, based on Novamin©, is one such example as it contains BG, but no fluoride, in its glass composition; so, soluble fluoride needs to be added in the formation of the dentifrice [10]. To achieve maximum benefits of fluoride, it should be released and deposited slowly on to the surface of the tooth [10]. The presence of fluoride inside the BG composition ensures slow and sustained release of fluoride with the possibility of forming long-lasting FAP, rather than fluorite [6]. Previously, Farooq et al. [27] synthesized new fluoride-containing BG compositions, which formed FAP in Tris buffer solution at a faster rate than 45S5. Another study on the effect of adding fluoride

to BG composition showed that fluoride softens the glass, and is thus more bioactive [28]. Therefore, the superiority of the two experimental BG dentifrices in tubule occlusion potential can be attributed to the presence of fluoride in the BG structure.

Zinc, though present only in small amounts in the human body, plays an important role in cell division, growth, and bone metabolism [29]. Considering all these benefits, zinc was included in the experimental BG-containing dentifrices, the effect of which is evident in the results of Energy-dispersive X-ray analysis. The in vitro nature of this study could be considered a limitation to obtaining fully reliable results, as the in vivo conditions could be quite different from the in vitro conditions. Another limitation could be the manual counting of blocked tubules, which could be false due to human error. However, every attempt was made to minimize the counting error by standardizing all the variables. Well-controlled clinical trials need to be conducted to further analyze the effect of these experimental dentifrices in actual dynamic in vivo conditions. The results of this study should be considered only as tentative findings, which need to be followed up by long-term assessment of the tested dentifrices.

Conclusion

We conclude that dentifrices containing fluoride-zinc-BG have better tubule occlusion potential compared to other dentifrices. The results of these experimental dentifrices are comparable to those of BioMinF© and are in conformity with the prevailing opinion in the literature that BGs containing fluoride and zinc occlude the tubules efficiently, and hence can be used for the treatment of DH.

Disclosure Statement

The authors have no conflicts of interest to declare.

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