FLUORIDE
Quarterly reports

Effects of Various Remineralization Agents on Surface and Subsurface Enamel Microhardness Following Demineralization

Unique digital address (Digital object identifier [DOI] equivalent):
www.fluorideresearch.online/epub/files/264.pdf]

Gamze KES[1], Cansu ATALAY[1*],
Meserret BASEREN[1],

[1] Department of Restorative Dentistry, School of Dentistry, Hacettepe University, Ankara, Turkey.

***Corresponding author:**
Cansu Atalay, Assoc. Prof.
Department of Restorative Dentistry, School of Dentistry, Hacettepe University, Ankara, Turkey. 06100
Phone: (+90) 5057502977
E-mail: cansuatalaydr@gmail.com

Accepted: 2024 Mar 1
Epub as e264: 2024 Mar 1

ABSTRACT

Purpose: The effects of different remineralization agents applied on enamel samples were evaluated by using surface and subsurface microhardness analysis.

Methods: Sixty bovine incisor enamel samples were fabricated. After the initial microhardness values were measured, enamel samples are divided into 3 equal parts, and assigned as the treatment area (T), the adjacent area (A) and the control area (C) (covered with an acid-resistant varnish). Samples were subjected to pH cycle for 5-days, and then microhardness measurements were repeated. The specimens were randomly divided into 5 groups according to the fluoride-releasing materials applied, as follows: Group 1: BioMin F; Group 2: Colgate Triple Effect; Group 3: FCP COMPLEX; Group 4: Fluor Protector; Group 5: Artificial saliva (control). Following the applications, surface and subsurface measurements were performed. The data were analyzed by one-way analysis of variance (ANOVA) and post-hoc Tukey test.

Results: BioMin F toothpaste provided the greatest increase in surface microhardness, followed by FCP COMPLEX, Colgate Triple Effect and Fluor Protector, respectively. Among the materials tested, BioMin F and Colgate toothpastes exhibited the highest increase in subsurface microhardness values, with FCP COMPLEX solution following closely in second place.

Conclusions: BioMin F showed the best results in the surface microhardness assessment, while both toothpastes (BioMin F and Colgate) showed similarly positive results in the subsurface microhardness assessment.

Key-words: fluoride, BioMin F, FCP COMPLEX, adjacent enamel.

INTRODUCTION

Recently, important developments in the diagnosis and treatment of early-stage caries lesions have changed the philosophy of caries treatment in dentistry. Invasive approaches based on restoration and extraction have left their place to preventive applications. The aim of modern dentistry is to establish individual preventive programs and to detect caries early in order to protect dental health and provide better conditions [1].

After the concept of remineralization came into the dentistry agenda, many methods and products have been developed to date. Fluoride is an agent that is accepted as the main component in the remineralization process, but its ability to support remineralization is limited by the presence salivary or plaque calcium and phosphate ions in adequate amounts [2,3]. Therefore, in cases of individual factors such as dry mouth, the presence of calcium and phosphate ions may limit enamel remineralization with topical application of fluoride ions. Although the caries-preventive effect of fluoride has been demonstrated by many studies [4,5], research on its use with other agents to increase this anti-caries effect continues. Materials that have proven to be more effective than other topical remineralizing agents, such as bioactive glass, have been used with fluoride to increase the remineralization capacity of fluoride [6,7]. In addition, a solution that increases fluoride precipitation due to the calcium and phosphate ions it contains has been produced and has been the subject of current studies [8,9].

As well as the effectiveness of the method and product used for protective purposes, it is also important that it reaches everyone with individual applications that do not require professional application, are easy to apply and cost-effective [10]. The use of toothpastes is the simplest, cheapest and most common method among many remineralization methods. In recent years, in addition to the effective use of toothpastes, a new toothpaste has been launched to provide increased remineralization with a combination of fluoride and bioactive glass. The manufacturers claim that it has the ability to cause sustained release of calcium, phosphate, and fluoride ions, resulting in enhanced remineralization of tooth structure and occlusion of dentinal tubules to relieve sensitivity [11].

The objective of this in vitro study was to assess the remineralization abilities of various toothpaste formulations, including fluoride and bioactive glass (BioMin F), standard fluoridated toothpaste (Colgate Triple Action), a solution containing fluoride, calcium, and phosphate (FCP COMPLEX), as well as a fluorinated varnish (Fluor Protector). This assessment was conducted through microhardness analysis and applications on demineralized bovine enamel. The null hypothesis posited that there would be no significant difference in surface hardness among the tested materials at different depths within the tooth tissue.

MATERIAL AND METHODS

Experimental design

For this in-vitro study, enamel blocks (4mmx9mmx3mm, n=60) were obtained from bovine incisors following the removal of soft tissue residues, and kept in 0.1% thymol solution. All samples were embedded in acrylic blocks and the enamel surface of the blocks was ground using a polishing device (Buehler Phoenix Beta, Lake Bluff, Illinois, USA) with 320, 600 and 1200 grids of Al_2O_3 papers under water cooling.

After enamel blocks were examined under a light microscope for cracks, scratches, or hypoplasia, they were subjected to the initial surface microhardness analysis. The Vickers microhardness test was carried out in a digital microhardness tester (Shimadzu HMV-G, Shimadzu Corp., Japan) using a diamond indenter with 980.7 mN force and a dwell time of 15 s for five indentations across each specimen. The Vickers hardness number (VHN) was calculated as the mean of the five readouts taken.

In all specimens, enamel surfaces were divided into 3 parts. These sections were respectively; Treatment (T), Adjacent (A) (adjacent part of the treatment surface) and the remaining three -third surface area covered with a polish that was not be affected by acid.

pH-Cycling Regimen

The specimens were submitted to a pH-cycling regimen for 5 days at 37°C according to Vieira et al [12]. They were subjected daily to alternated immersions in demineralizing solution (2.0 mM $Ca(NO_3)2.4\ H_2O$, 2.0 mM $NaH_2PO_4.2H_2O$, 0.077 mM acetate buffer, 0.02

ppm F, pH = 4.7) for 6 h and in remineralizing solution (1.5 mM $Ca(NO_3)2.4H_2O$, 0.9 mM $NaH_2PO_4.2H_2O$, 150 mM KCl, 0.1M buffer solution, 0.03 ppm F, pH=7.0) for 18 h.

After the pH cycle, microhardness measurements were repeated for each sample from the enamel surface, which was not covered, at intervals of 100 µm, and the mean of the values was recorded.

Experimental Groups

The specimens were randomly divided into 5 groups according to the fluoride-releasing materials applied, as follows (Table 1):

G1-BF: BioMin F toothpaste containing 530 ppm fluoride and bioactive glass was applied to the treatment surfaces for 2 minutes, 2 times a day for 30 days by the help of micro brush. After each application, the specimens were washed with distilled water and kept in the artificial saliva solution until the next application.

G2-CTE: Colgate Triple Effect toothpaste with 1450 ppm fluoride content was applied to the treatment surfaces as in Group 1. Each sample was washed with distilled water after application and kept in artificial saliva.

G3-FCP: FCP COMPLEX solution was prepared by mixing sodium fluoride, calcium chloride and phosphoric acid in a 6: 10: 1 molar ratio. The fluoride concentration (9000 ppm) of the solution was adjusted by adding distilled water. FCP COMPLEX solution was applied to the treatment surfaces and left for 20 seconds and then washed with distilled water for 10 seconds. This process was repeated 4 times once a week. Among the applications, the samples were kept in artificial saliva.

G4-FP: Fluor Protector varnish (1000 ppm) was applied to the treatment surfaces as a thin layer according to the manufacturer's instructions, kept for 1 minute and stored in artificial saliva after application.

G5-C: Since the samples in this group will be considered as a negative control group, after demineralization, they were stored in artificial saliva without any application during the experiment.

Artificial saliva solution used in the present study was prepared according to the formula 14.4 mM NaCl, 16.1 mM KCl, 0.3 mM Cl2.6H2O, 2.9 mM K2HPO4, 1.0 mM CaCl2.2H2O, 0.10 g/100 ml Sodium carboxymethylcellulose, at pH 4.7 [13].

Surface Microhardness Measurement

For post-treatment surface hardness measurements, the surface of the specimens was cleaned with the help of acetone, and then surface microhardness measurements were made for each group as previously mentioned. Five indentations at different distances with 100 µm interval were made in the treatment surface and their average was recorded. For the adjacent (A) surfaces, 5 measurements were made from the three areas which were 150 µm, 300 µm and 450 µm away from the treatment area and their averages were recorded. Comparison of the surface microhardness change was made for the four regions of each sample, as follows: The treatment area (T), Adjacent-150 µm (A-150), Adjacent-300 µm (A-300), Adjacent-450 µm (A-450).

The calculation of the microhardness change percentage (MCP) formed in the demineralized enamel after the procedure was made with the following formula for each test area.

MCP%: (Test Area - Demineralized Area) x100 / Demineralized Area

Subsurface Microhardness Measurement

After the completion of the surface microhardness measurement, the specimens were cut with a microtome device under water cooling in the buccolingual direction from the midpoint of the samples to measure the subsurface microhardness at various depths. One of the two sample halves obtained after each cutting was polished with 320, 600 and 1200 grit sandpapers. Subsurface microhardness values were obtained from these polished surfaces. Measurements were performed at the treatment area (T), adjacent-150 µm, adjacent-300 µm, adjacent-450 µm and the control area (C) which covered with acid-resistant varnish. Considering that "Ideally, the first depth measured should be made as close as possible to the outer enamel surface" (19), the first measurement was carried out at a depth of 20 µm. This first measurement was followed by the depths of 50µm, 100µm and 200µm. After that all sub-surface microhardness values of each sample were compared with the values at the same depth from the C area of the same specimen. With the formula below, the percentage of microhardness loss for each depth (20µm,50µm,100µm and 200µm) was calculated separately and the values obtained were compared.

Formula of the sub-surface microhardness change (SSMC);

SSMC%: (Control Area - Test Area) x 100 / Control Area

Statistical Analysis

The statistical analysis of the data obtained from the study was carried out at Biostatistics Department of Hacettepe University using the software program SPSS 23.0 (SPSS Inc., Chicago IL, USA). The normality of the data was examined by Shapiro-Wilk test.

Two-way analysis of variance (ANOVA) was performed to compare the data obtained after demineralization and application of test materials. One-way analysis of variance was applied to compare the differences between the groups as the interaction was significant. One-way analysis of variance was performed with repeated measurements to compare differences within the group. The 'Post Hoc Tukey' test was applied to compare which groups the difference was. Results were evaluated at $p < 0.05$ significance level and 95% confidence interval.

RESULTS

Surface Microhardness Evaluation

The results of surface microhardness evaluations are presented in Table 2. The values in the treatment area (T) were significantly higher compared to those in the adjacent areas ($p < 0.05$), as observed with A-150>A-300>A-450.

Considering the treatment area and all the distances from the adjacent area, the highest increase in surface microhardness was observed in G1-BF, followed by other groups as G3-FCP > G2-CTE > G4-FP > G5-C ($p<0.05$). This order of increase was also valid when treatment and adjacent areas were evaluated separately ($p <0.05$).

Subsurface Microhardness Evaluation

The sub-surface microhardness loss of the adjacent surfaces was as follows; A-450>A-300>A-150>T ($p <0.05$). This indicates that the subsurface hardness loss increased with distance from the treatment area. Considering the average of all depths and distances, it was observed that G5-C and G4-FP showed similar hardness loss ($p>0.05$), followed by the G3-FCP, G2-CTE and G1-BF, respectively ($p<0.05$).

The sub-surface microhardness loss values at the treatment surface are displayed in Table 3. No significant differences were observed between the 1st and 2nd groups at all depths, as well as between the 4th and 5th groups at depths of 20, 100, and 200 µm.

For the A-150 distance, the loss of substance at the depths of 20, 50 and 200 µm was G5> G4> G3> G2> G1, while the order at 100 µm was G4> G5> G3> G2> G1 ($p <0.05$). In these rankings, the difference between groups 1 and 2 and between groups 4 and 5 were not statistically significant ($p> 0.05$). (Figure 1,a)

For the A-300 distance, the loss of substance at the depths of 20, 100 and 200 µm depths was G4> G5> G3> G2> G1 ($p <0.05$), while the order at 50 µm was G5> G4> G3> G2> G1. ($p <0.05$). In these rankings, the difference between groups 1 and 2 and between groups 4 and 5 was not statistically significant ($p> 0.05$). (Figure 1,b)

For the A-450 distance, at every depth, G4, G5> G3, G2, G1 ($p <0.05$). The difference between 1st, 2nd and 3rd Groups and 4th and 5th Groups were statistically significant ($p< 0.05$). (Figure 1,c)

The average sub-surface microhardness loss across all treatment groups and distances was compared, revealing that the depths of microhardness loss values followed this order: 20 > 50 > 100 > 200 µm ($p < 0.05$). Consequently, it was observed that the hardness loss decreased as the depth increased.

Table 1. The materials and their compositions tested in the study.

Materials	Manufacturer/Lot no	Composition
BioMin F	BioMin Technologies Ltd., London, England (#701197)	Glycerin, silica, polyethylene glycol 400 (PEG 400), fluorocalcium phosphosilicate, sodium lauryl sulfate, titanium dioxide, aroma, carbomer, acesulfame potassium, fluoride <530 ppm
Colgate Triple Action	Colgate-Palmolive Co., Ltd., China (#920354)	Calcium carbonate, aqua, sorbitol, sodium lauryl sulfate, silica hydrate, sodium monofluorophosphate, aroma, cellulose gum, magnesium aluminum silicate, sodium carbonate, benzyl alcohol, sodium saccharin, sodium bicarbonate, eugenol, cl 74160, cl 74260
FCP COMPLEX	US Patent 8956596, February 15, 2015	Fluoride (9000 ppm): calcium: phosphoric acid; 6: 10: 1, distilled water
Fluor Protector	IVOCLAR VIVADENT AG, Schaan, Liechtenstein (#550579)	Bis {4- [2- (difluorohydroxyl) ethyl] -2-methoxy-cyclohexyl [N, N- (trimethylhexane-1,6-dil) dicarbamate] (9 mg) (Fluorosilane), Poly 2.2 - bis (hydroxymethyl), butanoltris [(3-isocyanato-4-methylphenyl) carbamate], ethylacetate isopentyl propionate, fluoride 1000 ppm

Table 2. Mean and standard deviation (SD) of surface VHN for each experimental group.

Groups	Treatment	Adjacent-150µm	Adjacent-300µm	Adjacent-450µm
G1-BF	20.92^{aA} (4.75)	$18,76^{aA}$ (8.25)	13.83^{aB} (7.01)	6.37^{C} (4.76)
G2-CTE	16.18^{bA} (4.30)	13.01^{bcB} (4.93)	10.35^{abB} (4.56)	4.77^{C} (3.74)
G3-FCP	17.94^{abA} (4.35)	14.74^{abB} (4.18)	12.09^{aB} (3.30)	6.03^{C} (2.78)
G4-FP	10.48^{cA} (3.10)	8.69^{cA} (4.55)	6.82^{bA} (5.86)	2.45^{B} (7.90)
G5-C	-1.30^{dA} (3.22)	-2.19^{dB} (5.02)	-3.02^{cB} (2.55)	-2.70^{aB} (5.49)

Different lowercase letters show significant differences in each column. Different capital letters show significant differences in each row. Standard deviation is shown in parentheses.

Table 3. Average values of the percentage of subsurface microhardness loss in the treatment (T) area of the groups at different depths (20µm, 50µm, 100 µm, 200µm).

Groups	20 µm	50µm	100µm	200µm
G1-BF	4.45 (4.91)[a]	4.57 (4.93)[a]	2.60 (4.33)[a]	2.16 (2.50)[a]
G2-CTE	4.84 (6.73)[a]	5.75 (6.26)[a]	4.77 (5.76)[a]	3.49 (5.44)[a]
G3-FCP	12.55 (6.65)[b]	11.58 (6.54)[b]	7.78 (5.68)[b]	5.70 (4.44)[b]
G4-FP	22.93 (5.50)[c]	18.83 (6.06)[c]	14.36 (4.98)[c]	9.13 (5.40)[c]
G5-C	24.36 (7.44)[c]	24.14 (5.44)[d]	15.51 (6.45)[c]	11.76 (3.65)[c]

Different lowercase letters show significant differences in each column. Different capital letters show significant differences in each row. Standard deviation is shown in parentheses.

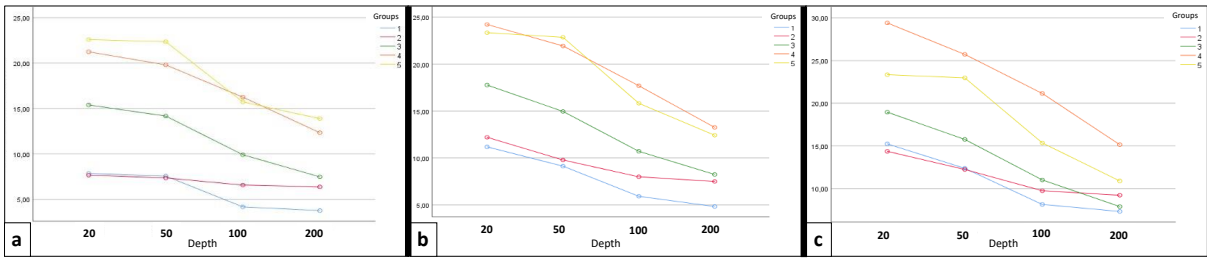

Figure 1. a. Graph showing percentage of subsurface microhardness loss in the A-150 area of the groups; **b.** Graph showing percentage of subsurface microhardness loss in the A-300 area of the groups; **c.** Graph showing percentage of subsurface microhardness loss in the A-450 area of the groups.

DISCUSSION

As a result of developments in preventive dentistry, studies focused on reducing the prevalence of caries and preserving the natural structure of the teeth have intensified. While the rate of caries is gradually decreasing in many developed countries, tooth decay remains a significant health problem in regions where protective applications and non-invasive techniques cannot be used effectively utilized [1]. In recent years, several new calcium phosphate-based delivery systems have been introduced to the dental market. These products have been shown contribute to the remineralization of non-cavitated caries lesions [14].

It has been reported that a two-component solution (1: sodium fluoride + phosphate; 2: calcium chloride + citric acid) can be used as an alternative to NaF solution. This solution provides higher fluoride precipitation on the enamel and dentin at a lower dose of fluoride. Transverse microradiography analysis has shown that this two-component solution restores lost minerals and reduces lesion depth, while NaF solution only reduces mineral loss [15,16].

As a result of the search for a new solution, a specific ratio of fluoride, calcium and phosphoric acid was discovered. This ratio (6: 10: 1) allows the three components to coexist in the solution without precipitation, and the solution was named FCP COMPLEX. In an in-vitro pH cycle model, it was found that this two-component solution is significantly more effective than NaF solution in remineralizing human enamel lesions [17].

It has been suggested that BioMin F, a new toothpaste containing fluoride and bioactive glass, can increase remineralization by continuously releasing calcium, phosphate and fluoride ions [18]. However, there are limited studies in the literature analyzing the remineralization potential of toothpastes that contain both fluoride and bioactive glass [19].

In the present study, remineralization potentials of four materials applied to demineralized enamel samples were evaluated using a surface microhardness test. Based on the results of this study, BioMin F showed the greatest increase in surface microhardness, regardless of the subgroups of distances in the treatment area and adjacent area. FCP COMPLEX followed closely behind BioMin F, while

Colgate and Fluor Protector had the least effect on surface microhardness.

In a systematic review [20]; it was demonstrated that bioactive glass-containing materials have the potential to effectively induce enamel remineralization, regardless of the mode of administration, when compared to control conditions and other topical remineralization treatments.

In a 2018 study, the efficacy of 1450 ppm fluoride-containing toothpaste and BioMin F toothpaste on demineralized enamel was evaluated in terms of microhardness, and Biomin F was found to be more effective [18]. The study also suggested that BioMin F toothpaste showed better enamel remineralization potential, which was attributed to the presence of fluoride and bioactive glass in its composition, as well as its slow and prolonged release of fluoride to obtain maximum benefits [21]. Similarly, in the present study, BioMin F was found to be more effective in increasing surface microhardness compared to 1450 ppm fluoride-containing toothpaste-Colgate Triple Effect.

The most effective fluoride regimen preventing caries involves daily application of topical fluoride in the form of toothpaste and mouthwash. In a study that evaluated 1100 ppm and 500 ppm toothpaste and 22600 ppm fluoride-containing varnish separately and in combination, toothpaste was applied twice a day for 10 days, while varnish was applied twice during the study period. The results showed that varnish application alone was not effective, but the single and combined use of toothpastes was successful [22]. Similarly, in the present study, the results showed that daily application of toothpaste and a weekly solution regimen were more effective in promoting remineralization compared to varnish application, which is consistent with the results of previous studies.

Complete recovery cannot be achieved when only the outer layer of an initial enamel lesion is remineralized, and thus complete remineralization cannot be attained. However, a combination of fluoride and other elements has been shown to have a positive effect on remineralization of all surfaces [23].

Among the remineralization agents used in this study, BioMin F and Colgate toothpastes were the most effective in compensating for the loss of subsurface microhardness, followed by FCP COMPLEX. However, Flour Protector did not show any remineralization effect beneath the surface. This may be attributed to the fact that agents with fluoride concentrations do not achieve complete repair beneath the surface, even though they provide hypermineralization on the surface [24].

Bakry et al. [25] evaluated the remineralization effect of a paste containing 9000 ppm topical fluoride solution and bioactive glass applied to demineralized enamel. According to the results of the study, the paste with bioactive glass improved the micromechanical properties of the sub-surface lesion of demineralized enamel. The high remineralization capacity of the bioactive glass observed in this study may be attributed to its high calcium and phosphate content [25]. Remineralizing agents containing calcium and phosphate have been reported to penetrate deeply and provide better remineralization for the entire depth of enamel subsurface lesions [2]. Similarly, in the present study, the bioactive glass-containing toothpaste showed the highest sub-surface remineralization compared to other materials.

In a study comparing the surface and subsurface effects and fluoride release of FCP COMPLEX and NaF solution applications on caries-affected dentine, a large amount of precipitation was observed on the surface in the FCP COMPLEX group, and a smoother dentin was observed in the section compared to other groups. The fluoride density examined was 14 times more in the FCP COMLEX group than in the NaF group. As a result, FCP-COMPLEX has been shown to significantly increase fluoride accumulation and inhibit demineralization [9].

A remineralization system that maintains sufficient concentrations of fluoride together with calcium and phosphate ions has the ideal effect. In such a system, mineral recovery takes place in the sub-surface layers instead of being limited to the enamel surface [26]. Both BioMin F toothpaste, which provides the most effective remineralization, and FCP COMPLEX solution, which shows similar values, contains fluoride, calcium and phosphate.

A study has shown that early caries can be evaluated using a surface microhardness test, while caries progression can be evaluated using a sub-surface microhardness test. In a sectional analysis, it was observed that as the depth increased, the size of the lesion decreased and the hardness and mineral gain increased [27]. Based on this study, we found that the order of microhardness loss was 20 µm > 50 µm > 100 µm > 200 µm.

In the present study, surface and sub-surface microhardnesses were evaluated at the treatment area and adjacent distances of 150µm, 300µm and 450µm. The general average of all groups showed that the remineralization order decreased with distance, with the highest values observed at the treatment area with surface and sub-surface microhardness analyses. In the control group where no remineralizing agent was

applied, there was no significant difference between the microhardness values at the distances.

One of the limitations of this in-vitro study was the use of bovine teeth. The reasons for choosing bovine teeth were their ease of obtaining large numbers of samples of sufficient quality and the ability to standardize age, nutrition, and other environmental conditions (such as fluoride uptake) that are difficult to control in human teeth. However, despite these advantages, the chemical composition, structural and morphological properties of bovine teeth cannot be fully compared with human teeth, which may have affected the results. Another limitation of this study is that the in vitro method does not fully reflect the oral environment and demineralization process. Factors such as human saliva, nutritional habits and dental pellicle found in in vivo and in situ studies make the experimental conditions compatible with the oral environment. In addition, the lack of a parameter such as SEM or laser fluorescence to support microhardness measurement or the lack of a TMR analysis, which is accepted as the gold standard in the evaluation of mineral change, is a limitation of this study. Given these limitations, the results of this study evaluating the effects of various materials on remineralization of demineralized enamel surfaces and sub-surfaces should be supported by additional in-vitro studies and clinical studies in the light of these findings.

CONCLUSIONS

Within the limitations of this in vitro study, Biomin F may be an effective option for promoting surface and subsurface remineralization compared to other tested remineralization agents. On the other hand, fluoride varnish may not be a good alternative to ensure subsurface remineralization. The findings emphasized the proportional relationship between the remineralization effect of the applied remineralization agent in the treatment area and the adjacent area, highlighting the importance of selecting the agent accordingly.

FUNDING

Not applicable.

CONFLICT OF INTERESTS

None.

REFERENCES

[1] Brostek AM, Walsh LJ. Minimal intervention dentistry in general practice. *Oral Health Dent Manag.* 2014;13(2):285-294.

[2] Reynolds EC, Cai F, Cochrane NJ, et al. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *J Dent Res.* 2008;87(4):344-348. DOI 10.1177/154405910808700420.

[3] ten Cate JM. New agents for caries prevention: introduction to ICNARA 2. *Adv Dent Res.* 2012;24(2):27. DOI 10.1177/0022034512453726.

[4] Twetman S, Axelsson S, Dahlgren H, et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontol Scand. 2003;61(6):347-355.

[5] Twetman S, Petersson L, Axelsson S, et al. Caries-preventive effect of sodium fluoride mouthrinses: a systematic review of controlled clinical trials. Acta Odontol Scand. 2004;62(4):223-230.

[6] Milly H, Festy F, Andiappan M, Watson TF, Thompson I, Banerjee A. Surface pre-conditioning with bioactive glass air-abrasion can enhance enamel white spot lesion remineralization. *Dent Mater.* 2015;31(5):522-533. DOI 10.1016/j.dental.2015.02.002.

[7] Milly H, Festy F, Watson TF, Thompson I, Banerjee A. Enamel white spot lesions can remineralise using bio-active glass and polyacrylic acid-modified bio-active glass powders. *J Dent.* 2014;42(2):158-166. DOI 10.1016/j.jdent.2013.11.012.

[8] Atomura J, Inoue G, Nikaido T, Yamanaka K, Uo M, Tagami J. Influence of FCP-COMPLEX on bond strength and the adhesive-artificial caries-affected dentin interface. *Dent Mater J.* 2018;37(5):775-782. DOI 10.4012/dmj.2017-336.

[9] Mashiko R, Inoue G, Nikaido T, Tagami J. Morphological evaluation of artificial caries-affected dentin after applying FCP-COMPLEX. *J Oral Sci.* 2017;59(3):343-350. DOI 10.2334/josnusd.16-0312.

[10] Adair SM. Evidence-based use of fluoride in contemporary pediatric dental practice. *Pediatr Dent.* 2006;28(2):133-142; discussion 192-138.

[11] BioMin™ (homepage of the Internet). BioMin™ F toothpaste. Available from: https://biomin.co.uk/products/biomintm-f-toothpaste

[12] Vieir AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF. Fluoride dose response in pH-cycling models using bovine enamel. *Caries Res.* 2005;39(6):514-520. DOI 10.1159/000088189.

[13] Poggio C, Lombardini M, Vigorelli P, Ceci M. Analysis of dentin/enamel remineralization by a CPP-ACP paste: AFM and SEM study. *Scanning.* 2013;35(6):366-374. DOI 10.1002/sca.21077.

[14] Elkassas D, Arafa A. Remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles

on artificial caries like enamel lesions. *J Dent.* 2014;42(4):466-474. DOI 10.1016/j.jdent.2013.12.017.

[15] Takagi S, Liao H, Chow LC. Effect of a low-fluoride-content, two-component rinse on fluoride uptake and on de--and remineralization of enamel lesions: an in vitro study. *Caries Res.* 2001;35(3):223-228. DOI 10.1159/000047460.

[16] Chow LC, Takagi S, Carey CM, Sieck BA. Remineralization effects of a two-solution fluoride mouthrinse: an in situ study. *J Dent Res.* 2000;79(4):991-995. DOI 10.1177/00220345000790041601.

[17] Chow LC, Takagi S, Shih S. Effect of a two-solution fluoride mouthrinse on remineralization of enamel lesions in vitro. *J Dent Res.* 1992;71(3):443-447. DOI 10.1177/00220345920710030401.

[18] Alhussain AM, Alhaddad AA, Ghazwi MM, Farooq I. Remineralization of artificial carious lesions using a novel fluoride incorporated bioactive glass dentifrice. *Dent Med Probl.* 2018;55(4):379-382. DOI 10.17219/dmp/97311.

[19] Ali S, Farooq I, Al-Thobity AM, Al-Khalifa KS, Alhooshani K, Sauro S. An in-vitro evaluation of fluoride content and enamel remineralization potential of two toothpastes containing different bioactive glasses. *Biomed Mater Eng.* 2020;30(5-6):487-496. DOI 10.3233/BME-191069.

[20] Taha AA, Patel MP, Hill RG, Fleming PS. The effect of bioactive glasses on enamel remineralization: A systematic review. *J Dent.* 2017;67:9-17. DOI 10.1016/j.jdent.2017.09.007.

[21] ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. *Acta Odontol Scand.* 1999;57(6):325-329. DOI 10.1080/000163599428562.

[22] Amaechi BT, Mathews SM, Ramalingam K, Mensinkai PK. Evaluation of nanohydroxyapatite-containing toothpaste for occluding dentin tubules. *Am J Dent.* 2015;28(1):33-39.

[23] Iijima M, Du C, Abbott C, Doi Y, Moradian-Oldak J. Control of apatite crystal growth by the co-operative effect of a recombinant porcine amelogenin and fluoride. *Eur J Oral Sci.* 2006;114 Suppl 1:304-307; discussion 327-309, 382. DOI 10.1111/j.1600-0722.2006.00324.x.

[24] Ogaard B, Rolla G, Arends J. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. *Am J Orthod Dentofacial Orthop.* 1988;94(1):68-73. DOI 10.1016/0889-5406(88)90453-2.

[25] Bakry AS, Marghalani HY, Amin OA, Tagami J. The effect of a bioglass paste on enamel exposed to erosive challenge. *J Dent.* 2014;42(11):1458-1463. DOI 10.1016/j.jdent.2014.05.014.

[26] Krishna MT, York M, Chin T, et al. Multi-centre retrospective analysis of anaphylaxis during general anaesthesia in the United Kingdom: aetiology and diagnostic performance of acute serum tryptase. *Clin Exp Immunol.* 2014;178(2):399-404. DOI 10.1111/cei.12424.

[27] Queiroz CS, Hara AT, Paes Leme AF, Cury JA. pH-cycling models to evaluate the effect of low fluoride dentifrice on enamel de- and remineralization. *Braz Dent J.* 2008;19(1):21-27. DOI 10.1590/s0103-64402008000100004.