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EFFICACY OF A NOVEL FLUORIDE CONTAINING BIOACTIVE GLASS BASED DENTIFRICE IN REMINERALIZING ARTIFICIALLY INDUCED DEMINERALIZATION IN HUMAN ENAMEL

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ABSTRACT: The aim of the study was to compare the remineralization potential of a novel dentifrice consisting of a fluoride-containing bioactive glass (BiominF®) with that of a dentifrice containing bioactive glass (BG) and sodium monofluorophosphate (Novamin®). Fifteen enamel blocks were divided randomly into three groups: 1: (*n* = 3; control, no treatment, storage in artificial saliva for 24 hr), 2: (*n* = 6; Novamin® toothpaste group), and 3: (*n* = 6; BiominF® toothpaste group). Toothpaste slurries were prepared by mixing 1 g toothpaste with 5 mL artificial saliva (AS). The specimens were exposed to 6 wt% citric acid (pH, 2.2) for 5 min to mimic demineralization, and specimens in groups 2 and 3 were then stored in the toothpaste slurries for 5 min and 24 hr (*n* = 3 each). Mean enamel volume changes were evaluated by micro–computed tomography, and mean surface loss or gain was investigated using a profilometer. The Wilcoxon rank sum test and one-way analysis of variance with post-hoc testing were used to identify significant differences (*p* < 0.05). BiominF® performed better than Novamin® in terms of remineralization after 5 min and 24 hr, as observed by micro-CT. The surface roughness values of all specimens decreased significantly after exposure to AS and the toothpaste mixtures (*p* < 0.05), with BiominF® specimens showing greater reduction after 5 min. A toothpaste comprised of a fluoride-containing BG showed promising potential to promote remineralization of demineralized human enamel.

Keywords: Bioactive glass; Demineralization; Enamel; Fluoride; Remineralization.

1. INTRODUCTION

Dental caries is a disease that initiates with demineralization of the tooth structure, caused by the acids produced by the cariogenic bacteria residing in dental plaque.[1] Although the prevalence of dental caries has decreased worldwide in the past four decades, this disease remains among the most common reasons for seeking dental treatment.[2] The composition of dental plaque varies among individuals,[3] but the most effective method suggested for its removal remains mechanical cleaning with the use of effective cleansing agents, such as toothpastes.[4]

Toothpastes are everyday oral care products that could be classified as drugs.[5] Several ingredients are incorporated into toothpastes to produce cleansing, antibacterial effects, and the remineralization of demineralized tooth structures.[6] Fluoride, calcium, phosphate, sodium, and magnesium are of particular interest, as they have many beneficial effects in the human body.[7] Fluoride in excessive quantity can damage human health [8] and teeth (especially in infancy)[9,10] but in correct amounts, it causes mineralization of teeth[11] and has a beneficial cariostatic

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effect.[12,13] Replacement of the hydroxyl (OH^-) ion with fluoride (F^-) in the apatite crystal structure of enamel makes it less soluble and more resistant to acidic encounters.[14] Various modes of delivery of fluoride to the oral cavity have been proposed; these include its incorporation into mouthwashes, varnishes, gels, and toothpastes.[15] Bioactive glass (BG) is a biocompatible material, traditionally used because of its osteogenic properties,[16] but its use in dentistry has been encouraged recently due to its compositional resemblance to bone and dental enamel.[17] It is composed of 45% silica, 26.9% calcium oxide, 24.4% sodium oxide, and 6% phosphorus pentoxide in weight percentage [18] and lacks fluoride. NovaMin® is a patented ingredient based on the traditional BG composition that is included in several professional over-the-counter toothpastes formulated to relieve tooth sensitivity and promote enamel remineralization.[19] Recently, a BG-based toothpaste with fluoride and high-phosphate-content glass (BiominF®) was introduced to the market, and claims have been made 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.[20]

Toothpastes that effectively produce optimum remineralization are being researched globally, as they can reduce the need for unnecessary removal of healthy tooth structures, which occurs during routine restorative dental procedures. Therefore, this study was carried out to assess the enamel remineralization potential of BiominF®-based toothpaste and compare it with that of a toothpaste containing Novamin® as an active ingredient.

2. MATERIALS AND METHODS

Ethical approval was obtained before commencing the study and all the procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 and its later amendments. Thirty human molars that were extracted for periodontal reasons were cleaned to remove calculus and soft-tissue debris, and inspected under a stereomicroscope (Nikon SMZ800, Japan). Fifteen teeth that were free of white spot lesions, caries, and restorations were stored in 1% thymol solution and used within 1 month of storage. All teeth were sectioned at the cemento-enamel junction using a water-cooled diamond saw (Isomet® 5000 Linear Precision Saw; Buehler Ltd., IL, USA). To facilitate specimen re-positioning, all sections were embedded in self-cure acrylic resin using plastic rings, leaving the enamel surfaces exposed. Artificial saliva (AS) was prepared by mixing 0.400 g NaCl, 0.400 g KCl, 0.69 g NaH_2PO_4 H_2O, 0.795 g $CaCl_2$ H_2O, and 0.005 g Na_2 $9H_2O$ in 1000 mL deionized water, as described by Fusayama et al.[21] The pH of freshly prepared AS was adjusted to 7.0 by adding 1 M sodium hydroxide (NaOH).

2.1. Experimental groups

The teeth (N = 15) were divided randomly into three groups: group 1 (n = 3; control, no treatment, storage in artificial saliva for 24 hr), group 2 (n = 6; Novamin® toothpaste group), and group 3 (n = 6; BiominF® toothpaste group). The compositions of these toothpastes are shown in Table 1. Toothpaste slurries were prepared freshly on the day of application by mixing 1 g toothpaste with 5 mL AS, and 15 mL of this mixture was used in each individually labeled container.

Table 1. The composition of the tested toothpastes

Toothpaste	Manufacturer (Country)	Composition	Active ingredient
Novamin	Glaxosmithkline (UK)	Glycerin, PEG-8, Silica, (NovaMin), Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Titanium Dioxide, Aroma, Carbomer, Sodium Saccharin, Limonene	Novamin® (Calcium Sodium Phosphosilicate)
BiominF	BiominF Technologies Limited (UK)	Glycerin, Silica, PEG 400, Fluoro Calcium Silicate, Sodium Lauryl sulphate, Titanium dioxide, Aroma, Carbomer, Potassium Acesulfame Fluoride 1000 ppm	Biomin® (Fluoro Calcium Phosphosilicate)

2.2. Demineralization/remineralization procedure

For demineralization, 6 wt% citric acid with a pH of 2.2 was used. Enamel blocks were immersed in citric acid for 5 min, and then washed thoroughly with distilled water and air dried. The three control (group 1) specimens were then placed in AS for 24 hr. Three samples each from groups 2 and 3 were placed in their respective toothpaste slurries for 5 min, and three samples each were placed in these slurries for 24 hr. After remineralization for 5 min or 24 hr, specimens were again washed with distilled water and air dried.

2.3. Micro-computed tomography

The remineralization potential of the toothpastes was evaluated by analyzing volume changes in the dental enamel using micro–computed tomography (SkyScan 1172, version 1.5; Bruker Micro-CT, Kontich, Belgium). All specimens were scanned using the following parameters: 100-kV source voltage, 100-µA source current, 27.45-µm image pixel size, Al + Cu filter, TIFF image format, 1600-msec exposure, 0.700º rotation step, 3 frame averaging, 10 random movement, and 360º rotation. Raw images were reconstructed using the NRecon software (Bruker SkyScan, Aartselaar, Belgium).

For each specimen, a volume of interest was created manually by outlining the enamel boundary wall, with non-enamel objects excluded. Enamel volume (µm^3) was calculated by outlining the same area after each scan of the corresponding specimen using the CTscan software (Bruker SkyScan, Aartselaar, Belgium). Each specimen was scanned three times – a) at baseline (sound enamel), b) after demineralization, and c) after remineralization – and volume changes were compared.

2.4. Surface roughness

Following micro-CT scanning, at each experimental timepoint, surface roughness was measured as average profile roughness (Ra) using a Contour GT Optical Microscopes profilometer (Bruker, Tucson, AZ, USA). Each specimen was placed in a marked, fixed position for each measurement, and overview scans were performed to identify areas of repeated measurement at each timepoint. Three measurements were made on each specimen at each timepoint (baseline, after demineralization, and after remineralization), and the mean of the three readings was used for statistical analysis.

2.5. Statistical analysis

The SPSS statistical software package (version 19.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The Wilcoxon rank sum test and one-way analysis of variance, followed by post-hoc multiple comparison tests, were used. The significance level was set to $p < 0.05$.

3. RESULTS

Micro-CT examination demonstrated that the AS and tested toothpastes could remineralize demineralized enamel surfaces. Although the BiominF®-based toothpaste showed greater remineralization capability, remineralization efficacy did not differ significantly between toothpastes. BiominF® performed slightly better than Novamin® in terms of remineralization after 5 min and 24 hr exposure (Table 2).

Table 2. Mean enamel volumes at different time intervals observed through micro-CT

Group	Micro-CT volume		
	Baseline (mm^3)	Post-demineralization (mm^3)	Post-remineralization (mm^3)
Artificial saliva	13.65	13.10	13.37
Novamin 5 min	17.88	17.13	17.32
BiominF 5 min	18.28	18.04	18.21
Novamin 24 hr	17.71	17.25	17.35
BiominF 24 hr	16.28	15.47	15.80

No significant differences observed on intra and inter group comparisons

The demineralization procedure significantly increased the surface roughness values of all enamel specimens compared with baseline ($p < 0.05$). The surface roughness values of all specimens decreased significantly after exposure to AS and the toothpaste slurries for 5 min and 24 hr, demonstrating the remineralizing capabilities of saliva and the toothpastes ($p < 0.05$; Figures 1–2, Table 3).

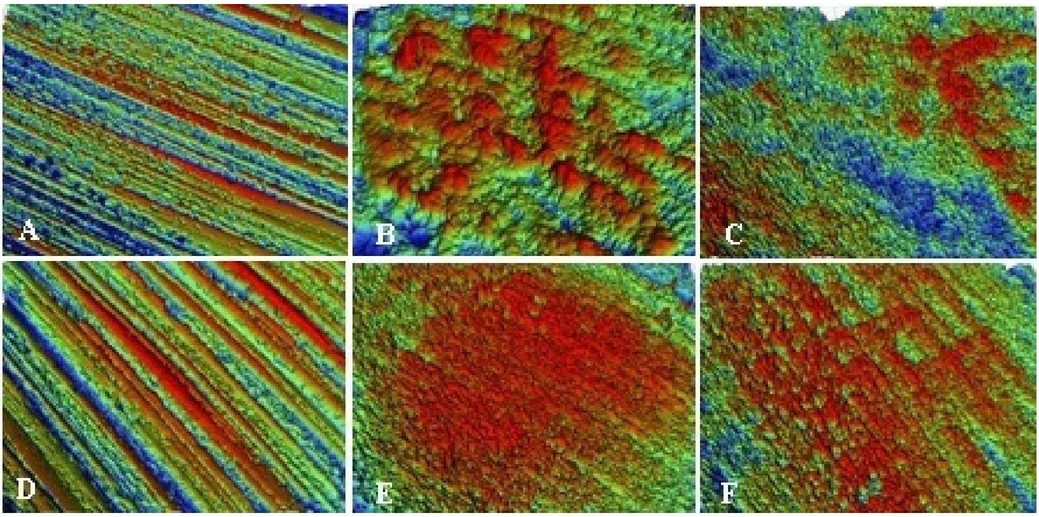

Figure 1. Enamel surface profiles for the Novamin® group: A) baseline, B) demineralized, C) after a 5-minute exposure to Novamin toothpaste, D) baseline, E) demineralized, and F) after 24-hours of exposure to Novamin toothpaste.

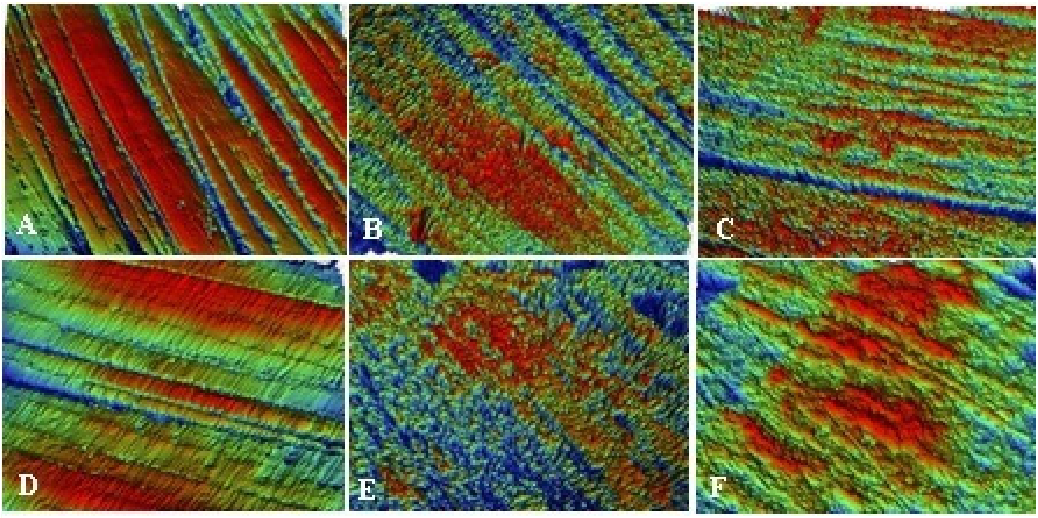

Figure 2. Enamel surface profiles for BiominF® group: A) baseline, B) demineralized, C) after a 5-minute exposure to BiominF toothpaste, D) baseline, E) demineralized, F) after a 24-hour exposure to BiominF toothpaste.

Table 3. Mean surface roughness values at different time intervals observed through a profilometer

Group	Surface roughness value (nm)		
	Baseline (Mean ± SD)	Post-demineralization (Mean ± SD)	Post-remineralization (Mean ± SD)
Artificial Saliva	405.24 ± 87.80	557.54 ± 71.31	472.36 ± 64.98*
Novamin 5 min	469.49 ± 151.91	767.95 ± 99.14	636.77 ± 100.66*
BiominF 5 min	521.17 ± 74.70	710.12 ± 118.13	599.6 ± 111.85*
Novamin 24 hr	519.14 ± 92.61	677.14 ± 120.64	560.47 ± 119.63*
BiominF 24 hr	499.51 ± 135.49	694.41 ± 130.12	556.76 ± 109.77*

*A statistically significant decrease in the surface roughness of the demineralized specimens following remineralization is present ($p < 0.05$)

4. DISCUSSION

In this study, the storage of specimens in mixtures of toothpaste and AS resulted in enamel volume regain. This finding reveals that the composition of these toothpastes adds to the existing mineral pool in AS. The BiominF®-based toothpaste showed better remineralizing capability than did the Novamin®-based toothpaste, according to micro-CT and surface roughness evaluations, especially after 5 min. This finding could be attributed to a difference in composition; the Novamin® toothpaste is based on a conventional 45S5 (Bioglass®) composition, which contains no fluoride,[22] whereas the BiominF® toothpaste contains a high-phosphate fluoride with BG, which has several benefits. When a BG is exposed to an aqueous solution like saliva, ionic exchange reactions occur and the glass begins to dissolve, releasing calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions, which results in the formation of hydroxycarbonated apatite.[23] Although based on BG composition, BiominF® differs slightly in that it releases fluoride ions in addition to Ca^{2+} and PO_4^{3-}, resulting in the formation of fluorapatite, which is quite desirable for various dental applications due to its increased acid resistance.[24]

Novamin® contains sodium monofluorophosphate, which is hydrolyzed in water to yield fluoride and PO_4^{3-} ($PO_3F_2^- + OH^- \rightarrow HPO_4^{2-} + F^-$).[25] The amount of fluoride available in the oral cavity for remineralization is questionable, as the fluoride–phosphate is cleaved further by the enzyme alkaline phosphatase.[26] BiominF®, on the other hand, contains fluoride in its BG composition with high PO_4^{3-} content, which could serve as a source of the delivery of all essential ions (Ca^{2+}, PO_4^{3-}, and F^-) together to form fluorapatite, rather than fluorite (CaF_2).[27] Brauer et al.[28] showed that high PO_4^{3-} content helps to maintain network connectivity of the glass and ensures the formation of fluorapatite, which is more desirable for dental clinical applications.

Another potential advantage of the addition of fluoride to BG is the achievement of a slow, sustained release of fluoride. Traditional fluorides incorporated in toothpastes are usually washed away quickly by the salivary flow.[29] Fluoride should be deposited and released slowly to achieve maximum benefit.[30] Farooq et al.[31] developed fluoride-containing BG compositions that formed apatite in Tris buffer solution within 6 hr, which was faster than observed for 45S5 (Bioglass®; 24 hr). Therefore, the superior remineralization potential of BiominF® could be attributed to the presence of fluoride, in addition to BG, in its composition.

In previous studies, researchers have used bovine enamel as a substitute for human enamel.[32, 33] In our study, we utilized extracted human molars to replicate realistic *in-vivo* conditions. This approach constitutes a study strength, but it also explains the small sample, as extracted teeth that are free from caries and other pathologies are difficult to obtain.

Dental enamel is composed of approximately 90% hydroxyapatite (HAP) crystals (by volume).[34] In environments supersaturated with minerals (e.g., toothpaste in saliva), increased remineralization of dental enamel is thus expected. The potential of a supersaturated environment can be estimated using its pH value. After preparation, the pH values of groups 2 and 3 (7.3 and 7.6, respectively) were higher than neutral (7.0), indicating good remineralization potential.

An earlier study produced similar results, showing highly effective enamel remineralization of such toothpastes through the formation of a protective layer on the demineralized enamel surface.[35] The results of the present study also show better remineralization in both BG groups. The presence of BG in saliva causes the release of sodium ions and increases the pH, resulting in more precipitation and formation of a calcium HAP layer on the external surface of hard tissue.[36]

Researchers have used various techniques, including those involving physical cross-sectional measurement and the examination of standard CT images, to measure enamel volume.[37] These techniques, however, are somewhat problematic; the cross-sectional method is destructive and standard CT produces low-resolution images, which are unsuitable for reliable measurement.[36] Micro-CT is a non-destructive technique that provides high resolution three-dimensional images, and many researchers prefer it for the measurement of enamel thickness/volume.[37,38] For this reason, we used micro-CT for the measurement of enamel volume changes in this study.

BiominF® showed better performance than Novamin® in decreasing surface roughness values, especially after 5 min. This could be the result of BiominF® particles adhering to the enamel surfaces and delivering essential remineralizing ions (calcium, phosphate, and fluoride) immediately.[39]

To our knowledge, this study is the first to compare the remineralization potential of BiominF® and Novamin® in a head-to-head trial. The positive results of this study could serve as a basis for future quantitative and clinical studies of the effects of these dentifrices under more vigorous *in-vivo* conditions.

CONCLUSION

BiominF® toothpaste showed a substantial potential to promote remineralization of demineralized human enamel. Future *in-vivo* studies are recommended to assess its clinical effectiveness.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

REFERENCES

1 Featherstone JDB. Dental caries: a dynamic process. Aust Dent J 2008;53:286-91.

2 Petersen PE, Bourgeois D, Ogawa H, Estupinan-Day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bull World Health Organ 2005;83:661-9.

3 Filoche S, Wong L, Sissons CH. Oral biofilms: Emerging concepts in microbial ecology. J Dent Res 2010;89:8-18.

4 Collins WJ, Walsh TF, Figures K. Handbook for dental hygienists. 4th ed. Bristol, UK: Butterworth-Heinemann;1998; pp 272-3.

5 Regos J, Hitz HR. Investigations on the mode of action of triclosan, a broad-spectrum antimicrobial agent. Zentralbl Bakteriol Orig A 1974;226:390-401.

6 Maldupa I, Brinkmane A, Rendeniece I, Mihailova A. Evidence based toothpaste classification, according to certain characteristics of their chemical composition. Stomatologija 2012;14:12-22.

7 Oyewale OA. Estimation of the essential inorganic constituents of commercial toothpastes. J SciInd Res 2005;64:101-7.

8 Zazouli MA, Mahvi AH, Dobaradaran S, Barafrashtehpour M, Mahdavi Y, Balarak D. Adsorption of fluoride from aqueous solution by modified *Azolla filiculoides*. Fluoride 2014;47(4):349-58.

9 Dobaradaran S, Mahvi AH, Dehdashti S, Abadi DRV. Drinking water fluoride and child dental caries in Dashtestan, Iran. Fluoride 2008;41(3):220-6.

10 Mahvi AH, Ghanbarian M, Ghanbarian M, et al. Determination of fluoride concentration in powdered milk in Iran 2010. Br J Nutr 2012;107(7):1077-9.

11 Faraji H, Mohammadi AA, Akbari-Adergani B, et al. Correlation between fluoride in drinking water and its levels in breast milk in Golestan Province, Northern Iran. Iran J Public Health 2014;43(12):1664-8.

12 Aghaei M, Karimzade S, Yaseri M, Khorsandi H, Zolfi E, Mahvi AH. Hypertension and fluoride in drinking water: case study from West Azerbaijan, Iran. Fluoride 2015;48(3)252-8.

13 Mahvi AH, Zazoli MA, Younecian M, Nicpour B, Babapour A. Survey of fluoride concentration in drinking water sources and prevalence of DMFT in the 12 years old students in Behshar City. J Med Sci 2006;6:658-61.

14 Mohammed RN, Lynch MJR, Anderson P. Effects of fluoride concentration on enamel demineralization kinetics *in vitro*. J Dent 2014;42:613-8.

15 Ripa LW. A critique of topical fluoride methods (dentifrices, mouthrinses, operator-applied, and self-applied gels) in an era of decreased caries and increased fluorosis prevalence. J Public Health Dent 1991;51:23-41.

16 Hench LL, Splinter RJ, Allen WC, Greenlee TK. Bonding mechanisms at the interface of ceramic prosthetic materials. J Biomed Mater Res 1971;5:117-41.

17 Farooq I, Imran Z, Farooq U, Leghari A, Ali H. Bioactive glass: A material for the future. World J Dent 2012;3:199-201.

18 Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res 2013;4:78-83.

19 Kumar A, Singh S, Thumar G, Mengji A. Bioactive Glass Nanoparticles (NovaMin®) for Applications in Dentistry. IOSR-JDMS 2015;14: 30-5.
20 BioMin™ [homepage of the Internet]. BioMin™ F toothpaste. Available from:.https://biomin.co.uk/products/biomintm-f-toothpaste [cited 2017 Nov 16].
21 Fusayama T, Katayori T, Nomoto S. Corrosion of gold and amalgam placed in contact with each other. J Dent Res 1963;42:1183-97.
22 Greenspan DC. NovaMin® and tooth sensitivity-an overview. J Clin Dent 2010; 21:61-5.
23 Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res 2013;4:78-83.
24 Robinson C, Shore RC, Brookes SJ, Strafford S, Wood SR, Kirkham J. The chemistry of enamel caries. Crit Rev Oral Biol Med 2000;11:481-95.
25 Schrödter K, Bettermann G, Staffel T, Wahl F, Klein T, Thomas Hofmann T. Phosphoric Acid and phosphates. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim, Germany: Wiley-VCH;2008.
26 Setnikar I, Ringe JD, Fluoride compounds. Pharmacokinetics and bioavailability. Arzneimitteltherapie 1995;13:73-9.
27 Mneimne M, Hill RG, Bushby AJ, Brauer DS. High phosphate content significantly increases apatite formation of fluoride-containing bioactive glasses. Acta Biomater 2011;7:1827-34.
28 Brauer DS, Karpukhina N, O'Donnell MD, Law RV, Hill RG. Fluoride-containing bioactive glasses: effect of glass design and structure on degradation, pH and apatite formation in simulated body fluid. Acta Biomater 2010;6:3275-82.
29 Aasenden R, Brudevold F, Richardson B. Clearance of fluoride from the mouth after topical treatment or the use of a fluoride mouthrinse. Arch Oral Biol 1968;13:625-36.
30 Ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand 1999;57:325-9.
31 Farooq I, Tylkowski M, Müller S, Janicki T, Brauer DS, Hill RG. Influence of sodium content on the properties of bioactive glasses for use in air abrasion. Biomed Mater 2013;8:065008.
32 Kielbassa AM, Hellwig E, Meyer-Lueckel H. Effects of irradiation on in situ remineralization of human and bovine enamel demineralized *in vitro*. Caries Res 2006;40:130-5.
33 Tschoppe P, Kielbassa AM, Meyer-Lueckel H. Evaluation of the remineralizing capacities of modified saliva substitutes in vitro. Arch Oral Biol 2009;54:810-6.
34 Goldberg M, Septier D, Le'colle S, Chardin H, Quintana MA, Acevedo AC, et al. Dental mineralization. Int J Dev Biol 1995;39:93-110.
35 Milleman LJ, Milleman RK, Clark EC, Mongiello AK, Simonton CT, Proskin MH. NUPRO sensodyne prophylaxis paste with Novamin® for the treatment of dentin hypersensitivity: a 4-week clinical study. Am J Dent 2012;25:262-8.
36 Swain MV, Xue J. State of the art of micro-CT applications in dental research. Int J Oral Sci 2009;1:177-88.
37 Olejniczak AJ, Smith TM, Wang W, Potts R, Ciochon R, Kullmer O, et al. Molar enamel thickness and dentine horn. Am J Phys Anthropol 2008;135:85-91.
38 Smith TM, Harvati K, Olejniczak AJ, Reid DJ, Hublin JJ, Panagopoulou E. Brief communication: dental development and enamel thickness in the Lakonis Neanderthal molar. Am J Phys Anthropol 2009;138:112-8.
39 BioMin: armour for teeth [homepage on the Internet]. How to brush your teeth for the best results. Available from: https://www.biomintoothpaste.com.au/consumer-2/how-to-brush-your-teeth-for-the-best-results/ [cited 2017 Nov 20]