Comparative Fluoride Release and Antimicrobial Analysis of Commercial and Experimental Bioactive Glass/Nano-Oxide-Based Dentifrices

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Abstract

Objectives The objectives were to measure fluoride release and assess the antimicrobial behavior of fluoride-doped nano bioactive glass (F-nBG) and nano zinc oxide (ZnO)-enriched novel dentifrices.

Materials and Methods Experimental dentifrices were synthesized by incorporating ZnO nanoparticles and F-nBG (1.5 wt% and 4 wt%) as active ingredients. The fluoride release behavior of suspensions and elutes of samples were analyzed by ion selective electrode. Antimicrobial activity and minimum bactericidal concentration against Streptococcus mutans and Lactobacillus casei were evaluated. Microbial stability against contamination was also assessed by a challenge test.

Results The fluoride release behavior of experimental dentifrices was higher than that of commercial dentifrices and was dependent on filler loading. The fluoride release was more from suspensions than elutes. Zones of inhibition (ZOIs) and minimum bactericidal concentration values for novel dentifrices showed direct proportionality with filler loading, and effectiveness was exhibited against both strains. Experimental dentifrices exhibited effective antibacterial potential, which could possibly be due to release of sufficient fluoride and zinc ions in aqueous media from F-nBG and ZnO present in their formulations.

Conclusion Combination of F-nBG and ZnO may provide a multi-benefit approach for simultaneously treating early white spot lesions, reducing bacterial growth, and providing core plaque control.

Introduction

Brushing teeth with fluoridated toothpaste has been widely practiced worldwide for dental caries prevention, as it removes biofilm mechanically, which adds to the therapeutic effects of fluoride. Other ingredients for functions such as whitening, antiplaque, antibacterial, antitartar, and erosive prevention can be added.¹ Availability and stability of fluoride in dentifrices are basic requirements for effectiveness against tooth decay.²,³ Fluoride ions can inhibit...
the production of bacterial acids in dental plaque due to the influx of hydrogen fluoride into bacterial cells, and the dissociation to the H+ and F- ions in the cytoplasm. For caries control, dentifrice formulation should contain a minimum of 1000 parts per million (ppm) fluoride, which must be in a soluble form to impart the anticaries effect. The factors which control the soluble fluoride content in dentifrice are its composition (i.e., fluoride source) and storage conditions (i.e., temperature and aging). It is reported that low-pH toothpastes containing 500 to 500 ppm fluoride showed similar results on enamel demineralization as the one containing 1100 ppm fluoride.

Dentifrice composition plays a crucial role in disabling free fluoride ions, which may lead to creation of a low-solubility product with a diminished anticaries outcome. Currently, there are no regulations to specify how much of total fluoride should be maintained in a dentifrice formulation. There has been a growing interest in the use of “smart” bioactive materials (i.e., amorphous calcium phosphate, hydroxyapatite, bioactive glass, etc.) for tooth remineralization. It is reported that the remineralization effect of nano-hydroxyapatite crystals is generally limited to the surface of the initial carious lesion. Bioactive glass is one such material being considered as a novel dental material with remineralization potential. Some in vitro studies have shown that bioactive glass-containing dentifrices minimized dentin hypersensitivity by dentinal tubule occlusion and apatite formation.

Recently, fluoride-doped bioactive glass nanoparticles (F-nBG) have been synthesized by our group, and the long-term fluoride release behavior of F-nBG and its effect on pH was evaluated. Zinc oxide (ZnO) powder has been added as a preservative in dentifrices, which, in aqueous suspension, not only inhibits dentin demineralization but also induces antimicrobial action by yielding zinc ions (Zn2+) and reactive oxygen species. The purpose of this study was to assess and compare the antimicrobial efficacy and fluoride release behavior from the suspensions and elutes of experimental dentifrices and a commercial dentifrice.

**Materials and Methods**

In this study, all chemicals were analytical grade. Initially, basic ingredients were mixed in optimized ratios and allowed to mix homogeneously. Then 1.5 wt% titanium dioxide (Sigma Aldrich; St. Louis, MO, United States) and 3 wt% zinc oxide nanoparticles were added in increments to avoid agglomeration. The ZnO nanoparticles were synthesized by our group as described previously. The scanning electron microscopy (SEM) image and X-ray diffraction (XRD) pattern of prepared ZnO nanoparticles are shown in Fig. 1. The morphological pattern showing the nanostructure of prepared ZnO and the diffractogram is in accordance with JCPDS N 00-036-1451 (standard X-ray peaks of zinc oxide). F-nBG (5% mol. concentration of F in F-BG) was used in this study and incorporated in experimental dentifrices in two ratios: 1.5% and 4% wt/wt. Colgate Cavity Protection (Colgate-Palmolive, Guildford, UK) was used as the control. The composition of both the control and the experimental dentifrices is given in Table 1.

**Fluoride Release Analysis**

The fluoride research protocol used in this study was modified from the one suggested by Pearce. The study was performed in quadruplicates. From each group, a 100 mg dentifrice sample was homogenized in 10 mL deionized water to form dentifrice suspensions, from which half of the suspension was split in two centrifuge tubes, each containing 2.5 mL suspension. The remaining half dentifrice suspension was centrifuged (Hettich EBA 20; Hettich, Tuttingen, Germany) at 6000 rpm for 10 min (2461 × g) to remove the insoluble fluoride bound to filler particles to extract elute into two centrifuge tubes, each containing 2.5 mL of elute. The same procedure was repeated for all dentifrice groups.

Then 2.5 mL of 2M HCl (BDH Chemicals; Hull, East Yorkshire, UK) was added to all the centrifuge tubes containing the dentifrice elute and suspension samples, which were then conditioned at 45°C for 1 hour followed by an addition of 5 mL of 1M NaOH (Sigma Aldrich) and 1 mL TISAB III reagent (Hanna Instruments; Woonsocket, Rhode Island, United States). Finally, the samples were analyzed using pre-calibrated ion selective electrode (ISE) potentiometry (Hanna HI3222 pH/ISE meter and fluoride electrode; Hanna Instruments, Woonsocket, Rhode Island, United States).

**Antimicrobial Susceptibility Test**

For the antimicrobial susceptibility test, freeze-dried microbial strains *Streptococcus mutans* (ATCC-25175) and *Lactobacillus casei* (ATCC-393) were obtained from American Type Culture Collection (ATCC; Manassas, VA, United States). Dehydrated culture media was obtained from Oxoid (Basingstoke, Hampshire, England) and Merck Millipore (Darmstadt, Germany). Microorganisms were revived from freeze-dried vials in their respective culture media—MRS (de Man Rogosa) broth for *L. casei* and BHI (Brain Heart Infusion) broth for *S. mutans*—as per American type culture collection (ATCC) guidelines. For the test procedure, 2 g of selected dentifrice (Table 1) was mixed in 2mL of sterile deionized water, and this yielded a 1:1 dilution. The same procedure was applied to prepare three dilutions of each dentifrice in the ratios of 1:1, 1:2, and 1:4, respectively. Dentifrice slurries were vortexed in an electric shaker (Heidolph REAX 2000; Gemini BV Laboratory, DG Apeldoorn, the Netherlands). For inoculum preparation, three to five well-isolated, similar colonies were selected and transferred to tubes containing 5 mL of 0.9% saline. The suspensions were then adjusted to a density equivalent to 0.5 standard of the McFarland Turbidity Scale (DensiCHEK plus; bioMérieux, NC, United States). Within 15 min of the preparation of the adjusted inoculum suspensions, it was swabbed evenly over the entire surface of the respective agar plates of prepared media to create a bacterial lawn.

**Agar Well-Diffusion Assay**

A sterile cork borer was used to create four wells in each of the agar plates. The punched agar circles were picked with a sterile needle and removed from the plates. The wells were 8 mm in diameter and 4 mm in depth and were placed at distances of 20 mm in each of the plates. Then, 0.2 mL of the three...
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Table 1  Grouping and composition of dentifrices used in the study

| Dentifrice                                      | Abrasive               | Other ingredients (%) wt | Fluoride agent Percentage | Group name |
|------------------------------------------------|------------------------|---------------------------|---------------------------|------------|
| Colgate cavity protection (Colgate-Palmolive, Guildford, UK) Lot-number: 10141222 | Calcium carbonate (CaCO₃) | Water, Glycerin, Sodium lauryl sulfate, Sodium saccharin, Cellulose gum, Flavoring agent, Tetrasodium pyrophosphate | SMFP 0.76 w/w (0.15 w/v) | CD         |
| Experimental dentifrices                        | Calcium carbonate (CaCO₃) 27% wt | Water ~ 36%, Glycerol ~ 32%, Sodium benzoate ~ 1%, Methylcellulose ~ 1%, Flavoring agent ~ 1%, Sodium lauryl sulfate ~ 2%, Water ~ 36%, Titanium dioxide (TiO₂) 1.5%, Zinc oxide (ZnO) nanoparticles 3% | F-nBG with 5% mol fluoride 1.5% F-nBG (w/w) | ED1        |
|                                                |                        |                           | F-nBG with 5% mol fluoride 4% F-nBG (w/w) | ED2        |

Abbreviation: SMFP, sodium monofluorophosphate.

Fig. 1  SEM image and X-ray diffractogram of synthesized zinc oxide. SEM, scanning electron microscope.
dilutions (1:1, 1:2, and 1:4) of each dentifrice and a control containing deionized water were poured into separate wells in each agar plate using a micropipette (Genex Beta; Guangdong China). The pH of prepared, solid agar mediums used for this study was similar to the physiological pH of plaque.17

All the plates were made in triplicate, and S. mutans was incubated in both aerobic and anaerobic conditions and tested on Mueller–Hinton agar plates. In the case of L. casei, the sets of triplicate were tested in microaerophilic as well as predominant anaerobic conditions on chocolate blood agar plates (Gas-Pak sachet-Oxoid AnaeroGen; Thermo Fisher Scientific, Bartlesville, OK, United States).

Determination of Minimum Bactericidal Concentration
Susceptibility break points are expressed as minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC) using appropriate dilution methods, whereby two-fold dilutions of the experimental and commercial groups were prepared as 1:1, 1:2, 1:4, 1:8, and 1:16 (2 g/2 mL yielded a 1:1 dilution). MBC was determined using modified broth dilution method for dentifrices.18 Then, 1 mL of each dilution of dentifrice was mixed with 1 mL of respective broth to yield a defined volume of 2 mL in each of the five test tubes. A test tube without dentifrice, containing 2 mL of deionized water for each bacterial strain, was used as control.

All the test tubes, including controls, were inoculated using a sterile wire loop. The test tubes inoculated with S. mutans were incubated aerobically for 24 hours at 37°C, whereas the test tubes inoculated with L. casei were incubated for 48 hours at 37°C. To ensure reproducibility, this step was repeated twice. Following incubation, all test tubes were sub-cultured to Mueller–Hinton agar (S. mutans) and chocolate blood agar plates (L. casei) in duplicate. The plate which showed no visible growth was taken as MBC and reported in g/mL of the corresponding dilution.

Statistical Analysis
SPSS version 22 (IBM; Armonk, New York, United States) was used to calculate the means and standard deviations of the groups along with differences within and between the groups by one-way ANOVA, and a posthoc Tukey’s test was used for pairwise comparison between groups.

Results
Fluoride Release Analysis
The fluoride release in ppm for both the elutes and the suspensions of all the study groups are presented in Fig. 2. Among elutes of the dentifrices, maximum mean fluoride release (± standard deviation) was detected in ED2 (13.22 ± 0.09 ppm), followed by ED1 (9.74 ± 0.05 ppm), and the control dentifrice (6.28 ± 0.05 ppm). Among suspensions of the study dentifrices, the maximum mean fluoride release was exhibited by ED2 (14.20 ± 0.09 ppm), followed by ED1 (10.07 ± 0.09 ppm), and the control group (6.63 ± 0.05 ppm). In both elutes and suspensions of experimental dentifrices, a rise in mean fluoride release was observed with a rise in F-nBG filler content. The posthoc Tukey’s test showed a statistically significant difference

Fig. 2 Mean fluoride elusion values with standard deviations (error bars) in the elutes and suspensions of the experimental dentifrices.
between the mean fluoride release of elutes of all the study
dentifrice groups and between the mean fluoride release of
suspensions of all study dentifrice groups (p = 0.000).

Antimicrobial Analysis
Antimicrobial Susceptibility Test: Agar Well Diffusion
Assay
Following incubation, resultant zones of inhibition were
observed on the agar plates. The clear and circular halos sur-
rounding the wells were observed. The zone of inhibition
against S. mutans appeared after 24 hours, and the inhibi-
tion zone against L. casei appeared after 48 hours. The inhibi-
tion zones increased in a dose-dependent manner for the
dilutions 1:1, 1:2, and 1:4 of dentifrice slurries. The nega-
tive control wells containing deionized water produced no
observable inhibitory effect for any of the bacteria in each
of the triplicate sets of agar plates. The assessment of anti-
bacterial potential against caries-causing odonto-pathogens
was done by recording the diameters of zones of inhibition
(ZOIs) (mm) using a Vernier caliper (∗Table 2). One-way
ANOVA (analysis of variance) revealed that only ED1 and CD
indicated statistical significance (p < 0.05). Pair-wise com-
parisons using a posthoc Tukey’s test revealed differences
between the concentration groups. ED1 and ED2 were statis-
tically insignificant (p > 0.05) for both bacterial strains under
all conditions.

Determination of Minimum Bactericidal Concentration
ED2 showed increased effectiveness and was bactericidal
(Aerobic) even at the dilution of 1:16, similar to CD. In the
case of L. casei, ED1 was effective up to a dilution 1:4 only
and growth was observed on plates at a dilution of 1:8. This
finding also corresponded with the smallest mean ZOIs
observed for ED1 against L. casei using agar well-diffusion
assay (∗Table 2). The remaining dentifrices were bactericidal
(L. casei) up to a dilution of 1:8. For both bacterial strains,
marked growth was observed on the plated controls.

**Table 2** Mean diameter and SDs of ZOI against microbial strains

| ZOIs against S. mutans | Experimental | Mean ± SD | Mean ± SD | Mean ± SD |
|------------------------|-------------|-----------|-----------|-----------|
|                        | Dilution 1:1 | Anaerobic | Anaerobic | Anaerobic |
| ED1                    | F-BG 1.5%   | 22.43 ± 0.61 | 21.8 ± 1.29 | 20.07 ± 1.46 | 17.83 ± 1.43 | 17.27 ± 0.90 |
| ED2                    | F-BG 4%     | 25.10 ± 0.69 | 23.23 ± 1.45 | 21.90 ± 1.51 | 20.16 ± 0.84 | 17.84 ± 1.03 |
| CD                     | SMFP 0.76%  | 29.83 ± 0.23 | 29.83 ± 1.31 | 25.83 ± 1.31 | 26.5 ± 1.47 | 21 ± 0.81 |

| ZOIs against L. casei | Experimental | Microaerophilia | Anaerobic | Microaerophilia | Anaerobic | Microaerophilia | Anaerobic |
|-----------------------|-------------|-----------------|-----------|-----------------|-----------|-----------------|-----------|
| ED1                   | F-BG 1.5%   | 10.5 ± 0.41 | 11.17 ± 1.02 | 8.17 ± 0.62 | 6.5 ± 0.41 | 7.0 ± 0.82 | 5.83 ± 0.24 |
| ED2                   | F-BG 4%     | 13.1 ± 0.54 | 15.33 ± 0.85 | 10.17 ± 1.03 | 11.0 ± 0.816 | 8.67 ± 0.47 | 8.83 ± 0.62 |
| CD                    | SMFP 0.76%  | 16.33 ± 1.25 | 17.67 ± 0.47 | 11.33 ± 0.62 | 14.33 ± 0.47 | 10.4 ± 0.43 | 11.33 ± 0.47 |

Abbreviation: ZOI, zone of inhibition.

Discussion
In the current study, evaluation of the proportions of F-nBG
fillers in dentifrices that yield maximum fluoride elusion
among novel dentifrices was performed. Upon increasing the
F-nBG filler content in experimental dentifrice groups, an
increase in fluoride release rates in both elutes and suspen-
sions of dentifrices was observed, whereas a direct propor-
tionality of fluoride release rate with the F-nBG filler loading
in novel dentifrices was observed.

Dentifrices are used in suspension form during tooth
brushing procedures; therefore, the fluoride elusion in den-
tifrice suspensions is of greater clinical significance than the
fluoride elusion in the dentifrice elutes. However, no remark-
able difference was observed in fluoride release values of
elutes and suspensions of the same group. This may possibly
be due to instant release of fluoride ions upon dissolution
of fluoride compounds used in experimental dentifrices in
aqueous media. Regarding the bactericidal effect of fluoride,
it is reported that fluoride release of 20 ppm exhibits direct
bactericidal action.10 However, due to the buffering action of
saliva, this amount drops and may not be as relevant clini-
cally. The elutes and suspension of ED2 showed 13.72 ppm
and 14.20 ppm and close to the required amount.

In a previous study conducted by our group, F-nBG par-
ticles with 5 wt% fluoride showed burst fluoride release for
the initial 9 days.11 As dentifrices are used to brush teeth for
only a few minutes, therefore, the burst fluoride release by
F-nBG filler particles with 5 wt% fluoride is of clinical signif-
icance in dentifrices. Addition of fluoride to bioactive glass
Although the commercial dentifrices used in this study remain unclear. ISO Standard 11609 states that NaF/SMFP dentifrices comprising total fluoride content and intent of the recommended methodology of the American Microbiological Society (ASM). For antimicrobial analysis, slurries of all the included dentifrices were made in various dilutions, as the level at which antimicrobial properties are buffered or lost in dilution by saliva is important. Agar well diffusion tests revealed that inhibition zones decreased in a dose-dependent manner for the dilutions 1:1, 1:2, and 1:4 of dentifrice slurries, further supporting the direct proportionality of filler loading of F-nBG. No increase in zone size was observed after 48 hours. All tested dentifrices exhibited greater antibacterial activity against the major cariogenic pathogen, that is, S. mutans, than L. casei. With regard to the evaluation of the facultative nature of S. mutans, the recorded diameters of inhibition zones in both aerobic and anaerobic conditions were similar, whereas the inhibition zones recorded for L. casei were larger in the case of anaerobic conditions when compared with the CO₂ jar.

The inhibition zones observed for ED2 were greater than ED1 because of greater fluoride content along with ZnO and consequent fluoride release. The ZOIs for CD were slightly elevated when compared with the inhibition zone for ED2, most likely due to incorporation of anticalculus agent—tetrasodium pyrophosphate in the commercial dentifrice—for enhanced cavity protection. According to previous studies, some dentifrices containing such agents may provide reduction in biofilm formation by approximately 15%.

MBC values of tested dentifrices revealed that all the test dentifrices had greater effectiveness against S. mutans compared with L. casei. It also revealed that 3 wt% ZnO and an increased concentration of F-nBG directly corresponded to increased bactericidal potential of experimental dentifrices. MBC values for ED2 (4 wt% F-nBG) were same as that for CD (Colgate Cavity protection). It is important to consider that MIC values are bacteriostatic, whereas MBC values are bactericidal as they corresponded to subcultured plates which showed absence of bacterial growth. Further, in vitro studies need to be performed to assess the tooth remineralization potentials of these dentifrices to gauge their efficacy in relieving enamel white spot lesions and dentin hypersensitivity. Finally, cytotoxicity testing and in vivo usage tests should be performed to introduce the most suitable composition commercially.

Conclusion

It is concluded that in dentifrices, mainly the composition of the fluoride source, governs fluoride release behavior. Ionic fluoride in elutes and suspensions of both novel dentifrices was directly proportional to F-nBG filler loading. Among novel dentifrices, maximum mean fluoride release
was detected in elutes and suspensions of group ED2, which contained the highest filler loading, that is, 4 wt% F-nBG. Filler loading matched the fluoride release of the commercial dentifrice.

Statistical analysis indicated that ED2 (F-nBG 4 wt%,) demonstrated similar antimicrobial potential to that of an ordinary, non-tetra sodium pyrophosphate-containing fluoride dentifrice in terms of ZOIs for both bacterial strains under all growth conditions. The MBC obtained for ED2 was also similar to obtained MBC values for CD. Incorporation of F-nBG and ZnO provide a multi-benefit approach to simultaneously treating early white spot lesions, reducing bacterial growth, and providing core plaque control.

Conflict of Interest
None declared.

References
1. Cviki B, Lussi A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur J Oral Sci 2015;123(3):179–185
2. Baglar S, Nalcaci A, Tastekin M. The effect of temperature change on fluoride uptake from a mouthrinse by enamel specimens. Eur J Dent 2012;6(4):361–369
3. Ricomini Filho AP, Tenuta LM, Fernandes FS, Calvo AF, Kusano SC, Cury JA. Fluoride concentration in the top-selling Brazilian toothpastes purchased at different regions. Braz Dent J 2012;23(1):45–48
4. Guven Y, Ustun N, Tuna EB, Aktoren O. Antimicrobial effect of newly formulated toothpastes and a mouthrinse on specific microorganisms: an in vitro study. Eur J Dent 2019;13(2):172–177
5. Cury JA, Oliveira MJ, Martins CC, Tenuta LM, Paiva SM. Available fluoride in toothpastes used by Brazilian children. Braz Dent J 2010;21(5):396–400
6. Nascimento HRd. Ferreira JMS, Granville-Garcia AF, Costa EMdDB, Cavalcante ALA, Sampaio FC. Estimation of toothpaste fluoride intake in preschool children. Braz Dent J 2013;24(2):142–146
7. Missel EM, Cunha RF, Vieira AE, Cruz NV, Castilho FC, Delbem AC. Sodium trimetaphosphate enhances the effect of 250 p.p.m. fluoride toothpaste against enamel demineralization in vitro. Eur J Oral Sci 2016;124(4):343–348
8. Zafar MS, Khurshid Z, Najeeb S, Zohaib S, Rehman IU, Therapeutic applications of nanotechnology in dentistry. In: Andronesu E, Grumezescu A, eds. Nanostructures for Oral Medicine. Elsevier;2017:833–862
9. Erol-Taygun M, Zheng K, Bocaccarini AR. Nanoscale bioactive glasses in medical applications. Int J Appl Glass Sci 2013;4:136–148
10. Curtis AR, West NX, Su B. Synthesis of nanobioglass and formation of apatite rods to occlude exposed dentine tubules and eliminate hypersensitivity. Acta Biomater 2010;6(9):3740–3746
11. Gul H, Zahid S, Zahid S, Kaleem M, Khan AS, Shah AT. Sol-gel derived fluoride-doped bioactive glass powders: Structural and long-term fluoride release/ph analysis. J Non-Cryst Solids 2018;498:216–222
12. Kanel SR, Al-Abed SR. Influence of pH on the transport of nanoscale zinc oxide in saturated porous media. J Nanopart Res 2011;13:4035–4047
13. Takatsuka T, Tanaka K, Iijima Y. Inhibition of dentine demineralization by zinc oxide: in vitro and in situ studies. Dent Mater 2005;21(12):1170–1177
14. Pasquet J, Chevalier Y, Pelletier J, Couval E, Bouvier D, Bolinger MA. The contribution of zinc ions to the antimicrobial activity of zinc oxide. Colloids Surf A Physicochem Eng Asp 2014;457:263–274
15. Ahtzaz S, Nasir M, Shahzadi L, et al. A study on the effect of zinc oxide and zinc peroxide nanoparticles to enhance angiogenesis-pro-angiogenic grafts for tissue regeneration applications. Mater Des 2017;132:409–418
16. Pearce EI. A laboratory evaluation of New Zealand fluoride toothpastes. N Z Dent J 1974;70(320):98–108
17. Balouiri M, Sadiki M, Ihsnoua SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal 2016;6(2):71–79
18. Inetianbor J, Ehiowemwenguan G, Yakubu J, Ogodo A. In vitro antibacterial activity of commonly used toothpastes in Nigeria against dental pathogens. J Adv Sci Res 2014;2:40–45
19. DeSchepper EJ, White RR, von der Lehr W. Antimicrobial effects of glass ionomers. Am J Dent 1989;2(2):51–56
20. Featherstone JD. Prevention and reversal of dental caries: role of low level fluoride. Community Dent Oral Epidemiol 1990;27(1):31–40
21. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369(9555):51–59
22. Rabiei SM, Nazparvar N, Azizian M, Vashaee D, Tayebi L. Effect of ion substitution on properties of bioactive glasses: a review. Ceram Int 2015;41:7241–7251
23. Benzian H, Holmgren C, Bujs M, van Loveren C, van der Weijden F, van Palenstein Helderman W. Total and free available fluoride in toothpastes in Brunei, Cambodia, Laos, the Netherlands and Suriname. Int Dent J 2012;62(4):213–221
24. Ericsson Y. Fluorides in dentifrices. Investigations using radio-active fluoride. Acta Odontol Scand 1961;19:41–47
25. Ingram GS. The reaction of monofluorophosphate with apatite. Caries Res 1972;6(1):1–15
26. Gran P, Brudveold F, Asasend H, Monofluorophosphate interaction with hydroxyapatite and intact enamel. Caries Res 1971;5(3):202–214
27. Hashizume LN, Lima YB, Kawaguchi Y, Cury JA. Fluoride availability and stability of Japanese dentifrices. J Oral Sci 2003;45(4):193–199
28. Bardal PA, Olimpio KP, da Silva Cardoso VE, de Magalhães Bastos JR, Buzalaf MA. Evaluation of total pH and soluble and ionic fluoride concentrations in dentifrices commercially available in Brazil. Oral Health Prev Dent 2003;1(4):283–289
29. Conde NC, Rebelo MA, Cury JA. Evaluation of the fluoride stability of dentifrices sold in Manaus, AM, Brazil. Pesquisa Odontol Bras 2003;17:247–253
30. ISO 11609. Dentistry — Dentifrices — Requirements, test methods and marking. 3rd ed., 2017–06
31. ISO 7405. Dentistry — Evaluation of biocompatibility of medical devices used in dentistry. 3rd ed., 2018–11
32. Grossman E, Hou L, Bollmer BW, et al. Triclosan/pyrophosphate dentifrice: dental plaque and gingivitis effects in a 6-month randomized controlled clinical study. J Clin Dent 2002;13(4):149–157
33. Pedrazzi V, Corsi LP, Pedrazzi H, Netto EI, Nascimento Cd, Issa JP. Clinical evaluation of residual tetrasodium pyrophosphate released from two different anticalculus flosses. Braz Dent J 2015;26(2):116–120
34. Aamer A, Abdul-Hafeez M, Sayed S. Minimum inhibitory and bactericidal concentrations (MIC and MBC) of honey and bee propolis against multi-drug resistant (MDR) Staphylococcus sp. isolated from bovine clinical mastitis. Altern Integr Med 2014;3(4):1000171
35. Moran J, Addy M, Wade W. Determination of minimum inhibitory concentrations of commercial toothpastes using an agar dilution method. J Dent 1988;16(1):27–31