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SHORT COMMUNICATION

In vitro effect of sodium trimetaphosphate additives to conventional toothpastes on enamel demineralization

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Abstract
Objective The aim of this study was to evaluate the ability of conventional toothpastes (1100 ppm F) supplemented with sodium trimetaphosphate (TMP) in demineralization.
Material and methods Blocks of enamel were selected and then divided into seven experimental groups of 12: toothpaste without F and TMP (placebo), toothpaste with 1100 ppm F (1100), and toothpaste with 1100 ppm F supplemented with TMP—1 % (1100 1 % TMP), 3 % (1100 3 % TMP), 4.5 % (1100 4.5 % TMP), 6 % (1100 6 % TMP), and 9 % (1100 9 % TMP). Blocks were subjected to five pH cycles (demineralizing/remineralizing solutions) at 37 °C and treated with toothpaste slurries twice daily, after which the blocks were maintained for 2 days in fresh remineralizing solution. Following treatments, surface hardness (SHf) and cross-sectional hardness were determined for calculating the integrated loss of subsurface hardness (ΔKHN). The fluoride present in the enamel was also measured.
Results The SHf and ΔKHN measurements showed that supplementation with 3 % TMP was the most effective ($p<0.001$) and showed greater concentration of F in the enamel ($p<0.001$).
Conclusion Addition of 3 % TMP to a conventional toothpaste (1100 ppm F) showed greater efficacy in reducing enamel demineralization.
Clinical relevance Fluoride toothpastes containing trimetaphosphate possess good anticaries potential required to reduce the prevalence of dental caries in high-risk patients.

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Keywords Phosphates · Fluorides · Demineralization · Toothpaste

Introduction

The development of dental caries has seen a growing reduction due to the use of fluoride (F) not only in public water supplies but also in other fluoride vehicles, such as toothpastes [1, 2]. The F acts as an important adjunct to the maintenance of the mineral balance of teeth, acting directly on the dynamics of the de-remineralization process, inhibiting demineralization, promoting remineralization, and forming calcium fluoride [3]. Despite the existence of numerous sources of F, there is currently a trend in polarization of caries, with 25 % of the subjects having dental caries in approximately 75 % of their teeth [4]. Studies have shown that this bias is particularly associated with socioeconomic factors [5, 6]. This indicates, therefore, that individuals such as children [7], teenagers, and people who do not have access to fluoridated water and dental services [8] are at a high risk of developing caries.

It is known that fluoridated toothpastes (1100 ppm F) are among the most widely used forms of topical administration in the world and contribute majorly to the reduction of tooth decay [3]; it would be beneficial to increase their efficacy, which would decrease the levels of disease. A method used to increase the effectiveness of toothpaste is the addition of phosphate salts. Studies have shown that the addition of polyphosphates to fluoride formulations with low fluoride concentrations increased their effectiveness against demineralization [9–17]. This effect is associated with the ability of phosphates to form complexes with cations such as Ca^{2+} and CaF^{+} and increase the ionic activity of neutral species such as

$CaHPO_4^0$ and HF^0 [10, 13, 15] that increase the diffusion coefficient of the enamel [18]. Among the polyphosphates, studies suggest that sodium trimetaphosphate (TMP) can reduce the solubility of hydroxyapatite mineral [19] thought to be dependent on the TMP/F ratio in the formulation [12, 17, 19, 20]. No studies, however, have evaluated the action of TMP in combination with conventional toothpastes (1100 ppm F). The TMP/F proportions have been studied at concentrations of 100 and 500 ppm F. Considering the above-mentioned synergistic effects of F and TMP on enamel demineralization when added to low-F toothpastes, it would be interesting to assess the combination of F and TMP in a conventional toothpaste (1100 ppm F), that is, if such a combination could be beneficial in reducing tooth demineralization, or if an increase in the concentration of fluoride in toothpastes would inhibit the synergistic action of TMP/F. Therefore, more studies are needed in order to increase the effectiveness of toothpastes for the reduction and prevention of polarization of dental caries among the less privileged.

Therefore, the aim of this in vitro study was to evaluate the effect of different concentrations of TMP added to fluoride toothpaste (containing 1100 ppm F) on enamel demineralization. The null hypothesis was that fluoride toothpastes (1100 ppm) with or without TMP have the same anticaries effect under in vitro conditions.

Materials and methods

Experimental design

For this study, enamel blocks (4 mm×4 mm, n=84) were obtained from bovine incisors kept in 2 % formalin, pH 7.0, for 30 days [21]. These blocks had their enamel surface sequentially polished, which allowed selection by determining the initial surface hardness (371 to 377 KHN; p=0.610). The experimental design was randomized, and the blocks were divided into seven groups (n=12 in each): toothpaste without fluoride and without TMP (placebo), toothpaste with 1100 ppm F (1100), and toothpaste with 1100 ppm F +1 % TMP (1100 1 % TMP), + 3 % TMP (1100 3 % TMP), + 4.5 % TMP (1100 4.5 % TMP), + 6 % TMP (1100 6 % TMP), and +9 % TMP (1100 9 % TMP). The blocks were subjected to pH cycling [22] spanning 7 days and treated with the different toothpastes twice a day for 1 min. After pH cycling, the surface hardness and integrated loss of surface hardness along with the F concentration present in the enamel were determined.

Formulation, determination of pH, and concentration of F in toothpastes

The toothpastes were prepared using the following ingredients: titanium dioxide, carboxymethyl cellulose, methyl p-hydroxybenzoate sodium, saccharin, mint oil, glycerin, abrasive silica, sodium lauryl sulfate, and deionized water. TMP-containing toothpastes were prepared (Aldrich Chemistry, CAS 7785-84-4, China) at concentrations of 1, 3, 4.5, 6, and 9 % with the addition of F at a concentration of 1100 ppm in the NaF form (Merck, CAS 7681-49-4, Germany). Moreover, a toothpaste without the addition of TMP and F (placebo) and another with 1100 ppm F but no TMP were also prepared. The F concentrations and the pH of the prepared toothpastes were measured [14, 23]. The mean (SD) concentration of total (TF) and ionic (FI) fluoride (n=3) for the placebo toothpaste were 9.5 ppm (1.1) and 9.7 ppm (0.4), respectively. For toothpastes with 1100 ppm F, the mean values (SD) between groups were 1162.0 ppm (44.1) and 1157.2 ppm (16.8), ranging between 1111.0 and 1183.9 ppm F. The mean pH among groups was 7.3 (0.3) with values ranging from 6.8 to 7.7.

pH cycling and treatment with toothpastes

The blocks were subjected to five pH cycles during 7 days, at a constant temperature of 37 °C [22]. The blocks were kept in a demineralizing solution (DE) (6 h; calcium and phosphate, both 2.0 mmol/L, in 75 mmol/L acetate buffer, pH 4.7; 0.04 μg F/mL, 2.2 mL/mm^2) followed by immersion in a remineralizing solution (RE) (18 h; 1.5 mmol/L calcium; 0.9 mmol/L phosphate; 150 mmol/L KCl in 0.02 mol/L cacodylic buffer, pH 7.0; 0.05 μg F/mL, 1.1 mL/mm^2). The treatment consisted of a 1-min soak under agitation in 2 mL/block of toothpaste/deionized water slurries (1:3 w/w), between immersion in the DE and RE solutions (twice a day). Deionized water rinses were performed between each step. The blocks were kept in fresh RE solution during the last 2 days of the procedure.

Analysis of enamel hardness

The surface hardness was measured using a 5114 MicroMet micro-hardness tester (Buehler, Lake Bluff, USA) with Knoop type indenter, and with a static load of 25 g for 10 s. Five indentations separated by a distance of 100 μm were made in the central region of each block (SHi). After the pH cycling, five other indentations were performed (SHf) with a distance of 100 μm from the SHi. For the cross-sectional hardness analysis, the block was sectioned at the center and one of the halves was treated with the acrylic resin and polished. A series of 14 indentations at distances of 5, 10, 15, 20, 25, 30, 40, 50, 70, 90, 110, 130, 220, and 330 μm from the external surface of the enamel was made in the central area of the block using a 5-g load for 10 s [11, 13]. The integrated area hardness (KHN×μm) of the enamel lesion was calculated using the trapezoidal rule (GraphPad Prism, version 3.02) and subtracted from the integrated area of the hardness of the sound enamel, thus yielding the integrated loss of subsurface

hardness (ΔKHN) [24, 25]. The integrated area of the differential profile (i.e., the hardness at each depth from fluoride toothpastes minus hardness values from the placebo) was determined in two depth zones (5–10 and 20–130 μm) in the lesion, to yield ΔIH values [11, 25].

Analysis of the F concentration present in enamel

Blocks (2 mm×2 mm) obtained from the remaining longitudinally sectioned blocks were attached to a mandrel and fixed to a modified microscope with an electronic digital micrometer (Starrett, São Paulo, SP, Brazil). A layer of enamel (57.5± 0.05 μm) was removed using a self-adhesive polishing disc of 400-grit silicon carbide (Buehler, Lake Bluff, IL, USA) fixed to the bottom of polystyrene crystal tubes (J-10 Injeplast, Paraná, Brazil) [16, 26]. The tubes, after addition of 1.0 mL HCl (1.0 mol/L), were agitated for 1 h followed by the addition of 1.0 mL NaOH (1.0 mol/L) [11, 16, 20]. For analysis of F, a previously calibrated 9409BN-specific electrode (Thermo Scientific, Beverly, MA, USA) was used. Samples and standards were buffered with TISAB II (total ionic strength adjustment buffer) at a 1:1 ratio.

Statistical analysis

For statistical analysis, we used the Sigma Plot program (version 12.0), with a level of significance set at 5 %. The SHf, ΔKHN, ΔIH, and F values were considered as variables in the enamel. The data were tested for normality (Shapiro–Wilk test) and homoscedasticity (Cochran). Data from the SHf, ΔKHN, and F values were homogenous and were subjected to one-way analysis of variance, followed by Student–Newman–Keuls' test. The resulting ΔIH were subjected to two-way analysis of variance (toothpaste and depth zone) followed by the Student–Newman–Keuls' test.

Results

The results of the SHf (Fig. 1a) showed that the addition of 3 % TMP produced the maximum effect in reducing demineralization ($p<0.001$). Concentrations of TMP above 3 % caused a reduction in the surface hardness values (Fig. 1a). The mineral loss in depths (ΔKHN) showed a pattern similar to that of surface hardness analysis (Fig. 1b) with 3 % TMP yielding lower demineralization ($p<0.001$). Compared to blocks treated with 1100 ppm F, those treated with 4.5, 6, and 9 % TMP showed smaller lesions (ΔKHN) ($p<0.001$). The addition of TMP resulted in little change in enamel hardness in the outer region of the lesion (5–10 μm; Fig. 1c), and the effect of toothpastes with 1, 4.5, 6, and 9 % of TMP was similar to that of 1100 ppm F ($p>0.001$). Demineralization was lower in the inner part of the lesion (20–130 μm) for toothpastes containing TMP when compared to 1100 ppm F

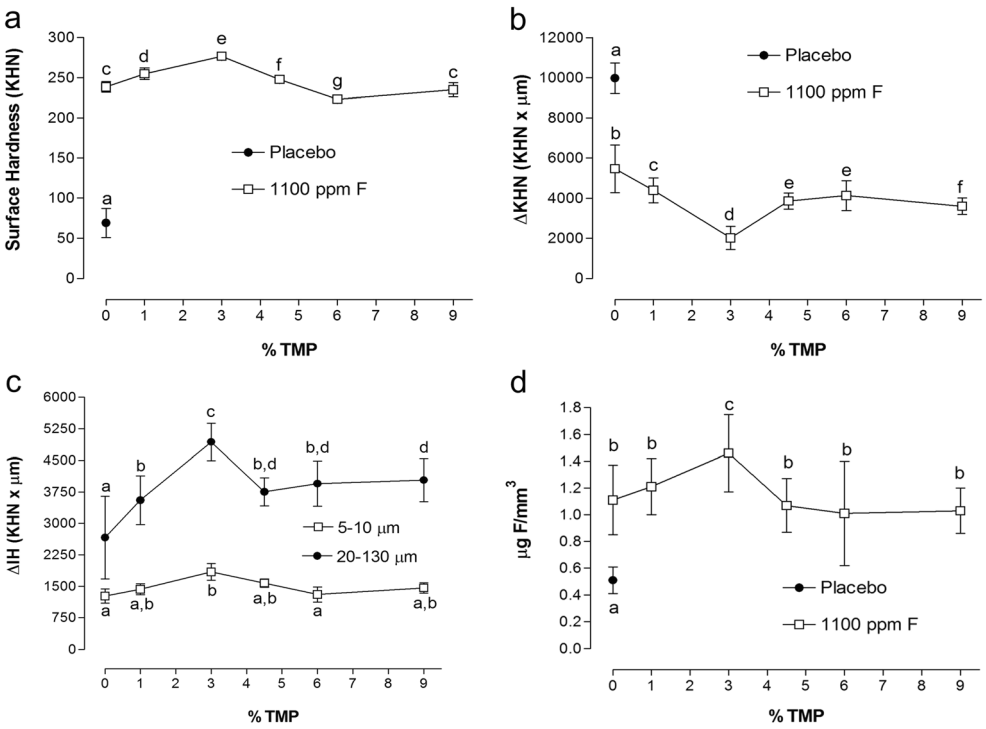

Fig. 1 Mean values ($n=12$) from **a** surface hardness (SHf) after pH cycling, **b** integrated loss of subsurface hardness (ΔKHN), **c** integrated hardness of differential profiles (ΔIH) at two zones (5–10 and 20–130 μm), and **d** fluoride (F) present in the enamel. *Distinct lowercase letters* indicate the differences among groups (Student–Newman–Keuls, $p<0.001$). *Vertical bars* denote the SD

toothpaste ($p<0.001$). The addition of 3 % TMP to 1100 ppm F toothpaste (Fig. 1d) provided the highest F presence in the enamel ($p<0.001$). No difference in the amount of fluoride in the enamel was observed between the toothpastes containing 1, 4.5, 6, and 9 % of TMP and 1100 ppm F toothpaste ($p>0.001$).

Discussion

The present study showed that the addition of TMP to 1100 ppm F toothpaste increases its ability to reduce mineral loss in an in vitro caries model. As observed in previous studies [16, 17], the TMP/F combination increased the capacity of toothpaste to reduce demineralization of the enamel. The reduction of mineral loss was 61 % with the addition of TMP compared to 3 % with conventional toothpaste (1100 ppm F) as determined from the hardness data on longitudinal sections (ΔKHN). These results can be explained by the adsorption of TMP to the enamel forming a protective layer against the diffusion of acid into the enamel [27]. However, when analyzing the surface hardness data, the reduction was lower at 16 %. Studies show that the action of fluoride is on the outermost regions of the carious lesion subsurface [17, 28].

As the effect of TMP is related to its ability to adsorb on the enamel, it is clear that the TMP:F molar ratio has a strong influence on the resulting anticaries effect [10, 12, 17]. The TMP/F combination affects the process of demineralization and remineralization in deeper regions of the carious lesions [11, 17, 20, 25]. In the present study, it was observed that demineralization was lower in depths (mainly at 20–130 μm) when TMP was present in the formulations, which is consistent with previous findings [11, 17, 25] (Fig. 1c). While in the outer part of the lesion (5–10 μm) only a small additional effect was produced by the presence of TMP, a greater effect was observed in the depths of the lesion. The interaction of Ca^{2+} and CaF^{+} with TMP (cross-linking) leads to reticular formation on the enamel by binding sites on the negative PO_4^{3-} in the cyclic structure. At acidic pH, these linkages are ruptured, releasing Ca^{2+} and CaF^{+} [10, 13, 15], which can react with $H_2PO_4^{3-}$, leading to the formation of $CaHPO_4^{0}$ and HF^{0}. These neutral species present a diffusion coefficient a thousandfold higher than that of ionic calcium in depths in the caries lesion [18]. The addition of 1 and 3 % TMP to fluoridated toothpaste increased the concentration of fluoride in the enamel by 8 and 31 %, respectively. A previous in vitro study showed an increase of around 26 % when 3 % TMP was added to toothpaste with a lower concentration of fluoride (500 ppm).

Though it was reported that TMP and F do not compete for the same binding sites on the enamel surface [29], increasing the concentration of TMP above 3 % reduced the presence of fluoride in the enamel (Fig. 1d). Since the adsorption of polyphosphates occurs quickly [30] and can compete with the adsorption of fluoride, TMP and F must be combined in a suitable molar ratio. The synergistic effect of TMP and F appears to be dependent upon their incorporation in the dentifrices in an appropriate proportion. Combining 500 ppm F with TMP resulted in better efficacy when compared to a toothpaste with 1100 ppm F with a molar ratio of 1.2 to 3.7 [16]. With 1100 ppm F toothpaste, the equilibrium adsorption of TMP and fluoride reaches maximal effectiveness with 3 % TMP (TMP/F 1.7), unlike with 500 ppm F. Above 3 % TMP ratios (TMP/F 2.5, 3.4, and 5.1), the bonding strength of hydroxyapatite with TMP exceeds the level of reducing the anticaries synergistic effect of F. When the fluoride concentration is increased to 3000 ppm, the bonding strength with enamel is more than that with 3 % TMP with no resulting improvement in anticaries effect [17].

Conclusions

We conclude from the toothpaste formulations tested in this in vitro study that the addition of 3 % TMP to a conventional toothpaste (1100 ppm F) shows better efficacy in reducing demineralization in bovine enamel. However, the results of our study cannot be considered definitive because the pH cycling model, although simulating the development of caries under controlled conditions, has limitations. Thus, in situ remineralization protocols and in vivo studies are needed to obtain more conclusive findings. Thus, the null hypothesis was rejected.

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Declaration of interests The authors transfer all copyright ownership of the manuscript to the Clinical Oral Investigation. The authors Marcelle Danelon and Alberto Carlos Botazzo Delbem hold a patent request for a product used in the study, by the National Institute of Industrial Property—INPI/SP, on April 29, 2008, under number 018080026091, PI0801811-1, and published on January 11, 2011. All authors approved the publishing of the manuscript.

References

1. Marinho VCC, Higgins JPT, Logan S, Sheiham A (2003) Topical fluoride (toothpastes, mouth rinses, gels, varnishes) for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 4:CD002782
2. Marinho VCC, Higgins JP, Sheiham A, Logan S (2004) Combinations of topical fluoride (toothpastes mouth rinses, gels or varnishes) versus single topical fluoride for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 1: CD002781

3. Buzalaf MAR (2011) Fluoride and the oral environment. Monogr Oral Sci 22:178 p. Karger (Basel, Switzerland). ISSN 0077−0892
4. Narvai PC, Castellanos RA, Frazão P (2000) Prevalence of caries in permanent teeth of school children in the city of São Paulo, SP, 1970-1996. Rev Saúde Públ 34:196–200. doi:10.1590/S0034-89102000000200015
5. Sogi GM, Bhaskar DJ (2002) Dental caries and oral hygiene status of school children in Davangere related to their socio-economic levels: an epidemiological study. J Indian Soc Pedod Prev Dent 20:152–157
6. Gillcrist JA, Brumley DE, Blackford JU (2001) Community socio-economic status and children's dental health. J Am Dent Assoc 132: 216–222
7. Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G et al (2007) Trends in oral health status: United States, 1988-1994 and 1999-2004. Vital Health Stat 248:1–92
8. Ditmyer M, Dounis G, Mobley C, Schawarz E (2011) Inequalities of caries experience in Nevada youth expressed by DMFT index vs. Significant Caries Index (Sic) over time. BMC Oral Health. doi:10.1186/1472-6831-11-12
9. Amaral JG, Sassaki KT, Martinhon CCR, Delbem ACB (2013) Effect of low-fluoride dentifrices supplemented with calcium glycerophosphate on enamel demineralization in situ. Am J Dent 26:75–80
10. da Camara DM, Miyasaki ML, Danelon M, Sassaki KT, Delbem AC (2014) Effect of low-fluoride toothpastes combined with hexametaphosphate on in vitro enamel demineralization. J Dent 42: 256–262. doi:10.1016/j.jdent.2013.12.002
11. Danelon M, Takeshita EM, Sassaki KT, Delbem ACB (2013) In situ evaluation of a low fluoride concentration gel with sodium trimetaphosphate in enamel re-mineralization. Am J Dent 26:15–20
12. Manarelli MM, Vieira AE, Matheus AA, Sassaki KT, Delbem AC (2011) Effect of mouth rinses with fluorides and trimetaphosphate on enamel erosion: an in vitro study. Caries Res 46:506–509. doi:10.1159/000331929
13. Manarelli MM, Delbem ACB, Lima TMT et al (2014) In vitro remineralizing effect of fluoride varnishes containing sodium trimetaphosphate. Caries Res 48:299–305. doi:10.1159/000356308
14. Moretto MJ, Magalhaes AC, Sassaki KT, Delbem AC, Martinhon CC (2010) Effect of different fluoride concentrations of experimental dentifrices on enamel erosion and abrasion. Caries Res 44:135–140. doi:10.1159/000302902
15. Pancote LP, Manarelli MM, Danelon M, Delbem AC (2014) Effect of fluoride gels supplemented with sodium trimetaphosphate on enamel erosion and abrasion: in vitro study. Arch Oral Biol 59:336–340. doi:10.1016/j.archoralbio.2013.12.007
16. Takeshita EM, Castro LP, Sassaki KT, Delbem ACB (2009) In vitro evaluation of dentifrice with low fluoride content supplemented with trimetaphosphate. Caries Res 43:50–56. doi:10.1159/000196507
17. Takeshita EM, Exterkate RA, Delbem AC, ten Cate JM (2011) Evaluation of different fluoride concentrations supplemented with trimetaphosphate on enamel de- and re-mineralization in vitro. Caries Res 45:494–497. doi:10.1159/000331209
18. Cochrane NJ, Saranathan S, Cai F et al (2008) Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res 42:88–97. doi:10.1159/000113161
19. Souza JAS, Amaral JG, Moraes JCS, Sassaki KT, Delbem ACB (2013) Effect of sodium trimetaphosphate on hydroxyapatite solubility: an in vitro study. Braz Dent J 24:235–240. doi:10.1590/0103-6440201302000
20. Favretto CO, Danelon D, Castilho FCN, Vieira AEM, Delbem ACB (2013) In vitro evaluation of the effect of mouth rinse with trimetaphosphate on enamel demineralization. Caries Res 47:532–538. doi:10.1159/000350229
21. Delbem ACB, Cury JA (2002) Effect of application time of APF and NaF gels on microhardness and fluoride uptake of in vitro enamel caries. Am J Dent 15:169–172
22. Vieira AE, Delbem AC, Sassaki KT, Rodrigues E, Cury JA, Cunha RF (2005) Fluoride dose response in pH-cycling models using bovine enamel. Caries Res 39:514–520. doi:10.1159/000088189
23. Delbem AC, Bergamaschi M, Rodrigues E, Sassaki KT, Vieira AE, Missel EM (2012) Anticaries effect of dentifrices with calcium citrate and sodium trimetaphosphate. J Appl Oral Sci 20:94–98. doi:10.1590/S1678-77572012000100017
24. Spiguel MH, Tovo MF, Kramer PF, Franco KS, Alves KM, Delbem AC (2009) Evaluation of laser fluorescence in the monitoring of the initial stage of the de-/re-mineralization process: an in vitro and in situ study. Caries Res 43:302–307. doi:10.1159/000218094
25. Danelon D, Takeshita EM, Peixoto LC, Sassaki KT, Delbem ACB (2014) Effect of fluoride gels supplemented with sodium trimetaphosphate in reducing demineralization. Clin Oral Investig 18:1119–1127. doi:10.1007/s00784-013-1102-4
26. Weatherell JA, Robinson C, Strong M, Nakagaki H (1985) Microsampling by abrasion. Caries Res 19:97–102. doi:10.1159/000260835
27. van Dijk JW, Borggreven JM, Driessens FC (1980) The effect of some phosphates and a phosphonate on the electrochemical properties of bovine enamel. Arch Oral Biol 25:591–595
28. Altenburger MJ, Schirrmeister JF, Wrbas KT, Klasser M, Hellwig E (2008) Fluoride uptake and re-mineralization of enamel lesions after weekly application of differently concentrated fluoride gels. Caries Res 42:312–318. doi:10.1159/000148164
29. Gonzalez M (1971) Effect of trimetaphosphate ions on the process of mineralization. J Dent Res 50:1056–1064
30. Anbar M, Farley EP, Denson DD, Maloney KR (1979) Localized fluoride release from fluorine-carrying polyphosphonates. J Dent Res 58:1134–1145