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Importance of bioavailable calcium in fluoride dentifrices for enamel remineralization

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ABSTRACT

Objectives: To compare remineralization of enamel subsurface lesions by fluoride dentifrices with added calcium in a double-blind, randomized, cross-over, *in situ* study.

Methods: Human enamel with subsurface lesions were prepared and inserted into intra-oral appliances worn by volunteers. A slurry (1 g toothpaste/4 ml H_2O) was rinsed for 60 s, 4 times per day for 14 days. Seven toothpastes were tested: (i) 1450 ppm F (NaF), (ii) 5000 ppm F (NaF), (iii) 1450 ppm F (MFP) with calcium sodium phosphosilicate (CSP), (iv) 1450 ppm F (MFP) with $CaCO_3$/Arg, (v) 1150 ppm F (SnF_2) with amorphous calcium phosphate (ACP), (vi) 1100 ppm F (NaF) with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and (vii) 5000 ppm F (NaF) with functionalized tri-calcium phosphate (TCP). Total (acid soluble) and bioavailable (water soluble) calcium, inorganic phosphate and fluoride levels of the dentifrices were measured using ion chromatography (F/MFP) and spectrophotometry (Ca and inorganic phosphate). Enamel lesion mineral content was measured using transverse microradiography. Data were statistically analysed using a linear mixed model.

Results: All calcium and fluoride containing toothpastes released > 90% of bioavailable fluoride and were superior to the respective fluoride alone toothpastes in remineralization of enamel subsurface lesions. The level of remineralization followed the order: CPP-ACP/1100 ppm F > ACP/1150 ppm F = TCP/5000 ppm F > 5000 ppm F = $CaCO_3$/Arg/1450 ppm F = CSP/1450 ppm F > 1450 ppm F. Bioavailable calcium levels significantly correlated with enhanced remineralization of enamel subsurface lesions.

Conclusions: Bioavailable calcium in fluoride dentifrices enhanced remineralization of enamel subsurface lesions.

1. Introduction

Dental caries is initiated *via* the demineralization of tooth hard tissue by organic acids from the fermentation of dietary carbohydrates by dental plaque bacteria [1]. The use of fluoride-containing dentifrices is an important strategy in the control of dental caries [2]. A major anticariogenic mechanism of the fluoride ion is to drive remineralization of caries-affected tooth hard tissue in the presence of bioavailable calcium (Ca^{2+}) and phosphate (PO_4^{3-}) ions to form fluorhydroxyapatite [3]. However to remineralize with fluorhydroxyapatite [$Ca_{10}(PO_4)_6(OH)_{2-2x}F_{2x}$] a molar excess of bioavailable Ca^{2+} and PO_4^{3-} ions to the F ion are required [4].

During the caries process PO_4^{3-} is removed from equilibrium with tooth mineral as HPO_4^{2-} and H_2PO_4- and the Ca^{2+} removed as calcium complexes (*e.g.* calcium lactate, $CaHPO_4^{\circ}$, $CaH_2PO_4^{+}$) [5,6]. Bioavailable Ca^{2+} and PO_4^{3-} ions can be provided by saliva but even in otherwise healthy people, lifestyle, diet and other factors can affect salivary calcium bioavailability. Since the mid 1990s there has been an increase in the consumption of soft drinks which contain not only fermentable carbohydrate (sugars) but many also contain calcium-complexing food acids (*e.g.* citric and phosphoric acids) which can act to further reduce the bioavailability of salivary Ca^{2+} [7,8]. The increase in global consumption of these drinks is associated with an increase in the global prevalence of dental caries and erosion [9–11]. The requirement for a molar excess of Ca^{2+} and PO_4^{3-} ions to F^- ion to remineralize with fluorhydroxyapatite helps to explain why remineralization of enamel and dentine lesions *in situ* has been reported to be limited by the bioavailability of calcium and phosphate ions [4,12–14].

This limitation of the bioavailability of calcium and phosphate ions on the application of topical fluorides has resulted in innovation in

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Table 1
Total and soluble Ca, Pi and F ion levels of the dentifrices.

Test Paste	μmol/g paste					
	Ca[c]		Pi[d]		F (F + MFP)[e]	
	Total[a]	Soluble[b]	Total	Soluble	Total	Soluble
Colgate (Arg/$CaCO_3$)	4771 ± 12	8.8 ± 0.1 (0.3%)	138 ± 1	62.1 ± 0.1	77.8 ± 2.1	74.3 ± 0.2 (96%)
GSK (CSP)	202 ± 13	13.6 ± 0.2 (6.7%)	134 ± 7	64.6 ± 1.0	77.2 ± 1.6	75.4 ± 1.2 (98%)
Clinpro (TCP)	8.6 ± 0.1	0.8 ± 0.1 (9.3%)	8.6 ± 0.2	2.2 ± 0.3	263.8 ± 2.6	240.9 ± 10.8 (91%)
Enamelon (ACP)	201 ± 8	57.7 ± 3.1 (28.7%)	22.1 ± 1.8	21.9 ± 2.2	56.2 ± 0.4	53.9 ± 0.4 (96%)
MI One (CPP-ACP)	428 ± 8	351 ± 13 (82.0%)	331 ± 6	284 ± 4	54.9 ± 2.5	54.4 ± 1.6 (99%)
1450 ppm F	ND[f]	ND	ND	ND	73.1 ± 1.4	69.4 ± 1.3 (95%)
5000 ppm F	ND	ND	ND	ND	261.0 ± 2.2	236.4 ± 5.4 (91%)

[a] Total (acid soluble) calcium, phosphate and fluoride levels of the dentifrice products were determined in triplicate by adding 1 g of product to 19 ml of 1 N HNO_3 in a 50 ml centrifuge tube and mixing overnight. The samples were centrifuged at 1000*g* for 15 min. Samples of the supernatants were diluted with distilled/deionised water.

[b] Soluble (water soluble) calcium, phosphate and fluoride levels of the dentifrices were determined in triplicate by adding 1 g of product to 19 ml of distilled/deionised water in a 50 ml centrifuge tube. The samples were mixed vigorously on vortex for 60 s and then centrifuged at 1000*g* for 15 min. Samples of the supernatants were diluted with distilled/deionised water.

[c] For calcium analysis 500 μL of diluted sample was added to 500 μL of 1 M HCl and 1.0 ml $LaCl_3$ (2%, w/v) prior to analysis on a Varian® AA2240 atomic absorption spectrophotometer at a wavelength of 422.7 nm.

[d] For inorganic phosphate analysis 100 μl of diluted sample was added to 500 μL of Malachite Green colour reagent containing ammonium molybdate in HCl followed by 20 μL of 1.5% Tween and then vigorously vortexed. These solutions were analysed after 30 min using a Varian 50 Bio ® UV–vis light spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at a wavelength of 660 nm.

[e] Fluoride (F and MFP) content was determined using a Dionex ICS-3000 ion chromatography system (Dionex Corporation, CA, USA) equipped with an Ion Pac AS18 anion column and a ICS3000 conductivity detector. Samples were diluted with deionised water and filtered through a 0.2 μm filter (Millex-FG, Millipore, MA, USA) before analysis.

[f] ND Not Detected.

fluoride-containing dentifrices that also contain calcium phosphate technologies. A range of calcium phosphate technologies has been developed to enhance the ability of fluoride to promote remineralization. These technologies can be divided into the following types: (1) crystalline, such as functionalized tricalcium phosphate (TCP) [15] and calcium carbonate/dicalcium phosphate with arginine (Pro Argin) [16]; (2) bioglasses, such as calcium sodium phosphosilicates (CSP, NovaMin) [17]; (3) unstabilized salts such as the amorphous calcium phosphate technology (ACP) [18]; and (4) phosphopeptide-stabilized complexes such as casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) [13].

However, a concern on adding calcium to dentifrice formulations is the unwanted reactions between calcium, fluoride and phosphate to form poorly soluble phases in the paste on storage and/or delivery thereby reducing the bioavailability of, not only the fluoride ion, but also the calcium ion [19]. Hence it is imperative to compare these new calcium and fluoride dentifrices with fluoride alone dentifrices for fluoride, calcium and phosphate ion bioavailability as well as for their ability to remineralize enamel subsurface lesions *in situ*.

The aim of this study was to determine the bioavailability of fluoride, calcium and phosphate ions and the efficacy in remineralization of enamel subsurface lesions *in situ* of five commercially available fluoride dentifrices with added calcium phosphate technologies in comparison with conventional fluoride-alone dentifrice formulations. The null hypothesis was no significant difference in remineralization efficacy between the dentifrice formulations with the same level of fluoride.

2. Materials and methods

2.1. Dentifrices

The dentifrices purchased for the study included (1) Maximum Cavity Protection containing $CaCO_3$/Arg/$CaHPO_4$ and 1450 ppm F as Na_2MFP (Colgate); (2) Sensodyne Protect and Repair containing calcium sodium phosphosilicate (CSP, NovaMin) and 1450 ppm F as Na_2MFP (GSK); (3) Enamelon containing ACP and 1150 ppm F as SnF_2 (Premier Dental); (4) ClinPro 5000 containing TCP and 5000 ppm F as NaF (3 M ESPE); (5) MI One containing CPP-ACP and 1100 ppm F as NaF (GC America); (6) 5000 ppm F as NaF control (generic) and (7) 1450 ppm F as NaF control (generic).

2.2. Participants

Eight healthy adults living in Melbourne, Australia with a fluoridated (0.9 ppm F), reticulated water supply participated in this double-blind, randomized, cross-over study. The participants were recruited from staff and students of the University of Melbourne and provided informed, written approval to participate in the study. Study inclusion criteria were: age 18–60 years; at least 22 natural teeth; unstimulated whole salivary flow rate of ≥ 0.2 ml/min and chewing gum-stimulated whole salivary flow rate ≥ 1.0 ml/min. Study exclusion criteria were: currently using antibiotics or medications that may affect salivary flow rates or a history of severe oral disease. The study was approved by the University of Melbourne Human Research Ethics Committee (HREC #1646383) and the work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The number of participants required for this study was based on power calculations using the results of previous studies [4,20].

The required sample size was calculated using the G*Power Version 3.1 sample size package [21] and was based on a repeated measures analysis of variance with 7 levels, an effect size of 0.97, a correlation, ρ, between any pair of treatment means of 0.5 and a non-sphericity correction ε of 0.5. The effect size of 0.97 was based on detecting differences between ΔZd-ΔZr means of 70 (fluoride control and fluoride plus calcium phosphate technology) and a common standard deviation of 100 within groups. The non-sphericity correction adjusts for heterogeneity in the variances of the repeated measures. With a 5% significance level and a power of 90% at least 6 subjects were required. To allow for subject attrition eight subjects were recruited for the study.

2.3. Intra-oral appliances and enamel subsurface lesions

Extracted human third molars were sterilized, enamel slabs cut and