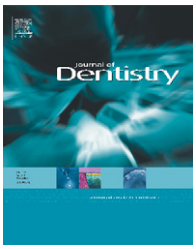
available at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/jden



The effect of casein phosphopeptide–amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion

S. Ranjitkar [a], J.M. Rodriguez [b], J.A. Kaidonis [a], L.C. Richards [a], G.C. Townsend [a], D.W. Bartlett [b,*]

[a] School of Dentistry, The University of Adelaide, Adelaide, SA 5005, Australia
[b] Department of Prosthodontics, King's College London Dental Institute, Guy's Hospital, London SE1 9RT, UK

ARTICLE INFO

Article history:
Received 26 September 2008
Received in revised form
19 November 2008
Accepted 19 November 2008

Keywords:
Abrasion
Erosion
CPP–ACP
Remineralization
Lubrication

ABSTRACT

Objective: In addition to its role as a remineralizing agent in preventing dental caries, calcium product (CPP–ACP) delivered as a mousse (Tooth Mousse®, TM) can reduce erosion of enamel and dentine. The aim of this study was to determine whether CPP–ACP could also reduce erosive tooth wear involving toothbrush abrasion.

Methods: Flat, polished enamel and dentine specimens ($n = 72$) were subjected to 10 wear regimes, with each regime involving erosion in 0.3% citric acid (pH 3.2) for 10 min followed by toothbrush abrasion in a slurry of fluoride-free toothpaste and artificial saliva (1:3 ratio by weight) under a load of 2N for 200 cycles. The specimens were immersed in artificial saliva for 2 h between wear regimes. In the experimental group 1, TM (containing CPP–ACP) was applied at the beginning of each wear episode for 5 min whereas TM– (without CPP–ACP) was applied in the experimental group 2. No mousse was applied in the control group.

Results: TM significantly reduced enamel wear (mean ± S.E., 1.26 ± 0.33 μm in the experimental group 1 vs 3.48 ± 0.43 μm in the control group) and dentine wear (2.16 ± 0.89 μm in the experimental group 1 vs 10.29 ± 1.64 μm in the control group), and dentine wear was significantly less in the experimental group 1 than in the experimental group 2 (5.75 ± 0.98 μm).

Conclusion: The finding that TM reduced erosive tooth wear involving toothbrush abrasion, probably by remineralizing and lubricating eroded tooth surfaces, may have implications in the management of tooth wear.

1. Introduction

A combination of erosion with attrition or abrasion, referred to as erosive tooth wear, can result in more destructive wear than if these processes occur independently.[1,2] Eroded enamel has a shallow, softened subsurface layer, with crystals being thin and vulnerable to mechanical wear.[3,4] Similarly, eroded dentine has a demineralized, subsurface layer[5] which is susceptible to wear by toothbrush abrasion.[6]

Normal tooth brushing habits with toothpaste are unlikely to produce excessive wear over a lifetime but the superimposed influence of acids is known to increase wear rates.[6]

* *Corresponding author at*: King's College London Dental Institute, Floor 25, Guy's Tower, London Bridge SE1 9RT, UK. Tel.: +44 20 7188 5390; fax: +44 20 7188 1792.
E-mail address: david.bartlett@kcl.ac.uk (D.W. Bartlett).
0300-5712/$ – see front matter.
doi:10.1016/j.jdent.2008.11.013

Previously suggested strategies for preventing erosive tooth wear by toothbrushing include the application of a protective resin coating on teeth[7] or of topical fluoride, which increases the resistance of tooth structure against erosive wear.[8] It is not always practical to eliminate the causes of tooth wear in individuals, so it is desirable to develop other effective preventive strategies to manage tooth wear.[7]

Recent *in vitro* studies have indicated that Tooth Mousse® (GC Asia Pty. Ltd., Japan), containing a phosphopeptide that stabilizes amorphous calcium phosphate (CPP–ACP) may reduce dental erosion caused by citric acid[9,10] and an acidic sports drink.[11] These findings support the observation that Tooth Mousse® (TM) reduces erosion from wine in both enamel and dentine.[9] Recently, Ranjitkar et al.[12] reported that attritional wear of dentine was almost eliminated *in vitro* with continuous application of TM compared with hydrochloric acid lubricant (pH 3.0) and deionized water lubricant (pH 6.1). Furthermore, intermittent application of TM also reduced dentine wear in both acidic and near neutral environments, highlighting its lubricating and remineralizing properties in reducing erosive dentine wear.[12] However, the effectiveness of TM in reducing erosive tooth wear involving toothbrush abrasion has not been assessed.

The aim of this study was to determine whether TM could reduce enamel and dentine wear under *in vitro* conditions simulating toothbrush abrasion after an exposure to dietary acid. It was hypothesized that CPP–ACP contained in a mousse would reduce enamel and dentine wear under these conditions.

2. Materials and methods

2.1. Sample preparation

Twelve intact, human third molars with unknown history were randomly selected from a pool of extracted teeth that had been stored in saturated aqueous thymol solution at 4 °C to reduce deterioration in storage, following approval from the Guy's Ethical Committee (reference no. 04/Q0704/57). Enamel specimens were obtained from the mesial, buccal, distal and lingual surfaces of the mid-coronal portion of each tooth using a diamond blade (Diamond wafering blade XL 12205, Benetec Ltd., London, UK). Similarly, dentine specimens were obtained from the upper third radicular portion of the same teeth. Each specimen was then mounted in a brass specimen holder using chemically cured acrylic resin, with a reference stainless steel ring placed around the periphery to enable measurement of erosion depths. An outer layer of 400 μm of enamel and 700 μm of dentine was removed, based on protocols developed by Ganss et al.,[8] by polishing the specimens flat progressively with 800, 1200, 2400 and 4000 grit silicon carbide sandpapers (SiC-Paper, Struers A/S, Copenhagen, Denmark) on a rotating polishing machine (Struers Labopol-1, Denmark).

2.2. Study design

Enamel specimens ($n = 36$) and dentine specimens ($n = 36$) were randomly allocated into two experimental groups and one control group so that each group ($n = 12$) contained equal numbers of specimens from the mesial, buccal, distal and lingual surfaces of teeth. CPP–ACP delivered in a mousse was applied to both enamel and dentine specimens in experimental group 1 (TM), whereas the mousse with the same formulation but without CPP–ACP (TM−) was applied intermittently in experimental group 2. No mousse was applied in the control group.

The effectiveness of TM and TM− in reducing enamel and dentine wear was tested in an abrasion-erosion model using a toothbrushing machine to reproduce the oral environment.[13] All specimens were bathed in artificial saliva for 2 h prior to experimentation. Artificial saliva was prepared according to the protocol used by Eisenburger et al.[14] and contained the following ingredients in deionized water: $CaCl_2{\cdot}2H_2O$ 0.7 mmol/l; $MgCl_2$ 0.2 mmol/l; KH_2PO_4 4.0 mmol/l; HEPES buffer (acid form) 20.0 mmol/l; KCl 30.0 mmol/l, with the pH being adjusted to 7.0. Enamel and dentine sections were subjected to 10 wear regimes, with each regime involving erosion in 0.3% citric acid (pH adjusted to 3.2 using NaOH buffer at 23.8 °C) for 10 min followed by toothbrush abrasion in a slurry of fluoride-free toothpaste and artificial saliva (1:3 ratio by weight) under a load of 2N for 200 cycles at 100 rpm. The reference stainless steel rings were covered with an adhesive tape to prevent abrasion by toothbrushing.

In experimental group 1, TM was applied for a total of 5 min following manufacturer's recommendation. After an initial application for 3 min, TM was diluted to a 30% solution with artificial saliva during the remaining 2 min to simulate the washing effect of saliva intra-orally. The same protocol was used for experimental group 2 using TM−, and for control group but without the mousse. At the end of each wear regime, TM and TM− were gently rinsed off the surface with tap water for 2 min and then air-dried. Specimens were subjected to erosion in 0.3% citric acid for 10 min. The titratable acidity of the acid solution, measured as the volume of 0.1 mol NaOH required to raise the pH of the solution to pH 7, was 5.8 ml. The specimens were then washed with water for a further 1 min and blot-dried gently using paper towels.

The specimens were subjected to toothbrush abrasion in a toothbrush machine using previously published protocols.[15] The tooth sections were placed on a mounting base and bathed in a slurry of fluoride-free toothpaste (Kingfisher natural toothpaste, Kingfisher, Norwich, UK) and artificial saliva (1:3 ratio by weight) before being subjected to toothbrushing with soft-bristled toothbrush (Oral-B® Plus Size 40, Oral B, UK). The brush heads were replaced after a total of 4000 wear cycles. At the end of this stage, the specimens were washed for 1 min and blot-dried gently using paper towels and then stored in artificial saliva for 2 h before the next wear regime began.

2.3. Assessment of tooth wear

Impressions of each specimen were obtained in polyvinyl siloxane (''Blue'' Light Body-Fast Set, 3 M ESPE Express™, St. Paul, USA) before the first wear episode and after the final wear episode as described by Sundaram et al.[16] Then, they were scanned on a Xyris 2000TL non-contacting laser profilometer (Taicaan® Technologies, Southampton, UK), which consisted

of a transmitter emitting a 785-nm red laser beam with a spot size diameter of 30 μm. The accuracy (maximum resolution) of the scanner was 0.1 μm and its reliability under repeated measurements was 0.3 μm. Data acquisition and analysis was performed with Boddies v1.81 software (Taicaan® Technologies, Southampton UK). Tooth wear was calculated by measuring the difference in step heights of tooth surfaces before and after the erosion–abrasion regime using the surrounding stainless steel disc as a reference plane, and a mean of three measurements was recorded for each specimen. Negative replicas were used to quantify wear depths because clear acrylic resin around enamel and dentine specimens could not be detected by the laser beam. Furthermore, the accuracy of the scanning process was dependent on the homogeneity of surface colour of polyvinyl-siloxane material, with blue colour providing more accurate surface profiles than other colours.

2.4. *Statistical analysis*

Based on power calculations (assuming an effect size = 0.55; type I error probability, $\alpha = 0.05$; power, $1 - \beta = 0.80$ for comparison of mean values between three samples displaying intermediate dispersion of individual values), 12 enamel and 12 dentine specimens were included in each sample. A linear mixed model analysis was designed using SAS software (Proc Mixed, SAS 9.1, SAS Institute Inc., Cary, USA) to determine whether there were significant differences in wear depths between the three experiments described. This model accounted for clustering of data from different surfaces of the same teeth. Data were log-normally distributed, so they were transformed to natural logarithms for statistical analysis. There was no significant interaction between hard tissue type (enamel vs dentine) and wear category (experimental groups 1 and 2 and control group), so this possible source of variation was excluded from the final model. Statistical significance for the final model was set at the 0.05 probability level.

3. Results

Fig. 1 shows the mean (S.E.) of enamel and dentine wear in different groups. The values for enamel wear were 1.26 μm (0.33 μm) in experimental group 1 (with TM application), 2.41 μm (0.50 μm) in experimental group 2 (with TM− application) and 3.48 μm (0.43 μm) in the control group (Fig. 1). The mean values of dentine wear were 2.16 μm (0.89 μm) in experimental group 1 (with TM application), 5.75 μm (0.98 μm) in experimental group 2 (with TM− application) and 10.29 μm (1.64 μm) in the control group. Intervention involving TM and TM− was found to have a significant effect on both enamel and dentine wear ($p < 0.01$). Post-hoc multiple comparison tests for enamel wear showed that the mean wear depth in experimental group 1 (TM) was significantly less than that in the control group ($p < 0.001$). Post-hoc multiple comparison tests for dentine wear also showed that mean wear depth in experimental group 1 (TM) was significantly less than those in experimental group 2 (TM−) ($p < 0.01$) and control group ($p < 0.001$).

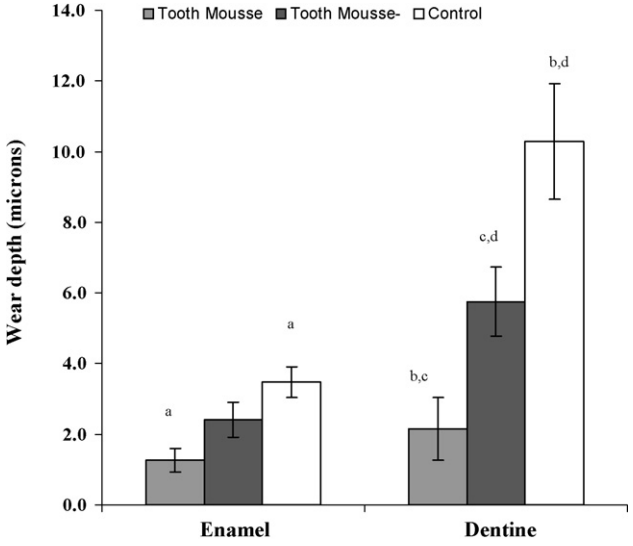


Fig. 1 – Comparison of wear depths ± S.E. (μm) in enamel and dentine specimens between three groups. [a,b,c]Pairwise comparisons using a mixed model indicate significant differences in mean log(wear depths) between experimental group 1 (Tooth Mousse®) and control group for both enamel and dentine ($p < 0.001$),[a,b] and between experimental group 1 (Tooth Mousse®) and experimental group 2 (Tooth Mousse-) for dentine ($p < 0.01$)[c]. [d]Borderline significance in the difference in mean log(wear depths) between experimental group 2 and control group for dentine ($p = 0.06$)[d].

4. Discussion

The toothbrush erosion/abrasion model used in this study is similar to that applied by Vieira et al.[13] to investigate the effect of fluoride treatment on erosive enamel wear after 600 toothbrushing cycles. To simulate the intra-oral environment, toothbrush abrasion occurred in the presence of a toothpaste-artificial saliva slurry and a 2 h remineralization period with artificial saliva was included between wear regimes. It has been suggested that treatment of eroded enamel with artificial saliva for 2 h partially rehardens enamel *in vitro*,[14] but Vanuspong et al.[17] indicated that this is less likely to occur in dentine. Nevertheless, Attin et al.[18] have observed that delaying toothbrushing by at least 30 min after an erosive regime *in situ* provides some protection against toothbrush abrasion. A total of 600 brushing strokes applied during three wear regimes on bovine teeth was reported by Vieira et al.[13] to correspond to around 2 years of wear. The present model, and those used by most previous researchers,[13,19] have used reciprocal toothbrush action against stationary tooth specimens, but Parry et al.[20] suggested that the movement of reservoirs containing tooth specimens and toothpaste slurry against stationary toothbrushes would better maintain slurry homogeneity at the wear interface. Parry et al.[20] also reported that toothbrushing forces selected in previous *in vitro* studies ranged from 0.1N to 8.8N. However, a force of around 2N has been used by most researchers to simulate normal toothbrushing force *in vivo*.[13,21] To detect minute amounts of tooth

wear using profilometry, flat and polished specimens were used in the present study. However, it should be noted that natural tooth surfaces erode more slowly than polished surfaces and that coronal dentine erodes faster than radicular dentine.[22]

Dentine was observed to be more susceptible to erosive wear by toothbrush abrasion than enamel, and this finding is consistent with results of previous *in vitro* and *in situ* studies.[20,23] Dentine is more susceptible to erosion than enamel at around pH 3.0,[24] and it is likely that softened dentine (including collagen matrix) is removed at a faster rate than softened enamel at this pH value. However, enamel specimens have been shown to wear faster than opposing dentine specimens at pH 1.2,[25] implying that findings on the relative erosive wear of enamel and dentine at around pH value of 3.0 should not be extrapolated to situations involving toothbrush abrasion immediately after gastric regurgitation.

TM (containing CPP–ACP) resulted in significantly less erosive wear of enamel and dentine sections than TM− (without CPP–ACP) or no TM (control specimens) (Fig. 1). The effect of the TM− probably reflects the lubricating potential of its ingredients (such as glycerol) in wear reduction, and the differences in wear depths between the experimental group 1 (TM) and experimental group 2 (TM−) are likely to reflect the remineralizing potential of CPP–ACP in reducing erosive tooth wear (Fig. 1). However, these findings should be interpreted with some caution because of relatively small differences in wear depths between different groups.

The potential of CPP–ACP as an anticariogenic agent has been reported both *in vitro* and *in situ*,[26,27] with CPP–ACP preventing demineralization and promoting remineralization of subsurface carious lesions in enamel and dentine.[28] CPP–ACP maintains saturation levels of calcium and phosphate at the tooth surface and provides a reservoir of neutral ion pairs ($CaHPO_4^0$), which inhibit demineralization and promote the formation of hydroxyapatite crystals inside carious lesions.[29] CPP–ACP is also detectable in the plaque matrix and the surface of bacterial cells of subjects 3 h after consuming CPP–ACP-containing mouthrinse or chewing gum.[26] The mechanisms by which CPP–ACP reduces erosive tooth wear are unclear. However, the finding that TM increases hardness of enamel eroded by cola drink[30] implies that its erosion-inhibiting potential probably involves remineralization action. Unlike the process of remineralization of carious lesions, eroded tooth structure is likely to be repaired by deposition of mineral into the porous zone rather than crystal regrowth.[14] This hypothesis is consistent with the observation that superficial granular structures were noted to form on the enamel surface, probably representing remineralized enamel structure, after treatment with a sports drink containing CPP–ACP.[11]

Previous studies have observed that non-fluoridated toothpaste results in greater erosive tooth wear than fluoridated toothpaste,[31] and that the level of protection also varies with fluoride formulation[32] A non-fluoridated toothpaste was used instead of fluoridated toothpaste in the present study because fluoride has been found to act synergistically with CPP–ACP in remineralizing carious lesions,[33] potentially making it confounding variable. However, it is possible that the effect of TM in reducing erosive tooth wear may have been overestimated in the present study. Future studies are needed to compare the effectiveness of Tooth Mousse and fluoride, when used individually or in combination, in preventing erosive tooth wear.

On the basis of this investigation, it appears that TM application may help reduce erosive tooth wear of coronal enamel and cervical dentine by toothbrush abrasion. These findings provide a basis for future *in situ* studies and clinical trials that will determine the true potential of TM in preventing erosive tooth wear.

Acknowledgements

This project was completed during the tenure of the NHMRC (National Health and Medical Council of Australia) Dental Postgraduate Research Award by the first author. It was also supported by research grants from the Australian Dental Research Foundation Inc. and GC Asia Pty. Ltd., and by travelling awards to the first author from the University of Adelaide and the NHMRC of Australia. The authors also wish to thank Prof Tim Watson for providing laboratory facilities to complete this study, Ms Nancy Briggs for conducting statistical analysis, and Mr Peter Pilecki and Mr Richard Mallet for providing technical assistance.

REFERENCES

1. Davis WB, Winter PJ. The effect of abrasion on enamel and dentine and exposure to dietary acid. *British Dental Journal* 1980;**148**:253–6.
2. Meurman JH, Sorvari R. Interplay of erosion and abrasion in tooth wear and possible approaches to prevention. In: Addy M, Embery G, Edgar W, Orchardson R, editors. *Tooth wear and sensitivity: clinical advances in restorative dentistry*. London: Martin Dunitz Ltd.; 2000. p. 171–80.
3. Eisenburger M, Hughes J, West NX, Jandt KD, Addy M. Ultrasonication as a method to study enamel demineralisation during acid erosion. *Caries Research* 2000;**34**:289–94.
4. Featherstone JD, Lussi A. Understanding the chemistry of dental erosion. *Monographs in Oral Science* 2006;**20**:66–76.
5. White I, McIntyre J, Logan R. Studies on dental erosion: an *in vitro* model of root surface erosion. *Australian Dental Journal* 2001;**46**:203–7.
6. Addy M, Shellis RP. Interaction between attrition, abrasion and erosion in tooth wear. *Monographs in Oral Science* 2006;**20**:17–31.
7. Azzopardi A, Bartlett DW, Watson TF, Sherriff M. The surface effects of erosion and abrasion on dentine with and without a protective layer. *British Dental Journal* 2004;**196**:351–4.
8. Ganss C, Klimek J, Brune V, Schurmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine *in situ*. *Caries Research* 2004;**38**:561–6.
9. Piekarz C, Ranjitkar S, Hunt D, McIntyre J. An *in vitro* assessment of the role of Tooth Mousse in preventing wine erosion. *Australian Dental Journal* 2008;**53**:22–5.
10. Rees J, Loyn T, Chadwick B. Pronamel and Tooth Mousse: an initial assessment of erosion prevention *in vitro*. *Journal of Dentistry* 2007;**35**:355–7.

11. Ramalingam L, Messer LB, Reynolds EC. Adding casein phosphopeptide-amorphous calcium phosphate to sports drinks to eliminate *in vitro* erosion. *Pediatric Dentistry* 2005;**27**:61–7.
12. Ranjitkar S, Narayana T, Kaidonis J, Hughes T, Richards LC, Townsend GC. The effect of casein-phosphopeptide-amorphous calcium phosphate on erosive dentine wear. *Australian Dental Journal*, in press.
13. Vieira A, Lugtenborg M, Ruben JL, Huysmans MC. Brushing abrasion of eroded bovine enamel pretreated with topical fluorides. *Caries Research* 2006;**40**:224–30.
14. Eisenburger M, Addy M, Hughes JA, Shellis RP. Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. *Caries Research* 2001;**35**:211–5.
15. Azzopardi A, Bartlett DW, Watson TF, Sherriff M. The measurement and prevention of erosion and abrasion. *Journal of Dentistry* 2001;**29**:395–400.
16. Sundaram G, Wilson R, Watson TF, Bartlett D. Clinical measurement of palatal tooth wear following coating by a resin sealing system. *Operative Dentistry* 2007;**32**:539–43.
17. Vanuspong W, Eisenburger M, Addy M. Cervical tooth wear and sensitivity: erosion, softening and rehardening of dentine; effects of pH, time and ultrasonication. *Journal of Clinical Periodontology* 2002;**29**:351–7.
18. Attin T, Siegel S, Buchalla W, Lennon AM, Hannig C, Becker K. Brushing abrasion of softened and remineralised dentin: an *in situ* study. *Caries Research* 2004;**38**:62–6.
19. Attin T, Deifuss H, Hellwig E. Influence of acidified fluoride gel on abrasion resistance of eroded enamel. *Caries Research* 1999;**33**:135–9.
20. Parry J, Harrington E, Rees GD, McNab R, Smith AJ. Control of brushing variables for the *in vitro* assessment of toothpaste abrasivity using a novel laboratory model. *Journal of Dentistry* 2008;**36**:117–24.
21. Ganss C, Schlueter N, Hardt M, von Hinckeldey J, Klimek J. Effects of toothbrushing on eroded dentine. *European Journal of Oral Sciences* 2007;**115**:390–6.
22. Ganss C, Klimek J, Schwarz N. A comparative profilometric *in vitro* study of the susceptibility of polished and natural human enamel and dentine surfaces to erosive demineralization. *Archives of Oral Biology* 2000;**45**:897–902.
23. Hooper S, West NX, Pickles MJ, Joiner A, Newcombe RG, Addy M. Investigation of erosion and abrasion on enamel and dentine: a model *in situ* using toothpastes of different abrasivity. *Journal of Clinical Periodontology* 2003;**30**:802–8.
24. West NX, Hughes JA, Addy M. Erosion of dentine and enamel *in vitro* by dietary acids: the effect of temperature, acid character, concentration and exposure time. *Journal of Oral Rehabilitation* 2000;**27**:875–80.
25. Ranjitkar S, Kaidonis JA, Townsend GC, Vu MA, Richards LC. An *in vitro* assessment of the effect of load and pH on wear between opposing enamel and dentine surfaces. *Archives of Oral Biology* 2008;**53**:1011–6.
26. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *Journal of Dental Research* 2003;**82**:206–11.
27. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: an *in vitro* study. *Australian Dental Journal* 2008;**53**:34–40.
28. Rahiotis C, Vougiouklakis G. Effect of a CPP–ACP agent on the demineralization and remineralization of dentine *in vitro*. *Journal of Dentistry* 2007;**35**:695–8.
29. Reynolds EC, Walsh LJ. Additional aids to remineralisation of tooth structure. In: Mount GJ, Hume WR, editors. *Preservation and restoration of tooth structure*. Queensland: Knowledge Books and Software; 2005. p. 111–8.
30. Tantbirojn D, Huang A, Ericson MD, Poolthong S. Change in surface hardness of enamel by a cola drink and a CPP–ACP paste. *Journal of Dentistry* 2007;**36**:74–9.
31. Bartlett DW, Smith BG, Wilson RF. Comparison of the effect of fluoride and non-fluoride toothpaste on tooth wear *in vitro* and the influence of enamel fluoride concentration and hardness of enamel. *British Dental Journal* 1994;**176**:346–8.
32. Lussi A, Hellwig E. Risk assessment and preventive measures. *Monographs in Oral Science* 2006;**20**:190–9.
33. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, *et al*. Fluoride and casein phosphopeptide-amorphous calcium phosphate. *Journal of Dental Research* 2008;**87**:344–8.