J Clin Periodontol 2004; 31: 434–438 doi: 10.1111/j.1600-051X.2004.00497.x
Printed in Denmark. All rights reserved

Copyright © Blackwell Munksgaard 2004

Journal of Clinical Periodontology

An in situ model to study the toothpaste abrasion of enamel

Andrew Joiner, Matthew J. Pickles, Carolyn Tanner, Elizabeth Weader and Peter Doyle

Unilever Oral Care, Port Sunlight Laboratory, Merseyside, UK

Joiner A, Pickles MJ, Tanner C, Weader E, Doyle P: An in situ model to study the toothpaste abrasion of enamel. J Clin Periodontol 2004; 31: 434–438. doi: 10.1111/j.1600-051X.2004.00497.x. © Blackwell Munksgaard, 2004.

Abstract

Objectives: In order to understand the clinical relevance of dentifrice abrasivity on the dentition in vivo, an in situ enamel wear model has been developed.

Materials and Methods: Polished human enamel blocks were indented with a Knoop diamond, attached to dentures and worn by adult volunteers for 24 h per day. The blocks were brushed for 30 s, twice per day with dentifrices of known relative dentine abrasivity (RDA) and relative enamel abrasivity (REA). The dentifrices used were either dentifrice A (RDA = 85, REA = 3.4), dentifrice B (RDA = 189, REA = 2.0) or dentifrice C (RDA = 132, REA = 42.7). After 28 days, the blocks were removed and the geometry of each Knoop indent was remeasured. From the baseline and post-treatment values of indent length, the amount of enamel wear was calculated from the change in the indent depth.

Results: The median values for enamel wear of dentifrices A, B and C were -0.02, 0.01 and $-0.48\,\mu$m, respectively. The differences between dentifrice C and dentifrices A and B were of statistical significance.

Conclusion: This study has demonstrated the usefulness of an in situ technique for investigating the relationship between the abrasivity of a dentifrice in vitro and the wear of enamel in situ.

Key words: abrasivity; dentifrice; enamel; in situ

Accepted for publication 13 July 2003

Tooth wear in vivo has a multi-factorial aetiology involving a number of inter-related processes. It is used to describe the processes of erosion, attrition and abrasion, although the effect of one factor – most notably erosion – is often dominant (Smith & Knight 1984). Erosion is defined as the chemical dissolution of teeth by acids, attrition is the wear of tooth against tooth, abrasion is the wear of teeth by physical means other than teeth (Bartlett & Smith 2000).

It is widely accepted in the dental profession that some degree of abrasivity must be tolerated in a dentifrice if satisfactory cleaning of the teeth is to be achieved (Stookey et al. 1982, Forward 1991). The abrasivity of a dentifrice formulation is commonly described in terms of its relative dentine abrasivity (RDA) and relative enamel abrasivity (REA) (Hefferren 1976). These in vitro methods investigate the ability of a slurry of a dentifrice to remove radioactive dentine or enamel during a brushing protocol relative to a standard abrasive or dentifrice. This has proven to be important, as the abrasive nature of a given product must be determined to ensure that during use by the consumer the oral hard tissues are not compromised. The International Standards Organization (ISO, 1995) has stated that for dentine, the abrasivity of the test formulation should not exceed 2.5 times the reference abrasive, in other words, RDA must not exceed 250. For enamel, the ISO has not set a maximum REA value for a normal use dentifrice that has a pH$>$5.5. Despite RDA and REA values often being quoted as a measure of dentifrice abrasivity, the clinical significance of RDA and REA has not been fully evaluated.

Although studies in vivo concerned with various tooth wear processes alone or combined are difficult, studies in situ which allow interactions to take place within the oral environment while combining the sensitivity of laboratory analysis should be possible. Indeed, a number of methods in situ to study tooth wear via erosion of dentine or enamel by soft drinks have been described (West et al. 1998, Amaechi et al. 2000, Clasen et al. 2000).

The aim of the current work was to study the clinical relevance of RDA and REA values for a number of dentifrices and to this end an in situ model was developed.

Materials and Methods

Subjects

The panel consisted of 12 adult volunteers with full dentures. Exclusion criteria included subjects not in full health, pregnant, or those unable to attend the regular dental surgery appointments. Informed consent was obtained and the protocol reviewed by an Independent Ethics Committee.

Preparation of inserts

The roots of human pre-molars, extracted for orthodontic purposes from either gender, were removed by using a diamond abrasion wheel. The lingual part of the tooth was then flattened to approximately 3 mm thick using the high abrasive disc, turned over and the facial surface then polished with the abrasive disc to flatten the surface. The polished sections were cut on a diamond wire cutter into approximately $3 \times 3 \times 2$ mm blocks such that one block was produced per tooth and labelled individually with a number.

The blocks were then polished successively with 3, 1 and 0.25 μm diamond polishes (Kemet International Ltd, Maidstone, UK) and indented four times with a Knoop diamond using a Buehler Micromet Microhardness Indenter (Buehler, Lake Bluff, IL, USA). The blocks were finally sterilised by γ irradiation prior to the in situ phase.

Study protocol

The study was a factorial crossover design and tested the hard tissue wear effect of three commercial dentifrices on enamel with differing RDA and REA values as shown in Table 1. The RDA and REA values were determined using the standard methods (Hefferren 1976). Each of the two test periods were 28 days in length with no wash out between the two test periods as new substrates were inserted for each leg.

One enamel block was randomly chosen and placed in a recess created in the upper posterior buccal surface of the full denture of a panellist so that the enamel surface was flush with the denture surface. The panellists were asked to remove their dentures for cleaning twice per day with one of the randomly assigned dentifrices using a soft toothbrush ensuring the enamel block was brushed for 30 s. Subjects were also instructed to wear their dentures continually for 24 h and not to use any other toothpaste, mouthwash, dental floss or denture cleaner for the duration of the study.

Table 1. Mean ($\pm$ standard error) RDA and REA values of dentifrices ($n = 8$)

Toothpaste	RDA	REA
A	85 ± 3	3.4 ± 0.3
B	189 ± 4	2.0 ± 0.7
C	132 ± 2	$42.7 \pm 3.0^{*}$

*Values differ significantly ($p < 0.05$) as determined by Student–Newman–Keul's analysis.

Wear measurement

The lengths of the Knoop indents were measured before the in situ phase of the study using the microhardness machine. After removal from the denture, the inserts were carefully cleaned with ethanol using a cotton bud swab and the indent lengths remeasured with the microhardness machine. The change in indent depth (Δd) was calculated from the change in indent length (Δl) using (see Fig. 1):

$$\begin{aligned} \Delta d &= 0.5\Delta l / \tan 86.25 \\ &= 0.032772\ \Delta l \end{aligned}$$

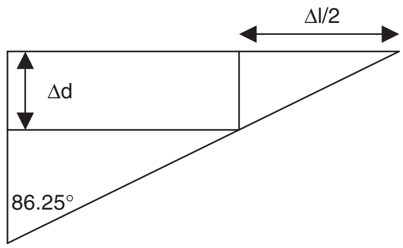

Fig. 1. Geometry of knoop indent before and after abrading the surface.

The amount of enamel abrasion was thus given by the average change in indent depth (Δd).

Results

For the enamel blocks brushed with either paste A or B, the indents were relatively easily found and remeasured post in situ. One sample from the dentifrice A group was unmeasurable due to the presence of what appeared to be a coating of polymethyl methacrylate that was used to fix the enamel block to the denture. One sample from the dentifrice B group was lost due to the sample fracturing upon removal from the denture. For the samples brushed with dentifrice C, it was more difficult to find the indents due to the increased level of scratching. Indeed, for one sample, the scratching was so severe that no clearly defined indents could be identified and measured and thus was excluded from further analyses. Typical optical microscope images of the enamel surfaces brushed with dentifrices A and C are shown in Figs 2 and 3, respectively. Enamel surfaces brushed with dentifrice B were qualitatively similar to dentifrice A.

The mean change in indent length (Δl) for all samples is given in Table 2. The calculated enamel wear values (Δd) and the overall median value for each dentifrice is also given in Table 2. It is apparent that the effects of dentifrices A and B on enamel wear are negligible whereas

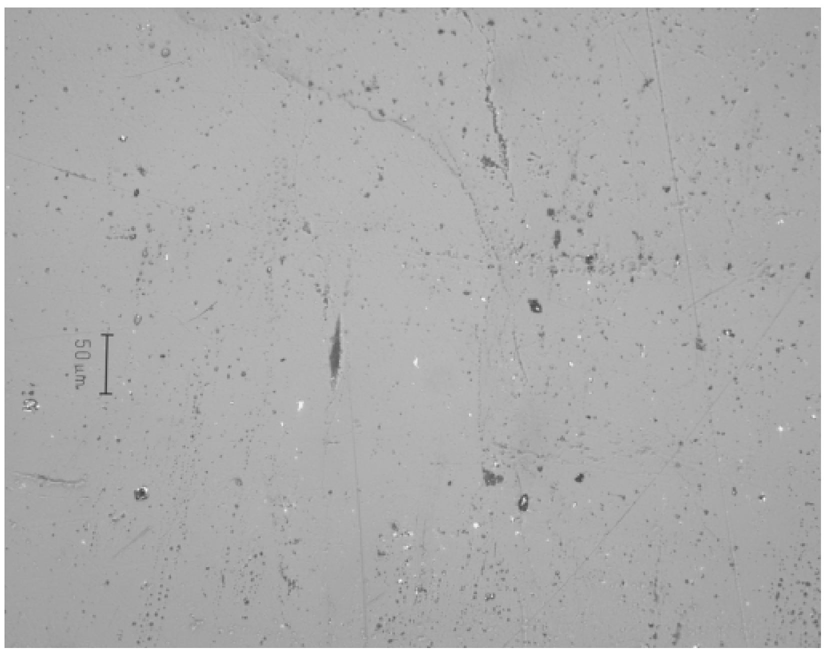

Fig. 2. Enamel sample brushed with dentifrice A for 28 days in situ.

Fig. 3. Enamel sample brushed with dentifrice C for 28 days in situ.

Table 2. Mean baseline and change in enamel indent length (Δl) and calculated enamel wear (Δd)

Dentifrice/ sample number	Mean baseline indent length (SD) (μm)	Mean change in indent length (Δl) (SD) (μm)	Mean enamel wear (Δd) (μm)
A1	68.4 (1.4)	– 1.9 (4.3)	– 0.06
A2	70.6 (2.4)	– 0.7 (2.0)	– 0.02
A3	70.7 (1.7)	– 0.6 (1.7)	– 0.02
A4	69.5 (1.6)	– 0.4 (3.7)	– 0.01
A5	71.2 (1.7)	– 1.2 (1.9)	– 0.04
A6	72.6 (2.2)	1.3 (1.5)	0.04
A7	71.6 (1.0)	– 1.7 (6.4)	– 0.06
	Mean = 70.7 (1.4)		Median = – 0.02
B1	69.2 (1.1)	– 2.8 (2.7)	– 0.09
B2	69.9 (3.0)	– 1.3 (0.4)	– 0.04
B3	69.4 (2.2)	8.5 (6.0)	0.27
B4	69.9 (2.1)	0.2 (1.8)	0.01
B5	70.9 (1.6)	4.7 (2.9)	0.15
B6	71.9 (1.1)	4.2 (2.0)	0.14
B7	71.7 (3.1)	– 0.4 (0.3)	– 0.01
	Mean = 70.4 (1.1)		Median = 0.01
C1	67.2 (0.2)	– 2.7 (2.4)	– 0.09
C2	67.0 (1.4)	– 3.0 (2.0)	– 0.10
C3	69.2 (1.6)	– 4.2 (4.0)	– 0.14
C4	69.4 (2.6)	– 23.4 (9.4)	– 0.77
C5	69.5 (1.2)	– 39.7 (10.2)	– 1.30
C6	70.3 (1.4)	– 14.7 (2.6)	– 0.48
C7	69.3 (2.1)	– 24.5 (6.4)	– 0.80
	Mean = 68.8 (1.2)		Median = – 0.48

Table 3. Specific paired comparisons for enamel brushed with three different dentifrices in situ

Comparison	Permutation test
A v B	NS
A v C	$p < 0.0255$
B v C	$p < 0.0427$

dentifrice C gave a much greater level of enamel wear which was of statistical significance (permutation test) (Table 3).

Discussion

In general, in situ models are important since they allow interactions to take place among bacteria, saliva and hard tissues in the oral environment, while retaining the sensitivity of laboratory analysis (Wefel 1990). The use of in situ models for the study of de- and remineralisation processes is well established (Manning & Edgar 1992, White et al. 1992, ten Cate 1994). These models are commonly used and recognised as valuable tools for caries researchers, for example, in the identification of novel anti-caries agents and the regulatory screening of commercial product variations (Schafer 1989, White, 1992). In recent years, there has been the introduction of in situ models that reflect the processes involved with stain formation (Joiner et al. 1995) and erosive wear (West et al. 1998, Amaechi et al. 2000, Clasen et al. 2000). The erosive wear in situ models consist of slabs of enamel mounted in the human mouth with intra-oral appliances and subjected to cariogenic or erosive conditions (e.g. orange juice), after which tissue loss was recorded by microradiography, microhardness or surface profilometry.

Historically, there have only been a few reports describing in situ models that have the potential to evaluate the effects of dentifrice abrasive wear of hard dental tissues. The first, described by Cowell (1974) used gold, circumferential clasps cemented to lower premolars in vivo to attach 2 mm square sections cut from the cemento-enamel junction of extracted teeth. Davis (1978) used a flattened enamel/dentine block set into the labial surface of an upper incisor porcelain crown. This was examined periodically by taking replicas of the test area. Kodaka et al. (2001) described dentine samples mounted on an intra-oral appliance that were brushed daily with a sensitive teeth dentifrice and the dentine wear was measured with a scanning laser microscope. Our approach, using full denture wearers is clearly advantageous to the earlier reported methods since it is much less invasive to the subject, quick, simple and offers the potential to study a larger number of inserts and products.

The results of the current study show that the wear of enamel by dentifrices A and B is similar and very small reflecting their relatively low yet similar REA values (Table 1). It appears that RDA values have little influence on enamel wear in situ since dentifrices A and B having quite different RDA values (85 and 189, respectively). The greater loss of enamel due to brushing with dentifrice C versus either dentifrice A or B is clearly reflected by the significantly higher REA value for the former. This difference is probably due to the relative hardness of the dentifrice abrasive system compared with the hardness of the dentine and enamel substrate.

For the in vitro REA measurement, a calcium pyrophosphate abrasive system is used as a standard (Hefferren 1976) and the abrasive wear to enamel is given an arbitrary value of 10. All test dentifrices are then given an enamel wear measurement relative to this standard. Thus dentifrices with an REA <10 are less abrasive to enamel than the calcium pyrophosphate standard. It is clear from our data that dentifrices with REA <10 gave negligible enamel wear whereas dentifrice C with a significantly higher REA gave measurable enamel wear in situ. Whether, the in situ wear caused by dentifrices of this order of magnitude of REA have any clinical relevance to enamel wear in vivo is currently not known and requires further study.

The approach of using changes of indent geometries has previously been described for measuring the wear of denture acrylic (Murray et al. 1986) and enamel (Jaeggi & Lussi 1999, Singleton et. al. 1999). In the denture acrylic study described by Murray et al. (1986), the denture acrylic was indented with a Vickers diamond to give a range of indent sizes. The amount of wear was categorised depending on the visibility of the indents after brushing with a dentifrice. It was found that in situ wear was lower than a corresponding in vitro study. In the study described by Jaeggi & Lussi (1999), it was possible to measure the extent of abrasive wear of erosively softened human enamel after being worn in situ for up to 1 h followed by a 30 s brushing with a dentifrice of RDA = 77 and REA = 4.5. The levels of enamel wear were typically between 0.19 and 0.26 μm. These values are significantly higher than for dentifrice A in our study (which has similar RDA and REA values) reflecting the profound effect, which an erosive challenge can have on tooth wear. In the in vitro study described by Singleton et al. (1999), polished enamel was indented with a Knoop diamond and the effect of two dentifrice products with REA values of 2.5 and 36 were reported to give an enamel wear of 0.40 and 0.62 μm, respectively, after brushing for 6 min. For the high REA paste, this is a change comparable to that found in our in situ study. However, for the low REA paste, the loss of enamel in vitro is much greater than for this in situ study. It is hypothesised that this difference may be because the in situ enamel slabs become coated with the acquired pellicle derived from saliva (Dawes et al. 1963, Sonju & Rolla 1973) and thus giving a protective layer to the underlying enamel. Indeed, it is documented that the acquired pellicle plays a large role in the protection of the tooth by providing an interface between the tooth surface and the external environment (Meckel 1968, Tabak et al. 1982, Meurman & ten Cate 1996). Therefore, due to the influence of the acquired pellicle, the data obtained from an in situ study perhaps gives a truer indication of the in vivo tooth wear situation than of any data obtained from in vitro studies.

Pellicle thickness and composition has been shown to vary around the mouth (Hannig 1997, Carlen et al. 1998, Amaechi et al. 2000, Clasen et al. 2000). In particular, Hannig (1997) has shown that the pellicle thickness formed on enamel inserts after 6 h positioned buccally is approximately 1.0 μm while that lingually is only approximately 0.1 μm. In addition, it has been shown that there are ultrastructural differences between the two pellicles. These differences may give rise to differences in protection to tooth wear processes caused by abrasive action. Indeed, Amaechi et al. (2000) suggested that the variation in pellicle thickness within the dental arches may be a determinant factor for the site specificity of dental erosion, and also confirmed that pellicle does provide some protection for teeth from erosion. Thus, the location of any in situ inserts for wear studies needs to be carefully considered and future work needs to consider the variation of insert location in order to understand more fully the protective effects of pellicle thickness against the action of dietary factors and toothpaste abrasives.

References

Amaechi, B. T., Higham, S. M. & Edgar, W. M. (2000) Development of an in-situ model to study dental erosion. In: *Tooth Wear and Sensitivity*, eds. Addy, M., Embery, G., Edgar, W. M. & Orchardson, R., 1st edition, pp. 181–188. London: Martin Dunitz Ltd.

Bartlett, D. & Smith, B. G. N. (2000) Definition, classification and clinical assessment of attrition, erosion and abrasion of enamel and dentine. In: *Tooth Wear and Sensitivity*, eds. Addy, M., Embery, G., Edgar, W. M. & Orchardson, R., 1st edition, pp. 87–92. London: Martin Dunitz Ltd.

Carlen, A., Borjessen, A. C., Nikdel, K. & Olsson, J. (1998) Composition of pellicles formed in vivo on tooth surfaces in different parts of the dentition, and in vitro on hydroxyapatite. *Caries Research* **32**, 447–455.

Clasen, A. B. S., Hannig, M. & Sonju, T. (2000) Variations in pellicle thickness: a factor in tooth wear? In: *Tooth Wear and Sensitivity*, eds. Addy, M., Embery, G., Edgar, W. M. & Orchardson, R., 1st edition, pp. 181–188. London: Martin Dunitz Ltd.

Cowell, C. R. (1974) An appliance for the study of tooth tissue in vivo. *British Dental Journal* **137**, 61–62.

Davis, W. B. (1978) The cleaning, polishing and abrasion of teeth by dental products. In: *Cosmetic Science*, ed. Breuer, M. M., **Vol. 1**, pp. 39–81. London: Academic Press.

Dawes, C., Jenkins, G. N. & Tonge, C. H. (1963) The nomenclature of the integuments of the enamel surface of the teeth. *British Dental Journal* **115**, 65–68.

Forward, G. C. (1991) Role of toothpastes in the cleaning of teeth. *International Dental Journal* **41**, 164–170.

Hannig, M. (1997) Transmission electron study of in vivo pellicle formation on dental restorative materials. *European Journal of Oral Science* **105**, 422–433.

Hefferren, J. J. (1976) Laboratory method for assessment of dentifrice abrasivity. *Journal of Dental Research* **55**, 553–573.

International Standards Organization (1995) Dentistry – toothpastes-requirements, test methods and marking, ISO 11609.

Jaeggi, T. & Lussi, A. (1999) Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. *Caries Research* **33**, 455–461.

Joiner, A., Jones, N. M. & Raven, S. J. (1995) Investigation of factors influencing stain formation utilizing an in situ model. *Advances in Dental Research* **9**, 471–476.

Kodaka, T., Kuroiwa, M., Okumura, J., Mori, R., Hirasawa, S. & Kobori, M. (2001) Effects of brushing with a dentifrice for sensitive teeth on tubule occlusion and abrasion of dentin. *Journal of Electron Microscopy* **50**, 57–64.

Manning, R. H. & Edgar, W. M. (1992) Intra-oral models for studying de- and remineralization in man: methodology and measurement. *Journal of Dental Research* **71**, 895–900.

Meckel, A. H. (1968) The nature and importance of organic deposits on dental enamel. *Caries Research* **2**, 104–114.

Meurman, J. H. & ten Cate, J. M. (1996) Pathogenesis and modifying factors of dental erosion. *European Journal of Oral Science* **104**, 199–206.

Murray, I. D., McCabe, J. F. & Storer, R. (1986) Abrasivity of denture cleaning pastes in vitro and in situ. *British Dental Journal* **161**, 137–141.
Schafer, F. (1989) Evaluation of the anticaries benefit of fluoride toothpaste using an enamel insert model. *Caries Research* **23**, 81–86.
Singleton, S. J., Doyle, P. & Huntington, E. (1999) In-vitro technique for measuring the abrasivity of commercially available dentifrices on human enamel. *Journal of Dental Research* **78**, 497 (IADR *Abstract*s).
Smith, B. G. N. & Knight, J. K. (1984) A comparison of patterns of tooth wear with aetiological factors. *British Dental Journal* **157**, 16–19.
Sonju, T. & Rolla, G. (1973) Chemical analysis of the acquired pellicle formed in two hours on cleaned human teeth in vivo. *Caries Research* **7**, 30–38.
Stookey, G. K., Burkhard, T. A. & Schemehorn, B. R. (1982) In vitro removal of stain with dentifrices. *Journal of Dental Research* **61**, 1236–1239.
Tabak, L. A., Levine, M. J., Mandel, I. D. & Ellison, S. A. (1982) Role of salivary mucins in the protection of the oral cavity. *Journal of Oral Pathology* **11**, 1–17.
Ten Cate, J. M. (1994) In situ models, physicochemical aspects. *Advances in Dental Research* **8**, 125–133.
Wefel, J. S. (1990) Effects of fluoride on caries development and progression using intra-oral models. *Journal of Dental Research* **69**, 626–633.
West, N. X., Maxwell, A., Hughes, J. A., Parker, D. M., Newcombe, R. G. & Addy, M. (1998) A method to measure clinical erosion: the effect of orange juice consumption on erosion of enamel. *Journal of Dentistry* **26**, 329–335.
White, D. J. (1992) The comparative sensitivity of intra-oral, in vitro, and animal models in the 'profile' evaluation of topical fluorides. *Journal of Dental Research* **71**, 884–894.
White, D. J., Faller, R. V. & Bowman, W. D. (1992) Demineralization and remineralization evaluation techniques – added considerations. *Journal of Dental Research* **71**, 884–894.

Address:
Andrew Joiner
Unilever Oral Care
Port Sunlight Laboratory
Quarry Road East
Bebington
Wirral CH63 3JW
UK
Fax: +44 151 641 1806
E-mail: andrew.joiner@unilever.com

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.