BIOLOGY OF TOOTH WEAR: PREVENTIVE STRATEGIES

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CHAPTER 8

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CHAPTER 9

APPENDICES

9.1 Publications

9.1.1 Research papers directly related to research for this PhD

9.1.1.1 Papers accepted for publication

1. **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.
   Arch Oral Biol 2008; 53: 1011-1016.

   [This paper relates to work completed during the earlier phase of the PhD. This paper provides basic information about the role of third body components in lubricating the wear interface, which forms the basis of research protocol used in the PhD project. Permission has been obtained from the Elsevier to reproduce this paper in this thesis.]

2. **Ranjitkar S**, Kaidonis J, Richards LC, Townsend GC. The effect of CPP-ACP on enamel wear under strong erosive conditions.
   Arch Oral Biol 2009, 54: 527-532.

   [This paper relates to the role of CPP-ACP in reducing enamel wear under a heavy load of 100N at a pH of 1.2 simulating gastric regurgitation, and these findings are covered in Chapter 3. Permission has been obtained from the Elsevier to reproduce this paper in this thesis.]

3. **Ranjitkar S**, Rodriguez JM, Kaidonis JA, Richards LC, Townsend GC. Bartlett DW. The effect of casein phosphopeptide - amorphous calcium phosphate on erosive enamel and dentine wear by toothbrush abrasion.
   J Dent 2009; 37: 250-254.

   [This paper relates to the role of CPP-ACP in reducing toothbrush abrasion of both enamel and dentine in an erosive environment. The findings related to enamel wear prevention are covered in Chapter 4. As this thesis focuses on enamel wear prevention, findings on dentine wear prevention are not included in Chapter 4. Permission has been obtained from the Elsevier to reproduce this paper in this thesis.]

4. **Ranjitkar S**, Narayana T, Kaidonis JA, Hughes TE, Richards LC, Townsend GC. An in vitro assessment of the effect of casein phosphopeptide- amorphous calcium phosphate on erosive dentine wear.
   Aust Dent J 2009; 54: 101-107.

   [This project was conducted as an honours project by T Narayana, who was supervised by other co-authors. The findings relating to lubricating and remineralizing potential of Tooth Mousse® from this project have formed a foundation for the development of experimental protocols for the PhD project. Permission has been obtained from the editor of the Australian Dental Journal to reproduce this paper in this thesis.]

5. Piekarz C, **Ranjitkar S**, Hunt D, McIntyre J. An in vitro assessment of the role of Tooth Mousse in preventing wine erosion.
   Aust Dent J 2008; 53: 22-25.

[This paper arose from a selective research project involving C Piekarz. Other co-authors supervised this project. The findings of this project have also formed a basis for the development of experimental protocols for the PhD project. Permission has been obtained from the editor of the Australian Dental Journal to reproduce this paper in this thesis.]

6. Nguyen C, **Ranjitkar S**, Kaidonis J, Townsend G. A qualitative assessment of non-carious cervical lesions in extracted human teeth.
Aust Dent J 2008; 53: 46-51.

[This paper arose from an honours project involving C Nguyen, who was supervised by other co-authors. This paper highlights the potential role of erosion and toothbrush abrasion in the aetiology of non-carious cervical lesions. The findings of the PhD project involving the role of CPP-ACP in reducing toothbrush abrasion in an erosive environment (Chapter 4/ Paper 2 of this section) is relevant to the management of non-carious cervical lesions. Permission has been obtained from the editor of the Australian Dental Journal to reproduce this paper in this thesis.]

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Dent Anthrop 2004; 17: 70-74.

[This paper relates to the validity and reliability of the 3D profilometer mentioned in Chapter 3 of this thesis. The editor of Dental Anthropology has indicated that the journal does not require the author to transfer copyright for reproduction in this thesis, and that authors retain the copyrights of their papers.]

Ranjitkar, S., Kaidonis, J.A., Townsend, G.C., Vu, A.M. and Richards, L.C. (2008)
An in vitro assessment of the effect of load and pH on wear between opposing enamel and dentine surfaces.
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The effect of casein phosphopeptide-amorphous calcium phosphate on erosive dentine wear

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ABSTRACT

Background: Erosive tooth wear is a growing concern in clinical dentistry. Our aims were to assess the effect of Tooth Mousse (TM) in managing erosive dentine wear *in vitro*.
Methods: Opposing enamel and dentine specimens from 36 third molar teeth were worn under a load of 100 N for 75 000 cycles in electromechanical tooth wear machines. In experiment 1, TM was applied continuously at the wear interface and the mean dentine wear rate was compared with those of specimens subjected to continuous application of hydrochloric acid (HCl, pH 3.0) and deionized water (DW, pH 6.1) as lubricants. In experiment 2, specimens were subjected to TM application every 1600 cycles at both pH 3.0 and 6.1, and the mean dentine wear rates were compared with those of specimens worn with continuous application of HCl and DW lubricants.
Results: Dentine wear was reduced significantly with continuous application of TM compared with HCl and DW lubricants. Specimens prepared with continuous TM application displayed smooth wear facets, whereas more pronounced microwear details were observed with HCl and DW lubricants.
Conclusions: Both remineralization and lubrication seem to contribute to reduction in dentine wear associated with TM application, although lubrication appears to have a more pronounced effect.

Key words: Attrition, erosion, lubrication, prevention, remineralization.

Abbreviations and acronyms: CPP-ACP = casein phosphopeptide-amorphous calcium phosphate; DW = deionized water; HCl = hydrochloric acid; SEM = scanning electron microscopy; TM = Tooth Mousse.

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INTRODUCTION

Tooth wear can result occasionally in extensive loss of enamel and dentine, leading to dentinal sensitivity and pulpal exposure.[1] Dental professionals have a responsibility to identify individuals at risk of tooth wear and to preserve the long-term health of the teeth.[2] The management of tooth wear may involve various regimes, including dietary advice, stress management, nightguards, application of protective resin coating, use of fluoride and complex restorative work.[3] Given that there has been an increase in the prevalence of erosive tooth wear (referring to tooth wear by erosion combined with mechanical factors, such as attrition and toothbrush abrasion), and an increase in the longevity of teeth in recent decades, clinical management should focus on early detection and prevention before a restorative approach is considered.[4]

Previous reports have indicated that topical fluoride can protect enamel and dentine against erosion[5,6] and a combination of erosion and toothbrush abrasion,[7–9] but not against attritional wear between opposing enamel and dentine specimens.[10] Recently, Tooth Mousse (GC Corporation, Japan) whose principal ingredient is an anticariogenic remineralizing agent CPP-ACP (casein phosphopeptide-amorphous calcium phosphate nanocomplexes) has been recommended for the management of dental erosion.[11] For example, CPP-ACP has been shown to reduce erosion by citric acid[12] and white wine,[13] and to reduce the erosive potential of acidic sports drink.[14] CPP-ACP has also been reported to reduce erosive enamel wear involving heavy attrition (at pH 1.2),[15] and to reduce erosive enamel and dentine wear involving toothbrush abrasion (at pH 3.0).[16] However, there is a lack of research investigating its ability to reduce erosive dentine wear involving attrition.

The aims of this study were: (i) to compare the rates of dentine wear with continuous application of TM, under conditions simulating heavy attrition, with those obtained without TM in an erosive environment (pH 3.0 simulating an acidic diet) and in a relatively neutral environment (pH 6.1); and then (ii) to investigate whether intermittent application of TM under these conditions reduced dentine wear. It was hypothesized that TM application would result in reduced dentine wear compared with no TM application, and that wear rates would be lower with continuous TM application compared with intermittent application.

MATERIALS AND METHODS

Sample preparation

The method of sample preparation, the design of the tooth wear machines and assessment of tooth wear using a 3D profilometer has been described previously.[17] The protocol for collection of extracted human teeth was approved by the University of Adelaide Human Research Ethics Committee (H/27/90). Freshly extracted, intact third molar teeth with unknown history were sectioned mesio-distally along their longitudinal axis with a Buehler Isomet diamond saw (Buehler, Dusseldorf, Germany), and enamel from the buccal halves of the crowns was carefully removed with high-speed diamond burs to expose the dentine surface. This resulted in two specimens per tooth, with the enamel specimen having the original contour of the palatal/lingual surface of the crown and the dentine specimen retaining the contour of the original dentino-enamel junction of the buccal surface of the crown. Opposing enamel and dentine specimens were then mounted on scanning electron microscopic (SEM) stubs, adjacent to three 2 mm diameter spherical steel reference balls, using autopolymerizing acrylic resin (Vertex-Dental BV, Zeist, The Netherlands). The specimens were randomly allocated to study groups. Power studies indicated that samples of between 5 and 10 were required in each group. Most experiments commenced with a sample of 10 specimens but a variable number of these failed and were excluded, resulting in a final sample size of 36.

Mechanical testing

Dentine specimens were worn against opposing enamel specimens from the same teeth in one of the two randomly selected electromechanical wear machines designed to simulate heavy attrition under a fixed load of 100 N at a rate of 80 cycles per minute.[18] Given that micro-hardness of mantle dentine (dentine within 1 mm from the dentino-enamel junction) is significantly less than that of underlying dentine,[19] dentine specimens were worn in two phases. A primary wear phase of 7200 cycles under a load of 32 N with deionized water as lubricant ensured that wear facets of similar surface areas were prepared well into dentine before its wear characteristics were assessed. All wear measurements for the purpose of the present study were obtained under a load of 100 N during a secondary wear phase that was subdivided into three secondary stages (S1, S2 and S3) each of 25 000 wear cycles.

Data for dentine wear rates were examined in three separate experiments that are outlined in Table 1. The first experiment was designed to investigate whether TM reduced dentine wear. The wear rates of dentine specimens worn with continuous application of TM at pH 7.2 (group A, n = 7) were compared with those of specimens worn with continuous application of hydrochloric acid (HCl) at pH 3.0 (group B, n = 9) and deionized water (DW) at pH 6.1 (group C, n = 6) derived in our previous study.[17] Deionized water is a standard lubricant in tribological studies of dental materials.[20] The HCl and DW lubricants were supplied to the wear interface by a gravity-fed drip system at the rate of 0.5 mL/min). The second experiment was designed to investigate the effect of continuous and intermittent application of TM on dentine wear under a neutral/near neutral environment by comparing the wear rates in group A (with continuous TM application, pH 7.2) with those in group D (n = 6) subjected to wear with DW (pH 6.1) with intermittent TM application. After the specimens in group D were worn for 1600 cycles with DW lubricant, the tooth wear machines were stopped and the specimens were washed gently with water for 40 seconds and dried with air for

Table 1. Comparison of the experimental design between study groups

Experiments	Comparisons	Identical parameters	Variable parameters
1†	Groups A vs B vs C	Continuous application of lubricants	Type of lubricants (TM vs HCl vs DW)
2‡	Groups (B vs E) and (C vs D)	pH of the lubricants (HCl and DW)	TM vs no TM for both HCl and DW lubricants
3¶	Groups A vs D	Neutral/near neutral pH values	Continuous vs intermittent application of TM

Abbreviations: TM = Tooth Mousse (pH 7.2); HCl = hydrochloric acid (pH 3.0); DW = deionized water (pH 6.1).
Dentine specimens were worn in tooth wear machines at a load of 100 N for 75 000 cycles with continuous application of TM in group A (in a neutral environment), HCl in group B (in an acidic environment) and DW in group C (in a near neutral environment). Specimens were worn with HCl in group E and with DW in group D, but tooth wear machines were stopped every 1600 cycles for intermittent application of TM.
†Comparison of the effect of continuous application of TM vs HCl vs DW lubricants on dentine wear.
‡Comparison of the effect of intermittent application of TM at pH = 3.0 and 6.1 on dentine wear.
¶Comparison of the effect of continuous vs intermittent application of TM under similar pH values on dentine wear.

20 seconds. TM was applied to the dentine surface with a microbrush for 5 minutes, then left at the surface during subsequent wear episodes. Although the pH values of DW (6.1) and TM (7.2) are slightly different, they are comparable for the purpose of this study because the rate of dissolution of enamel by DW has been shown to be minimal and is of no practical significance.[14,21]

The third experiment was designed to investigate the effect of intermittent application of TM in reducing dentine wear under both acidic and near neutral environments. As part of this experiment, dentine specimens in group E (n = 8) were worn using protocols similar to those in group D but with HCl lubricant instead of DW lubricant. Comparison of wear rates between groups B and E, and between groups C and D, yielded information about the effect of intermittent application of TM on dentine wear in an acidic environment and a near neutral environment, respectively.

Assessment of dentine wear

Quantitative analysis

The dentine specimens were scanned after the primary wear phase, and between each secondary wear stage, using a 3D profilometer (PIX-4, Roland DG, Tokyo, Japan) supplied with 'Dr. PICZA' software. The data were then imported to purpose-written MATLAB (version 6.5) software designed to quantify tooth wear.[22] The maximum resolution of the profilometer in X and Y axes is 50 μm and that in the Z axis is 25 μm. The validity of this system for calculating volumes of objects with complex geometry is greater than 90 per cent, and its intra- and inter-examiner reliability is very high.[22]

Qualitative analysis

Negative impressions of the dentine specimens were obtained using a light-bodied polyvinyl siloxane impression material (Imprint II, 3M-ESPE Corporation, St Paul, USA), and their positive replicas were prepared in epoxy resin for scanning electron microscopy (SEM) assessment at a magnification of ×200.

Statistical analyses

Mixed linear models were developed (Proc Mixed; SAS 9.1, Cary, USA, 2003) separately for the three experiments. These models accounted for the autoregressive covariance between successive measures on the same dentine specimens. Pairwise mean comparisons, adjusted for multiple testing (Tukey-Kramer), were conducted within each model. Significance was set at the 0.05 probability level.

RESULTS

Quantitative analysis

Mean values and standard deviations for dentine wear rates at different secondary wear stages are shown in Table 2. When data were analysed to investigate the effect of continuous application of lubricants on dentine wear, the nature of TM application (continuous vs intermittent), secondary wear phase (S1 vs S2 vs S3), and the interaction between type of lubricant (TM, HCl and DW) and secondary wear phase (S1, S2 and S3) were all associated with significant reduction in dentine wear ($p < 0.05$) (Fig 1). Pairwise comparisons of means between types of lubricants indicated significant reduction in wear rates with TM compared with HCl and DW lubricants (TM < HCl = DW), and pairwise comparisons of means between secondary wear stages indicated significantly lower wear rates in S1 and S2 stages compared with S3 stage (S1 = S2 < S3). Figure 1 also illustrates the interaction between type of lubricant and wear stage, which was significant primarily due to an increased wear rate in the S3 stage with lubrication by HCl.

When data were analysed to investigate the effect of continuous and intermittent application of TM at neutral/near neutral environment, continuous TM application resulted in an overall lower mean wear rates (0.02 ± 0.06 mm^3 per 1000 cycles, mean ± SE adjusted for factors in the linear mixed model)

Table 2. Comparison of rates of dentine wear (mm^3 per 1000 cycles) across three secondary wear stages (S1, S2 and S3) in various study groups

Secondary wear stages	Study groups														
	Group A			Group B			Group C			Group D			Group E		
	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD
S1	7	0.02	0.02	9	0.31	0.13	7	0.40	0.15	6	0.16	0.11	8	0.30	0.08
S2		0.02	0.02		0.32	0.20		0.46	0.18		0.15	0.03		0.25	0.09
S3		0.02	0.01		0.51	0.21		0.53	0.18		0.20	0.13		0.36	0.16

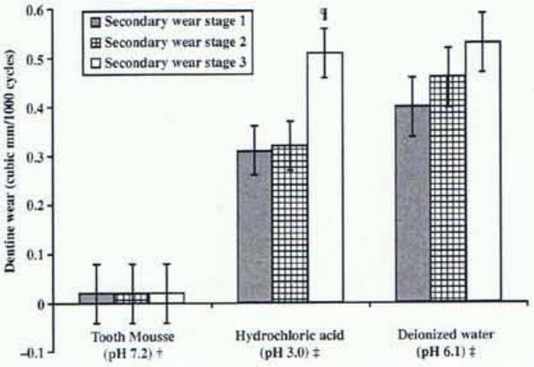

Fig 1. Comparison of dentine wear rates with continuous application of Tooth Mousse (group A), hydrochloric acid (group B) and deionized water (group C) at a load of 100 N for 75 000 cycles. Error bars represent standard errors of the mean wear rates. †,‡Significant differences in the overall wear rates between TM and HCl or DW lubricant (TM† < HCl‡ = DW‡) ($p < 0.001$). §Significant interaction between lubricant and wear stage due to an increased wear rate in the S3 stage for dentine lubricated by HCl lubricant ($p < 0.05$).

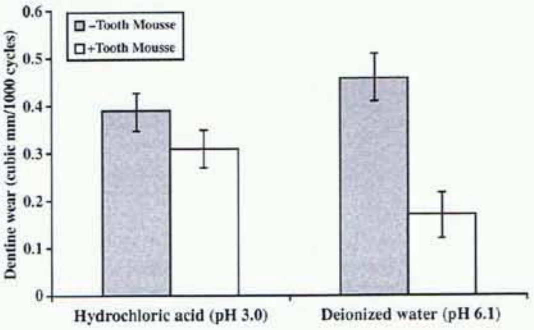

Fig 2. Comparison of dentine wear rates with intermittent and no application of Tooth Mousse at an acidic environment (group B vs group E) and a near neutral environment (group C vs group D). The four columns represent wear rates for group B, group E, group C and group D, respectively (from left to right). Error bars represent standard errors of the mean wear rates. There was a significant pairwise difference in wear rates between methods of TM application (TM application < no TM application) ($p < 0.001$).

compared with those of intermittent application (0.17 ± 0.05 mm^3 per 1000 cycles) ($p < 0.001$).

When data were analysed to investigate the effect of intermittent application of TM at acidic and near neutral/neutral environments, method of TM application (TM vs no TM), secondary wear stages (S1 vs S2 vs S3) and the interaction between pH values (acidic and near neutral environments) and TM application (TM and no TM) contributed to significant reduction in dentine wear ($p < 0.05$) (Fig 2). Pairwise comparisons of means between methods of TM application indicated significant reduction in wear rates with TM application compared with no application (application < no application), and pairwise comparisons of means between secondary wear stages indicated significantly lower wear rates in S1 and S2 stages compared with S3 stage (S1 = S2 < S3). Figure 2 illustrates the effect of interaction between pH and TM application on dentine wear rates. The mean wear rate at pH 6.1 in the presence of TM was significantly less than those at pH 6.1 in the absence of TM, or at pH 3.0 in the absence of TM.

Qualitative analysis

The specimens in group A (with continuous TM application) had very smooth and polished surfaces, with minimal striations due to the wear process, and appeared different from specimens in other groups (Fig 3). Specimens in group B (with continuous HCl application) (Fig 4) and group C (with continuous DW application) (Fig 5) displayed striations corresponding

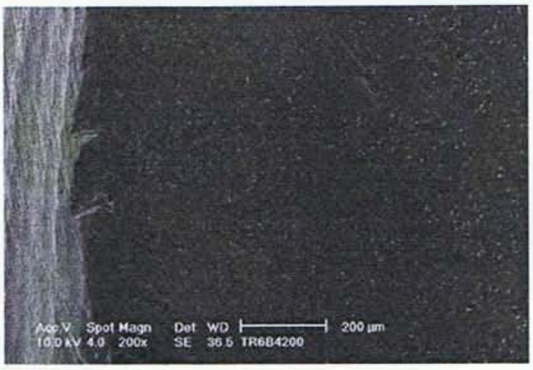

Fig 3. SEM micrograph of an epoxy replica of a dentine specimen in group A worn at load = 100 N with Tooth Mousse as the sole lubricant after 75 000 wear cycles (×200). Note the smooth surface compared with other groups (Figs 4–7).

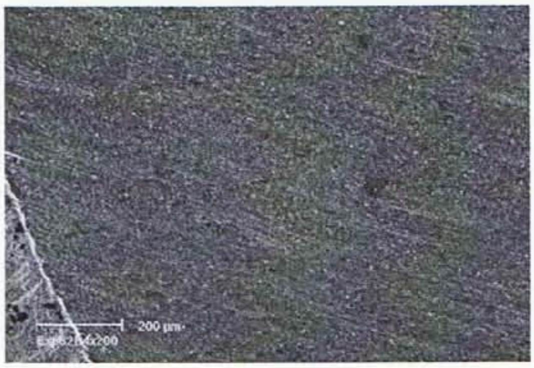

Fig 4. SEM micrograph of an epoxy replica of a dentine specimen in group B worn at load = 100 N and pH = 3.0, but without Tooth Mousse application, after 75 000 wear cycles (×200). Note the rough surface with some striations.

Fig 5. SEM micrograph of an epoxy replica of a dentine specimen in group C worn at load = 100 N and pH = 6.1, but without Tooth Mousse application, after 75 000 wear cycles (×200). Note the very rough surface, with marked striations.

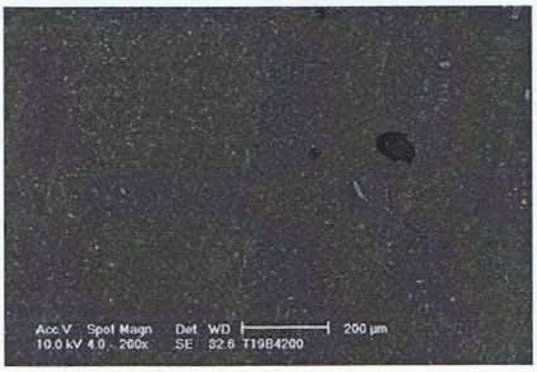

Fig 7. SEM micrograph of an epoxy replica of a dentine specimen in group E worn at load = 100 N and pH = 6.1 after 75 000 wear cycles, with Tooth Mousse applied intermittently (×200). Note the smooth surface compared with Fig 5.

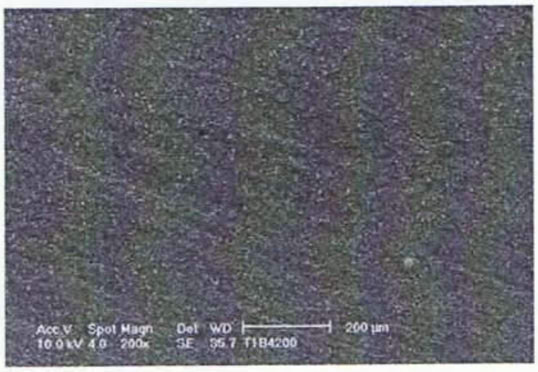

Fig 6. SEM micrograph of an epoxy replica of a dentine specimen in group D worn at load = 100 N and pH = 3.0 after 75 000 wear cycles, with Tooth Mousse applied intermittently (×200). Note the presence of some striations due to the wear process. The globular structures present on the wear facet are artefacts.

to gouges on the dentine surface made by microscopic particles of mineralized tissue (third bodies) at the wear interface and by rough surfaces of the opposing enamel specimens. However, specimens in group C displayed very rough striations and appeared clearly different to specimens from all the other groups. Specimens in group D (with intermittent TM application along with DW) (Fig 6) displayed striations similar to those in group B (Fig 4). Specimens in group E (with intermittent TM application along with HCl) (Fig 7) appeared smoother and more polished than those in group C (Fig 5).

DISCUSSION

This study has shown that TM can reduce dentine wear under *in vitro* conditions involving heavy attrition. Our experimental design is relevant to wear occurring between large areas of exposed dentine and opposing enamel surfaces, e.g., in patients with Class II Division II malocclusions where severely eroded palatal surfaces of maxillary incisor teeth with exposed dentine can wear against mandibular incisor teeth with enamel present on the incisal edges. However, our findings are not relevant to patients with exposed dentine on posterior teeth surrounded by an enamel rim.

The rationale behind selection of the number of wear cycles for secondary wear stages, loads and pH values has been reported in our previous study.[17] Little is understood about the frequency of sleep bruxism episodes with jaw movements and associated bite force. In a sleep bruxism study, Lavigne *et al.*[23] observed an average of 30 bruxism episodes with grinding noise in individuals per night. Thus, our turnover rate of 1600 cycles may represent around 1.8 months of wear in heavy bruxists, but caution is needed in extrapolating the findings of the present study to clinical situations because of the modification of the wear rates *in vivo* by salivary factors and by variations in episodes of bruxism and acid intake.[23,24]

Dentine wear rates were much lower with continuous application of TM than with continuous application of HCl lubricant in an acidic environment (pH 3.0) and DW lubricant in a near neutral environment (pH 6.1). The findings of faster rates of dentine wear in the S3 stage compared with S1 or S2 stages are consistent with those of our previous study showing that dentine near the pulp (deep dentine) wears faster than dentine near the dentino-enamel junction (superficial dentine).[17] Intermittent application of TM also reduced dentine wear in both the acidic and relatively neutral environments, although the benefit was greater in the latter. These findings open up possibilities for a new approach to tooth wear prevention, and indicate that TM could be used as an alternative to nightguards in certain situations.

Further studies are needed to compare the effectiveness of CPP-ACP paste, nightguards and other lubricating agents (e.g., artificial saliva products) in tooth wear prevention, and to investigate whether there could be an additive effect of different preventive strategies on tooth wear. Clinically, the constant presence of CPP-ACP paste on the tooth surface seems highly desirable. The finding that CPP-ACP could be detected in dental plaque three hours after individuals chewed CPP-ACP-containing gum,[25] implies that traces of TM may adhere to the tooth surface even after it appears to have been washed off by saliva and contribute to wear reduction. Further research is needed to investigate the duration of retention of TM on the tooth surface under various intra-oral conditions.

The mechanism by which CPP-ACP reduces erosive tooth wear is unclear, but its anticariogenic potential, including prevention of demineralization and enhancement of remineralization of sub-surface carious lesions in both enamel[25–27] and dentine[28] has been well documented. Recent studies have indicated that the anticariogenic properties of CPP-ACP may be responsible for prevention of enamel erosion.[14,29] Given that erosion is predominantly a surface phenomenon and that it is likely to result in only a shallow, softened subsurface layer compared with carious lesions,[4,24,30,31] remineralization of eroded lesions is likely to involve repair by deposition of mineral into the porous zone rather than growth of eroded crystals.[32] In the present study, TM was not diluted to a slurry and this could have restricted its potential to remineralize eroded lesions. However, its application as a paste was chosen to simulate use in the home or clinic with close-fitting trays.

Our findings of greater reduction in dentine wear with continuous application of TM compared with HCl and DW lubricants, and with intermittent application under a near neutral environment compared with an acidic environment (Figs 1 and 2), highlight the lubricating potential of TM which appears to be more pronounced than its remineralizing effect. It is hypothesized that CPP-ACP nanocomplexes and other ingredients of the TM formulation (e.g., glycerol) provide lubrication at the wear interface, thus producing very smooth, polished wear facets (Fig 3). The ingredients of TM also seem to adhere better to the tooth surface in a relatively neutral environment than in an acidic environment. The role of third bodies in reducing enamel wear has been highlighted by Kaidonis *et al.*[33] who found that calcium fluoride (CaF) powder and CaF/olive oil slurry produced polished wear facets and reduced attritional wear compared with dry (no additional) lubrication.

Further studies are needed to clarify the nature of lubricating and remineralizing properties of CPP-ACP paste in reducing tooth wear, and to compare its effectiveness with nightguards and other remineralizing products (including topical fluoride). Such information will be important in designing better strategies for tooth wear prevention. The findings of the present study also point to the need for clinical trials to investigate the effect of CPP-ACP in preventing tooth wear *in vivo*.

CONCLUSIONS

This study highlights the potential role of CPP-ACP in the clinical management of tooth wear. Dentine wear was almost eliminated with continuous application of TM, and the wear rate was lower at pH 6.1 than at pH 3.0 with intermittent application of TM. Our findings suggest that remineralization and lubrication both play a role in dentine wear prevention associated with TM application, although lubrication appears to have a more pronounced effect. These findings point to the need for clinical trials to investigate the effect of CPP-ACP on tooth wear *in vivo*. Further studies are also needed to compare the effectiveness of CPP-ACP in wear prevention with those of existing preventive strategies, including topical fluoride and nightguards.

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ADRF RESEARCH REPORT

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An *in vitro* assessment of the role of Tooth Mousse in preventing wine erosion

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ABSTRACT

Background: The recommendation that Tooth Mousse (TM), an anticariogenic remineralizing agent, is effective for controlling dental erosion in professional wine tasters is not evidence-based. The aim of this *in vitro* study was to determine the effectiveness of TM in reducing erosion of coronal enamel and radicular dentine/cementum simulating a typical wine judging session.

Methods: Enamel and dentine/cementum from buccal halves (experimental sample) and palatal halves (control sample) of human maxillary premolar teeth were subjected to 1500 one-minute exposures (cycles) to white wine (pH = 3.5). TM was applied every 20 cycles to the experimental sample, but not to the control sample. Paired t-tests were used to determine whether there were significant differences in erosion depths between the experimental and control samples.

Results: Mean erosion depths were significantly shallower in the experimental sample than the control sample for both enamel (34.4 µm versus 49.2 µm, respectively) ($p < 0.05$) and dentine/cementum (143.2 µm versus 203.7 µm, respectively) ($p < 0.01$).

Conclusions: TM may have significant role in the management of wine erosion, which is under-recognized as an occupational hazard by the Australian wine industry. Future studies are needed to compare the relative benefits of TM and other remineralizing agents, when used individually or in combination, in preventing dental erosion.

Key words: Tooth wear, wine tasting, occupational hazard, demineralization, remineralization.

Abbreviations and acronyms: CPP-ACP = casein phosphopeptide-amorphous calcium phosphate; TM = Tooth Mousse.

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INTRODUCTION

Wine erosion is an occupational hazard in professional wine tasters, particularly in wine judges who may taste up to 200 wines per day for four consecutive days.[1] Wine is acidic in nature (pH = 3.0–4.0) and contains organic acids with high erosive potential, such as tartaric, malic and lactic acids and smaller amounts of citric, succinic and acetic acids.[2,3] Furthermore, tooth structure eroded and softened by acids from extrinsic and intrinsic sources becomes susceptible to further wear by attrition and toothbrush abrasion.[4,5] Long periods of wine tasting can also overwhelm normal salivary protection of the oral environment, leading to extensive tooth wear and severe dentine hypersensitivity.[2,6]

Dental professionals have a responsibility to identify individuals at risk of erosion and to offer appropriate preventive measures.[7,8] Professional recommendations for prevention of erosion include use of fluoride products, modification of toothbrushing habits and application of protective resin coating to teeth.[9,10] These preventive strategies provide some degree of protection against dental erosion, but improved strategies are needed to further diminish the risk of abrasive/erosive insult on teeth.[10]

Tooth Mousse (TM) (manufactured by the GC Corporation, Japan), contains a new anticariogenic remineralizing agent CPP-ACP (a casein phosphopeptide that stabilizes amorphous calcium phosphate nanocomplex) and has superior remineralizing properties compared with fluoride alone.[11,12] Recent evidence shows that a single application of TM can reduce enamel erosion against citric acid,[13] and that CPP-ACP can reduce erosive potential of an acidic soft drink.[14] Furthermore, TM has been found to reduce tooth wear from attrition in both acidic and near neutral environments.[15] Current recommendations for the management of wine erosion include using oral products containing CPP-ACP (e.g., TM and Recaldent chewing gum) and fluoride,[9,16] although such recommendations are not evidence-based. Thus, more research is needed to better understand the role of TM in reducing wine erosion.

The aim of this *in vitro* study was to determine the effectiveness of TM in reducing erosion of coronal enamel and radicular dentine/cementum. It was hypothesized that TM would prevent wine erosion in both enamel and dentine/cementum.

MATERIALS AND METHODS

Six intact extracted human maxillary premolar teeth were sectioned longitudinally along the mesiodistal axis and then painted with an acid resistant varnish, leaving two 3 × 3 mm windows on coronal and radicular surfaces. Buccal halves of teeth were treated as experimental samples (n = 6) and palatal halves were treated as control samples (n = 6). White Riesling wine (pH = 3.5 at 21.2°C) was chosen in this study because it is more erosive than champagne, claret style wine and red wine.[17] Both experimental and control samples were dipped in wine and artificial saliva alternately for one minute each using a dipping machine and this cycle was repeated 1500 times. The solutions were stirred continuously and maintained at an average room temperature of 21.3°C (ranging from 18.0°C to 24.2°C). One litre of artificial saliva (pH = 6.5 at 21.2°C) contained the following ingredients in deionized water: 4.766 g Hepes free acid buffer, 0.1225 g K_2HPO_4, 11 mg NaF and 1.5 mL Stock $CaCl_2$. To ensure that the solutions did not become overly contaminated with extraneous chemicals, specimens were gently dried by using a desk fan for 30 seconds between dipping cycles. The solutions were also replaced every 200 cycles. The pH of the solutions remained consistent throughout the experiments, and the specimens were not desiccated during the drying stage.

In the experimental samples (n = 6), TM was applied every 20 cycles for four minutes and then washed off thoroughly for two minutes before the next cycle was continued. The experimental conditions are relevant to the sequence of wine exposure and Tooth Mousse application that might occur *in vivo*. In the control sample (n = 6 each), enamel and dentine/cementum specimens were eroded using similar protocol but no TM was applied. One dentine/cementum specimen was excluded from this study because polarized light microscopy revealed marked hypercementosis in this specimen.

Erosion depths were measured on cross-sections of erosion lesions by using a Leica MZ16 FA stereomicroscope (Heerbrugg, Switzerland) for enamel (x25.0) and by using an Olympus BX51 transmission polarized light microscope (Olympus Corporation, Tokyo, Japan) for dentine (x8.0) as described previously.[17,18]

Statistical analysis

Paired t-tests were used to determine whether there were significant differences in erosion depths for enamel and dentine/cementum between the experimental and control samples. Statistical significance was set at the 0.05 probability level. These tests were analysed using the statistical package SPSSX (version 13.0, SPSS Inc, Chicago, USA).

RESULTS

Mean erosion depth for enamel in the experimental sample (34.4 ± 3.65 μm, mean ± SE) was significantly less than that in the control sample (49.2 ± 4.57 μm) ($p < 0.05$). Mean erosion depth for dentine/cementum in the experimental sample (143.2 ± 13.63 μm) was also significantly less than that in the control sample (203.7 ± 10.09 μm) ($p < 0.01$). Overall, erosion depths in dentine/cementum specimens were four times greater than those in enamel specimens, and TM reduced erosion depths in both enamel and dentine/cementum by around 30 per cent (Table 1).

DISCUSSION

Our data show that dentine is more susceptible to wine erosion than enamel, supporting the premise that erosion proceeds at a faster rate once dentine is exposed.[19] Enamel erosion predominantly involves loss of surface volume, with the formation of only a shallow subsurface layer of approximately 1 μm depth in most situations.[20] In contrast, dentine/cementum erosion is characterized by minimal surface loss and a thick zone

Table 1. Comparison of erosion depths (μm) between experimental and control samples in enamel and dentine/cementum

Enamel			Dentine		
n	Experimental sample, mean ± SE	Control sample, mean ± SE	n	Experimental sample, mean ± SE	Control sample, mean ± SE
6	34.4* ± 3.65	49.2* ± 4.57	5	143.2** ± 13.63	203.7** ± 10.09

Paired t-tests show significant difference in erosion depths between experimental and control samples at p < 0.05* and p < 0.01**.

of demineralized organic dentine matrix having similar histological appearance to that of root caries.[18] Despite these physical differences in the outcomes of erosive demineralization between enamel and dentine/cementum, the present study showed that TM was equally effective in reducing wine erosion in both hard tissues (Table 1). Our findings along with those of other erosion preventive studies[13,14] point to the need for more comprehensive, well-designed clinical trials to determine the effectiveness of TM in the prevention of dental erosion. Further studies are also needed to determine the optimum frequency and mode of application of TM for preventing wine erosion.

Current recommendations for self-application of TM for wine tasters include application of TM after wine tasting sessions, or on the morning of the tasting event.[16] Wine tasting involves a very detailed evaluation of the aroma, bouquet, taste, flavour, body and astringency of wine,[3] and it is important that anti-erosive products do not alter the taste. Ramalingam *et al.*[14] reported that the addition of CPP-ACP did not alter the taste of an acidic sports drink. Our preliminary survey of eight professional wine tasters (unpublished data) also indicated that TM did not affect the taste one to two hours after initial application, but a detailed investigation is needed to confirm this.

The mechanism by which TM reduces erosion is unclear, although its anticariogenic properties, involving prevention of demineralization and enhancement of remineralization, are well-documented.[6,12] By maintaining saturation levels of calcium and phosphate at the tooth surface, CPP-ACP provides a reservoir of neutral ion pair ($CaHPO_4^0$) that inhibits enamel demineralization and promotes formation of hydroxyapatite crystals inside carious lesions.[12] It is hypothesized that, in addition to the prevention of erosive demineralization, TM also remineralizes (repairs) eroded enamel and dentine crystals. This hypothesis is supported by an observation that superficial granular structures, probably representing remineralized enamel crystals, formed on the enamel surface after treatment with a sports drink containing CPP-ACP.[14]

The present study did not compare the erosion-inhibiting effect of TM with that of fluoride as it is difficult to make direct comparisons between our findings and those for fluoride[21] due to methodological differences. Recent reports have indicated that frequent application of fluoride protects enamel against erosion by wine[17] and soft drinks.[22] Intensive application of fluoride has also been shown to reduce enamel and dentine erosion by strong acids,[23] even under conditions simulating gastric reflux.[24] Future studies are needed to compare the relative benefits of CPP-ACP and fluoride, when used individually or in combination, in preventing dental erosion.

Extensive erosion as a result of wine tasting may have dento-legal implications.[25] In Sweden, identification of wine tasting as an occupational hazard led to the provision of free preventive dental treatment by the state-owned company 'Systembolaget' to its employees.[26] This occupational hazard is under-recognized by the Australian wine industry. Further epidemiological studies are needed to investigate the prevalence of dental erosion in Australian wine tasters and to widely disseminate evidence-based measures to minimize erosive damage to their teeth.

CONCLUSION

Within the limitation of the present *in vitro* study, it is concluded that TM is effective in reducing wine erosion in both enamel and dentine/cementum. These findings have positive implications in the control and prevention of occupational hazard caused by wine erosion in professional wine tasters. Further studies are needed to compare the relative benefits of TM and fluoride, when used individually or in combination, in preventing dental erosion.

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SCIENTIFIC ARTICLE

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A qualitative assessment of non-carious cervical lesions in extracted human teeth

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ABSTRACT

Background: Opinions vary about the causes of non-carious cervical lesions (NCCLs). They have been attributed to toothbrush abrasion, acid corrosion (commonly termed dental erosion), and abfraction. The purpose of this study was to examine the microwear details of NCCLs in a collection of extracted human teeth using scanning election microscopy (SEM).

Methods: Negative replicas of large NCCLs in 24 extracted human teeth were obtained in polyvinylsiloxane impression material (Light Body Imprint ™II, 3M ESPE) and viewed under SEM.

Results: All NCCLs extended from the cemento-enamel junction to the root surface and they displayed a variety of wedge-shaped appearances. There was evidence of both abrasion and corrosion in 18 of the 24 teeth (75.0 per cent), abrasion only in one tooth (4.2 per cent) and corrosion only in five teeth (20.8 per cent). Horizontal furrows with smooth edges and minor scratch marks, characteristic of abrasion and corrosion, were noted in 13 teeth (54.2 per cent).

Conclusions: Based on microscopic assessment of a sample of extracted teeth, it appears that abrasion and corrosion are common associated aetiological factors in the formation of NCCLs.

Key words: Abrasion, erosion, abfraction, scanning electron microscopy.

Abbreviations and acronyms: CEJ = cemento-enamel junction; FEA = finite element analysis; NCCLs = non-carious cervical lesions; SEM = scanning election microscopy.

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INTRODUCTION

The term non-carious cervical lesion (NCCL) refers to the loss of tooth structure at the cemento-enamel junction (CEJ) of teeth by wear processes unrelated to bacterial action.[1] NCCLs can present in a variety of forms, including shallow grooves, broad saucer-shaped lesions and large wedge-shaped lesions.[1] It has been reported that NCCLs occur in up to 85 per cent of individuals, with their prevalence and severity increasing with age.[2] Large NCCLs with exposed dentine can cause dentine hypersensitivity[3,4] and increase the risk of pulp exposure or tooth fracture.[5]

There are numerous theories regarding the aetiology of NCCLs, including toothbrush abrasion, corrosion (commonly termed dental erosion) and abfraction,[1,6–8] and this has led to confusion over their management. Recommended treatment options include monitoring, adhesive restorations, diet modification, tooth cleaning regimes, and even occlusal adjustment to prevent further progression.[5,9] Toothbrush abrasion has been considered to be a cause of NCCLs since the mid-twentieth century,[1,5] but McCoy[10] proposed that bruxism had a primary aetiological role. The abfraction hypothesis states that concentration of tensile forces at the cervical region of teeth, as a result of cuspal flexure from heavy occlusal loading, leads to micro-crack formation cervically by disrupting bonds between hydroxyapatite crystals in enamel and dentine.[10,11] These cracks are then believed to be further susceptible to abrasion and corrosion.[12] However, there is a general lack of firm clinical evidence to support the abfraction hypothesis, and non-clinical models supporting this remain unrealistic.[13] In this context, assessment of microwear details of NCCLs may provide some insight into their aetiology.

Detailed studies investigating microwear features of NCCLs are scarce. A previous study using scanning electron microscopy (SEM) described the morphology of NCCLs at a magnification of ×15, and the authors suggested that the wedge-shaped lesions might have been caused by abfraction.[14] In another investigation conducted on six individuals, Bevenius *et al.*[15] noted horizontal or oblique scratches and grooves of varying depth and regularity in both saucer- and wedge-shaped NCCLs. The authors stated that the NCCLs were of idiopathic origin, although they noted horizontal scratch marks in enamel and dentine that were characteristic of abrasion.[3] In contrast, corrosion results in a much smoother appearance of the tooth surface, with enamel displaying honeycomb structures[1] and dentine displaying an undulating or rippled surface at a high magnification.[16] Abrasion and corrosion occurring in combination produce a smoother dentine surface compared with abrasion alone.[4] Although the detailed micromorphology of abfraction lesions remains unclear, an *in vitro* study noted fracture and chipping of enamel in the cervical region of premolar teeth subjected to occlusal stress under neutral and acidic conditions.[8]

By examining the microwear details of NCCLs in a collection of extracted human teeth, we aimed to clarify the nature of the factors involved in their aetiology.

MATERIALS AND METHODS

Twenty-four extracted human teeth with large cervical lesions were selected from a collection of teeth extracted as part of general dental treatment at the Adelaide Dental Hospital. The protocol for collection of extracted teeth was approved by The University of Adelaide Human Ethics Committee (H/27/90). These teeth had been stored in Savlon, an antiseptic agent, which complied with infection control practices at the Adelaide Dental Hospital.

After the teeth were gently handwashed with water and air-dried, extraneous material from the NCCLs was removed by obtaining impressions in alginate (3M ESPE Palgat™ Plus Quick, Seefeld, Germany), as described in a previous study.[17] The NCCLs were then categorized as wedge-shaped or scooped lesions by visual examination. Negative replicas of the NCCLs were obtained using polyvinylsiloxane impression material (3M ESPE Imprint™ II Garant™ Light Body, St Paul, USA), and then examined under SEM at various magnifications. Each NCCL was examined for presence of abrasion and corrosion in nine different fields or regions, except when they were obscured by artefacts. To determine whether there was any major variation in the micromorphology in different regions of the NCCLs, each lesion was divided into upper, middle and lower thirds and also into mesial, central and distal thirds. The nine fields were examined for each specimen to determine whether there was evidence of abrasion only, corrosion only, or a combination of both. Representative micrographs showing evidence of abrasion, corrosion or a combination of both were selected for presentation in this report. The NCCLs examined did not display evidence of damage as a result of extraction. Replication of microwear details from natural teeth into silicone rubber impressions and epoxy dyes has been validated at a resolution of less than 1 µm in a previous study.[18]

RESULTS

The NCCLs were all on buccal surfaces and extended from the CEJ to the root surface. They displayed a variety of appearances (Fig 1). There was no major variation in the micromorphology observed across all nine fields of each NCCL. All lesions showed evidence of abrasion and/or corrosion. Using SEM, abrasion was characterized by the presence of horizontal scratch marks (Fig 2) while corrosion was characterized by a smooth surface (Fig 3). When both abrasion and corrosion occurred in combination, scratch marks (Fig 4) were not as pronounced as those produced by abrasion only (Fig 2).

There was definite evidence of both abrasion and corrosion in 18 of the 24 teeth (75 per cent), abrasion only in one tooth (4.2 per cent) and corrosion only in five teeth (20.8 per cent) (Table 1).

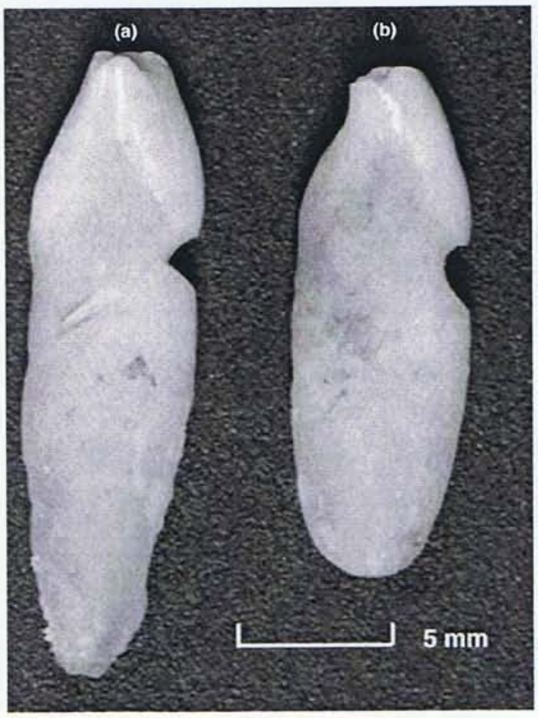

Fig 1. Variation in the appearance of non-carious cervical lesions (NCCLs) that extend from the cemento-enamel junction (CEJ) to the subjacent root surface in a mandibular right lateral incisor (a) and in a mandibular left central incisor (b).

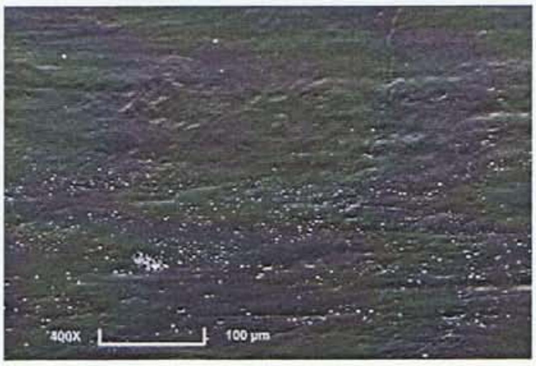

Fig 2. A micrograph showing evidence of abrasion within an NCCL characterized by presence of parallel horizontal scratch marks (×400 magnification). The white spots are artefacts, produced by scattering of electron beams incident on the irregular surface of the NCCL.

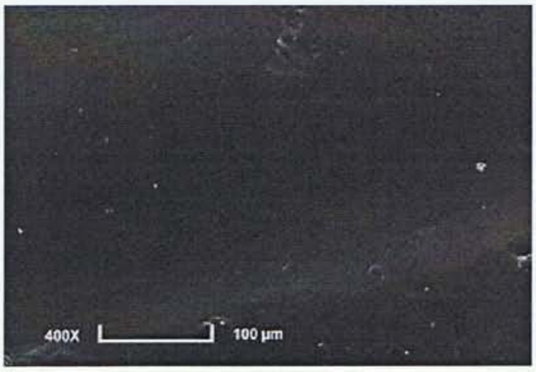

Fig 3. A micrograph showing evidence of corrosion in dentine within an NCCL characterized by smooth surface (×400 magnification).

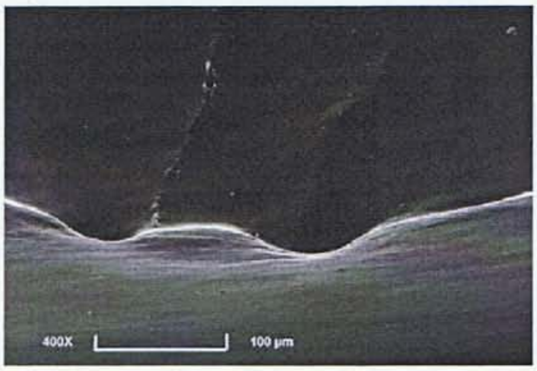

Fig 4. A micrograph showing evidence of abrasion and corrosion occurring concurrently in an NCCL (×400 magnification). The wavy line at the centre of the micrograph represents the upper margin of the NCCL, and horizontal scratch marks in the NCCL (below the wavy line) appear relatively smoother.

Horizontal furrows were noted in 13 (54.2 per cent) teeth. Their widths varied considerably from around 5–250 µm, and some of them ran mesio-distally almost

Table 1. Summary of wear characteristics of non-carious cervical lesions (NCCLs)

Wear characteristic	Number of teeth	Per cent (%)
Both abrasion and corrosion	18	75.0
Abrasion only	1	4.2
Corrosion only	5	20.8
Total	24	100.0

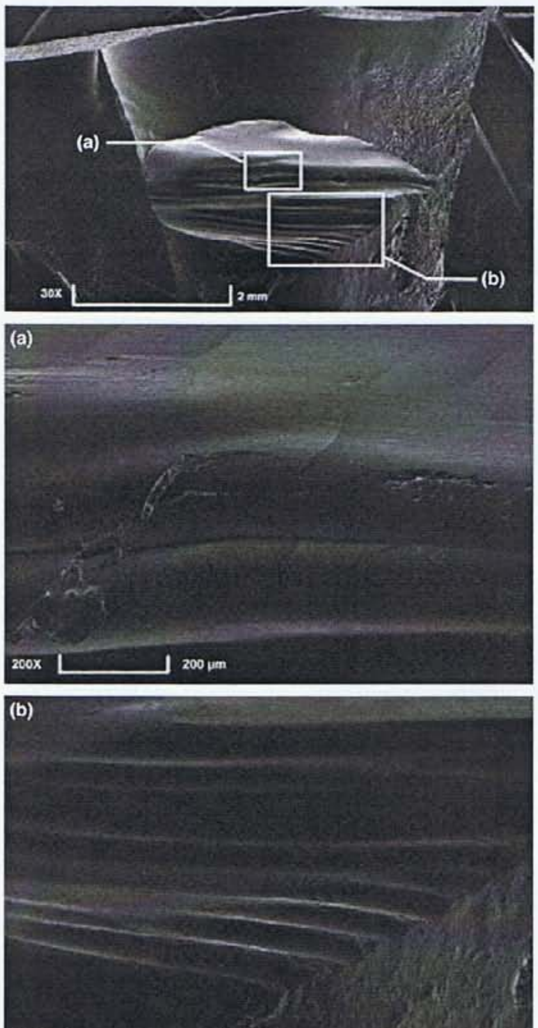

Fig 5. A micrograph of an NCCL showing numerous horizontal furrows at a magnification of ×30. Enlarged areas of this micrograph are shown in (a) and (b). Horizontal furrows in (a) are around 100–250 µm wide (×200 magnification) and those in (b) are around 20–100 µm wide (×100 magnification). Minor horizontal scratch marks towards the top-half of the micrograph (a) are characteristic of both abrasion and corrosion occurring concurrently. There are some artefacts on the lower left part of micrograph (a). Horizontal furrows in (b) appear smooth, which is characteristic of corrosion.

along the entire length of the NCCLs. The horizontal furrows generally appeared smooth, characteristic of corrosion, but minor horizontal scratch marks were also noted to occur adjacent to these furrows, indicating a combination of abrasion and corrosion (Fig 5).

DISCUSSION

Although several wedge-shaped NCCLs with sharp internal line angles were observed in the present study, a detailed classification of different morphological forms was not undertaken. A separate study is being conducted by our research group to develop a detailed classification system for NCCLs according to their morphological forms and to associate these with aetiological factors. Evidence of abrasion and/or corrosion was noted in all NCCLs in the present study, but extensive crack networks that would be expected in abfraction-induced NCCLs were not found. However, some researchers have implied that such crack networks may occur only on cervical enamel.[19–21] Crack networks on the cervical enamel, as described by Palamara *et al.*,[8] need to be verified clinically before the abfraction hypothesis can be validated. In another *in vitro* study, artificial NCCLs produced in extracted premolar teeth under axial loading in an acidic environment had rounded outlines similar to those formed *in vivo*.[22] Micrographic assessment of these NCCLs revealed an extensively corroded enamel, cracks in dentine coronal to the NCCL, concentric steps extending from the outer surface towards the centre and an extensively "rippled" appearance of the dentine surface.[22] These features were not found in any of the NCCLs in this study or in previous *in vivo* studies.[15,18] This implies that considerable caution is needed when extrapolating findings based on artificial NCCLs to the clinical situation.

The present study also provides a comprehensive description of horizontal furrows. These are less pronounced than the concentric steps or deep grooves described in artificial NCCLs.[22,23] One previous *in vivo* study has presented micrographs of NCCLs showing horizontal furrows but does not provide any explanation.[15] In the present study, a large proportion of NCCLs (54.2 per cent) displayed evidence of horizontal furrows of varying sizes. We propose that smaller furrows (5 μm in width) may coalesce to form larger ones (up to 250 μm in width), and that this process continues to further increase the size of NCCLs. The streamlined or smooth appearance of horizontal furrows, with few scratch marks, suggests involvement of both abrasion and corrosion in their formation, although corrosion is more likely to be a predominant wear process.

Litonjua *et al.*[23] noted in their *in vitro* study that toothbrush abrasion began apical to the CEJ, progressed to dentine, and then undermined cervical enamel. These findings are supported by our observation that NCCLs extended from the CEJ to the root surface (Fig 1). In contrast, the abfraction hypothesis states that cervical enamel is the initial site of progression of NCCLs, and that tensile forces generated by cuspal flexure result in microfractures of enamel and dentine crystals in the cervical region.[11] This hypothesis provides an explanation for possible mechanisms involved in the breakdown of hydroxyapatite crystals in cervical enamel where enamel crystals are aligned approximately perpendicularly to the dentino-enamel junction.[24] However, the majority of hydroxyapatite crystals in dentine are orientated in a different direction, approximately perpendicular to the long axis of dentinal tubules. Dentine also contains areas of randomly orientated crystals.[25] The abfraction hypothesis does not provide any explanation about how wedge-shaped NCCLs can form by breakdown of hydroxyapatite crystals in dentine. It seems likely that tensile stresses acting on teeth may have different effects on enamel and dentine.

The abfraction hypothesis is based primarily on computerized models of finite element analysis (FEA),[19–21] but most FEA models do not incorporate periodontal ligament or alveolar bone. A few models incorporating these structures have provided some support for the abfraction hypothesis,[19,26] whereas others have indicated that periodontal tissues are likely to dissipate occlusal forces from the cervical area.[27] Most researchers have modelled enamel, dentine, periodontal ligament and alveolar bone as structures having identical physical properties in all planes (isotropic) rather than structures having different physical properties in different planes (anisotropic).[20,21] Using an FEA model, it has been shown that anisotropic enamel structure dissipates tensile stress in the cervical region of teeth more effectively than an isotropic structure.[28,29] Thus, the effect of tensile forces on the cervical region of teeth in most FEA models may not accurately depict the actual clinical situation.[2,30] Further research is needed to clarify whether modelling of dentine as an anisotropic structure in FEA leads to reduction in cervical strain and plastic deformation of teeth due to occlusal loading.

Only a few *in vitro* studies have investigated factors responsible for the formation of artificial NCCLs.[22,23] Whitehead *et al.*[22] reported that artificial NCCLs formed in eight per cent of premolar teeth under axial loading at a pH of 3.0, providing some support for the role of occlusal factors in the formation of NCCLs. However, given the low prevalence of NCCLs in their study, the authors stated that the simplistic model of occlusal stress and acid corrosion was invalid. Moreover, Litonjua *et al.*[23] have shown that artificial NCCLs can be produced in premolar teeth by toothbrush abrasion with a slurry of toothpaste regardless of whether the teeth are loaded or not.

Clinical and anthropological studies may provide more fruitful approaches for understanding the aetiology of NCCLs. A critical review of abfraction has indicated that the force, frequency and method of toothbrushing are significant factors in the aetiology of NCCLs.[2] Some researchers have suggested that observed associations between occlusal wear due to attrition and presence of NCCLs provide support for the abfraction hypothesis,[5] but these associations are weak in some instances.[31,32] Even when such associations have been strong, occurrences of wedge-shaped NCCLs were found to be associated with both occlusal attrition and corrosion.[7] Furthermore, the finding that NCCLs occur most frequently on buccal surfaces of teeth and rarely on lingual surfaces has been attributed to variation in salivary protection against corrosion at different intra-oral sites rather than occlusal stress.[33] An example of formation of an NCCL on a maxillary premolar with no opposing tooth has also been reported.[34] Furthermore, no NCCLs have been reported in the heavily worn dentitions of Australian Aboriginals, except for interproximal grooving caused by processing kangaroo sinews.[35] Aaron[36] found that NCCLs did not occur in the dentitions of American Indians living in the 11th and 17th centuries, and he implied that NCCLs were probably a product of modern lifestyle factors, including overzealous toothbrushing and acidic diet, rather than tooth flexure.

Overall, the findings of the present investigation, along with those of other *in vitro*, clinical and anthropological studies, indicate that abrasion and corrosion are important factors involved in the formation of NCCLs. Although there are a few *in vitro* studies that support the abfraction hypothesis, clinical evidence linking occlusal stress to the formation of NCCLs is weak.[2] Longitudinal *in vivo* studies based on large samples are needed to elucidate the initial progression of NCCLs, and to test the validity of the abfraction hypothesis. Further *in vitro* studies that investigate the effect of toothbrushing in a corrosive environment could also improve our understanding of the aetiology of NCCLs. It is our view that occlusal adjustment should not be used as part of the management of NCCLs until the abfraction hypothesis is confirmed by stronger clinical evidence.

CONCLUSIONS

Based on microscopic assessment of a sample of extracted human teeth, the present study has confirmed that abrasion and corrosion are common associated aetiological factors in the formation of wedge-shaped NCCLs. Horizontal furrows of varying sizes were identified in about half of the teeth examined, confirming the presence of both abrasion and corrosion. Further clinical studies are needed to clarify how NCCLs are initiated and to provide a sound basis for the clinical management of NCCLs. Until stronger clinical evidence is generated to support the abfraction hypothesis, occlusal adjustment for their management is not recommended.

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A System for the Acquisition and Analysis of Three-Dimensional Data Describing Dental Morphology

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ABSTRACT: Accurate, reproducible three-dimensional (3D) data provide an important contribution to our ability to describe, compare and understand dental morphology but the existing technology is often expensive or has technical limitations. Recently available, inexpensive 3D profilometers interfaced with standard personal computers offer the potential to overcome some of these problems. This technical note describes a system that uses a 3D profilometer and purpose written software to analyse changes in dental morphology resulting from tooth wear. The validity of the derived data was determined by comparing data derived from scans of objects of known dimensions with calculated volumes. These differences were less than 10% from objects that were difficult to scan because of their geometry and were commonly less than 5%. The reproductibility, expressed as intra- and inter-observer coefficients of variation, was less than 1%. The potential applications of systems of this type are outlined. *Dental Anthropology* 2004;17(3):70-74.

A number of systems for the acquisition and analysis of three-dimensional (3D) data have been developed (Roylet *et al.*, 1983; Delong *et al.*, 1985; McDowell *et al.*, 1988; Hewlett *et al.*, 1992) specifically for use in studies of dental morphology. In most cases the developers have recognised difficulties including hardware limitations, computational complexity or cost. In studies of dental morphology the practical problems are usually associated with the complex morphology of teeth that can make it impossible to scan parts of a surface from some directions or lead to with difficulties in defining appropriate reference points and planes. In some cases "internal" reference points (*i.e.*, anatomical features or prepared reference markers) are defined and scanning can extend either to a limit defined by another reference point or a predetermined distance. Alternatively, in some studies appropriate "external" reference points (eg points on the specimen mount) can be defined.

This paper describes the application of widely available hardware and software packages to provide an affordable system for acquiring 3D coordinates from the surface of a dental crown and subsequently for comparing three-dimensional data derived from these coordinates. As part of the development process, the system was validated by comparison of calculated data with the known dimensions and volumes of standard objects. The reproducibility of the derived data, both within and between observers, was also determined from repeated measurements.

To illustrate the application of the system, we have measured the loss of tooth occurring during simulated tooth wear. However, the software and hardware have the flexibility to provide valid, reproducible data in a broad range of studies of morphology.

MATERIALS AND METHODS

For data acquisition a 3D scanner (PIX-4, Roland DG, Tokyo, Japan) interfaced with a personal computer was used to record the heights (Z) of surface mesh points (X and Y). In this system an active piezo sensor detects contact between its stylus and the scanned surface (Fig. 1). The X and Y mesh steps can be set between 50μ and 5.00 mm in 50μ steps and the Z-axis direction has a resolution of 25μ. The "Dr.PICZA" software (Roland DG, Tokyo, Japan) provided with the scanner is a Windows or MAC OSX-based tool that allows the scan area to be defined to accommodate the dimensions of the specimen and the scanning resolution to be set according to the user's needs. This decision involves balancing the need for high resolution against the size of the resultant data set and the scanning duration, both of which are increased with increasing resolution. In addition, a lower limit and the approximate X and Y coordinates of the highest point of the specimen can be defined to further optimise the size of the data set and shorten the scanner's calibration and scanning times. The software allows basic manipulation and visualization of the data (Fig. 2) and has the facility to export data in a range of

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Fig. 1. Scanner with specimen mounted on scanning table.

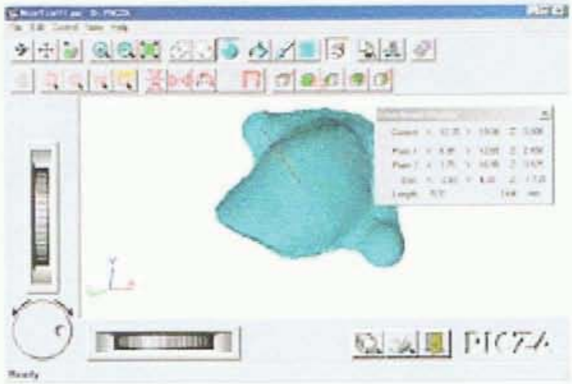


Fig. 2. Data visualization and co-ordinate display from "Dr Picza" software.

formats for subsequent analysis.

In our example, we were aiming to measure the changes in dental crown volume resulting from simulated tooth wear. We therefore mounted our specimens (in this case either the buccal of lingual halves of human tooth crowns) with three reference markers (2 mm diameter titanium spheres) equally spaced around the specimen. After each period of simulated wear the specimen was re-scanned and the volume of the crown above the reference plane compared with previous volumes. Because the predicted changes were relatively small (expected to be of the order of 20 mm^3) we chose the highest scanning resolution (*i.e.*, 50μ for the X and Y matrix and 25μ for the height (Z)). The derived data set was exported as a text file for detailed analysis.

For data analysis, a purpose-written software package was developed using MATLAB (version 6, The Mathworks Inc, Natick MA, U.S.A.). The package accepts data from "Dr PICZA" in the form of (X, Y, Z) triples, where the X values are the west-east coordinates and the Y values the north-south coordinates. To make optimum use of MATLAB and its graphic facilities, we converted the data set to a regular mesh grid and saved the Z-values to a matrix (Z). The menu-driven software package then provides a series of options for defining the reference plane, graphing the data in 3D and deriving data describing the volume of the scanned object and the surface area and the height of the highest point on the object from the reference plane in cases where this is of interest.

In our example, we needed to find the volume bounded by two surfaces: the tooth surface and a planar surface defined by the three external reference points. The data transferred from Dr PICZA were plotted using the MATLAB routines and the maximum heights of

Table 1. Three-way analysis of variance comparing 10 repeated measures from two scans of a single specimen performed by three independent observers

Source	Sum Squares	d.f.	Mean Square	F-Ratio	P-Value
Repeated measures	8.7303	9	0.97003	37.89	<0.001
Observers	0.0287	2	0.01437	0.56	0.5803
Scans	0.0003	1	0.00026	0.01	0.9214
Repeat-x-Observer	0.8173	18	0.04541	1.77	0.1169
Repeat-x-Scan	0.1028	9	0.01143	0.45	0.8914
Observer-x-Scan	0.1011	2	0.05054	1.97	0.1678
Error	0.4608	18	0.02560		
Total	10.2414	59			

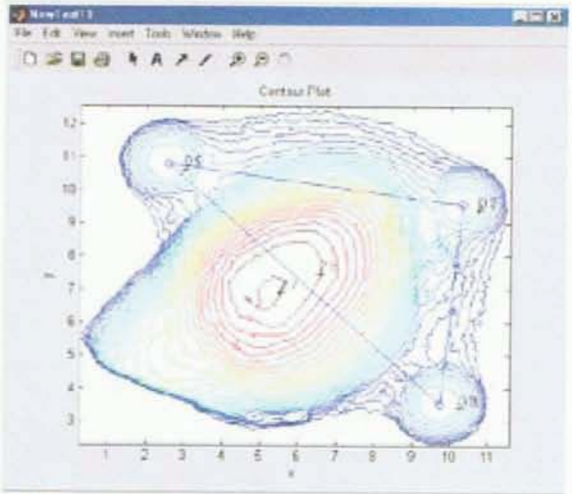


Fig. 3. Data plot and reference plane identification using purpose-written software.

the three reference points were identified (Fig. 3). The volume of the part of the specimen above the reference plane was calculated.

Because 3D objects can on occasions include undercut areas, the package has an option to allow the reference plane to the re-aligned so that the calculations do not include parts of the specimen for which there are no data.

In our example this was undertaken by selecting the appropriate option from the menu, inspecting the graphical display and deciding on an appropriate realignment to avoid the undercut and recalculating the data (Fig. 4).

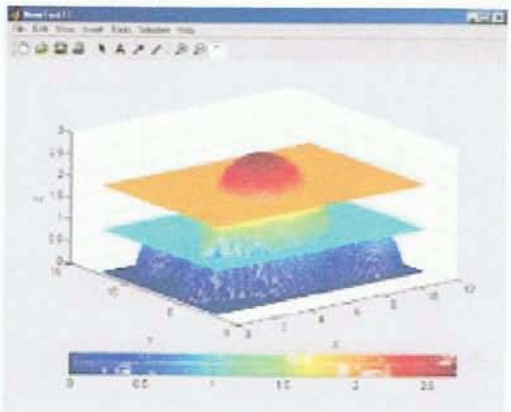
To establish the validity of the data obtained, objects of know dimensions were scanned and the calculated volumes compared with the volumes derived from the scanner data.

To establish the reproducibility of the method, intra-observer variation was assessed by repeated analysis by one observer (PZ) and inter-observer variation was determined by comparing data derived by different

Does the object have undercut areas (Y/N)? y
Please give lift value of the referenceplane, which you can estimate from the contour diagram you have drawn.
Lift value = 1.012

Max distance of the remnant to reference plane is: 0.7130 mm
The convex surface area of the remnant is: 2.4011 mm^2
The volume of the remnant is: 7.6169 mm^3

If you want to see the 3-D picture of the original object and reference plane together, please press 'Y', otherwise just press <Enter>.
Selection (Y/N): y



Congratulations! Your measurement of NewTest11 is done.

Matrix: Distance*, Surface*, Volume

B = 0.7130 2.4011 7.6169

Fig. 4. Example of menu-driven adjustment to reference plane height to avoid undercut areas on specimen.

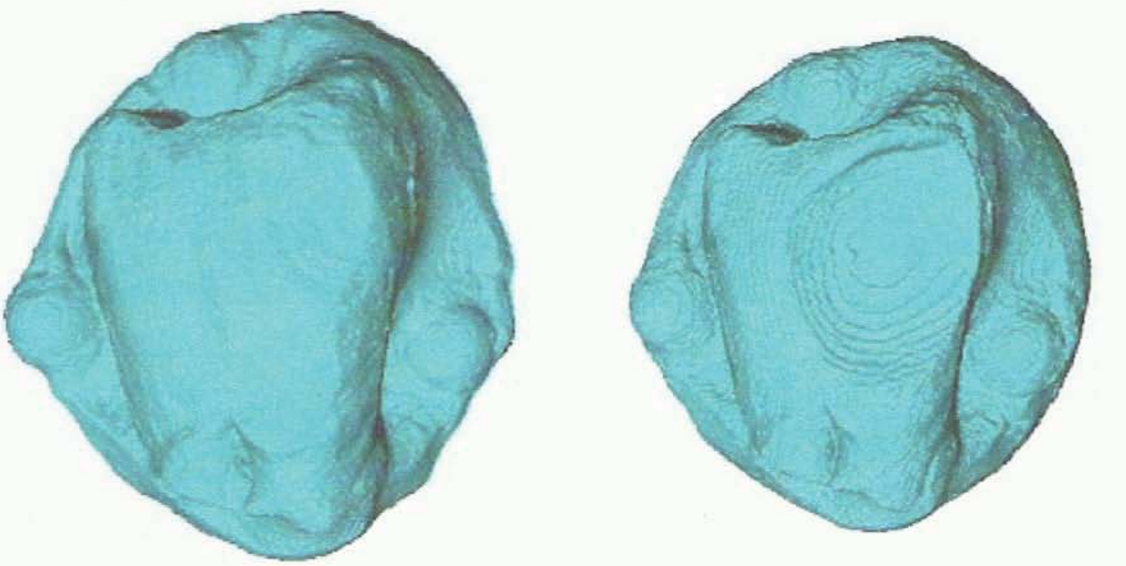


Fig. 5. (*left*) Data plot for specimen after 7,000 cycles of wear. (*right*) Data plot for specimen after 112,000 cycles of wear.

observers.

RESULTS

Scanning a relatively simple object (for example, a hemisphere of diameter 18.0 mm) gave a volume of 1583.27 mm^3 compared with a calculated volume of 1526.81 mm^3. The difference (56.45 mm^3) represents 3.7% of the true volume. A smaller, more complex object (the small cylindrical projection on a Lego® building block which has parallel sides and hence can have small undercut areas if the block is not mounted exactly horizontally) had a theoretical volume of 32.66 mm^3 and a volume derived from the scanning data of 35.45 mm^3 representing a difference of 8.5%.

The intra-observer variation in calculated volume was small with the coefficient of variation (100 x standard deviation/mean) being 0.90% for the most experienced observer and 0.91% for the least experienced observer.

To determine whether inter-observer variation or differences between repeated scans of the same object contributed significantly to the observed variation in repeated measures, one specimen was scanned on two occasions and each of these scans was analysed 10 times by three independent observers. A three-way analysis of variance (Table 1) revealed no significant variation between observers ($p=0.58$) or between repeated scans ($P = 0.92$) and no significant interaction between any of the considered factors suggesting that the performance of experienced and inexperienced observers was similar.

In our study of tooth wear the buccal surface of an extracted human tooth was subjected to 7000 cycles at the rate of 80 cycles per minute under a load of 3.2 kg with water at pH 7 used as a lubricant in an electro-mechanical tooth wear machine (Kaidonis *et al.*, 1998) to produce a wear facet (Fig. 5a). The specimen was scanned and the volume of the dental crown above the plane defined by the three 2mm diameter ball markers that were used as "external" reference points was calculated. This was compared with volume of the specimen after it had been subjected to a further 105,000 cycles of wear (Fig. 5b). The volume of enamel lost due to wear during this experiment (21.85 mm^3) was calculated by comparing the first volume (149.74 mm^3) with the final volume (127.89 mm^3).

DISCUSSION

Based on our assessment of the validity and reproducibility of the measurements derived using this system, we believe that it provides an affordable and reliable method for the acquisition of 3D data for the comparison of dental morphology. Like most systems it is limited in its ability to deal with undercut areas that makes it important to carefully select the initial orientation of the specimen and define an appropriate reference plane to avoid undercuts. Also, the acquisition of data from larger specimens at the highest resolution can be time consuming with high resolution scanning of a whole dental arch taking up to 30 or more hours.

The costs involved in setting up the system are relatively small compared with some other systems. If a suitable personal computer and a licensed copy of the MATLAB package are available then the total hardware set up cost should be less than $US 1,200 compared with more than $US 100,000 for some commercial laser-based systems. The purpose-written MATLAB-based software package is available on request from the authors.

The validity of the data derived using the system was established by comparing volumes derived by scanning with the calculated volumes of objects of known dimensions. This indicated that calculated and scan-derived volumes differed by between 3.7 percent and 8.5 percent depending on the size and geometry of the specimen. Interpreting this information was complex for a number of reasons. In the case of the sphere (a computer-mouse ball), the difference between the scanned and calculated volumes was relatively small and challenged our ability to accurately measure the ball. A difference in radius of the ball of the order of 0.1 mm would result in a difference in volume of more than 3.4% and made it difficult to determine which of the calculated and scan-derived data was the more valid. In the case of the Lego® building block the differences were larger because the object presented some obvious and some hidden challenges and represented a "worst case" in terms of the ability of the system to derive valid data. The obvious challenge was the geometry of the object that, with its parallel sides, required precise orientation to avoid undercuts. In addition, the curve-fitting procedures that were used to define the surface were not ideally suited to objects of this type. The hidden challenge was the surface morphology of the object. The face of the projection on the block included the manufacturer's trademark etched into the surface. This was not obvious on observation and therefore not included in the calculations but was obvious on the enlarged scan and would contribute to the difference between the calculated and scan-derived volumes.

The reproducibility of the data was assessed by repeated measures of a test specimen by different observers. The intra- and inter-observer errors were all small with coefficients of variation for repeated measures being less than 1.0% for all scans and observers, and with no significant differences between observers or repeated scans.

Based on our experience, we believe that the system described is an affordable, valid and reliable method for obtaining 3D data for the description and comparison of dental morphology.

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9.1.1.2 Papers/manuscripts to be submitted for publication

1. **Ranjitkar S**, Kaidonis JA, Richards LC, Townsend GC. The effect of CPP-ACP on enamel erosion under conditions simulating acid regurgitation.
   Aust Dent J.

   [This paper forms Chapter 5 of this thesis and will be submitted for publication after further experiments involving fluoride have been conducted. This paper has not been included in this section to avoid duplication.]

2. **Ranjitkar S**, Lewis A, Kaidonis J, Marino V, Richards LC, Townsend GC. Spectral analysis of the enamel surface treated with CPP-ACP.
   Aust Dent J.

   [This paper forms Chapter 6 of this thesis. It covers preliminary findings on the spectral analysis of the enamel surface after treatment with CPP-ACP, and it will be submitted for publication after experiments on a larger sample have been conducted. This paper has not been included in this section to avoid duplication.]

9.1.2 Research abstracts

Abstract of a paper presented at the 86th General Session of the International Association for Dental Research (IADR), Toronto, 2008

Presentation date: Friday 4 July, 2008

2500 The role of Tooth Mousse in reducing erosive tooth wear

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In addition to its role as a remineralizing agent in preventing dental caries, recent evidence has shown that Tooth Mousse (TM, GC Corporation, Japan) can reduce dental erosion. Objective: Our aim in this study was to determine whether TM could 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 immersion 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 200gm for 200 cycles. The specimens were immersed in artificial saliva for 2hrs between wear episodes. In experimental group 1 (n = 12 each for enamel and dentine), TM was applied at the beginning of each wear episode for 5min whereas Tooth Mousse without the remineralizing agent (TM-) was applied in experimental group 2 (n=12 each for enamel and dentine). No TM or TM- was applied in the control group (n = 12 each for enamel and dentine). Results: A linear mixed model showed that intervention involving TM and TM- had a significant effect on both enamel and dentine wear (p<0.01). The mean wear depth for enamel in experimental group 1 (mean ± SE, 1.26 ± 0.33µm) was significantly less than that in the control group (3.48 ± 0.43µm) (p<0.001), but not significantly different from that of experimental group 2 (2.41 ± 0.50µm) (p>0.05). The mean dentine wear in experimental group 1 (2.16 ± 0.89µm) was significantly less than those in experimental group 2 (5.75 ± 0.98µm) (p<0.01) and control group (10.29 ± 1.64µm) (p<0.001). Conclusion: Our findings that TM can reduce erosive tooth wear, probably by remineralizing and lubricating eroded tooth surfaces, have clinical implications in the management of tooth wear.

Abstract of a paper presented at the 42nd annual meeting of International Association for Dental Research (IADR)-Continental European and Israeli Divisions, Thessaloniki 2007

Presentation date: Friday 28 September, 2007

0375 The role of Tooth Mousse in preventing enamel wear

S. RANJITKAR, J.A. KAIDONIS, G.C. TOWNSEND, and L.C. RICHARDS, The University of Adelaide, Australia

In addition to its role as a remineralizing agent in preventing dental caries, recent evidence has shown that Tooth Mousse (TM), manufactured by GC Corporation, Japan, may reduce enamel wear under conditions simulating attrition and acid regurgitation. Objective: Our aims were to determine whether TM could reduce enamel wear under different in vitro conditions and to clarify its likely mode of action. Methods: Buccal and lingual enamel halves of human third molar teeth were worn against each other in a purpose-built electromechanical tooth wear machine under a load of 10.0 kg. In experimental sample 1, enamel specimens (n = 12) were worn for around 10,000 cycles in the presence of hydrochloric acid (pH = 1.2). The machine was stopped every two minutes (160 cycles of wear) and specimens were washed and dried before TM was applied for four minutes. Specimens were washed again and dried before the cycle was continued. This protocol was also followed for experimental sample 2 (n = 12) using Tooth Mousse containing the same formulation but without the remineralizing agent, CPP-ACP (TM-). In the control sample (n = 12), the specimens were worn at pH = 1.2, but no TM or TM- was applied. Wear rates were compared between the samples using a one-way ANOVA. Results: There were no significant differences in rates of enamel wear between samples 1 and 2 (0.44 ± 0.05 vs 0.63 ± 0.06 mm3 per 1,000 cycles), but both these rates were significantly less than that in the control sample (0.92 ± 0.11 mm3 per 1,000 cycles) ($p<0.05$). Conclusions: Tooth Mousse with and without the remineralizing agent reduced enamel wear under conditions simulating attrition and acid regurgitation. We suggest that this is related to the lubricating agents in the TM formulation (for example, glycerol).

Abstract of a paper presented at the 84th general session of the International Association for Dental Research (IADR), Brisbane 2006

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2424 An in vitro study of wear prevention in dentine

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Tooth wear is a significant problem facing clinicians, and several approaches are being used to manage it. Objective: This study aims to quantitatively and qualitatively test the efficacy of Tooth Mousse® in managing dentine wear under highly controlled conditions. Methods: Eight dentine specimens from the lingual halves of third molar teeth were worn against enamel antagonists under a load of 9.95kg in an electro-mechanical tooth wear machine with hydrochloric acid lubricant (HCl) (pH=3), and with regular Tooth Mousse® application. A further eight dentine specimens were worn with Tooth Mousse® as the sole lubricant. Dentine wear was quantified by measuring reduction in dentine volume using a Dr PICZA 3D Scanner (PIX-4) and a MATLAB software package (version 6, The Mathwork Inc, Natick MA, USA), and assessed qualitatively by examining epoxy resin replicas under a scanning electron microscope. These data were then compared using ANOVA with data from two control experiments conducted under a load of 9.95kg with deionised water and HCl (pH=3) as lubricants, but no Tooth Mousse® was applied. Results: Dentine specimens worn with Tooth Mousse® as the sole lubricant exhibited minimal wear, and had very smooth and shiny wear facets. Those worn with HCl (pH=3) with regular Tooth Mousse® application showed less wear than both the control groups. Their wear facets were not obviously different from facets of control specimens worn with HCl (pH=3), but were smoother than those of specimens worn with deionised water as the lubricant. The control group worn with HCl (pH=3) exhibited less wear than that worn with deionised water, and also displayed smoother facets. Conclusions: This study has shown that Tooth Mousse® is capable of reducing dentine wear, and has also highlighted the importance of lubricants in reducing wear. Further research is required to clarify its clinical usefulness of Tooth Mousse® in this context.

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2428 Enamel wear prevention under conditions simulating bruxism and acid regurgitation

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Tooth wear is a growing public health problem and there is a need to better understand its aetiology and management. Objective: Our aim was to investigate the effectiveness of frequent applications of Tooth Mousse (GC Corporation) in preventing enamel wear in vitro, under conditions simulating bruxism and acid regurgitation. Methods: Sixteen human third molar teeth were sectioned longitudinally in a mesio-distal direction, and enamel halves of the same teeth were worn against each other in a purpose-built electromechanical tooth wear machine under a load of 10.0 kg with hydrochloric acid lubricant (pH = 1.2) for around 10,000 test cycles. In the experimental sample (n = 8), the machine was stopped every two minutes (160 cycles of wear) and the specimens washed and dried. Tooth Mousse was then applied for four minutes. The specimens were further washed and dried before the cycle was continued. The same protocol was followed for the control specimens (n = 8) but no Tooth Mousse was applied. Tooth wear was quantified by measuring reduction in enamel volume per 1,000 cycles using a Dr PICZA 3D Scanner (PIX-4) and MATLAB software package (version 6, The Mathwork Inc, Natick MA, USA). Wear rates were compared between the samples with an unpaired t-test. Qualitative assessment was also carried out using Scanning Electron Microscopy (SEM). Results: The rate of enamel wear was significantly lower in the experimental sample (0.41 mm3 per 1,000 cycles) than in the control sample (1.01 mm3 per 1,000 cycles) ($p<0.01$). Enamel wear facets in the experimental sample were also found to be smoother than those in the control sample. Conclusions: Frequent application of Tooth Mouse is effective in reducing enamel wear under conditions simulating bruxism and acid regurgitation, probably due to its lubrication properties. These findings open up new possibilities for the prevention of tooth wear.

Abstract of a paper presented at the 45th Annual Meeting of Australian/New Zealand Division of the International Association for Dental Research (IADR) 2005

Presentation date: Tuesday 27 September, 2005

0087 Characteristics of dentine wear: an in vitro study

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Previous studies have described the nature of wear in enamel and various restorative materials under conditions simulating attrition and erosion. Objective: The aim of this study was to investigate the pattern of dentine wear under various loads and with different lubricants. Methods: 53 human third molar teeth were sectioned longitudinally in a mesio-distal direction. After removing enamel from one half of the teeth, dentine surfaces were worn against enamel in a purpose-built electromechanical tooth wear machine at different loads (3.2, 6.2 and 9.95 kg), and with different lubricants (water at pH 7.0 and hydrochloric acid solutions at pH 1.2 and 3.0). Tooth wear was quantified by measuring reduction in dentine volume over time using Dr PICZA 3D Scanner (PIX-4) and MATLAB software package (version 6, The Mathwork Inc, Natick MA, USA). Qualitative assessment was also carried out using Scanning Electron Microscopy (SEM). Results: Dentine wear increased with increasing loads at each pH level ($p<0.05$). Dentine wear rate was greatest at pH 1.2 ($p<0.05$) and there was a trend for the wear rate at pH 3.0 to be less than that at pH 7.0 under all loads. Dentine was noted to wear more slowly than enamel at loads of 3.2 kg and 9.95 kg at pH 1.2 ($p<0.05$). SEM analyses showed extensive surface destruction of dentine wear facets due to erosion at pH 1.2, whereas dentine wear facets were smoother at pH 3.0 compared with pH 7.0. Conclusions: This study provides evidence of different wear mechanisms in dentine under different experimental conditions. Dentine wear rate is linear with load but its relationship with pH is more complicated. This project was supported by the National Health and Medical Research Council of Australia (Grant no: 207803).

Abstract of a paper presented at the 45th Annual Meeting of Australian/New Zealand Division of the International Association for Dental Research (IADR) 2005

Presentation date: Monday 26 September, 2005

0038 A qualitative assessment of non-carious cervical lesions in human teeth

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Opinions vary about the causes of non-carious cervical lesions (NCCLs). They have been attributed to excessive tooth brushing (abrasion), effects of exogenous or endogenous acids (corrosion), and flexion of teeth from occlusal loads (abfraction). Objective: Our aims were to examine the micro-wear detail of NCCLs in a collection of extracted teeth under scanning election microscopy (SEM) and to look for evidence of abrasion and corrosion in these lesions. Methods: Twenty-four extracted teeth with NCCLs were selected from a collection in the Adelaide Dental School. Negative replicas of NCCLs were obtained in polyvinyl impression material (Light Body Imprint ™II Garant™, 3M ESPE) and viewed under SEM. Results: All NCCLs were located below the cemento-enamel junction (CEJ) and they displayed a variety of wedge-shaped appearances. Abrasion was characterized by the presence of horizontal scratch marks, presumably caused by toothbrush and/or dentrifice, while corrosion was characterized by a smooth amorphous surface which at times displayed open dentinal tubules. There was evidence of abrasion in 19 (79.2%) teeth and corrosion in 23 (95.8%) teeth. Eighteen (75.0%) of the 24 teeth displayed evidence of both abrasion and corrosion, whereas 6 (25%) teeth displayed evidence of either abrasion or corrosion. Horizontal furrows were noted in 13 (54.2%) teeth. These furrows had smooth edges, with a corrosive appearance, but no evidence of abrasive scratches. They ranged in width from 5-250 µm. All NCCLs with furrowing displayed evidence of corrosion. Conclusion: Based on microscopic assessment of a sample of extracted teeth, it appears that abrasion and corrosion are common associated aetiological factors in the formation of wedge-shaped NCCLs. This project was supported by the Dental Board of South Australia.

9.2 Other academic activities during PhD candidature

- **Scholarships and awards**
  - NHMRC Dental Postgraduate Award –2005/08
  - NHMRC travelling award – 2007
  - GC Travelling award – 2008
  - The Graham Mount ESPE Prize –2005

- **Research grants**
  - Ranjitkar S, Lewis A, Hall C, Townsend GC, Kaidonis JA, Richards LC, Smart R. ADRF. Characterisation of enamel surface treated with Tooth Mousse and Tooth Mousse Plus by using mass spectrometry (ToF-SIMS). 2009 - $7,000
  - Ranjitkar S, Hall C, Lewis A, Townsend GC, Kaidonis JA, Richards LC, Smart R. ADRF. Changes in the nanomechanical properties of eroded enamel after treatment with Tooth Mousse, Tooth Mousse Plus and fluoride. 2009 - $7,000
  - Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC, Bartlett D, Lewis A. ADRF. The role of Tooth Mousse and Tooth Mousse Plus in reducing enamel wear by corrosion (erosion), $8,848 - 2008
  - Ranjitkar S, Bartlett D, Townsend GC, Richards LC, Kaidonis JA, Lewis A. ADRF. Prevention of enamel wear by Tooth Mousse under conditions simulating toothbrush abrasion in an acidic environment, $3,200 – 2007/08
  - Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC, Lewis A. G C Asia Pty Ltd. Prevention of cervical wear under conditions simulating toothbrush abrasion in an acidic environment, $7,401 – 2007/08
  - Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC, Bartlett D, Lewis A. Dentsply Pty Ltd. The role of Tooth Mousse and Tooth Mousse Plus in reducing enamel wear by corrosion (erosion), $1,500- 2008
  - Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC, Lewis A. ADRF. Biology of tooth wear: preventive strategies, $5,500- 2006/07
  - Ranjitkar S, Kaidonis JA, Townsend GC, Richards LC. The University of Adelaide. Biology of tooth wear: preventive strategies, $1,800 –2006
  - Hunt D, McIntyre J, White I, Ranjitkar S. School of Dentistry Research Fund, University of Adelaide. The longitudinal study of dental erosion in oenology students, $3,000 – 2006

- **Visiting Research Fellow**
  - King's College London, The University of London, 2007

- **Class meetings / lecturing**
  - Invited lecturer on dental erosion for the Riverland Seminar by the Australian Dental Association Inc (South Australian Branch), 2009
  - Invited lecturer on "the risk of dental erosion in professional wine tasters" to the oenology students in the Waite's Campus, The University of Adelaide, 2008
  - Lecturer on dental materials (five sessions) for 3rd year students, The University of Adelaide, 2009
  - Lecturer on tooth wear (three sessions) for 3rd year dental students, The University of Adelaide, 2007
  - Tutoring and two lectures in Head and Neck Anatomy in the second year Adelaide BDS curriculum, 2004

- **Co-supervisor for student projects**
  - T Narayana - awarded first class honours (BScDent, 2006)
    T Narayana also a recipient of prestigious Leonard Hanson Prize for submitting the best summer vacation research report Australia wide (Australian Dental Research Foundation Inc, 2005)
  - C Nguyen - summer vacation project
  - A Kanter - summer vacation project, 2007/08
  - B Yap - summer vacation project, 2008/09
  - T Pang - final year selective project 2009

- **Other research activities**
  - The longitudinal study of dental erosion in oenology students. ADRF and The University of Adelaide. Chief investigators - Hunt D, McIntyre J, White I, Ranjitkar S, Kaidonis J. Adelaide, 2004 – 2008
  - Townsend G C, Richards LC, Messer LJB. Teeth and faces of Australian twins: a longitudinal study. NHMRC. Adelaide, 2002-2006 (clinical investigator)

- **Contribution to student assessment at the University of Adelaide**
  - Oral assessor for the selection of first year dental students, 2007-
  - Oral assessor for the selection of bridging students in the undergraduate dental curriculum, 2006

- **Committee membership at the University of Adelaide**
  - Postgraduate Student Association, 2006
  - Graduate School Committee, Dental School, 2006 (Postgraduate student representative)

- **Seminar and conference participation (oral and poster presentations)**
  - 86th General Session of the IADR (International meeting), Toronto, 2008
  - 42nd Annual Meeting of the IADR (European and Israeli Divisions), Thessaloniki, 2007
  - 84th General Session of the IADR (International meeting), Brisbane, 2006
  - 45th Annual Scientific meeting of the IADR (ANZ division), Queenstown, 2005
  - 31st Australian Dental Congress and Exhibition, ADA, Adelaide, 2005