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The potential for dental plaque to protect against erosion using an *in vivo-in vitro* model – A pilot study

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Abstract

Background: Tooth erosion is a problem for professional wine tasters (exogenous erosion from frequent exposure to wine acids) and for people with gastro oesophageal reflux disease (GORD) and bulimia who experience frequent reflux of gastric contents into the mouth (endogenous erosion from mainly HCl). The objective in this study was to determine whether plaque/pellicle could provide teeth with any protection from two common erosive acids, using an *in vivo-in vitro* technique.

Methods: Tiles of human tooth enamel and root surfaces were prepared from six extracted, unerupted third molar teeth and sterilized. Mandibular stents were prepared for six volunteer subjects and the tiles bonded to the buccal flanges of these stents. They were worn initially for three days to permit a layer of pellicle and plaque to form over the tile surfaces, and for a further 10 days of experimentation. Following cleaning of the plaque/pellicle layer from the tiles on the right side flange, all the tiles were submerged in either 0.06M HCl or white wine for an accumulated time of 600 and 1500 minutes, respectively. Depths of erosion were determined using light microscopy of sections of the enamel and root tiles. SEM of the lesion surfaces was carried out to investigate the nature of erosive damage and of plaque/pellicle remnants.

Results: Retained plaque was found to significantly inhibit dental erosion on enamel, from contact with both HCl and wine, compared with that resulting following its removal. However, it was found to provide no significant protection on root surfaces. SEM analysis of the tile surfaces revealed marked etching of enamel on the cleaned surfaces, and considerable alteration to the appearance of remaining plaque and pellicle on most surfaces.

Conclusion: Within the limitations of numbers of specimens, dental plaque/pellicle provided a significant level of protection to tooth enamel against dental erosion from simulated gastric acids and from white wine, using an *in vivo-in vitro* model. It was unable to provide any significant protection to root surfaces from these erosive agents. Possible reasons for this difference are explored.

INTRODUCTION

The role of natural protection factors in inhibiting carious demineralization of teeth has been well demonstrated.[1] Even though plaque is most frequently credited for its role in harbouring the acidogenic bacteria which ferment carbohydrate to produce caries-causing acids, there is strong evidence that it also contributes to caries control and repair through its buffering capacity and its storage of fluoride ions.[2]

Bevenius and L'Estrange,[3] and Young[4] also noted the high correlation between salivary protective factors and susceptibility to, and location of, dental erosion. Dental erosion is caused by strong acids which have their origin either in gastric fluid (endogenous erosion) or from dietary, therapeutic or industrial sources (exogenous erosion). Clinically, dental erosion is most frequently evident on the facial, lingual or occlusal surfaces of teeth, and least so on the interproximal surfaces below the contact points. The latter are those surfaces where pellicle and plaque remain for the longest time without threat of removal by natural or physical cleaning processes.

The pellicle layer of salivary glycoproteins and proteins forms relatively quickly on enamel and root cementum, and is usually around 10μm thick.[2] It serves among other roles as a diffusion barrier by its permi-selective nature, restricting the transport of acid ions in, and of calcium and phosphate ions out of the hard tissue, and can thus influence the solubility behaviour of the enamel surface. Plaque also can act as a diffusion barrier, and as a store for high concentrations of calcium, phosphate and fluoride ions.[2] For this reason, it was decided to investigate more fully the possible role

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of pellicle/plaque in protecting both enamel and root cementum/dentine against erosive demineralization.

The aims of the experiment were to assess the potential of the pellicle/plaque layer on both enamel and root cementum/dentine to protect against erosive demineralization by simulated gastric acid and by wine, using an *in vivo-in vitro* model.

MATERIALS AND METHODS

Tooth specimens

Six extracted, unerupted third molar teeth of unknown origin, with intact crowns and roots, were selected for preparation of enamel and root cementum/dentine tiles. Each of the crowns and roots were sectioned into four tiles to give approximately 3x3mm of surface area. The enamel and root tiles from the same teeth were kept together at all times to ensure that test and control tiles would later be from the same tooth and thus carry similar background concentrations of chemicals which might affect the demineralization process. All tiles were sterilized in ethylene oxide at the central sterilizing department of a local major teaching hospital, properly aired with sterility maintained, and placed in a sterile moist environment at 4ºC until required.

Subjects and appliances

Six volunteer subjects were selected from students and staff of the Dental School at The University of Adelaide on the basis of having good dental health, normal stimulated salivary flow rates measured using routine clinical methods, most teeth present and time available to participate in the study. Impressions were taken to enable heat and vacuum adapted mandibular stents (Erkodent Erich Kopp Gmbh, Germany) to be prepared for each subject. The stents were trimmed to incorporate a buccal flange on the right and left sides. Two enamel and two root tiles from the same tooth were attached onto the buccal flanges of both the right and left sides using a light cured resin (Triad Gel, Dentsply, York, UK). The stents were adjusted for comfort for each subject, and any rough edges of the tiles were smoothed to minimize tissue trauma. A thin line of unfilled resin was painted longitudinally over the centre of the exposed surface of each tile as a locating and standardizing device (Fig 1).

Fig 1. Surface of bonded tiles showing the central line of protective resin.

The subjects agreed to use only non-fluoride containing toothpastes and non-fluoride containing spring water for three days prior to and during the course of the experiment. The stents were worn for three days prior to the experiment to initiate the build up of pellicle and plaque. They were cleaned with non-fluoride tooth paste, except for the tiles. Each stent was kept in deionized distilled water (DDW) in special personalized containers when not being worn. The project was approved by the Committee for the Ethics of Human Experimentation of the University of Adelaide.

Erosive solutions

Two categories of erosive solution were used. The first was 0.06M HCl, with $CaHPO_4$ added to a concentration of 2.2mM (pH: 1.4). This has been previously used to simulate gastric acid mixed with salivary components which may impact on demineralisation.[5] The second solution was a cask of dry white wine (Riesling) which was used as the exogenous erosive agent (pH: 3.2).

Method

Each day of the experiment, the stents were worn by the subjects during the morning and collected in their storage containers just before lunch-time. The right hand side tiles on each stent were cleaned with a new toothbrush to remove thick plaque and pellicle and the toothbrush disposed. This side on each appliance served as the 'unprotected' side, the left side tiles remaining covered with pellicle and plaque being the 'protected' side. Standards of infection control set by the Adelaide Dental Hospital were maintained throughout handling of the stents.

The stents from three of the subjects (A, B and C) were placed in a bath of simulated gastric acid for one hour each lunch-time, the remainder from subjects D, E and F being placed in a bath of wine for two and a half hours. These times were chosen on the basis of information from previous experiments[5,6] as resulting in sufficient depth of erosion in unprotected enamel and root cementum/dentine surfaces to permit a significant level of protection to be demonstrated. After these immersion times, the stents were washed thoroughly in DDW and given back to the subjects to wear the remainder of the working day, after which they were cleaned except for the tiles and stored in fresh DDW at 4ºC overnight. These procedures were maintained for 10 working days. During this time, stents from subjects A, B and C were subject to HCl erosion for a total of 600 minutes, while those from subjects D, E and F were subject to wine erosion for a total of 1500 minutes.

Preparation of specimens for analysis of the results of erosion

The buccal flanges were cut from the stents and tiles removed, with care being taken not to disturb the tooth surface deposits remaining. They were stored in DDW at 4ºC while awaiting analysis. One sample from each flange was used for SEM analysis, the remainder being used to measure the depth of erosion.

Determination of depth of erosion

Enamel

Erosion of enamel results in the surface dissolution of apatite mineral, leaving a confined area and depth of loss of enamel. To measure the depth of erosion, each enamel tile was hemi-sectioned using a Buehler Isomet Low Speed Diamond Saw perpendicular to the resin dividing line. The stereomicroscope was then used with an eyepiece measuring graticule, which had been standardized against a mm scale divided into fifty 20µm segments, to measure the depth of erosion resulting on each enamel surface. The protected surface provided an intact surface against which to measure the depth of erosion. This method is similar to that used by Jones *et al.*[5]

Root cementum/dentine

As erosion of root cementum/dentine results in demineralization of these structures, leaving the hydrated collagen intact, it is necessary to cut thin sections of the eroded root, and view these under a transmission microscope to permit measurement of the depth of demineralisation.[7] The root tiles were cut into 150µm thick sections, again perpendicular to the resin dividing line, using the Buehler Isomet sectioning machine with a diamond wafering blade. The depths of erosive demineralization of root cementum/dentine were then examined using an eyepiece graticule on an Olympus BH2 transmission microscope.

SEM examination of eroded surfaces

One set of enamel and root tiles from each flange were fixed in a solution containing glutaraldehyde (1.25 per cent), sucrose (4 per cent) and paraformaldehyde (4 per cent) in phosphate buffered saline (PBS) at pH: 7.2, and dehydrated using critical point drying in preparation for scanning electron microscopy. Each was then mounted on a metal stub and coated with carbon before examination using a Phillips 3300 FE SEM at 10kV. This enabled surface changes to the enamel and cementum/dentine to be detected, and any changes to the physical appearance of any pellicle/ plaque remaining to be determined. Magnification levels varied from x313 to x10 000.

RESULTS

Depths of erosion recorded

The mean depths of erosion recorded in enamel from both 0.06M HCl and from wine are presented in Table 1, with those from root cementum/dentine presented in Table 2. The microscopic appearance of both enamel

Table 1. Mean depth of erosion of enamel in µm ± SD (N=3) when protected (P) and unprotected (U) against wine and HCl challenge

	Wine	Wine	HCl	HCl
	P	U	P	U
Mean	60.0	114	201.6	368.0
SD	±39.4	±37.8	±23.1	±119.0
Significance	P=.0002.		P=0.04.	

Table 2. Mean depth of erosion of dentine in µm ± SD (N=3) when protected (P) and unprotected (U) against wine and HCl challenge

	Wine	Wine	HCl	HCl
	P	U	P	U
Mean	115	125	235	260
SD	±33.5	±30.6	±57.6	±37.9
Significance	NS		NS	

Fig 2. Section through root showing eroded areas.

Fig 3. Surface view of section through eroded enamel.

and root sections which permit depths of erosion to be determined, is seen in Figs 2 and 3.

Enamel

The 'protected' samples had an average depth of erosion of 60µm compared with 114µm in 'unprotected' samples when exposed to wine. Enamel samples treated with HCl also demonstrated reduction of erosion by about a half when plaque was used as protection. On the 'protected' side approximately 201.6µm of erosion was observed, while the average on the 'unprotected' side was 368µm.

The differences between 'protected' and 'unprotected' specimens were statistically significant for enamel using both erosive agents, when tested by the Student t test analysis (P=.0002 for wine, 0.04 for HCl).

Root surface cementum/dentine

Dentine samples challenged with wine showed little or no protection from erosion by plaque. 'Protected' samples had an average depth of erosion of 115µm compared with 'unprotected' samples for which the average depth was 125µm. There was also little
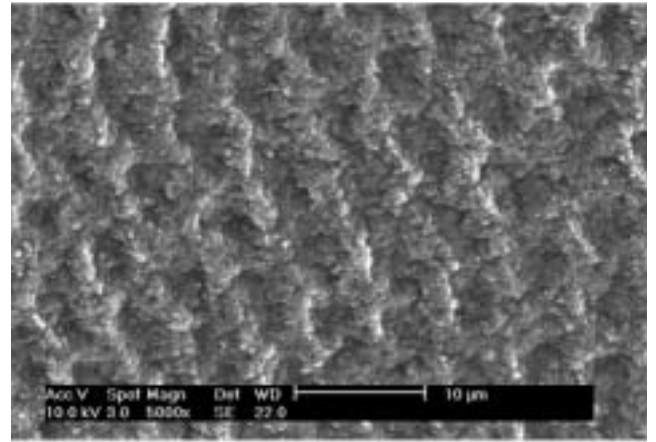
Fig 4. Enamel treated with 0.06M HCl.

Fig 4b.i protected enamel showed gradations of etching, from. early degradation of prism structure (x5000).
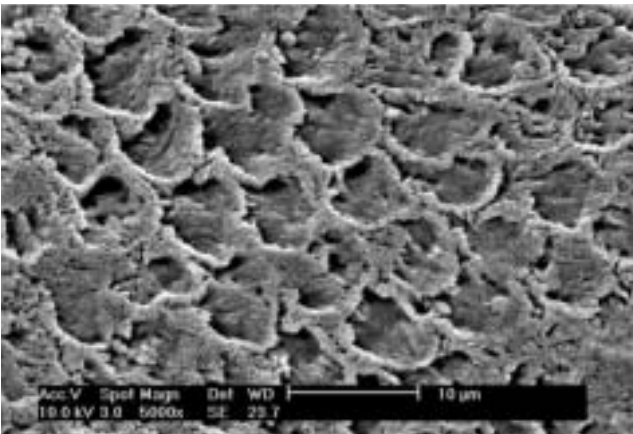

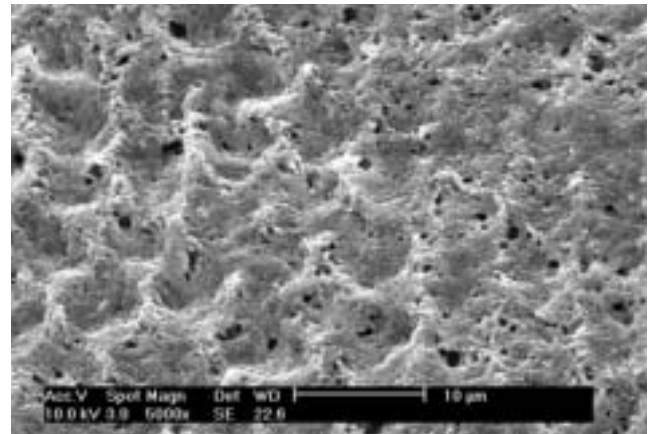**Fig 4a.** unprotected enamel (x5000).

Fig 4b.ii to early signs of breaching of prisms (x5000).

protection from plaque for root cementum/dentine samples treated with HCl. The 'protected' samples had an average depth of erosion of 235µm compared with 260µm for 'unprotected' samples. These differences were not statistically significant using the test described above.

Scanning electron microscopic analysis of the enamel and root surfaces

The following observations were made:

i. Enamel treated with 0.06M HCl (Fig 4)

Unprotected enamel (x5000) was very etched (Fig 4a). No pellicle or plaque was present on any views. The protected enamel (x5000) showed gradations of etching, from early degradation of prism structure (Fig 4b.i), to early signs of breaching of prisms in Fig 4b.ii (x5000), with scattered remnants of pellicle still attached as in Fig 4b.iii (x313). Some of the etched enamel (x5000) appeared as severely demineralized as that on the unprotected enamel (Fig 4b.iv).

ii. Root surface treated with 0.06M HCl (Fig 5)

Unprotected roots (x5000) showed remnants of pellicle binding with a few scattered bacteria. The cementum structure appeared degraded (Fig 5a). The protected root (x5000) revealed degraded plaque with sheets of pellicle and underlying cementum, bacteria still attached (Fig 5b).
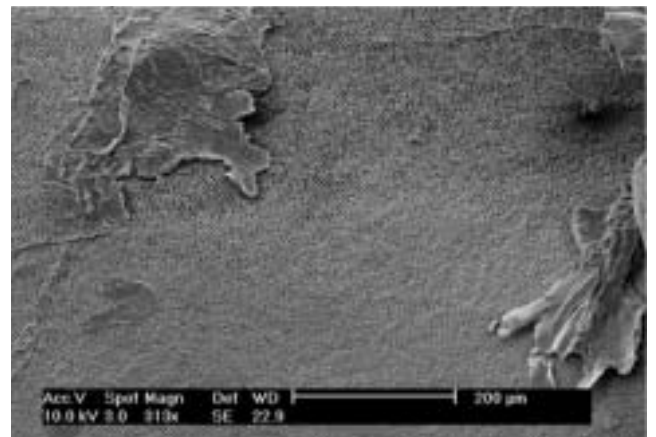

Fig 4b.iii with scattered remnants of pellicle still attached (x313).

iii. Enamel treated with wine (Fig 6)

Unprotected enamel (x313) was very clean with no pellicle. Partially degraded perikymata were visible on the enamel surface (Fig 6a). The protected enamel (x5000) showed intact plaque still present, making it impossible to view the condition of the enamel surface (Fig 6b).

iv. Root surface treated with wine (Fig 7)

Unprotected root cementum (x5000) appeared with remnants of pellicle binding, and degraded cementum
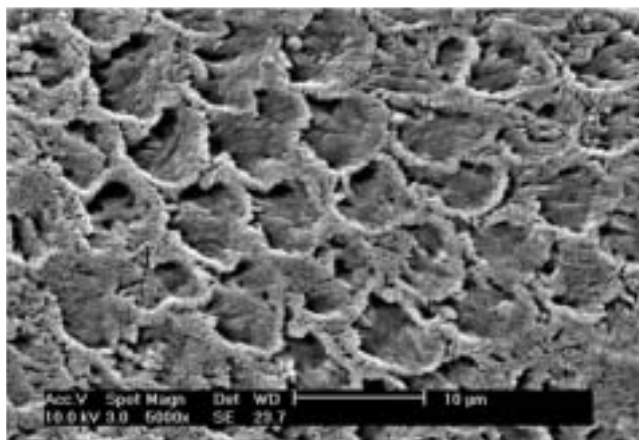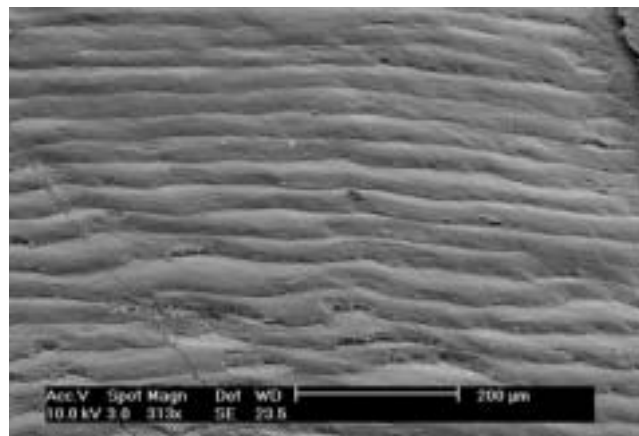
Fig 4b.iv severely demineralized enamel (x5000).

Fig 6. Enamel treated with wine.
a. unprotected enamel (x313).
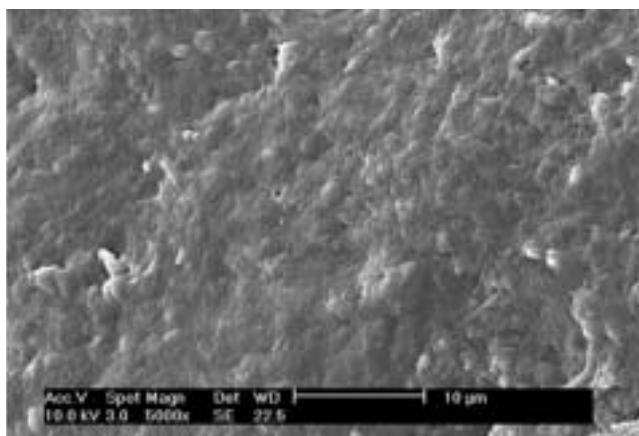

Fig 5. Root surface treated with 0.06MHCl.
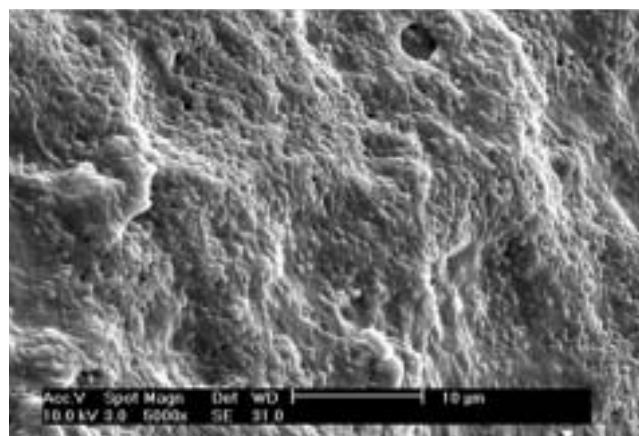**a.** unprotected roots (x5000).
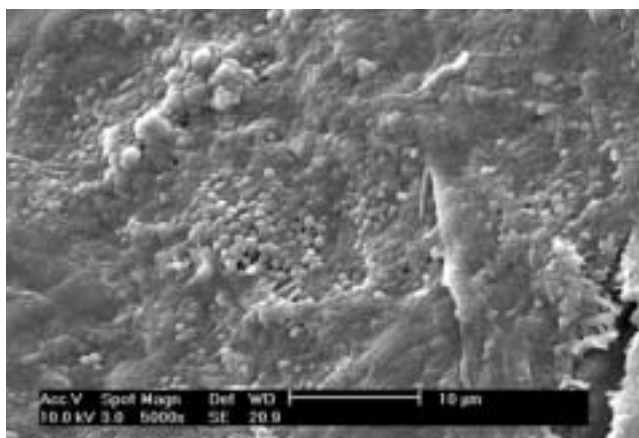

Fig 6b. protected enamel (x5000).

Fig 5b. protected roots (x5000).

(Fig 7a). The protected root cementum appeared either with intact plaque as in Fig 7b.i (x10 000) or in some cases, with partially disrupted plaque with greatly reduced bacterial presence and dislodged sheets of pellicle, as seen at x5000 magnification in Fig 7b.2.

DISCUSSION

Even though the number of specimens was not high, the results obtained were sufficiently consistent to allow interesting comparisons to be made between gastric acid and wine erosion, both on human enamel

and on root surface cementum/dentine. This study repeated that previously carried out by Zid *et al.*[8] on the effects of wine erosion on enamel and root cementum/dentine. In that study, the highly protective effects of plaque/pellicle on enamel against erosion by wine were recorded, along with little protection of root cementum/dentine. This result was considered of such interest that it needed to be re-tested. These results confirm those of that study relating to wine erosion.

As might be expected, the depth of erosion resulting from exposure of 'unprotected' surfaces both of enamel and dentine to 0.06M HCl was significantly greater than that resulting from wine, even though the times of exposure were adjusted to cater for the stronger acid (600 minutes for HCl and 1500 minutes for wine). The depth of erosion caused by 0.06M HCl in unprotected enamel is considerably greater than that in unprotected root cementum/dentine. This differs from the situation with caries, where the rate of acidic demineralization in root surfaces is consistently greater (around 50 per cent greater) than in enamel.[9] It also differs from the results obtained from wine erosion in this study. The depths of erosion resulting from exposure of 'unprotected' surfaces both of enamel and dentine to wine were fairly similar. This result differs from that obtained by Mok *et al.*,[6] where the depth of erosive demineralization *in vitro* following a relatively similar exposure time,
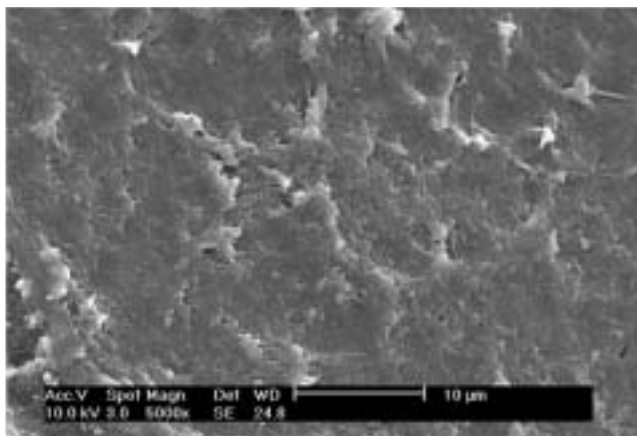
Fig 7. Root surface treated with wine.
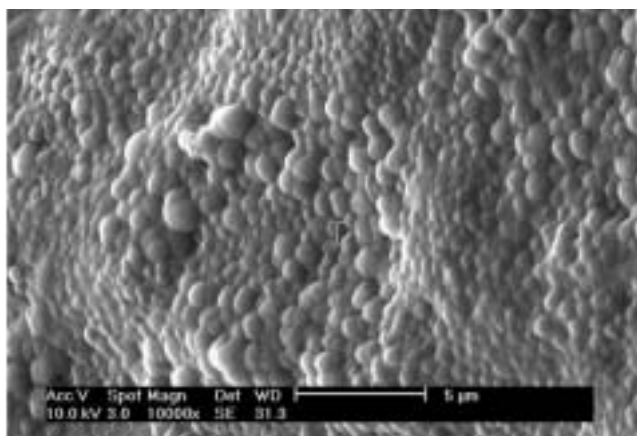**a.** unprotected root (x5000).

Fig 7b.i protected root.
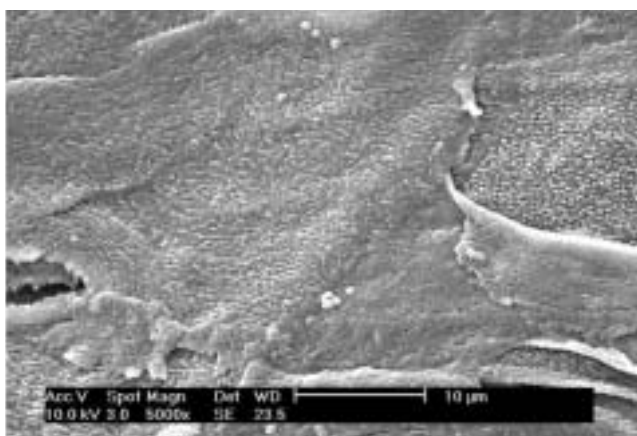cementum with intact plaque (x10 000).

Fig 7b.ii partially disrupted plaque (x5000).

though with a slightly less acidic wine, was double the depth of erosion in root surfaces compared with that in enamel. However, in the latter situation, the enamel and root surfaces had been cleaned, and had had no immediate previous contact with plaque and pellicle.

The differing rates of erosion caused by HCl and wine in enamel and root surfaces is interesting. It appears that root surfaces can resist demineralization by strong acids more successfully than enamel, whilst the reverse holds for weaker acids, e.g., wine, and in

simulated carious lesion generation. This aspect needs further investigation.

The SEM examination of the eroded surfaces, including remnant plaque/pellicle, also provide interesting comparisons.

The most valuable evidence to emerge from this examination was that the plaque/pellicle layer, which had been left to protect the tooth, appeared to have been largely removed from both enamel and root surfaces by the 0.06MHCl by the end of the experiment. It was likely that the HCl would eventually hydrolyze the plaque and pellicle glyco-proteins and eliminate most of the bacteria. However, the fact that this pellicle and plaque still helped to reduce the erosive damage to enamel and root cementum/dentine before it was lost is of particular interest. Wine had a much reduced effect on plaque/pellicle, some deterioration in plaque structure only being evident in a few cases.

It was not possible to determine any visual differences in the structure of plaque/pellicle when present on enamel compared with that present on root cementum/dentine in those cases where it was retained. This method did not provide information on difference in thickness of the plaque, which may be a factor contributing to the lack of protection of root surfaces against erosion.

The nature of the erosive damage from both types of acid is very different, again as might be expected. Apart from the more aggressive loss of enamel, the HCl resulted in a marked attack on the enamel prism core, as is seen with acid etching by 37 per cent H_3PO_4. Wine did not result in this type of attack, appearing to cause a much more gentle etch, with thin layers of surface enamel being removed. With prolonged exposure though, this may have been more pronounced.

Interpretation of the results
Clinical significance

For enamel, the results have implications for erosion reduction practices, though need further confirmation *in vivo*. An almost halving of the depth of erosion by the presence of plaque/pellicle, in combination with chemical control measures such as concentrated topical fluorides,[5,6] would provide significant protection against both causes of erosion of enamel. The plaque may also help to reduce the potential for severe staining of enamel by the anthocyanins and tannins of red wines, though its ability to achieve this is not known, and needs investigation.

For example, wine assessors might be advised to not brush their teeth the morning immediately before a day of continuous wine tasting. They would, however, be advised to brush effectively around an hour after finishing the day's tasting activity, this delay being intended to allow saliva to help partially stabilize damaged tooth surfaces. This should be followed by comprehensive oral hygiene on retiring for the night,

when a concentrated fluoride gel would also be self-applied.

In relation to protection of enamel against endogenous erosion, it is more difficult to see how this information can be used beneficially for the patient, unless the erosive challenge occurs at a regular time in the morning or evening, as it may do in the case of pregnancy, or some eating disorders. In such cases, patients might be advised to alter their brushing routines so as to leave a layer of plaque present at the most vulnerable time. It would certainly be advisable to leave any layers of calculus present while such a problem was occurring, provided it was for a limited time period, as there is evidence that calculus has an even greater buffering capacity than plaque.[2] In both cases, this should be one of a number of approaches to erosion control, and be recommended along with concentrated topical fluoride gels or varnishes, use of bicarbonate mouth rinses and concentrated calcium phosphate (CPP-ACP) materials. In very severe cases, physical protection with resins or GICs might be indicated.

The failure of the plaque/pellicle layer to protect root cementum/dentine to the same extent as enamel is quite puzzling. This result implies that older professional wine-makers or assessors with exposed root surfaces may still need a number of extra measures to assist with protecting such surfaces from prolonged sessions of wine assessment.

Exploring possible reasons for the deficiency in plaque/pellicle protection of root cementum/dentine

It is difficult to explain this result. One possibility to consider is that the nature and/or quality and/or quantity of plaque and pellicle might be different on cementum compared with enamel. Initial colonization by plaque-forming organisms on root cementum is similar to that on enamel, but the process occurs more rapidly.[2]

Colonization does not take place in a particular pattern on root surfaces.[10] Hence it would seem that there should be more plaque on root dentine, therefore imparting a higher degree of protection. Results of this study have shown differently. Further studies need to be carried out to analyse the quantity and quality of plaque growth on root cementum/dentine compared to enamel.

The more likely explanation relates to the potential for plaque to store large quantities of calcium, phosphate and fluoride ion both from saliva and from dissolution products of tooth mineral. This enables plaque to exert a significant buffering capacity against acidic challenges, described by Thylstrup and Fejerskov[2] as having up to 10 times the buffering capacity of saliva. As the concentration of apatite mineral is very high in enamel (87 per cent by volume), and this apatite is rapidly dissolved from enamel by acids, it is to be expected that overlying plaque would rapidly become saturated with dissolution products,

enabling the plaque to exert a strong buffering effect on the erosive acids. In root cementum/dentine, the apatite mineral occupies only around 47 per cent by volume. It is also more slowly dissolved by HCl in root cementum/dentine than in enamel. Hence, the plaque may not reach the same level of saturation as it does over enamel, and thus may exert a much lower buffering capacity to the erosive challenge. This matter requires further investigation.

CONCLUSION

Despite the small sample size, this study has demonstrated that plaque/pellicle protection is effective in reducing the effects of enamel erosion by wine and gastric acids, but has little effect on root cementum/dentine, using this model. These results need further testing, preferably clinically within ethical bounds, to enable their implications for erosion control to be confirmed.

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