SCIENTIFIC ARTICLE

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Studies on dental erosion: An in vitro model of root surface erosion

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Abstract

Most research on dental erosion has focused on erosion in enamel. However, the increasing retention of teeth into old age has increased the potential for root surface erosion. The nature and kinetics of root surface erosion have not yet been adequately explored.

The objective of this project was to develop an in vitro model of root surface erosion in order to investigate the polarised light microscopy of controlled erosion and the histology and rate of demineralisation of this process in root cementum/dentine.

The results demonstrate a process quite different in outcome from that in enamel erosion. Erosion results in loss of volume of enamel. However, it leaves a root surface softened though with little volume loss so long as the demineralised collagen remains hydrated. The polarised light histology was similar to that seen in root surface caries. The rate of erosive demineralisation of tooth roots by selected acids was investigated, with differing rates of demineralisation similar to that seen in enamel erosion observed. The process of erosion showed evidence of significant diffusion control.

Key words: Erosion, root surface, histology, rate of demineralisation, root caries.

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INTRODUCTION

In recent years, the phenomenon of dental erosion has aroused a great deal of clinical and research interest. This interest has almost exclusively focused on erosion of dental enamel and the causative factors of this erosion. In vitro studies[1-2] described the effects of erosive substances on the potential for abrasion of exposed tooth roots. A major review of erosion hardly mentions erosion on tooth root surfaces.[3]

However, if certain strong acids are able to dissolve apatite from enamel, it might be expected they would have the same effect on exposed root dentine/cementum. In the high caries risk clinic at the Adelaide Dental Hospital, a number of cases of suspected clinical root erosion were detected.[4] These cases involved elderly people who suffered from frequent gastric acid reflux. In such cases, after a period of active gastric reflux, the exposed root cementum/dentine became very soft (leathery) to touch with a blunt probe. Following a few weeks of topical fluoride therapy, these surfaces returned to relatively firm or normal condition.

The lesions could not be detected by visual examination alone and increased hypersensitivity was the usual reason for visiting a dentist. The affected roots were usually on both lingual and facial surfaces of maxillary posterior teeth and occasionally on anterior teeth. Such lesions were often very dark in colour. The diets of most of these patients were not considered cariogenic and patients' oral hygiene was reasonable. Hence, these lesions were considered to be largely a result of periodic erosive demineralisation of the exposed root cementum/dentine, followed by periods of remineralisation. On tactile examination, in terms of their texture, the lesions differed little from incipient root caries. The objective of this study was to develop a simple in vitro model of root surface erosion, employing the methods previously used to study enamel erosion.[5] This model was used to investigate the polarised light histology of the resultant lesions and to determine the rate of demineralisation with differing erosive acids.

MATERIALS AND METHODS

Root preparation

One hundred intact roots of teeth, which had been sectioned from crowns through the cemento-enamel junction buccolingually, were gently scraped of superficial debris and soft tissue, cleaned and stored in doubly distilled water (DDW), at 40°C, to which had been added thymol (0.1 per cent) as a preservative. Each root had two windows 3×1mm of surface cementum left exposed, the remainder being covered by a water- and acid-resistant varnish.

Erosive test substances

In this study, it was decided to use acids which cause quantifiable levels of erosion in enamel as test erosive

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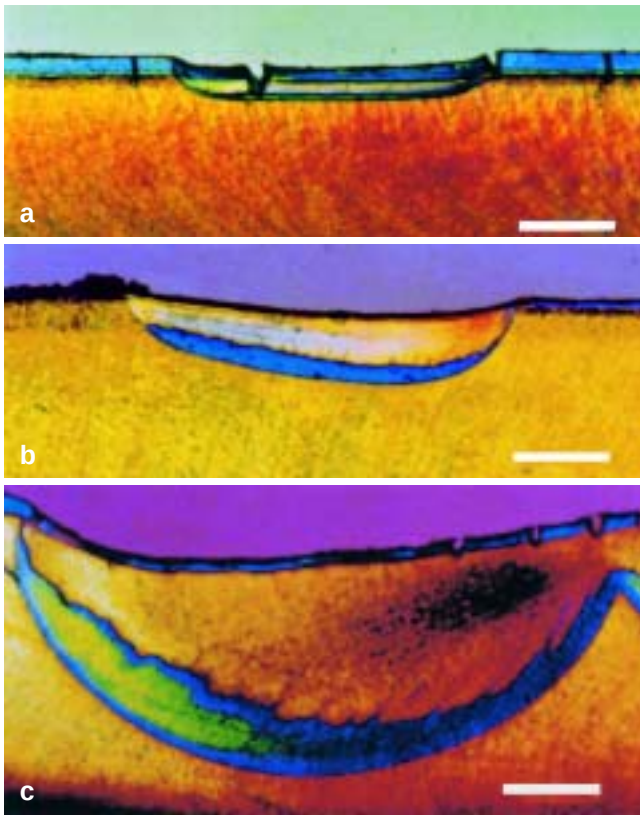

Fig 1. Histologic profile of 0.06mol/L HCl erosion lesions with sections immersed in quinoline, after (a) 4h, (b) 24h, (c) 168h (7 days) of demineralisation (×50, Bar=300µ).

acids.[5] The first acid was 0.06mol/L HCl with 2.2mmol/L $CaHPO_4$ added, which was found to simulate the effects of endogenous erosion on enamel by gastric acids. A solution of 5 per cent citric acid with 2.2mmol/L $CaHPO_4$ added was intended to represent the action of lemon juice mixed with some salivary components present in unstimulated saliva. Acetic acid at a range of concentrations was also tested. The acetic acid tested consisted of an acetate buffer initially containing 0.5mol/L acetic acid and 2.2mmol/L $CaHPO_4$. The pH of separate samples was adjusted to 2.5, 3.5 and 4.5 using glacial acetic acid or sodium hydroxide. The solution at pH 2.5 was intended to simulate the erosive effects of vinegar (pH 2.8) and that at pH 4.5 provided conditions similar to those used by Featherstone[6] to generate artificial carious lesions in tooth roots. This would enable some comparison to be made between artificially generated root caries and erosion.

Continuous erosion test model

Three individual roots were placed in 40ml of each of the five test erosive substances and incubated at 37°C:

(a) 0.06 mol/L HCl+2.2mmol/L $CaHPO_4$ (pH 1.22) for 2, 4, 7, 12, 24, 48 hours; 3 days, 7 days and 14 days.

(b) 5 per cent citric acid+2.2 mmol/L $CaHPO_4$ (pH 1.76) for 4, 7, 12, 24, 48 hours; 3 days, 7 days and 14 days.

(c) Acetate buffer (pH 2.5) for 1, 2, 3, 7, 14 days.

(d) Acetate buffer (pH 3.5) for 2, 3, 7, 14 days.

(e) Acetate buffer (pH 4.5) for 3, 7, 14 days.

After these periods of time, roots were removed, washed in DDW and stored in a moist environment awaiting analysis. Some samples, which had been previously photographed, were wiped with acetone to remove the protective varnish, enabling further photographs of demineralised surfaces to be made.

Measurement of depth of demineralisation against time of exposure

Using a thin diamond blade, roots were sectioned through the middle of the exposure windows and polished to result in sections approximately 100mm thick. Sections were immersed in DDW and analysed at 50 times magnification using an Olympus polarising light microscope with a graduated eyepiece scale. From a line formed by the crossed wires in the scale connecting the remaining unaffected surface cementum adjacent to the lesion, the greatest depth of demineralisation was recorded in each section and the results tabulated. Mean depths from all three specimens within a category of erosive demineralisation were calculated and the rate of change of depth with time of exposure recorded graphically. Standard deviations from means of the three root specimens were calculated and also recorded.

Analysis of histological pattern of demineralisation

To determine the nature of the changes in structure observed in the demineralised component of the cut sections, DDW and quinoline immersed sections were analysed using an Olympus polarising light microscope. The two distinctive zones of demineralisation which were apparent when the sections were immersed in quinoline were observed and photographed.

RESULTS

Histological pattern of demineralisation

Examination of sections from differing categories of specimens, when immersed in DDW using polarised light microscopy, showed similar evidence of reduced density of mineral with time in the demineralised zones, though with tubular structure intact in the deeper part of the lesion. This reduced density was similar to that seen when roots are subjected to artificial carious demineralisation, as seen when acetate buffer at pH 4.5 was used as the demineralising agent (Fig 3). Immersion in quinoline resulted in similar depth of demineralisation and pattern of two zones of differing birefringent sign, as seen when intact roots are subjected to artificial carious demineralisation (Fig 1-3).

The patterns of zones did not vary greatly irrespective of whether demineralisation was achieved using HCl, citric or acetic acid, the depth of zones varying only with time, concentration and strength of acid. A further observation was that erosive demineralisation extended laterally through cementum and dentine from the line

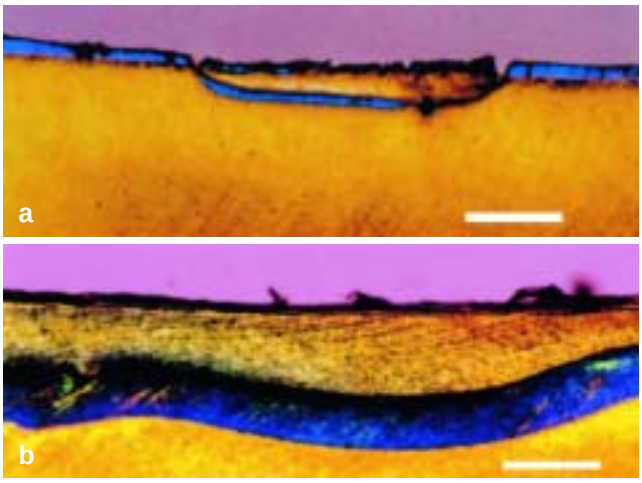

Fig 2. Histologic profile of citric acid erosion lesions with sections immersed in quinoline, after (a) 12h, (b) 168h (7 days) of demineralisation (×50, Bar=300μ).

of protection by varnish. It appeared that this lateral spread of demineralisation proceeded at a similar rate to its movement depthwise into the dentine structure, resulting in a joining of demineralisation zones, which had initially been 1mm apart, beneath adjacent exposed cementum windows in the more advanced cases of erosion (Fig 1-3).

Rate of erosive demineralisation using differing categories and concentrations of erosive acids

The results in Table 1 and Fig 4 show an increase in rate and depth of demineralisation of exposed roots with increasing strength of acid and, in the case of acetic acid, increasing concentration. Irrespective of the strength of acid, after three days the demineralisation rate slowed to be comparable for each category. That is, after this time, the rate of continuing demineralisation with HCl was little greater than for citric or acetic acid, even though the aggregate depth of erosion was greater with the stronger acids because of the increased erosion demonstrated during the initial hours of exposure.

Diagnostic appearance of root surface erosion

The appearance of a root surface before acidic challenge and after erosion had occurred to a depth of approximately 1mm is seen in Fig 5. This appearance was conditional on the root being kept moist.

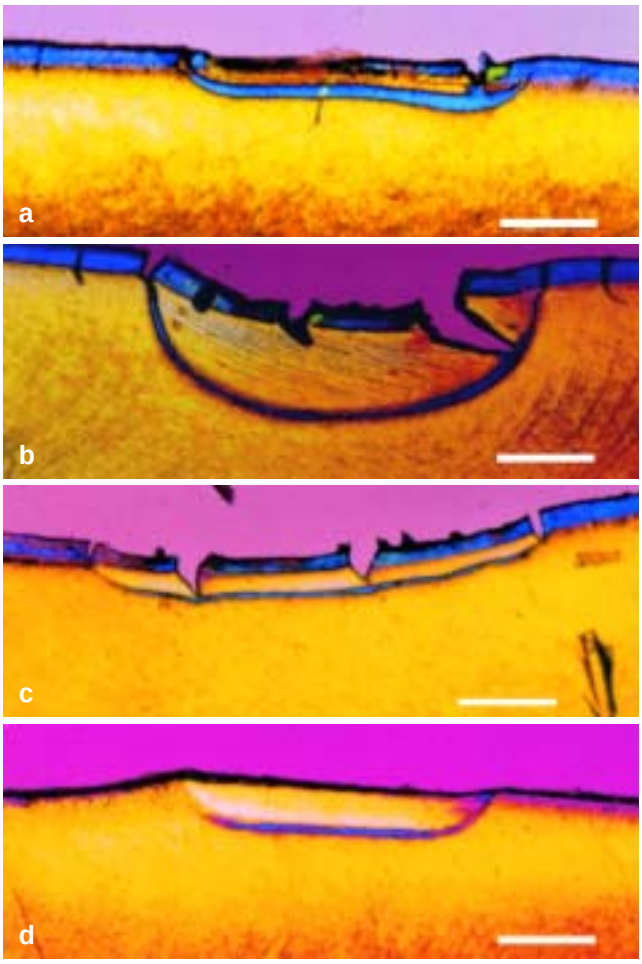

Fig 3. Histologic profile of acetic acid erosion lesions with sections immersed in quinoline, at the following concentrations, after the following demineralisation times (×50, Bar=300μ): pH 2.5 (a) 48h, (b) 168h (7 days); pH 3.5 (c) 72h (3 days); pH 4.5 (d) 168h (7 days).

DISCUSSION

Demineralisation of intact roots by all categories of erosive acid resulted in polarised light histological patterns similar to those seen in artificial root caries, only the rate and resultant depth being greater.[6-7]

The histological pattern of two zones of opposite birefringent sign seen in all categories of erosive demineralisation was of particular interest. These zones of differing birefringence, also seen in root surface caries, did not represent zones of differing pore size as occurs with such zones seen in polarised light

Table 1. Mean depths (±SD) of demineralisation (μ) of roots using 0.06mol/L HCl, 5 per cent citric acid and acetic acid at pH 2.5, 3.5 and 4.5 against time (h) (all solutions containing 2.2mmol/L $CaHPO_4$, all sections immersed in water).

Time (h)	HCl	Citric acid	Acetate pH 2.5	Acetate pH 3.5	Acetate pH 4.5
2	130±20	0	0	0	0
4	180±15	143±31	0	0	0
8	267±17	228±26	0	0	0
12	322±29	343±31	0	0	0
24	372±87	347±55	250±88	0	0
48	612±11	440±75	240±50	220±51	0
72	755±84	696±186	280±77	205±50	124±27
168	925±165	760±113	640±63	304±50	230±28
336	1340±87	1013±207	760±111	420±60	307±50

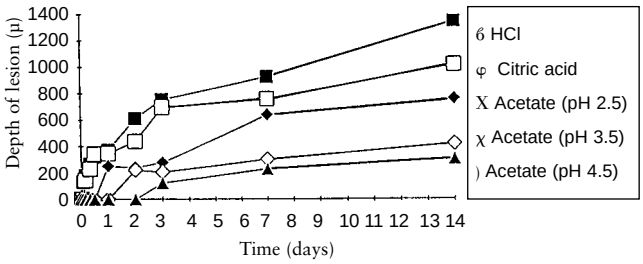

Fig 4. Mean depths of demineralisation (µ) of roots using 0.06mol/L HCl, 5 per cent citric acid and acetic at pH 2.5, 3.5 and 4.5 against time (days) (all solutions containing 2.2mmol/L $CaHPO_4$, all sections immersed in water).

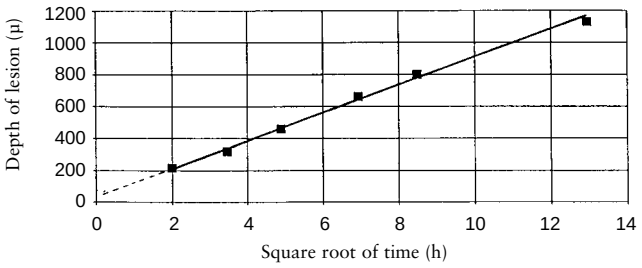

Fig 6. Diffusion control data for 0.06mol/L HCl: Lesion depths against square root of time (h).

microscopy of caries in enamel. In dentine, they have been found to represent different degrees of mineralisation of collagen, first described by Von Ebner in 1894 as being present in pre-dentine before it became fully mineralised.[8] Von Ebner described the phenomenon as the phenol reaction, proposing that when collagen was only partially mineralised, phenol and other aromatic molecules were able to bind to the collagen and result in a reversed birefringent pattern in this partially mineralised zone. Recent investigations led to the conclusion that this so-called phenol reaction also occurred when sufficient demineralisation of dentine had taken place to permit phenol or similar aromatic molecules such as quinoline to bind to collagen.[9] However, the fact that this phenol reaction zone did not continue into the surface section of the lesion was due to such a high level of demineralisation occurring in this region as to cause the collagen bundles to collapse in on each other and thus exclude the binding of these aromatic molecules. Full mineralisation also excludes the binding of these molecules to collagen. It was estimated that this phenol reaction resulted in those sections of demineralised dentine in which from 1-40 per cent of mineral had been lost.

The data on the rate of erosive and carious demineralisation seen in Fig 4 suggest that a similar type of diffusion control mechanism to root surface caries may operate.[7] A calculation of the rate of depth change when HCl was the erosive acid against the square root of time revealed a linear relationship characteristic of a diffusion controlled process (Fig 6). That is, the buildup of high concentrations of apatite dissolution products, in particular calcium and phosphate, at the acid ion diffusion front begins to exert a significant level of inhibition of further demineralisation of apatite by the acid ions.

A further explanation could be that the erosive solutions progressively became more saturated with calcium and phosphate levels, with respect to those in the apatite mineral of the root, and their ability to penetrate into the root surface was reduced. In order to test this possibility, a further brief investigation was carried out in one of the categories of erosion (0.06mol/L HCl+2.2mmol/L $CaHPO_4$). Extra root samples were placed in 40ml containers of this agent and each time a set of samples was collected at two, four, 12, 24, 48 and 72 hours and at seven days, all the roots were placed in a fresh solution of the erosive agent. Figure 7 shows the depth of resultant erosive demineralisation compared to the level of demineralisation when the roots remained in the same solution for the whole experiment. In the short term, the results indicate there appears to be little difference between the two categories. However, by seven days, the rate of continuing demineralisation was not so markedly suppressed when fresh solutions were used. This would indicate that some degree of suppression of erosive demineralisation resulted from an increased level of saturation of the erosive acids with dissolved calcium and phosphate ions. However, there is still evidence of a significant level of reduction in rate of continuing demineralisation even when the samples are periodically introduced to fresh erosive solutions. This supports the conclusion that diffusion control contributes significantly to this result.

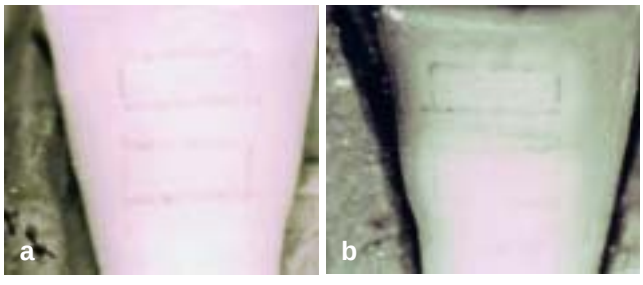

Fig 5. Appearance of roots (a) prior to acidic challenge and (b) following erosive demineralisation to approximately 1mm depth with HCl, the collagen being maintained well hydrated (erosion lesion outlined).

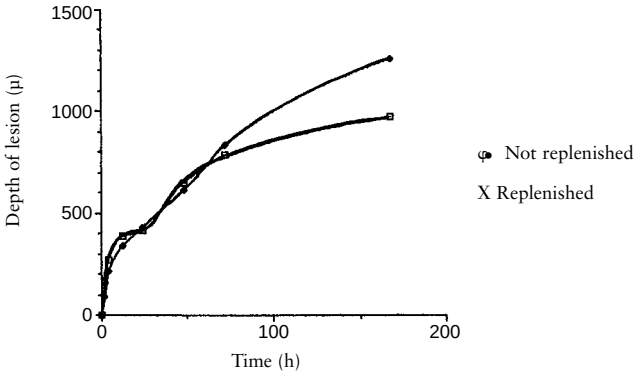

Fig 7. Rate of 0.06mol/L HCl erosion in roots when the erosive solution is replenished at each time block completion compared to that following retention of the same erosive solution.

Photographic evidence of sample roots prior to acidic challenge and following demineralisation, even when lesions greater than 1mm in depth resulted, showed no major visible difference in appearance, provided the remaining collagen remained well hydrated. Thus, if no other factors cause damage to the demineralised collagen, both erosive and incipient carious lesions of roots are clinically difficult to detect and require tactile evidence of deformability of the residual collagen before a diagnosis can be made. This also points to the difficulty of making a differential diagnosis between eroded and carious root lesions. In both cases, dehydration of the demineralised collagen resulted in a rapid contraction of the collagen, with a resultant depression remaining. Clinically, such demineralised surfaces are frequently damaged by abrasion or bacterial proteolytic action or become heavily stained, thus permitting easier detection.

The important distinction between root surface and enamel erosion is that, in the latter, there is loss of volume of enamel, whereas in root erosion, when the mineral component is wholly or partially dissolved, the collagen structural protein remains. As long as the remaining collagen structural proteins hydrate and are not damaged, physical shape and appearance are maintained. During this time, the root erosion area is very susceptible to abrasion damage caused by tooth-brushing, coarse foods or dental instruments.

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