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Exposure Time of Enamel and Dentine to Saliva for Protection against Erosion: A Study in vitro

S. Wetton J. Hughes N. West M. Addy

Division of Restorative Dentistry, University of Bristol, Dental School, Bristol, UK

Key Words
Enamel · Dentine · Tooth wear · Dental erosion · Saliva · Salivary pellicle

Abstract
Previous research, mainly on enamel, supports a protective role for salivary pellicle against erosion. Pretreatments have tended to be lengthy (24 h or more) and of questionable relevance to the regular intake of acidic food and drink by many individuals. The aim of this study in vitro was to determine the protective effect of salivary pellicle formed on enamel and dentine over time periods up to 4 h. Flattened, polished human enamel and dentine specimens were pretreated with unstimulated human saliva from a single donor for 2 min, 30 min (enamel only), 1, 2, or 4 h. Controls were exposed to water for the same times. Specimens were then exposed to 0.3% citric acid, pH 3.2 for 10 min with stirring. This cycle was carried out 12 times. Tissue loss was measured by profilometry after 3, 6, 9 and 12 cycles. For enamel, statistically significant protection was found at ≥1 h. For dentine, significant protection was achieved at 2 min. Salivary pellicle offered proportionately greater protection to enamel than dentine. Cautiously extrapolating these in vitro data suggests that pellicle should offer erosion protection to individuals who imbibe acidic drinks at frequencies of 1 h or less.

Tooth wear, as a potential threat to the longevity of the human deciduous and permanent dentitions, has attracted increasing research interest in recent years [for reviews see Mair, 2000; Bartlett and Smith, 2000; Addy and Hunter, 2003]. Of the processes involved in tooth wear, arguably erosion or, perhaps more correctly, chemicophysical wear, has been most studied [for reviews see Imfeld, 1996; Nunn, 1996, 2000; Zero and Lussi, 2000]. A number of acidic substances, from intrinsic and extrinsic sources, have been implicated in dental erosion [Zero and Lussi, 2000]. Notable amongst these aetiological factors have been soft drinks, which is perhaps not surprising given their increasing intake over recent decades by developed nations [British Soft Drinks Association, 1991; O'Brien, 1993, Dugmore and Rock, 2004]. As with other diseases and conditions, which affect the teeth, erosion appears to show considerable individual and site-specific variation [Hall et al., 1999; Nunn, 2000]. A whole host of factors, contributing to this variation, have been suggest-

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Martin Addy
Division of Restorative Dentistry, Dental School
Lower Maudlin Street
Bristol BS1 2LY (UK)
Tel. +44 1179 284 506, Fax +44 1179 284 100, E- Mail Martin.Addy@bristol.ac.uk

ed. One such factor, which has featured in debates on variability of erosion, is the salivary pellicle [Sonju Clasen et al., 2000]. There is much evidence that salivary pellicle inhibits demineralisation of enamel and dentine, drawn from studies in situ and in vitro [Moreno and Zahradnik, 1979; Meurman and Frank, 1991; Hannig, 1998, 1999; Amaechi et al., 1999; Hall et al., 1999; Hannig and Balz, 1999, 2001; Sonju Clasen et al., 2000; Young et al., 2000]. The protection could be physical, chemical or both.

A majority of studies reporting that pellicle is protective against erosion have been concerned with enamel, usually bovine, although some studies have used human enamel. These studies also used pellicle formation times of many hours, often 24 h, or many days [Hall et al., 1999; Hannig and Balz, 1999, 2001; Hara et al., 2003]. However, one study [Hannig et al., 2003] failed to show additional protection beyond 2 h of pellicle formation, even though pellicle thickness and amino acid composition are known to increase as a function of time [Lie, 1977; Skjörland et al., 1995; Sonju Clasen et al., 1997; Hannig, 1999]. Since acid exposure thins or even removes pellicle relatively quickly [Hannig and Balz, 2001; Hannig et al., 2003], lengthy formation times are not relevant to persons most at risk from erosion, namely individuals who imbibe acid fluids frequently throughout the day. The aim of the present study in vitro was to determine the shortest time of exposure to saliva, which would result in a reduction in erosion of enamel and dentine compared to water-pretreated controls. The null hypothesis was that erosion protection afforded by saliva to enamel and dentine would not be influenced by the time of exposure to saliva, between 2 min and 4 h.

Materials and Methods

Preparation of Specimens

The detailed methods for the preparation of the enamel and dentine specimens have been described previously [West et al., 1998]. Pieces of enamel and dentine were cut from human third molars, which had been extracted from individuals aged 18–35 years. The dentine pieces were placed into moulds measuring 30 × 30 × 5 mm with the root surface downwards. Enamel pieces were placed into moulds measuring 8 × 6 × 2 mm with the outer enamel surface downwards. The moulds were then filled with epoxy resin, which was allowed to set for at least 24 h. Specimens were then removed from the moulds and polished using 1,200 grit abrasive discs in a lapping and polishing machine, with water lubrication, to expose a window of enamel or dentine with an acceptance profile of ±0.3 μm, measured by contact profilometry. The profilometer (Surfometer, Planar Products Ltd., Sunbury on Thames, UK) had a diamond stylus with a tip radius of 20 μm and a head velocity of 10 mm min^{-1}. The force of the stylus varied linearly with deflection at a rate of 8 mg μm^{-1} up to a maximum of 1 g at 100 μm. Specimens were then taped with parallel strips of PVC tape to leave a window of enamel or dentine 2 mm wide. Groups of six specimens were allocated to each treatment.

Treatments

Unstimulated saliva was obtained from the same 28-year-old male (S.W.) throughout the day on experimental days by allowing saliva to dribble into screw cap 30-ml polystyrene universal containers at room temperature. Collection was always at least 1 h after any intake of food or drink and collected saliva was used immediately. Groups of specimens were soaked in 20 ml saliva or water for 2 min, 1, 2 and 4 h, removed and rinsed in running tap water for 30 s and then placed in 200 ml of 0.3% citric acid, adjusted to pH 3.2 with NaOH at 35°C, with constant, overhead stirring at 270 rpm [for details see Eisenburger et al., 2000]. After 10 min, to simulate a sipped drink, specimens were removed and washed in water as before. The cycle of exposure to water or saliva, then acid was repeated a total of 12 times. After 3, 6, 9 and 12 cycles the tape was removed and tissue loss measured across two zones of the window using the contacting profilometer. Following measurement, specimens were re-taped to expose the same window and the cycles continued. As negative controls, two groups of enamel and dentine were cycled through water or saliva and then soaked in water for 10 min: a total of 12 cycles being performed. In a supplementary experiment a group of enamel specimens was assessed for erosion protection after 30 min exposure to saliva. Measurements were only taken after the 12th cycle.

Statistical Methods

The sample size of 6 specimens per group was chosen to provide 80% power to demonstrate a minimum 10% difference between saliva- and water-treated groups at the 95% probability level. Descriptive statistics (mean and standard deviation) are given for erosion by cycles and between enamel and dentine. The erosion of saliva-treated enamel and dentine after the 12th cycle was compared with that in the water-treated controls by unpaired t tests.

Results

Erosion of enamel for the saliva- and water-pretreated specimens is shown in table 1 and for dentine in table 2. The control enamel and dentine specimens exposed to water or saliva and then 'eroded' in water showed no measurable change and specimens all remained at their baseline measurements. For all enamel and dentine groups, the first mean increment of loss at cycle 3 was the greatest, except enamel treated for 2 min with water. Thereafter the pattern was, in most cases, approximately linear. At cycle 12, for water-treated groups, with the exception of the 2-min pretreatment, the mean loss of enamel was always greater than dentine. With saliva pretreatment, erosion was similar in enamel and dentine at cycle 12.

Table 1. Erosion depths (μm) of enamel specimens after pretreatment for various times with saliva or water

Cycles	Saliva pretreatment time							
	2 min		60 min		120 min		240 min	
	saliva	water	saliva	water	saliva	water	saliva	water
0	0.10 (0.05)	0.11 (0.09)	0.04 (0.09)	0.10 (0.05)	0.17 (0.08)	0.08 (0.08)	0.07 (0.09)	0.12 (0.11)
3	6.14 (0.71)	4.53 (0.82)	4.60 (1.23)	6.05 (1.09)	3.49 (0.55)	5.26 (0.48)	4.18 (0.74)	5.08 (0.82)
6	9.95 (1.73)	10.12 (0.49)	7.57 (0.60)	10.35 (2.03)	6.12 (1.04)	9.41 (0.99)	7.86 (1.35)	11.47 (2.17)
9	12.48 (6.23)	15.46 (0.99)	9.64 (1.22)	15.86 (2.28)	10.08 (1.40)	13.94 (2.35)	11.03 (1.32)	16.13 (1.89)
12	18.60 (1.56)	18.99 (0.76)	12.38 (1.92)	21.92 (2.68)	11.40 (1.44)	17.18 (1.49)	13.50 (2.42)	20.1 (2.66)
p value[1]	0.59		<0.001		<0.001		0.002	

Values are expressed as means (SD).
[1] Comparison of saliva- and water-treated enamel specimens after 12 cycles.

Table 2. Erosion depths (μm) of dentine specimens after pretreatment for various times with saliva or water

Cycles	Saliva pretreatment time							
	2 min		60 min		120 min		240 min	
	saliva	water	saliva	water	saliva	water	saliva	water
0	0.10 (0.06)	0.04 (0.11)	0.05 (0.10)	0.04 (0.07)	0.06 (0.07)	0.08 (0.12)	0.06 (0.15)	0.07 (0.15)
3	8.58 (0.76)	7.17 (1.03)	5.28 (0.72)	7.26 (1.17)	6.12 (0.56)	7.03 (0.75)	5.48 (0.82)	6.17 (0.49)
6	11.76 (1.49)	11.24 (1.27)	9.23 (1.08)	10.69 (1.73)	9.55 (0.50)	10.72 (1.73)	8.94 (1.48)	10.69 (1.12)
9	15.32 (0.82)	16.28 (1.96)	11.68 (0.35)	12.67 (1.94)	11.67 (1.02)	14.40 (1.98)	10.70 (1.46)	14.38 (1.66)
12	17.56 (0.91)	20.97 (3.45)	13.59 (0.75)	15.77 (2.17)	12.73 (1.03)	15.55 (1.97)	12.48 (1.58)	16.60 (1.92)
p value[1]	0.049		0.042		0.011		0.002	

Values are expressed as means (SD).
[1] Comparison of saliva- and water-treated dentine specimens after 12 cycles.

For enamel, after every cycle and pre-exposure time, with one exception (2 min saliva versus water at cycle 3), erosion depths in samples pretreated with saliva were lower than in water-treated controls. At cycle 12 there was no significant difference in enamel erosion between water and saliva groups for 2-min pretreatment time, but significantly less erosion depth in saliva-treated samples than in water-treated samples at 1, 2 and 4 h. The average percentage reduction in erosion at 1, 2 and 4 h saliva pretreatment was 37% (range 33–44%). In the separate 30-min pretreatment time experiment, the erosion depth in saliva-treated specimens was 19.5 (SD 4.05) μm, and this was not significantly different from any water controls ($p > 0.05$).

For dentine at cycle 12, erosion was always less in saliva-treated samples than in water-treated samples. At cycle 12 the differences between saliva and water pretreatments were significantly different for all pretreatment times. The actual mean protection in proportional terms was at 1 h greater for enamel compared to dentine: 44% compared to 14%.

Discussion

The cyclical model was used to amplify the possible protective effect of saliva and to simulate the intake of soft drinks over a period of several days. Such a protocol has been used in a number of studies in situ and in vitro on the erosive potential of commercially available soft drinks [e.g. West et al., 1998]. The method used in the present study employed a single source of saliva. This was

considered essential as the variable under study was saliva exposure time and therefore the saliva needed to be as standardised as possible. Pooled saliva could have improved the logistics of the study in respect of available volumes of saliva, but accurate and reproducible pooling from different individuals would be difficult to standardize with any degree of certainty.

Studies in situ have clearly shown that individuals vary in the amount of erosion experienced with the same acid exposure [e.g. Hall et al., 1999]. One factor to explain this may be individual variation in saliva and pellicle formation. Relevant to this may be the observation that a saliva chlorhexidine tea staining model in vitro revealed different degrees of staining dependent on the individual providing the saliva [Sheen et al., 2001]. It would be of interest now to determine whether erosion was influenced by different individuals' saliva in the model. Some evidence that this is a variable can be derived from published studies in vitro and in situ with ex vivo challenge [Hall et al., 1999]. Further in respect of the model, the acid challenge was in a stirred environment. The intake of soft drinks produces a flow of liquid over the tooth surface the rate of which influences the erosion process [Eisenburger and Addy, 2003] and therefore in a study of this type is a variable, which must also be standardized.

The data from the present study on enamel are consistent with a previous investigation [Hannig et al., 2003] in that 1-hour pretreatment of specimens with saliva afforded the maximum protection with no further reduction in erosion after longer pretreatments. The shorter pretreatment times, 2 and 30 min, did not provide protection against erosion. Certainly, pellicle does form on enamel after very short exposure times to saliva, but the layer is thin [Hannig, 1999]. Hannig [1999] also noted that pellicle thickness was a function of time with initial layers (2 h) electron dense but the later formed layers appeared more loosely arranged. Hannig [1999] suggested that such outer layers could be more susceptible to shear forces. Based on further work from this group [Hannig et al., 2003] these outer layers appeared to afford little additional protection against erosion over the first formed pellicle layer. The thickness of pellicle appeared to be not only a function of time but was influenced by the position in the mouth (tooth and tooth surface), which may be relevant to proximity of the salivary glands' duct openings or frictional effects of oral mucosae, particularly the tongue [Hannig, 1999; Amaechi et al., 1999; Hannig and Balz, 2001]. Indeed, it has been suggested that the predilection of palatal aspects of upper incisors for erosion was due to the thinning effect of the tongue on pellicle [Milosevic and Dawson, 1996; Amaechi et al., 1999]. Alternatively, the tongue might remove acid-softened enamel as demonstrated in a recent study [Gregg et al., 2004].

The present study supports the idea that an optimum pellicle thickness must be achieved to afford protection against an acid challenge such as the ingestion of a soft drink. The data, if consistent with what would occur in vivo, are therefore encouraging and suggest that, except at the extremes of acid intake, frequency and duration, the maximum pellicle protection is likely to be achieved or re-achieved between acid challenges that are an hour or more apart.

The same conclusion can be drawn for pellicle protection of dentine from erosion, an area, which has received little attention [Hall et al., 1999]. Some protection was achieved with only 2-min saliva pretreatment, but maximum protection appeared to occur with 1-hour pretreatment. At present the reason for the difference in the degree of protection by saliva between enamel and dentine can only be a matter of conjecture. One possibility is that the saliva penetrates the tubule system to produce not only a pellicle layer on the dentine surface and possibly within the dentine but a meniscus of viscous liquid at the tubule orifices. Although the differences within time periods were significant between saliva and no saliva pretreatment of dentine, the proportionate saliva protection increased from approximately 15% at 2 min and 1 h to 18 and 25% at 2 and 4 h, respectively.

Data from in vitro studies must be extrapolated with caution to effects in vivo. The present investigation, however, is in agreement with others in vitro and in situ that salivary pellicle or saliva pretreatment does protect dentine and proportionately more enamel from acid erosion. In conclusion, this study, which attempted to titrate exposure time with saliva to erosion protection for both human enamel and dentine, indicated optimum effects for enamel at 1 h and probably less than 1 h for dentine.

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