The Effect of Saliva Derived from Different Individuals on the Erosion of Enamel and Dentine

A Study in vitro

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Abstract
The aim of this study was to determine if saliva from 14 subjects afforded different levels of protection to human enamel and dentine against erosion in vitro. Test specimens were exposed for 2 h to saliva and control specimens to water for 2 h followed by citric acid for 10 min. This cycle was carried out 12 times. Tissue loss measured by contact profilometry was highly significantly different between subjects. Erosion was significantly reduced by pre-treatment with saliva from 7 subjects (enamel) or 6 subjects (dentine). Saliva from 1 subject resulted in significantly more enamel erosion than control.

Conclusion: Saliva from different donors affords different levels of protection against erosion.

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Introduction
Studies in situ have shown that participating subjects show considerable differences in the amount of enamel and dentine specimen erosion experienced despite consuming soft drinks under similar standardized conditions [for review see Hughes et al., 1999b]. The subject variation in some studies reached 10-fold differences between high and low eroders [Hughes et al., 1999b; Pontefract et al., 2001]. Additionally, erosion studies in vitro, which mimicked the protocols in situ, showed loss of enamel and dentine many orders of magnitude greater than recorded on specimens in situ [Hughes et al., 1999a, b]. Many factors, possibly acting together, may explain this variation in erosion of dental tissues between subjects and between study environments. One possible variable is saliva and the derived salivary pellicle [Hall et al., 1999; Sonju Clasen et al., 2000; Hara et al., 2006]. It is possible that salivary pellicles may differ between individuals and sites in the mouth and such differences may be physical and/or chemical [Amaechi et al., 1999; Lamkin et al., 2001]. Also, the majority of studies in vitro on erosion did not use saliva in the model.

There are a number of studies supporting the widely accepted contention that salivary pellicle offers some protection against erosion [Moreno and Zahradnik, 1979; Meurman and Frank, 1991; Hannig, 1998; Amaechi et al., 1999; Hall et al., 1999; Hannig and Balz, 1999, 2001; for reviews see Sonju Clasen et al., 2000; Young et al., 2000; Hara et al., 2006]. The protocols in a number of these studies allowed pellicle formation over many hours, even many days, which can be criticized on the grounds of...
clinical relevance to regular intakes of dietary acid. One study, however, reported no difference in protection for pellicles formed over 2 h compared to 24 h [Hannig et al., 2003]. A more recent study in vitro using saliva from a single donor tended to confirm the 2-hour finding although some significant protection was afforded to enamel at 1 h and to dentine at 2 min [Wetton et al., 2006]. The aim of the present study in vitro was to determine whether saliva from different individuals afforded different levels of erosion protection to enamel and dentine specimens.

Materials and Methods

Preparation of Enamel and Dentine Samples
Caries-free human third molar teeth extracted from patients aged 18–35 years were used to prepare enamel and dentine specimens. Teeth were soaked in 20,000-ppm sodium dichloroisocyanurate solution for a minimum of 24 h, then cleansed of any residual soft tissue remnants using a scalpel. The crown was sectioned from the root. Each crown, for enamel specimens, and root, for dentine specimens, was cut into 4–6 portions. Each portion had an outer face of enamel or dentine. The portions were then placed in a mould and embedded in epoxy resin. After 24 h hardening time the specimens were removed and polished on an automatic lapping and polishing machine up to 1,200 grit to expose a flat window of enamel or dentine with surface profiles within ±0.3 μm using a contacting profilometer (Surflometer Planar Products Ltd., Sunbury on Thames, UK). The dimensions of the blocks (8 × 6 × 2 mm) conformed to those of a stainless steel dye used to hold specimens for profilometry. After base-lining, a zone of enamel or dentine approximately 2 mm wide was delineated by placing two parallel strips of adhesive PVC insulating tape. A total of 84 enamel and 84 dentine specimens were prepared and 6 of each randomly allocated to each subject.

A group of 14 dentate subjects with no relevant medical or pharmacotherapy histories (9 female, 5 males, age range 23–60 years) participated in the study. At least 20 ml unstimulated saliva was collected from individual subjects by dribbling into 1.5-cm plastic caps, one 2 mm wide was delineated by placing two parallel strips of adhesive PVC insulating tape. A total of 84 enamel and 84 dentine specimens were prepared and 6 of each randomly allocated to each subject.

Immediately after collection two 5-ml aliquots of a subject’s saliva were added to two 3 × 1.5-cm plastic caps, one containing the enamel and the other the dentine specimens allocated to that subject, for 2 h at room temperature. Caps were gently shaken by hand every 30 min. Specimens were then removed from the saliva, washed under running tap water and then placed in a 500-ml glass beaker containing 200 ml 0.3% citric acid, pH 3.2 at 35°C with overhead paddle stirring at 270 rpm. After 10 min specimens were removed and rinsed under tap water. The remaining saliva for the subject was stored at 5°C for approximately 3 h and used in an identical afternoon cycle after equilibrating to room temperature. The cycles of saliva collection, morning and afternoon treatments were repeated 6 times to give a total of 12 cycles. When not in a cycle enamel and dentine specimens were stored on damp tissue paper in a small sealed jar. Fresh saliva was collected for each of the 6 treatment days.

Immediately after every third cycle, the tape was removed and tissue loss was measured by profilometry. Each profile was taken from just within the taped zone on one side across the exposed window to just within the opposite taped zone. Two mean depth values, each calculated from 100 measurements, were taken from each specimen and averaged. Tape was re-applied after each profilometry reading. Control specimens were exposed to tap water instead of saliva with profilometry measurements taken after cycle 12 only. For logistical reasons, affecting both the subjects and the cycling method/number of specimens, it was only possible to enter 1 or 2 subjects at a time into the 6-day regimen, which was continuous except for weekends. To control for this, the water controls were run at two different time points during the whole experiment.

Statistical Methods
The erosive effect of each donor’s saliva was characterized by the median losses of dentine and enamel at the 12th cycle based on 6 specimens. For the control series median enamel and dentine losses over the 12 cycles at cycle 12 were calculated. The distribution of the data was non-Gaussian. Non-parametric analysis of variance (Kruskal-Wallis) was performed to test differences between the 14 subjects in the potential of their saliva to affect erosion. Pairwise non-parametric tests (Mann-Whitney) assessed differences between each subject’s saliva in turn and water. A scatter plot was constructed to relate median enamel and dentine losses for the 14 subjects, and the Spearman correlation calculated. Finally, the progression of median dentine and enamel loss with each subject’s saliva through 3, 6 and 9–12 cycles for each subject was plotted and visually appraised for pattern.

Results
For 1 subject only the median value was based on 5 specimens each of dentine and enamel, 1 enamel and dentine specimen being lost to study. For most subjects the progress of erosion over time appeared approximately linear for enamel. For dentine the pattern for most subjects was one of reduced erosion at successive 3 cycles. For erosion depth at cycle 12, differences between the 14 subjects were highly significant both for enamel (Kruskal-Wallis test: χ² = 50.2; p < 0.001) and dentine (χ² = 71.3; p < 0.001). Using the median values for erosion with water of 23.5 μm for enamel and 19.5 μm for dentine, all subjects had values similar to or less than water for both substrates (3–55% less for enamel and 6–36% less for dentine), except for 1 subject who had a median loss of 31.5 μm for enamel (significant 34% increase; p = 0.0002) and 20.0 μm for dentine (non-significant 2% increase; p = 0.55). Pairwise comparisons revealed that saliva from 7...
subjects was associated with significantly reduced erosion of enamel compared to water, with p values ranging from 0.002 to 0.0001. Likewise saliva from 6 subjects was associated with significantly reduced erosion of dentine compared to water, with p values ranging from 0.024 to 0.001.

The scatter plot of enamel loss versus dentine loss for the 14 subjects’ saliva is shown in figure 1. The correlation coefficient was quite high at 0.62 (p < 0.02), indicating that in general, saliva associated with relatively high degrees of erosion of enamel was also associated with relatively high degrees of erosion of dentine. This was also borne out by the fact that 5 subjects’ saliva was associated with significantly reduced erosion of both enamel and dentine compared to water, whilst 1 subject’s saliva tended to enhance erosion of both materials.

Discussion

In common with previous studies in vitro in this area, we did not add protease inhibitors to the saliva. It is clear that pellicle contains proteolytic fragments as well as intact proteins [Lendenmann et al., 2000]. Excessive proteolysis during storage of the saliva used in the second exposure on each day was minimized by storage in the cold.

It is possible that our measurements of erosion depth for dentine would be subject to error, if the profilometer stylus did not fully penetrate the demineralized layer which forms at the surface. While the extent of this error requires further investigation, previous work [Vanuspong et al., 2002] showed that the depth of dentine erosion measured by this method increased according to severity of the erosive challenge, and recent work in our laboratory [Shellis, unpublished] has shown that profilometric depth of dentine erosion is highly correlated with dissolution rate.

The majority of previously cited studies concerned with enamel erosion, often using bovine enamel, have reported protection from salivary pellicle formed in vitro or in situ. Less data are available on salivary pellicle protection against dentine erosion although these all report a protective effect [Hall et al., 1999; Wetton et al., 2006]. It is, however, difficult to make comparisons with other studies where the subject was not the unit of the research and very small numbers of subjects provided data. This study employed a 2-hour salivary contact time with enamel and dentine based on the work of Hannig et al. [2003] and Wetton et al. [2006]. Furthermore, 2 h would seem clinically relevant to individuals who consume four or more soft drinks in a day: a not unusual practice by a large proportion of the UK population, particularly the young [British Soft Drinks Association, 1991]. The protocol thus attempted to simulate the action of the first drink on a 2-hour established pellicle, followed by the re-establishment of a similar pellicle over the next 2 h before the second challenge, and so on. The data did indeed reveal a highly significant difference in the protection afforded to enamel and dentine by pellicle derived from different individuals’ saliva, and also when compared to water controls.

For enamel all but 1 subjects’ saliva offered some protection, although this was not statistically significant in all cases. Interestingly, saliva from 1 exceptional subject increased the erosion of enamel considerably. An explanation for this is not clearly apparent because even if the saliva from this subject did not form any substantial pellicle, similar erosion to the control would have been expected.

For dentine, again, the differences between subjects were highly significant. In mean terms also, all subjects had less erosion than control, except for the individual whose saliva afforded increased erosion to enamel (and a non-significant increase in erosion of dentine). Although

![Fig. 1. Scatter plot of median enamel and dentine depths of erosion for each subject (○) donating saliva and water control (●).](image-url)
only 6 subjects had significantly less erosion than control, the correlation coefficient for erosion of dentine and enamel for the same subjects supported the idea that the protection afforded enamel by any one source of saliva will be similar for dentine.

In conclusion, saliva from different sources offers differing protection against acid erosion of enamel and dentine using a model in vitro. It would be of interest to repeat the study using pellicles formed in situ. Studies of composition of pellicles associated with different effects on erosion would also be extremely interesting.

References