



ORIGINAL ARTICLE

The interplay of saliva, erosion and attrition on enamel and dentine

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Abstract Purpose: This investigation aimed to compare the protective role of saliva against erosion and attrition challenges.

Method: Polished enamel and dentine samples ($n = 160$) were prepared and randomly assigned to either the saliva or saliva-free group ($n = 40$ enamel and $n = 40$ dentine/group). Within each subgroup, they were allocated to four subgroups: negative control (deionized water exposure 10 min), erosion (0.3% citric acid 10 min), attrition (120 S of 300 g force), or combined erosion/attrition (0.3% citric acid 10 min then 120 S of 300 g force). Experimental cycles were repeated three times. Data analysis was performed using SPSS.

Results: The mean and standard deviation (SD) of step heights produced by the attrition and erosion/attrition groups in enamel in the saliva-free group were 5.6 μm (2.4) and 13.4 μm (2.8), respectively, while they were 2.4 μm (3.8) and 12.9 μm (3.5) in the saliva group, with no significant difference between the saliva and saliva-free groups. For dentine, the corresponding step heights were 25.2 μm (5.5) and 35.9 μm (7.9) for the saliva-free group, but 21.8 μm (5.3) and 27.3 μm (6.4) for the saliva group ($p < 0.001$).

Conclusion: There was a trend that saliva decreased wear, but this was only statistically significant for erosion/attrition dentine wear.

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1. Introduction

Saliva has a multifactorial protective role against erosive tooth wear within the mouth. Salivary actions include oral clearance, dilution, and buffering after the acid enters the oral cavity (Buzalaf et al., 2012). Surprisingly, contradictory clinical studies suggest reduced saliva flow and decreased buffering capacity linked with increased erosive tooth wear (Dynesen et al.,

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2008, Moazzez et al., 2004); however, others studies did not (Moazzez et al., 2000, Lussi et al., 2012).

In contrast, other researchers have reported on the protective role of the salivary pellicle. The pellicle initially works as a diffusion barrier, preventing acid from coming into direct contact with hydroxyapatite crystals (Carpenter et al., 2014), providing protection even during the early maturation phases (Hannig et al., 2004, Hannig et al., 2009). It achieves an initial thickness after 2–3 min and maintains that level for approximately 30 min. The thickness then triples and stabilizes at this size (Skjrand et al., 1995). This thickness has been observed to range between 0.3 μm and 1.06 μm and may be an essential indication of a site's vulnerability to dental erosion (Amaechi et al., 1999).

In vitro studies have observed that the salivary pellicle has a protective effect on erosion (Mutahar et al., 2017a,b), but saliva's role in attritional wear is underresearched. Several authors have found that saliva acts as a lubricating agent when tooth-on-tooth contact occurs, and reduced wear has been observed when artificial saliva is used (Vieira et al., 2006, Austin et al., 2010). However, natural saliva has yet to be investigated in erosive-attritive wear of both enamel and dentine. Dentine has a lower mineral concentration and modulus of elasticity than enamel, making it less vulnerable to erosion and more responsive to mechanical forces.

1.1. Aim

This study aimed to investigate the protection conferred by the salivary pellicle on enamel and dentine, to erosive and attritive wear processes.

1.2. Hypothesis

We hypothesize that salivary pellicle plays a protective role in enamel and dentine erosion and attrition.

2. Materials and methods

2.1. Teeth selection, storage, and preparation

Human molar enamel ($n = 80$) and dentine ($n = 80$) samples were prepared from the buccal surfaces of extracted intact caries-free 3rd molar teeth (REC ref 12/LO/1836). Teeth were stored in sodium hypochlorite (20,000 ppm) for 72 h before being sectioned and mounted in self-cured bisacryl resin (Protemp™4, 3 M ESPE, Seefeld, Germany). Samples were polished and ultrasonicated (GP-70, Nusonics, Lakewood, USA) in 100 ml deionized water (pH 5.8) for 15 min to remove the smear layer. Each sample was wiped with alcohol to remove organic debris remnants and air-dried for 24 h at room temperature. Ten percent of all samples were randomly selected and scanned using a 2 μm laser spot sized red light confocal scanning profilometer (Taicaan, XYRIS 2000, UK) with 0.01 μm height in a low precision mode in a raster scanning pattern at 10 μm steps to ensure flatness tolerance $\pm 0.2 \mu\text{m}$. PVC adhesive tape was used to divide these samples into 1:3 ratios, leaving a 1 mm \times 3 mm window of exposed enamel and two protected zones of reference.

2.2. Sample allocation and grouping

Each enamel or dentine sample was randomly allocated to a wear challenge with a salivary pellicle and salivary remineralization period (Group A, 40 enamel samples and 40 dentine samples) or a deionized water control (Group B, 40 enamel samples, and 40 dentine samples). Within each group, 10 enamel and 10 dentine samples were further allocated to four wear challenges. Subgroup 1 was exposed only to deionized water, subgroup 2 was exposed to erosion challenges, subgroup 3 was exposed to attrition challenges, and subgroup 4 was exposed to both erosion and attrition challenges. Sample allocation can be seen in Fig. 1.

For antagonist preparation ($n = 40$), intact premolars extracted for orthodontic reasons (REC ref 12/LO/1836, $n = 20$) were sectioned into two, to produce 40 premolar cusp tips. These were embedded in bisacryl composite, and each one was bonded to the end of a toothbrush (Sensodyne Search 3.5, Brentford, Middlesex, England) using cyanoacrylate adhesive ($n = 40$), which could be used in a toothbrushing machine. The lubricant used during attrition cycles was artificial saliva at pH 7 prepared according to previously published protocols (Eisenburger and Addy, 2002a, O'Toole 2015).

2.3. Citric acid preparation

Citric acid solution (0.3%) was prepared using 3.0 g anhydrous citric acid powder (Sigma Aldrich, Poole, Dorset UK) measured on a digital scale. The powder was added to 1 L of deionized water. The pH of the solution was adjusted to 3.2 using 0.1 M sodium hydroxide buffer (4 g 99% NaOH in deionized water). The titratable acidity of the solution was calculated as 18.0 ml, indicative of the volume of sodium hydroxide required to neutralize the pH to 7.

2.4. Saliva collection

Stimulated whole mouth human saliva was collected from healthy volunteers (Northampton REC, REC ref: 14/EM/0183) who had not eaten or drunk anything for 1 h prior to donation. Volunteers were asked to chew flavorless paraffin wax for 10 min, and saliva was expectorated into a preweighed 20 ml polypropylene tube. Saliva was frozen at $-80 \text{ }^\circ\text{C}$ within 15 min of collection. Before the experiment, saliva was thawed overnight on ice at a temperature of $4 \text{ }^\circ\text{C}$. The saliva was pooled and shaken in a vortex shaker to resuspend particles that had separated during the process and then stored in 8 ml aliquots following previous protocols (Mutahar et al., 2017a,b) while nonsaliva group samples were stored in 8 ml of deionized water for 24 h. All samples were removed from their solutions and bottle rinsed with deionized water before being subjected to one of four treatments.

2.5. Wear challenge

Both enamel and dentine samples were immersed in either 100 ml deionized water or 100 ml of 0.3% citric acid solution for the control and erosion groups for 10 min using Orbital Shaker S05 (Stuart Scientific, UK) with constant gentle agitation at 62.5 rpm. For the attrition regime, samples were

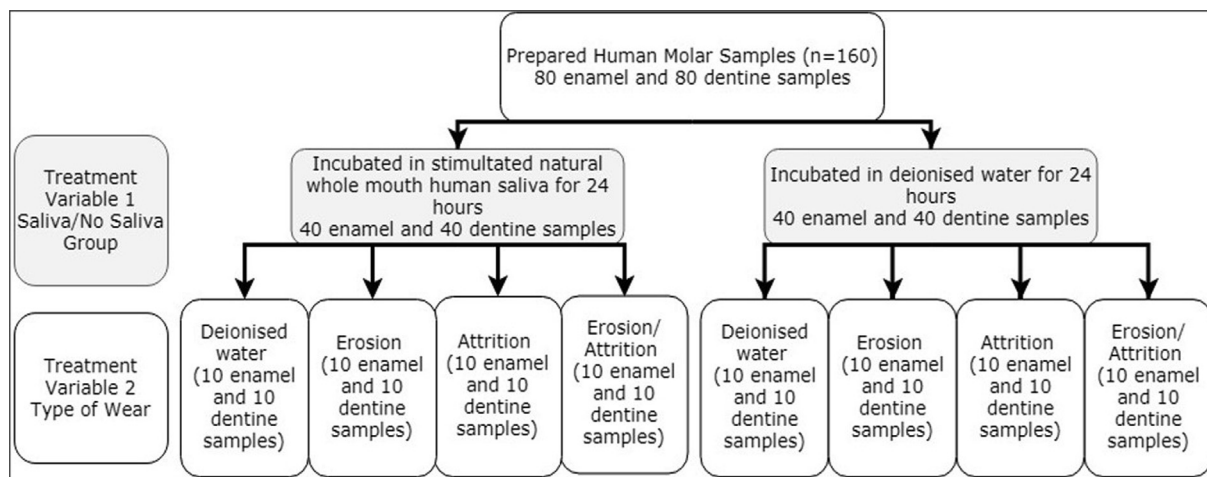


Fig. 1 Sample allocations.

mounted in the tooth brushing machine using artificial saliva as the lubricant, and 120 linear strokes of 300 g of enamel attrition were delivered to each sample over 2 min. For the attrition/erosion groups, samples initially underwent an erosive challenge in 100 ml of 0.3% citric acid solution for 10 min using Orbital Shaker S05 (Stuart Scientific, UK) with constant gentle agitation of 62.5 rpm and then immediately followed by attrition challenges.

Following each challenge, the samples were washed with deionized water for 30 s. Pellicle-covered samples (Group A) were immersed in 8 ml of saliva per sample, whereas pellicle-free samples (Group B) were immersed in 8 ml of deionized water, each for 60 min. Following the 60-minute immersion, the samples were washed for 30 s with deionized water, thereby completing one experimental cycle. A total of three cycles were completed and then the materials were allowed to dry overnight in a perforated foam dish before scanning.

2.6. Scanning

All samples were scanned with a red light confocal laser profilometer with a spot size of 2 μm (Taicaan, XYRIS 2000, UK). In addition, a 5 \times 3 mm region of the sample was scanned in medium precision mode using a raster scanning pattern and a stepover of 10 μm , integrating the wear scar and reference areas on each side. The mean step height for each sample was computed by averaging 10 step height readings per sample in surface metrology software (Boddies®, Taicaan Technologies, Southampton, UK).

Enamel surface texture changes were qualitatively assessed using scanning confocal microscopy in white light reflection mode (Noran Instruments, Middleton, WI, USA) in conjunction with a x40 objective lens (M-Plan SLWD Brightfield \times 20/0.35NA) and an automatic Z-stage piezoelectric controller (E-662. SR LVPZT Piezo Amplifier/Position Servo Controller, Physik Instrumente, Germany). For each sample, an 85 μm Z stack at 0.5- μm intervals was acquired using proprietary image acquisition software (Micromanager v1.4.22, Open Imaging; Inc. San Francisco, CA, USA). The resulting stack of 533 μm \times 533 μm 2D images was automatically processed by ImageJ to produce a pseudotopography image for qualitative analysis.

2.7. Data analysis

Data were normally distributed, and a two-way ANOVA was used for each of the two factors (saliva and wear challenge). Fisher's protected least significant difference test was used to control the overall significance level of the tests. Based on previous investigations and estimated differences of 1 μm between groups, a sample size of 10 per group would be needed to detect a 2- μm difference per wear challenge/saliva combination using a two-sided test at 80% power, at a 5% significance level.

3. Results

The mean step heights (SD) produced by the controls, erosion, attrition, and erosion/attrition groups in enamel in the pellicle-free group were 0.1 μm (0.0), 10.0 μm (1.0), 5.6 μm (2.4), and 13.4 μm (2.8), respectively, while the mean step heights produced in the saliva were 0.1 μm (0.0), 9.0 μm (1.1), 2.4 μm (3.8), and 12.9 μm (3.5) respectively. There were no differences between samples exposed to saliva and those exposed to deionized water ($p = 0.192$). However, all differences between the different wear processes were statistically significant ($p < 0.001$). These results are shown in Fig. 2.

The mean step heights (SD) produced by the control, erosion, attrition group and the erosion/attrition group in dentine in the absence of saliva were 0.2 μm (0.1), 10.2 μm (2.8), 25.2 μm (5.5), and 35.9 μm (7.9), respectively, while the mean step heights produced in the saliva group were 0.1 μm (0.3), 9.2 μm (2.2), 21.8 μm (5.3), and 27.3 μm (6.4) respectively. Although there was a trend that saliva resulted in decreased wear, this difference was only statistically significant for erosion attrition wear subgroup ($p < 0.001$). All differences between the wear processes within each group were statistically significant ($p < 0.001$). The results are presented in Fig. 3.

4. Discussion

This is the first study investigating the impact of natural saliva on erosive-attritive wear on enamel and dentine. Natural saliva exhibited a significantly reduced step height on dentine during the erosion/attrition wear process, but a similar effect was



Fig. 2 Enamel Profilometric Step Height Loss (in microns).

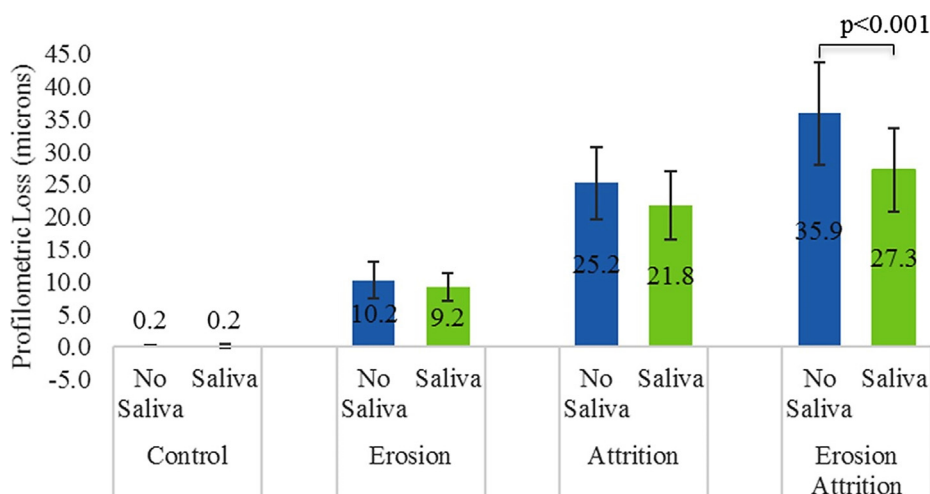


Fig. 3 Dentine Profilometric Step Height Loss (in microns).

observed for any other subgroup on enamel or dentine. The individual mean step heights for enamel and dentine showed a combined total for erosion/attrition, suggesting cumulative erosion/attrition. Why natural saliva influenced erosion/attrition unlike other regimes is not easy to understand. The confidence in the data from the profilometer was high, as step heights were above 9 μm and the standard deviations were reasonably tight for biological samples. Although, it is difficult to understand whether saliva action is a result of remineralization or lubrication.

Previous studies have shown that the salivary pellicle offers protection against erosion for both enamel and dentine (Mutahar et al., 2017a,b, Wetton et al., 2006). However, Hannig et al. concluded that the acquired pellicle’s protective qualities against an erosive challenge of the dentinal surface are limited. This is due to the pellicle’s ability to operate as an ion-permeable network rather than a barrier. Saliva’s proteolytic enzymes and matrix metalloproteinases may potentially contribute to the wear process.

In this study, although there was a trend that the pellicle reduced step height, the differences were not significant. This may indicate that the pellicle offers less protection than

previously anticipated under these experimental conditions. Due to its higher organic matrix content, dentine is more susceptible to both attrition (Austin et al., 2010) and abrasion (Lippert et al., 2017), and it was this group where most of the wear was created. In vitro, the pellicle has previously been shown to produce a protective effect on dentine within 2 min of exposure compared to 60 min with enamel (Wetton et al., 2006). This could be why it was able to offer the most protection under the most aggressive wear conditions. The lack of a significant protective effect against only attrition is interesting. Kaidonis et al. observed that under low loading forces, attritional wear on enamel using any form of lubricant was decreased. As the loads were maintained at a low level in this study, it would suggest that any type of lubricant would protect against attritive wear in enamel. A reason for this effect may be that we used natural enamel cusps from unworn premolar teeth (extracted for orthodontic reasons). This factor may have been the reason that an increased wear challenge was observed compared to other groups who polished the cusps of their teeth (Wiegand et al., 2017, Eisenburger and Addy, 2002a,b).

Another interesting observation was that the two wear processes were cumulative rather than synergistic when erosion

was followed by attrition. This may be due to the low loading involved in this study designed to simulate light contact of teeth during mastication instead of heavy bruxism. Higher loads generate significantly greater wear in a nonlinear fashion (Gibbs et al., 1981). In addition, authors have observed a threshold effect whereby increased loading will dramatically increase the rate of tooth wear (Kaidonis et al., 1998, Eisenburger and Addy, 2002a,b).

4.1. Limitations

This study has limitations since it is an *in vitro* evaluation that will never duplicate the oral settings. Moreover, polished dental samples were used to obtain precise profilometric wear measures. However, it has been shown that polished enamel and dentine surfaces wear at a greater rate than natural dental surfaces (Ganss et al., 2000), and pooled human saliva was used, which is subject to variation. The collection, freezing, thawing, and pellicle creation process may lead to proteolysis, resulting in decreased protection. However, the protocol used in this study has been previously validated, albeit with more severe erosion challenges (Mutahar et al., 2017a,b). To minimize the effects of excessive proteolysis during saliva storage at -80°C immediately upon collection and thawing, samples over ice were collected, which could have affected salivary properties.

5. Conclusions

The study findings may exaggerate the protective effect of saliva against wear processes. The protective effect may be more relevant for dentine surfaces. Although the combination of erosion and attrition caused the most wear, erosion for enamel surfaces and attrition for dentine surfaces caused much of the wear. Interestingly, combining erosion and attrition resulted in wear similar to the individual wear processes, suggesting that wear at this load is cumulative rather than synergistic for attrition. More studies are needed to determine whether these effects are evident at higher loading forces.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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