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Tribocorrosion of dental tissues: the role of mucin

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1 Abstract

Artificial saliva can benefit tooth degradation management; this study assesses the role of mucins in this process using a combination of techniques to assess wear and surface degradation. Different neutral and acidic artificial saliva solutions were investigated, focusing on the protective capability of mucins. Static immersion and reciprocating ball-on-flat tribometer tests were performed on bovine enamel samples. The calcium released into the test solutions was assessed using Atomic Absorption Spectrophotometry (AAS) and enamel surfaces were examined using Vertical Scanning Interferometry (VSI). The addition of mucins reduced the coefficient of friction and wear scar volumes for neutral pH solutions. In acidic conditions the addition of mucin did not affect the lubrication, however it significantly reduced the calcium release under tribological conditions.

2 Introduction

Dental erosion, or what engineers would refer to as corrosion, and tooth loss due to wear are areas of concern in modern dentistry [1]. Moderate tooth wear has been observed for people between the ages 16-34 years in the Adult Dental Health Survey of 2009, which results from a combination of tooth dissolution (corrosion) from dietary and other acids, abrasion from food particles/toothpaste and direct tooth grinding/sliding [2]. This is significantly more than what has been observed in previous surveys and may be linked to the increased consumption of acidic foods/beverages [1]. Dental erosion can be defined as the chemical dissolution of hard dental tissues excluding the impact of acids produced by bacteria [3]. Corrosion in this case considers all chemical degradation and material loss of dental hard tissues, with dissolution, demineralisation and dental erosion falling within this umbrella term. Therefore the direct impact grinding/sliding of teeth within a corrosive environment can be termed as tooth tribocorrosion.

Corrosion of tooth enamel can occur in two stages. The initial stage of corrosion causes enamel softening by demineralisation, which can be reversed [4]. Hydrogen ions within acidic solutions directly attack phosphate or carbonate groups of the hydroxyapatite prisms which make up the structure of tooth enamel, as shown in equation (1) [22]. This releases calcium and phosphate ions into the oral environment, and if acids are removed by saliva, these components can lead to remineralisation of the teeth. Enamel softening also occurs within the subsurface depending on the severity and frequency of acid exposure [24]. Should the enamel remain in contact with an acid, this

can lead to a layer-by-layer loss of surface enamel, which cannot be reversed [4].

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6\left(HPO_4{}^{2-}\right) + 2H_2O \quad (1)$$

Saliva naturally protects teeth from material loss by enhancing lubrication and wear resistance of enamel as it contains numerous electrolytes and proteins. These components allow for remineralisation of softened enamel, neutralisation of acidic substances and the adsorption of a protein barrier film on the enamel surface, known as the pellicle [5]. The pellicle is a protective film that forms on the surfaces of teeth due to protein-surface electrostatic interactions which serves as a chemical diffusion barrier and a boundary-lubricating film [5-6]. This can reduce the diffusion of calcium and phosphate ions into the surrounding environment as well as delaying the diffusion of an acid into the enamel [7]. Pathological conditions, such as Sjögren's syndrome or diabetes, certain medications and radiotherapy of the head and neck may lead to symptoms of Xerostomia (dry mouth syndrome); which can negatively affect the production and flow of saliva [8]. This ultimately reduces protection against corrosion, which might heighten tooth material loss when considering the addition of the mechanical impact and sliding of mastication. One solution to this would be to use an artificial saliva substitute to retain some of the lost protective characteristics of saliva. Artificial saliva compositions have been shown to influence the remineralisation process and aid the re-hardening of enamel after corrosive challenge [9-10]. The presence of mucins, a protein found within saliva, can have a positive influence with respect to this [9-10]. Artificial saliva's influence on demineralisation and corrosion resistance have also been shown to be comparable to natural saliva in in-vitro studies [10-12].

It is unclear as to what components of artificial saliva's are necessary for the protection of enamel against tribocorrosion. The majority of studies in the area utilize static corrosion models, where enamel samples are immersed in either an acid or artificial saliva, followed by subsequent surface hardness measurements and/or calcium ion release into solution [10-11, 13]. While this does provide information on the effects of remineralisation and demineralisation of artificial saliva and acid solutions respectively, it focuses on the *corrosion* aspect of overall tooth material loss. However, in reality a combination of mastication in a corrosive environment may enhance tooth material loss from these interactions and potentially their synergetic effects between mechanical and chemical wear. An artificial saliva would need to be able to protect enamel much like a natural saliva does by creating a protein layer, the pellicle, on the surfaces of teeth. The pellicle serves to protect enamel from both mechanical and chemical forms of wear, so being able to artificially build a layer with similar properties would be a step forward to improving current therapies. It's therefore important to consider the biological implications of tooth loss by studying how it can reduce the impact of tribocorrosion.

Being able to maximize protection against tooth tribocorrosion is an area of growing interest for effective enamel protection [24]. Knowledge of this is essential for improved artificial saliva substitutes for the future of tribocorrosion testing and the development of enhanced commercial preventative therapies [1, 5, 24]. In order to engineer saliva substitutes the underlying mechanisms of degradation need to be understood. This study aims to examine the protective capabilities of elementary artificial saliva compositions on the surfaces of tooth enamel to assess their protective roles under tribocorrosive conditions.

3 Materials and methods

3.1 Bovine enamel samples

Bovine lower mandibles were locally sourced from a nearby abattoir and were collected on the day of cattle processing. Teeth were extracted from the mandible on the same day and sterilized in a 10% formalin solution for 7 days at room temperature. Teeth were then stored in deionised water at 4° C until required as this has been shown to have the least effect on reducing the surface microhardness of enamel [13-14].

A diamond bur rotary cutter was used to section teeth into approximately 3 mm x 3 mm slabs for testing. All sectioning was carried out under a stream of deionised water. Slabs were then embedded in resin and samples were carefully ground (with silicon carbide paper of grit sizes 600 and 1200) to expose a clean and level enamel surface. This surface was then polished (with a 3 µm and ¼ µm diamond suspension) to attain an approximate surface R_a of less than 0.2 µm. After polishing, samples were thoroughly rinsed with deionised water and the surface was wiped with 70% isopropanol before testing [13, 25].

3.2 Artificial saliva solutions

Tribocorrosion tests were designed to determine the dissolution of teeth with and without mechanical interactions. Static corrosion tests were conducted to determine pure tooth dissolution without the involvement of mechanical interactions. Basic compositions of an artificial saliva environment were chosen to assess their influence on tooth tribocorrosion. Compositions would therefore examine different pH and ionic solutions, with and without the addition of Porcine Gastric Mucin (PGM, Sigma Aldrich), values as highlighted in Table 1.

The purpose was to determine the role of mucin in reducing tooth material loss and dissolution by itself, in a neutral ionic environment and in an acidic one. As there are numerous formulations of artificial saliva used in the literature, for this study, a PBS solution was chosen as a simple salt-only artificial saliva [11]. Citric acid at a pH of 3.1 would be used to simulate an acidic environment that might be experienced after consuming an acidic beverage or substance *in-vivo* [13, 15].

Table 1 *Test solutions used in this study to simulate artificial saliva.*

Solutions	Artificial saliva type	pH	Composition per 100 mL
Deionised water	Control	6.5	100 mL deionised water
0.2% Mucin	Protein only	6.5	100 mL deionised water 0.2 g Porcine Gastric Mucin, (PGM)
PBS	Salt only	7.4	100 mL Phosphate Buffered Saline solution
PBS & 0.2% mucin	Protein and salt	7.4	100 mL Phosphate Buffered Saline solution 0.2 g PGM
Citric acid	Acid control	3.1	100 mL 1.75 mM citric acid solution
Citric acid & 0.2% mucin	Acid and protein	3.1	100 mL 1.75 mM citric acid solution 0.2 g PGM

3.3 Static corrosion tests

An assessment of the pure corrosion was conducted on bovine enamel samples. Samples (n = 3) were tested for each artificial saliva solution listed in Table 1. Samples were immersed in 300 µL of the artificial saliva solution over a test duration of 35 minutes. The time was chosen to equal the test duration of tribological testing, which is covered in the next sub-section. For mucin tests in a neutral pH environment, samples were pre-treated in the chosen mucin solution for 30 minutes to permit any mucin surface adsorption. For the mucin tests in the acidic environment, samples were immersed in the DIW + mucin solution for 30 minutes prior to acid + mucin solution tests. This would prevent excessive surface morphology changes prior to the tribological tests and therefore the immersion protocol was replicated in the static tests. Pre-treatment solutions were disposed of and test solutions were refrigerated at 4 ºC prior to calcium release analysis.

3.4 Tribocorrosion tests

A nanotribometer (Anton Paar, NTR^3) was used to provide the mechanical interactions by simulating the sliding of teeth after impact using a reciprocating ball on flat configuration, displayed on the schematic in Figure 1. A Ø3 mm Yttria stabilized Tetrahedral Zirconia Polycrystalline (Y-TZP) ball (Goodfellow Cambridge Ltd, UK) was chosen as the antagonist material to imitate an artificial tooth's cusp. ISO TS 14569-2:2001 [16] suggests the use of an alumina antagonist for testing, however Y-TZP is more appropriate as this material is used for artificial crowns [34]. Similar to static tests, samples were immersed in 300 µL of the artificial saliva solution for the test duration, and were then refrigerated at 4 ºC prior to calcium release analysis.

Test conditions were chosen as a selection of the conditions used in in-vitro testing and in the ISO TS 14569-2:2001 for the testing of dental materials [16], which are displayed in Table 2. Mastication pressures of molars have been observed between 4 – 40 MPa for foods of different elastic properties [32-33] and some methods in ISO TS 14569-2:2001 [16] take this into account. However in the literature contact pressures within 200 – 400 MPa are observed for enamel wear testing [16-17, 31]. A normal load of 10 mN was therefore chosen to provide a contact pressure up to 140 MPa depending on the mechanical properties of the enamel surface. This was designed to provide more realistic

contact pressures for mastication, below the ranges used in the literature for in-vitro wear testing.

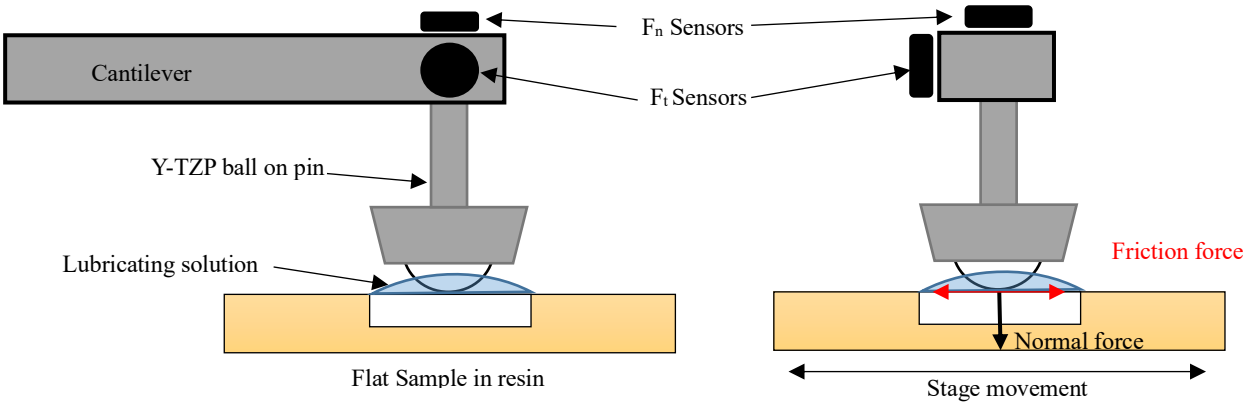

Figure 1 Schematic of nanotribometer configuration

The nanotribometer measured the raw coefficient of friction of the reciprocating motion. Forward and reverse traces were acquired and converted into a friction loop which represented a single cycle. The friction loop was normalised and the central region of the loop was used to calculate a mean coefficient of friction for that cycle. This ensured only the dynamic friction was used for comparison, negating the acceleration/deceleration of the pin during the reciprocating motion. This process was done for all test cycles. Figure 2 outlines this determination.

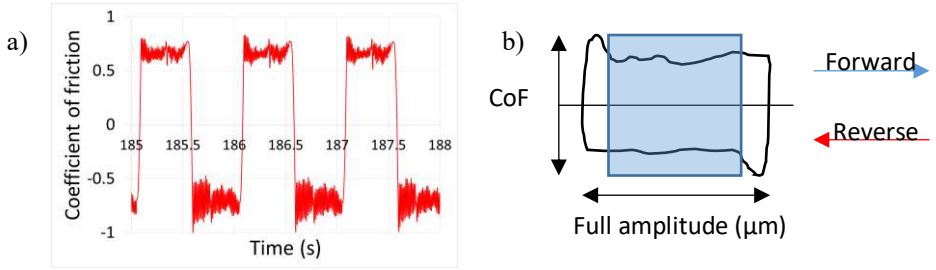

Figure 2 a) Example of raw friction measurement and b) the selected area within the friction loop for mean friction calculation.

Table 2 *Tribometer test parameters [16-18].*

Loading type	**Constant**
Load (mN)	10
Temperature (°C)	20 - 25
Sliding amplitude (µm)	1000
Test duration	2000 cycles
Sliding frequency (Hz)	1

3.5 Calcium ion release analysis

Total calcium ion release was analysed as a principal measure for total degradation of the enamel surface. Post-test solutions were assessed with Atomic Absorption Spectrophotometry, (AAS, Agilent, 200 series AA). This allowed quantification of the total calcium dissolution into the immersion solutions from testing. The volume of the retrieved test solutions were initially measured and then diluted with 5 ml of MilliQ water (>18MΩ Purity) for analysis. Prior to sample testing, the absorbance was calibrated using a bulk standard calcium solution of 800 ppm and the use of SIPS, (sample introduction pump system). SIPS diluted the standard such that a calibration curve with 7 points was produced. Test sample solutions were then measured after this curve was established. Baseline measurements of all artificial saliva solutions were also completed and this value was subtracted from the test sample solutions. After determining the calcium concentration of the diluted solution with AAS, the initial concentration was then determined from the AAS concentration, the diluted volume and the initial volume prior to dilution.

3.6 Surface wear scar and profile analysis

The samples from the tribocorrosion tests were removed, rinsed thoroughly with deionised water and wiped with 70% isopropanol. Vertical scanning interferometry, (VSI, Bruker, NPFlex) was used to take post-test images of the wear scar profile and overall volume lost. Scar depth and widths would be taken from a section profile at the centre of these scars and the overall volume loss would be calculated using the post processing software, Vision 64 (Bruker).

Raw data was processed by initially flattening the surface while masking the wear scar area. Scar profiles were then taken and the depth, width and length of the scar were measured. The scar depth was determined as the distance between the flattened surface and the lowest point of the wear scar at the central scar width cross section. Scar width was measured from this cross section too as the distance between the opposing scar edges at the flat reference surface. The length was also measured in a similar way, but from a scar length cross section.

To determine the volume of the scars, the VSI images were further processed. Images adjusted such that the flat reference surface possessed a depth value of $Z = 0$ μm. The image was masked so that only the scar and edges were applied to the volume calculation function. This provided the negative volume from the surface, which determined the wear scar volume.

3.7 Statistical analysis

Data presented shows a mean value for each variable ($n = 3$) ± SD. A students t-test was used to assess the significance of observed differences between samples for friction, calcium release, scar profile and scar volume results. Paired tests were performed on Microsoft Excel using a two-tail distribution and differences were statistically significant if $p < 0.05$.

4 Results

4.1 Friction behaviour

Figure 3 shows the mean coefficient of friction for each artificial saliva solution over test. This is displayed as a line with an error shadow representing the standard deviation.

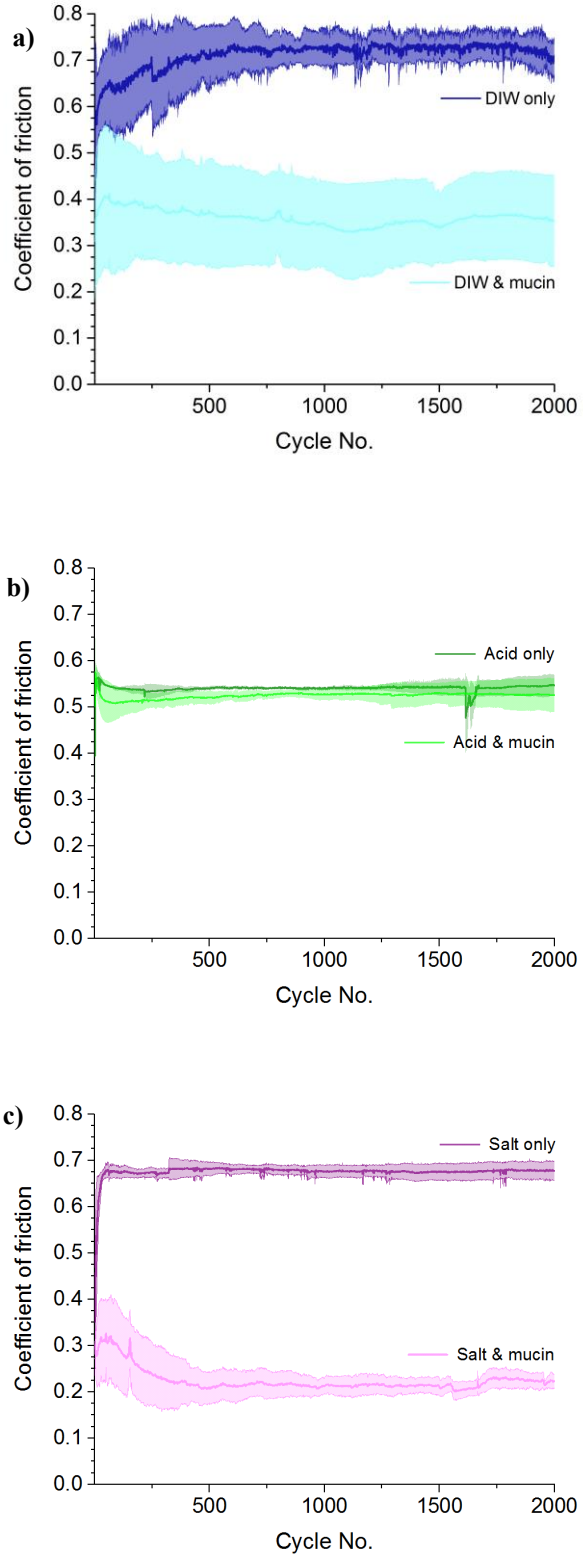

Figure 3 Coefficient of friction of enamel with error shadows for a) deionised water (DIW) and 0.2% mucin only solutions, b) pH 3.1 citric acid only and pH 3.1 citric acid & 0.2% mucin solutions and c) PBS salt only and PBS salt & 0.2% mucin solutions.

Figures 3a) and 3c) show a clear reduction in the coefficient of friction with the addition of mucin into deionised water and salt only (PBS) solutions, a change from approximately μ= 0.72 to 0.36 and μ= 0.68 to 0.22 respectively. There is also more deviation from the average coefficient of friction for deionised water based solutions compared to all others. In contrast to this the addition of mucin to citric acid did not have a significant effect on reducing the coefficient of friction, as presented in Figure 3b).

Figure 4 shows the mean coefficient of friction over the last 500 cycles of testing for all solutions with standard deviation bars. This comparison highlights that mucins have an effect in significantly reducing the coefficient of friction in the neutral pH solutions.

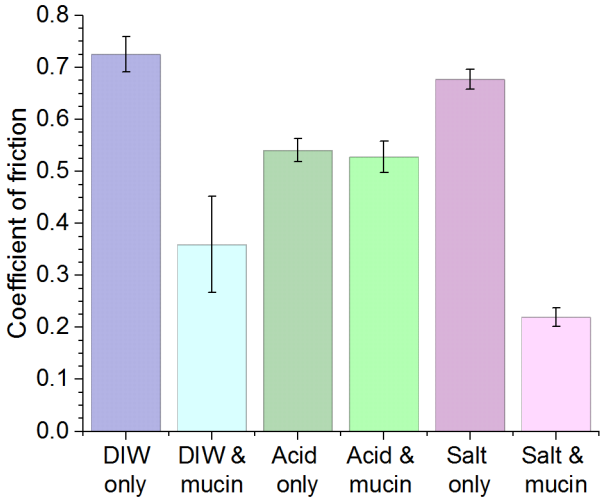

Figure 4 Mean coefficient of friction of last 500 cycles with SD bars for enamel in all solutions.

4.2 Static corrosion vs dynamic corrosion

Figure 5 shows the Atomic Absorption Spectrophotometry (AAS) analysis of all solutions (n = 3) for both static and dynamic tests. As expected, the highest concentration of calcium release was observed for citric acid in tribological conditions. The lowest calcium concentration was observed for deionised water in static conditions.

Student t-tests were employed to compare static and tribocorrosion tests as well as the addition of mucins to solution. A significant increase in calcium release between static and dynamic tests was observed for the acid only (p value < 0.02) solutions and a significant decrease in calcium release was observed for salt and salt + mucin solutions (p value < 0.01).

The presence of mucin in citric acid also significantly reduces the calcium release under tribological conditions (p value < 0.05). For all other solutions, the presence of mucin has increased the calcium concentration in post-test solutions. It was acknowledged that these differences were a

few magnitudes smaller than what was observed for citric acid solutions. Significant differences for static tests were observed between DIW and DIW + mucin solutions (p value < 0.03) and salt only compared with salt + mucin solutions (p value < 0.02).

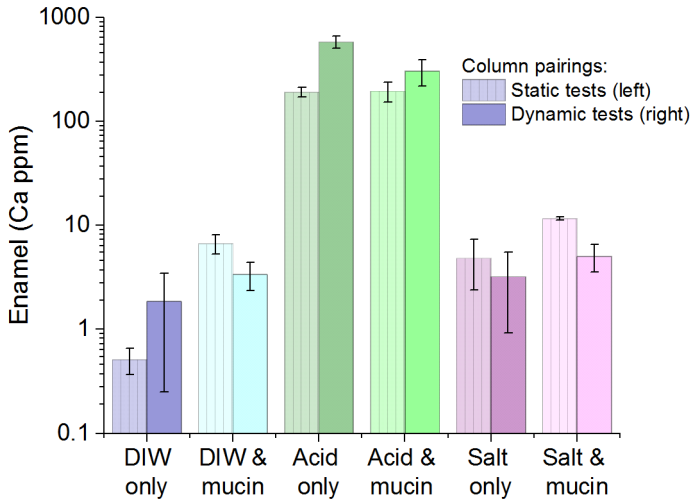

Figure 5 Calcium release into post-test solution in pairs of static and dynamic tests. Static tests are displayed as paler, patterned column on the left of the pairing, dynamic tests are displayed in the darker, non-patterned column on the right of pairing.

4.3 Tribocorrosive wear behaviour of bovine enamel

Vertical scanning interferometry examination shows there is some variation in the wear scar characteristics, Figure 6. Table 3 shows the data on the wear scar characteristics in terms of maximum depth, length of scar and maximum width of scar taken at the centre of all samples. The scar profile width for deionised water is larger than the DIW + mucin wear scar, 0.059 mm and 0.027 mm respectively. In salt-only conditions the scar width is much smaller, 0.038 mm, and the salt + mucin wear scar is much smaller, 0.012 mm, which is clearly seen in Figure 6 a-d).

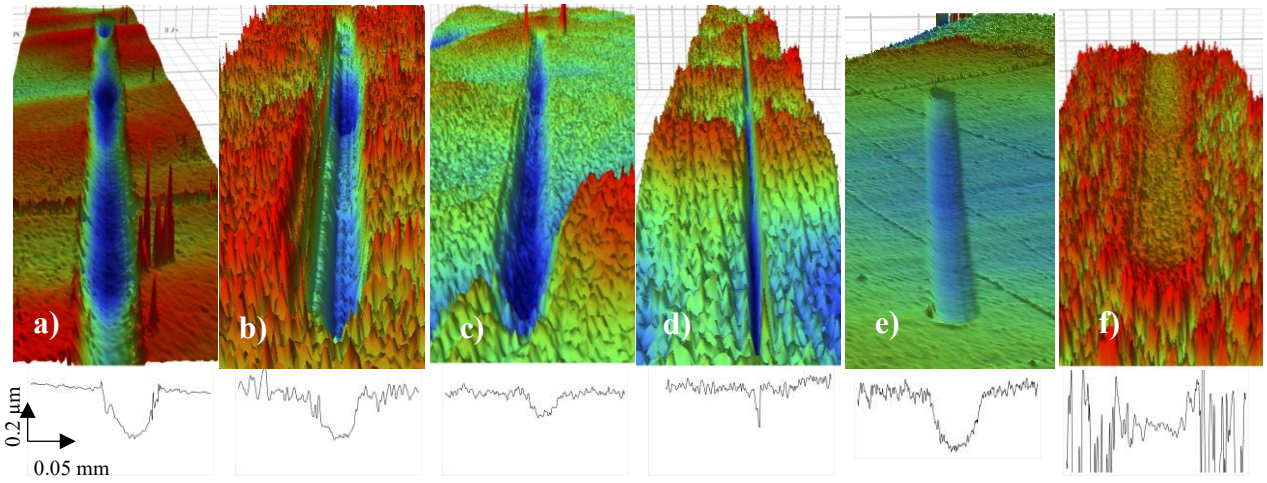

Figure 6 Vertical scanning interferometry images and cross section profiles of enamel wear scars after dynamic tests. a) deionised water, b) 0.2% mucin only, c) PBS salt only, d) PBS salt & 0.2% mucin, 3) pH 3.1 citric acid only, f) pH 3.1 citric acid & 0.2% mucin. Cross section profiles are all displayed to the same scale.

The scar profile depth follows a similar pattern. The depth of the deionised water scar is slightly smaller than DIW + mucin scar, 0.232 µm and 0.131 µm respectively. The citric acid wear scar width, 0.060 mm is similar to that of deionised water, however its scar depth is much larger at a maximum depth of 0.315 µm. Another feature of interest is the scar profile for the acid and mucin scar in particular. Unlike the other profiles which exhibit a trough like scar feature, the surfaces either side of the acid/mucin wear track are much more corroded and irregular while this is not the case for the acid only scar. These features prevented an accurate calculation of the scar volume as the reference plane could not be clearly determined.

Table 3 *Maximum width, length and depth of wear scars.*

Solution	**Length (mm)**		**Width (mm)**		**Depth (µm)**	
	Average	**SD**	**Average**	**SD**	**Average**	**SD**
DIW (n=3)	0.800	0.046	0.059	0.007	0.232	0.068
DIW + mucin (n=3)	0.864	0.018	0.027	0.010	0.131	0.093
PBS (n=3)	0.854	0.007	0.038	0.001	0.116	0.002
PBS + mucin (n=3)	0.796	0.112	0.012	0.003	0.067	0.046
Citric acid (n=3)	0.935	0.001	0.060	0.004	0.315	0.027
Citric acid + mucin (n=3)	0.781	0.072	0.114	0.013	1.215	0.347

Wear volume analysis shows that the mucin only and salt/mucin solutions influence on the tribological wear, shown in Figure 7. The largest wear volume was observed with the acid only solution, while the salt and mucin solution showed the lowest scar volume. Student t-tests were performed and significant volume reductions were observed for the salt only solution compared with deionised water (p value < 0.02) and for the salt/mucin solution compared with deionised water (p value < 0.01).

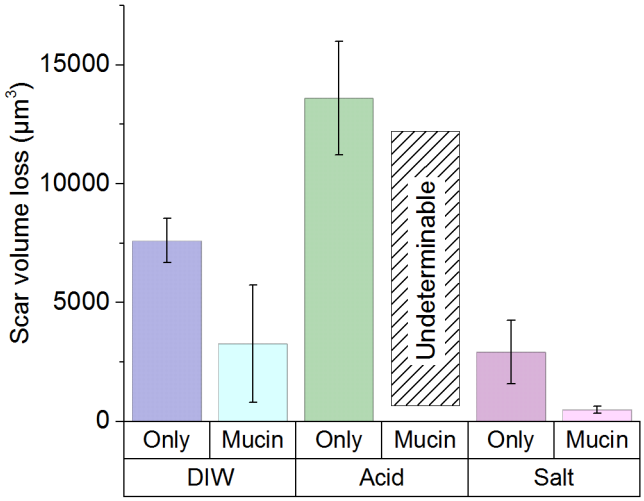

Figure 7 Estimated wear volume of enamel wear scars.

Upon examination of the whole sample, the citric acid wear scar is clearly defined in the centre, shown in Figure 8. Around the scar, there is a smooth area and outside of this is a rougher corroded enamel area before reaching the untouched enamel boundaries (in red). The overall volume lost appears to be dominated by corrosion from the citric acid, causing large-scale demineralization and dissolution over the test duration. This is also highlighted in Figure 9 which compares the wear scar volume to the total visible volume lost from the enamel surface under acid only conditions.

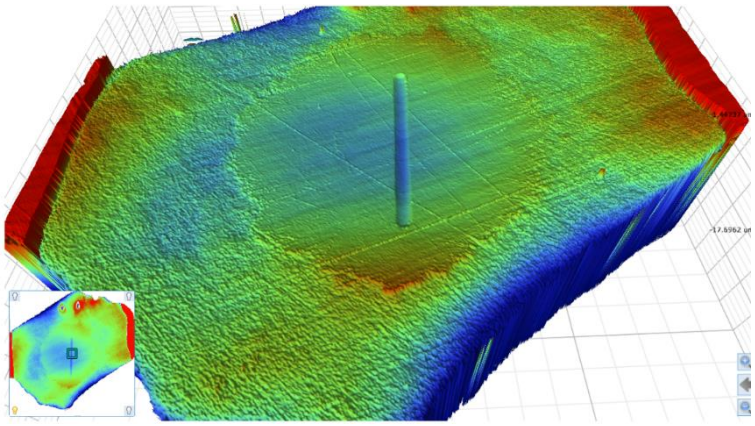

Figure 8 Vertical scanning interferometry of pH 3.1 citric acid wear area.

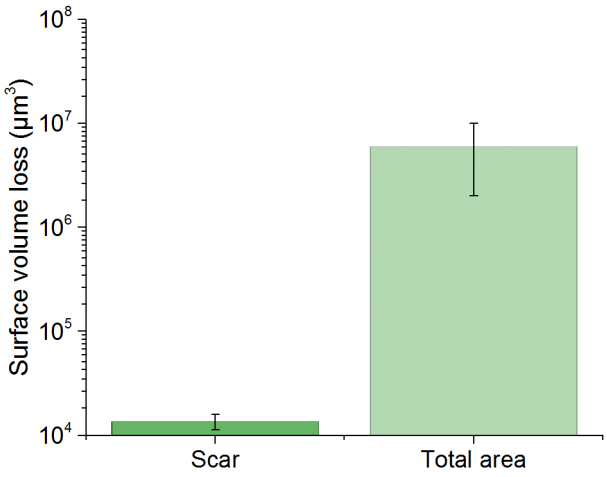

Figure 9 pH 3.1 Citric acid comparison to scar volume only and total affected area.

5 Discussion

To further develop preventative therapies that maximise the protection of tooth enamel, the contributions of overall tooth degradation need to be understood. This is especially the case in corrosive and tribocorrosive environments from interactions with citric acid which have been previously investigated [13, 23, 25-26, 35-40]. This present study aimed to demonstrate how different compositions of artificial saliva can impact tooth degradation of bovine enamel in static and dynamic conditions. In general, increased degradation was observed in a tribocorrosive environment compared to a corrosion only one and that the solution composition influences the degree of surface wear observed. The addition of porcine gastric mucins to artificial salivas was also observed to influence certain aspects of degradation and that the protein behaved differently depending on the experimental conditions and environment.

5.1 Influence of static vs dynamic conditions on overall tooth degradation

Previous work on tooth tribocorrosion has primarily focused on depth profiling of the corroded surface and the wear scar profile as an estimate to the overall wear [28, 36-37]. Other studies have also performed pre and post-test micro indentations to examinine the changes to surface hardness and modulus properties [13, 25, 36, 38]. These methods investigated the impacts of a corrosive environment in different conditions, but not the contributions to the overall degradation. In the present study the measurement of calcium release from static and dynamic experiments provided further insight into the contributions to the total degradation of tooth enamel in an acidic environment. This technique has been observed in corrosion only studies [11, 23, 39-40] and has shown a linear relationship with changing surface micro hardness [39]. In the present study, significantly more calcium is released in a tribocorrosive environment compared to corrosion alone. This result supports

the hypothesis that combining mechanical interactions with a corrosive environment can be more detrimental to the tooth enamel. As mentioned previously, enamel within a corrosive environment begins to soften due to demineralisation of the hydroxyapatite prisms [22,24]. The enamel surface also begins to roughen and form a 'honeycomb' like structure as the core of enamel rods corrode at a greater rate compared to the outer regions of the rod [13,25]. This ensures the enamel surface is more susceptible to material loss from mechanical interactions after it has been attacked by an acid [41]. In the present study, the additional calcium release may therefore be attributed to the mechanical sliding of the YTZP cusp against the softened enamel where the corrosion is enhancing the surface wear and, subsequently, tooth degradation.

These results add an additional perspective to the field of tooth tribocorrosion where controversy exists from study to study. This may arise from primarily examining the wear scar characteristics without considering additional affects corrosion may have. Wu et al [36] observed higher wear volume loss under distilled water lubrication while low volume loss was seen with a pH 3.2 citric acid. Under similar lubrication conditions this was also seen by Eisenburger et al [37] and it was generally concluded that enamel on enamel wear was higher in neutral pH conditions compared to the low pH conditions. On the other hand, Weigand et al [28] performed similar sliding tests with different materials pairs, including enamel on enamel and ceramic on enamel, and found that tribocorrosion in citric acid yielded the most surface wear, approximately 70 times more that the mineral control solution for the ceramic on enamel pairing. From only examining the wear scar characteristics in the present study, scar width under deionised water and citric acid conditions are the same, while the depth slightly increased in acid conditions. Upon examination of the scar volume, the acid scar volume is the larger than deionised water and PBS solution scars. These results are in agreement with what has been observed with Weigand et al [28]. Other studies which consider acetic acid as the lubricating solution also show contrasting results in terms of wear rate examination, where on the one hand a lower wear rate was observed in pH 3 acetic acid compared to deionised water [26], whereas on the other hand the reverse was seen [27]. This highlights the variation in tribocorrosion studies and the requirement for additional measures to break down the contributions to overall degradation which have been eluded to and employed in the present study. This contribution would be the sub-surface corrosion resulting from acid penetration into the surface which has previously been seen up to a depth of 12 μm [24, 30]. Therefore, it may be assumed that the calcium release is a measure of overall tooth degradation (Ca_T) which is a combination of surface volume lost (V_T) plus sub-surface corrosion. This has been decribed by Figure 10 as a schematic of the degradation contributions for a corrosion only environment and a tribocorrosive environment.

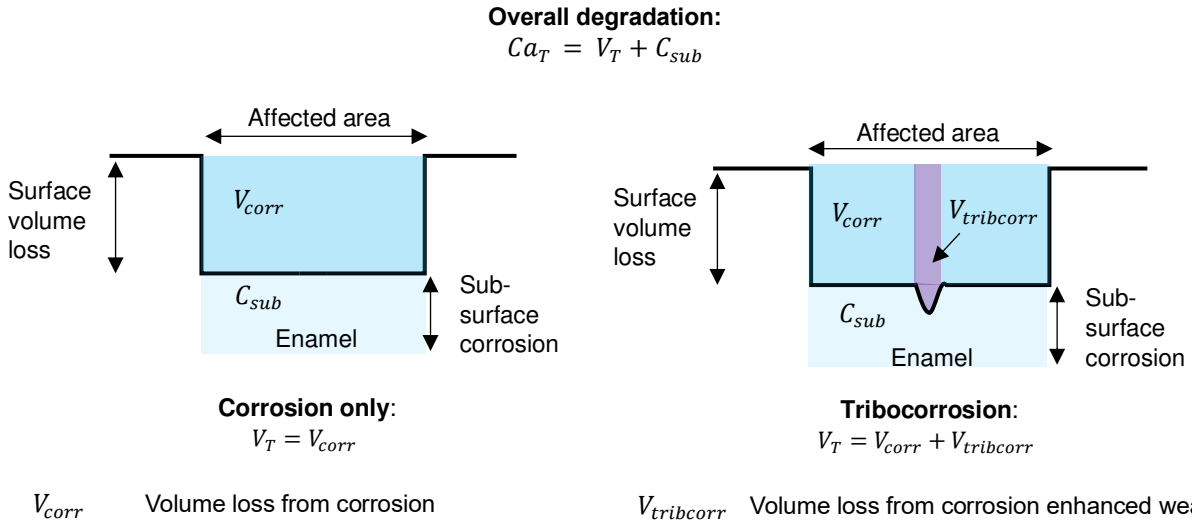

Figure 10 Schematic of suggested wear contributions of enamel under corrosive and tribocorrosive conditions (below pH 5.5).

5.2 Influence of mucins on overall tooth degradation

The application of a mucin component in an artificial saliva has been used in a number of in-vitro studies on enamel [9-12, 29, 42], focusing of aspects of demineralisation before, or, remineralisation after corrosive challenge and influences on tribological behaviour. Much like with tooth tribocorrosion, there are conflicting studies which argue what type of saliva in most beneficial in terms of protection [9-12, 19, 23, 42].

Batista et al [11] investigated the protective ability of artificial salivas by pre-treating enamel surfaces and measuring the surface hardness change after corrosion in pH 2.5 hydrochloric acid. This included a mucin containing artificial saliva, other artificial salivas without mucin and human saliva for in-vitro pre-treatment procedures. No significant differences were observed between the pre-treatment solutions, all of which being comparable to the protective capability of human saliva in-vitro. On the other hand, a mucin containing artificial saliva provided similar protection to human saliva under demineralisation conditions compared to the same artificial saliva without mucin and the deionised water control [10]. Bauman et al [23,40] also examined the interplay between mineral ions and proteins in pre-treatment solutions. A dialysed human saliva, a protein only solution, was compared to a salt only artificial saliva, artificial saliva/dialysed saliva combination and human saliva [23]. The protein only solution provided superior protection after corrosion cycles in terms of surface hardness and calcium release compared to human and other artificial salivas. It was hypothesised that less competition exists between protein and mineral ions in solution which facilitates a better level of surface coverage and therefore protection from demineralisation. A later study also showed that artificial saliva solutions which were under saturated with respect to calcium were likely to hinder protection from demineralisation [40]. Mucin within an artificial saliva has also been seen to reduce

remineralisation, despite being comparable to human saliva [12]. This may be partly be a result of the mucin-surface interactions forming a viscous layer reducing diffusion of calcium ion to the enamel surface [12]. It may also relate to the mucin-ion interactions where calcium ions are complexed by the mucins which is more prevalent in a neutral to alkaline pH environment [19-20].

In the present study, only the interactions between the test solution and enamel were examined and all solutions used were under saturated with respect to calcium ion concentration, i.e. no remineralisation took place. It was observed that neutral pH solutions containing mucin, salt or both yielded an increased level of calcium release compared to deionised water alone. Assuming there is no competition between salts and mucins in the single component solutions [23], direct surface interaction occurs with the calcium ions on the enamel surface [43]. Sodium and potassium ions within the PBS may substitute with calcium, releasing free calcium ions into the test solutions [21-22]. In the presence of PBS salts and mucins, any subsequent free calcium ions may be complexed by free mucins in the solution [19-20]. Despite this observed increase in calcium release from solution interactions, this does not translate across to the friction and wear behaviour. These results suggest the addition of mucin within a dynamic neutral pH environment enhances enamel protection with significantly improved lubrication and less surface damage. These results are in agreement to similar studies which examined different mucin types in PBS solutions compared to human saliva [29, 42]. In the present study the addition of PBS salts fortified the enamel surface under dynamic test conditions. Despite being under saturated with respect to calcium ions, the combination of phosphate, sodium and potassium ions in PBS and their interactions with the surface had a role to play in reducing the surface wear. In the acidic pH environment the mucin does not appear to have a significant impact on enamel protection. These results suggest that the mucin is influenced in some way by the mechanical interactions between the YTZP ball and the enamel surface as seen by a significant reduction in calcium release. However, these results cannot explain why this may be occurring, especially as there is no effect of mucin on the coefficient of friction. The majority of previous work has examined the influence of proteins and mineral ion interplay in a corrosion only setting, either after corrosive attack and surface remineralisation. However, to further understand the influence of these solution components in a dynamic environment, future work is needed that focuses on surface interactions in static and dynamic conditions and how they influence overall degradation.

6 Conclusion

The tribocorrosion of bovine enamel is affected by a network of mechanical, chemical and biological influences. The environment not only influences the tribological properties of enamel, but also the way mucins interact with one another and the surfaces. The key conclusions from this study are summarised below:

- In a neutral pH environment the addition of mucins can be used to improve lubrication and reduce the interacting surface wear, especially with the addition of salts.
- In an acidic pH environment the mucins did not influence static dissolution or lubrication.
- In both environments, the additional mechanical interactions had an effect on reducing the overall calcium in the presence of mucins.
- It was also acknowledged that the contributions of wear could not be determined from the experiments in this study which will be further researched in future investigations including: surface mechanical properties over time, changes in surface chemistry and crystal structure, depth of corrosion and tribo-film analyses.

7 *References*

[1] Lussi, A. et Jaeggi, T. 2008. Erosion – diagnosis and risk factors. *Clin Oral Invest.* **12** (suppl) pp.S5-S13.

[2] White, D., Pitts, N., Steele, J., Sadler, K. et Chadwick, B. 2011. 2: Disease and related disorders – a report from the adult dental health survey 2009. NHS Information centre for health and social care. Pp.1-55.

[3] Rakhmatullina, E., Bossen, A., Höschele, C. Wang, X., Beyeler, B., Meier, C. et Lussi, A. 2011. Application of the specular and diffuse reflection analysis for in vitro diagnostics of dental erosion: correlation with enamel softening, roughness, and calcium release. *Journal of Biomedical Optics* **16**(10) pp. 1-12.

[4] Lussi, A., Schlueter, N., Rakhumatullina, E. et Ganss, C. 2011. Dental erosion - an overview with emphasis on chemical and histopathological aspects. *Caries Research*. **45** (suppl 1) pp.2-12.

[5] Lendenmann, U., Grogen, J. et Oppenheim, F.G. 2000. Saliva and dental pellicle: a review. *Adv Dent Res*. **14**: pp22-28.

[6] Berg, I.C., Rutland, M.W. et Arnebrant, T. 2003. Lubricating properties of the initial salivary pellicle – an afm study. *Biofouling*. **19**(6) pp365-369.

[7] Siqueira, W.L. Margolis, H.C., Helmerhorst, E.J. Mendes, F.M. et Oppenheim, F.G. 2010. Evidence of intact histatins in the in vitro acquired enamel pellicle. *J Dent Res.* **89** pp.626-630.

[8] Villa, A., Connell, C.L. et Abati, S. 2014. Diagnosis and management of Xerostomia and hyposalivation. *Therapeutics and Clinical Risk Management*. **1** pp.45-50.

[9] Ionta, F.Q., Mendoça, F.L., Oliveira, G.C., Alencar, C.R., Honório, H.M., Magalhães, A.C. et Rios, D. 2014. In vitro assessment of artificial saliva formulation on initial enamel erosion remineralisation. Journal of dentistry. 42 pp.175-179.

[10] Jordão, M.C., Ionta, F.Q., Bergantin, B.T., Oliveira, G.C., Moretto, M.J., Silva, T.C. et Rios, D. 2017. The effect of mucin in artificial saliva on erosive rehardening and demineralisation. *Caries research.* **51** pp.136-140.

[11] Batista, G.R., Rocha Gomes Torres, C. Sener, B.m Attin, T. et Weigand, A. 2016. Artificial saliva formulations versus human saliva pretreatment in dental erosion experiments. *Caries Research*. **50**. Pp78.86.

[12] Hara, A.T., González-Cabezas, C., Creeth, J. et Zero, D.T. 2008. The effect of human saliva substitutes in an erosion-abrasion cycling model. *Eur J Oral Sci.* **116** pp. 552-556.

[13] Zheng, J., Xiao, F., Qian, L.M. et Zhou, Z.R. 2009. Erosion behavior of human tooth enamel in citric acid solution. *Tribology International.* **42**(11-12) pp. 1558-1564

[14] Aydin, B., Pamir, T., Baltaci, A. Orman, M.N. et Turk, T. 2015. Effect of storage solution on microhardness of crown enamel & dentin. *Eur J Dent.* **9**(2) pp. 262-266.

[15] Baker, N.J Parker, D.M., Addy, M., Hughes, J.A., West, N.X. et Macdonald, E. 1998. Reduction of the erosive potential of low calorie soft drinks. *Journal of Dental Research.* 77 pp. 846 [special issue B, abstract 1713].

[16] International Standards Organisation. 2001. Dental materials – guidance on testing of wear – Part 2: wear by two- and/or three body contact. ISO TS 14569 – 2:2001

[17] Zhou, Z.R., Yu, H.Y., Zheng, J., Qian, L.M. et Yan, Y. 2013. Biomechanics of human teeth. Dental biotribology. Springer Science+Business Media New York.

[18] Rilo, B., Fernandez, J., Silve, L.D., Martinez, A. et Santana, U. 2001. Frontal-plane lateral border movements and chewing cycle characteristics. *Journal of Oral Rehabilitation.* **28**(10) pp.9390-936.

[19] Meyer-Lueckel, H., Hopfenmuller, W., Klinggraff, D. et Kielbassa, A.M. 2006. Micro radiographic study on the effects of mucin-based solutions used as saliva substitutes on demineralised bovine enamel in vitro. *Archives of Oral Biology.* **51**. pp.541-547

[20] Kuver, R. et Lee, S.P. 2003. Calcium binding to biliary mucins is dependent on sodium ion concentration: relevance to cystic fibrosis. *Biochemical and Biophysical Research Communication.* **314** pp. 330-334.

[21] Secilmis, A., Dilber, E., Gokmen, F., Ozturk, N. et Telatar, T. 2011. Effect of storage solutions on mineral contents of dentin. *Journal of Dental Sciences*. **6** pp. 189-194.

[22] Featherstone, J.D.B. et Lussi, A. 2006. Understanding the chemistry of Dental Erosion. *Lussi A (ed): Dental Erosion. Monogr Oral Sci.* Basel, Karger. **20** pp.66-76.

[23] Baumann, T., Kozik, J., Lussi, A. et Carralho, T.S. 2016. Erosion protection conferred by whole human saliva, dialysed saliva, and artificial saliva. *Scientific Reports*. **6**(34670). Pp1-8.

[24] Wu, Y.Q., Arsecularantne, J.A et Hoffman, M. 2017. Attrition corrosion of human dental enamel: a review. *Biosurface and Biotribology.* **3** pp. 196-210.

[25] Zheng, J., Xiao, F., Zheng, L., Qian, L.M. et Zhou, Z.R. 2010. Erosion behavior of human tooth enamel at different depths. *Tribology International.* **43**(7) pp. 1262-1267.

[26] Kaidonis, J.A., Richars, L.C., Townsend, G.C. et Tansley, G.D. 1998.Wear of human enamel: a quantitative in vitro assessment. *J Dent Res.* **77**(12) pp.1983-1990.

[27] Shabanian, M. et Richards, L. 2002. In vitro wear rates of materials under different loads and varying pH. *The journal of prosthetic dentistry.* **87**(6) pp.650-656.

[28] Weigand, A., Credé, A., Tschammler, C., Attin, T. et Tauböck, T.T. 2017. Enamel wear by antagonistic restorative materials under erosive conditions. *Clin Oral Invest*. **21** pp. 2689-2693.

[29] Mystkowska, J., Karalus, W., Sidorenko, J., Dąbrowski, J.R. et Kalska-Szostko, B. 2016. Biotribological properties of dentures lubricated with artificial saliva. *Journal of Friction and Wear.* **37**(6) pp.746-754.

[30] Cougot, N., Doullard, T., Dalmas, F., Pradelle, N., Gauthier, R., Sanon, C., Grogogeat, B., Colon, P. et Chevalier, J. 2018. Towards quantitative analysis of enamel erosion by focused ion beam tomography. *Dental Materials*. **34** pp.e289 – e300.

[31] Zheng, J. et Zhou, Z.R. 2007. Friction and wear behaviour of human teeth under various wear conditions. *Tribology International*. **40** pp.278-284.

[32] Anderson, D.J. 1956. Measurement of Stress in Mastication. I. J.D.Res. 35(5) pp.644-670.

[33] Dejak, B., Młotkowski, A. et Romanowicz, M. 2003. Finite Element Analysis of Stress in Molars during Clenching and Mastication. *The Journal of Prosthetic Dentistry.* **90**(6) pp.591-597.

[34] Wang, H.C., Stack, M.M., Li, W.L., Hong, T.F. et Wang, M.C. 2010. On the construction of wear maps for Y-TZP dental ceramics in aqueous environments: pH, exposure time and impact angle effects. *Tribology International*. **43**(12) pp.2258-2267.

[35] West, N.X, Hughes, J.A. et Addy, M. 2000. Erosion of dentine and enamel in vitro by dietary acids: The effect of temperature, acid character, concentration and exposure time. *Journal of Oral Rehabilitation*. **27** pp. 875-880.

[36] Wu, Y.Q., Aresecularatne, J.A. et Hoffman, M. 2015. Effect of acidity upon attrition-corrosion of human dental enamel. *Journal of the Mechanical Behaviour of Biomedical Materials.* **44** pp. 23-34.

[37] Eisenburger, M. et Addy, M. 2002. Erosion and attrition of human enamel in vitro Part 1: Interaction effects. *Journal of Dentistry*. **30** pp. 341-347.

[38] Zhang, Y., Arsecularatne, J.A. et Hoffman, M. 2015. The effects of three different food acids on the attrition-corrosion wear of human dental enamel. *Journal of Physics D: Applied Physics.* **48** pp. 1-10.

[39] Carvalho, T.S., et Lussi, A. 2015. Susceptibility of enamel to initial erosion in relation to tooth type, tooth surface and enamel depth. *Caries Research.* **49** pp. 109-115.

[40] Bauman, T., Bereiter, R., Lussi, A. et Carvalho, T.S. 2017.The effect of different salivary calcium concentrations on the erosion protection conferred by the salivary pellicle. *Scientific Reports.* **7**(12999) pp. 1-9.

[41] Weigand, A., Köwing, L. et Attin, T. 2007. Impact of brushing force on abrasion of acid-softened and sound enamel. *Archives of Oral Biology.* **52**(11) pp. 1043-1047.

[42] Klimek, J., Hellwig, E. et Ahrens, G. 1982. Flouride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds in vitro. *Caries Research*. **16** pp. 156-161.

[43] Hannig, M. et Joiner, A. 2006. The structure, fuction and properties if the acquired pellicle. Duckworth RM (ed) : The teeth and their environment. *Monogr Oral Sci.* Basel, Karger. **19** pp. 29-64.