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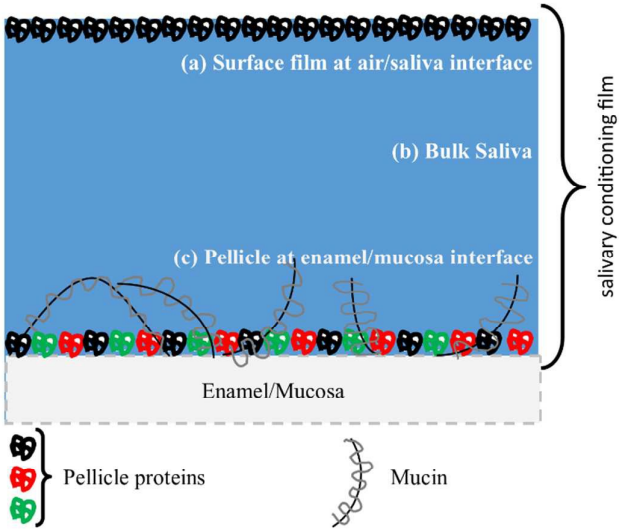

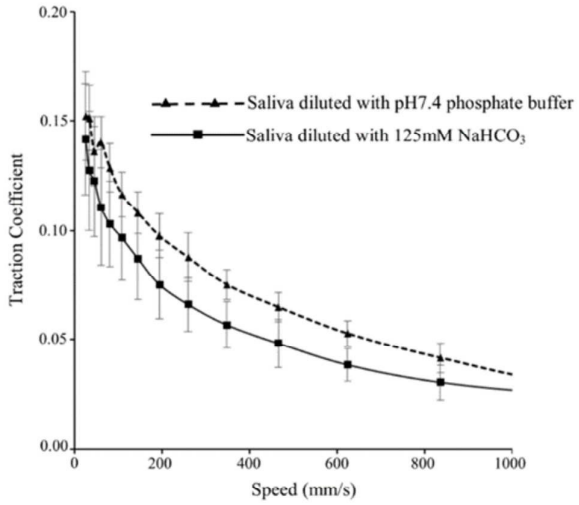

This study suggests that changes in the lubricative properties of saliva are likely to be due to changes at the interfaces of the salivary conditioning film (i.e. air/saliva interface or enamel/saliva or mucosa/saliva interface) as opposed to any changes in the bulk viscosity of saliva, when exposed to sodium bicarbonate. Exposure to chemicals that can modulate the interfacial properties of the salivary conditioning film could also be partly responsible for changes in mouth feel perception.

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ARTICLE

Structural modifications of the salivary conditioning film upon exposure to sodium bicarbonate: Implications for lubrication and mouthfeel.

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The salivary conditioning film (SCF) that forms on all surfaces in the mouth plays a key role in lubricating the oral cavity. As this film acts as an interface between tongue, enamel and oral mucosa, it is likely that any perturbations to its structure could potentially lead to a change in mouthfeel perception. This is often experienced after exposure to oral hygiene products. For example, consumers that use dentifrice that contain a high concentration of sodium bicarbonate (SB) often report a clean mouth feel after use; an attribute that is clearly desirable for oral hygiene products. However, the mechanisms by which SB interacts with the SCF to alter lubrication in the mouth is unknown. Therefore, saliva and the SCF was exposed to high ionic strength and alkaline solutions to elucidate whether the interactions observed were a direct result of SB, its high alkalinity or its ionic strength. Characteristics including bulk viscosity of saliva and the viscoelasticity of the interfacial salivary films that form at both the air/saliva and hydroxyapatite/saliva interfaces were tested. It was hypothesised that SB interacts with the SCF in two ways. Firstly, the ionic strength of SB shields electrostatic charges of salivary proteins, thus preventing protein crosslinking within the film and secondly; the alkaline pH (≈8.3) of SB reduces the gel-like structure of mucins present in the pellicle by disrupting disulphide bridging of the mucins via the ionization of their cysteine's thiol group, which has an isoelectric point of ≈8.3

Introduction

Stimulated whole mouth saliva (sWMS) is a complex aqueous fluid that contains a mixture of proteins, bacteria, sloughed mucosal cells, organic and inorganic material [1]. The saliva that humans produce has some interesting viscoelastic properties that may affect mouthfeel[2]. For example, one of the main functions of saliva is to provide lubrication in the oral cavity [3]. This facilitates speech, the consumption of food and also ensures that attrition between contacting hard and soft tissue of the mouth is minimised[4]. Without adequate saliva in the mouth to produce well lubricated mucosal surfaces, individuals can suffer impediment of speech, dental caries, gum disease and increased mucosal membrane damage [5, 6]. The salivary conditioning film (SCF) that forms on all surfaces in the mouth can be thought of as an assembly of three unique structures; the salivary film that forms at the air/saliva interface, the salivary pellicle that forms at the enamel/saliva (or mucosa/saliva) interface and the bulk saliva that resides between these two interfaces (figure 1). The interplay between these structures modulates lubrication and consequently has the potential to affect mouthfeel too. As the SCF acts as an interface between tongue, enamel and oral mucosa, it is likely that it plays a significant role in sensory perception such as: smoothness, cleanliness and dryness[7, 8]. Although there are only a few studies that identify physical parameters associated with the sensory perception[2, 9, 10] it is reasonable to assume that perturbations to the structures of the SCF can potentially modulate lubrication and thus mouthfeel. Our previous work showed that certain cleaning agents (e.g. sodium dodecyl sulphate (SDS) and sodium tripolyphosphate (STP)) were able to modify the salivary pellicle that forms at the solid/liquid interface, either by competing with pellicle proteins at the surface, or by interacting with pellicle proteins directly[11]. In each case, conclusions were made possible partly due to the chemical structure of the agents being tested and the surface chemistry of the adsorbents. The removal of the salivary pellicle, via exposure to SDS, also observed by Santos et al., [12] resulted in a loss of lubrication[13]. This could potentially lead to a change in mouthfeel perception, as is often experienced after exposure to oral hygiene products. For example, consumers that use dentifrices containing a high concentration of sodium bicarbonate (SB) often report a clean mouth feel after use; an attribute that is clearly desirable for all oral hygiene products.

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However, unlike SDS and STP, the chemical structure of SB is innocuous, with no regions of high charge density or any regions of hydrophilicity or hydrophobicity. Consequently, understanding the mechanisms by which SB potentially interacts with the SCF to alter lubrication in the mouth is significantly more challenging. It is possible that the high ionic strength of SB in some dentifrice products, for example up to 67% in Parodontax®, could play a role[14, 15]. It could also be that the relatively high alkalinity (pH 8.3) of SB may be influential in disrupting the SCF[16-19]. Therefore, in this study, we exposed saliva and SCF to high ionic strength and alkaline solutions to independently test the impact on the physical characteristics of the SCF that influences oral lubrication. Characteristics including bulk viscosity of saliva and the viscoelasticity of the interfacial salivary films that form at both the air/saliva and hydroxyapatite/saliva interfaces were tested (See Figure 1).

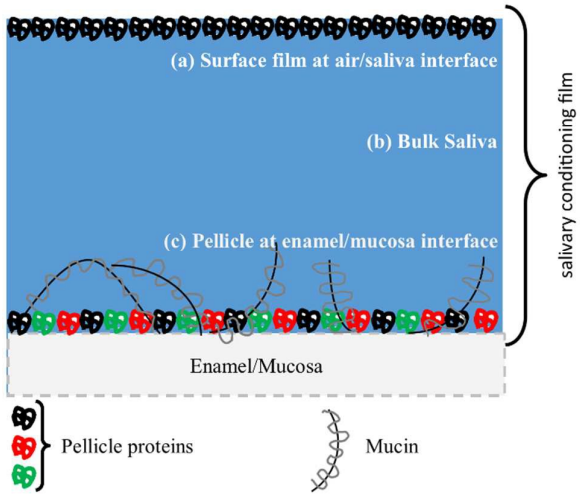


Figure 1 Simplified 2-D outline of the key structures of the 'salivary conditioning film' in the mouth. These structures are considered key for oral lubrication: (a) Surface film at air/saliva interface (b) Bulk saliva (c) Pellicle at enamel/mucosa interface (adapted from Yakubov[3]). Each structure was measured independently *in-vitro,* so that (a) was measured via an AR-G2 rheometer (b) via an AR2000 Advanced Rheometer and (c) via a QCM-D. The overall lubrication properties of the salivary conditioning film was measured via a Mini traction machine.

Materials & Methods

Saliva collection

Saliva collection was undertaken according to a protocol previously assessed by an independent ethics panel (reference number: 2013/2014 – 67 HT registered online at ClinicalTrials.gov ID: NCT02188238; Protocol ID: IFR01-2014-HRGC). Individual saliva samples were obtained from healthy, non-smoking, male and female volunteers, ranging in age from 20 to 50 years. Volunteers refrained from eating 1 h prior to donation, and rinsed their mouths twice with 10 ml of bottled, still water (Waitrose, Bracknell, UK). Volunteers then chewed on flavour-free gum (Cafosa Gum, Barcelona, Spain) and expectorated stimulated whole mouth saliva (sWMS) into a sterile collection tube. Salivas were kept in ice upon expectoration, and were used immediately for study; and therefore it was deemed that no protease inhibitors were required. Moreover we aimed to mimic the behaviour of the in vivo pellicle as closely as possible, including potential proteolysis of the pellicle and pre-cursor proteins.

Lubrication of saliva

The change in the lubrication potential of saliva when exposed to SB was investigated via a Mini traction machine. Tribology measurements were carried out using a mini traction machine (MTM, PCS Instruments, London, UK). The equipment was setup with a steel ball and silicone elastomer discs (Samco Silicone Products, Warwickshire, UK) as the contact surfaces. The traction coefficient of saliva samples were measured over a range of rotational speeds from 1 to 1000 mm/s and a normal force of 1 N and 2 N at 25°C. The tongue can move at speeds of 200 mm/s [20] and apply loads between 0.01 and 90N [21] (For further instrument details refer to Myant et al. [22]). One subject provided fresh samples that were diluted by a factor of 7/8 (87.5% saliva) to contain 125 mM SB or 12.5 mM pH 7.4 phosphate buffer, as the control. 12 ml of the prepared saliva sample was placed into the MTM dish and the run was started immediately. Six repetitions within the same sample alternating ascending and descending speed were carried out. This was repeated another two times with two different samples so that three independent repeats were used to compare differences in the lubrication of the two salivary solutions tested.

Subsequently, the following physical properties of saliva were also tested:

- Surface viscoelasticity of saliva at air/saliva interface (investigated via AR-G2 rheometer)
- Bulk viscosity of saliva (investigated via AR-2000 rheometer)
- Pellicle mass and thickness at hydroxyapatite/saliva interface (investigated via QCMD)

Surface viscoelasticity of saliva at air/saliva interface

The viscoelasticity of saliva's surface film was measured using an AR-G2 rheometer (TA Instruments, Hertfordshire, UK) fitted with an interfacial geometry. Four samples (at 87.5% saliva) were tested: saliva containing 125 mM SB, saliva containing 125 mM NaCl, saliva containing 125 mM phosphate buffer pH 8.3 and finally, saliva containing 12.5 mM phosphate buffer pH 7.4, as the control. 8 ml of the prepared saliva solution was placed in a 44 mm diameter glass dish and an aluminium bicone (20 mm diameter, 6.0° cone angle) was aligned exactly

at the saliva/air interface. Once the bicone was in position an oscillating sinusoidal torque was applied to the bicone and the resultant strain values measured by means of a proximity detector. The time-dependent stress and strain values were used to calculate the elastic modulus (G') of the salivary film. The samples were all measured at a frequency of 0.2 Hz and 0.1% strain at 25°C and repeated 5 times per experiment.

Bulk viscosity of saliva

The bulk viscosity of saliva was measured using an AR2000 Advanced Rheometer (TA Instruments) equipped with an acrylic cone (60 mm diameter, 1.0° cone angle) and plate. Four samples (at 87.5% saliva) were tested: saliva containing 125mM SB, saliva containing 125 mM NaCl, saliva containing 125 mM phosphate buffer pH 8.3 and finally, saliva containing 12.5 mM phosphate buffer pH 7.4, as the control. 1 ml of the prepared saliva solution was placed in between the plate and cone then measurements were undertaken in constant flow mode between shear rates of 10 to 1000 s^{-1} at 25°C and repeated 5 times per experiment. The viscosity of saliva samples were recorded at a shear rate of 48 s^{-1}, as this rate was used as an approximation of the shear rate that the mouth is often exposed to [23].

Pellicle hydrated mass at hydroxyapatite/saliva interface

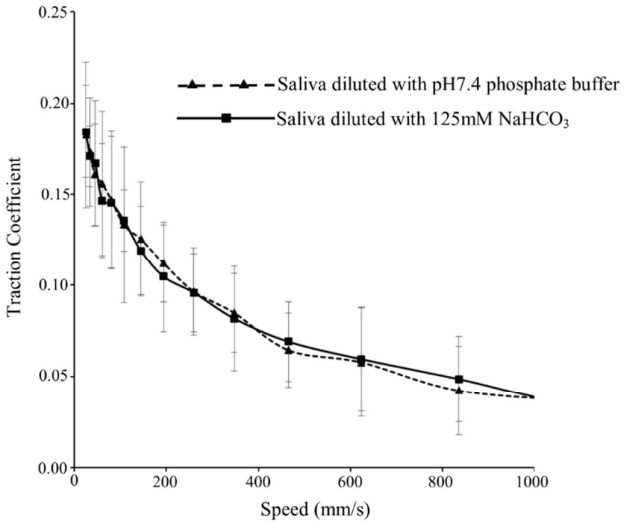
The measurements were performed using a D300 QCMD (Q-Sense AB, Vastra Frolunda, Sweden) with a QAFC 302 axial flow measurement chamber maintained at 36.8°C. Hydroxyapatite coated AT-cut piezoelectric quartz crystals sandwiched between gold electrodes (QSX-303, Q-Sense AB) were used as the substrata (sensors were used once only and not reused). Changes in the frequency of the oscillating sensor were related to the changes in the hydrated mass adsorbing on to the quartz crystal sensor using the Sauerbrey model[24]. The Sauerbrey model was considered the most conservative model to use, as this gives the lowest value of hydrated mass that the pellicle could be. A secondary parameter known as dissipation, which measures the adsorbed films capacity to dampen the sensor's frequency of oscillation was also recorded. This gives a qualitative understanding of the adsorbed films viscoelastic properties[25]. During the entire course of the experiment the chamber was filled with liquid, thus preventing any air/liquid interfacial phenomena affecting the data. Upon injection of 1 ml of undiluted sWMS, pellicle formation was monitored for 60 min. Subsequently, the pellicle was rinsed with 2 ml of 0.1M phosphate buffer solution (pH 7.4) to remove loosely adsorbed material. After 10 min, the remaining pellicle was exposed to a treatment step which consisted of 2 ml of 0.5 M sodium bicarbonate or 2 ml of 0.5 M sodium chloride or 2 ml of 0.5 M phosphate buffer pH 8.3 or 2 ml of 0.1 M phosphate buffer solution as a control. Another 10 min was given before rinsing with the phosphate buffer solution. This was repeated three times and the difference between the first buffer rinse and the final buffer rinse was calculated and compared between them (Figure 5). This protocol was repeated 5 times per experiment.

Results

Lubrication

Figure 2 shows the changes in lubrication in the boundary and mixed regimes and the differences in these lubrication regimes between the saliva diluted with buffer and saliva diluted with SB at a load force of 1 N and 2 N. Overall the trend in the data showed that the saliva containing SB reduced the friction in the boundary and the mixed regime when a load force of 2 N was applied. However, it was only in the mixed regime that this difference was revealed to be statistically significant ($p<0.05$). For example at a disc speed of 347 mm/s the traction coefficient (unitless) for the saliva control (buffer) was 0.075 ± 0.005 whereas the saliva containing SB was lower at 0.057 ± 0.005. A significant difference was also observed at 623 mm/s where the traction coefficient for the saliva control (buffer) was 0.053 ± 0.001 relative to a lower traction coefficient for the saliva containing SB at 0.038 ±0.004. In both cases the saliva reduced the friction coefficient between the two surfaces ~~slightly~~ less than has been reported in other studies. For example, Bongearts et al. and Rosetti et al. [9, 13] both observed a friction coefficient of 0.01 in the boundary-mixed lubrication regime. This difference is likely a consequence of experimental variances between the studies. Intriguingly no difference in traction coefficient of the two salivas was observed at 1 N.

(a)



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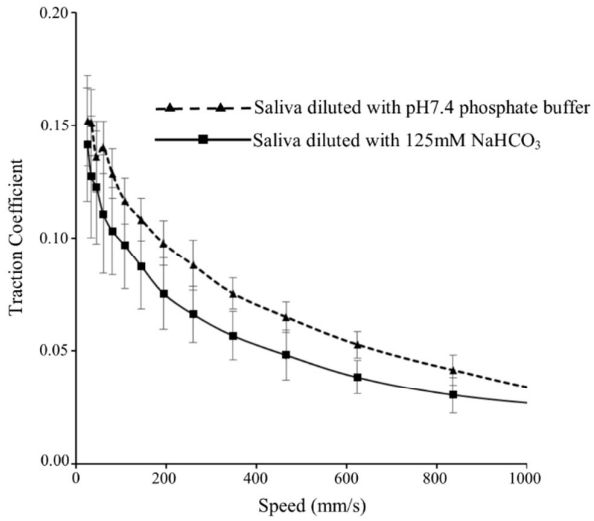

Figure 2 Traction coefficient curves comparing stimulated saliva diluted with phosphate buffer (n=3) against saliva diluted with SB (n=3) at a load force of (a) 1 N and (b) 2 N. At 1 N there was no significant difference between the two salivas' potential to reduce the traction coefficient between the steel ball and the PDMS disc. Whereas at 2 N, the saliva containing SB appears to reduce the traction coefficient relative to the saliva containing phosphate buffer, indicating an increase in lubrication.

Salivary pellicle elasticity at the air/saliva interface

Saliva that was diluted with buffer only (i.e. control) formed the strongest film at the interface between the saliva and air, relative to saliva samples diluted with NaCl, pH 8.3 buffer and SB (Figure 3a). Although saliva is unlikely to reside in the mouth for the full 60 minutes that was observed in these experiments, differences in pellicle strength at the air/saliva interface start to appear after 10 minutes adsorption where the early stages of film formation at the interface begin.

(a)

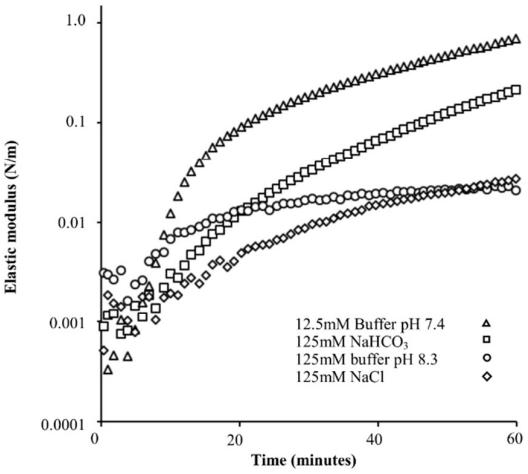

Figure 3 a Representative graph that displays the formation of the salivary pellicle at the air/saliva interface and the difference in the elastic modulus (i.e. 'strength' of the pellicle) of four saliva samples diluted with either SB, NaCl, phosphate buffer pH 7.4 or a phosphate buffer at pH 8.3.

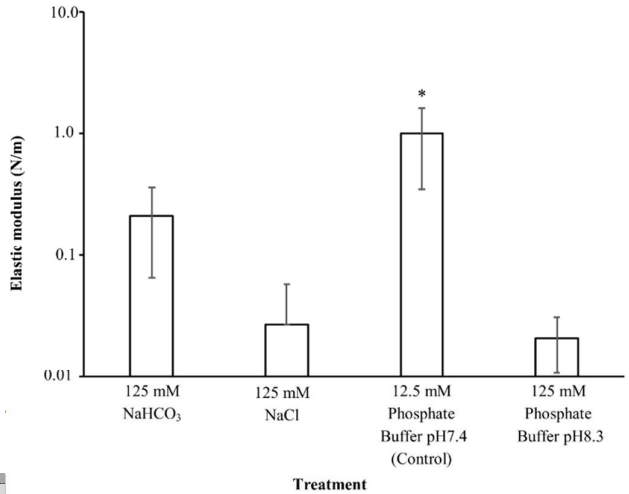

Figure 3 b Bar chart that displays the differences in the elastic modulus (i.e. 'strength' of the pellicle) of four saliva samples diluted with SB, NaCl, phosphate buffer pH 7.4 and a phosphate buffer at pH 8.3 (n=5) after 60 minutes adsorption.

Salivary proteins, mainly statherin [26], begin to form an elastic structure at the saliva/air interface. The formation of this film was significantly reduced when saliva contained NaCl (0.027 ± 0.03 mN/m), pH 8.3 buffer (0.021 ± 0.01 N/m) and to a lesser degree SB (0.2 ± 0.17 N/m). In fact, the addition of NaCl and pH 8.3 phosphate buffer to saliva had the most impact; where a 100 fold reduction in film strength was observed (≈ 0.02 N/m compared to 0.98 N/m for the control). Whilst, the addition of SB to saliva also reduced the strength of the film but only by a 10 fold decrease in strength (0.2 N/m compared to 0.98 N/m for the control) (Figure 3b).

Bulk viscosity

No significant difference in the bulk viscosity of saliva between the control saliva (0.0026 ± 0.001 Pa.s), the saliva containing NaCl (0.0023 ± 0.0007 Pa.s), SB (0.0024 ± 0.0009 Pa.s) or the saliva containing pH8.3 buffer (0.0022 ± 0.0007 Pa.s) was observed (see figure 4). Considering that the bulk viscosity of water (0.001 Pa.s) is a similar order of magnitude to saliva, with or without SB, may indicate that film formation at the interfaces of the SCF plays a more significant role in salivary lubrication.

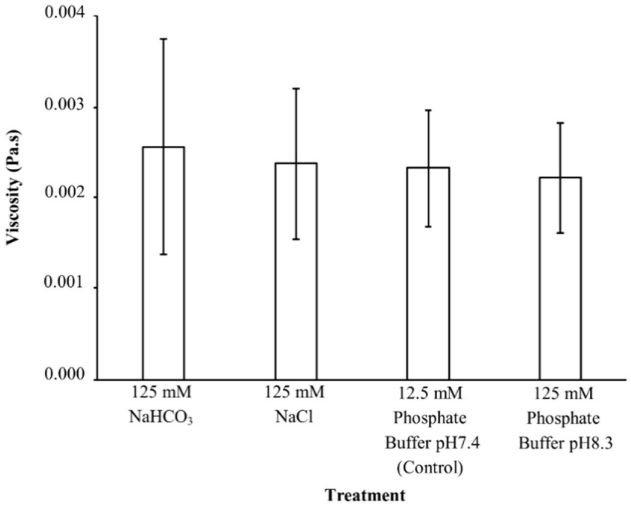

Figure 4 Bar chart comparing bulk viscosity (Pa.s) of four saliva samples diluted with SB, NaCl, phosphate buffer pH7.4 and a phosphate buffer at pH 8.3 (n=5). The data appears to show no significant difference between the four saliva samples' viscous properties.

Salivary pellicle displacement at the hydroxyapatite/saliva interface

Figure 5 shows the hydrated mass of the salivary pellicle derived from a stimulated saliva sample adsorbing onto a hydroxyapatite

coated sensor. The mean hydrated mass for all salivary pellicles tested (n=20) was 1248 ±163 ng/cm^2 almost identical to previous observations for pellicle mass on hydroxyapatite sensors [11, 27]. Subsequent displacement of the pellicle was measured after exposing the pellicle to SB, pH7.4 phosphate buffer, pH 8.3 phosphate buffer and a NaCl solution (See Figure 6).

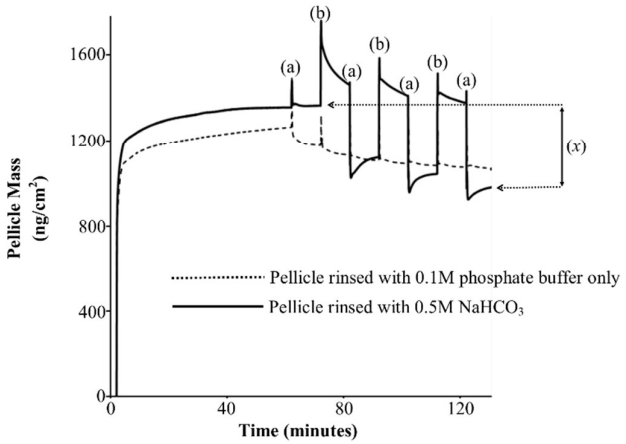

Figure 5 A representative graph that displays the hydrated mass of the adsorbing salivary pellicle (0-60 min); and the hydrated mass of the pellicle after exposure to SB (bold line) or a phosphate buffer only (dotted line). The amount of pellicle displaced after three rinse steps of (b) 0.5 M SB was measured by the difference of the first and the final (a) phosphate buffer rinses (x). This experiment was repeated using a 0.5 M NaCl and a pH 8.3 phosphate buffer solution.

0.5 M SB removed more of the salivary pellicle from the hydroxyapatite surface than the other three solutions. In fact, SB removed 413 ± 132 ng/cm^2 of pellicle, more than double the removal of pellicle after exposure to NaCl 166 ±50 ng/cm^2. The phosphate buffer pH 8.3 removed the least amount of pellicle (31 ± 68 ng/cm^2) followed by the pH 7.4 phosphate buffer control (116.3 ± 81.36 ng/cm^2), although the difference between the latter two was not statistically significant.

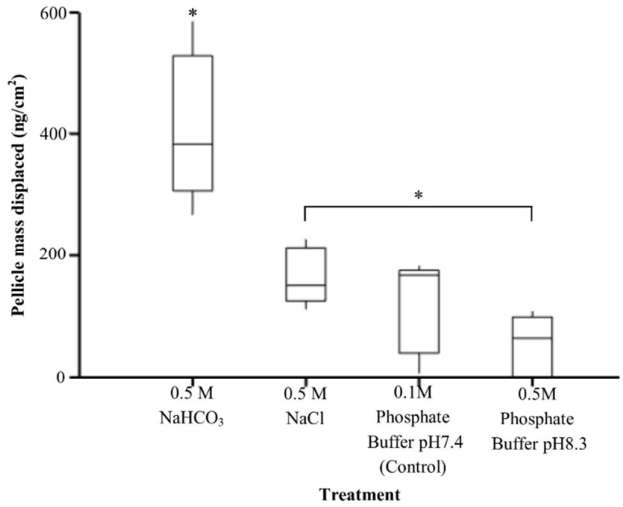

Figure 6 Box plot that shows the amount of pellicle displaced after exposure to: 0.5 M SB, 0.5M NaCl, 0.1 M phosphate buffer pH 7.4 (control) and a 0.5 M phosphate buffer at 8.3 (n=5).

Salivary pellicle structure at the hydroxyapatite/saliva interface

Figure 7 gives a qualitative viscoelastic analysis of the adsorbed salivary pellicle derived from a sWMS adsorbing onto a hydroxyapatite coated sensor. By comparing the ratio between Δf and ΔD the viscoelastic properties of the pellicle with respect to the induced energy dissipation of the sensor per coupled unit mass was observed. The mean Δf/ΔD ratio for all salivary pellicles tested (n=20) was -9 ± 1.3. A slightly higher ratio (more elastic) relative to previous work by Ash et al. [11, 27]. Potentially, a more realistic figure considering the adsorption took place on unused sensors each run. The changes in the Δf/ΔD ratio (i.e. viscoelasticity) after exposing the pellicle to SB, pH 7.4 phosphate buffer, pH 8.3 phosphate buffer and a NaCl solution were compared in (Figure 8). The results showed that the salivary pellicle, after exposure to SB, pH 8.3 phosphate buffer and NaCl, had become softer relative to the pellicle that was exposed to the control pH 7.4 phosphate buffer.

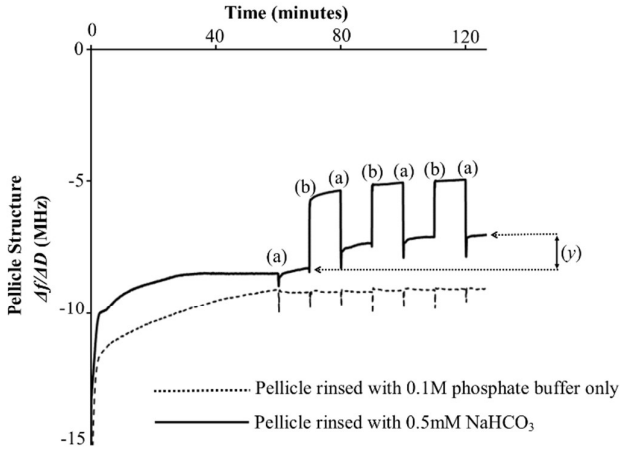

Figure 7 A representative graph that displays the structure of the adsorbing salivary pellicle (0-60 min) and after exposure to SB (bold line) or pH 7.4 phosphate buffer only (dotted line). The change in structure of the pellicle after three rinse steps of (b) 0.5 M SB was measured by the difference of the first and the final (a) phosphate buffer rinses (y). This experiment was repeated using a 0.5 M NaCl and a pH 8.3 phosphate buffer solution.

The largest decrease in the Δf/ΔD ratio (i.e. an increase in pellicle softness) took place when the pellicle was exposed to a pH 8.3 phosphate buffer (-2.2 ± 0.8 MHz). An interesting result considering that this solution was relatively ineffective at displacing the pellicle from the surface (Figure 6). The pellicle also became softer relative to the pH 7.4 phosphate buffer control (-0.1 ± 0.4 MHz) after exposure to NaCl (-1.6 ± 0.8 MHz) and SB (-1.4±0.7 MHz). In other words, the pellicle has become less elastic (e.g. softer) relative to the control upon exposure to SB, pH 8.3 phosphate buffer and NaCl. However, this change in pellicle structure is independent of pellicle displacement (Figure 5 and 6).

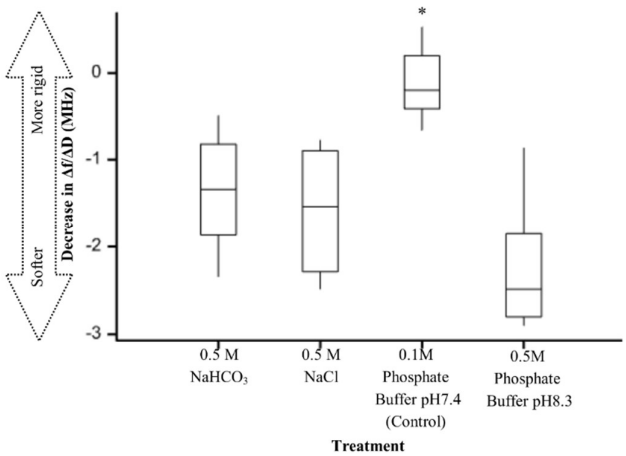

Figure 8 Box plot of the salivary pellicle structure after exposure to four solutions: 0.5 M SB, 0.5 M NaCl, 0.1 M phosphate buffer pH 7.4 (control) and a 0.5 M phosphate buffer at 8.3. (n=5). The softness of the pellicle was increased after exposure to SB, NaCl and pH 8.3 relative to the phosphate buffer control.

Discussion

It is widely recognised that changes in the physical properties of saliva can modulate saliva's potential to lubricate the mouth [3, 10, 28, 29]. Lubrication of the mouth is partly influenced by the rheological characteristics of the SCF, which is composed of three primary structures, two interfacial salivary films and the bulk components of saliva. The interplay between these structures dictates the lubrication potential of the SCF [30]. Consequently, a structural change to any one of the independent structures of the SCF has the potential to modulate the lubrication properties of saliva and therefore mouthfeel perception. The lubrication properties of saliva have been previously characterised by adsorbing saliva to hydrophobic PDMS substrates [9, 13]. A friction coefficient of order 0.01 was observed in the boundary-mixed lubrication regime. This was somewhat lower than the friction coefficient observed in this study (0.15 – 0.03), which likely reflects the different methodology employed. For example, a steel ball was used herein, as opposed to a PDMS ball used in the other study and the dilution of saliva can also impact frictional properties of saliva too [31, 32]. Nevertheless, from the perspective of this work alone, a small increase in lubrication upon the addition of SB to saliva was observed when a load force of 2 N was applied. However at a lower load (1 N) no difference between the two saliva samples was observed. This phenomenon has also been observed by Prinz et al. [33] who suggested that this was due to a smoothing of the soft surfaces when higher loads were applied. Therefore, a certain amount of load force maybe required to obtain significant differences in the traction coefficient of the saliva samples tested that was not applied here. Alternatively, it could be that the saliva samples were not given sufficient time to adsorb to the respective surfaces. For example, Zhang et al. [34] observed that one minute adsorption time of saliva to enamel surfaces resulted in the lowest friction coefficient and significantly decreased enamel wear loss. Importantly however, despite experimental differences between studies, any modulation to the lubricous nature of the SCF has the potential to trigger a change in mouthfeel perception[10, 35-37]. Consequently, changes in the three primary structures of the SCF, i.e. bulk viscosity and interfacial salivary films, upon the addition of SB was investigated. In addition, NaCl, pH 8.3 and pH 7.4 phosphate buffers were also tested to elucidate whether the interactions observed were a direct result of SB its high alkalinity or its ionic strength.

No differences in bulk viscosity between saliva samples containing 125 mM SB, 125 mM NaCl, pH 8.3 or pH 7.4 phosphate buffers were observed. This was an important observation of the study as it suggested that the key interaction affecting the lubrication of saliva containing SB was likely to be due to interfacial changes in the SCF (i.e. air/saliva interface or hydroxyapatite/saliva interface). In support of this supposition early work by Reeh et al. [38] highlighted that little correlation exists between the viscosity of certain solutions and their lubricating potential. It has also been observed in more recent work that saliva reduces the friction coefficient by two orders of magnitude compared to water [13]. This is particularly interesting considering that the bulk viscosity of saliva observed in this study resides around 0.002 Pa.s at a shear rate of 50 s^{-1}, which is in the same order of magnitude as that of water (0.001 Pa.s). In fact it is perhaps the unusual rheology of saliva (i.e. high elastic and low viscous modulus) that is responsible for the lubricious nature of the fluid. This would explain why a number of saliva substitutes, that match the viscosity of saliva, are unable to match the lubricating properties of human saliva, due to the difficulties in replicating the complex interfacial properties of whole saliva [39].

With this in mind, the elastic properties of the interface between air and saliva was probed. Unlike the bulk viscosity of saliva, clear differences were observed depending on the solution added to the saliva. For example, the addition of 125 mM NaCl and the 125 mM pH 8.3 phosphate buffer reduced the strength of the film by almost 2 orders of magnitude relative to the saliva control (from 0.98 N/m to ≈ 0.02 N/m). However, the addition of 125 mM SB to saliva was only able to reduce the strength of the film from 0.98 N/m to ≈ 0.2 N/m. Thus, 125 mM NaCl and 125 mM pH 8.3 PB significantly prevent a strong film from forming relative to the buffer control. However, the addition of 125 mM SB still appears to permit a film to from at the air/saliva interface, albeit at a lower elastic modulus relative to the control. This suggests that not only does the ionic strength of a solution impact the formation of the salivary film at the air interface, but also, that the type of salt will affect the formation of the film to different

magnitudes. For example, 125 mM NaCl was more effective at preventing film formation at the air/saliva interface than the same concentration of SB. An additional peculiarity observed was that the 125 mM pH 8.3 phosphate buffer was more effective at reducing the strength of the film at the air/saliva interface than 125 mM SB which also had a pH of 8.3. It appears that the impact of ionic strength and pH on the formation of the salivary film at the saliva/air interface was diminished when present as a bicarbonate salt relative to the 125 mM pH 8.3 phosphate buffer salt.

It is accepted that high concentrations of salt solutions can shield the positive/negative charges of proteins [14, 39, 40]and by doing so, either prevent proteins from reaching the air/saliva interface or from interacting with each other when they eventually reach the saliva/air interface. It was not possible form this work to determine exactly which of these processes was taking place but the results clearly show how the ionic concentration of a solution can prevent the formation of the film at the air/saliva interface. It may be that the degree to which the elastic strength of the salivary film is reduced may impact the degree to which the lubrication of the salivary film can be altered. The results showed a decrease in the strength of the film at the air/water interface upon the addition of SB to saliva, this reflected a small decrease in the traction coefficient at a load force of 2 N but not at 1 N. Consequently, it may be that the reduced formation of the film at the air/saliva interface in the presence of SB was not sufficient to alter significantly the lubrication properties of saliva, as one might expect, upon the disturbance of this film. However, correlating an interpretation between the two different instruments should be viewed with caution. Furthermore, overall lubrication is a function of the interplay between a number of physical properties of SCF, including the salivary film (pellicle) that forms at the hydroxyapatite/saliva interface as described below.

The salivary film (pellicle) that forms at the hydroxyapatite/saliva interface has an independent physical structure distinct from the salivary film that forms at the air/saliva interface. For example, while the film at the air/saliva interface is thought to be predominantly composed of statherin, the composition of the salivary pellicle that forms at the hydroxyapatite/saliva interface, is more diverse and complex, with presence of high-molecular weight mucins, amylase, cystatins, statherin and other acidic proline-rich proteins. This distinction is important as the types of proteins present in the respective interfaces (air/saliva or hydroxyapatite/saliva) potentially lubricate in different regimes. For example, statherin is thought to act as a boundary lubricant during dental contact [41, 42]whereas the salivary mucins present in the pellicle are thought to lubricate oral surfaces in the mixed regime [15]. Consequently, the interaction between a pre-adsorbed salivary pellicle and SB may be somewhat different. Therefore, the final step was to observe the displacement and structural changes to the pellicle that took place at the hydroxyapatite surface upon exposure to 0.5 M SB, 0.5 M NaCl, 0.5 M phosphate buffer pH 8.3 and a 0.1 M phosphate buffer control pH 7.4.

It was clear that SB displaced the largest quantity of salivary pellicle from the hydroxyapatite surface, whereas phosphate buffer pH8.3 removed the least. Curiously the pellicle at the hydroxyapatite surface was resistant to displacement by pH 8.3 buffer, despite it having a significant impact on the formation of the salivary film at the air/saliva interface. Furthermore, although the pH 8.3 buffer only displaced a negligible quantity of pellicle from the HA surface, it had a significant impact on its structure; becoming softer (relative to the control) after exposure to the pH8.3 buffer. This would suggest that pellicle displacement was independent of pellicle structure, and thus a reduction in pellicle hydrated mass did not always equate to a more rigid pellicle. Interestingly, Vijay et al. [43] also observed that the extensional rheology of saliva was significantly altered upon exposing saliva to increasing concentrations of sodium bicarbonate and sodium hydroxide.

The salivary pellicle is currently accepted as being formed of a dense basal layer, rich in low molecular weight proteins, and a more diffuse outer layer, rich in larger molecular weight mucins [44]. The cysteine residues within mucins participate in establishing disulphide bonds and are considered to play an important role in the ability of mucins to form gel like structures [17-19, 45]. Since, the pKa of the thiol group of cysteine is typically ~8.3 it could be that exposing the pellicle to a pH 8.3 buffer prevents the aggregation of mucins to form a gel like network, resulting in a more open, softer network. Evidence to support this supposition comes from work that describes how the aggregation of mucins is prevented via cysteine-specific enzymes or reducing agents such as dithiolthreitol[45, 46]. However, the addition of high concentrations of salt has also been observed to prevent the formation of gel like structures and therefore physical changes in the pellicle could also be triggered by electrostatic interactions, often involving carbohydrate side chains of mucins [47]. This would also explain why not only exposing the salivary pellicle to pH 8.3 buffer but also to 0.5 M NaCl increased pellicle softness, despite displacing negligible quantities of pellicle from the hydroxyapatite surface.

From this work it is possible to hypothesise that changes in the SCF observed in this study may directly influence lubrication and subsequently mouthfeel changes. However, these changes may only be the initial stages of structural alterations to the SCF. It is likely that the redevelopment of a salivary film or pellicle on top of the altered salivary film/pellicle may influence mouthfeel to a greater degree. For example, it has been shown that the salivary pellicle that reforms after re-exposure to fresh saliva may have a different physico-chemical structure relative to an undisturbed pellicle [48]. As the salivary pellicle coats all the surfaces of the mouth, including the mechanoreceptors, it is evident that any modulating effects to the pellicle structure could trigger a change in mouthfeel perception. Perhaps then, in terms of the oral cavity and

lubrication, the pellicle re-exposed to saliva, after undergoing a structural alteration, would be a more relevant system to explore in the future.

Conclusions

It is likely that interfacial rheology of saliva makes an important contribution to oral lubrication; and exposure to chemicals that can modulate the interfacial properties of saliva could also have implications for mouthfeel changes. SB not only impacts the preformed pellicle at the hydroxyapatite surface but it also reduces the strength of the film at the air saliva interface. It is likely that this phenomenon occurs via two ways: firstly, the ionic strength of SB shields electrostatic charges of the pellicle proteins, thus preventing protein crosslinking; secondly, the alkaline pH of SB reduces the gel-like structure of mucins present in the pellicle by disrupting disulphide bridging of mucins via the ionization of cysteine's thiol group. Curiously, no change in the bulk viscosity of saliva was observed in this study, which suggests that the complex interfacial properties of saliva play a significant role in lubricating the oral cavity.

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