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Protein–saliva interactions govern the structure and lubrication of salivary films

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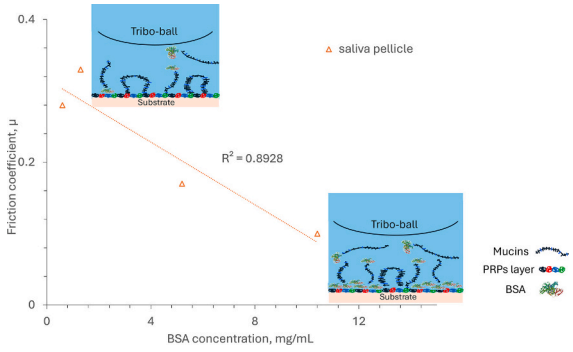
HIGHLIGHTS

- Dynamic Tribology Protocol shows increased protein reduces friction on saliva pellicle.
- Adsorbed mass and viscoelasticity confirm protein-saliva interaction.
- Impact of protein concentration on friction is not differentiated on whole saliva film.
- Dynamic Tribology Protocol enables probe into impact of salivary film layers.

GRAPHICAL ABSTRACT



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ABSTRACT

Hypothesis: Saliva is essential for oral lubrication and is influenced by interactions with foods, beverages and pharmaceuticals and their components. We hypothesize that increasing protein concentration increases protein adsorption and therefore reduces measured friction coefficient on the saliva coated tribopair.
Experiments: In this study, we employed our dynamic tribological protocol (DTP) to measure friction coefficient of model bovine serum albumin (BSA) solutions on *ex vivo* human whole saliva (HWS), saliva pellicle and proline-rich protein (PRP) components of the salivary film. This approach advances current methods that overlook saliva's complexity by testing on bare polydimethylsiloxane (PDMS) surfaces or using whole saliva. Quartz crystal microbalance with dissipation (QCM-D) monitored the mass and viscoelastic properties of these adsorbed layers.
Findings: We find that BSA concentrations >5.4 mg/ml correlate with decreased friction coefficients across bare PDMS and PDMS coated with the salivary pellicle and PRP layers, suggesting increased protein adsorption. This contrasts with friction measured with whole saliva, which showed no significant difference between samples from 0.6 to 10.4 mg/ml BSA concentration. QCM-D revealed substantial changes in the mass and viscoelastic properties of the adsorbed layers, highlighting a concentration-dependent interaction between BSA and salivary

Abbreviations: β-LG, β-Lactoglobulin; BSM, Bovine submaxillary mucin; BSA, Bovine serum albumin; DTP, Dynamic tribology protocol; HWS, Human whole saliva; MTM, Mini traction machine; PBS, Phosphate buffered saline; PDMS, Polydimethylsiloxane; PGM, Porcine gastric mucin; PRPs, Proline-rich proteins; QCM-D, Quartz crystal microbalance with dissipation; SDS, Sodium dodecyl sulphate; WPI, Whey protein isolate..

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proteins. These interactions suggest that BSA modifies the structural properties and enhances lubrication on the saliva pellicle, impacting oral processing of foods, beverages and pharmaceuticals. These findings expand understanding of salivary lubrication mechanisms and provide an enhanced method for investigating saliva-protein interactions. This offers insight into biophysical changes at oral surfaces during food and pharmaceutical intake, informing the design of products optimized for delivery, mouthfeel, and consumer satisfaction.

1. Introduction

Oral lubrication is a key function of saliva aiding both oral health and food consumption. By forming an adsorbed salivary protein film on oral surfaces, the excellent lubricity of saliva supports speaking, mastication, swallowing and taste perception [1–3]. During food or beverage consumption, the lubricity of saliva may be lost due to contact and interaction with food ingredients as well as changes in solution conditions, such as pH and ionic strength [4–6]. Protein components are known to influence both measured lubricity and mouthfeel of food and beverages [5,7–12]. However, the mechanism driving the loss of lubricity and the influence of protein concentration on lubrication is not clear. Here we aim to assess the influence of the concentration of model protein solution on the lubricity of saliva.

It is known that a remarkably low friction coefficient is achievable in the presence of an adsorbed film of human whole saliva measured *in vitro*. The highly lubricious salivary film is composed of a multilayered structure [4,13–16], which forms in less than 10 min [17–19]. This film is composed of the bulk saliva and adsorbed saliva pellicle, which in turn is composed of two parts. Firstly, a rigid layer of predominantly proline-rich protein (PRP) layer that tightly binds to the substrates and is resistant to detergent washing [14,20,21]. Secondly, the mucin-rich layer is able to bind to the PRP layer enhancing lubrication and load-bearing capacity of the adsorbed film [4,14,15]. In the absence of the PRP layer, the mucin layer on its own was poor in wear resistance [22]. Understanding how dietary or pharmaceutical proteins interact with these distinct salivary layers and which salivary layers provide differentiation in measured friction is therefore important to the field for determining changes in lubrication relevant to mouthfeel.

To measure the change in lubricity of saliva upon interacting with food and beverages, Bongaerts, et al. (2007) [23] introduced a novel tribological method to assess the lubricity of an *ex vivo* (*i.e.* expectorated) whole salivary film formed by adsorption of expectorated saliva onto a substrate. The Mini Traction Machine (MTM) with polydimethylsiloxane (PDMS) ball and disk tribo-surfaces with an adsorbed film of human whole saliva was used to measure the friction coefficient at a fixed low entrainment speed in the boundary regime. We focus on the boundary regime as it is well known that the surface chemistry, the interaction between the surface and the lubricant has more significant impact on boundary lubrication than in the mixed or hydrodynamic regimes [24]. This approach has been used to examine a variety of food and beverages including: tea with and without milk [9]; model dairy protein beverage [5], custard, yogurt and thickened cream [25], and on different substrates including porcine tongue, agarose gel and PDMS [5,8,26].

Another approach to including saliva in tribological measurement of food involves the *in vitro* or *ex vivo* formation of a bolus to assess the impact of saliva - food interactions on the lubricity *in vitro,* presented in the format of a 'Stribeck curve' [27,28]. The *in vitro* method with a bolus is not sensitive to variation in lubricity. As demonstrated by Campbell, Foegeding and Van De Velde (2017) [27], who showed that by measuring friction as a function of entrainment speed, known as a 'Stribeck curve', on the expectorated bolus the difference in lubrication in the presence of saliva cannot be discriminated between the model dairy protein (whey protein isolate, WPI) beverages. This method is different from the *in vivo* oral environment, where an adsorbed saliva film is present on oral surfaces. Further details of this field can be found in reviews by Pradal and Stokes (2016) [29], Sarkar and Krop (2019) [30] and Shewan, et al. (2019) [31] who discuss a range of tribological protocols utilizing *ex vivo* saliva. Although the interactions and lubricity of whole saliva is reviewed, the multilayer structure of saliva film is not considered despite its importance in oral lubrication [32,33]. This disconnection between the experimental measurements and the complex *in vivo* salivary film further motivates the need for methodologies that can probe individual salivary layers.

Recent studies by Wooster, et al. (2023) [34,35] examined the interactions between saliva and protein stabilized emulsions using a novel oral microscope. They propose that droplets interact with the salivary film and oral surfaces through protein adsorption and saliva displacement, dependent on the protein charge. They suggest that both dairy and plant proteins can also modify lubrication due to creation of large aggregates of emulsion droplets that further interact with saliva coated papillae, thereby affecting mouthfeel. Their observations *in vivo* indicate that competitive adsorption and restructuring of the salivary film are key factors. However, the role of protein concentration alone, particularly within specific salivary layers, remains unclear. This motivates the present study, which uses tribology and quartz crystal microbalance with dissipation to provide insight into salivary multi-layers to examine how protein concentration influences adsorption, structural changes, and lubrication.

Studies have been carried out to investigate the impact of β-lactoglobulin (β-LG) protein concentration on friction of saliva (or mucin) coated substrates in acidic beverages. Vardhanabhuti, Cox, Norton and Foegeding (2011) [5] showed that friction on an adsorbed human whole saliva film increased rapidly after adding β-LG (0 to 4%) at low pH 3.5, which corresponded to astringency. But increasing β-LG concentration did not alter the rate of increase in saliva lubricity. The authors highlight that a static lubrication study may not capture the complexity of the dynamic oral environment. The study of Çelebioğlu, Gudjónsdóttir, Chronakis and Lee (2016) [8], found that lower friction coefficient was measured with higher concentration of β-LG-Porcine Gastric Mucin (PGM) mixture at low pH, but the protein concentration did not influence the lubricity of β-LG-PGM mixture at neutral pH. Their investigation showed that the β-LG dominated the surface adsorption after interacting with the mucins. These studies only use one fraction of saliva or do not consider the multiple-layer structure of saliva film, which limits that ability to determine the mechanism of action that drives lubricating behavior of saliva in the presence of protein. Therefore, a systematic investigation that considers both protein concentration and the distinct salivary film structure is required to clarify these effects.

Bovine serum albumin (BSA) is a component of cow's milk, which occurs at 6 wt% of whey protein [36]. BSA is used as a food additive because of its emulsifying properties [37], and is also a versatile carrier for pharmaceuticals because of its high stability, water solubility and easy accessibility [38]. BSA has been widely used as a model protein to investigate adsorption behavior in drug delivery [39,40] and tribological behavior in artificial joint applications [41,42], but there is little research into the influence of BSA on lubricity of saliva. In this study, we use BSA solution with concentrations ranging from 0.6 to 10.4 mg/ml as a model protein at neutral pH conditions to investigate the influence of the protein concentration on the lubricity of *ex vivo* saliva pre-adsorbed onto a PDMS substrate. In addition to investigating the effect of BSA concentration on interaction with saliva, novelty is introduced by using the Dynamic Tribology Protocol (DTP) with *ex vivo* saliva prepared in three ways to expose the influence of the whole saliva film, saliva pellicle and the detergent resistant PRP layer on the lubrication response

of salivary layers. Quartz Crystal Micro-balance with Dissipation (QCM-D) is used to characterize the structure change of the saliva film after exposure to BSA solutions. By combining structural and tribological measurements, this study tests whether protein concentration drives competitive adsorption and changes in salivary film structure.

2. Materials and methods

2.1. Sample preparation

Bovine serum albumin (BSA, ≥98%, Sigma-Aldrich, Co., USA) is dissolved at 50 °C in 70 mM phosphate buffered saline (PBS, pH 7.4, without calcium and magnesium, MP Biomedicals, LLC,USA) at four concentrations: 0.6 mg/ml (the concentration of BSA in skim milk [43]), 1.3 mg/ml (the average protein level in saliva [44]), 5.2 mg/ml and 10.4 mg/ml. 70 mM was chosen because the structure of saliva film is influenced by ionic strength [4,45] and this ionic strength is close to the upper limit of saliva's natural variation in-mouth [45,46]. Ultrapure water was used in all experiments. Protein samples prepared from powder were stored in the fridge for 12 h to ensure they were completely rehydrated prior to measurement.

2.2. Collection of human whole saliva

Acid stimulated human whole saliva (HWS) was used in this study to provide a lower friction and a more stable saliva film than mechanically stimulated saliva [19]. The saliva collection method was the same as that used in our previous study [19]. Briefly, this process required the donor to abstain from food and beverages, except water, for 90 min prior to saliva collection. The donor expectorated after at least 30 s rinsing their mouth with water. Then twice 2 ml of lemon juice (99.9% lemon juice, Coles Own Brand, Australia) was used to swill around the mouth for 15 s and expectorated, and a further expectoration to waste continued for 30 s. A sterile Nylon mesh cell strainer (40 μm, BIOLOGIX) was used to collect saliva and remove the food debris and cells. Then the saliva was stored below 4 °C for no more than 30 min until used. To minimise biological variability, the saliva from a single 40 year old, male donor was used in this study.

2.3. Tribology measurement

2.3.1. 'Stribeck curve' (speed-dependent friction)

Friction coefficient is measured on the Mini Traction Machine (MTM, PCS instruments LTD, U.K.) using the method from Bongaerts, Rossetti and Stokes (2007) [23]. The measurement temperature was 35 °C, which is the average surface temperature of the oral mucosa. Polydimethylsiloxane (PDMS) was used to form soft rubbing contacts, consisting of a disk (diameter 22.5 mm, thickness 4 mm) and ball (radius of 9.3 mm). PDMS, (Sylgard 184, Dow Corning) was cured at 65 °C with base to curing agent ratio at 10 to 1 by mass. 1 N normal load (W) was applied between the rubbing surfaces. During the measurement, entrainment speed ($(U = U_{ball} + U_{disk})/2$) varied from 0 to 1000 mm/s at logarithmic intervals. Slide-to-roll ratio of 50% was used where $SRR = |U_{ball} - U_{disk}|/U$, and U_{ball} is the surface velocity of the ball and U_{disk} is the surface velocity of the disk. The friction coefficient (μ), defined as the quotient of friction force (F) and load ($\mu = F/W$), was measured and averaged five times at every speed, and the measurement was repeated in triplicate for each sample.

2.3.2. The time-dependent friction (dynamic tribology protocol)

The lubrication responses of BSA solutions in contact with pre-adsorbed saliva layers (saliva pellicle and PRP layer) on PDMS surfaces were measured with the protocol modified from our previous study [19]. In order to observe the transitory lubrication behavior of BSA proteins in contact with the saliva pellicle and detergent-resistant PRP layer respectively, a step was added following the HWS film formation on the substrate and before the sample addition. In the additional step, there were two options: 'A' for lubrication on the saliva pellicle, and 'B' for lubrication on the PRP layer. The sequence steps are summarised below, with the full description given in Fan, Shewan, Smyth, Yakubov and Stokes (2021) [19]:

1. Friction coefficient of dry PDMS on PDS (no lubricant) (1 min).
2. 50 ml water added to the pot to measure wet friction coefficient (2 min) before draining the water from the PDMS disk surface. Excess water remained under the tribo-pair to maintain humidity.
3. A thin track of saliva film was formed and adsorbed on the disk by pipetting 300 μl of HWS on to the disk in front of the contact. After saliva addition, friction coefficient was measured (10 min).
4. **A.** To form the **saliva pellicle**: the saliva-coated substrate was rinsed with 150 ml PBS solution to remove the bulk, non-adsorbed components of HWS. The 'baseline' friction coefficient was then measured at 1 N in 50 ml PBS (5 min). After measurement, all PBS was drained.

   B. On the **detergent-resistant PRP layer**: after rinsing the HWS saliva film with 150 ml of 70 mM PBS solution, 100 ml of 10 mM sodium dodecyl sulphate (SDS) was used to rinse the saliva pellicle and 50 ml of SDS was used to submerge the tribo-pairs for 1 min *in situ*. SDS is a highly surface-active anionic surfactant, which was previously shown to remove the hydrated part of the salivary pellicle (mainly mucins), leaving stiff and tightly bound small molecular weight protein layer (mainly PRP) [33,47]. 150 ml of PBS solution was then used to rinse off all SDS and de-adsorbed mucins, and the friction coefficient was measured as 'baseline' of PRP layer in 70 mM PBS solution for 5 min.
5. 50 ml protein sample was added and friction measured (10 min).

The procedure for measuring friction coefficient on whole saliva film (saliva pellicle + bulk saliva, which was used in many previous studies [5,9,25]) does not include step 4, as preliminary experiments showed that there was no difference in measured lubricity observed on the whole saliva film between BSA solutions with different concentrations (results are shown in the supplementary document Table S1). Three repeats were conducted for all measurements at 35 °C and SRR of 50% with a constant rotational speed of 5 mm/s and 1 N load. The statistical significance of the DTP results was evaluated with a one-way analysis of variance (ANOVA) by SPSS Statistics (IBM Corp., Version 26).

2.4. Lubrication on bare PDMS

The time-dependent friction coefficient was also measured with BSA solutions on bare PDMS surfaces for 10 min after 4 min friction coefficient measurement with 70 mM phosphate buffered saline (PBS) solution to observe the lubrication behavior. Three repeats were conducted for all measurements at 35 °C and SRR of 50% with a constant rotational speed of 5 mm/s and 1 N load.

2.5. Rheology measurement

A stress-controlled rheometer (AR-G2, TA Instruments, U.K.) was used with a solvent evaporation trap to measure the flow curves of BSA samples. The geometry of 60 mm stainless steel 1° cone-and-plate was chosen for a stress-controlled measurement from 0.01 to 1 Pa at 35 °C (60 s equilibration time). Each sample was measured in triplicate. Samples removed from 4 °C storage were equilibrated for 30 min at room temperature (around 25 °C) prior to the measurement.

2.6. Quartz crystal micro-balance with dissipation (QCM-D) measurement

The mass and viscoelastic property change of the adsorbed film was monitored by the QCM-D (Q-Sense™, model E4, Biolin Scientific, Swe-

den). QCM-D is a sensitive tool to detect the alteration in the resonance frequency and dissipation of the oscillation energy, by alternately switching on and off the driving voltage of the piezoelectric quartz resonator over time. The resonance frequency and decay of the oscillation amplitude are measured when the voltage is switched on and off respectively, and two parameters (frequency change (Δf) and dissipation change (ΔD)) are yielded per odd overtone up to 13th. Dissipation is given by $D = \frac{1}{\pi} f \tau$, using the oscillation frequency (f) and the time constant of the decay (τ).

The adsorbed film is modelled as a Voigt viscoelastic element with a complex elastic modulus (G_f) as:

$$G_f = \mu_f + i2\pi f \eta_f = G'_f + G''_f \quad (1)$$

determined from the film shear elastic modulus (μ_f), frequency (f), film viscosity (η_f), and apparent storage (G'_f) and loss modulus (G''_f) of the film. The expressions for signal changes due to film adsorption in liquid media can be characterised as:

$$\frac{\Delta f}{f} = -\frac{h_f \rho_f}{h_0 \rho_0}\left\{1 - \eta_f \rho_b \times \frac{(\eta_f/\rho_f)\omega^2}{{\mu_f}^2 + \omega^2 {\eta_f}^2}\right\} \quad (2)$$

$$\Delta D = \frac{h_f}{h_0 \rho_0}\left\{\eta_b \rho_b \times \frac{\mu_f \omega}{{\mu_f}^2 + \omega^2 {\eta_f}^2}\right\} \quad (3)$$

where $\omega = 2\pi f$, density of quartz crystal (ρ_0), bulk liquid viscosity (η_b), bulk density (ρ_b) (here input 0.74 mPa.s and 1.001 g/cm^3 for PBS bulk liquid viscosity and density respectively), adsorbed film thickness (h_f), film density (ρ_f), film shear elastic modulus(μ_f) and film viscosity (η_f) [48,49]. Here, $h_f$is obtained from the fit to Voight model and ρ_f is chosen to be 1150 kg/m^3 according to the previous study [45]. The sensed mass (m_{Voigt}) was obtained from:

$$m_{Voigt} = h_f \rho_f \quad (4)$$

This technique has previously been used to detect the mass and viscoelastic property changes of adsorbed saliva film [33,45].

Gold sensors (Testbourne Ltd., UK) with a fundamental resonance frequency of 4.95 MHz were coated with polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) to ensure that the substrate used was the same for both QCM-D and tribology experiments. The method for PDMS spin coating onto QCM-D sensors is derived from that used by Macakova, Yakubov, Plunkett and Stokes (2010) [45]. The specific method used here to get a consistent thickness of the PDMS coating layer, required 15 μl of 5% *w*/w PDMS-toluene solution for spin-coating onto cleaned gold QCM-D sensors in the spin coater (S.P.S. Vertriebs GmbH, Germany) with 3 s acceleration to 3000 rpm and maintain at this speed for 15 s. The coated PDMS layers were cured in an oven at 120 °C overnight. The thickness of this hydrophobic PDMS film is 3–5 nm as measured by spectroscopic ellipsometer (α-SE™, J.A. Woollam Co., NE).

During QCM-D measurement, the flow rate was maintained by a peristaltic pump (Ismatec IPC-N 4, Cole-Parmer GmbH, Germany) at 200 μl/min, and temperature was set at 35 °C. To ensure the cleanliness of the sensor surface prior to experimentation, ultrapure water and 1% SDS solution were alternated in the washing process until the measured frequency and dissipation of ultrapure water before and after wash were at the same level. The fluid injection sequence was similar to the steps followed in the DTP measurement as shown in Table S2 with the addition of a final PBS rinse step to enable direct comparison with the PBS baseline. The changes in thickness and viscoelastic properties of the adsorbed salivary layers before, upon BSA addition and after BSA rinse were modelled using a Voigt viscoelastic model fitting with commercially available software, Dfind (Q-Sense®, Biolin Scientific, Sweden).

3. Results and discussion

3.1. Tribology measurement with BSA solutions

3.1.1. 'Stribeck curve' (speed-dependent friction)

The 'Stribeck curve' is presented in Fig. 1 for each BSA concentration measured on the PDMS tribo-pair in the absence of saliva. For all solutions, the boundary regime and mixed regime are observed in the 'Stribeck curve' within the entrainment speed range of 0 to 1000 mm/s. The 'Stribeck curve' for PBS buffer was not significantly different from water, which indicates the PBS buffer did not significantly change any lubricating property of the tribo-pair. All the 'Stribeck curves' of BSA solutions were lower than those of water and PBS buffer, and the friction coefficient decreased with increasing BSA concentration in the boundary regime, as shown by the friction coefficient at 5 mm/s entrainment speed in Table 1. That indicates that the BSA adsorbs on the substrate and contributes to lubrication of the tribo-pair. We hypothesize that the increased BSA concentration enables more BSA protein to adsorb on the tribopair surface, which will be investigated further using QCM-D in '*Surface property change monitored by QCM-D', below.*

3.2. The time-dependent friction (dynamic tribology protocol)

The measured friction coefficient on the saliva pellicle, detergent-resistant PRP layer (*i.e.* SDS-washed pellicle, as described in methods - time-dependent friction (dynamic tribology protocol), step 4B) and bare PDMS are plotted against time in Fig. 2. Fig. 2 A shows the friction coefficient plotted against time for four BSA solutions on saliva pellicle across all 5 steps of the dynamic tribology protocol (DTP). When saliva was added to the PDMS substrate, the friction coefficient sharply dropped from around 1.6 to less than 0.1 because of the formation of an adsorbed saliva film, which provided extreme lubrication to the tribo-surfaces. The adsorbed film remained stable on the PDMS substrate as shown by the horizontal curve of measured friction coefficient against time up to 780 s. After the adsorbed whole saliva film was rinsed by PBS buffer and PBS was added to the chamber, the bulk saliva was removed and there was only saliva pellicle remaining on the PDMS substrate. The duration after PBS addition and before BSA solution addition is referred to as the PBS 'baseline'. The adsorbed saliva pellicle in PBS buffer provides an exceptionally low measured friction coefficient (μ) in the range of 0.05 to 0.07. Since the 70 mM PBS buffer did not change the structure of the saliva pellicle [33], this slight increase in friction coefficient is caused by the wear of the adsorbed pellicle on the tribo-pair. As indicated in Fig. 2, $\mu_{BSA\text{-}start}$ is the friction coefficient when BSA was added,

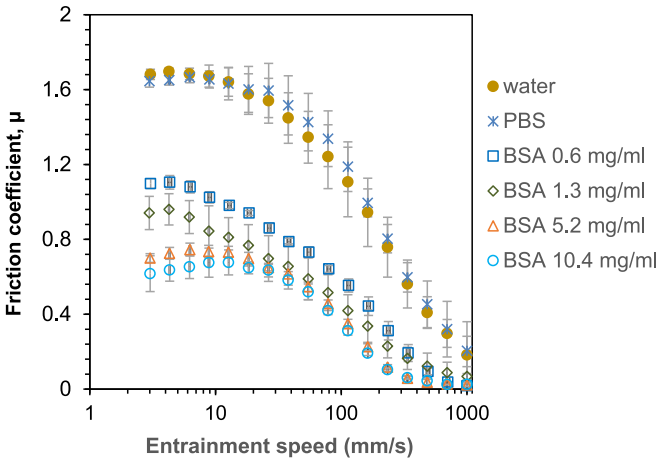

Fig. 1. The 'Stribeck curve' (speed-dependent friction) of BSA solution at four concentrations of 0.6 mg/ml (□), 1.3 mg/ml (◊), 5.2 mg/ml (Δ) and 10.4 mg/ml (○), water (●) and PBS buffer (Ж) are for reference. Curves show an average of three measurements.

Table 1
The comparison of friction coefficient of 'Stribeck curve' at 5 mm/s entrainment speed and viscosity between BSA solutions at four concentrations. Three repeats were completed for each measurement.

	Friction coefficient of 'Stribeck curve' at 5 mm/s	η, viscosity (mPa.s)	n, power law index of viscosity
0.6 mg/ml	$1.09^{b} \pm 0.03$	0.83 ± 0.02	0.96 ± 0.01
1.3 mg/ml	$0.94^{c} \pm 0.08$	0.83 ± 0.02	0.96 ± 0.02
5.2 mg/ml	$0.74^{d} \pm 0.03$	0.85 ± 0.03	0.93 ± 0.04
10.4 mg/ml	$0.64^{d} \pm 0.06$	0.91 ± 0.07	0.93 ± 0.05
PBS	$1.66^{a} \pm 0.03$	0.71 ± 0.01	1.00 ± 0.01

Remark: The data is presented in mean value ± standard deviation. $^{a–d}$ at column of Friction coefficient of 'Stribeck curve' at 5 mm/s, every data point with same superscript is not significantly different to other data points with same letters (Tukey's HSD test; $p < 0.05$).

and $\mu_{BSA\text{-}end}$ is the friction coefficient at the end of measurement with BSA. After addition of the BSA solution, the friction coefficient slightly increased but remained low, $\mu_{BSA\text{-}start} < 0.14$, as shown in Table 2. At the end of these measurements, the friction coefficient of BSA solution at 10.4 mg/ml $\mu_{BSA\ end} = 0.10 \pm 0.02$ is the lowest, compared to BSA solution at 0.6 mg/ml and 1.3 mg/ml where $\mu_{BSA\ end} = 0.28 \pm 0.08$ and 0.33 ± 0.11 respectively, which are the highest values with no significant difference between them. The friction coefficient of BSA solution at 5.2 mg/ml is in the middle. Where PBS without BSA solution was added measured friction at $\mu_{BSA\ end}$ was between 1.3 mg/ml and 5.2 mg/ml, as shown in supplementary Fig. S1. This suggests that at higher BSA concentrations the deposition of BSA on the saliva coated surface is sufficient to overcome the removal of saliva due to wear between the ball and disk.

Fig. 2 B shows the measured friction coefficient for the BSA solutions on the PRP layer. Friction coefficient measured with PBS buffer until BSA addition is referred to as the PBS 'baseline'. It has been shown that the mucins are removed by SDS from the saliva pellicle and only the PRP layer remains adsorbed on the PDMS substrate [33]. The measured friction coefficient of the PBS 'baseline' suggests that the lubrication ability of the PRP layer is poor because the friction coefficient sharply increases from less than 0.1 to around 1.2, which is close to the friction coefficient measured with water on the tribo-pair (around 1.4). The lubricity of this PRP layer is significantly less than the whole saliva or saliva pellicle, which is consistent with the results of the previous study [33]. After BSA is added to the PRP layer, the friction coefficient suddenly drops from around 1.2 to less than 1.0, see Table 2. This suggests that the BSA contributes to the lubrication between the PDMS tribo-surface coated with the PRP layer. For the four BSA solutions, the friction coefficient on this layer decreases against time and the slope of the friction coefficient becomes increasingly negative in the sequence of 10.4 mg/ml > 5.2 mg/ml > 1.3 mg/ml > 0.6 mg/ml. This suggests that BSA interacts with the PRP layer and/or PDMS substrate, which contributes to the lubrication of the tribo-pair. Lubricity increases with increasing BSA concentration under these experimental conditions.

Fig. 2 C shows the measured friction coefficient on bare PDMS. It presents the same lubrication trend with BSA concentration as that on the PRP layer but with slightly higher friction coefficient (see Table 2). As expected, the results for friction coefficient, in Fig. 2 C, at the end of measurement with BSA on bare PDMS are similar to that found from the 'Stribeck curve' (Fig. 1) in boundary regime at entrainment speed of 5 mm/s for each BSA concentration (listed in Table 1). Comparing the $\mu_{BSA\text{-}end}$ on bare PDMS with that on PRP layer (Table 2) shows the friction coefficient is slightly lower in the presence of the PRP layer than with bare PDMS. Although this difference is not statistically significant, the consistent trend suggests a small contribution to lubricity from the PRP. which is consistent with the previous study [33]. In their study, the PRP layer also presents limited lubrication ability. The results here also suggest that the lubrication with BSA solutions on PRP layer is dominated by the surface property of bare PDMS.

To ensure that the effect of saliva variability was minimized, the change in lubrication ($\Delta\mu_{BSA} = \mu_{BSA\text{-}start} - \mu_{BSA\text{-}end}$), was calculated. It was found that the lubrication change ($\Delta\mu_{BSA}$) after BSA sample addition, increases with increasing concentration on saliva pellicle, and positively correlates with the concentration in a linear relationship on PRP layer and bare PDMS ($R^2 = 0.94$ and 0.99 respectively), as shown in Fig. 3.

Comparing the lubrication response of these four BSA solutions on different surfaces, $\mu_{BSA\text{-}end}$ is in a sequence, where friction coefficient of bare PDMS > PRP layer > saliva pellicle. The lowest $\mu_{BSA\text{-}end}$ is obtained from the saliva pellicle suggesting that the presence of mucins is important to the lubrication of saliva pellicle. This is in agreement with Yakubov, Macakova, Wilson, Windust and Stokes (2015) [33] who show that mucins provide the most significant contribution to the lubricity of saliva. The protein structure may also contribute to the lubricity of different protein induced layers. When BSA solution was added to the PRP layer, a slight increase in lubricity was observed but not nearly as much as that of mucins on PRP layer (saliva pellicle). BSA has a globular structure [40,50], but mucins with a brush-like structure maintain hydration and prevent surfaces from rubbing [4,14]. On saliva pellicle, PRP layer and bare PDMS, the $\mu_{BSA\text{-}end}$ decreases with the increasing the concentration of BSA solutions. The result suggests that with increasing BSA concentration the mass and thickness of the adsorbed BSA layer increases leading to a lower friction coefficient. The mass and thickness of the adsorbed layer was measured by the quartz crystal microbalance with dissipation (QCM-D). Increasing solution viscosity with increasing BSA concentration may also play a role.

3.3. Viscosity of BSA solutions

BSA samples display a power-law index ranging from 0.93 to 1.00 indicating Newtonian behavior, and fall within a small viscosity range (0.83 to 0.91 mPa·s as shown in Table 1) suggesting that bulk viscosity is not likely to contribute to differences in measured friction coefficient, so that the viscosity is calculated as an average of all data points for three repeats in the shear rate range of 30–260 s^{-1}. The viscosity of BSA solutions with concentrations of 0.6 mg/ml, 1.3 mg/ml and 5.2 mg/ml are similar and slightly lower than that of BSA at concentration of 10.4 mg/ml.

3.4. Surface property change monitored by QCM-D

Representative plots showing the measured frequency change (Δf) and dissipation change (ΔD) (5th, 7th, 9th overtones) as a function of time for the pre-adsorbed saliva layers (saliva pellicle and PRP layer) and bare PDMS are presented in Fig. 4, repeats are shown in Fig. S2.

All measurements start with water followed by PBS injection where Δf drops and ΔD rises subtly. Fig. 4 A and B show the change of Δf and ΔD throughout the measurement process with the saliva pellicle (formed at step 3) and the BSA (added at step 5) at 1.3 mg/ml and 10.4 mg/ml respectively. Before the injection of BSA solution at step 5, there is no statistically significant difference in the measured value of Δf and ΔD. In step 3, a sharp decrease of Δf and rapid increase of ΔD occurred, indicating formation of a soft viscoelastic saliva film on the substrate [45,51]. When 70 mM PBS was injected to rinse the adsorbed saliva film in step 4 (PBS baseline), Δf increased and ΔD decreased slightly due to the removal of non-adsorbed bulk phase saliva. When the BSA was injected in step 5, Δf increased and ΔD decreased more for 10.4 mg/ml BSA solution than for 1.3 mg/ml BSA solution. An overshoot in Δf was observed for 10.4 mg/ml BSA, which may be indicative of surface protein rearrangement, as previously observed for β-casein on polystyrene coated gold sensors but not observed for rigid BSA [40]. Given the

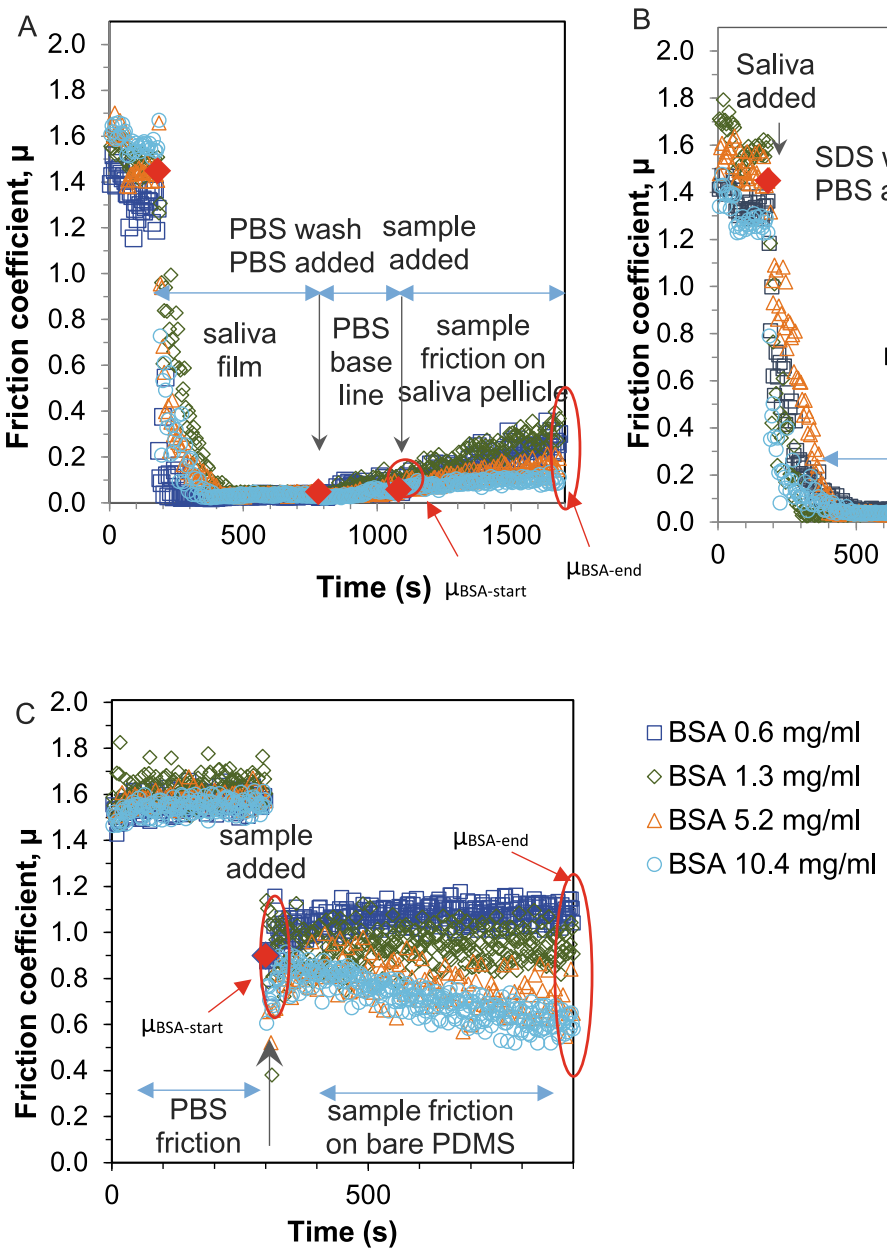

Fig. 2. *The friction coefficient, μ, against time of BSA samples at 5 mm/s entrainment speed, 35 °C with the concentration of 0.6 mg/ml (□), 1.3 mg/ml (◇), 5.2 mg/ml (Δ) and 10.4 mg/ml (○). A is the result on saliva pellicle, B is the result on PRP layer, C is the result on bare PDMS. Data points are an average of three measurements. Uncertainty is calculated and shown for key data points in* Table 2.

absence of this overshoot on the PRP layer or bare PDMS, Fig. 4 D and E, and the absence of the overshoot for BSA on bare polystyrene [40] it is likely that this rearrangement is driven by saliva protein-BSA interactions and BSA adsorption on the surface.

After the saliva pellicle was rinsed by PBS to remove the bulk saliva, in step 6 (PBS final rinse), Δf increased and ΔD decreased more for 10.4 mg/ml BSA solution than that for 1.3 mg/ml BSA solution. This result indicates that more interaction occurs between saliva components of the saliva pellicle and BSA at the highest concentration (10.4 mg/ml) than at the lower BSA concentration (1.3 mg/ml).

Fig. 4 C and D present the change of Δf and ΔD for the samples of BSA at 1.3 mg/ml and 10.4 mg/ml with the PRP layer. During the saliva film formation (step 3) and bulk saliva removal (step 4), the change of Δf and ΔD follow the same trend as that in Fig. 4 A and B. When 10 mM SDS was injected in step 5, both Fig. 4 C and D shows that Δf rose and ΔD decreased until reaching an equilibrium. At step 6 (PBS baseline), after PBS was injected, Δf increased and ΔD decreased quickly and reached a plateau close to the zero baseline in step 1, indicating that some components of the adsorbed saliva layer, mostly mucins, were removed leaving a rigid adsorbed layer. This is in agreement with previous work, which showed that SDS removed the large hydrated molecular components responsible for high dissipation, mainly mucins, and left the tightly adsorbed stiff proteinaceous PRP layer with low dissipation [33,47]. At step 7, when 1.3 mg/ml BSA was injected (Fig. 4 C), Δf increased and ΔD decreased slightly and remained largely consistent into step 8 after rinsing with PBS. After the PBS final rinse at step 8, Δf increased to a higher level and ΔD decreased to a lower level than that before the addition of BSA on the PRP layer. The change of Δf and ΔD for BSA at 10.4 mg/ml in step 7 and step 8, shown in Fig. 4D, is more significant than that for BSA at 1.3 mg/ml in Fig. 4 C, indicating that more BSA participates in the interaction with the PRP layer at a higher concentration.

Table 2
The comparison of friction coefficient of BSA at 4 concentrations on saliva pellicle, PRP layer and bare PDMS. Three repeats were completed for each measurement.

		$\mu_{PBS\ end}$	$\mu_{BSA\ start}$	$\mu_{BSA\ end}$	$\Delta\mu_{BSA}$
Saliva pellicle	0.6 mg/ml	0.07 ± 0.01	0.09 ± 0.03	0.28^{ab} ± 0.08	−0.19
	1.3 mg/ml	0.09 ± 0.02	0.11 ± 0.02	0.33^{a} ± 0.11	−0.22
	5.2 mg/ml	0.07 ± 0.02	0.08 ± 0.02	0.17^{c} ± 0.09	−0.09
	10.4 mg/ml	0.07 ± 0.03	0.06 ± 0.02	0.10^{d} ± 0.03	−0.04[3]
	PBS	0.09 ± 0.02	0.10 ± 0.02	0.22^{bc} ± 0.05	−0.12
PRP layer	0.6 mg/ml	1.17 ± 0.01	0.93 ± 0.05	0.91^{a} ± 0.04	0.02
	1.3 mg/ml	1.21 ± 0.06	0.93 ± 0.04	0.88^{b} ± 0.06	0.05
	5.2 mg/ml	1.22 ± 0.04	0.85 ± 0.03	0.66^{c} ± 0.06	0.19
	10.4 mg/ml	1.14 ± 0.02	0.77 ± 0.05	0.51^{d} ± 0.11	0.26
Bare PDMS	0.6 mg/ml	1.58 ± 0.02	1.00 ± 0.05	1.09^{a} ± 0.03	−0.09
	1.3 mg/ml	1.63 ± 0.07	0.92 ± 0.10	0.96^{b} ± 0.05	−0.04
	5.2 mg/ml	1.58 ± 0.02	0.76 ± 0.06	0.69^{c} ± 0.10	0.07
	10.4 mg/ml	1.56 ± 0.03	0.83 ± 0.07	0.61^{d} ± 0.03	0.22

Remark: the friction coefficient data is presented in mean value ± standard deviation. $^{a–d}$ within $\mu_{BSA\ end}$ grouped by surface, every data point with different superscript is significantly different to other data points with different letters (Tukey's HSD test; $p < 0.05$) . $\Delta\mu_{BSA} = \mu_{BSA\ start} - \mu_{BSA\ end}$.

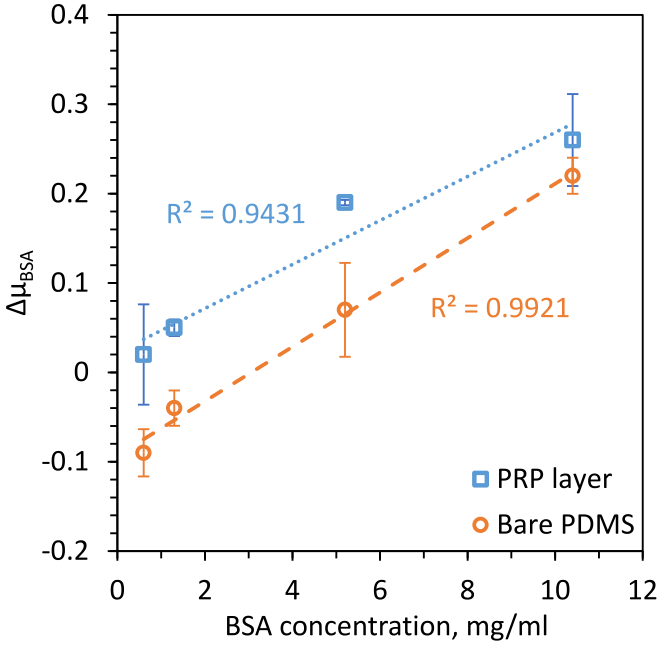

Fig. 3. The correlation between the change in friction coefficient ($\Delta\mu_{BSA} = \mu_{BSA\text{-}start} - \mu_{BSA\text{-}end}$), $\mu_{BSA\text{-}start}$ is the friction coefficient when BSA was added and $\mu_{BSA\text{-}end}$ is the friction coefficient at the end of measurement with BSA, and concentration of BSA samples on the PRP layer (□) and bare PDMS (○). Data points are an average of three repeats.

Fig. 4 E and F show the change of Δf and ΔD for BSA at 1.3 mg/ml and 10.4 mg/ml on bare PDMS substrate. After injection of BSA in step 3, Δf decreases sharply and ΔD increases, and slightly recovers after the PBS injection in step 4 (PBS final rinse), and the recovered level of Δf and ΔD with 10.4 mg/ml BSA solution is higher than that with 1.3 mg/ml. The final ΔD in Fig. 4 F is similar to that in Fig. 4 E, and the final level of Δf in Fig. 4 F is lower than that in Fig. 4 E indicating that more BSA is adsorbed to the substrate when a higher concentration of BSA is available for adsorption, see the mass data in Table 3 on bare PDMS. After PBS rinse, 3.60 ± 0.33 mg/m^2 remains with sample of 1.3 mg/ml BSA solution and a higher mass of 4.18 ± 0.14 mg/m^2 for 10.4 mg/ml BSA solution. The mass and shear modulus of the adsorbed layer on PDMS substrate, obtained by fitting the Voigt viscoelastic model as described in the method of Quartz Crystal Micro-balance with Dissipation (QCM-D) measurement, are summarised in Table 3.

For BSA solution at 1.3 mg/ml, the mean value of mass adsorption on bare PDMS is 3.6 mg/m^2, which is consistent with the result in previous study (3.8 mg/m^2) with 1 mg/ml BSA solution on hydrophobic polystyrene surface [40]. The adsorbed mass of BSA solution at 10.4 mg/ml on bare PDMS is 4.18 mg/m^2, which is higher than that of 1.3 mg/ml BSA solution, which supports our hypothesis that the decreased friction coefficient in the boundary regime on bare PDMS, as shown in the tribology measurement result of 'Stribeck curve' (speed-dependent friction) and the time-dependent friction (dynamic tribology protocol), may be attributed to the increase of BSA adsorption on PDMS substrate at higher BSA concentration.

As shown in Table 3, on saliva pellicle, for the 1.3 mg/ml BSA solution, the mass of pre-adsorbed saliva pellicle decreased from 16.22 ± 1.52 mg/m^2 at step 4 (PBS baseline) to 13.56 ± 0.78 mg/m^2 after injection of BSA solution at step 5. It maintained the same level (13.22 ± 0.32 mg/m^2) at step 6 (PBS final rinse). For 10.4 mg/ml BSA solution, the mass of pre-adsorbed saliva pellicle also decreased from 16.87 ± 0.21 mg/m^2 at step 4 (PBS baseline) to 13.68 ± 0.06 mg/m^2 after injection of BSA solution at step 5 and further reduced to 12.44 ± 0.01 mg/m^2 at step 6 (PBS final rinse). The reduction of mass suggests that the interaction between BSA and saliva pellicle increased with the increased BSA concentration and removed some components of the saliva pellicle.

On the PRP layer, the mass value of the PBS 'baseline' at step 6 is 7.52 ± 1.28 mg/m^2, which was measured after rinsing with 10 mM SDS solution and PBS buffer, that is 50% lower than that of the saliva pellicle (at step 4, PBS baseline, 16.22 ± 1.52 mg/m^2 for 1.3 mg/ml BSA solution and 16.87 ± 0.21 mg/m^2 for 10.4 mg/ml BSA solution), indicating the highly hydrated mucin-rich layer was removed by SDS. The removed part includes mucins and or their complexes with PRP, statherin and other salivary proteins [52,53]. Comparing the mass and modulus data of the adsorbed PRP layer on the substrate before BSA injection (step 6, PBS baseline) and after BSA removal by PSB rinse (step 8, PBS final rinse), the mass loss is 0.75 ± 0.07 mg/m^2 and 1.39 ± 0.73 mg/m^2 for 1.3 mg/ml and 10.4 mg/ml BSA solution respectively, which suggests that the interaction between BSA and PRP layer also increased with the BSA solution concentration and removed some components of the saliva pellicle.

The change in mass and modulus suggests that an interaction occurs between BSA, saliva pellicle and the PRP layer. This interaction may occur as a hydrophobic interaction between BSA, which is hydrophobic at neutral pH [54], mucins, which have both charged and polar fractions as well as hydrophobic residues [55] and PRP, which contains hydrophobic proline residues [53,56]. This interaction formed a softer but not tightly adsorbed heterotypic complex which was removed by PBS rinse and increases the elasticity of the complex, which contributes to the decrease of measured friction coefficient with higher BSA concentration on the PRP layer. These results are consistent with the recent *in vivo* studies [34,35], which suggested that the protein adsorption and restructuring of both emulsion droplets and saliva interfaces influence oral lubrication. The decrease in friction with increasing BSA concentration supports the role of protein adsorption in modifying surface properties.

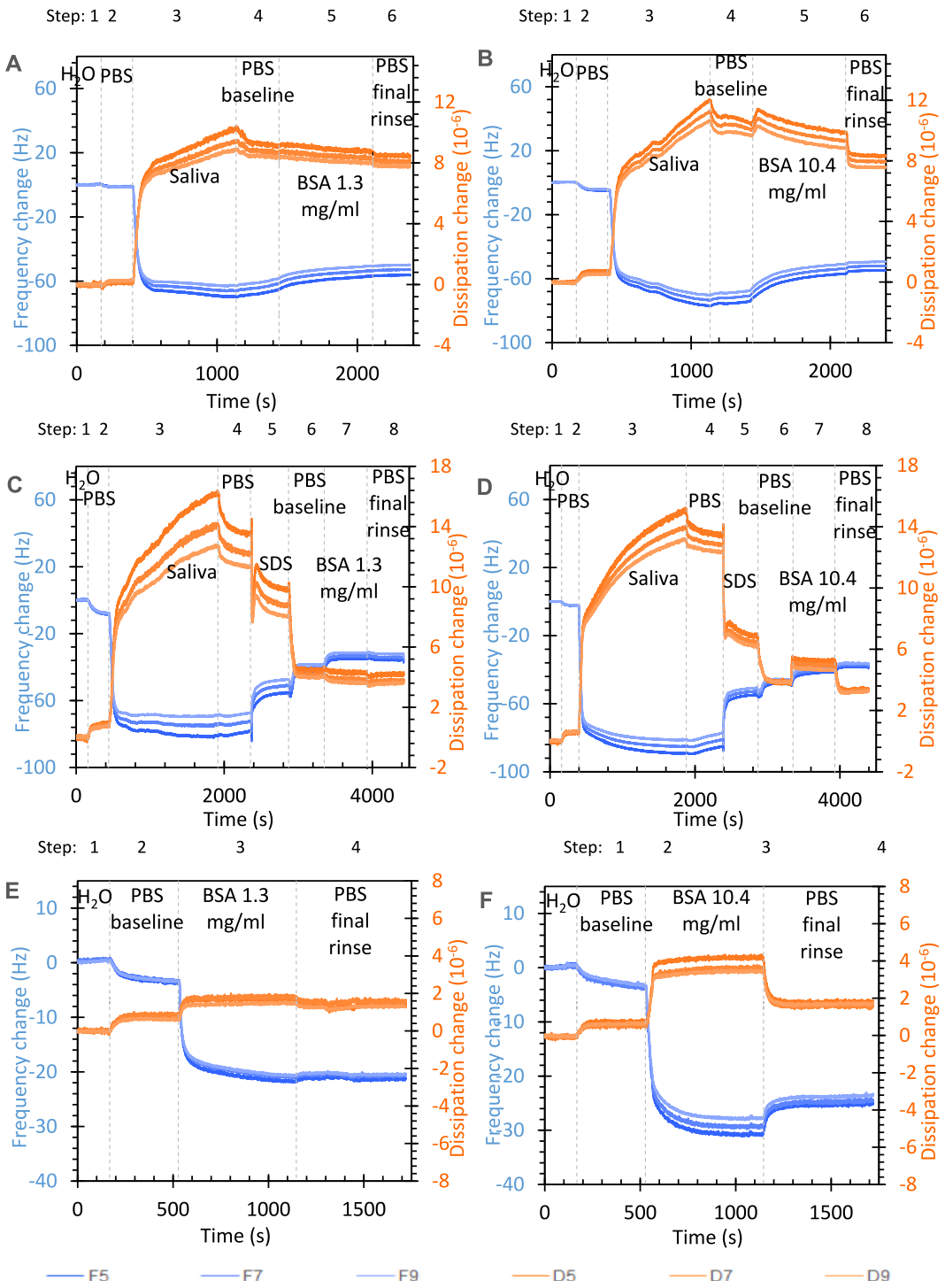

Fig. 4. QCM-D measurement of frequency (blue) and dissipation change (orange) against time at 35 °C. A, C and E are the result of the whole process with 1.3 mg/ml BSA solution and B, D and F are the result of the whole process with 10.4 mg/ml BSA solution on saliva pellicle, PRP layer, and bare PDMS respectively at 5th 7th and 9th overtone. Process steps with different fluids are separated by dotted lines. Plots show a single representative measurement at each condition, repeats are shown in Fig. S2 . Measurements were repeated at least 3 times at each condition with uncertainty shown in Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

In this study, our dynamic tribological protocol [19,23] was used to capture the lubricity change of the pre-adsorbed *ex vivo* saliva pellicle and PRP layer when they are exposed to bovine serum albumin protein. The results supported the hypothesis that increasing protein concentration increases protein adsorption and reduces measured friction coefficient on the saliva coated tribopair. The novel step of removing the bulk saliva to form the salivary pellicle enabled differentiation of measured friction as a function of BSA concentration, which could not be observed with whole saliva film in this study or with other proteins such as β-lactoglobulin [5,8] or expectorated whey protein beverage bolus [27]. Importantly, the analysis of interactions between the BSA solution and the adsorbed saliva fractions show a reduction in mass of the saliva

Table 3
The calculated mass and elastic modulus of the adsorbing layer on substrate before and after adding BSA solutions according to Voigt viscoelastic model fitting. At least three repeats were measured at each concentration.

		1.3 mg/ml				10.4 mg/ml			
		PBS baseline	Sample	PBS rinsed	Δ	PBS baseline	Sample	PBS rinsed	Δ
		Step 2	Step 3	Step 4		Step 2	Step 3	Step 4	
Bare PDMS	Mass, mg/m^2	–	3.94 ± 0.13	3.60 ± 0.33	3.60 ± 0.33	–	5.83 ± 0.15	4.18 ± 0.14	4.18 ± 0.14
	Modulus, kPa	–	1102 ± 159	1542 ± 109	1542 ± 109	–	1496 ± 26	1573 ± 133	1573 ± 133
		Step 4	Step 5	Step 6		Step 4	Step 5	Step 6	
Saliva pellicle	Mass, mg/m^2	16.22 ± 1.52	13.56 ± 0.78	13.22 ± 0.32	−2.99 ± 0.81	16.87 ± 0.21	13.68 ± 0.06	12.44 ± 0.01	−4.43 ± 0.21
	modulus, kPa	478 ± 27	447 ± 26	473 ± 8	−4.82 ± 18.76	434 ± 31	373 ± 12	459 ± 41	24 ± 9
		Step 6	Step 7	Step 8		Step 6	Step 7	Step 8	
PRP layer	Mass, mg/m^2	7.53 ± 0.15	7.04 ± 0.69	7.08 ± 0.13	−0.75 ± 0.07	7.52 ± 1.28	7.30 ± 0.37	5.99 ± 0.38	−1.39 ± 0.73
	modulus, kPa	549 ± 47	507 ± 24	534 ± 23	−14.55 ± 8.67	624 ± 42	473 ± 4	751 ± 22	126 ± 37

Remark: Δ = PBS final rinse – PBS 'baseline', if it is minus value "-", it means the value after PBS rinse is less than that at PBS 'baseline', mass lost. Because the measured data of PBS fluid at step 2 is a reference for the Voigt viscoelastic model fitting in all measurements, there is no data for PBS 'baseline' with bare PDMS.

pellicle after BSA addition, indicating that an interaction occurred between salivary proteins and BSA that is positively correlated with the BSA concentration. This enhances past studies showing the impact of concentration on adsorption of BSA on bare polystyrene surfaces in single protein systems [40]. In comparison with our earlier work [57], demonstrating protein-protein interactions between saliva and dairy proteins that increase friction, this study shows that BSA adsorption reduces the friction coefficient. The measurement of adsorbed mass and viscoelasticity using QCM-D in combination with a dynamic tribology measurement, offers a mechanistic understanding of competitive adsorption as a driver for measured friction coefficient after protein-saliva interactions. Compared with the current *in vivo* observations on emulsions [34,35], which show complex restructuring of the salivary film and fat deposition, the present study isolates the effect of protein concentration. It shows that competitive adsorption within the salivary pellicle alone can significantly change lubrication, independent of emulsion structure or fat content.

This study demonstrates that the dynamic tribology protocol, using an adsorbed saliva pellicle, is a valuable method for detecting changes in saliva's lubricating properties when it interacts with proteins. The influence of other dairy proteins, such as individual casein proteins and micellar caseins, could be tested in the future work. This approach of adsorbing biofluids to the surface and removing the remaining free components may enable the elucidation of small changes in friction that may otherwise be obscured by the presence of the bulk biofluid. This may be applied to *ex vivo* biofluid measurements in a range of areas where lubrication is vital, including human joint models, artificial joints, oral care, and pharmaceutical interactions with oral, nasal, ocular and other mucosal surfaces. The rapidly developing field viewing foods as colloidal soft-matter systems [58] also invites the use of this approach for investigation of the mouthfeel of novel colloidal food proteins, especially to reduce the astringent perception of plant-based and precision fermented proteins currently in high demand to meet the protein needs of the world population [59].

Statement of studies with human subjects

The experiments involving salvia were guided by the approved ethical clearance "Lubrication, Rheology, and Physicochemical Properties of Expectorated Saliva, and its Effect on the Properties of Foods, Beverages, Oral care products and Bio-materials – 19/11/2020 AMENDMENT" (#2019000972).

CRediT authorship contribution statement

Nengneng Fan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Heather M. Shewan:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Gleb E. Yakubov:** Supervision, Methodology, Funding acquisition, Conceptualization. **Jason R. Stokes:** Writing – review & editing, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: (Nengneng Fan reports financial support was provided by Australian Government Research Training. Jason Stokes reports financial support was provided by Fonterra Co-operative Group Limited. Jason Stokes reports financial support was provided by Australian Research Council. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcis.2026.140568.

Data availability

Data will be made available on request.

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