Advances in Colloid and Interface Science 273 (2019) 102034

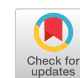
Contents lists available at ScienceDirect

Advances in Colloid and Interface Science

journal homepage: www.elsevier.com/locate/cis

Historical Perspective

Human saliva and model saliva at bulk to adsorbed phases – similarities and differences



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ARTICLE INFO

Article history:
4 July 2019
Available online 31 August 2019

Keywords:
Human saliva
Model saliva
Mucin, pellicle
Lubrication
Tribology

ABSTRACT

Human saliva, a seemingly simple aqueous fluid, is, in fact, an extraordinarily complex biocolloid that is not fully understood, despite many decades of study. Salivary lubrication is widely believed to be a signature of good oral health and is also crucial for speech, food oral processing and swallowing. However, saliva has been often neglected in food colloid research, primarily due to its high intra- to inter-individual variability and altering material properties upon collection and storage, when used as an *ex vivo* research material. In the last few decades, colloid scientists have attempted designing model (*i.e.* 'saliva mimicking fluid') salivary formulations to understand saliva-food colloid interactions in an *in vitro* set up and its contribution on microstructural aspects, lubrication properties and sensory perception. In this Review, we critically examine the current state of knowledge on bulk and interfacial properties of model saliva in comparison to real human saliva and highlight how far such model salivary formulations can match the properties of real human saliva. Many, if not most, of these model saliva formulations share similarities with real human saliva in terms of biochemical compositions, including electrolytes, pH and concentrations of salivary proteins, such as α-amylase and highly glycosylated mucins. This, together with similarities between model and real saliva in terms of surface charge, has led to significant advancement in decoding various colloidal interactions (bridging, depletion) of charged emulsion droplets and associated sensory perception in the oral phase. However, model saliva represents significant dissimilarity to real saliva in terms of lubricating properties. Based on in-depth examination of properties of mucins derived from animal sources (*e.g.* pig gastric mucins (PGM) or bovine submaxillary mucin (BSM)), we can recommend that BSM is currently the most optimal commercially available mucin source when attempting to replicate saliva based on surface adsorption and lubrication properties. Even though purification *via* dialysis or chromatographic techniques may influence various physicochemical properties of BSM, such as structure and surface adsorption, the lubricating properties of model saliva formulations based on BSM are generally superior and more reliable than the PGM counterpart at orally relevant pH. Comparison of mucin-containing model saliva with *ex vivo* human salivary conditioning films suggests that mucin alone cannot replicate the lubricity of real human salivary pellicle. Mucin-based multi-layers containing mucin and oppositely charged polyelectrolytes may offer promising avenues in the future for engineering biomimetic salivary pellicle, however, this has not been explored in oral tribology experiments to date. Hence, there is a strong need for systematic studies with employment of model saliva formulations containing mucins with and without polycationic additives before a consensus on a standardized model salivary formulation can be achieved. Overall, this review provides the first comprehensive framework on simulating saliva for a particular bulk or surface property when doing food oral processing experiments.

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https://doi.org/10.1016/j.cis.2019.102034
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1. Introduction

Saliva secreted by the salivary glands, is one of the most important bodily fluids that enables maintaining oral and general health [1]. Besides oral lubrication, microbial defence functions, saliva plays an important role in the transport of food from the oral cavity to the oesophagus and stomach, buffering action and water balance. The composition and properties of saliva varies significantly depending on the salivary glands from which the saliva is secreted, cardiac rhythm as well as age, gender, diet, medicines, and type of stimulations, such as chewing, citric acid *etc* [2,3]. Adults generally secrete about 0.5–1 L of saliva on a daily basis, of which 80% is associated with food oral processing [4]. Besides acting as a solvent for the flavours and tastants present in the consumed foods, the physicochemical and biochemical interactions (*e.g.* electrostatic, ionic, enzymatic) of saliva with food structure at colloidal to nano-scale can inevitably influence the taste, texture, mouthfeel and sensory appreciation [5–11].

Saliva is a complex fluid that possesses intriguing material properties, such as low viscosity with excellent lubrication properties, which engineers can only envy. Such properties are postulated to be associated with the complex internal structure of saliva across length scales, which has remained poorly understood and controversial to date, though the first hypothesis about the presence of structural components in saliva dates back to 1908 [12]. The combined fluid present in oral cavity is conventionally described as “whole saliva”, “mixed saliva” or “oral fluid”. Whole saliva is a clear, slightly acidic (pH ranging from 6.24–7.36) exocrine secretion contributed by different salivary glands located all over the oral cavity [13–15]. Although saliva is an aqueous dispersion with 99% continuous phase, the presence of ~0.3 wt% mucin, a high-molecular-weight (0.5–20 MDa) anionic glycosylated protein is often claimed to render saliva its rheological (viscosity, elasticity, stickiness), unique water-holding and lubrication properties [2,5,6,16,17].

Beyond bulk saliva, the significance of the absorbed layer of saliva *i.e.* the “saliva pellicle” on oral lubrication [18,19] has been recently appreciated. Salivary pellicle is a supramolecular film with a complex architecture predominantly formed by salivary proteins on enamel or mucosal surfaces [20]. It is hypothesized mucin multilayer networks are formed by complexation of mucins on their own as well as in synergistic combination with other salivary proteins yielding a hydrated lubricating salivary pellicle [21–23], however, the true structure of salivary pellicle has remained still elusive. The advancement of saliva research field has accelerated over the last few decades with the advent of novel quantitative approaches, such as high performance liquid chromatographic and mass spectrometric techniques, proteomics, genomics and bioinformatics allowing a systematic biochemical characterization of numerous cellular, organic and inorganic components in saliva. Many of these components can act as biological markers that may be used in the early detection and monitoring of oral and general disease, suggesting the enormous potential of saliva as a non-invasive diagnostic tool [4].

In particular, saliva research has gained significant momentum in food colloids community due to the recognition of role of saliva in oral tribology *i.e.* studies involving friction, lubrication and wear occurring in mouth [17]. Indeed, *ex vivo* experiments using real human saliva are often limited by the inter- and intra-individual variability, ethical constraints and instability upon collection and storage [2]. Therefore, attempts have been made to design simple saliva-mimetic fluids that can simulate the ionic and enzymatic composition of human saliva which have generated useful fundamental insights on electrostatic and biochemical interactions of food with saliva [5,6,8,9,11,24–29]. Many, if not most, of these model saliva formulations have used commercially available pig gastric mucin (PGM) with few formulations employing bovine submaxillary mucin (BSM) as a substitute for salivary mucins. Although the presence of mucins can enable matching the viscosity of real human saliva under orally-relevant shear rates [25], they have been largely unsuccessful in contributing to other material properties of real saliva, such as lubrication and elasticity. It is worth noting that a fundamental understanding of structure-property relationships in real human saliva is crucial, before a *true blueprint* can be developed to guide the formulation and design of a standardized model saliva for *in vitro* oral processing experiments.

Renewed research interests in understanding saliva is also driven by the attempts to provide sustained therapies for population suffering from ‘Xerostomia’, which is clinically defined as a subjective complaint of “dry mouth”. The currently available salivary substitutes that are commonly used by dry mouth patients are composed of thickeners, such as carboxymethyl cellulose, xanthan gum [30,31], that are far from the composition of real human saliva. Not surprisingly, these salivary substitutes are sub-optimal in replicating the lubricating properties of saliva and thus only give a symptomatic relief with short-lived wetting effects in patients suffering from xerostomia [32–34]. Some researchers have indicated that the key issue of using salivary substitutes in xerostomia management is the insufficiency of such formulations to form an effectively adsorbed salivary pellicle on oral surfaces [35].

Since Decher [36] revealed the “layer-by-layer assembly” research in the early 1990s, this novel approach has gained substantial attentions in designing *in vitro* mucoadhesion experiments and salivary substitutes. The multilayer films that may be relevant for salivary substitutes are the ones built by depositing alternative layers of anionic molecules of mucins derived from various animal sources, such as PGM and BSM and cationic molecules on a charged substrate. The charge, porosity, thickness and other film properties of multilayers can be tailored by careful selection of the cationic polymers [37]. These mucin multilayer networks are showing early promises for engineering a biomimetic salivary pellicle [38], with few of those electrostatiically-driven networks being also studied for their lubricating properties [39,40]. Progress on saliva research has been well-described in other excellent reviews [2–4,13,19,41,42], summarizing the oral biology, biochemistry,

functions and mechanical properties. However, to our knowledge, there exists no review that has discussed the structure and properties of saliva, whether being present in bulk or in adsorbed phases, to specifically understand the fundamental similarities and dissimilarities between real saliva and engineered model saliva formulations.

Hence, this review aims to provide a well-balanced, systematic synthesis of recent works aimed at understanding saliva from both bulk and interfacial standpoints and comparing them with engineered bulk model saliva and mucin-based structure, respectively. Firstly, we discuss the multiscale structures and rheological properties of real human saliva in bulk phase to clearly highlight what is understood so far. Then, we have examined the model saliva formulation highlighting their similarities and differences to real human saliva. Although, high elongation viscosity of saliva is an important aspect of real human saliva [43], particularly relevant for swallowing action, we have not covered this aspect owing to scant literature in extensional rheological characterization of model saliva formulation. In addition, we have only focussed on model saliva formulations in literature that have some similarities in their biochemical composition to real human saliva with respect to ions and salivary proteins. Artificial saliva formulations, such as glycerine-containing Biotène®-OralBalance, carboxymethylcellulose-containing Luborant®, polymer-coated liposomes that are specifically designed as salivary substitutes for xerostomia or hyposalivation patients containing no salivary proteins [32–34,44] are beyond the scope of this review.

Specifically, we have conducted an in-depth examination of the structural, physicochemical and lubrication properties of commercially available mucins from animal sources (PGM and BSM) that are used in those model formulations. This insight will enable us to identify the types and degree of purification of mucins that should be used in *in vitro* oral processing experiments and to discuss their limitations when attempting to replicate the lubrication properties of real human saliva. Following the discussion on properties in the bulk phase, we critically analysed the structure of salivary pellicle present in the adsorbed phase and its role on oral lubrication. Lastly, we have analysed the mucin-adsorbed layers as well as briefly discussed the mucin-based multi-layer approaches in literature, which might be promising in future for engineering biomimetic salivary pellicle. To our knowledge, this is the first review that serves as a preeminent source of fundamental knowledge on colloidal aspects of real and model saliva. In addition, this review highlights key principles to underpin engineering of biomimetic saliva (bulk + adsorbed phase) in the future for performing standardized *in vitro* oral processing experiments. Table 1 lists the abbreviations used in this review.

Table 1
Abbreviations and symbols.

AES	Anion exchange chromatography
AFM	Atomic Force Microscopy
BOAS	Beads-on-a-string
BSM	Bovine submaxillary mucin
CLSM	Confocal laser scanning microscopy
LbL	Layer-by-layer
G′	Elastic modulus
G″	Viscous modulus
MTM	Mini traction machine
MUC5B	High-molecular-weight mucins
MUC7	Low-molecular-weight mucins
PDMS	Polydimethylsiloxane
PGM	Porcine gastric mucin
PRP 1	Proline-rich protein 1
SEM	Scanning electron microscopy
sIgA	Secretory immunoglobulin A
TEM	Transmission electron microscopy
θ_w	Water contact angle
β-lg	β-lactoglobulin
μ	Friction coefficient
η	Viscosity
η_0	Zero-shear viscosity
γ	Surface tension
γ_s	Total surface free energy
ρ	Density

2. Bulk saliva — structure and rheological properties

2.1. Human saliva

The complexity of saliva has captured the attention of many researchers from various disciplines. Bulk saliva is postulated to contain several phases that simultaneously coexist including non-Newtonian weak gel-like phase, air dispersed in continuous ionic aqueous phase, as well as a typical colloidal system including globular micelles, epithelial cells, lipoid materials dispersed in an aqueous media [1,6,45,46]. We have observed centrifuged fresh unstimulated human saliva using macroscopic imaging, confocal laser microscopy (CLSM) and cryo-scanning electron microscopy (cryo-SEM) at different magnifications (Fig. 1A-D). The CLSM image allows the visualization of saliva (Fig. 1B) without any freezing step and shows some dense areas of micron-sized aggregates (in red) dispersed in aqueous medium, which was also seen previously [2]. Such aggregates dyed with Rhodamine B must be proteinaceous and can be considered to be associated with homo- or hetero-typic oligomers to heteroprotein complexes of mucin-mucin or mucin-other small molecular salivary proteins associated *via* ionic forces (*e.g.* Na^+, Ca^{2+} ions), electrostatics interactions, hydrogen bonding, and/or hydrophobic interactions.

However, the CLSM images did not show any classical *network-like* structure of saliva, as was clearly evident at a lower length scale using cryo-SEM (Fig. 1C). This is in agreement with previous literature [2,47,48], where CLSM was unable to probe into the '50-200 nm salivary protein threads' as visible in the cryo-SEM images (Figs. 1C-D). This weak gel-like structure of saliva has been attributed to a hydrogel formed by highly glycosylated mucins (MUC5B) involving a complex set of charge-mediated carbohydrate-carbohydrate interactions, calcium-induced crosslinks, disulphide bonds and hydrophobic interactions) [45,49,50]. Interestingly, globular structures of nearly 100–500 nm (Fig. 1C, shown by arrows) were evident that created some sort of knots in the otherwise web-like network. These have been termed previously as "salivary micelles" [51–53] that contained mucins (MUC7) and other salivary proteins, such as secretory immunoglobulin A (sIgA), lactoferrin, amylase, proline-rich protein (PRP) and lysozyme [54]. The fact that MUC5B is a protagonist in gel forming property of saliva was not evident in these micelles [54]. The zeta-potential of saliva micelles has been reported to range from −13 to −17 mV [51], highlighting the important role of anionic proteins such as mucins containing sialic acid (pK_a ~ 2.6) and sulfate groups (pK_a < 1) [55], overshadowing the quantitative charge contribution of the cationic proteins, such as lysozyme (pI ~11) [56] and lactoferrin (pI ~8.5) [57] at neutral pH. Existence of micelles in saliva has been compared to casein micelles in milk, however, the exact structural organization of these salivary micelles and their roles in material properties of saliva have remained elusive to date.

The structure of saliva at four-times higher magnification (Fig. 1D) appears to be highly woven network interconnected by threads, corroborating with the visual image showing some degree of stretchability (Fig. 1A). Interestingly, the average values of material properties of saliva, such as, density (ρ ~ 1000 kg m^{-3}) and zero-shear-rate viscosity (η_0 ~ 1 mPa.s) are comparable to that of water [2,58]. The surface tension of saliva is lower than water (γ ~ 50 mN m^{-1}), which suggests the presence of some surface active salivary proteins [59]. In addition, unlike Newtonian water, saliva is a non-Newtonian fluid that elongates to form long-lived "beads-on-a-string" (BOAS) morphology (Figs. 2A and B) *i.e.* a fluid necklace containing intermittent pattern of beads laced up together [60]. Interestingly, the lifespan (t_c) of the thread of saliva (with an initial radius R of ~ 1 mm) has been calculated to be

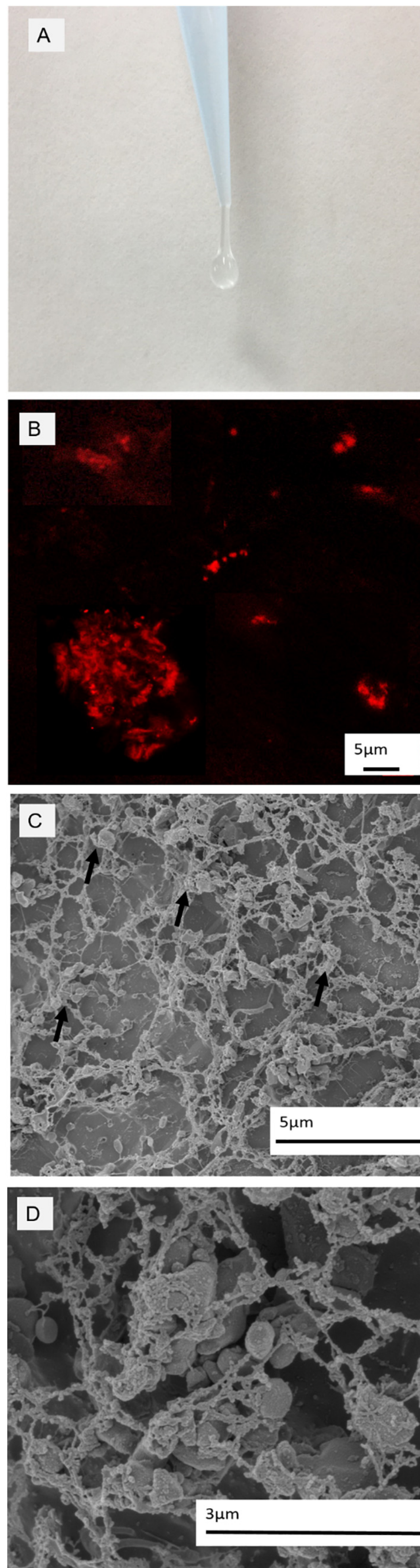

Fig. 1. Multi-scale images of fresh unstimulated human saliva after centrifugation with macroscopic image (A), confocal laser scanning image (CSLM), where salivary proteins are stained red using Rhodamine B (B), and cryogenic-scanning electron micrographs (cryo-SEM) at 20,000 × (C) and 80,000 × (D) magnification, respectively. The black arrows indicate the globular salivary micelles. Human saliva (A-D) was collected from healthy participants (n = 15 participants) in the morning, at least two hours since eating and drinking (Ethics number: MEEC 16–046, University of Leeds, UK), pooled and was centrifuged at 4,400 rpm for 3 min and the supernatant was used for microscopic imaging.

significantly longer than a simple Rayleigh estimate for an inviscid fluid $\left(t_c = \sqrt{\frac{\rho R^3}{\gamma}} \approx 4ms\right)$ [60], but may be smaller than that of Newtonian viscous threads, such as honey that scales with viscosity $\left(t_v = \frac{\eta_o R}{\gamma}\right)$. However, such Newtonian viscous threads do not form a long-lived BOAS structure like saliva. The role of the different forces in the dynamics of thinning and break-up of these thread-like filaments is given primarily by two dimensionless parameters: the Ohnesorge number ($O_h \equiv {}^{t_v}/_{t_c} = n_o/\sqrt{\rho R \gamma}$), which represents the ratio of viscous to inertial forces (when the characteristic velocity scale is set as equal to R/t_c), and the Deborah number ($D_e = {}^{\lambda}/_{\tilde{t}}$), which represents the ratio of the

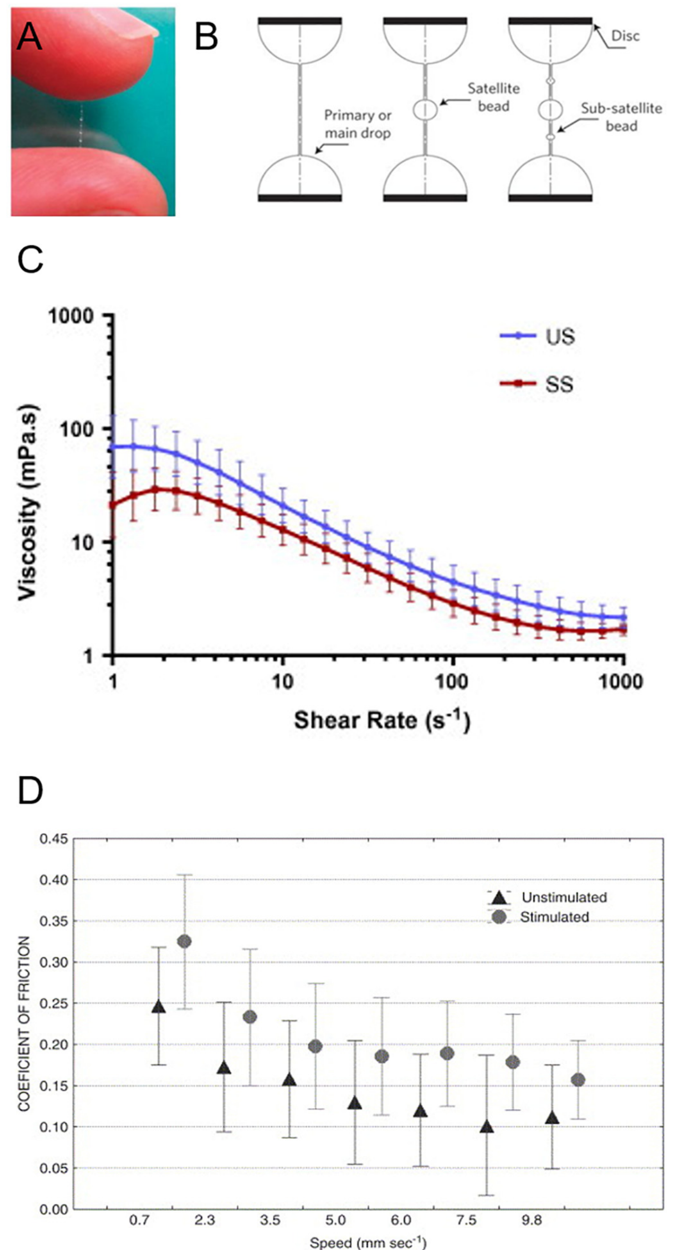

Fig. 2. Properties of human saliva *i.e.* forming a 'beads-on-a-string (BOAS) morphology when a drop of saliva is stretched between a thumb and forefinger (A), with schematics of viscoelastic filaments exhibiting different BOAS morphologies (B), viscosity values of human saliva at various shear rates, where US is unstimulated saliva; SW is stimulated saliva (n = 30 participants) (C), and friction coefficients of unstimulated or stimulated saliva when sheared between pig's oesophagus and tongue surface at sliding speeds ranging from 0.7 to 9.8 mm/ s (D), respectively. In case of stimulated saliva, mechanical stimulation was achieved by chewing a 5 cm × 5 cm piece of Parafilm®. Images (A) and (B) [60], (C) [66] and (D) [68] in the panels are reproduced with permission.

relaxation time λ of the polymer molecules to the characteristic process time $\tilde{t}$. For saliva, the mechanism of BOAS phenomenon has been attributed to this subtle balance between capillary, viscous, elastic and fluid inertial forces. The dynamics of this bead formation can alter dramatically if any of these forces becomes dominant over the others.

Besides such intriguing rheological properties, bulk saliva enhances the wettability of the otherwise hydrophobic oral surfaces [17]. For example, the contact angle between the piglet tongue surface *ex vivo* and distilled water is 77 ± 4° [61], similar to the values obtained for the human gingival surfaces (θ_{Water} ~ 72–79°) *in vivo* [62]. However, coating of piglet tongue surfaces with saliva (*ex vivo*) makes them significantly more hydrophilic (50.5 ± 2.4°) as compared to that of the uncoated tongue surfaces (77.3 ± 4.1°) [61]. Ranc and co-workers [61] also compared the mean value of the total surface free energy (γ_s) of the piglet tongues surfaces obtained with/without salivary coating based on Oss's acid–base theory using contact angle measurements. Oss's acid–base theory [63] is the sum of a non-polar Lifshitz–van der Waals component (γ_s^{LW}) that includes the London dispersion forces, the Debye induction and Keesom orientation contributions and a polar Lewis acid–base component (γ_s^{AB}). The polar Lewis acid–base component (γ_s^{AB}) is equal to $2\sqrt{\gamma_s^{+}+\gamma_s^{-}}$, where, the surface parameters γ_s^{+} and γ_s^{-} are the Lewis acid component (the electron-acceptor parameter) and Lewis base component (the electron-donor parameter), respectively. Coating with saliva increased the total surface free energy of the tongue surface significantly up to 37.5 ± 1.4 mJ/m^2 (1.4 times with respect to the uncoated counterpart) [61]. Such increase in total surface free energy was imparted only by the Lewis acid–base component (γ_s^{AB}) acid γ_s^{+} and γ_s^{-} increased by factors of 4.7 (1.4 ± 0.3 mJ/m^2) and 2.4 (38.7 ± 2.8 mJ/m^2) as compared to their uncoated surfaces counterparts, respectively. This suggests that saliva not only enhances the wettability of the tongue surface but also imparts a stronger basic character.

Interestingly, stimulation (*e.g.* citric acid, mechanical stimulation by chewing a parafilm) can result in different compositions of saliva [58,64] and consequently influence its physicochemical and material properties. Basically, unstimulated saliva is mainly secreted from sublingual and submaxillary glands, while stimulated saliva is secreted 80% by the parotid gland, latter containing little or no mucin [65]. Table 2 lists the physicochemical properties of stimulated and unstimulated saliva.

Table 2
Physicochemical and material properties of unstimulated and stimulated human saliva.

Parameters	Unstimulated saliva	Stimulated saliva	References
Key secretory salivary glands	Sublingual, Submaxillary	Parotid	[142]
Parotid (% by volume)	28	53	[13,143,144]
Sublingual/ Submaxillary	68	46	
Minor	4	1	
Flow rate (mL/ min)	0.12–0.46	0.2–2	
pH	6.0–7.5	7.42–8	[15,142,145]
Protein content (mg/ mL)	1.26–1.42	0.97–1.11	[142,143]
Amylase (IU/ L × 10^3)	454–516	422–580	[143]
Sodium (meq/L)	5.36–5.73	7.27–10.08	
Potassium (meq/L)	19.33–21.48	18–18.39	
Calcium (meq/L)	1.93–2.39	2.17–2.35	
Magnesium (meq/L)	0.58–0.63	0.53–0.56	
Surface tension (γ)	58.98	59.69	[66]
Viscosity η (mPa.s)[a]	0.86–1.5	1.14–1.16	[142]
Viscosity η (mPa.s)[b]	1.22–1.24	1.15-1.17	
storage modulus G′ (mPa)[b]	1.77–2.11	1.27-1.41	
Viscoelasticity	More	Less (described as "water-like")	[58]
Friction coefficient (μ) at sliding speeds 0.7–10 mm/s, Load 0.34 N, pig mucosa-based tribopairs)	0.11–0.24	0.16–0.33	[68]

[a] η measured with cone-and-plate geometry at 100 s^{-1}
[b] η measured with capillary setup at 95 s^{-1} and shear strain of 1.1

Generally speaking, the viscosity of stimulated saliva is lower as compared to the unstimulated counterpart at orally relevant shear rates of 10–500 s^{-1}, although both of them show shear-thinning behaviour attributed to the breakup of the mucin-induced networks upon shearing [66] (Fig. 2C). Interestingly, the surface tensions of stimulated and unstimulated saliva are reported to be similar (Table 2). It might be attributed to the presence of salivary proteins, such as calcium-binding 'statherin' present even in stimulated saliva (parotid as well as whole), which has been demonstrated to be surface-active that moves rapidly to the air interface reducing the interfacial tension (γ ~ 55 mN m^{-1}) and forms a calcium-enriched viscoelastic film with elastic modulus (G′) of 60.8 mNm^{-1}), and the viscous modulus (G″) of 12.9 mNm^{-1} within 15 min [67]. Tribological measurements using oesophagus and pig tongue surface as tribopairs at speeds ranging from 0.7 to 9.8 mm/s suggest that the unstimulated saliva is a significantly better lubricant with lower friction coefficients (μ) as compared to that of stimulated saliva [68] (Fig. 2D). Therefore, for *ex vivo* oral processing experiments using human saliva, it is extremely important to specify whether the saliva is collected with or without stimulation.

Although real human saliva can be considered as the "gold standard" in oral processing experiments, there are issues in using saliva as a research material. Besides the ethical constraints, inherent variabilities and biological risks, saliva undergoes significant physicochemical changes that translates in loss of its mechanical properties. Viscosity and lubrication properties of saliva alter dramatically upon pooling, freezing and thawing [2,3,49,69]. For instance, a key experimental approach used in experiments involving real human saliva is to pool saliva from healthy donors and then store it frozen at −20 to −80 °C [2,66,70]. During the freeze-thawing process, salivary proteins bind to calcium ions and precipitate out as Ca^{2+}-induced protein aggregates [1]. Consequently, the colloidal and mechanical properties change enormously, which make it challenging to use real human saliva for research purposes.

2.2. Model saliva formulation

Due to the difficulties encountered during collection, handling, storage of real human saliva, model saliva has been preferred as a research material by colloid scientists. Model saliva formulations have been developed with an aim to simulate the ionic concentrations, pH and to a certain extent the viscosity using mucins from animal sources [24,25,71–73]. Depending upon the need of experiments, in some cases model saliva formulation may contain α-amylase [28,29,74] particularly when dealing with starch-based experiments. From ionic composition viewpoint, model saliva formulations contain ions ranging from three key components ($NaHCO_3$, NaCl, KCl) up to nine components [75]. The model saliva formulation (Table 3) prepared by Sarkar and co-workers [25] based on composition used in previous dental studies [72,73,76] is one of the most cited formulation (>200 times, Google Scholar), most likely due to its interesting similarity to bulk human saliva from electrostatics and apparent viscosity perspectives.

Table 3
Chemical composition of model saliva formulations (Reproduced with permission from [25]).

Chemical name	Chemical formula	Concentration (g/L)
Sodium chloride	NaCl	1.594
Ammonium nitrate	NH_4NO_3	0.328
Potassium phosphate	KH_2PO_4	0.636
Potassium chloride	KCl	0.202
Potassium citrate	$K_3C_6H_5O_7.H_2O$	0.308
Uric acid sodium salt	$C_5H_3N_4O_3Na$	0.021
Urea	H_2NCONH_2	0.198
Lactic acid sodium salt	$C_3H_5O_3Na$	0.146
Porcine gastric mucin Type II		Varying concentrations (0–30 g/L)
Water		Make upto volume

Ex vivo experiments conducted by Silletti and co-workers [77] suggested that real human saliva causes depletion flocculation in weakly negatively-charged emulsions, such as those stabilized by β-lactoglobulin (β-lg) with homogenously dispersed flocs, whereas bridging flocculation occurs in positively-charged emulsions, such as those stabilized by lysozyme with larger densely packed structures (Figs. 3A and B). Interestingly, *in vitro* experiments by Sarkar and co-workers [25] demonstrated similar behaviour with model saliva (referred in the literature as 'artificial saliva', where such depletion flocculation in β-lg-stabilized emulsions and bridging flocculation in lactoferrin-stabilized emulsions (Figs. 3C and D) were attributed to the interactions of the charged droplets with negatively charged mucin present in the model saliva formulation. In fact the apparent viscosities of the weakly negatively charged emulsions and the positively charged emulsions mixed with real human saliva [77] or model saliva [25] were comparable. Similarly, droplet coalescence has been observed in hydrophobically-modified starch-stabilized emulsions in the presence of both real saliva (*in vivo* oral processing) [10] as well as model saliva containing α-amylase (*in vitro* experiments) [28]. Such similarities of bulk human saliva and model saliva in apparent viscosities and electrostatic charge have generated key insights into oral colloidal science that has also enabled mechanistic understanding behind some sensory perception [11,24,78,79]. For instance, bridging flocculation of positively charged emulsions observed in presence of model saliva containing mucins [25] or real saliva [77,78,80] was translated to 'dry' and 'rough' or 'astringent' mouthfeel perception during real oral processing [79]. Such sensory perception was attributed to precipitation of salivary mucins from oral surfaces by complexation with positively charged emulsions. On the other hand, depletion flocculation of negatively charged emulsions observed in presence of model saliva containing mucins [25] or real saliva [77] was translated into 'creamy', "fatty' and 'thick' mouthfeel perception. This suggests that matching the biochemical composition with ions and mucins to a certain extent and consequently replicating the surface charges of real saliva using mucins in model saliva in an *in vitro* experiment can generate powerful insights about physico-chemical mechanism behind sensory perception that is experienced during *in vivo* oral processing [6].

Comparison of the results from human saliva and purified MUC5B mucin solutions using confocal fluorescence recovery after photobleaching experiments suggested that the network properties of saliva cannot be replicated using purified MUC5B mucin at physiological concentration but requires at least 10–20 times higher concentration of MUC5B to mimic saliva [81]. This further indicates that the complex structural organization of real human saliva cannot be replicated by purified MUC5B mucin alone and interactions with other components within the human saliva are required to produce its mechanical properties even in bulk phase. Therefore, it is not surprising that the model saliva formulation containing only mucin in an ionic dispersion as discussed above does not reproduce the properties of natural human saliva, such as viscoelasticity or BOAS morphology.

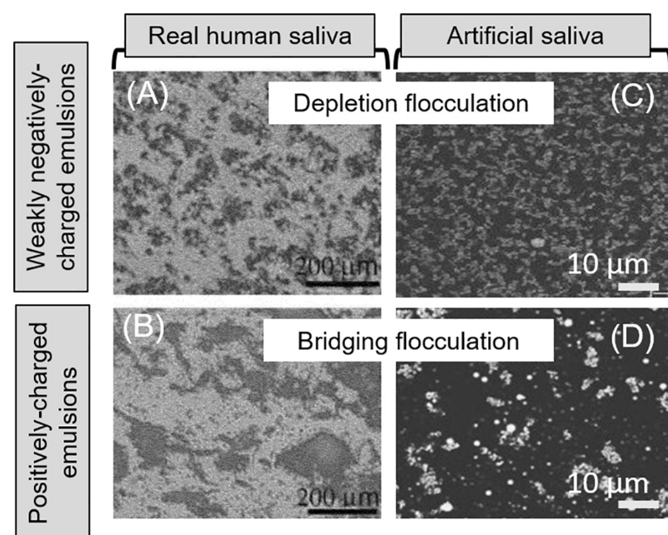

Fig. 3. Microscopic images of mixtures of human saliva and emulsions stabilized by β-lg (A) or lysozyme (B) and mixtures of artificial saliva and emulsions stabilized by β-lg (C) or lactoferrin (D) at neutral pH, respectively. Images (A), (B) [77] and (C), (D) [25] in the panels are reproduced with permission.

To summarize, no studies have considered salivary proteins other than mucin and α-amylase in their model formulations so far, and the these non-mucinous salivary proteins may play a key role in colloidal interactions with food structure that has remained underestimated in the literature. Since model saliva includes a different source of mucin as compared to human salivary mucins, we have included a section entailing the types of mucins and their properties to identify mucin from which source should be used to answer a specific question during food oral processing experiments.

3. Mucins

Mucins are glycoproteins with molecular weights ranging from 200 kDa to 40 MDa [82], and can be classified as either membrane-bound or secreted. Both of them are highly glycosylated by *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, galactose, sialic acid and a few mannose and sulphate [83]. It has been reported that mucins constitute approximately 16% of the total protein in whole stimulated saliva [84]. Members of the mucin family can vary considerably in size, structure, degree of glycosylated modification, which result in their different physicochemical and material properties [85]. As for the scope of this review, we focus only on salivary mucins. For more detailed information on mucins, readers can refer to excellent previous reviews [55,85–89].

3.1. Salivary mucins

There are two major structurally and functionally distinct mucins in human whole saliva: MG1 mucin and MG2 mucin encoded by genes MUC5B and MUC7, respectively [90–92]. Salivary MUC5B mucin is a heavily glycosylated (approximately 80% of its weight comes from carbohydrates) with molecular weight of 2.5–3.0 MDa [92], while MUC7 is the smallest of the secreted mucins with less heterogeneous glycosylation [93]. In addition, MG1-derived oligosaccharides are reported to be much longer than those of MUC7 [94]. The MUC5B is the representative of gel-like mucin, which is the principal protein for constructing the structural framework of saliva, secreted by all the salivary glands except the parotid gland [82]. The MUC7 secreted by submandibular, sublingual and palatine salivary glands is the non-gel forming mucin that are present in the salivary micelles. Despite their differences resulting from carbohydrate content and charge density, they share the general structural features with other mucins present in different organisms. Generally, mucins are made up of different domains [85]. There are tandem repeat domains that are rich in serine, threonine and proline (STP repeats) that contain more than 60% of the amino acids. The lack of typical α-helix and β-sheets in mucin makes the repeat domains serve as a scaffold providing primary targets for *O*-linked oligosaccharides (oligosaccharides that link to oxygen atom in the serine and threonine) [55,95]. Varied copy numbers of these STP repeats give the variation of mucin lengths, glycosylation, and then lead to the different molecular weights.

In fact, commercially available mucins derived from animal sources, such as, bovine sabmaxillary mucin (BSM) and porcine gastric mucin (PGM) that are commonly used in academic research have some degree of similarity to MUC5B [85,96–98]. However, it is worth noting that there are still large differences between human salivary MUC5B and commercial animal source derived mucins (BSM and PGM). For

instance, Sandberg and co-workers [99] demonstrated that the adsorbed mass on hydrophobized silica using ellipsometry was 2.6 mg/m^2 for highly purified fraction of mucins from either BSM or PGM whilst 5.0 mg/m^2 for mildly purified MUC5B, the latter containing some albumin.

3.2. Commercial mucins derived from animal sources

As mentioned above, a few animal-derived mucins have long been commercially available (*e.g.* BSM or PGM) as provided by many manufacturers, and they have been employed to formulate model saliva. However, there is no consensus on the criteria so far in literature in designing model saliva formulations, such as type and concentration of mucin source, solvent, additives. To date, notable differences have been reported in the physicochemical characteristics, structure, absorption behaviour and lubrication properties of the two major commercial mucins, BSM and PGM. This section discusses the detailed differences between these two types of mucins in view of their structural, physicochemical, and material properties (Table 4), which is the first step when designing oral processing studies with either of these mucins in the model saliva formulations.

3.2.1. Influence of batch-to-batch variations and purification

One of very notable and possibly puzzling problems with BSM and PGM is that aggregation and physicochemical properties of mucins have been reported to vary significantly across studies in literature even for the same types of mucins. We suggest that this discrepancy might be closely associated with varying purity of the mucin samples. Firstly, many researchers have attempted to isolate and purify mucins from animal organs as opposed to using commercial alternatives, yet according to different procedures [100–105]. Thus, it is reasonable to assume that reported molecular weight, structure, and composition of mucins, even for the same types and including commercial mucins, would be affected by purification details. Secondly, among the commercially available mucins, researchers often employ mucin samples with varying purity, which are announced by manufacturers; examples include Type I (ceased to be produced) [106,107] versus Type I-S of BSM [26,39,40,108] or Type II [25,109] versus Type III [16,108,110] for PGM from Sigma-Aldrich®. PGM from Sigma-Aldrich® and commercially available AS Orthana® [110] appear to display noticeable differences in structure and composition as well. In addition, researchers from Sweden, Denmark, and USA have reported batch-to-batch variation with a wide range of contaminants in the same types of mucins too [111–113]. Finally, a few studies have further purified commercial mucins in order to improve the purity as well as to standardize the status, yet also according to different procedures, *e.g.* dialysis [16,26,111], anion exchange chromatography [112,114,115], size exclusion chromatography [116,117], or combination of both chromatographic techniques [98,106,118] and lastly without further purification [11,24,25,28,29,108,109,119–121]. Depending on the purpose of each study, all these different handling of mucin samples prior to experiments can be justified, and it is not an easy task to draw an agreement on the standardized purification method across different research groups. Therefore, it can be only emphasized that readers should be fully aware that all these different pre-treatments, or even the absence of any pre-treatment, may have a significant impact on the observed properties of the commercially available mucins.

3.2.2. Charge density

The most important difference between BSM and PGM is their sialic acid contents. Sialic acids, *i.e. N*-acetyl-neuraminic acids, the family of 9-carbon carboxylated sugar. Sialic acids ($pK_a = 2.5$) attached to both the polypeptide core and terminal groups of mucin, together with other acidic oligosaccharides (*i.e* sulfate, $pK_a = 1$) are responsible for the anionic nature of mucins [106]. Consequently, BSM with significantly higher sialic acid content is more negatively charged mucin than PGM (Table 4); BSM has consistently been reported to display more negative ζ-potential values as compared to those of PGM. In addition, this difference inevitably influences the pH-dependent adsorption behaviour of mucins onto nonpolar surfaces, as will be addressed below.

3.2.3. Structure

Various models of BSM and PGM have been hypothesized in the last 50 years, but their molecular structural models remain under debate to

Table 4
Physicochemical properties, structure and material properties of bovine submaxillary mucin (BSM) and pig gastric mucin (PGM).

Parameters	Bovine submaxillary mucin (BSM)	Pig gastric mucin (PGM)	References
Physicochemical properties and structure			
M_w, Molecular weight (MDa)	1.6–4.1	2.2–4.7	[80,103,117,146]
R_g, Radius of gyration (nm)	140–259	49–72	[80,105,146]
Feature of ordered degree	More obvious	Less obvious	[114]
Protein content (% by dry weight)	36.2–50	13.2–17	[106,146,147]
Negatively charged amino acids content (% by amino acid composition)	15	10	[147]
Positively charged amino acids content (% by amino acid composition)	10	10	[147]
Neutral amino acids content (% by amino acid composition)	54	68	[147]
Hydrophobic amino acids content(% by amino acid composition)	20	10	[147]
Carbohydrate content (% by dry weight)	63.8	82.9	[106]
Sialic acid content (% by total carbohydrate mass, obtained from Sigma Aldrich website)	9–24	0.5–1.5	
Isoelectric point (pI)	2–3		[105]
% of mucin agglomerates changes with decreasing pH from 7.4 to 3.0	0–58	43–83	[106]
D_h (nm) at pH 7.4	56.1 ± 8.9	77.8 ± 19.7	[114]
Sensitivity of D_h on lowering pH values	higher	lower	[114]
ζ-potential (mV) at pH 2.4	−1.92 to −2[a]	−0.95 to −3[a]	[106,114]
ζ-potential (mV) at pH 7.4	−5.99 to −21[a]	−5.47 to −9[a]	[106,114]
Tendency to form aggregates	lower	higher	[106,114]
Absorption behaviour			
Absorbed amount on PDMS at pH 7.4 (ng/cm^2)	54–143.3	87–98.2	[107,114]
Speed of adsorption at pH 7.4	higher	lower	[114]
Absorbed amount on PDMS at pH 2.4 (ng/cm^2)	116–128.9	68.7–77	[107]
Speed of adsorption at pH 2.4	similiar		[114]
Lubrication and viscoelasticity			
Viscoelastic property of film	more elastic/less viscous	less elastic/more viscous	[114]
Friction coefficient (μ) at pH 7.4 at speeds 0.25–10 mm/s, load 1 N, PDMS-PDMS tribopairs)	0.03–0.09	0.7–0.9	[114]
Friction coefficient (μ) at pH 7.4 at speeds 10–1000 mm/s, load 5 N, POM-PDMS tribopairs)	0.03–0.01[a]	0.58-0.1[a]	[39]
Friction coefficient (μ) at pH 7.4, load 0–25 N, PS-PS tribopairs)	~0.02[a]	0.01–0.12[a]	[107]

[a] Derived from figures presented in references.

date. Based on the "zipper-like" entanglement of bottle-brush branched mucins model predicted by Bloomfield [122], a dumbbell-like configuration (two globules per chain) has been proposed by Yakubov and co-workers [110] for PGM in bulk solution. By using AFM and transmission electron microscopy (TEM) experiments, they described this model with a 40–50 nm long peptide backbone chain flanked with 2.5 nm long carbohydrate side-chains and two globular terminal subunits of the approximately 9 nm radius. Later, Znamenskaya and coworkers [108] performed imaging of dried PGM to confirm this model by AFM. Both fiber-like and dumbbell-like structures were visualized for PGM. Their measurements showed a smaller size of PGM molecules, which can be explained by their different sample preparations as PGM molecules became flatter during dehydration. However, only fiber-like structures of BSM were observed in their AFM experiments [123]. It should be noted that different pre-treatment of mucin samples, including purification, can be partly responsible for this discrepancy too, as mentioned above.

3.2.4. Adsorption behaviour

A majority of adsorption studies of mucins to date have been carried out by employing nonpolar, hydrophobic surfaces as a target substrate to highlight the amphiphilic characteristics of mucin molecules, even though studies on polar surfaces are also available [26,123,124]. A few studies have directly compared the adsorption behaviour of PGM versus BSM. Both Madsen and co-workers [114] and Çelebioğlu and coworkers [39] demonstrated higher adsorbed mass of PGM as compared to BSM (Type I-S following a further purification [114] and as received Type I-S [39], both from Sigma-Aldrich®) onto polydimethylsiloxane (PDMS) surface under neutral environment, while this was contradicted with the report by Lee (Type I, Sigma-Aldrich®) [107]. Considering that BSM samples in these studies had known difference in the degree of purity, the observed difference might have a technical rather than a scientific origin.

Another study from Lee's group revealed that a further purification of commercially available BSM (Type III, Sigma-Aldrich®) by means of anion exchange chromatography (AES) led to a substantial decrease in the adsorbed mass [112]. This observation suggests that the adsorption behavior, notably adsorbed *amount*, of mucins is significantly affected by the degree of purity of mucin samples. For instance, impurities in less purified mucin samples may contribute to the adsorbed mass and/or aggregation of mucin-impurities may alter various physicochemical properties affecting the adsorption properties. The influence of changing the environmental pH from neutral to acidic on the adsorbed mass of mucins also showed a strong dependence on both type and purity. While the adsorbed mass of PGM was virtually uninfluenced or decreased slightly in most studies [39,107,114], the trends in the corresponding change of BSM are highly scattered [107,114]. As it is well known, adsorption of anionic mucins onto nonpolar, hydrophobic surfaces from bulk solutions results in the accumulation of the charges on the surfaces, which act as an additional barrier to suppress further adsorption [125]. Although both BSM and PGM are anionic macromolecules with various acidic moieties, BSM is expected to be more sensitive to pH change, which is consistent with more abundant negatively charged moieties than PGM throughout its structure [114] (Fig. 4). This may explain the relatively insignificant changes of the adsorbed mass of PGM in response to the pH change. Meanwhile, as commercial BSM is known to contain a significant amount of other proteins as impurities, the influence of change in pH on highly purified versus as-received BSM can be further influenced by the impact of pH on impurities. This requires further experimental verification and is currently under study. Lastly, from the assessment of their film viscoelasticity (Table 4), BSM was reported to form a more elastic film on PDMS surface than PGM, which may be further associated with BSM's stronger binding ability onto the hydrophobic surfaces. These differences with regard to the adsorption behaviours give more insight to their lubrication abilities and conformational changes upon adsorption [114].

3.2.5. Lubrication

Both from the measurements of the nanoscale and macroscale tribological devices, BSM has been reported to show superior lubricating properties as compared to PGM (Table 4). For nanoscale measurements, Lee [107] explored these two kinds of mucin coatings on polystyrene (PS) surface by AFM. Results suggested that both types of mucins can be used as potential lubricant under nanoscopic scale contacts, although BSM was more efficient than PGM in reducing the frictional forces between PS surfaces. The superior lubricating properties of BSM to PGM

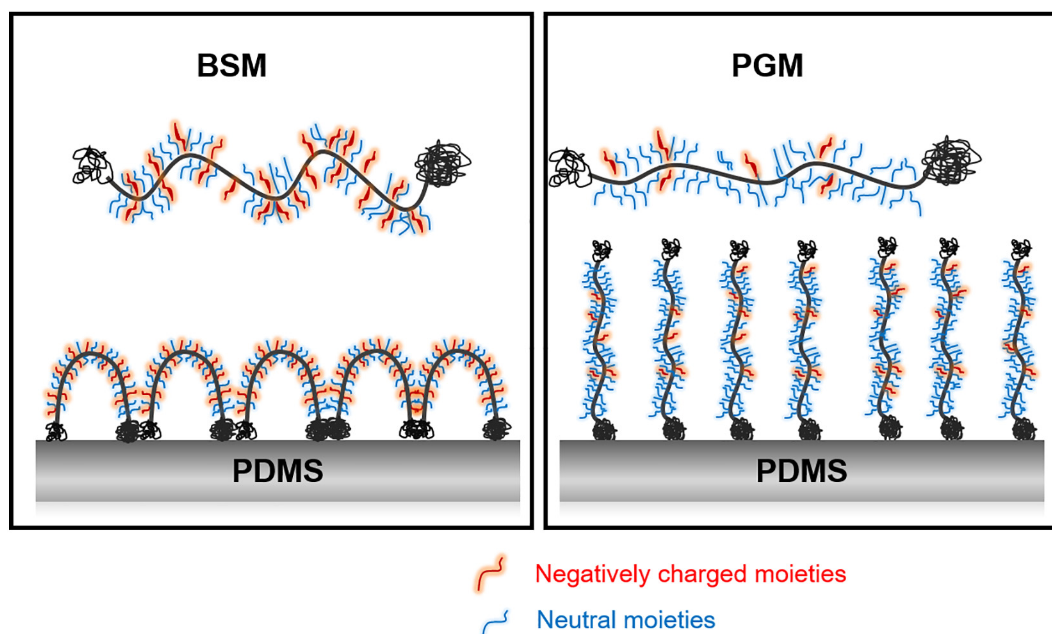

Fig. 4. Schematic illustration of bovine submaxillary mucin (BSM), pig gastric mucin (PGM) and their monolayer adsorption onto hydrophobic polydimethylsiloxane (PDMS) surfaces at neutral pH.

were more pronounced at macroscale contact, such as PDMS-PDMS interface as reported by Madsen and co-workers [114]. While the friction coefficients (μ) of BSM slightly increased from 0.03 to 0.09 with increasing speeds (from 0.25 to 100 mm/s), at pH 7.4, PGM was less lubricious with one order of magnitude higher μ values than BSM under the same conditions [114]. The contrasting lubricating capabilities between BSM and PGM can be correlated to their different adsorption behaviour onto the tribopair surface, *i.e.* nonpolar, hydrophobic surfaces, such as PDMS or PS. It is important to note that while adsorbed mass of mucins may play a role, it is not the determining factor for their lubricating properties. As mentioned above, the relative magnitude of adsorbed BSM and PGM varied significantly depending on the purity of mucin samples, but superior lubricity of BSM over PGM was consistently observed throughout all the studies to date. Moreover, further purification of BSM in a study by Nikogeorgos and co-workers [112] showed an improved lubricity despite a substantial decrease in the adsorbed mass after AES-based purification. These observations collectively point to that a vital parameter to determine the lubricity is not the adsorbed amount, but possibly the binding strength and the stability of the mucin films anchored on hydrophobic surfaces [114].

Çelebioğlu and co-workers [39] also confirmed that at macro-scale, BSM performed as a better lubricant than PGM using Mini Traction Machine (MTM) at pH 5 and pH 7.4 (Table 4). With increasing speeds from low (10 mm/s) to high (1000 mm/s), the μ values of BSM solution (1 mg/ mL) began to decrease, reaching as low as 0.03 at pH 7.4. The lubricating ability of PGM was minimal in the low-speed regime with a starting point of μ value of approximately 0.8 (in the case of BSM, μ = 0.24) [39].

In summary, if we focus on the lubricity, commercial BSM derived from Sigma-Aldrich® appears to be a better choice than PGM for model saliva formulations owing to its consistently reported superior lubricating capabilities at neutral pH, regardless of the purity. However, many other physicochemical properties of BSM have been reported to be not sufficiently reproducible. Based on the known information on the commercially available BSM across studies, it can be suggested that varying purity of BSM, either due to batch-to-batch variations by manufactures or different further purification, is chiefly responsible for it. Thus, it is important to have a full control on the purity and composition in model saliva formulation to expect a high reproducibility in all necessary properties.

4. Adsorbed salivary film — structure and tribological properties

4.1. Human salivary pellicle

The salivary pellicle is a complex proteinaceous film that adsorbs on all oral surfaces, and provides a barrier to dissolution of enamel by dietary acids, and also lubricates the mouth, facilitating the oral processing of food. Salivary pellicle formation is a highly selective adsorption process, where macromolecules, such as mucins, PRP, statherin, α-amylase, lactoferrin, cystatins, lysozyme, IgA, from whole saliva adsorb onto any oral surface [20,21,126]. Depending on the type of oral surfaces, salivary pellicles are divided into enamel (teeth) pellicle and mucosal (epithelia) pellicle. Compared to the well-established structure and protective role of enamel pellicle, the fundamental understanding of mucosal pellicle still remains in the early stage [19].

The term "mucosal pellicle" remains under discussion on its definition among different scientific fields, due to the varying degrees of attachment of salivary proteins and involvement of other components. In general, the mucosal pellicle consists of a bound protein-rich inner layer (immobile layer) anchored onto the substrates and a lubricious, loosely arranged outer layer (mobile layer) containing proteins, ions and foreign components [20,127,128]. The MUC5B and MUC7 are integral for the structure formation of mucosal pellicles but these mucin molecules alone are not sufficient to build the multicomponent films [23,127]. *Ex vivo* studies showed initially rapid kinetics of formation of mucosal pellicle was triggered by smaller molecules, such as statherin and PRP, interacting with the membrane-bound mucins. Then, the pre-adsorbed proteins affect the subsequent and more complex adsorption of other components (mainly MUC5B) in the mucosal pellicles [19,20,129]. Thickness of the pellicle varies from 30 to 100 nm depending on the location of oral cavity as the composition of pellicle is influenced also by the underlying substratum [42,130]. A bulk salivary layer keeps the pellicle regenerated and maintains its integrity involving the formation of a salivary film together with adsorbed pellicle beneath [129]. It should be noted that the *ex vivo* salivary pellicle (also termed as salivary conditioning films in literature) obtained by simple deposition of whole human saliva onto a surface might not have similar properties to those of human saliva *in vivo*, due to the dynamic nature-engineered architecture of the latter found on soft oral surfaces. This gives food researchers difficulties in designing *ex vivo* oral processing experiments [35] and relating the material properties with sensory perception. Nevertheless, the lack of fundamental studies on structure and properties of salivary pellicle makes it a rich area for future research.

Lubricity is probably one of the most important functions of saliva. When the bulk salivary film is largely squeezed out from the rubbing contact zones of tongue-oral palate, salivary pellicle adsorbed onto the oral surfaces acts as a protagonist in supporting the applied load by the oral contact surfaces and modulating the interfacial friction, which is key to achieve oral lubrication. Salivary pellicle has been proposed to have two structural layers: a tightly bound layer and a hydrated mucin-rich layer, the latter effectively entrapping water within the pellicle [23]. At relatively low loads and consequently low pressures (up to a few atm), $\mu \sim 0.03$ can be observed suggesting that the frictional dissipation pathways tend to be dominated by hydration lubrication [23,131–133]. This hydrated architecture of pellicle retain mobility in order to protect the oral surfaces from wear and friction and contribute to oral lubrication. An *ex vivo* experiment confirms the ability of salivary conditioning films to reduce coefficient of friction (μ) using colloidal-probe AFM against silica surface [22]. The μ of *ex vivo* saliva-coated silica was in the range of 0.03–0.66 at nanoscale, which is lower by at least a factor of 20 than the water-lubricated counterparts [22]. Even the addition of surface-active components may result in the loss of lubricity, and this robust salivary film can be rehydrated and replenished without losing its lubricity permanently [35]. Interestingly, Bongaerts and co-workers [35] also pointed out the pre-coated human saliva substrates are prone to degradation and dehydration, and thus have widely varying properties depending on the preparation method. For instance, dried human saliva may result in $\mu \approx 2$–3, highlighting the importance of hydration for efficient boundary lubrication by the salivary pellicle [35]. Moreover, ploughing of the deposited salivary conditioning films from the surfaces during applied shear may increase the μ values dramatically [131]. Thus, a simple transfer of human saliva onto substrates might not be an ideal *ex vivo* model to investigate the oral lubrication due to issues with reproducibility, besides the obvious challenges of inherent variability of human saliva [35].

4.2. Adsorbed model saliva film using mucin monolayer

Approaches of mimicking salivary pellicle in *in vitro* experiments include using BSM. For instance, Sarkar and co-workers have bio-functionalized the hydrophobic tribological substrates, such as PDMS ($\theta_{water} \sim 110°$), with BSM after O_2-plasma treatment, where the hydrophilicity was restored over weeks ($\theta_{water} \sim 47°$) [26]. Although this was an effective technique to change the hydrophilicity of the substrates, such mucin-based model salivary formulation show significant differences in lubricating aspects when compared to real human saliva [24,134]. On the other hand, model saliva containing PGM does show significantly higher μ values as compared to those of real unstimulated and stimulated human saliva in the boundary lubrication regime (sliding speed $<$10 mm/s) [24,68,134]. However, this particular comparison should be taken with caution as the lubrication of adsorbed model saliva

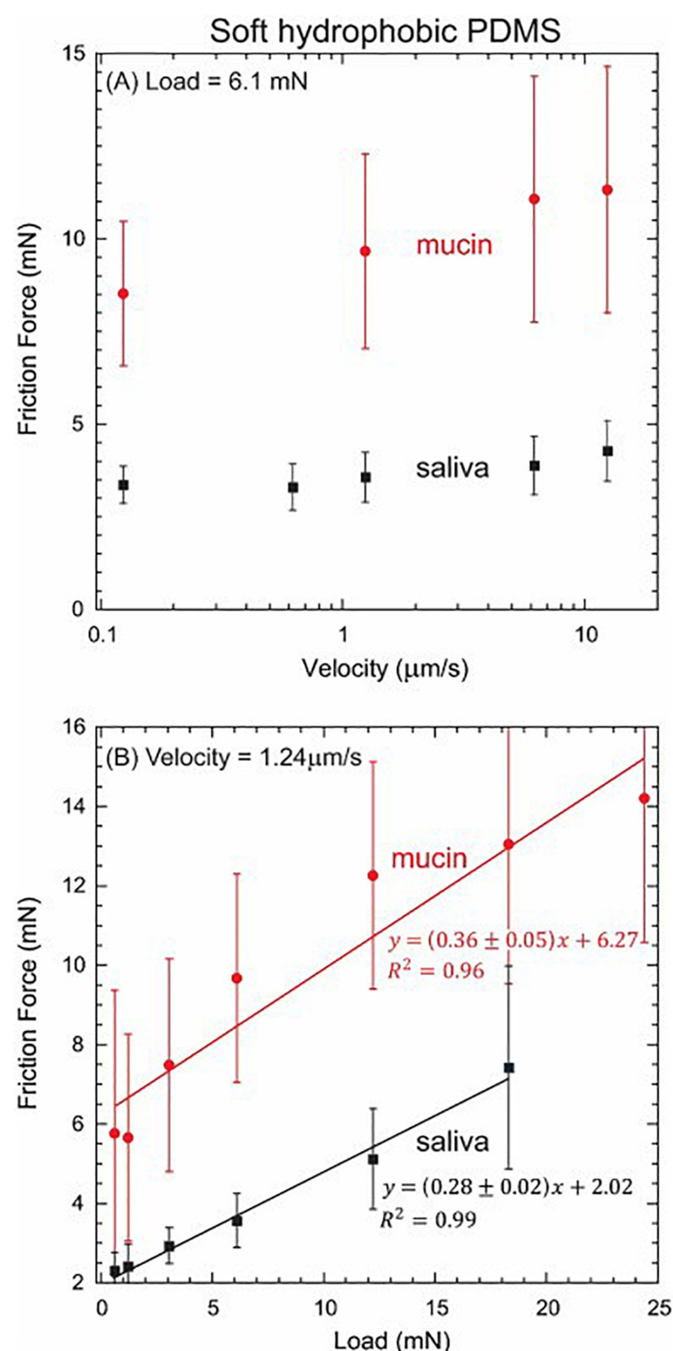

Fig. 5. Friction force (mN) as a function of velocity of soft, hydrophobic PDMS coated mica in presence of saliva or mucin as lubricant, at a load of 6.1 mN (A) and as a function of load at a velocity of 1.24 μm/s (B) (Reproduced with permission from [135]).

has been done using hydrophobic PDMS tribopairs, whereas real human saliva experiments were performed using pig mucosal contact surfaces.

To compare comparable contact surfaces, the lubrication effect of human salivary film and BSM monolayer film on PDMS-coated mica surfaces was recently determined using a surface force apparatus [135]. The friction force was more than twice as high in the presence of mucin than saliva within ca. 0.1 to 10 μm/s (Fig. 5A). Moreover, the μ obtained from the slope of friction force versus normal load graph (Fig. 5B) was also higher in presence of mucin (0.36) than saliva (0.28). Noteworthy is that salivary pellicle contains many acidic proline-rich proteins (PRP-1) that adsorb more on hydrophobic surfaces [136] and has a more pronounced lubricating effect as compared to mucin [137], the latter studied using AFM. In another study using nanoscale lubrication experiments (AFM), lubrication properties of salivary film was compared with its mucin-rich and PRP-1-rich fractions [110]. It was proposed that the low friction in salivary pellicle was a combinatorial effect of the hydrated brush-like layer formed by mucin, with low molecular weight components contributing to spreading, adsorption and strengthening of the salivary films on hydrophobic substrates. Thus, a more complex milieu of saliva, which is composed of mucins but also of other proteins, effectively reduces friction force and enhances the lubricity compared to mucin alone.

4.3. Adsorbed mucin multilayers – potential for biomimetic pellicle fabrication

It is worth noting that salivary pellicle in nature is created by a complex assembly of a range of proteins, and is still an unresolved structural research question at multiple length and time scales. There are some hypotheses of salivary pellicle being a multi-layered network [38,67]. Interestingly, layer-by-layer (LbL) assembly *i.e.* a classical technique of alternative depositions of anionic and cationic polymers or other polyelectrolytes from respective dilute solution with salivary proteins (*e.g.* mucin) as one of the chosen polymer can be a promising future strategy to create an effectively adsorbed biomimetic pellicle to study the dynamics of *in vitro* oral tribological experiments. As the driving force of these multi-layered architecture is electrostatic interaction, the properties of the resulting films (*i.e.*, thickness, adsorbed mass, roughness, wettability and viscoelasticity) can be precisely controlled by varying the self-assembly conditions, such as pH, ionic strength, polymer concentration and their charge ratio [138]. In addition, numerous studies demonstrated that the successfully built-up multi-layered systems are stable and uniform as compared to the monolayer [139]. Readers can refer to excellent reviews on LBL approaches (formation, properties) elsewhere for various applications from drug delivery, biomaterials, oral health, antibacterial coating to sensor technology [88,140].

To our knowledge, there are no study on mucin multilayer discussing the relationship between film thickness, roughness and the resultant lubrication in comparison to that of a real human salivary pellicle to date. Although mucin (BSM) monolayer adsorbed to PDMS surfaces has been used in tribological study of protein microgels with high relevance to food [26], there is no study in food tribology that has employed mucin multilayer-adsorbed tribopairs as contact surfaces to date. To explore its potential for model biomimetic salivary pellicle development, one first needs to compare the tribological properties of hydrophobic PDMS surfaces and mucin multilayer-adsorbed (hydrophilic) PDMS surfaces, in the absence and presence of food systems. In addition, such instrumentally acquired friction data should be compared with friction- or lubrication-associated sensory attributes, such as smoothness, pastiness, astringency *etc* [8,11,141]. A strong correlation (if obtained) between instrumentally acquired friction data and sensory dimensions may intuitively indicate adequate replication of the properties of the real salivary pellicle in these biomimetic surfaces.

5. Conclusions and future opportunities

Based on the comprehensive evidences gathered in this review, we can reiterate that there is no holy-grail model salivary formulation in literature that has fully replicated the bulk and surface properties of real human saliva yet. This is mainly associated with the fact that real human saliva has an extremely complex architecture with multiple proteins and consequently specialized properties, understanding of which is far from complete. Hence, designing one standardized formulation of real human saliva is not straightforward. Indeed, model saliva and real human saliva show similarities in food colloid-saliva interaction outcomes when the model saliva emulates the biochemical composition (*e.g.* ions, negatively charged commercially available mucin) and the electrostatic charge, to a certain extent. Such understanding has helped to decipher the physico-chemical mechanisms behind sensory

perceptions. However, considering the growing research interests on oral lubrication, model saliva studied mainly using less expensive and crude PGM is particularly problematic in replicating the lubrication and adsorption properties of saliva. Based on surface adsorption and lubrication studies from a wide pool of studies, we recommend that BSM appears to be the most optimal choice for the mucin type for model saliva in terms of lubrication properties. Further purification of BSM *via* dialysis or chromatographic techniques can help to generate better reproducibility and reliability for surface adsorption to hydrophobic surfaces that are commonly employed in oral tribology experiments. Despite that mucin is recognized as the chief contributor for the lubricating properties of real saliva, it is evident that aqueous solution of mucin alone cannot fully represent various physicochemical and biophysical properties of saliva. Systematic studies on designing mucin-multilayers with targeted tribological properties have to be investigated in future, particularly with respect to examining its potential to replicate human salivary pellicle. Tribological measurements in the presence of mucins and mucin-polycationic additive systems (multilayers) in comparison to *ex vivo* human salivary conditioning films are needed in first place to warrant its use in food tribology experiments.

Acknowledgements

The author (AS) would like to the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement n° 757993) for funding.

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