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ABSTRACT

Saliva plays a critical role in oral health, offering protection, aiding in digestion, and facilitating speech and swallowing. This study explores the biochemical composition of human saliva from healthy subjects, including total protein, glycoprotein, and calcium concentrations, in relation to its shear and time-dependent rheological properties. Eleven healthy, nonsmoking subjects were recruited, and salivary secretion rates were measured. Assays were used to determine concentrations of total protein, glycoproteins, and calcium, in addition to rheometry for evaluating the rheological properties of saliva. The results showed that unstimulated saliva, dominated by the mucins MUC5B and MUC7, displayed significantly higher viscosity and pronounced viscoelastic properties compared to stimulated saliva. Rheological analysis revealed saliva to be a viscoelastic material, exhibiting both elastic (solid-like) and viscous (liquid-like) responses. Shear thinning behavior was observed, where viscosity decreased with increasing shear rates, contributing to the fluid's ability to adapt to varying oral conditions. Furthermore, saliva exhibited thixotropy, a time-dependent material behavior characterized by structural breakdown under shear and recovery at rest. Calcium and glycoprotein levels were positively correlated with increased visco-elasticity, particularly with the storage modulus (G'), which reflects the ability of saliva to store elastic energy. These findings highlight the intricate relationship between the biochemical composition of saliva and its rheological properties, specifically its capacity for shear thinning, viscoelastic behavior, and time-dependent recovery, which are vital for its lubrication and protective functions in the oral cavity.

I. INTRODUCTION

Saliva has many important functions for oral health.[1] It covers and protects all soft and hard surfaces in the oral cavity, regulates pH, is important for oral clearance, helps bolus formation of food, and aids at mastication and swallowing.[2] Unstimulated saliva is rich in mucins MUC5B and MUC7 and secretory immunoglobulin A, and stimulated saliva has a high concentration of proline-rich proteins and amylase.[3] There are several reasons for a reduced salivary secretion rate of which side-effects of medicines are the most common.[4] Individuals with reduced salivary secretion rate often experience dry mouth, but there is no clear correlation between the experience of dry mouth and salivary secretion rate. This indicates that saliva composition and its functional properties are of importance for the experience of dry mouth.[2,3] Among individuals visiting primary health care in Sweden, the prevalence of dry mouth was 43.6%.[5]

A. Glycoproteins

Many of the proteins in saliva are glycoproteins, i.e., they consist of a protein core with a high content of glycan chains covalently attached to it by a post-translational modification called glycosylation.[6] Glycoproteins are rich in negatively charged groups of sialic and sulfate residues that further contribute to the rheological (flow) properties of the saliva.[7,8] Mucins are large glycoproteins and may contain one or more negatively charged sialic acid (N-acetylneuraminic acid, sialomucins) and/or sulfated sugars (SO3GlcNAc, sulfomucin). Sulfated and sialylated units with strong anionic character strengthen the water-holding capacity of the mucin molecule and its interaction with hard and soft tissues.[9] Thus, the structural properties of glycoproteins have roles in the immune defense, water-holding ability, and resistance against proteases.[8] As a result, they affect the physicochemical and functional qualities of the saliva, including viscosity, lubrication, and bacterial clearance.[8–10] In addition to mucins, saliva also contains other less complex glycoproteins such as agglutinin, proline-rich proteins, statherins, and histatins, which are present in serous secretions from the parotid gland.[11]

The most prevalent mucins in saliva are the soluble MUC7 with a molecular mass of 200–250 kDa and the gel-forming MUC5B with a molecular mass above 1 MDa[12] to 19–32 MDa.[13] Seventy percent of the mucins are secreted from the sublingual glands and by the labial and palatal minor salivary glands. The remaining 30% is secreted from the submandibular gland.[14] The level of MUC5B is 84% lower in stimulated whole saliva than in unstimulated whole saliva.[15]

B. Rheological properties of saliva

The rheological properties of saliva, such as its shear-thinning viscosity functions, extensional,[16] and viscoelastic properties, have been shown to be of high relevance for lubrication, bolus formation, mouthfeel, and taste perception, and have thus been the focus of extensive research.[17–21] Viscoelasticity describes a material exhibiting both solid and liquid-like characteristics, while shear thinning refers to rate-dependent viscosity. Mucins consist of 50%–80% carbohydrates[22] and are important for the viscoelastic properties of saliva. The removal of mucins causes a decrease in the viscosity of saliva to the level of water.[23] Large and highly glycosylated glycoproteins, with viscoelastic and protective properties, are vital in coating mucosal surfaces and are considered the main contributors to the rheological properties of saliva. Salivary secretion rate has a large influence on the experience of dry mouth,[24] along with factors such as pH and calcium ion concentration that affect the structure of MUC5B,[25,26] thus influencing lubrication efficacy and the experience of oral dryness.

In this study, we determine the correlation between the amount of total proteins, glycoproteins, and calcium, and the rheological properties of human saliva from healthy subjects with normal secretion rates. Rheological tests were performed to measure the viscoelastic, shear thinning, and time-dependent properties of saliva. In contrast to previous studies, we show that saliva also exhibits a pronounced thixotropic behavior, wherein the material structure undergoes time- and shear-dependent structural breakdown and recovery. With the introduction of thixotropy to the broad rheological analysis of saliva, we construct a non-dimensional number that describes the influence between the secretion rate and rheological properties of saliva with a power-law relationship.

II. MATERIAL AND METHODS

11 healthy, nonsmoking subjects (seven women and four men) with a mean age of 40 ± 9 years were recruited among volunteering employees at the Institute of Odontology, Sahlgrenska Academy, Gothenburg, Sweden. All the subjects were free from medications, without any diseases, and showed no signs of subjective or objective dry mouth problems. Collection of saliva was performed between 11 and 12 am before lunch. The subject first rinsed the mouth with 10 ml of tap water for one minute and had refrained from eating, drinking, and tooth-brushing at least 1 h before the collection of saliva.

Collection of unstimulated and stimulated whole saliva and determination of secretion rate.

For unstimulated whole saliva, the subjects sat on a chair leaning forward and allowing the saliva secreted to flow into an ice-cooled, graded plastic vial for 15 minutes.

Stimulated whole saliva was collected using paraffin wax. The subjects chewed on a piece of paraffin until it was soft and then swallowed once. Thereafter, saliva was collected for 5 min in an ice-chilled graded plastic vial. After the collection, the secretion rate was determined in ml/min for each study subject.

All the saliva samples were immediately transferred to Eppendorf vials, which were stored at −80 °C in Sahlgrenska's biobank with registration number 890.

A. Analysis of calcium

The samples were thawed on ice. Calcium concentration was determined using a calcium electrode (Thermo Scientific, Inc.) as described earlier.[5] 800 μl of saliva was mixed with 200 μl calcium ionic strength adjuster (ISA, Cat. No. 932011, Thermo Scientific, Inc.). In cases where the level of calcium in the saliva was out of range of the standard curve, samples were diluted and tests repeated with 700 μl of saliva and 300 μl of the ISA standard.

The free calcium concentration was calculated using a standard curve performed through serial dilution of the 100-ppm calibration standard (Cat. No. 923206), following the guidelines provided in the calcium ion-selective electrode user guide (Thermo Scientific Inc.).

B. Analysis of the total protein concentration

The total protein concentration was analyzed with the BCA Protein Assay Kit (Pierce, Rockford, IL, USA) with bovine serum

albumin as a standard. The saliva samples were diluted with distilled water to 1:2 and 1:4 and analyzed in duplicates. After the addition of reagents as described by the manufacturer, the plate was incubated for 30 min at 37 °C. The plate was thereafter read at 562 nm in an ELISA reader (Synergy 2, Biotek, Highland Park, Winooski).

C. Analysis of glycoprotein concentration

The Alcian Blue method was used to determine the amount of sulfated and sialylated glycoproteins. The method has previously been used to study intestinal mucous glycoproteins[27] and was applied to the saliva samples with some modifications.[28] Many glycoproteins are polyanionic containing both sialic acid and sulfate residues attached to the oligosaccharide structures. Those structures interact with Alcian Blue and form insoluble complexes.

A solution of 0.1 w/v Alcian Blue in 0.1 M sodium acetate acid buffer (pH 5.8) containing 25 mM $MgCl_2$ was used. Chondroitin 4 sulfate (Sigma Aldrich) was used as a standard[11] with concentrations ranging between 0.4 and 0.2 mg/ml. KH buffer (58.44 g/mol NaCl, 74.548 g/mol KCl, 120.361 g/mol $MgSO_4$, 110.978 g/mol $CaCl_2$, 136,086 g/mol KH_2PO_4, and 84.007 g/mol $NaHCO_3$) was used to dissolve the chondroitin 4 sulfate. Absorbance (A) was measured by spectrophotometer at 620 nm The concentration gradient of chondroitin sulfate was measured by using the Beer–Lambert law ($A = \varepsilon$ b C), which reveals the concentration (C) of sulfated and sialylated glycoproteins in saliva.

The saliva samples (100 μL) were mixed with 50 μL KH-buffer and incubated for at least one hour at room temperature to improve dissolution of the glycoproteins. Thereafter, 50 μL Alcian Blue was added to each analyte to a final volume of 200 μL. The analytes were incubated for 30 minutes at room temperature to allow sulfated and sialylated glycoproteins to form complexes with Alcian Blue solution. Then, samples were centrifuged at 3000 rpm for 15 min at 20 °C. Thereafter, the supernatant was removed, and each pellet was washed twice with 0.1 M sodium acetate buffer (pH 5.8) containing 25 mM $MgCl_2$ and precipitated at 4000 rpm at 20 °C for 10 minutes. The supernatant was discarded, and pellet was dissolved in 0.9% sodium chloride solution and was sonicated for 10 min at 50 °C. Finally, the properly dissolved analytes were measured at 620 nm in the spectrophotometer (Shimadzu UV-Visible UV-160).

D. Rheological analysis

Rheological properties were measured on an Anton Paar MCR702 MultiDrive (Anton Paar, Graz, Austria) rotational rheometer in single motor-transducer configuration. A parallel plate measuring geometry (50 mm diameter) was used with the gap set between 0.75 and 1 mm, to adjust for the amount of sample available and ensure proper contact with the plate geometry. After thawing the saliva samples, 2 ml was transferred to the measuring geometry and used for the experiments. The measuring temperature was set to 23 °C and the free surface of each sample was coated with 0.1% sodium dodecyl sulfate[29] after setting the measurement gap and left to rest for 300 s. To determine the limit of the linear viscoelastic regime, dynamic shear strain sweeps were performed on saliva samples at a constant angular frequency of $\omega = 6$ rad/s. Three types of tests were performed. Dynamic angular frequency sweeps and steady shear tests were performed in succession on the same sample to limit the material usage, with a 200 s relaxation time in-between. The angular frequency was varied between $\omega \in [0.1100]$ rad/s, while the shear rate range was $\dot{\gamma} \in [0.1500]$ s^{-1}. For both types of tests, the data were trimmed to exclude likely experimental noise. The steady shear data were fitted with the well-known power-law model,

$$\eta = K\dot{\gamma}^{n-1}, \tag{1}$$

with K being the consistency index—a measure of the magnitude of the viscosity—and n being the flow index—a measure of shear thinning, for $n > 1$. On a separate set of samples, thixotropy was measured in hysteresis loop tests, where the shear rate was increased from $\dot{\gamma} = 10^{-2}$ to 10^{2} s^{-1} and then decreased within the same range. The transient shear stress response, σ^{+}, was measured at constant time intervals of 10 s. Due to a complex time-dependent behavior described in the results section, thixotropy was quantified by calculating the area under the hysteresis loop for a portion of the shear rate range,

$$\sigma_y = \int_{t_0}^{t_a} \sigma^{+} d\dot{\gamma} - \int_{t_{end}}^{t_a} \sigma^{+} d\dot{\gamma}, \tag{2}$$

where t_a is the time corresponding to the shear rate limit of thixotropic behavior, as opposed to the apparent anti-thixotropic behavior recorded at higher shear rates. In effect, this is the lowest shear rate for which the transient stress on the upward curve is approximately equal to the transient stress on the downward curve.

E. Statistical analysis

Student's t-test was used to analyze differences in total protein, glycoprotein, and calcium concentrations and output/min between unstimulated and stimulated saliva. When calcium concentration was under the standard curve, the value was set to 1. The analysis of correlations between secretion rate of unstimulated/stimulated saliva and concentration of total protein, glycoprotein, calcium, viscosity, shear thinning, viscoelasticity, and thixotropy was analyzed with Pearson

TABLE I. Sex, age, and salivary secretion rates of the 11 subjects.

Subject	Sex	Age (years)	Unstimulated Secretion rate ml/min	Stimulated Secretion rate ml/min
1	M	28	0.9	2.8
2	F	47	0.6	2.2
3	M	35	0.3	2.4
4	F	46	0.7	2.4
5	F	51	0.5	3.0
6	F	47	0.3	1.2
7	M	50	0.5	1.9
8	F	27	0.5	2.0
9	M	28	0.5	2.6
10	F	43	0.7	4.0
11	F	33	0.5	1.5
	Mean ± SD	40 ± 9	0.5 ± 0.2	2.6 ± 0.8
	Median	43	0.5	2.4
	Range	27–51	0.3–0.9	1.2–4.0

TABLE II. Concentrations and outputs of total protein, glycoproteins, and calcium in unstimulated and stimulated saliva for the 11 subjects.

	Concentration			Output		
	Unstimulated	Stimulated	p-value	Unstimulated	Stimulated	p-value
Total protein (mg/ml)						
Mean ± SD	1.6 ± 0.8	2.7 ± 1.7	NS	0.8 ± 0.4	5.1 ± 2.4	$p < 0.001$
Median	1.4	2.5		0.7	4.6	
Range	0.7–3.3	1.3–7.1		0.3–1.4	1.9–10.3	
Glycoproteins (mg/ml)						
Mean ± SD	3.7 ± 2.3	4.8 ± 1.7	$p < 0.05$	2.0 ± 1.2	12.4 ± 6.1	$p < 0.001$
Median	3.6	5.2		2.2	12.7	
Range	0.4–7.9	0.9–7.1		0.2–4.0	2.1–22.4	
Calcium (mmol/L)						
Mean ± SD	0.26 ± 0.12	0.08 ± 0.07	$p < 0.001$	0.14 ± 0.07	0.21 ± 0.26	NS
Median	0.29	0.05		0.10	0.10	
Range	0.07–0.46	0.02–0.21		0.06–0.22	0.03–0.85	

correlation coefficient. Moreover, Pearson correlation coefficient was also used to analyze correlations between calcium, glycoprotein, total protein and viscosity, shear thinning, viscoelasticity, and thixotropy. A p-value < 0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

The unstimulated and stimulated salivary secretion rate of the 11 healthy subjects is shown in Table I. The secretion rate of stimulated (S) saliva was found to be almost five times the secretion rate of unstimulated (US) saliva. For stimulated saliva, there was a significant positive correlation between secretion rate and shear thinning ($r = 0.57$, $p < 0.05$) and a tendency to a positive correlation with viscoelasticity ($r = 0.50$, $p = 0.07$).

A. The amount of total proteins and glycoproteins

The mean total protein ($p = 0.06$) and glycoprotein ($p < 0.05$) concentration in unstimulated saliva tended to be lower than in the stimulated saliva. Similarly, the mean secretion rate of total proteins ($p < 0.001$) and glycoproteins ($p < 0.001$) was measured to be lower for unstimulated saliva compared to stimulated saliva, see Table II.

B. Calcium concentration and output

The mean concentration of calcium in the unstimulated saliva was significantly higher than in stimulated saliva ($p < 0.01$) (Table II). For eight of the nine subjects in whom calcium concentration was determined in both unstimulated and stimulated saliva, the concentrations were higher in unstimulated saliva.

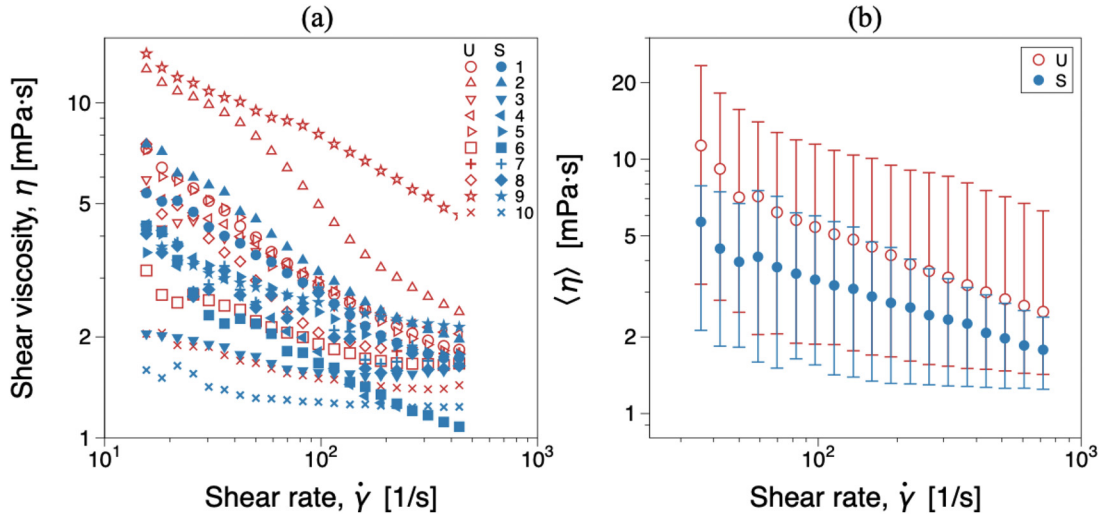

FIG. 1. (a) Shear viscosity functions and (b) mean shear viscosity functions of unstimulated and stimulated saliva samples.

TABLE III. Functional properties of unstimulated and stimulated saliva for the 11 subjects. For unstimulated saliva, data are missing for K, n (viscosity function), and $\tan(\delta)$ (viscoelasticty) for one subject. Hysteresis loop for unstimulated saliva could only be determined for four subjects.

	Unstimulated	Stimulated	p-value
K			
Mean ± SD	0.02 ± 0.02	0.01 ± 0.008	N.S (p = 0.06)
Median	0.03	0.01	
Range	0.002–0.05	0.002–0.03	
n			
Mean ± SD	0.64 ± 0.15	0.68 ± 0.20	N.S
Median	0.62	0.68	
Range	0.45–0.89	0.29–0.95	
$\tan\delta$			
Mean ± SD	0.74 ± 0.73	0.51 ± 0.26	N.S
Median	0.40	0.38	
Range	0.28–2.6	0.30–1.1	
S_y			
Mean ± SD	2.0 ± 0.9	1.0 ± 0.8	N.S (p = 0.07)
Median	2.0	0.9	
Range	0.9–3.1	0.2–2.9	

C. Rheological analysis

1. Steady-shear viscosity

Due to a higher concentration of MUC5B and MUC7 mucins, unstimulated (empty red symbols) saliva samples are more viscous compared to stimulated samples (filled symbols),[15] as shown in Fig. 1(a). Specifically, the viscosity of unstimulated saliva ranges from 2 to 20 mPa·s, whereas stimulated saliva displays a reduced viscosity range of 1 to 8 mPa·s, as illustrated in Fig. 1(b). Viscosity functions were modeled using the power law [Eq. (1)] to determine consistency index (K) and flow index (n). The higher viscosity of unstimulated saliva is reflected in its consistency index, which is twice that of stimulated saliva. The parotid gland has serous cells producing a watery saliva, while the sublingual and minor glands only have mucous cells giving a viscous saliva. The submandibular gland has both serous and mucous cells. The submandibular/sublingual gland contribute to 68% of the unstimulated saliva, the parotid glands with 28% and the minor salivary glands to 4%. When the glands are stimulated by chewing for example, the parotid glands produce 53% of the saliva, 46% from the submandibular/sublingual glands, and 1% from the minor glands.[30] Additionally, unstimulated saliva has slightly lower flow index (n) and a more pronounced shear-thinning behavior (Table III), which can be attributed partly to proteins getting adsorbed to the liquid–air interface,[31] increasing the density and viscosity of the adsorbed layer. Application of shear induces structural breakdown of this layer decreasing the viscosity.

2. Linear viscoelasticity

Viscoelasticity refers to the property of materials that exhibit both elastic (solid-like) and viscous (fluid-like) behavior when subjected to deformation. In viscoelastic materials, stress and strain responses are time-dependent, with elastic components storing deformation and viscous components dissipating energy. To characterize these properties, oscillatory shear tests are commonly used, where a sinusoidal strain is applied to the material, and the corresponding stress response is measured. The material's response is quantified by the storage modulus (G'), which represents the elastic, energy-storing behavior, and the loss modulus (G''), which reflects the viscous, energy-dissipating behavior. At low strain amplitudes in dynamic strain amplitude sweep tests, the storage (G') and loss (G'') moduli remain constant, independent of strain, indicating that the material is within its linear viscoelastic range (LVR). Subsequently, frequency sweep tests were performed within the LVR at a constant strain amplitude to assess the dependence of viscoelastic properties on oscillation frequency. Unstimulated and stimulated saliva show similar viscoelastic characteristics with a pronounced

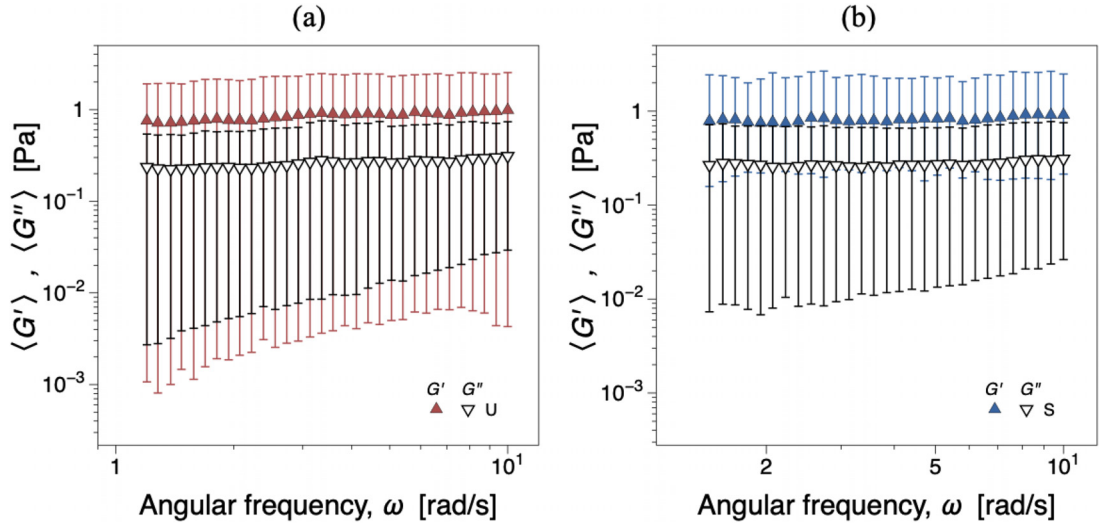

FIG. 2. Mean storage (G') and loss (G'') moduli of (a) unstimulated (U) and (b) stimulated (S) saliva samples obtained from oscillatory (angular) frequency sweep tests.

gel-like behavior ($\langle G' \rangle > \langle G'' \rangle$, $\tan(\delta) = 0.3$), see Figs. 2(a) and 2(b). For unstimulated saliva, there are statistically significant positive correlations between calcium concentration and viscoelasticity ($r = 0.72$, $p < 0.05$) and between glycoprotein concentration and viscoelasticity ($r = 0.57$, $p < 0.05$). Calcium is important for the unfolding of MUC5B,[26] which might indicate that an increased access to calcium contribute to unfolding of MUC5B, which in turn increases the ability of MUC5B to retain water, imparting viscoelastic properties to saliva.

For stimulated saliva, there is a tendency toward a negative correlation between glycoprotein concentration and viscosity ($r = -0.52$, $p = 0.08$). The seemingly parallel evolution of the dynamic moduli curves in the measured angular frequency range suggests that the high molecular weight long protein chains in saliva impart extremely long relaxation times to the material,[28,29] whose influence on thixotropic behavior is discussed in the following section.

a. Thixotropy and viscoelastic hysteresis. Thixotropy is a rheological property characterized by a material's ability to undergo structural breakdown over time when subjected to shear, followed by gradual recovery once the shear is removed.[32] This behavior is not only shear-dependent but also time-dependent, making it challenging to characterize due to the interplay between shear and time during rheological testing. One common method for assessing thixotropy is the hysteresis loop test. In this test, a logarithmically increasing shear rate is applied, followed by a gradual decrease, with a constant time step at each discrete shear rate. As the shear rate increases, the material's structure breaks down, leading to an increase in stress. Conversely, upon reducing the shear rate, the material partially recovers, resulting in lower stress levels and the formation of a hysteresis loop.

Human salivary glands produce two primary types of mucins: high molecular weight MUC5B ($M_w > 1$ MDa) and lower molecular weight MUC7 ($M_w = 200$–250 kDa).[15] Unstimulated saliva, Fig. 3(a),

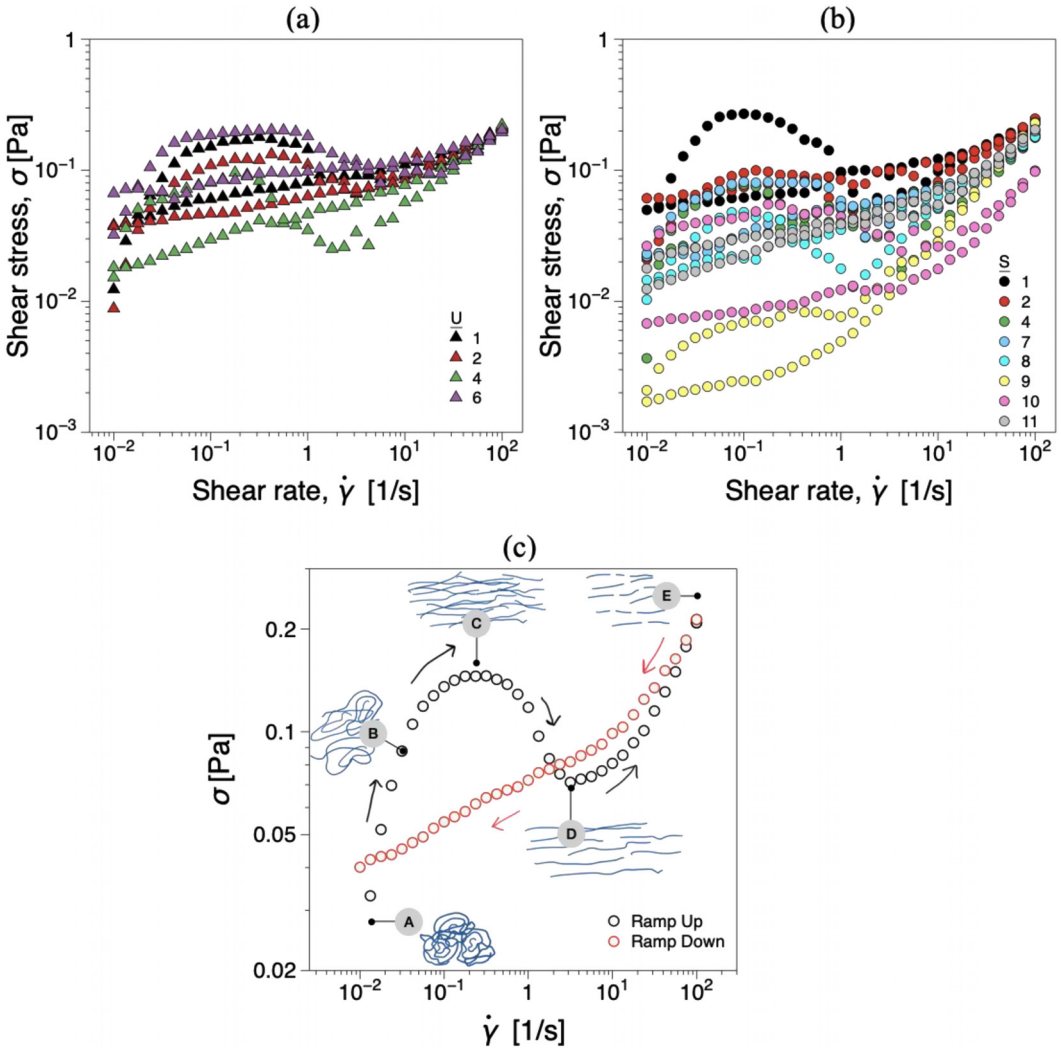

FIG. 3. Hysteresis loops of (a) unstimulated and (b) stimulated saliva samples. (c) Proposed morphological evolution of saliva microstructure in the hysteresis loop test (labels A through D).

having a larger content of MUC5B and MUC7 mucins, records higher stress magnitudes compared to stimulated saliva, Fig. 3(b), in hysteresis loop tests, in agreement with the viscosity data in Fig. 1.

We analyze the structural evolution of saliva in a representative thixotropic loop, shown in Fig. 3(c). When shear is applied in the hysteresis loop test, the structure of mucins and other proteins in the saliva samples breaks down as the stress increases. We propose that the (long chain) high molecular weight mucins exhibit significant elastic effects, resulting in a pronounced stress overshoot at lower shear rates, $\dot{\gamma} = 0.025\,\mathrm{s}^{-1}$ and $0.500\,\mathrm{s}^{-1}$, as the intertwined protein chains gradually start to disentangle [point A to B, Fig. 3(c)]. Upon increasing the shear rate further, the stress drops as the protein chains completely disentangle and align toward the flow direction [point C to D, Fig. 3(c)]. Subsequently, at the highest shear rate, the structure breaks down until the end of the ramp up cycle as the individual protein chains tear apart [point D to E, Fig. 3(c)]. As the shear rate is ramped down, the protein chains gradually recover, specifically the high molecular weight, gel-like, MUC5B mucin with long relaxation times,[33,34] resulting in a monotonically decreasing flow curve typical of shear thinning fluids that can be described by a power law model, $\sigma = K\dot{\gamma}^n$. When kept at rest for an extended period, the structure may completely recover and go back to its original state (point A), highlighting the thixotropic nature of saliva.

In addition to material relaxation, the adsorption of a rigid protein layer, approximately 100 nm thick, at the saliva–air interface over time enhances the stress response, imparting apparent shear-thickening characteristics to the material at higher shear rates. This dynamic interplay between the glycoproteins and the adsorbed protein layer underscores the complex rheological behavior of saliva under varying shear conditions and is responsible for the self-intersection of the hysteresis loop, as also observed for soft glassy materials.[35]

To study the interplay between the rheological properties of saliva, K and n from power law fits to viscosity functions, $\Delta G = G' - G''$ calculated from frequency sweep, thixotropic loop area $S_y = \int_{\dot{\gamma}_i}^{\dot{\gamma}_f} \sigma\, d\dot{\gamma}$, secretion rate R, and age of the subjects A can be incorporated into a non-dimensional number represented by Π, the Sialo number (based on Sialon, the Greek word for saliva) for a unit volume (V) of sample. Π is a relative measure of the shear thinning, viscoelastic, and thixotropic behavior of human saliva having a power law scaling with the secretion rate, R (see power law fits in Fig. 4). Π calculated for unstimulated and stimulated saliva samples is inversely and directly proportional to R, respectively.

We postulate that the proposed scaling power laws can be used to approximate the secretion rate of human saliva from the rheological parameters of the sample. Among the rheological parameters considered, hysteresis loop area has a dominant influence on R as Π scales up with the square of S_y. Therefore, the salivary secretion rate greatly influences the thixotropic behavior of saliva, which is a result of the time-dependent structural evolution and long relaxation times of the high molecular weight protein chains. A secretion rate ≤ 0.2 ml/min (shaded region in Fig. 4) is considered low, whereas a secretion rate below 0.1 ml/min often results in oral dryness.[36]

IV. CONCLUSIONS

The present study explored the biochemical composition of human saliva from healthy subjects, including total protein, glycoprotein, and calcium concentrations, in relation to its shear- and time-dependent rheological properties. Unstimulated saliva with high levels of the mucins MUC5B and MUC7, displayed significantly higher viscosity and pronounced viscoelastic properties compared to stimulated saliva. Rheological analysis revealed saliva to be a viscoelastic material, exhibiting both elastic (solid-like) and viscous (liquid-like) responses. Shear thinning behavior was observed, where viscosity decreased with increasing shear rates, contributing to the fluid's ability to adapt to varying oral conditions. Furthermore, saliva exhibited thixotropy, a time-dependent material behavior characterized by structural breakdown under shear and recovery at rest. Calcium and glycoprotein levels were positively correlated with increased viscoelasticity, particularly with the storage modulus (G'), which reflects saliva's ability to store elastic energy. The findings of the present study highlight the intricate relationship between the biochemical composition of saliva and its rheological properties, specifically its capacity for shear thinning, viscoelastic behavior, and time-dependent recovery, which are vital for its lubrication and protective functions in the oral cavity. We demonstrate that the rheological properties of saliva have a power law correlation with the secretion rate, which can be leveraged to estimate the rheological functions or the secretion rate itself, given the other parameters are known.

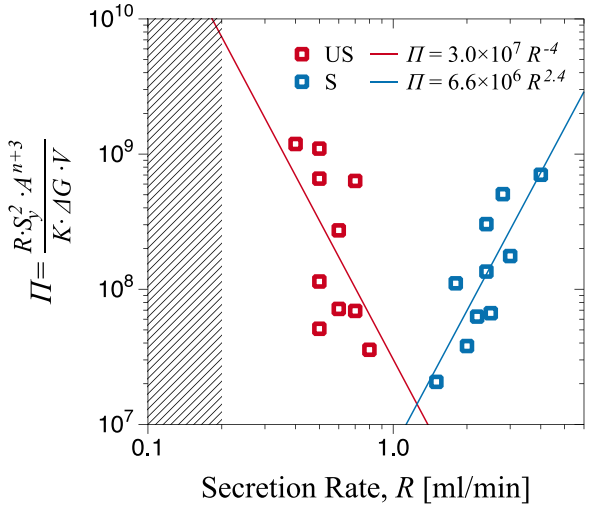

FIG. 4. Power law relationship between secretion rate and rheological properties of unstimulated and stimulated saliva samples. The hatched region represents a low unstimulated secretion rate, whereas $R \geq 1$ ml/min is considered a normal stimulated secretion rate.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

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DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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