Age- and Flow-dependency of Salivary Viscoelasticity

INTRODUCTION

Viscoelasticity is a rheological property of complex fluids having two components, viscosity and elasticity. Viscosity is related to energy dissipated during flow, while elasticity is related to energy stored during flow. Saliva contains various quantities of macromolecules (Briedis et al., 1980), which affect its rheological properties. Rheological measurements have been performed on saliva only for shear viscosity, with the use of a simple capillary viscometer (Waterman et al., 1988), but never for viscoelasticity (elongational viscosity), which is the more appropriate analysis for salivary biological and physiological properties (Schwarz, 1987; Aguirre et al., 1989a,b; Mellema et al., 1992). Saliva's viscoelastic, polymeric nature gives it its exceptional lubricant properties. Measuring salivary viscoelasticity is of paramount importance, because salivary viscosity has been associated with the development of oral diseases, such as dental caries. In both animal and human model studies, novel, improved methods to evaluate properties associated with salivary distribution and lubrication are of clinical interest. It is often assumed that salivary viscosity is directly related to factors such as dry weight of solids, protein or mucin content, glycoproteins, and/or proline-rich protein composition.

The elasticity-related flow behavior of polymeric liquids, which are macromolecular solutions of the type of saliva, is known to be dramatically different from that of the corresponding purely viscous Newtonian liquids, even at such low polymer concentrations as 10 ppm (parts per million). Therefore, flow behavior of saliva, which has clinical importance, cannot be fully understood or characterized without accounting for salivary viscoelasticity. The latter is the main goal of the present work. Salivary viscoelasticity almost certainly should be affected by the nature and content of the dissolved polymers.

Accordingly, one may expect to note different viscoelastic properties in saliva secreted from the different salivary glands, since submandibular/sublingual saliva contains much higher concentrations of mucins and glycoproteins than does parotid saliva. Such differences may also be expected to be found between saliva secreted under resting conditions (which contains relatively high concentrations of submandibular/sublingual saliva) and saliva secreted under stimulation. All such factors affecting salivary viscoelasticity were studied in the present work. Furthermore, various studies showed that many of the elderly have diminished salivary secretion (xerostomia), either due to the effects of medication or as a result of age-related physiological changes (Nagler and Hershkovich, 2005). Such changes could also alter viscoelastic properties of saliva in the elderly, since reduced lubricating ability is characteristic of xerostomia. It has been well-established that, in oral and dental conditions, there are age-related differences that may be closely related to different salivary properties. For example, teenagers have a higher caries rate than the elderly, while the elderly suffer from higher rates of periodontitis.
Therefore, an additional aim of the present work was to examine the correlation between age and variations in salivary rheological properties. Saliva secreted under different physiological conditions and/or from different salivary glands has different physiological characteristics. For example, while saliva secreted at rest comes mostly from the submandibular gland, the parotid gland under stimulation secretes proportionally more saliva than do the submandibular glands, and thus the two types of glands contribute equally to stimulated whole saliva (Nagler et al., 2002a,b).

The purpose of the current study was to examine salivary viscoelastic behavior characterized by the relaxation time affected by the above-mentioned factors. In particular, the measured relaxation times were correlated to salivary flow rates and composition under different physiological conditions, from different salivary glands, and at different ages. Measurements were made by means of an elongational thread viscometer (Stelter et al., 2000; Yarin et al., 2004), which was applied to and validated for polymer solutions and fuel simulants, but which had never been used in salivary research. The different rheological properties of whole, parotid, and submandibular/sublingual saliva revealed by the present work might contribute to a better understanding of the pathologies found in xerostomic and elderly individuals, in the context of salivary secretions whose rheological behavior is compromised and which are less capable of flowing freely to the oral sites where their protective and other functions are required.

**MATERIALS & METHODS**

**Study Design and Saliva Collection**

Salivary flow rates, viscoelastic relaxation times, and sialochemical analyses of total protein, sodium, and potassium were measured in whole-saliva samples collected from 11 young (aged 20.6 ± 1.6 yrs) and 22 elderly (aged 75.8 ± 7.0 yrs) healthy non-medicating, gender-matched, consenting individuals. The study was approved by our institution’s Human Studies Ethics Committee. In six of the young participants, selected at random, the relaxation times of whole, parotid, and submandibular/sublingual gland salivas were also analyzed at rest and under stimulation.

**Salivary Collections, Sialochemistry, and Flow Rate Analysis**

The salivary samples (whole, parotid, and submandibular/sublingual) were collected on ice according to a fully standardized procedure, and then subjected to sialochemical analysis as previously described (Nagler et al., 2002b). Briefly, unstimulated (collected at rest) saliva specimens were obtained in the morning. No oral stimulus was permitted for 90 min prior to the collection. We used Carlson-Crittenden cups to obtain parotid saliva, and concurrently collected submandibular/sublingual saliva by standard gentle suction from the floor of the mouth. Subsequently, we collected saliva samples similarly, under stimulation, by applying 2% citric acid solution to the tongue dorsum bilaterally at 30-second intervals. Total protein was measured by Lowry’s method, and the concentrations of Na and K were measured by flame photometry as previously described (Nagler et al., 2002b).

**Viscoelastic Relaxation Time**

In the present study, rheological behavior of the salivary samples was studied in uni-axial elongational flow. Measurements captured the dependence of the cross-sectional diameter of a liquid thread on time \( d = d(t) \), during elongational flow. The experimental set-up consisted of a high-speed camera, which captured the filament shape, a fixed bottom plate, and an upper plate, which could be quickly raised by an electromagnet (Fig. 1).

![Figure 1. Elongational viscometer.](image)

For measurement, a droplet (~ 10 μL) of a tested fluid was placed between the two plates. Then, the movable plate was raised, the droplet was stretched, and a liquid thread appeared. After the movable plate came to rest, the thread continued to thin. The elongational flow in the thread was driven by capillary forces, the capillary pressure in the thread being of the order of \( \sigma d/d \), where \( \sigma \) was the surface tension of the liquid and \( d \) was the thread diameter. In contrast, the capillary pressure in the end regions of the thread, in the vicinity of the plates (Fig. 1), was of the order of \( \sigma / R \), where \( R \) was the characteristic radius of those regions. Since \( R >> d/2 \), pressure drops from the thread center appeared toward the end regions. Therefore, the surface tension forces squeezed liquid out of the thread, and elongational flow (depicted by the arrows in Fig. 1b) was produced. An example of a sequence of snapshots presenting the self-thinning process undergone by the liquid thread after stretching stops is presented in Fig. 2.

The earlier theoretical description of the evolution of the thread diameter \( d \) yielded the following formula for the self-thinning threads of this type (Stelter et al. 2000):

\[
d = d_0 \exp(-t/\tau)
\]

where \( d_0 \) is the diameter value at the start of the self-thinning process, \( t = 0 \). The experimental results fitted the above equation, which allowed for evaluation of the relaxation time \( \tau \).

During the experiments, the initial droplet length in the \( x \) direction was about 0.5 mm, the thread length during self-thinning was kept constant at 3.1 mm, and the unstretched end sections of the filaments totaled 0.8 mm. The end plates were made of copper, with a diameter of 7 mm. The thread thinning diameter \( d(t) \), at the center of the thread, was recorded by a CCD camera at a frame rate of 1000 fps and a shutter speed of 1/2000. Measurements were conducted at room temperature, 26°C.
Statistical Analyses

For the various data obtained, medians, means, and standard errors were calculated. Due to the large physiological variability of parameters in saliva, median values were preferred. Since the sample sizes of the groups studied (young vs. old) were rather limited (fewer than 30 in each), and since the variability of salivary parameters was large, the non-parametric Wilcoxon rank-sum test was used for comparing the results between the two groups. Thus, the Wilcoxon rank-sum test (a non-parametric test also called the Mann-Whitney-Wilcoxon test) was used to examine the hypothesis that two independent samples came from distributions with equal medians. The null hypothesis was that both population medians were equal (and that the two samples were drawn from a single population).

RESULTS

Salivary Relaxation Time and Flow Rate as Related to Age

While the median whole salivary flow rate of the elderly group dropped significantly as compared with that of the young group, the median relaxation value increased, although not in a statistically significantly manner. The median flow rates of the young and elderly were 0.45 mL/min and 0.17 mL/min, respectively (mean ± SE values were 0.42 ± 0.17 mL/min and 0.16 ± 0.12 mL/min, respectively; p = 0.002) (Fig. 3A). The median relaxation times of the two were 2.24 ms and 3.45 ms, respectively (a 54% increase in the elderly group) (Fig. 3A).

Sialochemical Analysis

The median total protein concentration in the whole saliva collected from the elderly was 85.7 mg/dL, higher (by 48%) than that from the young group (p < 0.05). Similarly, the median concentration of potassium in the elderly was 25.9 mmol/L, significantly higher (by 47%) than in the young (p < 0.01) (Fig. 3B). The total protein and potassium mean ± SE concentrations in the elderly were 93.5 ± 46.0 mg/dL and 27.2 ± 1.47 mmol/L, respectively. In contrast, the salivary concentrations of sodium were not significantly different for the two groups.

Relaxation Times of Whole, Parotid, and Submandibular/Sublingual Saliva

The median relaxation times at rest of whole, parotid, and submandibular/sublingual saliva were 39.5 ms, 1.04 ms, and
42.1 ms, respectively. The difference between parotid and submandibular/sublingual values was significant (p = 0.05), while those between parotid and whole saliva, and whole saliva and submandibular/sublingual saliva, did not reach statistical significance.

The pattern of the median salivary relaxation times under stimulation of whole, parotid, and submandibular/sublingual saliva was similar to that observed at rest, although with more extreme differences. Under stimulation, the median relaxation times of the whole, parotid, and submandibular/sublingual saliva collected were 47.6 ms, 1.40 ms, and 399.0 ms, respectively. The differences between the salivary relaxation time values of parotid and whole, parotid, and submandibular/sublingual saliva were significant (p = 0.02), while the differences of submandibular/sublingual and whole saliva did not reach statistical significance (p = 0.20).

The experimental results obtained for the elongational flow of parotid saliva, collected at rest from a young individual, are presented in Fig. 4. Graphs a-f in Fig. 4 present the change of the liquid thread diameter over time for this young individual. We found the relaxation time by fitting the equation to the almost linear sections (in the semi-logarithmic frame) of the graphs between the vertical lines. The relaxation times at rest were obtained from Fig. 4 as: (a) whole saliva, $\theta = 1$ ms; (b) submandibular/sublingual, $\theta = 3.58$ ms; (c) parotid, $\theta = 1.08$ ms. Times under stimulation were: (d) whole saliva, $\theta = 3.46$ ms; (e) submandibular/sublingual, $\theta = 18.70$ ms; and (f) parotid, $\theta = 1.31$ ms. For comparison, the relaxation times for an elder person at rest were: whole saliva, $\theta = 77.5$ ms; submandibular/sublingual, $\theta = 35.88$ ms; and parotid, $\theta = 1.01$ ms. Times under stimulation were: whole saliva, $\theta = 340$ ms; submandibular/sublingual, $\theta = 592$ ms; and parotid, $\theta = 1.40$ ms.

**DISCUSSION**

Our most important finding was that different types of saliva were viscoelastic, and that viscoelasticity of whole, parotid, and submandibular/sublingual saliva could be measured and expressed by their relaxation times. While the relaxation time of parotid saliva was almost nil, which means that it was very close to that of an ordinary Newtonian liquid, that of submandibular/sublingual saliva was significantly higher, and more so under stimulation. Salivary comparison between the groups showed an age-related reduction in salivary flow rate, accompanied by an increase in salivary viscoelasticity and protein content. The increase in salivary potassium found among the elderly was typical of a salivary flow-rate reduction and reinforced our observations. This increased viscoelasticity of whole saliva in the elderly may result from a reduction in salivary watery content, which results in increased salivary protein concentration, as previously shown in similar conditions (Nagler et al., 1997; Nagler and Hershkovich, 2005). The significant difference in the viscoelasticity of the parotid and submandibular/sublingual saliva may have resulted from the difference in their protein profiles, such as mucins and...
glycoproteins, which are much more prevalent in submandibular/sublingual saliva (Aguirre et al., 1989; Nagler et al., 2002a). Following stimulation, the relaxation value of the submandibular/sublingual saliva increased ten-fold, while the relaxation value of the parotid saliva was not altered. This added credence to the relation suggested between the specific protein composition of the saliva and its rheological properties, since the stimulation-dependent increase in parotid proteins (which do not substantially affect salivary viscoelasticity) would not be expected to alter parotid salivary relaxation time. The opposite would be expected for submandibular/sublingual saliva, whose protein content increased following stimulation.

In any case, regardless of the mechanisms involved, the clinical significance is of paramount importance, because a salivary secretion whose rheological capacity is compromised is less capable of flowing freely to the oral sites where its protective and various other functions are required. Hence, we suggest that salivary viscoelasticity, expressed through salivary relaxation time, can become a ‘fingerprint’ for certain disease conditions, age-related and medication-related changes, etc., and should be examined further.

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REFERENCES


