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ORIGINAL ARTICLE

Viscosity and wettability of animal mucin solutions and human saliva

M-S Park, J-W Chung, Y-K Kim, S-C Chung, H-S Kho

Department of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, Seoul, Korea

OBJECTIVE: The purpose of this study was to compare viscosity and wettability between animal mucin solutions and human saliva.
MATERIALS AND METHODS: Human whole and glandular saliva, porcine gastric mucin, bovine submaxillary mucin, and a mucin-based saliva substitute were used. Viscosity was measured with a cone-and-plate digital viscometer, while wettability on acrylic resin and Co–Cr alloy was determined by the contact angle.
RESULTS: The viscosity of animal mucin solutions was proportional to mucin concentration, with the animal mucin solution of concentration 5.0 mg ml^{-1} displaying similar viscosity to stimulated whole saliva. A decrease in contact angle was found with increasing animal mucin concentration. For the saliva samples tested, viscosity increased in the following order: stimulated parotid saliva, stimulated whole saliva, unstimulated whole saliva, stimulated submandibular–sublingual saliva. Contact angles of human saliva on the tested solid phases were inversely correlated with viscosity. Contact angles of human saliva on acrylic resin were much lower than those of animal mucin solutions and of those on Co–Cr alloy ($P < 0.01$).
CONCLUSIONS: The effectiveness of animal mucin solutions in terms of their rheological properties was objectively confirmed, indicating a vital role for mucin in proper oral function as well as the development of effective salivary substitutes.
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Keywords: mucin; viscosity; wettability; contact angle

Introduction

The development of effective salivary substitutes requires an understanding of both the rheological and biological properties of natural human saliva, which is composed of a complex of macromolecules, primarily proteins and glycoproteins such as proline-rich proteins, α-amylase, mucins, statherins, cystatins and histatins (Mandel, 1987, 1989; Levine, 1993; Schenkels *et al*, 1995). Because surfaces within the oral cavity are in continual moving contact, a fundamental function of salivary proteins is to provide a lubricating film on the teeth and oral mucosa. This lubricative film allows food to travel easily through the digestive system and provides a smooth tissue surface with minimal friction (Mandel, 1989). Among the salivary proteins, mucus glycoproteins, or mucins, are primarily responsible for the lubricating and film-forming properties of human saliva (Tabak *et al*, 1982; Mellema *et al*, 1992; Van der Reijden *et al*, 1994; Christersson *et al*, 2000; Dodds *et al*, 2005).

The importance of saliva becomes readily apparent in individuals whose capacity for saliva production is diminished. For example, most xerostomic patients have difficulty in essential functions such as speech, taste, mastication and swallowing (Mandel, 1987; Sreebny and Valdini, 1988; Porter *et al*, 2004). Both intrinsic and extrinsic approaches are used to address the complaints of xerostomic patients (Levine *et al*, 1987). The intrinsic approach is to employ sialogogues, such as pilocarpine and cevimeline, in order to maintain or stimulate hypofunctional glands (Fox, 1987, 2004; Porter *et al*, 2004). The extrinsic approach, the only regimen for patients with completely impaired salivary glands, is to administer a saliva substitute (Levine *et al*, 1987; Levine, 1993). Current saliva substitutes are generally divided into two categories: carboxymethylcellulose (CMC)-based saliva substitutes and animal mucin-based saliva substitutes (Vissink *et al*, 1984, 1986; Hatton *et al*, 1987). Although these saliva substitutes may decrease some symptoms of oral dryness in xerostomic patients, the alleviating effects of today's commercially available substitutes are short-lived and, therefore, of limited benefit to patients (Levine *et al*, 1987; Olsson and Axell, 1991). Despite this, several studies have reported that mucin-based saliva substitutes are more effective than their CMC-based

Correspondence: Dr Hong-Seop Kho, Department of Oral Medicine and Oral Diagnosis, School of Dentistry and Dental Research Institute, Seoul National University, Yunkeun-Dong 28, Chongro-Ku, Seoul 110-749, Korea (ROK). Tel: +82-2-2072-2611, extn 3989, Fax: +82-2-744-9135, E-mail: hkho@plaza.snu.ac.kr
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counterparts (Vissink *et al*, 1983; Duxbury *et al*, 1989; Blixt-Johansen *et al*, 1992).

Previous clinical studies on the effectiveness of saliva substitutes have largely depended on subjective reports of xerostomic patients (Vissink *et al*, 1983; Duxbury *et al*, 1989; Olsson and Axell, 1991). Few objective data exist regarding the rheological (viscosity) and film-forming (wettability) properties essential for the proper functioning of any saliva substitute. Although an ideal saliva substitute mimics the rheological and biochemical properties of natural human saliva (Vissink *et al*, 1984), the addition of antimicrobials to a solution otherwise having rheological properties similar to human saliva may be an even better solution, and this approach is presently feasible.

We investigated the viscosity and film-forming property of animal mucin solutions, human saliva, and a commercially available, animal mucin-based saliva substitute. This study furthers our understanding of mucin's role in saliva substitutes and may assist in the development of effective saliva substitutes.

Materials and methods

Saliva collection

Human saliva was collected from 20 healthy donors, aged 25–35 years, between 9:00 and 11:00 AM. All subjects had refrained from eating or drinking for 2 h prior to collection. Unstimulated whole saliva (UWS) was collected for 10 min by the spitting method (after swallowing, saliva is collected with closed lips and then expectorated into a vessel one or two times per minute). Stimulated whole saliva (SWS) was collected for 5 min, and chewing of paraffin wax (1.0 g) was employed as a mechanical stimulus. Stimulated parotid saliva (SPS) was collected with the aid of a plastic suction cup (modified Lashley cup) placed directly over the Stensen's duct orifice. Stimulated submandibular–sublingual saliva (SSMSLS) was collected with a custom-made Block and Brottman collector (Block and Brottman, 1962). For the collection of glandular saliva, secretion was stimulated by applying 2% citric acid solution every 30 s to the lateral border of the tongue. All stimulated human saliva collected during the first 2 min was discarded. All human saliva samples were centrifuged at 3500 *g* for 10 min at 4°C; the resulting clarified supernatant fluid was used immediately for experiments.

Animal mucin solution and artificial saliva

Commercially available porcine gastric mucin (PGM) and bovine submaxillary mucin (BSM) (Sigma Chemical Co., St Louis, MO, USA) were dissolved in simulated salivary buffer (SSB, 0.021 M Na_2HPO_4/NaH_2PO_4, pH 7.0, containing 36 mM NaCl and 0.96 mM $CaCl_2$) (Bennick and Cannon, 1978) and distilled deionized water (DDW) at various concentrations (0.5, 1.0, 2.5, and 5.0 mg ml^{-1}). To investigate the effect of denaturation, animal mucins dissolved in SSB were boiled in a water bath for 10 min. Saliva Orthana (Orthana Kemisk Fabrik, Kastrup, Denmark), a commercially available porcine gastric mucin-based saliva substitute, was used for comparison with human saliva.

Measurement of viscosity

Viscosity measurement was conducted with a model LVT Wells-Brookfield cone-and-plate digital viscometer (Brookfield Engineering Laboratories, Stoughton, MA, USA). Shear rates were varied incrementally from 11.3 to 450.0 s^{-1} at six different speeds. All measurements were carried out at 37°C, and 0.5 ml volume of fluid was used in each test. The viscosity of each sample was measured five times.

Preparation of test specimens for contact angle measurement

Heat-cured acrylic resin, Paladent® 20 (Herareus Kulzer, Wehrheim, Germany), and cobalt–chromium alloy, Biosil® f (DeguDent, Hanau, Germany) were used as surface phases. Ten specimens of each material ($30 \times 30 \times 1.5$ mm) were prepared to have highly flat surfaces. For acrylic resin specimens, a sheet of wax 1.5 mm thick was pattern-adapted between two plates of glass. The glass and wax sandwiches were inserted into dental flasks, boiled for 5 min to soften and eliminate the wax, and heat-cured. The samples were ground with 600- and 800-grit silicon carbide sandpapers, followed by a felt disc with pumice. Co–Cr alloy specimens (composition in mass %; Co 64.8, Cr 28.5, Mo 5.3, Si 0.5, Mn 0.5 and C 0.4) were cast and finished in the same manner as would be the tissue surface of a removable partial denture framework, according to the manufacturer's instructions.

Measurement of contact angle

Measurement of contact angle was performed with a Phoenix 300 (Surface Electro Optics Co., Ansan, Korea). Contact angles were measured on the photographs as follows: 10 μl droplets of each liquid were positioned on the test specimens by means of a 1-ml syringe with a blunt point. After 30 s, a tangent to the droplet was drawn from the point of air–fluid–solid phase intersection. Contact angles between this tangent line and the dental material surface were calculated from enlarged photonegatives of the droplets. Measurements of contact angle were performed 10 times for each test solution.

Statistics

Student's *t*-test, ANOVA and Duncan's multiple range test were used to compare the mean values of viscosity and contact angle.

Results

Viscosity

The viscosity of human saliva was found to be inversely proportional to shear rate, a non-Newtonian trait of biological fluids. Mean viscosity values at various shear rates increased as follows: SPS, SWS, UWS and SSMSLS (Figure 1). Representative values are those at a shear rate of 90 s^{-1}: 1.33 ± 0.29,

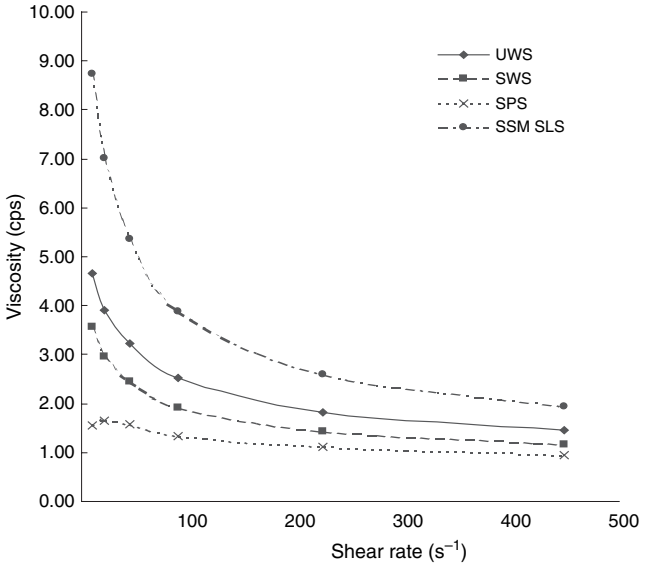

Figure 1 Viscosity values of human saliva at various shear rates. UWS, unstimulated whole saliva; SWS, stimulated whole saliva; SPS, stimulated parotid saliva; SSMSLS, stimulated submandibular–sublingual saliva

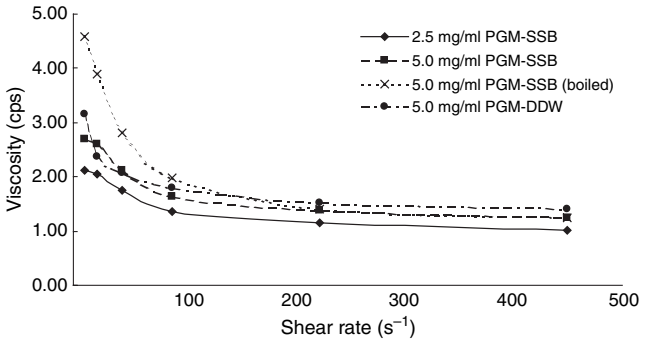

Figure 2 Viscosity values of PGM in different conditions. PGM–SSB, porcine gastric mucin in simulated salivary buffer; PGM–DDW, porcine gastric mucin in distilled deionized water

1.91 ± 0.54, 2.52 ± 0.59 and 3.88 ± 1.12, respectively.

The viscosity values for animal mucins dissolved in SSB were also dependent on shear rate (Figures 2 and 3). In all animal mucin concentrations, particularly at higher concentrations such as 2.5 and 5.0 mg ml^{-1}, viscosity values followed a pattern typical of a non-Newtonian fluid (data for 0.5 and 1.0 mg ml^{-1} not shown). An increase in viscosity was found with increasing animal mucin concentration, as expected. Boiled PGM dissolved in SSB (PGM–SSB) had a higher viscosity than PGM–SSB ($P < 0.01$ at shear rate of 90 s^{-1}), whereas the viscosity of boiled BSM dissolved in SSB (BSM–SSB) was constant regardless of shear rate. There was no significant difference between PGM–SSB and PGM dissolved in DDW (PGM–DDW). BSM–SSB, in contrast, had much lower viscosity than BSM dissolved in DDW (BSM–DDW) ($P < 0.01$ at shear rate of 90 s^{-1}).

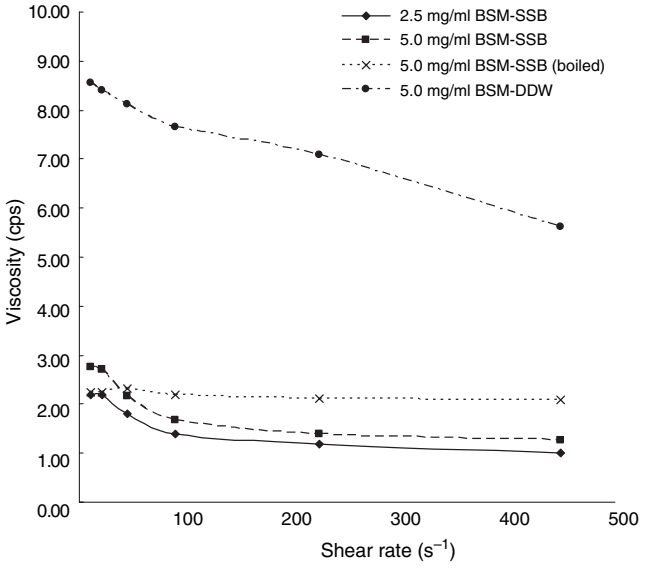

Figure 3 Viscosity values of BSM in different conditions. BSM–SSB, bovine submaxillary mucin in simulated salivary buffer; BSM–DDW, bovine submaxillary mucin in distilled deionized water

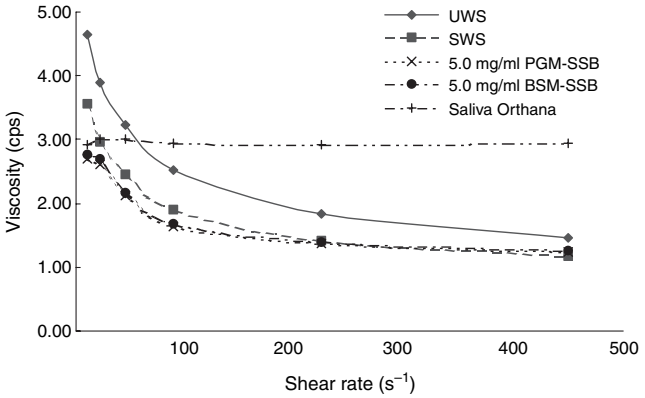

Figure 4 Viscosity values of human saliva, animal mucin solutions, and Saliva Orthana. UWS, unstimulated whole saliva; SWS, stimulated whole saliva; PGM–SSB, porcine gastric mucin in simulated salivary buffer; BSM–SSB, bovine submaxillary mucin in simulated salivary buffer

Comparing animal mucin solutions with human saliva, 5.0 mg ml^{-1} animal mucin dissolved in SSB displayed viscosity similar to SWS at shear rates of 90 and 225 s^{-1}. Viscosity for Saliva Orthana was constant regardless of shear rate and was significantly higher than that of human whole saliva at shear rates of 90 and 225 s^{-1} ($P < 0.01$) (Figure 4).

Contact angle

In human saliva, the mean contact angles on Co–Cr alloy fit a pattern exactly opposite that of the viscosities, namely decreasing in the order SPS, SWS, UWS and SSMSLS. Though the contact angles of human saliva on acrylic resin displayed a slightly different pattern from that on Co–Cr alloy, SPS had the highest contact angle on both substrates. For human saliva, contact angles on

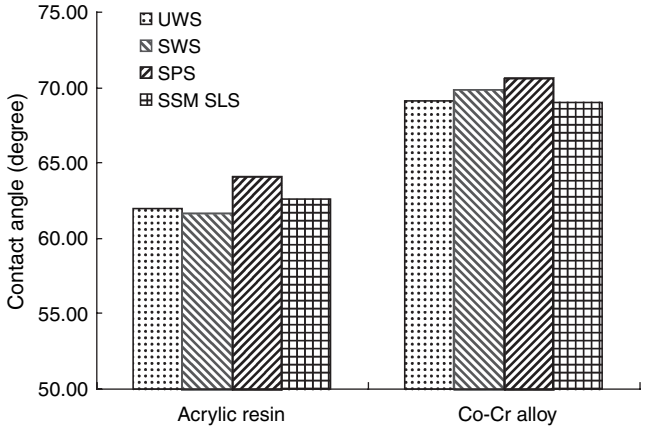

Figure 5 Contact angle of human saliva. UWS, unstimulated whole saliva; SWS, stimulated whole saliva; SPS, stimulated parotid saliva; SSMSLS, stimulated submandibular–sublingual saliva

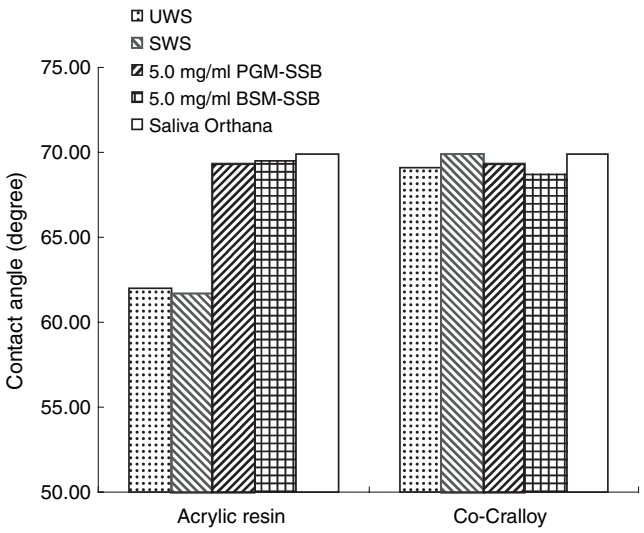

Figure 6 Contact angle of human saliva, animal mucin solutions, and Saliva Orthana. UWS, unstimulated whole saliva; SWS, stimulated whole saliva; PGM–SSB, porcine gastric mucin in simulated salivary buffer; BSM–SSB, bovine submaxillary mucin in simulated salivary buffer

acrylic resin were significantly lower than those on Co–Cr alloy ($P < 0.01$) (Figure 5).

A decrease in contact angle was found with increasing animal mucin concentration. Whereas there was no significant difference in contact angle between boiled PGM–SSB and PGM–SSB, the contact angle of boiled BSM–SSB was much higher than that of BSM–SSB. There was also no significant difference in contact angle between PGM–SSB and PGM–DDW, while BSM–SSB displayed a lower contact angle than did BSM–DDW, regardless of specimen surface at the concentration of 5.0 mg ml^{-1} (data not shown).

Comparing animal mucin solutions with human saliva, the contact angles between acrylic resin and human saliva solutions were significantly lower than those between acrylic resin and animal mucin solutions, including Saliva Orthana ($P < 0.01$) (Figure 6).

Discussion

Previous clinical studies have reported relatively weak correlations between subjective mouth dryness and objective sialometric values (Fox *et al*, 1985, 1987; Sreebny and Valdini, 1988). Such a correlation would indicate that the lubricative and hydration functions of saliva are dependent on saliva quality such as viscosity and film-forming property as well as quantity. The practical goal of developing salivary substitutes for xerostomic patients then, is to achieve a viscoelastic pattern similar to that of human whole saliva.

The efficacy of saliva as a lubricant is at least partially dependent on its viscosity and how this changes with shear rate (Waterman *et al*, 1988). According to our results, all animal mucin solutions, as well as human saliva, displayed viscoelastic properties, which is characteristic of macromolecular solutions. Solutions containing 5 mg ml^{-1} of animal mucin had viscosities similar to that of human SWS at shear rates that would exist during oral functions, such as swallowing or speech (from 60 to 160 s^{-1}) (Balmer and Hirsch, 1978). It is known that an excessively sticky salivary substitute gives rise to unpleasantness and difficulty in masticatory function (Glantz and Friberg, 1970; Vissink *et al*, 1984). Saliva Orthana, an animal mucin-based saliva substitute, has displayed higher viscosity than human whole saliva at clinically important shear rates, in particular above 60 s^{-1}. Considering the clinical preference of mucin-based saliva substitutes over traditional CMC-based formulations, which have comparatively higher viscosity values (Vissink *et al*, 1984; Hatton *et al*, 1987; Fox, 2004), high viscosity is not always desirable in terms of the function of the salivary substitute.

Because the wettability on oral structures and dental materials is indispensable for the maintenance of lubrication and denture retention, the film-forming property seems to have a greater impact on the clinical efficacy of saliva substitutes than does viscosity alone (Vissink *et al*, 1986; Christersson *et al*, 2000). The present study demonstrated that the contact angle of human saliva was inversely proportional to viscosity, although there was a slight difference according to solid phase. Specifically, human saliva displayed superior wettability on acrylic resin *vs* other animal mucin solutions. This finding contradicts a previous study that reported that both types of saliva substitute tested had better wetting properties on denture base resin than did natural human saliva (Kevser Aydin *et al*, 1997). These contradictions may be due to the differences in composition of the mucin-based saliva substitute used or to the finishing method of the solid phase.

Human saliva displayed better wettability on acrylic resin than on Co–Cr alloy, which coincides with a previous report (Sipahi *et al*, 2001). The good wetting of the acrylic resin by human saliva is of clinical importance because good wettability can improve the retention of removable dentures (Monsenego *et al*, 1989). Surface treatment of intra-oral removable appliances for

enhanced wettability is thus a potentially important consideration for improving their effectiveness and retention in xerostomic patients.

The results of the present study showed that SSMSLS, abundant in salivary mucin, plays a crucial role in effective lubrication and wettability because of its high viscosity and good wettability. This supports previous reports of the important role of salivary mucins in proper oral function (Tabak *et al*, 1982; Mellema *et al*, 1992; Van der Reijden *et al*, 1994; Schenkels *et al*, 1996).

Boiled PGM–SSB displayed higher viscosity values than did PGM–SSB, whereas boiled BSM–SSB and Saliva Orthana displayed constant viscosity, regardless of shear rate. It has been theorized that the boiling may cause heat degradation and/or aggregation of animal mucin molecules. The difference in viscosity between BSM–DDW and BSM–SSB is attributable to the difference in ionic strength of the two solutions. This was established by a study on the relationship between ionic strength and viscosity wherein an approximately 25% decrease in intrinsic viscosity of canine tracheal mucin was found upon increasing the ionic strength from 50 to 250 mM (Litt *et al*, 1977). It was reported elsewhere that increasing the ionic strength from 35 to 235 mM resulted in an approximately 50% decrease in specific viscosity (Veerman *et al*, 1989). In addition, increasing ionic strength was reported to cause greater quantities of macromolecules to be adsorbed onto hydrophilic and hydrophobic solid surfaces (Vassilakos *et al*, 1992), which may partially explain the relatively lower contact angle in BSM–SSB *vs* BSM–DDW.

Under the various conditions of the present study, PGM and BSM displayed different patterns in viscosity and wettability, which may have been due to the difference in their molecular structures (Nordman *et al*, 1997; Jiang *et al*, 2000). Moreover, the characteristics of these commercially available mucins could have been altered during the purification processes, which may explain the different results of the two mucins and suggest that further studies are needed.

This study provided an objective observation of the effectiveness of animal mucin solutions in terms of their rheological properties, which is in accordance with previous reports of xerostomic patients' clinical preference of mucin-based saliva substitutes. For the development of even more effective saliva substitutes, additional studies focusing on the wettability of animal mucin solutions on oral mucosa and various dental materials are needed.

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