Time-dependent interfacial tension of whole saliva and saliva–bacteria mixes

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Using a tensiometer in accordance with the drop volume principle, the surface tension decrease with time was determined for whole and for 2%, 10%, and 50% aqueous solutions of saliva from one healthy donor. The reduction of surface tension with time was also measured for 10% and 20% saliva solutions with added samples of *Streptococcus salivarius* KRF2, *S. sanguis* KRF3, and *Actinomyces naeslundii* 2t-55. The results show that 1) there is a time dependence of the surface tension reduction of both whole saliva and diluted saliva, 2) an increase of the concentration of whole saliva in salivary solutions gives rise to larger and more rapid surface tension reduction, 3) the proteinaceous components of saliva appear to have a dominant contribution on surface tension in whole saliva and diluted saliva, and 4) the surface-active proteinaceous components in saliva have the abililty to dominate the air–saliva interface also in the presence of high concentrations of salivary bacteria. □ *Air/liquid interface; microorganisms; saliva surface tension; time dependence*

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As dental plaque is formed on surfaces precovered with so-called pellicle, the mechanisms of adhesion of pellicle components and subsequently also of oral microorganisms to teeth and dental restorations have attracted considerable interest over the years (for reviews, see Refs. 1–9).

Experimental data have shown that certain solutions of proteins and also of oral microorganisms behave like colloidal systems (10–12). Furthermore, since adsorption to solids, not only of the mentioned aggregates and microorganisms but also of individual protein molecules, is dependent on the surface chemical characteristics of the system, it is, of course, of interest and importance to study the fundamental interfacial behavior of saliva on solid surfaces (13). As natural tooth surfaces have a partly hydrophobic character (14), under physiologic conditions saliva is constantly exposed to microorganisms (15), and as the interfacial behavior of protein molecules on hydrophobic surfaces is related to their behavior at the air–water interface (16), a study was undertaken to determine the time-dependent surface tension decay of human saliva and salivary mixes with microorganisms. Furthermore, adsorption of salivary components at the air/saliva interface will affect gas bubble stability and also Langmuir–Blodgett-type deposition phenomena.

Materials and methods

Saliva

All saliva samples were donated by a 37-year-old female test subject, who considered herself to be in good general health and who was found to be in good oral health at clinical examination.

Before each experiment, between 0830 h and 0900 h, 10 to 20 ml of saliva was collected in a glass test tube. Secretion was stimulated only by masticatory movements, and no food or drink was allowed for 2 h before sampling. The samples were then diluted with a phosphate composition buffer to the predetermined concentration levels given below.

Bacteria

The bacterial strains used were *Streptococcus salivarius* KRF2, *S. sanguis* KRF3, and *Actinomyces naeslundii* 2t-55. The strains were stored frozen in double-strength skim milk at −80°C until used.

The bacteria were subcultured once before each experiment. Subculturing was performed on blood agar plates (Oxoid blood agar base no. 2) containing 6% citrate-treated horse blood (SVA, Håtunaholm, Sweden) for 20 h in $H_2 + CO_2$ (Gas Pak, BBL Microbiology Systems) at 37°C. Before each experiment bacterial strains were inoculated in 90 ml of Brain-Heart Infusion Broth (BHI lab 49 labm) and incubated overnight (for approximately 18 h) at 37°C. Then the cells were centrifuged for 10 min at a speed of 11000 rpm (centrifugal force = 14,500 *g*) and washed twice in 90 ml of Ringer's solution. The composition of Ringer's solution was 9.00 g sodium chloride, 0.42 g potassium chloride, 0.18 g calcium chloride, and 0.20 g sodium bicarbonate per liter solution. Finally, they were suspended in either 90 or 40 ml of this solution. The amount of microorganisms per 1 ml of suspension was estimated and calibrated by means of their optical density at 700 nm in a Hitachi 100–60 spectrophotometer. All chemicals used were of analytical purity grade or higher.

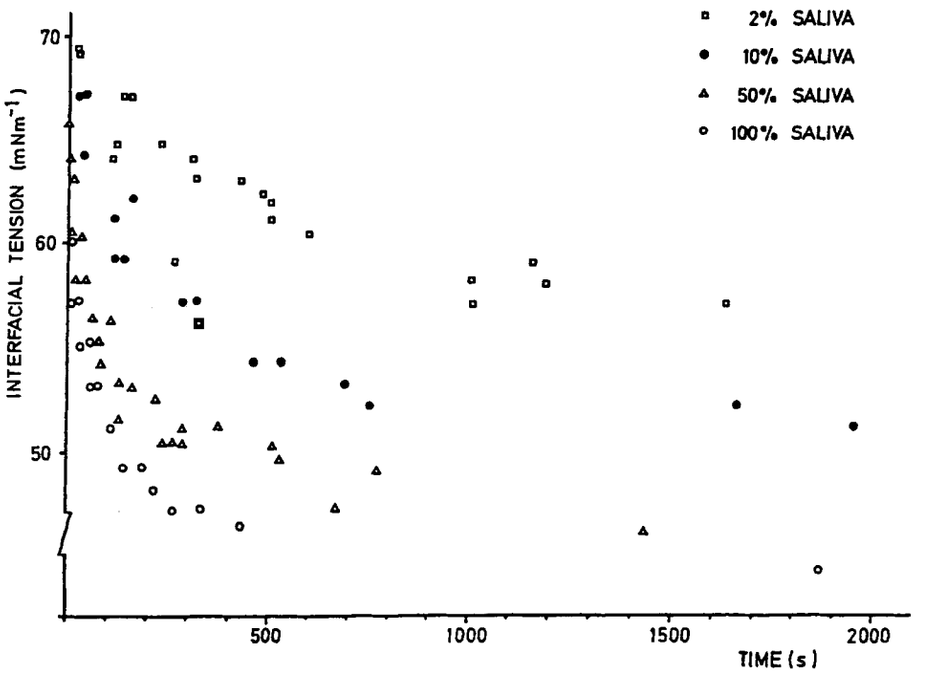

Fig. 1. Time dependence of the interfacial tension of whole saliva and 2%, 10%, and 50% aqueous solutions of such saliva with added buffer (0.005 M phosphate, 0.1 M NaCl at pH 7).

Before all experiments the glassware used was washed in concentrated sulphuric and nitric acid solution (1:1 by volume) and then rinsed with double-distilled water.

Tensiometer

The experiments were performed in a tensiometer based on the drop volume principle (17). The type of instrument used was originally described by Tornberg (18) and later modified by Arnebrant & Nylander (19). The details of this instrument are given by the latter authors (19), who also reported on the mathematical approach for calculation of interfacial tensions. With this instrument the time-dependent interfacial tension decrease was determined at 37°C for a range of saliva samples and salivary mixes. In brief, the experimental procedures were carried out in the following manner: a sample drop of a certain volume, corresponding to a predefined interfacial tension value, was syringed rapidly through a tube, and a drop formed on the tip. The time was then measured for the interfacial tension of the sample drop to fall to the level where it became detached. This procedure was repeated systematically for differing drop sizes and interfacial tension values, until sufficiently precise plots of the interfacial tension as a function of time could be created.

Experiments

To enable comparisons to be made with Sefton et al. (20), the surface tension decay with time was determined for whole saliva samples and for 2%, 10%, and 50% aqueous solutions of the whole saliva. The buffer used in these experiments had the following composition: 0.005 M phosphate and 0.10 M NaCl, pH 7.

In another series of experiments with Ringer solution as the suspending medium, the reduction of surface tension with time was measured for whole saliva solutions and for suspensions of each of the three types of microorganisms. Two concentrations of saliva–bacteria mixes were studied. One contained an added number of 10^8–10^9 of each of the three types of microorganisms per milliliter of tested dilution of 10% whole saliva. The other contained twice as many added microorganisms in 20% whole saliva, giving a constant relationship for the concentrations of original and added microorganisms in the two mixes. All experiments were carried out at pH 7.0 and repeated at three separate experimental sessions.

Studies of some errors of the method

The accuracy and precision of the method used for surface tension measurements were tested in the following manner:

a) Throughout this study the accuracy of the tensiometer and the precision of the method were tested in more than 60 experiments using deionized, redestilled water. At 37°C the measured surface tension of water was 70.59 ± 0.96 mNm^{-1}, corresponding well with data given in the literature (21).

b) The variance in surface tension between saliva

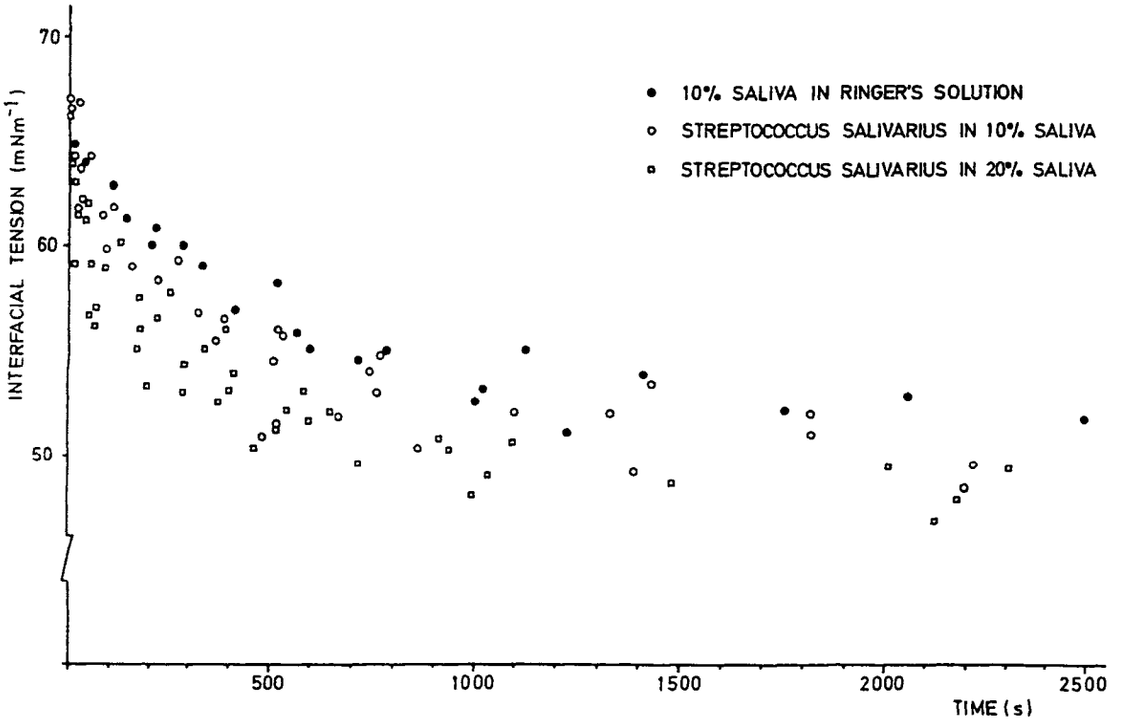

Fig. 2. Time dependence of the interfacial tension of 10% whole saliva in Ringer's solution and of suspensions of *Streptococcus salivarius*, 10^8–10^9 ml^{-1}; in 10% and 20% aqueous solutions of whole saliva.

samples donated at different experimental sessions was studied for 10% saliva concentrations in Ringer's solution. These measurements were performed at the beginning of each experimental session and occasionally also at their end. As the range of the observed surface tension values was moderate for given observation times (for example, 8.0 mNm^{-1} at approximately 1000 sec), and as the coefficients of variation were reasonable (less than 10%), it was concluded that from the chemical and compositional points of view, an acceptably stable secretional product was studied.

c) The efficiency of the methods used for washing the microorganisms was tested by measuring the surface tension decay of suspensions of the washed microorganisms and the suspending media (Ringer's solution) containing 1% solution of the broth itself. The surface tension values for the Ringer's solution and the three suspensions of microorganisms showed little variance (coefficients of variation between 0.4% and 0.7%). They showed little or no time dependence and were all close to that for pure water (range, 69.9–70.4 mNm^{-1}). The surface tension of the 1% broth solution, on the other hand, was time-dependent and reached a level of 60.5 mNm^{-1} after 3600 sec.

On the basis of these observations we concluded that the washing procedure used did not leave enough broth material on the surfaces of the microorganisms to appreciably affect the interfacial properties of the suspensions.

Results

The results of the measurements of the kinetics of surface tension decrease for the test solutions of whole saliva are given in Fig. 1. In Fig. 1 it can be seen that through the adsorption at the air–liquid interface of surface-active components, in concentrated whole saliva, the interfacial tension reached a value of 53 mNm^{-1} at 60 sec. A relatively stable value of around 45 mNm^{-1} was then reached after less than 1000 sec. As the concentration of whole saliva was reduced, the surface tension was observed to be higher and the leveling off took place after longer periods of time.

The results of the experiments with saliva–bacteria mixes are given in Fig. 2 for *S. salivarius*, in Fig. 3 for *S. sanguis*, and in Fig. 4 for *A. naeslundii*.

In Figs. 2 to 4 it can be seen that for all the tested bacterial strains, the data for the 10% saliva–bacteria mixes correspond well with those obtained for the 10% saliva–Ringer's solution alone. As the concentrations of saliva and added microorganisms were doubled, lower levels of surface tension were reached. Furthermore, these levels were reached more rapidly for the 20% saliva

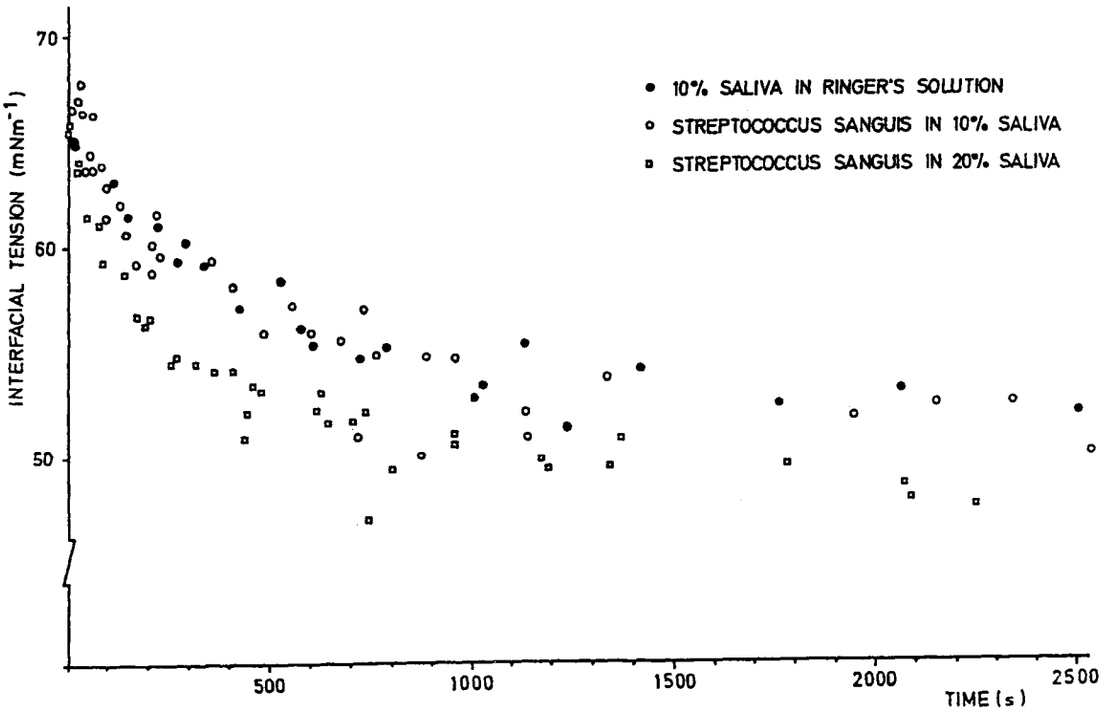

Fig. 3. Time dependence of the interfacial tension of 10% whole saliva in Ringer's solution and of suspensions of *Streptococcus sanguis* (10^8–10^9 ml^{-1}) in 10% and 20% aqueous solutions of whole saliva.

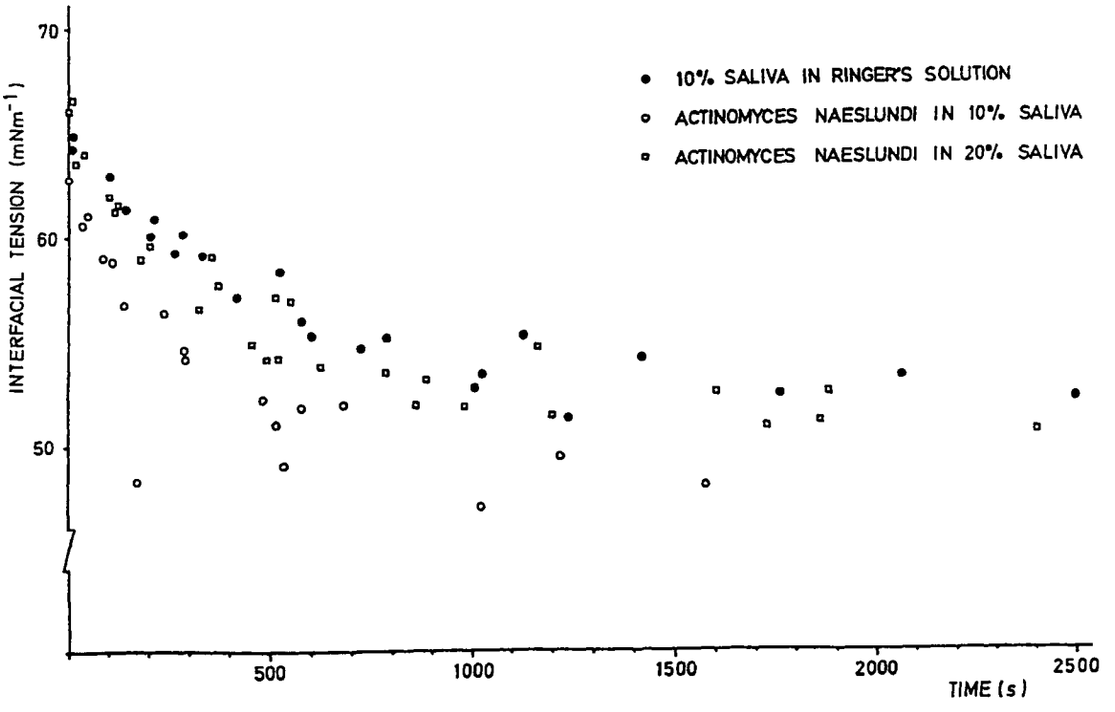

Fig. 4. Time dependence of the interfacial tension of 10% whole saliva in Ringer's solution and of suspensions of *Actinomyces naeslundii* (10^8–10^9 ml^{-1}) in 10% and 20% aqueous solutions of whole saliva.

concentrations than for the corresponding 10% ones. This shift is of the same type and magnitude as those observed with increasing saliva concentrations in buffer solution (Fig. 1).

From the data given in Figs. 1–4 it is obvious that for the saliva–bacteria mixes the observed surface tension reductions at the air–liquid interface depended more on the presence and concentration of the saliva and its surface-active components than on the amount, shape, or relative pathogenicity of suspended bacteria.

Discussion

This study was performed because, although numerous studies have been carried out on pellicle formation, pellicle composition, and bacterial adhesion to solid substrata, few studies have so far reported on the time-dependent interfacial properties of saliva. In the literature no reports have been found on the possible influence of microorganisms on surface tension of saliva. Bearing in mind that both plaque and pellicle contain relatively large amounts of salivary material, the interfacial tension of saliva and particularly its decay with time is of importance for the formation of these integuments.

To define the time-dependent properties of saliva and saliva-containing mixes, the so-called drop-volume method was used. This method, which has frequently been used in other fields of application (16, 19, 22, 23) was easy to apply for this study and was considered to be relevant for it. Shear forces shown in the used instrument were, for example, small and well within the physiologic range (15).

The samples of saliva used in these experiments were collected from the same test subjects at the beginning of each experimental session. This enables strict sampling procedures to be undertaken and further eliminated differences caused by, for example, individual variations in dietary and hygiene habits.

Certain differences in the salivary sample compositions must, however, still have been present, as some surface tension variations were observed within the sets of triple experimental session. These variations can be observed as a noticeable scatter of data points in the resulting diagrams (Figs. 1–4).

The general validity of this study is of course somewhat restricted by the fact that only samples from one test subject were studied. In this context it must, however, be remembered that only small interindividual variations have been reported in the limited number of studies so far presented for the surface tension of saliva in larger groups of patients (24). Furthermore, the results of this study also correspond well with those reported by Sefton et al. (20) in a similar experiment with saliva samples from another donor.

It is interesting to note that in spite of the complex composition of saliva, both the results of this study and those of Sefton et al. (20) generally correspond well with reported interfacial properties of food proteins and most pure proteins (22, 23). Further, in all these systems the rate of surface tension reduction decreased as the amount and conformation of surface-active molecules at the interface approached a steady state.

Larger, more rapid reduction in surface tension were observed at increased whole saliva concentrations, again pointing to similarities with aqueous protein solutions.

Bearing in mind these observations of kinetics and concentration dependence of salivary solutions and also the general composition of whole saliva (15), it is likely that proteinaceous salivary molecules were the ones diffusing to and dominating the air–liquid interface. It is well known from the literature that, for example, the proline-rich proteins (PRPs) constitute a very surface-active fraction (25, 26), and, furthermore, it has recently been shown that structures reminescent of the casein micelles in milk are formed by surface-active molecules within saliva (27).

Further, on the basis of the composition and the complex nature of whole saliva, the noted time dependence for surface tension was probably related to concentration effects, to exchange reactions, and to conformational changes taking place on adsorption at the liquid–air interface.

The low surface tension levels observed for salivary samples also correspond well with a report by Holterman et al. (28), who by means of ellipsometry showed large amounts of proteinaceous material to adsorb at saliva–air interfaces.

The great extent of surface activity of protein-containing salivary molecules explains their adhesive properties and the fact that they obviously adsorb spontaneously both to tooth surface and to other types of solid surfaces exposed in the oral cavity (29, 30).

The results of the experiments with time-dependent surface tension characteristics of saliva–bacteria mixes show that within the studied time frame the surface-active, probably proteinaceous, salivary molecules were able to totally dominate the air–liquid interface of the pure saliva-containing samples and of the mixes also in the presence of greatly increased amounts of microorganisms of different shapes, sizes, and clinical behaviors. This view is supported by the observed lack of appreciable surface activity for the tested microorganisms when studied in non-saliva-containing Ringer solution. Bearing in mind the general chemical nature of bacterial cell surfaces, it is not unlikely that these surface-active salivary molecules are also able to dominate saliva–bacteria interfaces and thus to monitor at least the early stages of bacterial adhesion to oral solid surfaces. This conclusion is well in line with those previously drawn by Glantz & Baier (31).

Here it must be recognized that this interfacial dominance of salivary proteinaceous molecules is relative. It can, for example, be changed by microorganisms with the ability to hydrolyze pellicle material or otherwise to alter a particular local oral environment in their own favor. Obviously, however, under the experimental conditions of this study, such an ability was not shown by any of the tested microorganisms in spite of the known

frequent association with dental plaque formation by at least two of them, *S. sanguis* and *A. naeslundii*. On the basis of observations made in this study, clinically significant interfacial effects could be expected from rinsing solutions with the ability to reduce the surface tension of water more rapidly and to lower levels than those recorded for concentrated whole saliva.

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