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Biochemical and physiological considerations for modeling biofilms in the oral cavity: A review

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ABSTRACT

This review describes some of the biochemical and physiological factors that should be considered in designing *in vitro* models of the oral cavity. The significance of the non-Newtonian properties and internal strucutres of saliva are reported as well as the ability of saliva to form biological films on all types of solid surfaces. The presence of compositional biochemical variations between absorbed salivary films is discussed for solid surfaces having different surface chemistries. The significance of the presence of small amounts of organic material in enamel and dentin is also stressed. Finally, by selecting other, more easily available secretional products than saliva, more relevant model systems for materials testing *in vitro* may be established than if only simple aqueous salt solutions are used.

INTRODUCTION

Before considering the isolated tooth in relation to restorative materials, it is important to discuss what happens when biomaterials are exposed to the oral cavity. It is well known that, after a brief time, every solid in the oral cavity is covered by salivary material generally known as pellicle. This material then participates in most surface-related physiological processes such as wear and adhesive contacts. To create relevant models of restorative procedures, it is therefore essential to know some of the basic properties of saliva and salivary pellicles, such as the general composition and rate of secretion or formation.

SALIVA

Under normal physiological conditions, saliva is present throughout the oral cavity. In humans, the secretion of saliva is a nerve-regulated process, which under waking conditions, progresses in the absence of obvious stimuli. Since the classic experiments of Pavlov, the existence of conditioned reflexes in the control of the salivary flow have been well known. The presence of food in the mouth is, for example, a powerful stimulus to salivation. For a review on this topic, see Jenkins (1978).

Saliva is a biochemically complex secretory product with a great deal of individual variations. Two persons with identical diets who donate saliva under identical conditions may produce salivas not only at different rates but also with different compositions. From a biochemical point of view, saliva has a variety of important biological functions that sometimes are of relevance in biomaterials experiments. Due to its limited stability, it is, however, difficult to store in sufficient amounts for most biomaterials model studies. This topic is covered in the previously mentioned review article (Jenkins, 1978).

SALIVARY STRUCTURES

Apart from its film-forming capacity, microbial control function and wear protection, saliva has other important functions, such as enzymatic activity, buffering, and control of water balance. This variety of salivary functions is so wide that it cannot be suitably explained by considering saliva as a simple solution. Instead, saliva should be regarded as having an internal structure and exhibiting behavior similar to that of a substance like a weak gel (Schwarz, 1987).

The presence of salivary structural components has been speculated since around the turn of this century (Fano, 1908), and a variety of descriptions of salivary structural features have been presented (Colombini, 1939; Hoepfner, 1943). However, until recently, a structural model for this low-viscosity, non-Newtonian body fluid has been lacking. In theory, it should be possible to explain the nature of structural elements in fluids such as saliva by the use of rheology. Detailed characterization has, however, been retarded by the fact that no sufficiently accurate instrument has yet been developed capable of precise rheological measurements of fresh whole saliva. This is probably because saliva is secreted in limited volumes, is bio-chemically unstable, and contains multiple large particles such as microorganisms and epithelial cells.

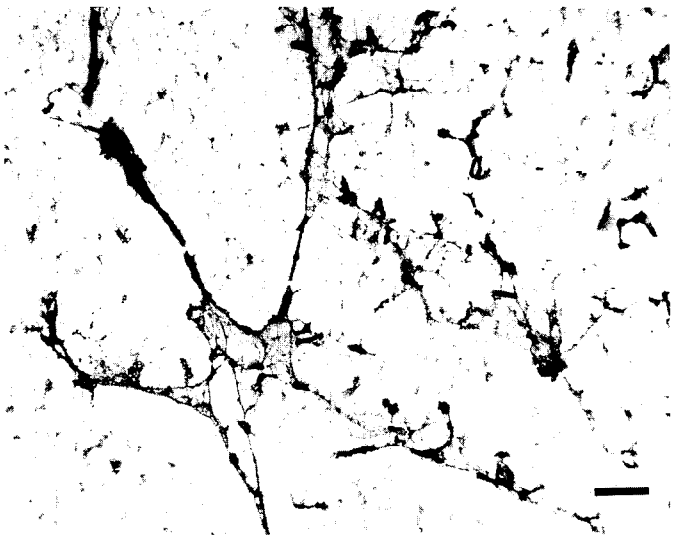

Fig. 1. Hematoxylin-eosin-stained section of whole saliva from a 32-year-old subjectively healthy female donor. The section shows the presence of a network created by seemingly amorphous structural components of various dimensions. Many of the network components meet at approximately 120° angles, and the figure also shows the presence of epithelial cells in contact with the network. Length of bar = 1 mm.

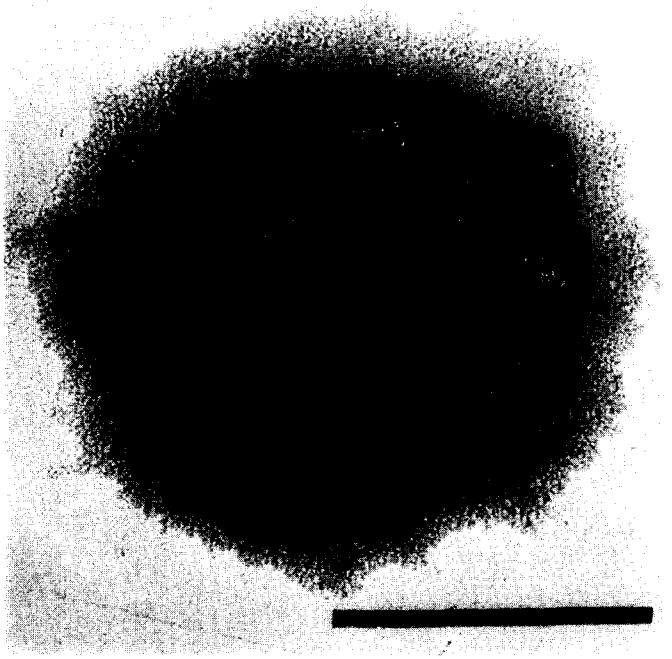

Fig. 2. Transmission electron micrograph of a 90- to 100-nm thick section of whole saliva from a subjectively healthy 51-year-old male donor. The picture shows the probable cross section of a major structural component with a comparatively thick foamlike outer zone and an inner, more electron-dense, foamlike core exhibiting granules with different shapes, sizes, and electron densities. Length of bar: 1 μm.

Due to the failure to obtain consistent rheologic data, alternative approaches have recently been developed. A method has been presented (Glantz *et al.* 1989a; 1989b; 1989c) for the preservation of intrinsic saliva structures by instantaneous gravitational immersion of samples directly into flasks of liquid nitrogen. After freezing, solid pieces of saliva can then be handled in the same way as any other frozen piece of tissue, *i.e.*, they can be cryogenically sectioned, fixed, stained, and examined by both light and electron microscopy.

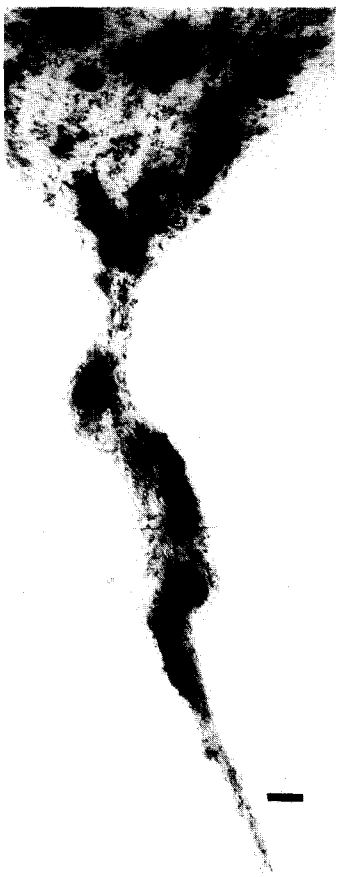

Fig. 3. Transmission electron micrographs of a paraformaldehyde-vapor-fixed and OsO_4 postfixed, approximately 10-μm thick section of whole saliva from a subjectively healthy 51-year-old male donor. The picture shows a branched major structural component with integrated structures, including multiple microorganism-like particles. Length of bar: 1 μm.

The results of the studies performed so far point uniformly in the same direction, that human saliva is a highly structured secretion product which perhaps is better described as a tissue than a fluid. At the light-microscope level, its main structural feature is a continuous filamentous network (Fig. 1). This network can be better observed in fractions of human parotid saliva (HPS) than in submandibular/sublingual (HSMSL) ones, suggesting that the primary network-forming substance may be the proline-rich proteins that are known to have extended chain (proline II) structures and dominate in HPS fractions. The network is not markedly affected by water dilution, implying that excess amounts of network-forming material exist at the time of secretion, and that hydrophobic stabilization (such as proline-proline interactions) may be present.

It was also repeatedly observed that when particulate biological materials such as desquamated epithelial cells, microorganisms, and foreign material are present, they are usually aggregated and preferentially retained inside the intrinsic salivary network (Fig. 3). Parts of the saliva filaments appeared to have undergone local expansion to accommodate this extraneous particulate material (Glantz *et al.*, 1991).

The particle wetting action of saliva has recently been demonstrated in a series of experiments where cell-free whole saliva was mixed with well-defined hydrophilic or

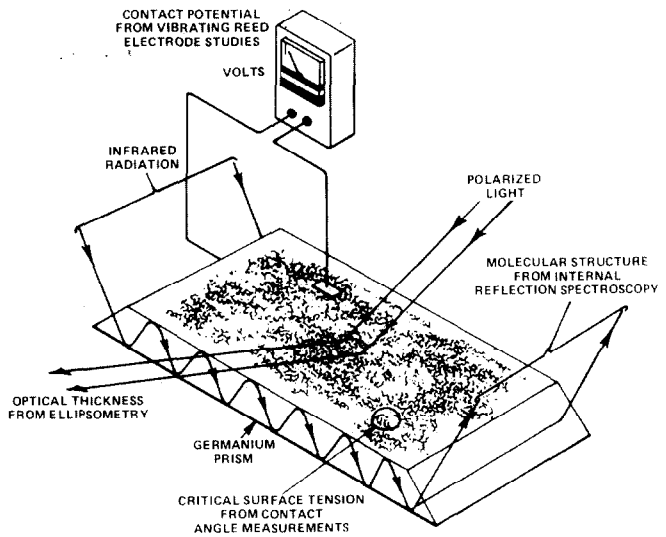

Fig. 4. **Physicochemical analytical methods applied to the characterization of oral films.**

hydrophobic particles of 0.9 mm diameter (Glantz *et al.*, 1995). The micrographs of the flash-frozen mixes of saliva and hydrophilic particles showed multiple particles all over the salivary network, while the mixes with the hydrophobic particles showed the presence of only few objects. Further, when observed, the particles were located only in the dense central regions of the salivary structures.

The observed difference is easy to accept, bearing in mind the high water content of saliva. The high water content of saliva might naturally also explain the difficulties of hydrophobic particles to bind to salivary filaments, but this does not explain why rapid coating of the hydrophobic particles with salivary protein — rendering them hydrophilic and more miscible — did not occur. Few such particles were in the micrographs, and the ones that were observed were found mainly in salivary network regions believed to possess surface chemical properties suitable for association with hydrophobic material. These regions were earlier identified in the transmission electron micrographs as the electron dense core material of the salivary network structures (Fig. 2), thought to contain water-insoluble proteins (Glantz *et al.*, 1991). The observations made from hydrophobic particle engulfment suggest that it is the most difficult mode of transportation for hydrophobic particles from the volume of mixed saliva samples into these core structures.

Based on available data, the following idealized model of four main saliva structures is proposed: 1) A continuous phase water-based electrolyte that acts mainly as a diluent for water-soluble substances and maintains the water balance of the oral mucosa and the mineral balance of the teeth; 2) A scaffold-like continuous network structure, based on the overall staining pattern observed, that is believed to comprise higher-molecular-weight salivary glycoproteins. Bearing in mind the comparatively high spreading pressures of such macromolecules, these glycoproteins should be implicated in virtually every type of oral adhesive event taking place in the presence of saliva. They should also serve as partial diffusion barriers and lubricants to protect against wear of the hard oral structures. In addition, they could participate in the buffering activity of saliva, together with certain electrolyte components; 3) Network core structures within the surface-active glycoproteinaceous filaments are likely to contain higher concentrations of other types of macromolecules, such as less water-soluble proteins. These may, for example, perform specific biochemical functions of saliva, requiring a protected environment. Results by the present authors show these inner-core components to be associated with the previously documented ability of saliva to aggregate epithelial cells and microorganisms; and 4) Dispersed droplets of lipoid material, with varying dimensions and distribution, are associated predominantly with water-insoluble foreign substances and desquamated epithelial cells.

PELLICLE AND BIOFILMS

With regard to the salivary pellicle, *in vivo* studies of oral films have provided detailed information about many of its chemical and physical properties through the use of the range of surface physicochemical analytical techniques shown in Fig. 4. The primary materials used for the adsorption of these films were silica test pieces and germanium prisms treated to give different types of surface chemical characteristics, representing both normal tooth surfaces and a range of restorative materials. The results of these studies basically show that the formation of oral films proceeds at high speed and is of a certain qualitative selectivity. That is, the layers deposited are similar to one another but obviously different from the bulk salivary pool. Of the salivary fractions, it is mainly the high-molecular-weight one that seems to be engaged in the formation of pellicles (Vassilakos *et al.*, 1992).

Additional information has been obtained by exposing test plates in both protected, stagnant regions of the oral cavity and in regions exposed to considerable shear forces. Test prisms left free in the vestibular sulcus, exposed to high shear, acquired the same type and amounts of films on their surfaces as when kept in special holders shielding them from shear forces. When the prisms with their adsorbed films were not rinsed free of excess clinging saliva after removal from the oral cavity, the dried films were thicker by about 10 to 40 nm. Furthermore, in these no-rinse experiments, no differences could be detected between the thicknesses of films formed on high-energy and low-energy surfaces. This is clearly not the case when the intraorally exposed prisms are rinsed with water; the retained films are relatively thicker on low-energy than on high-energy surfaces in the same time period. That is, the same amount of material seems to be adsorbed on both high- and low-energy surfaces. This, in turn, suggests that a more native configuration or coagulum exists for the molecules adsorbed on the low-energy surfaces. The results of recent studies also indicate that on high-energy materials, more potential protein sites can be surface-associated and also that the packing system is more complicated, with greater film densities on high-energy surfaces. This result corresponds with those from clinical experiments of growing plaque on different solids (Glantz, 1969).

A third observation made was that on test pieces which have been exposed in a stagnating system, particles such as microorganisms or biological debris adhered to the film-coated prisms much faster than when the prisms were exposed to shearing forces by the movement of oral mucosa or by saliva (Christersson *et al.*, 1988).

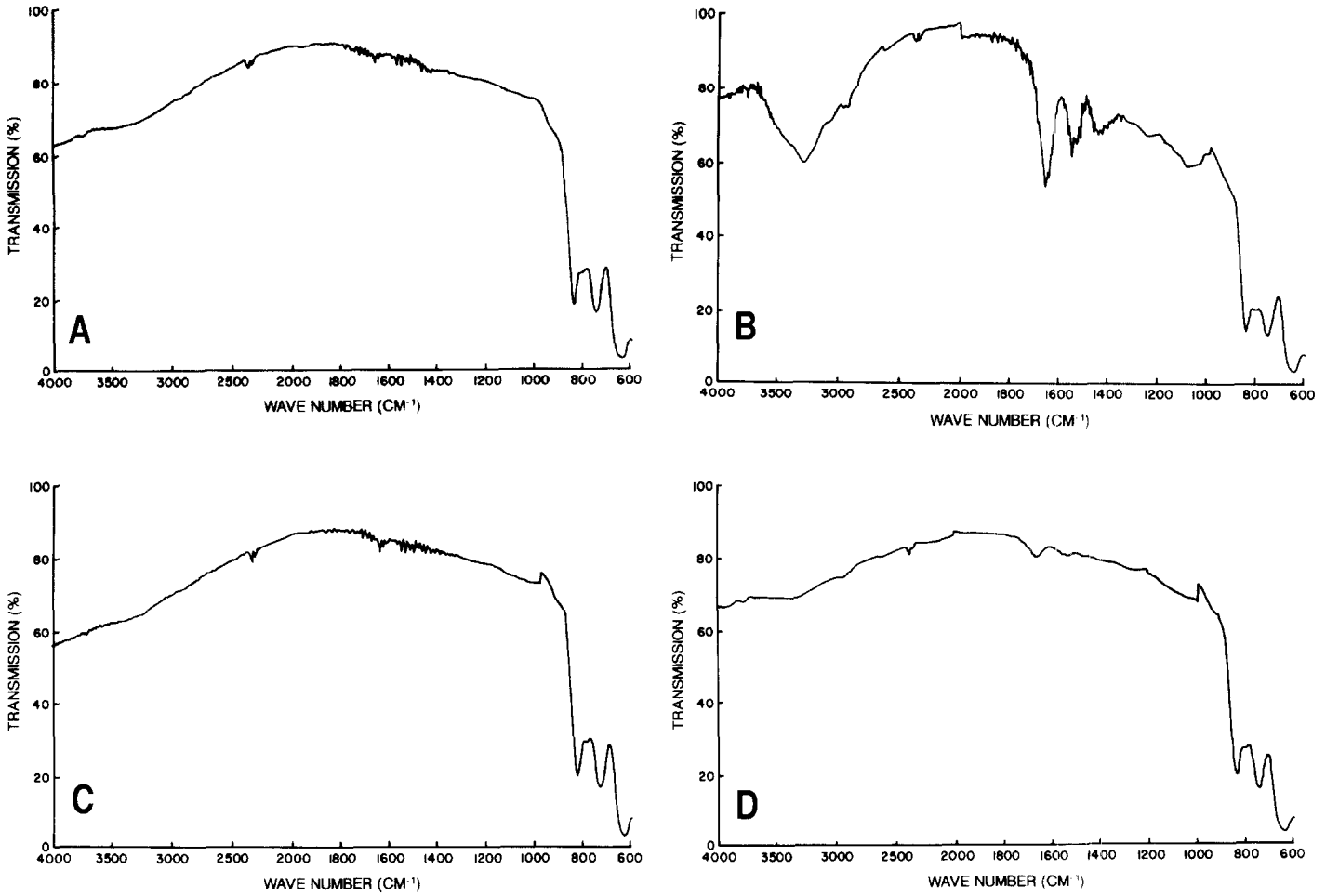

Fig. 5 A-D. Infrared spectra (A) of a clean germanium prism; (B) after 2 h exposure to whole human saliva; (C) following two HCl elutions, and (D) following five water elutions. Amide I and Amide II bands are shown at 1635 cm^{-1} and 1545 cm^{-1}, respectively.

BIOCHEMICAL MODELING OF PELLICLES

A series of systematic biochemical analyses of pellicle materials formed on different solids has been presented by Christersson (1991). Spectra obtained from the infrared spectroscopic examinations performed in this study are given in Figs. 5 A-D. In Fig. 5B, a typical spectrum from a saliva-coated germanium plate is depicted. Note the presence of the following absorbance bands: Amide I (C=O stretch) at 1635 cm^{-1}, Amide II (C-N stretch and N-H bend) at 1545 cm^{-1} and backbone N-H stretch at 3300 cm^{-1}, which are all representative of protein deposits. Similar spectra have been obtained throughout the 20-year course of studies where germanium has been exposed to whole human saliva (Baier, 1973).

Complete removal of pellicle material requires repeated HCl elutions (Fig. 5C), while water elutions alone — even when repeated up to five times — never entirely remove the proteinacious material (Fig. 5D).

The amino acid compositions of pellicles collected *in vivo* from tooth enamel (EP) and *in vitro* from germanium prisms (GeP) of medium- and low-critical surface tensions are shown in Table 1. In Table 1, it is noteworthy that the amino acid compositions of the HCl eluted GePs and the *in vivo* EPs collected by mechanical means are similar, yet differ from the amino acid profiles of the pellicle material incompletely recovered by water elutions (EP/dH_2O and GeP/dH_2O). These profiles were also distinctly different from the amino acid compositions of HPS, HSMSL and WHS (Table 2), which confirm the selective nature of the pellicle formation process.

It is interesting to note the close similarities in the amino acid compositions of the pellicles formed on the three different collector surfaces, the modified hydrophobic, low-energy surface, the medium-energy surface and the tooth enamel. It should, however, be pointed out that in these experiments, static deposition of saliva occurred, and none of the test surfaces were challenged by high shear forces during the rinsing procedure (Christersson, 1991).

The total protein amounts obtained from sampling various *in vivo*- and *in vitro*-formed pellicles are displayed in Table 3. Here, it can be seen that twice the amount of protein was recovered from a single germanium test surface (6.5 cm^2) of medium critical surface tension than was recovered *in vivo* by scraping all buccal surfaces in a full dentition (estimated to an area of 20 cm^2). Recognizing that the lower amounts was recovered from low-energy coated prisms, it is clear that natural enamel is surface-energetically in an intermediate position between clean, inorganic materials and organic coated ones.

TABLE 1. AMINO ACID COMPOSITION OF 2 h PELLICLE MATERIAL COLLECTED *IN VIVO* FROM TOOTH ENAMEL (EP) AND *IN VITRO* FROM GERMANIUM PRISMS (GeP) OF MEDIUM AND LOW CRITICAL SURFACE TENSIONS BEFORE AND AFTER ELUTION WITH DISTILLED WATER OR HCl

Asp	7.8	8.7	8.9	8.4	6.1
Thr	5.6	5.0	5.4	6.3	4.6
Ser	6.9	5.7	5.7	6.0	8.8
Glu/Glx	14.9	14.6	14.9	13.2	11.4
Pro	7.0	7.2	5.9	15.8	15.7
Gly	14.9	14.9	14.6	11.8	6.8
Ala	9.1	9.1	9.6	5.8	5.5
1/2Cys	0.2	0.1	0.2	0.9	1.3
Val	5.3	5.1	5.6	4.8	5.5
Met	0.6	0.1	-	0.9	0.2
Ile	3.5	3.6	3.7	2.8	3.4
Leu	7.5	7.3	7.5	5.9	8.0
Tyr	2.6	2.3	2.6	2.6	3.9
Phe	3.4	3.1	3.0	2.8	3.8
His	2.2	2.3	1.9	1.9	2.5
Lys	5.8	5.9	6.1	5.1	5.9
Arg	4.8	5.2	4.3	4.9	5.0

Results from carbohydrate analyses of the GeP-absorbed saliva samples are reported in Table 4. Since the saliva was not centrifuged prior to exposure to the germanium, the high content of glucose may be due to the contribution of excreted bacterial polysaccharides, such as dextran or mutans, or even traces of capsule material left on the surface. The glucose level may also depend on the presence of glycolipids, which are derived from secretions, cells or bacteria. Hexosamine and mannose are known constituents of salivary mucins and amylase, two proteins reported to be present in 2 h EP (Al-Hashimi and Levine, 1989).

The individual salivary components present in the 2 h GeP were, along with HPS, HSMSL and WHS, separated according to molecular weight by SDS-PAGE and so-called Western transfer blot (Christersson, 1991). From these experiments, it is apparent that the GeP absorbed material consists not only of selective proteins from the salivary pool, but also of proteins of the same general nature as those observed on natural tooth surfaces.

To further define the intraoral condition, a method has been developed to calculate the clinical adhesiveness of pellicle-covered tooth surfaces and restorative dental materials (Glantz *et al.*, 1980). These studies showed that, *in vivo*, the critical surface tensions for all tested surfaces and subjects were in the bioadhesive range of 32 to 50 mN/m (Jendresen and Glantz, 1980). The formation of organic films on solid surfaces in the oral cavity thus brings these to a common state of bio-adhesiveness (Jendresen and Glantz, 1981). The formation of organic films was also observed to have a masking effect on minor surface irregularities (Jendresen *et al.*, 1981). Generally speaking, these results again demonstrate that the speed of oral film formation is extremely high and that film qualities have a high degree of uniformity in composition on different kinds of surfaces despite some variations from one human being to another.

The only normally appearing clinical variable so far found to alter the surface chemical characteristics of the films are drops in pH. In *in vivo* studies, it has even been noted that the pH decrease created by chewing only a couple of pieces of orange is enough to increase the wettabilities of the films. Bearing in mind the general protective actions of oral films discussed previously, it is understandable that they are pH sensitive. If not, their protective effects on masticated food even after passage through the stomach, might delay digestive processes.

What types of actions or "driving forces" are responsible for the spontaneous formation of the oral biological films? This question cannot be answered precisely, given our present state of knowledge. It is well known, however, that in saliva and other biological fluids, there are organic macromolecules with hydrophilic, hydrophobic, and ionically charged groups such as sulfates, phosphates, amines, and carbonyls. Thus, it is likely that due to the comparatively high spreading pressures of such macromolecules, they cover the surfaces of all solids present. Spreading mechanisms are readily explained thermodynamically on the basis of dehydration and favorable entropy shifts (Norde, 1986).

Even if experimental access to the oral environment is comparatively simple, the establishment of *in vitro* models must continue as an integrated part of dental biomaterials research for a long time mainly for safety reasons and also for convenience. As teeth are integrated components in most oral restorative situations and thus in most models, it is appropriate to close this discussion of the biochemical and physiological aspects of biomaterial models with a reference to the major tooth structures, enamel and dentin.

ENAMEL AND DENTIN

Like other mineralized tissues, both enamel and dentin also contain organic matter with potential biochemical functions. Considerable variations can be found in the distributions of the organic components in enamel and dentin and also between their respective volumes. Certain systematic internal variations can also be found in these chemically complex, anisotropic tissues. For reviews, see Miles (1967), Lazzari (1976), and Berkovitz *et al.* (1989).

With regard to model studies and problems associated with the design of clinically relevant laboratory experiments of

TABLE 2: COMPARISON OF AMINO ACID COMPOSITIONS OF HUMAN SALIVAS AND 2 h *IN VIVO* (EP) AND *IN VITRO* (GeP) FORMED PELLICLES

Asp	8.4	7.1	8.0	7.8	8.7
Thr	4.4	1.0	2.8	5.3	5.0
Ser	4.5	4.2	3.9	6.4	5.7
Glu/Glx	15.2	18.4	17.3	14.9	14.6
Pro	18.1	28.4	18.5	6.8	7.2
Gly	13.8	18.2	13.8	14.1	14.9
Ala	4.9	2.5	3.6	8.9	9.1
1/2Cys	0.7	0.2	0.4	0.2	0.1
Val	4.4	2.0	4.0	5.3	5.1
Met	0.7	0.2	0.4	0.6	0.1
Ile	2.5	1.3	2.5	3.5	3.6
Leu	4.8	2.2	4.6	7.5	7.3
Tyr	2.5	1.3	3.8	2.6	2.3
Phe	2.6	1.3	3.2	3.2	3.1
His	2.1	1.7	3.3	2.0	2.3
Lys	5.0	5.1	3.9	5.8	5.9
Arg	4.5	4.6	5.6	4.8	5.2

TABLE 3: TOTAL AMOUNT OF PROTEIN PRESENT IN SAMPLES FROM PELLICLES FORMED *IN VIVO* ON BUCCAL TOOTH SURFACES (EP) AND ON GERMANIUM PRISMS (GeP) OF LOW AND MEDIUM CRITICAL SURFACE TENSIONS

EP	10	13
GeP of medium γ_c	25	27
GeP of low γ_c	4	5

TABLE 4: CARBOHYDRATE COMPOSITION OF 2 h GeP SAMPLES (MED γ_c)

Galactosamine	1.7	2.8
Glucosamine	6.2	8.6
Mannose	10.1	12.5
Glucose	82.0	76.1

restorative dental materials, there is really no reason to generate models for teeth as long as extracted ones are available. Even if certain breakdown has been reported to take place after extraction, the negative influence of such processes must be regarded as minimal as long as storage of the extracted teeth is carried out in a hydrated environment and suitably pressurized if, for example, dental adhesives are being studied (Brännström and Johnson, 1970; Elhabashy *et al.*, 1993; Krejci, *et al.*, 1993).

Surface studies of extracted human teeth show that the surfaces of ground but otherwise untreated enamel and dentin are covered with their respective low-energy, *i.e.*, organic, phases. In their basic surface chemical behavior, only minor differences could thus be found between these enamel and dentin surfaces, giving a good example of the fact that in a heterogeneous biological tissue such as a tooth, the composition of the surface does not necessarily reflect the composition of the bulk.

In spite of their hydrophilicities, the presence on teeth of these organic surface films with relatively low adhesiveness, probably explains why it is so comparatively difficult to adhere materials strongly to tooth surfaces that have not been etched or otherwise primed. After rather short exposures even to weaker acids such as lactic acid, human enamel surfaces become more resin-wettable and hydrophilic. Human dentin, with its relatively higher organic content, takes both stronger acid solutions and much longer exposure times before completely resin-wettable, penetrable hydrophilic surfaces are exposed. The use of the intrinsically low surface tension, water-compatible primer to enter these interfacial zones and establish a suitable "hybrid layer" underlines the success of modern "fourth-generation" dentin adhesives (Swift *et al.*, 1995).

The presence of low-energy surfaces on teeth is probably of physiological significance and essential for the maintenance of oral health. Bearing in mind the high adhesiveness of uncoated hydroxyapatite and also the high number of potentially adherent pathogenic or potentially pathogenic microorganisms normally present in saliva, it is desirable to possess the ability to form organic tooth surfaces with relatively low adhesiveness.

The clinical significance of these adhesive characteristics has been demonstrated in clinical studies where differences in so-called critical surface tension were found to have relevance for *in vivo* adhesion of dental plaque (Glantz, 1969). As a general observation, these results coincide with those from other biological fields. Baier (1973; 1975) and Dexter (1979) have described a similar relationship between the critical surface tension of exposed substrata and the number of retained cells on them. They also identified the least adhesive range of critical surface tension for solids to be entered around 25 mN/m (dyn/cm). Results by Schakenraad *et al.* (1986) and Christersson *et al.* (1989) have extended this relation to include the influence of critical surface tension values on other biological cell-surface interactions.

It is reasonable to assume that the major principles discussed here for the salivary filaments and functions observed are also valid for other secretory products. It also appears possible that these products may display similar structures and functional roles. By selecting more easily available secretory products, *e.g.,* whole milk, more relevant model systems may be established for *in vitro* experiments than if simple aqueous salt solutions are used.

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REFERENCES

Al-Hashimi I, Levine MJ (1989). Characterization of *in vivo* salivary-derived enamel pellicle. *Arch Oral Biol* 34:289-295.

Baier RE (1973). Occurence, nature, and extent of cohesive and adhesive forces in dental integuments. In: Lasslo A, Quintana RP, editors. Surface Chemistry and Dental Instruments. Springfield, Illinois: Charles C. Thomas Publisher, 337-391.

Baier RE (1975). Applied chemistry at protein interfaces. In: Baier RE, editor. Applied Chemistry at Protein Interfaces. Advances in Chemistry Series No. 145, Washington DC: American Chemical Society, 1-25.

Berkovitz BKB (1989). Handbook of Microscopic Anatomy. Vol. 6, Teeth. Berlin:Springer-Verlag,.

Brännström M, Johnson G (1970). Movements of the dentine and pulp liquids on application of thermal stimuli. *Acta Odontol Scand* 28:59-70.

Christersson CE (1991). On interfacial film formation and bacterial retention to solids. *Swed Dent J* 14:44-49, Suppl 77.

Christersson CE, Dunford RG, Glantz P-O, Baier RE (1989). Effect of critical surface tension on retention of oral microorganisms. *Scand J Dent Res* 97:247-256.

Christersson CE, Glantz P-O, Baier RE (1988). Role of temperature and shear force on microbial detachment. *Scand J Dent Res* 96:91-98.

Colombini N (1939). Ricerche sui cosiddette corpi filanti. Sulla diffusione libera di alcune sostanze coloranti. *Bolla Soc Ital Biol Sper* 14:453-456.

Dexter SC (1979). Influence of stratum critical surface tension on bacterial adhesion - *in situ* studies. *J Coll Interf Sci* 70:346-354.

Elhabashy A, Swift Jr EJ, Boyer DB, Denehy GE (1993). Effect of dentin permeability and hydration on the bond strengths of dentin bonding systems. *Am J Dent* 6:123-126.

Fano G (1908). Contributo allo studio corpi filanti. *Arch Fisiol* 5:365-370.

Glantz P-O (1969). On wettability and adhesiveness. *Odont Rev* 20:87-118, Suppl 17.

Glantz P-O, Jendresen MD, Baier RE (1980). A clinical method for the study of *in vivo* adhesiveness of teeth. *Acta Odontol Scand* 38:371-378.

Glantz P-O, Natiella JR, Vaughan CD, Meyer AE, Baier RE (1989a). Structural studies of human saliva. *Acta Odontol Scand* 47:17-24.

Glantz P-O, Wirth SE, Baier RE, Wirth JE (1989b). Electron microscopic studies of human mixed saliva. *Acta Odontol Scand* 47:7-15.

Glantz P-O, Friberg SE, Wirth SM, Baier RE (1989c). Thin-section transmission electron microscopy of human saliva. *Acta Odontol Scand* 47:111-116.

Glantz P-O, Attström RW, Meyer AE, Baier RE (1991). Interfacial phenomena with reference to the oral cavity. In: Bender M, editor. Interfacial Phenomena in Biological Systems. New York: Marcel Dekker Inc., 77-103.

Glantz P-O, Friberg SE, Christersson CE, Baier RE (1995). Surface and colloid chemical aspects of saliva particle interactions. *J Oral Rehab* 22:585-588.

Hoepfner H (1943). Über Strukturviskosität und Schaumvermögen des menschlichen Speichels. *Kolloid Z* 105:216-223.

Jendresen MD, Glantz P-O (1980). Clinical adhesiveness of the tooth surface. *Acta Odontol Scand* 38:379-383.

Jendresen MD, Glantz P-O (1981). Clinical adhesiveness of selected dental materials. *Acta Odontol Scand* 39:39-45.

Jendresen MD, Glantz P-O, Baier RE, Eick JD (1981). Microtopography and clinical adhesiveness of an acid-etched tooth surface. *Acta Odontol Scand* 39:47-53.

Jenkins GN (1978). The Physiology and Biochemistry of the Mouth. 4th ed. Oxford: Blackwell Scientific Publications, 238-259.

Krejci I, Kuster M, Lutz F (1993). Influence of dentinal fluid and stress on marginal adaptation of resin composites. *J Dent Res* 72:490-494.

Lazzari E (1976). Dental Biochemistry. 2nd ed. Philadelphia: Lea & Febiger.

Miles A (1967). Structural and Chemical Organization of Teeth. Vol. II. London: Academic Press.

Norde W (1986). Adsorption of proteins from solution at the solid-liquid interface. *Adv Coll Interf Sci* 25:267-340.

Schakenraad JM, Busscher HJ, Wildevuur RH, Arends J (1986). The influence of stratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. *J Biomed Mater Res* 20:773-784.

Schwarz WH (1987). The rheology of saliva. *J Dent Res* 66:660-664.

Swift Jr EJ, Perdigao J, Heymann HO (1995). Bonding to enamel and dentin: A brief history and state of the art. *Quintessence Int* 26:95-110.

Vassilakos N, Rundegren J, Arnebrant T, Glantz P-O (1992). Adsorption from salivary fractions at solid/liquid and air/liquid interfaces. *Arch Oral Biol* 37:549-557.