Characterization of oral in vivo films formed on different types of solid surfaces

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Studies were made of oral films formed *in vivo*, which had been allowed to form on fused silica and Ge-prisms during periods between 2 s and 2 h using a variety of physico-chemical methods. To produce surfaces of different qualities the silica and Ge-prisms had either been detergent-washed, glow discharge treated or covered with polydimethylsiloxane. The following simultaneous analytical techniques were performed on the adsorbed films: a. internal reflection infrared spectroscopy, b. ellipsometry, c. contact potential measurements, d. contact angle measurements, e. scanning electron microscopy and f. energy-dispersive x-ray analysis

The results of these studies show that the formation of oral films proceeds at high speed and is of a certain qualitative selectivity. The formed films were found to be stable over long periods of time, and only showed patches of adhering micro-organisms on some of the prisms which had been exposed in the oral cavity for 2 h.

Key-words: Biochemistry; physics; human

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Of the organic integuments, which form on tooth surfaces *in vivo*, plaque is recognized as a prime etiologic factor in caries as well as in periodontal disease (for reviews see 13, 15). Plaque is generally characterized as a closely packed aggregation of many kinds of microorganisms which are embedded in an organic matrix, composed of material derived from the diet, from biological fluids like the saliva and the gingival crevice fluid, or from the microorganisms themselves (20).

Plaque does not form directly onto surfaces in the mouth, but is originally separated from them by a distinct layer of the material commonly referred to as the acquired pellicle or the pellicle (24). Subsequently, bacteria and bacteria products are adsorbed to this organic layer, which is then transformed into plaque (14). The composition of dental pellicles has been studied extensively through the use of microscopic and/or biochemical methods (1, 8, 22, 29, 30).

The results of these studies indicate that the pellicle is mainly formed through selective adsorption of salivary proteins. Many models offer explanations of the mechan-

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isms involved in pellicle and plaque formation, including those reported by Bernardi & Kawasaki (9), Gibbons & Spinell (15), Kleinberg (19), and Sönju & Rölla (30).

When the mechanisms of pellicle formation are compared with those acting during formation of other types of biological films, there appears to be a common interfacial chemistry. The similarities are seen even when pellicle formation is compared with seemingly unrelated phenomena such as film formation on sutures, artificial organs or medical equipment contacting blood, or with the wide variety of marine fouling events. That is, most of the interfacial films, like the pellicle, are dominated by glycoproteins as the first spontaneously acquired conditioning layers. Such film formation is the prerequisite for subsequent cellular adhesive events (4). Recent studies of many types of biological and related interfaces have provided detailed information about those films through the use of a variety of surface physico-chemical analytical techniques, including internal reflection infrared spectroscopy, ellipsometry, and determinations of critical surface tensions and contact potential values (for a review, see 4). In previously reported studies of oral films, however, such methods have so far been used only to a limited extent (3).

Therefore, in order to characterize oral films better, such as the dental pellicle, and their *in vivo* formation, physico-chemical analyses of the surface material using multiple methods was begun.

MATERIALS AND METHODS

The primary materials used for the adsorption of oral films were fused silica discs and germanium prisms (type 6068, Wilks Scientific Corporation, S. Norwalk, Connecticut) which had been treated by a) detergent washing, b) glow discharge exposure according to the technique described by Baier & DePalma (5), or c) coating with polydimethylsiloxane, by a known method (7). The latter process forms a film which chemisorbs to silica and germanium, giving surfaces essentially dominated by closely packed methyl groups. The size of the germanium prisms was 12 x 5 x 1 mm, and they had 45° face angles. The fused silica discs were about 10 mm diameter by 5 mm thick.

Table 1 reports the surface treatments and the resultant surface energetic parameters for the germanium prisms used. The surface energies are reported in both the empirical category of critical surface tension as deduced from the approach of Zisman (31), and the thermodynamic categories of composite surface free energy, dispersion force, and polar force components, as calculated by the methods of Nyilas et al. (25)

The typical film thickness for the covalently bound siliconizing layer on the germanium prisms was 14 nm, with a range from 12.5 through 17.5 nm. Siliconization created a surface energetic state dominated by the van der Waals interactions of the closely packed methyl group constituents of the polysiloxane backbone. In contrast, the glow discharge treated surface were almost totally dominated by polar forces, while the simply detergent washed metallic specimens for intraoral exposure had about a 50–50 mix of polar and dispersion force components making up their total potential interactive boundary energy, as typifies most clean metal and ceramic surfaces (10, 11, 27).

The silica discs were worn in a test piece holder, where they were placed approximately parallel to and in front of the buccal surfaces of teeth nos. 34, 35 and 45, 46 Soon, however, the silica discs were abandoned in favor of the more versatile germanium prisms.

For time periods varying between 2 s and 2 h, two germanium prisms per test were worn intraorally in all acrylic mandibular bite splint, shown in Fig. 1, by one experi

Table 1. *Surface treatments and surface energies of germanium surfaces*

Surface treatment given to germanium	Surface energies				
	γ_c dyne/cm	S cm/dyne	γ_s dyne/cm	γ_s^d dyne/cm	γ_s^p dyne/cm
Siliconizing (methyl groups outer most)	22.5	−0.024	24.6	22.8	1.8
Detergent washed	38.6	−0.011	47.8	24.4	23.4
RFGDT*	51.2	−0.004	70.6	8.6	62.0

γ_c – critical surface tension

S – slope of the best straight line fit (determined by the method of least squares) through a plot of the cosine of the contact angles of a group of liquids against the liquid/vapor surface tension of the liquids

γ_s – composite surface free energy

γ_s^d – dispersion-force component of surface free energy

γ_s^p – polar-force component of surface free energy

* Radio frequency glow discharge treated

mental subject. In special holders situated buccally in regions 35/36 and 45/46 of the bite splint, the prisms were kept in the buccal extensions of the occlusal planes of these teeth. No food or drink was consumed during the experiments nor during 2 h before them. After each experiment, the prisms were rinsed with redistilled water to remove excess saliva "carry". The samples were then either immediately analyzed and/or stored for varying amounts of time. The initial 24 h of each storage period, which varied between one day and about seven weeks, always took place at 20–22 °C in a dessicator filled with dry silica.

The molecular structure of the films adsorbed on the germanium prisms was first deduced by an internal reflection technique described by Harrick (18). Secondly, as germanium is a material of high refractive index, the thickness and refractive indices of the films were determined by the ellipsometric technique (23). Further as germanium is also a conductive substrate, the determination of the contact potential was made from vibrating reed electrometer

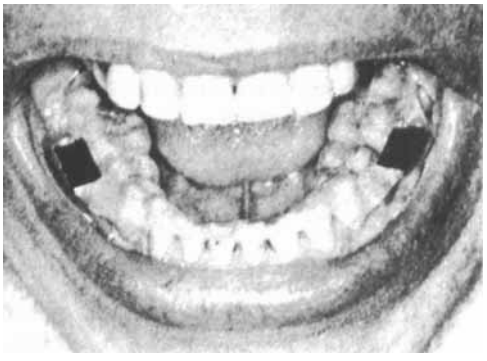

Fig. 1. Intraoral view of mandibular bitesplint, containing miniature internal reflection prisms in the buccal extensions of the occlusal planes of teeth 35/36 and 45/46.

studies using the type of experimental equipment originally designed by Bewig & Zisman (12). The fourth method used for characterization of the adsorbed material was to take contact angle data according to the technique described by Zisman (31). These data were then used to derive the critical surface tensions of the films. Finally, scanning electron microscopy and energy

dispersive x-ray analysis was applied to record the film's texture, and the presence, if any, of cellular deposits and associated inorganic elements. Before their microscopic examinations, which were performed at an angle of 45° with tilt correction, the film-covered prisms were coated with Au-Pd.

As all the analytical methods, except the two last mentioned ones, are nondestructive, on some of the films the nondestructive analyses were repeated at regular intervals after further storage. Fig. 2 illustrates some of the described physico-chemical analytical methods used to assess the interfacial structure and properties of the prism-collected materials.

Under the conditions used in this study germanium is a material, which has a good biocompatibility (for a review, see 26). When used in similar types of both biological and nonbiological systems it has proved no interference with the precisions of the analytical techniques used (4). The sensitivity for all of the methods is great enough to detect layers less than 1 nm thick, representing a fraction of a microgram of material on the prism surfaces (4).

RESULTS AND DISCUSSION

Fig. 3 typifies the findings from earlier experiments (4) in which larger, 50 x 20 x 1 mm, internal reflection prisms were carried intraorally, presenting a characteristic infrared spectrum for the adsorbed film upon clean (that is, detergent-washed) prisms kept in the oral cavity for 2 min. Almost identical infrared spectrum was obtained for the accumulated material when the incubation time was extended to 15 min. Certain differences in the results were both expected and obtained in the currently reported study, as a result of the shift to the use of smaller adsorbing solid surfaces, with different oral placement, and also as a result of the use of different subjects. For example, when using the larger prisms, more surface area was available and more internal reflections were produced within the specimens to give spectra with more intense bands and clearer contrast. On the other hand, the large size of the prisms of our earlier work prevented their mounting in relevant buccal or lingual positions in the mouth. Further, the sharp edges of the large unmounted prisms easily caused irritations to the subjects' tongues and buccal mucosae. This raised questions as to the representativeness of the acquired films previously examined, and to the likelihood of their having been formed from secretory components not normally available for adsorption. Further, the use of additional subjects, and even the same subject tested years later, raised questions of the generality of the prior findings that we have now set out to answer by using more subjects and adsorbent surfaces of different original characteristics.

Thus, Fig. 4 presents an internal reflection infrared spectrum of the material acquired on a glow discharge treated miniature prism, during 5 min of intraoral exposure. Comparing this spectrum with those spectra presented in Fig. 3, one can note the small prisms' more intense carbon dioxide adsorption band at 2350 cm^{-1} and the relatively less intense germanium lattice adsorptions at approximately 850 cm^{-1} and 750 cm^{-1}. These differences result from, first, the greater sample beam path length in the special mirror device required for use of the small prisms, and, secondly, from the considerably smaller mass of the miniature prisms. The relative intensities of the key diagnostic bands for proteins, the Amide I and II bands between 1600 and 1700 cm^{-1}, and 1500 and 1600 cm^{-1}. respectively are the similar features coupling the previous large prism work to the current miniature prism work. Note, as well, the relative absence of infrared absorption in the spectral region between 1000 and 1100 cm^{-1} in all these spectra, which point against strong adsorption of carbohydrate-

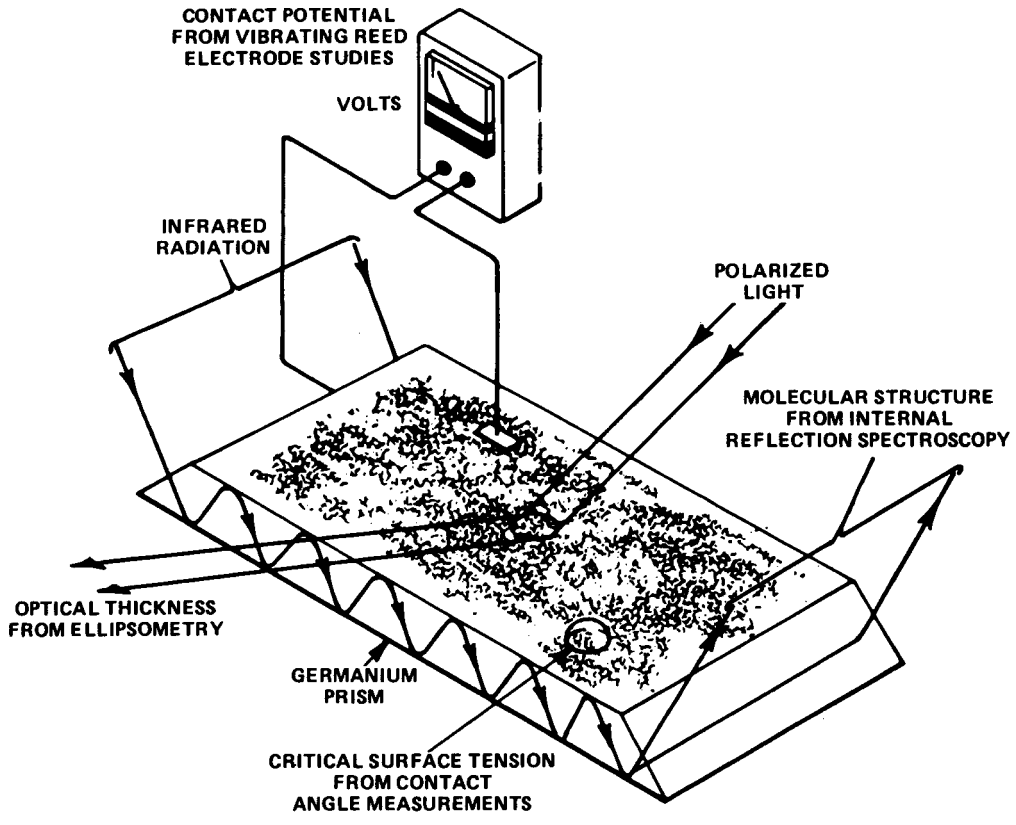

Fig. 2. Physico-chemical analytical methods applied to the characterization of films acquired on the prisms removed from the bitesplint shown in Fig. 1.

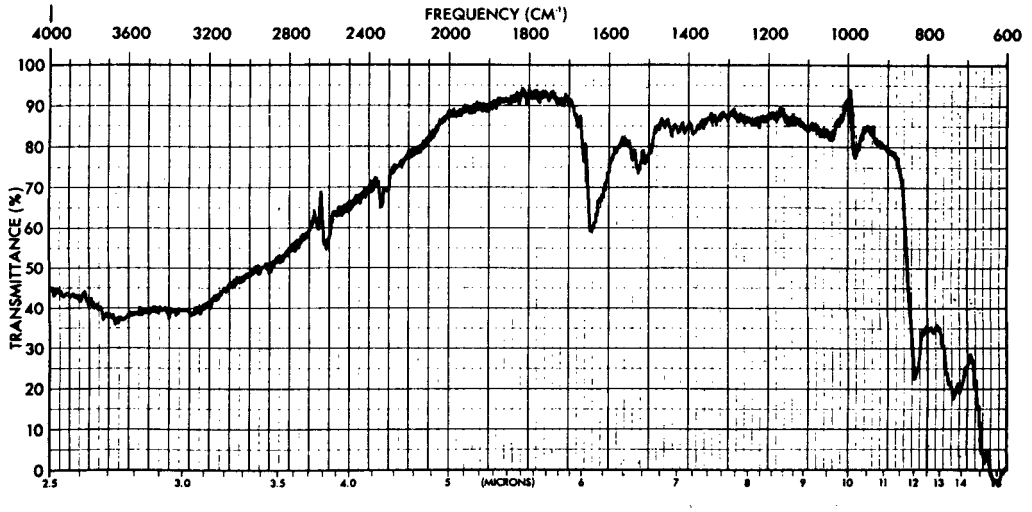

Fig. 3. Characteristic infrared spectrum of adsorbed film on clean prism placed intraorally for 2 minutes.

rich substances in the initially bound layers. When comparing Fig. 4 with the spectra obtained during longer periods of intraoral exposure, it was easily seen that very little change in the qualitative characteristics of the adsorbing components occurred during additional times of exposure. It was, for instance, noted that the measured average dry film thickness of the acquired material increased only from 6,8 to 7,7 mm during an additional 10 min adsorption time. Although Fig. 4 is for a glow discharge-treated, that is, scrupulously cleaned and high surface free energy, test object, it is to be noted that the results are not qualitatively different from those obtained with the same subject using simply detergent-washed specimens. The acquired films on the larger prisms did seem to be qualitatively different from those on similar detergent-washed miniature prisms, however. This qualitative difference in the type of initially bound components seems, at the present time, mainly to be subject related.

Results that are in some ways similar and in some ways in contrast to those just presented, are typified in Figs. 5 and 6 which are internal reflection infrared spectra for the films spontaneously acquired on the low surface energy (siliconized) prisms exposed intraorally in the same subject as Fig. 4. Fig. 5, in particular, characterizes a five-minute exposed test specimen which did acquire about the same mass of adsorbed protein as did the detergent-washed specimens used during the same experiment. As the recorded average dry film thickness of this was only 0.2 nm the adsorbed material must, however, have been organized in a nonuniform way. With continuing incubation time, as shown in Fig. 6 for a 2 h specimen, the qualitative nature of the matter adsorbing to that homogeneous solid surface in increasing amounts did not change much while the film thickness increased to 14,4 nm (Table 3). As will be discussed later, the adsorbed amounts, as determined by integration of the areas under the Amide I and Amide II adsorption bands for such films, and their ellipsometrically measured film thicknesses, showed the acquired material on low energy surfaces to be in fact, more ample than their counterparts formed on the higher energy substrates.

There are many additional points of interest to be taken from these studies with

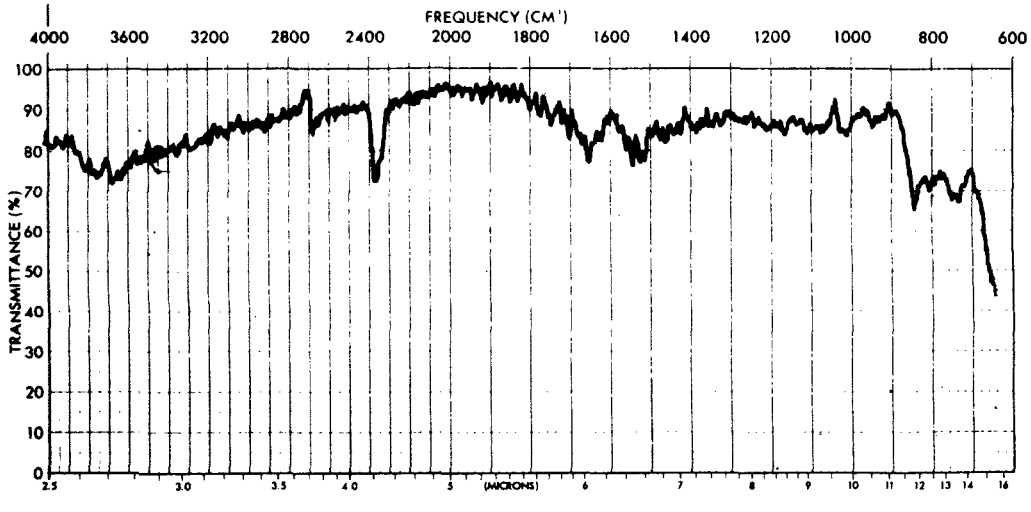

Fig. 4. Internal reflection infrared spectrum of material acquired on glow-discharge-cleaned (RFGDT), miniature prism during 5-minute intraoral exposure. Average dry film thickness (n = 1.5) : 6.8 nm.

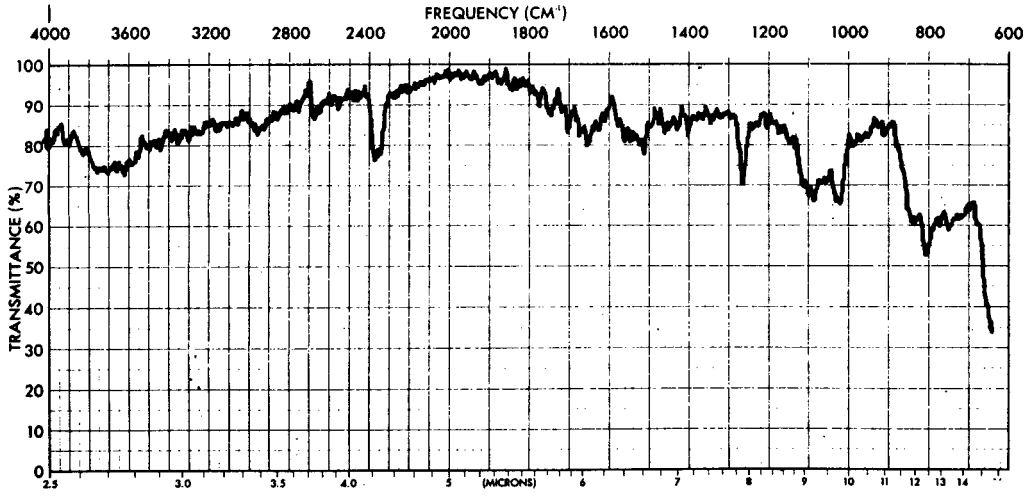

Fig. 5. Internal reflection infrared spectrum of material acquired on siliconized prism exposed intraorally for 5 minutes. Average dry film thickness (n = 1.5) : 0.2 nm.

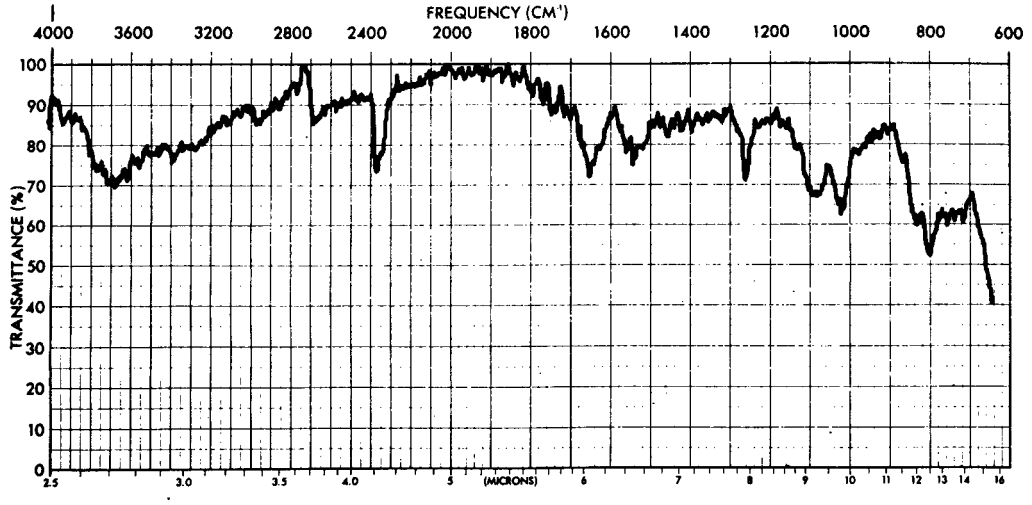

Fig. 6. Internal reflection infrared spectrum of material acquired on siliconized prism exposed intraorally for 120 minutes. Average dry film thickness (n = 1.5) : 14.4 nm.

the siliconized specimens tested. Note, particularly, the clarity of separation of the infrared adsorptions for the silicone itself (SiO: 1000–1150 cm^{-1}, Si-CH_3: 1260 cm^{-1}; and CH_3: 2950–2980 cm^{-1}) from the otherwise apparently similar bands for the adsorbed matter discovered first on the clean prisms. The small average dry film thickness of the most rapidly adsorbed proteinaceous matter on the siliconized materials probably reflects, low adhesion of the adsorbed matter. Clustering of the adsorbed mass could then occur relatively easily during dessication of the specimens prior to infrared and ellipsometric analysis. Detachment of the siliconizing coating is not a significant factor in these results, as demonstrated by comparing pre- and postexposure infrared and ellipsometric findings with thoroughly buffer-rinsed controls in separate experiments.

Fig. 6 and other spectra obtained for

exposure times ranging from 15 to 120 min, confirm the general increase of adherent proteindominated matter with increasing adsorption time. This is easily seen in the Amide I and Amide II absorption bands near 1650 cm^{-1} and 1540 cm^{-1}, respectively, and confirmed by the increase in average ellipsometric thickness of the films on otherwise constant silicone film thicknesses. It is important to note that, for these experiments, different prisms were used and independently prepared siliconizing coatings were covalently attached to them, attesting to the good reproducibility of the used cleaning, coating, and exposure techniques. Deficiencies of our study to date have been the use of the same volunteer subject throughout the study of low energy surfaces, and the limited number of replications of these findings even in that single subject.

In this context it is of importance to remember that the analytical techniques used in this study do not allow detailed determinations of the composition of the adsorbed proteinaceous material. The nature of the amino acids of the adsorbed proteins could therefore differ on the different solid surfaces as has been reported by Sönju & Glantz (30).

Turning to wettability tests of the disc and prism surfaces pre- and postexposure, the wetting behavior of clean fused silica discs prior to oral exposure showed very low contact angles for pure liquids with liquid-vapor surface tension of 30 dynes/cm or greater, giving variable critical surface tension intercepts from 30 dynes/cm to some 50 dynes/cm (depending upon the relative humidity of the room in which measurements were made). This behavior is typical of that for clean glass, metal, oxide, and ceramic specimens (10, 11, 27). In contrast, change in wettability behavior for this fused silica was observed after 2 hrs of intraoral exposure in the same volunteer whose oral deposits are characterized in the infrared spectra already presented in Figs. 4–6. The critical surface tension of this acquired film on these test specimens, between 20 and 30 dynes/cm, is situated in what is considered to be a biologically "abhesive" range (2, 4, 6). It should be noted that this critical surface tension value is different from the previous values recorded for earlier subjects and also different from the values for natural enamel surfaces (3, 17). For example, the wettability plots of the adsorbed surface films formed on

Table 2. *Preliminary matrix of film acquisition rates for prisms of different initial surface properties*

Exposure (min)	Film thickness (nm) on Ge-prisms							
	Clean			Methylated				
2/60	7,4							
1	7,1							
2	7,7							
5	6,8;	8,3		0,2;	17,3	17,5		
10	11,5							
15	7,7	9,3		6,6;	20,7			
20	12,5							
30	9,0;	9,4		12,5;	14,0			
45	13,2							
60	8,1							
75	11,5;	11,5;	11,7	8,9;	20,1;	21,2		
120	9,4;	9,4;	14,0	14,4;	17,3;	17,4;	17,5;	27,2

Table 3. *Variation in measured average film thicknesses for oral films stored dry on (A) clean and (B) siliconized substrates*

Storage Time (days)	Pellicle film thickness (nm) after storage	
	A	B
0	8,9	20,2
5	9,0	20,8
8	7,6	19,4
12	7,4	19,2
19	10,5	22,3
23	10,6	22,4

detergent-washed germanium prisms after 2 and 15 min of oral exposure in an earlier subject showed extrapolated critical surface tension intercepts in the bioadhesive range between 30 and 40 dynes/cm. The chemical contents of the films characterized by these wettability criteria are those already exemplified in Fig. 3.

Fig. 7 is a summary chart of the *in vivo* wetting behavior on four different human teeth, two each in two volunteers, obtained shortly after brushing with a fluoride-containing tooth paste and thorough rinsing. The critical surface tension intercept, above 30 dynes/cm, is again in the bioadhesive range.

A major portion of our work to date has dealt with establishing the rates and sequences of events occuring on solid surfaces in the oral cavity, as they might differ in some predictable or controllable manner depending upon the initial substrate surface properties. Table 2 presents a matrix of the film acquisition rates on prisms of different initial surface properties, as gleaned from our preliminary data. Beyond noting that the trend for film thickening is an erratic one, with the rates of increasing matter deposition found to be quite slow after the first few seconds of intraoral exposure, the other main feature has been that, on average, the acquired film thickness is greater on the low energy surfaces than on the detergent-washed or glow discharge treated, higher energy surfaces. Further, the acquired film on the low energy (siliconized) substrates gave new wettability data characterizing the polydimethylsiloxane layer. These latter observations reinforce the speculation that the adsorbed proteinaceous matter is in a considerably looser and more native configuration on the low energy substrates. This allows access of the diagnostic wetting liquids to the original solid boundary even in the presence of considerable, but not occlusive, overcoatings of salivary constituents. The same observation suggests that low surface energy materials in the oral cavity might be much more easily cleaned of built-up films of protein and, subsequently, of adherent formed elements because of the loose, weakly adherent film structure. Following from this, when exposed to functional shearing forces such low energy surfaces may achieve a state of long-term freedom from gross deposits. These data confirm the observation by Glantz (16) that the maximum amount of dental integument that can adhere to a solid surface depends on the magnitude of the critical surface tension of that surface.

Table 3 lists the ellipsometrically determined thickness data bearing upon the storage stability of films acquired on (A) clean and (B) siliconized substrates. It is necessary in our work to address these issues of film storage stability, since samples are to be acquired from large numbers of subjects eventually, sometimes at remote locations, and the samples shipped after moderate storage intervals to a central analytical laboratory. The listing in Table 3 shows that storage times of 8 and 12 days duration gave apparently minimum film thicknesses for these same specimens. It is likely that a change in the relative humidity in the measurement room, was the cause of this apparent change in film thickness because of its hygroscopic quality. The small apparent increases in film thickness during additional days of storage were certainly

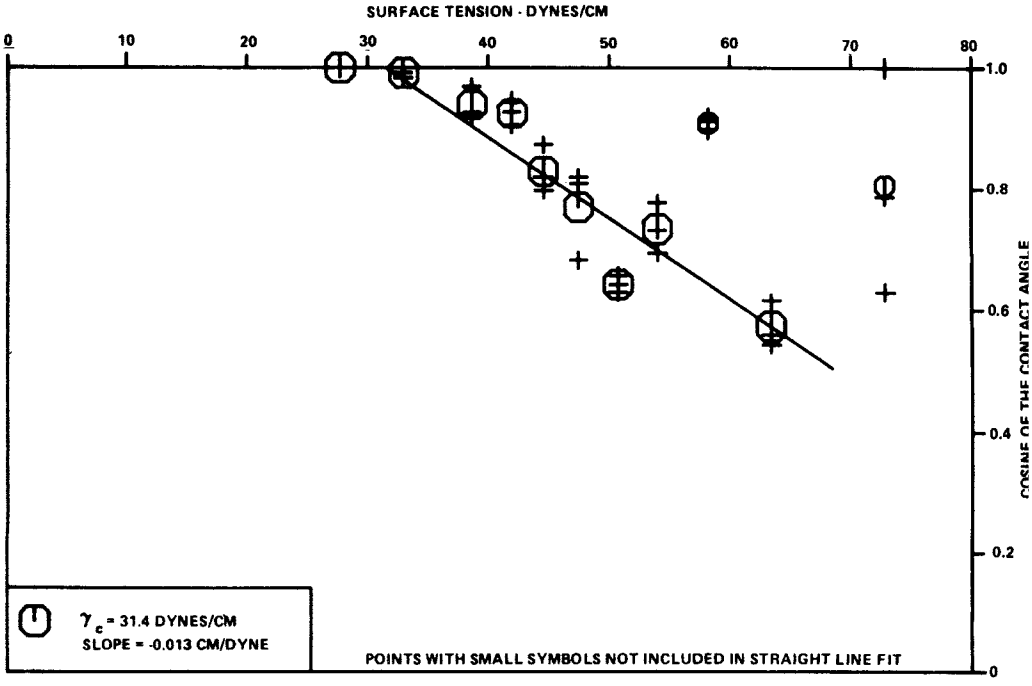

Fig. 7. Wettability of human teeth in situ. Average data from 4 teeth, 2 volunteers, shortly after vigorous brushing with fluoride-containing toothpaste and thorough rinsing.

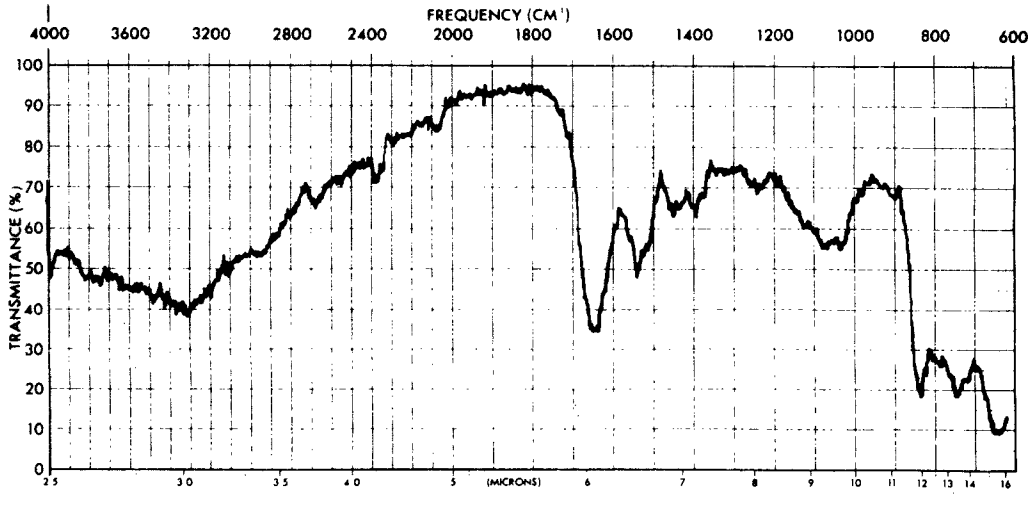

Fig. 8. Infrared spectrum of dehydrated whole saliva.

due to accumulation of contamination from multiple handlings of the prisms, as confirmed in the infrared spectra and by scanning electron microscopic inspection.

Fig. 8, an infrared spectrum of dehydrated whole saliva, supports the general findings that the acquired dental film is only a specific subfraction of the proteinaceous components available in the whole salivary pool. The spontaneous absorption

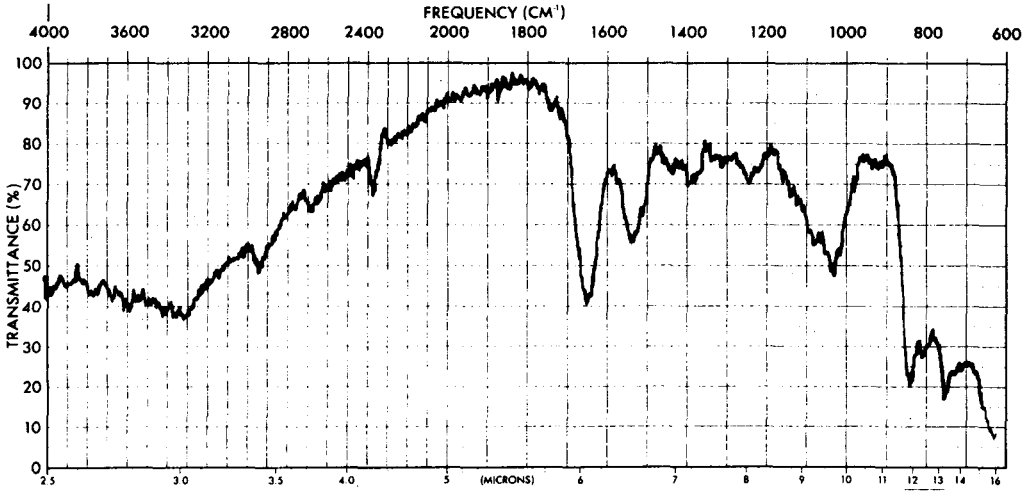

Fig. 9. Infrared spectrum of dehydrated dental plaque.

Fig. 10. SEM-picture (x 7000) showing adhering, mostly rod-shaped micro-organisms after two hour intraoral exposure of detergent washed Ge-prism.

process excludes the majority of dissolved components normally in the oral cavity. Note, for example, in Fig. 8 the small cyanate absorption band at about 2080 cm^{-1}, which varies to much greater intensities from time to time for saliva from the same subject as well as from subject to subject, but which was always missing in acquired film and plaque spectra in any of the subjects we have examined. Fig. 9 provides the infrared spectrum of dehydrated dental plaque taken from the same subject at the same time as the saliva characterized in Fig. 8. The sample was analyzed on the identical infrared instrument with exactly the same optical components and supporting windows, so that their baselines are exactly comparable. The plaque is, again, different from the acquired films but also considerably different from the saliva of the same subject. There is an increased proportion of carbohydrate with respect to protein – reflecting bacterial exudates, probably – and both sharper and increased hydrocarbon bands in the plaque spectrum. Also there are modest increases in the 750 cm^{-1} and 1250 cm^{-1} spectral regions, characteristic for lipids. The minimal increases in absorption intensities in these bands, however, suggest that actual bacterial population was low.

Although no illustrations are included here, the scanning electron micrographs of the acquired films showed that adherent micro-organisms were essentially absent during the first hour of exposure. Some of

the prisms exposed for two hours showed patchy appearences of mostly rod-shaped but also some sphere-shaped micro-organisms (Fig. 10). Generally speaking, this finding is in correlation with those of Lie (21) but not with those reported by Socransky et al. (28), who found micro-organisms on tooth surfaces immediately after cleaning them with pumice. Energy dispersive X-ray analyses performed at the same time showed that inorganic elemental abundances were not substantially different from those in the salivary pool. In particular, no excessive amounts of calcium were found.

The electrical measurements showed that all adsorbed salivary protein films increased the contact potentials of the tested specimens, but by widely varying amounts. Additional studies of the changes of the surface electrical properties during accumulation of oral deposits are therefore required before conclusions can be presented about the importance of surface electrical states and/or their perturbations.

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