Intraoral adhesion to a well defined surface

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One male subject was used as test person in an *in vivo*-study of oral films formed on well defined germanium prisms under unprovoked oral conditions as well as in the presence of sucrose or blood. The following analytical techniques were performed on the adsorbed films: a. internal reflection infrared spectroscopy, b. ellipsometry, c. scanning electron microscopy and d. energy-dispersive x-ray analysis.

The results show that in the created moderately stagnated intraoral system the formation of biological films was similar in different parts of the oral cavity. The process was not found to be markedly influenced by the presence of blood. Sucrose, on the other hand, was found to increase both the overall film thickness and the carbohydrate-content of the films.

The attachment of microorganisms was found to be a process proceeding at a comparatively slow rate especially in the presence of sucrose and when compared with biological debris. The majority of the first adhering microorganisms were found to be rod-shaped.

Key-words: Physics; biochemistry; human; biological films

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The initial step in the formation of dental plaque includes the irreversible attachment of plaque-forming microorganisms. A number of theories have been presented on the nature of the mechanisms responsible for intraoral adhesion of such microorganisms (4, 6, 7, 11, 14, 16).

The ranges of action are short for all adhesive forces ($< 10^{-8}$ m) and especially for those that can exist in biological systems. Therefore, after adsorption of only a monolayer of most biological macromolecules, the adhesive properties of the exposed parts of these molecules completely dominate over those of the underlying solid onto which they have been adsorbed. For a review of surface chemical aspects on bioadhesion see Baier (2).

In clinical experiments using *in vivo*-contact angle measurements (8) the significance of this phenomenon has been demonstrated by Jendresen & Glantz (9) and Jendresen, Glantz, Baier & Eick (10), who observed that a range of dental materials as well as acid etched and natural human enamel surfaces gained the same clinical adhesiveness after adsorption of salivary film material.

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Consequently, it is essential that studies of intraoral adhesion are performed in the presence of relevant film forming material, and that other factors with influence on the adhesion of particles, such as salt concentrations, are also kept at relevant levels (5).

As these conditions are extremely difficult to fulfill in *in vitro*-situations, it was thought worth-while to perform in vivo-studies of intraoral adhesion to well defined germanium surfaces under unprovoked as well as some provoked conditions.

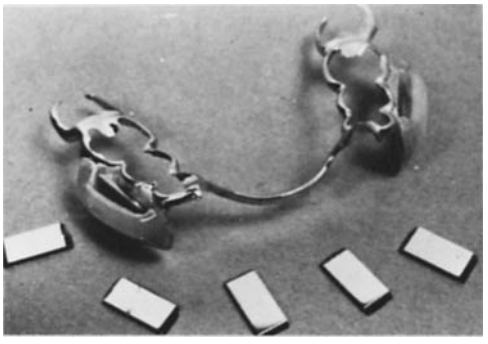

Fig. 1. Ge prisms and intraoral holder for such prisms used to collect salivary films in oral areas of moderate stagnation.

MATERIALS AND METHODS

One male subject aged 42 was chosen as the test person. He considered himself to be in good general health, and upon clinical examination was found to be in good oral health.

Using standard clinical and laboratory procedures the holder shown in Fig. 1 was cast in a Co-Cr alloy, type Vitallium (Austenal Inc.). The holder allowed for two miniature Ge prisms type 6068 (Wilks Scientific Corp., S. Norwalk, Connecticut) to be kept in well defined positions, one approximately parallel to and about 2 mm in front of the buccal surface of teeth nos. 35 and 36, the other in the same relation to teeth nos 45 and 46. Due to the presence of double Co-Cr-girders approximately 1 mm in front of the prisms the flow of saliva was restricted, although not stopped in the space occupied by the prisms.

Before the beginning of every experiment the prisms to be used were cleansed and sterilized with glow discharge (12) and then treated with a detergent, using the method described by Baier (3). Baseline infrared absorption spectra were then obtained on the cleaned prisms (3).

Pairs of Ge prisms were then worn in the mouth of the test person for one 2 hr and one 4 hr period, during which no food or drink was consumed. An additional 2 hr experiment was performed, during the final 60 min of which the test person held in his mouth a teaspoon measure of sucrose, which was swallowed and renewed every 10 min.

Finally, two 15 min experiments were also performed, where three prisms were kept free in the oral cavity, one on each side in the mandibular vestibular sulcus, and the third one on the base of the tongue. During the first of these experiments no special precautions were taken except that no food or drink was consumed, whilst during the second experiment, as well as for 15 minutes immediately before it, the test person vigorously brushed his periodontal tissues thereby causing profuse bleeding to occur.

At the end of all oral exposures the prisms were rinsed under a stream of distilled water in order to remove excess salivary material. The prisms were then kept in a desiccator for about 24 hours and finally analysed using internal reflection infrared spectroscopy, ellipsometry, scanning electron microscopy and energy dispersive x-ray analysis. The details of these analytical techniques have been reported by Baier & Glantz (4).

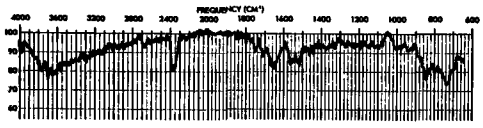

Fig. 2. Internal reflection infrared spectrum of oral film material acquired on germanium prism exposed for 15 min under unprovoked conditions. Note the corbon dioxide adsorption band at 2350 cm^{-1} resulting from the great sample beam path length in the special mirror device required for use of the germanium prisms, and the germanium lattice adsorptions at approximately 850 cm^{-1} and 750 cm^{-1}. The relative intensities of the key diagnostic bands for proteins, the Amide I and II bands, are visible at 1600 – 1700 cm^{-1} and 1500 – 1600 cm^{-1} respectively.

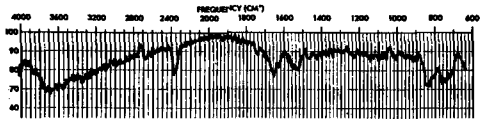

Fig. 3. Internal reflection infrared spectrum of oral film material acquired on germanium prism exposed for 15 min during profuse periodontal bleeding. Note similarities with the spectrum of Fig. 2.

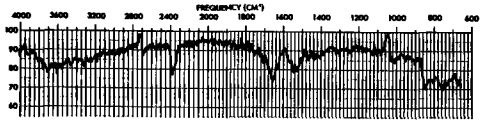

Fig. 4. Internal reflection infrared spectrum of oral film material acquired on germanium prism exposed for 2 hr under unprovoked conditions. Note similarities with the spectra of Figs. 2 and 3.

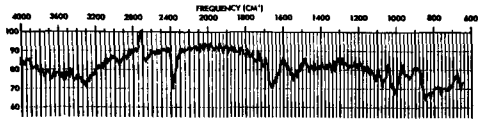

Fig. 5. Internal reflection infrared spectrum of oral film material acquired on germanium prism exposed for 2 hr, during the final 60 min of which in the presence of sucrose. Note additional C-OH adsorption band at approximately 1040 cm^{-1}, and CH_2/-CH_3 adsorption band at just below 3000 cm^{-1}.

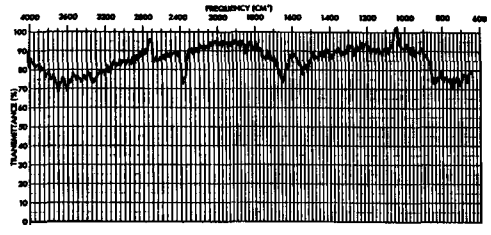

Fig. 6. Internal reflection infrared spectrum of oral film material acquired on germanium prism exposed for 4 hr under unprovoked conditions. Note similarities with the spectra of Figs. 2 – 4.

A study of a possible error of the method

The clinical adhesiveness of miniature Ge prisms was studied by allowing three test persons, aged 25 to 28 years and in clinically good oral health to carry 2 such prisms each in their mandibular vestibular sulcus for time periods between 15 and 20 minutes. The prisms were then removed and rinsed with distilled water. Finally, both the prisms and the teeth of the test persons were exposed to clinical contact angle measurements using the liquids and techniques described in detail by Glantz, Jendresen & Baier (8).

For the individual test person no statistically significant differences could be detected between the clinical adhesiveness of the films formed on normal tooth surfaces and Ge prisms. Statistically significant differences ($p < 0.001$) could, on the other hand, be observed between this characteristic of the films formed by different test persons. The results of this study of a possible error of the method agree with those reported by Jendresen & Glantz (9).

RESULTS AND DISCUSSION

When the ellipsometric (Table 1) and IR-data (Figs. 2–6) were examined, they were found to support the findings by Baier & Glantz (4). Thus, the formation of intraoral films is obviously a uniform process involving protein containing material, which initially proceeds at a high rate. Under the experimental conditions of the present study, the process seemed to be completed within 2 hrs. Furthermore, the data indicated that the presence of sucrose –

but not that of blood ⟷ may influence on the types and amounts of film material adsorbed. When compared with the unprovoked 2 hr prisms, the prisms in the sucrose experiment showed film thickness increases of 2.5 to 14 nm. Moreover, the films in the sucrose experiment showed increases in the IR-absorbance at 1000–1050 cm^{-1} – thought to be caused by increased C–OH stretching vibrations in the films on these prisms. Increased absorbance was also observed immediately under 30000 cm^{-1}. This was thought to reflect $-CH_2$; $-CH_3$ stretching vibrations (Fig. 5). It thus appeared that the sucrose rinse caused an increased film thickness as well as an enrichment of carbohydrates in the films. As free sucrose would be easily washed away during the distilled water rinse, it is likely that either the initial concentration of free sucrose was high in the film or that it had bound to some original portion of the film material. If this happens, the carbohydrate side chains of the glycoproteins creates the most likely portions to be used.

The SEM-pictures taken under unprovoked conditions showed that very few microorganisms adhered to the film covered prisms after only 15 minutes' exposures, but the patches of microorganisms were observed both after 2 and 4 hrs. No major differences could, on the other hand, be observed between the numbers of adhering organisms at these two time periods. Slightly fewer microorganisms were in fact judged to be present on the 4 hr films as compared to the 2 hr ones. In all these prisms the total surface coverage of microorganism-like particles was judged to be less than 10 per cent and definitely less than the coverage of particles judged not to have microbial origins.

In the experiment with bleeding periodontium there was a large number of adhering particles without microbial shapes (Fig. 7). These comparatively large and often irregularly shaped particles, among which not one erythrocyte could be observed, were believed to have mucosal origin and to have been worn off from this tissue during the vigorous toothbrushing.

As has been indicated above, at the prolonged, but unprovoked, exposures large amounts of adhering irregularly shaped particles were also observed as in for example Fig. 8. This is taken from one of the 4 hr films and shows many irregularly shaped particles, which upon energy dispersive x-ray analysis turned out to consist of some type or types of noncalcified biological material. As no food or drink was con-

Table 1. *Film thickness (nm) on Ge-prisms exposed in the oral cavity for varying amounts of time (min) under unprovoked as well as provoked conditions*

Time of exposure (min)	Oral conditions	Film thickness (nm)
15	Unprovoked	7.2; 7.1; 8.7*
15	Bleeding periodontium	6.4; 7.2; 5.3*
120	Unprovoked	11.1; 10.9
120	Sucrose rinsing during final 60 min	13.6; 25.2
240	Unprovoked	17.2; 8.8

*Prism held on the base of the tongue

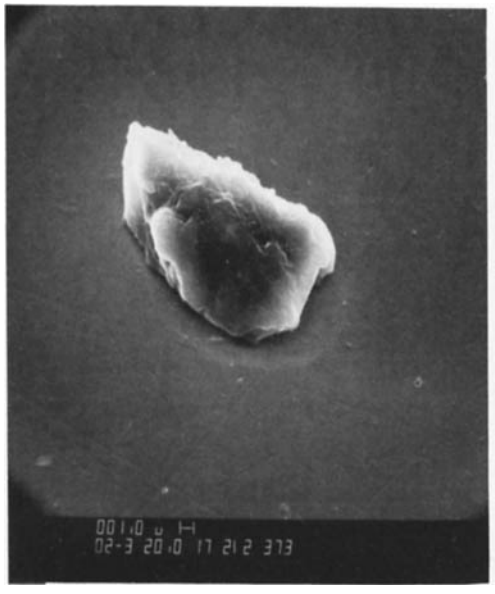

Fig. 7. SEM-photograph (original magnification 200 X) showing irregularly shaped particle adhering to film covered Ge prism at the end of a 15 min. intraoral exposure with bleeding periodontium.

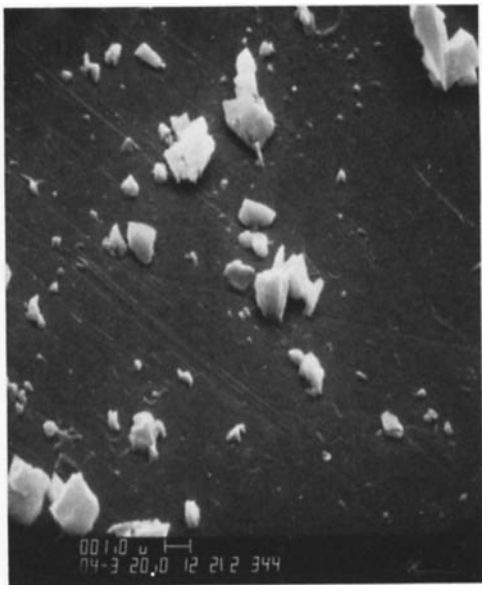

Fig. 8. SEM-photograph (original magnification 400 X) showing irregularly shaped particles adhering to film covered Ge prism at the end of a 4 hr unprovoked intraoral exposure. Simultaneously performed dispersive x-ray analysis gave the following most prominent absorption bands: 1.186 keV (Ge Lα-band); 2.123 keV (Au Mα-band); 2.838 keV (Pd Lα-band); 9.885 keV (Ge Kα-band) and 11.100 keV (Ge Kß-band).

sumed during the experiment nor for 2 hrs before it, it was concluded that, as in the case of the bleeding periodontium, the oral mucosa was the most likely origin of these particles, and that they were the result of the natural turnover of cells in this type of tissue. Conversely, other adhering irregular particles, such as the one found on one of the unprovoked 2 hr films (Fig. 9), turned out to be both mineralized and sulphur-containing.

Very typic particles, like the one shown in Fig. 10, were occasionally found both on the 2 and the 4 hr films. As far as these particular particles are concerned, their size and anatomical form could indicate that they are parts of a tongue filament. Their exact origin is, however, unknown.

As to the particles judged to be of microbial origin, their overall frequency was, as has been indicated above, found to be limited and definitely lower than that of material judged to be biological debris.

More microorganisms were judged to be present on the unprovoked 2 hr films than on the 4 hr ones, which in their turn were found to inhabit markedly more microorganisms than the thick 2 hr sucrose rinsed films. On the other hand, good agreement was found between the amounts of adhering microorganisms on the pairs of simultaneously exposed prisms. In this context it should be noted that the number of our observations on the amount of adhering microorganisms was too small to validate any general conclusions.

Even if it is impossible to give the approximate sucrose concentrations in the vicinity of the prism surfaces, the results of the experiments indicate that, in spite of its film-thickening properties, sucrose can hardly have any significant promoting influence on the pri-

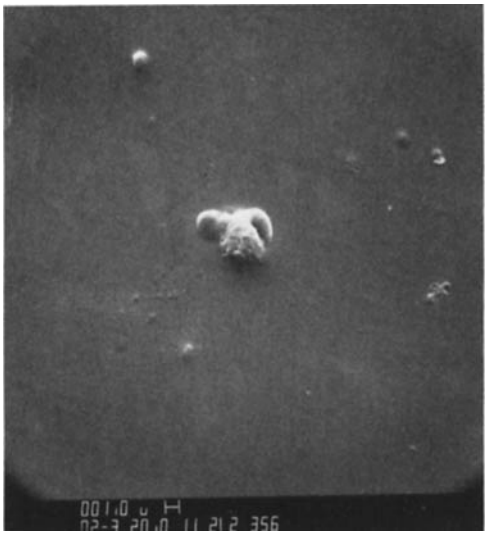

Fig. 9. SEM-photograph (original magnification 200 X) showing particle adhering to film covered Ge prism at the end of a 2 hr unprovoked intraoral exposure. Simultaneously performed dispersive x-ray analysis gave the following most prominent absorption bands: 1.186 keV (Ge Lα-band); 2.308 keV (S Kα-band); 2.838 keV (Pd Lα-band); 3.690 keV (Ca Kα-band); 4.012 keV (Ca Kß-band); 9.885 keV (Ge Kα-band); 11.100 (Ge Kß-band).

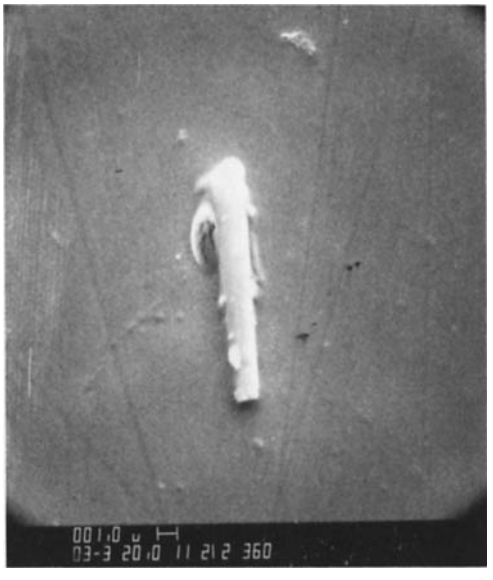

Fig. 10. SEM-photograph (original magnification 300 X) showing rounded bar-like particle adhering to film covered Ge prism at the end of a 2 hr unprovoked intraoral exposure.

mary *in vivo*–attachment of oral microorganisms to solid surfaces. This is in agreement with the results of *in vitro* experiments recently performed by Miyasaki & Newbrun (13). Further, the results of the experiment with bleeding periodontium indicate that blood can hardly have any strong promoting influences on microbial adhesion either.

The frequencies of the morphologically different types of micro-organisms were found to be different on the prisms exposed during the various time periods. Thus, on the two unprovoked 2 hr prisms rod-shaped microorganisms were dominating (Fig. 11). On the 4 hr films, however, several more sphere-shaped particles were observed (Fig. 12). These general observations agree with those reported by Baier & Glantz (4). As can further be seen on Fig. 12, around some of the coccoid particle groups a halo was observed. This halo possibly indicates that inside its periphery a biochemical transformation and addition of material has taken place as a consequence of microbiological metabolism. This theory is supported by the fact that in higher magnification the halo-surrounded group of particles were clearly seen to be covered by a film (Fig. 13). As on the very same prism particles of judged non-microbial origin were found not to have such coverage (Fig. 8), it was concluded that microorganisms in Figs. 12 and 13 had surrounded themselves and the area next to them with a film of sufficient cohesive characteristics to resist removal by rinsing with distilled water. Polysaccarides are a type of substances that could give such film characteristics. In areas thus covered, the adhesive properties could very well be altered, thereby perhaps facilitating or hindering local adherence of new microorganisms. It should also be noted that the film shown in Fig. 13 was dried out.

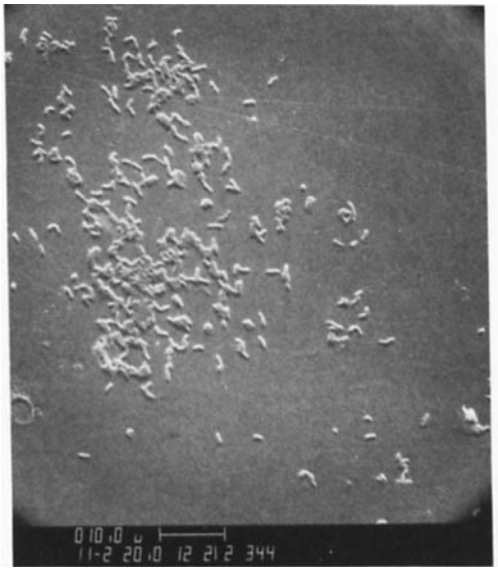

Fig. 11. SEM-photograph (original magnification 11 X) showing mostly rod shaped particles, judged to be microorganisms, adhering to film covered Ge prism at the end of a 2 hr unprovoked intraoral exposure.

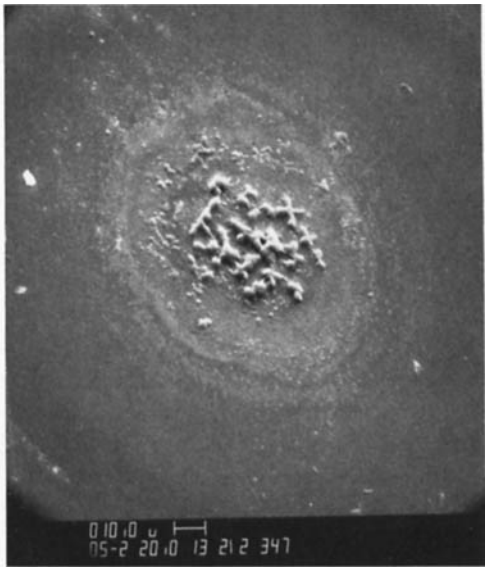

Fig. 12. SEM-photograph (original magnification 50 X) showing particles, judged to be microorganisms, adhering to film covered Ge prism at the end of a 4 hr unprovoked intraoral exposure. Note the halo surrounding the particles at an approximate distance of 20 μm.

In its original oral environment it is likely that the film material occupied a larger volume.

If the above described situation is present, and because of the fact that it is virtually impossible nondestructively to remove all adsorbed material from a solid surface under clinical conditions, the mechanisms responsible for the primary adhesion of microorganisms to intraoral solid should not be studied on surfaces previously colonized by microorganisms. Instead, like in this study, well defined surfaces, primarily characterized and shown to be free from any microbiological contamination, should be used.

As long as comparatively non-corrosive and definitely non-toxic (1, 15) solids like germanium are used, as has been demonstrated in this study, the adhesive properties of the adsorbed oral films are identical to those formed on enamel. Therefore, in our opinion film covered germanium prisms pro-

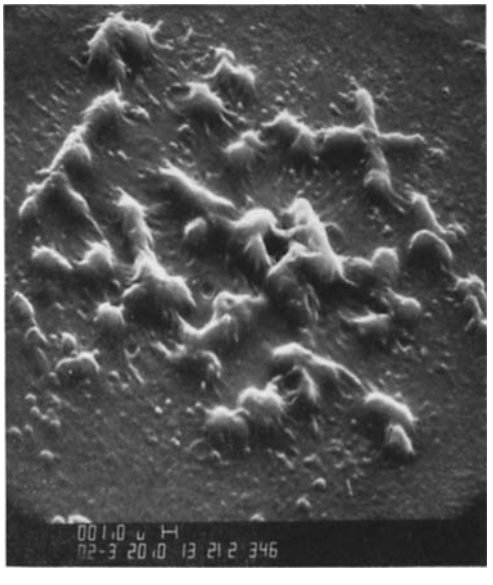

Fig. 13. SEM-photograph (original magnification 200 X) of same group of particles as in Fig. 12. Note the coating on the mostly sphere shaped particles, judged to be microorganisms. Note also that these particles were observed on the same prism as those shown in Fig. 8.

vide a relevant model system for the study of primary intraoral adhesive contacts between microorganisms and solid surfaces; a model system that further allows for the consecutive use of a variety of highly specific analytical techniques.

Summing up the results of this *in vivo*-study, it shows that in the oral cavity of a person in good oral health and in a moderately stagnated oral system the formation of biological films is obviously a process that is similar in different parts of the oral cavity and not markedly disturbed by the presence of blood. Sucrose, however, was found both to increase the overall film thickness and to increase the carbohydrate-content of the films.

Further, in this moderately stagnated system, bearing in mind the high number of microorganisms normally present in saliva, the attachment of such organisms was found to be a process proceeding at a comparatively slow rate especially on the sucrose-rinsed prisms. This fact, and the observation that the majority of the first adhering microorganisms were rod-shaped, support our previously presented theory that under clinical conditions the primary adhesion of oral microorganisms to primarily uninfected solid surfaces is a process of low chemical specificity (4, 7).

After the establishment of such adhesion, however, there seems to be possibilities for appearence of microbial growth variations that may sometimes alter the conditions for further interfacial events to take place in that particular area.

In our continuing studies of these problems special attention will be paid to systematic variations of the surface chemical characterization of the used solid surfaces and to variations in the shear rates of saliva and the rinsing solutions used.

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