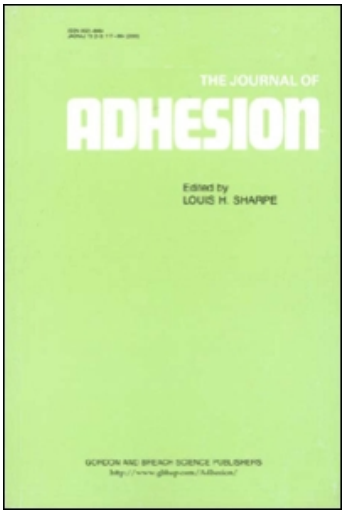

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Recent Studies on Nonspecific Aspects of Intraoral Adhesion

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Recent Studies on Nonspecific Aspects of Intraoral Adhesion

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There is usually a high number of microorganisms present in the oral cavity, yet it takes some time for the first microorganisms to attach *in vivo* on clean solid surfaces. Similar patterns of microbiological adhesion can be observed *in vitro* and, conversely, *in vivo* attachment of non-biological colloidal particles occurs readily. This indicates that primary physical forces rather than biological specificities are responsible for initial adhesion of microorganisms and similarly shaped particles to teeth and restorations. Under physiological conditions, oral adhesive events are probably based on general types of physico-chemical interactions, and might be mainly controlled by the properties of the spontaneously adsorbed biological films that precede particle attachment. A variety of surface physico-chemical techniques, monitoring formation of biological films and attachment of biological, as well as nonbiological particles of colloidal dimensions both *in vivo* and *in vitro* on various solid surfaces, indicated that the formation of oral biological films proceeds at high speed and precedes particle attachment. Further, when different surfaces were clinically exposed to saliva and covered with such biological films, their significant original differences in surface properties could no longer be detected. Continuing differences in strength of particle adhesion to these surfaces must result, therefore, from differences in binding of the initial salivary films.

KEY WORDS Adsorbed films; dental adhesion; high-energy surfaces; low-energy surfaces; attachment of microorganisms; influence of salivary films.

INTRODUCTION

Forces of cohesion, as well as of adhesion, play active roles in the oral cavity both in pathological processes and in the restoration of damage produced by such processes.

In restorative dentistry it is an accepted fact that direct contacts have to be established between vital oral tissues and prosthetic devices such as crowns, fillings and implants to secure the long-term successful function of these devices. One must also prevent the invasion and attachment of so-called plaque forming microorganisms which appear in gaps between restorative dental materials and remaining vital tissues like teeth, the oral mucosa or the alveolar bone. When accumulated in sufficient numbers, plaque organisms are able to induce pathological conditions such as dental caries, stomatitis or periodontal diseases.

Cells and microorganisms do not, however, adhere directly onto solid surfaces in the mouth, but are originally separated from these surfaces by distinct layers of organic material.[1] When dealing with dental plaque, this layer is commonly referred to as the pellicle.[2] The composition of dental pellicles has been studied extensively through the use of microscopic, biochemical and surface chemical methods.[3,4,5,6,7,8] The results from these studies indicate that the pellicle is mainly formed through selective deposition of protein-containing material of salivary origin. Many models offer explanations of the mechanisms involved in pellicle and plaque formation, including those reported by Bernardi & Kawasaki,[9] Gibbons & Spinell,[10] Kleinberg,[11] Sonju & Rolla,[7] and Leach.[12]

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.[4] The similarities are seen not only when pellicle formation is compared with film formation prior to general cellular attachment, but also with seemingly unrelated phenomena such as film formation on medical equipment contacting blood, or with the wide variety of marine fouling events.[1] That is, most of these interfacial films are dominated by glyco-proteins as the first spontaneously acquired "conditioning" layers.

REVIEW OF RECENT EXPERIMENTS

A. Spontaneous adsorption of "conditioning" biofilms

Recent *in vivo*-studies of oral films have provided detailed information through the use of numerous surface physico-chemical analytical techniques.[1,4] The primary materials used for the adsorption of these films were silica test pieces and germanium prisms which had been treated by a) detergent washing, b) glow discharge exposure, or c) coating with polydimethylsiloxane (to give surfaces essentially dominated by closely packed methyl groups).

Prior publications report the surface treatments and the resultant surface energetic parameters for the various types of test surfaces used.[4,13,14] The surface energies are reported in both the empirical category of critical surface tension, as deduced from the approach of Zisman,[15] and the thermodynamic categories of composite surface free energy, dispersion force, and polar force components, as calculated by the method of Nyilas, Morton, Cumming, Lederman, Chiu and Baier.[16]

For time periods varying between 2 seconds and 2 hours, silica or germanium prisms were worn by human subjects intraorally. The test pieces were placed either in specially constructed bite splints of varying design or in the vestibular sulcus. No food or drink was consumed during the experiments nor for about 2 hours before them. After some of the experiments, the prisms were rinsed with redistilled water to remove excess clinging saliva; after others, the samples were directly transferred to a desiccator, where the samples were kept for 24 hours at a temperature of 22–24°C, before analysis.

The molecular structure, thickness, critical surface tension, contact potential, and morphology of the adsorbed films were determined using the previously described analytical methods.[4] Finally, on some of the samples, energy dispersive x-ray analysis was performed to record the presence, if any, of associated inorganic elements.

All these studies show basically the same result, *i.e.*, that the formation of oral films proceeds at high speed and is of a certain qualitative selectivity. That is, the deposited layers are similar to one another but obviously different from the bulk salivary pool.

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 10–40 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; then, the retained films are relatively thicker on low-energy than on high-energy surfaces, at the same time as roughly 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 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 experiments of growing plaque on various types of solids under clinical conditions.[13]

A third observation made in these experiments is that, on test pieces which have been exposed in a stagnating system, particles such as microorganisms or biological debris adhere to the film-coated prisms much faster than when the prisms are exposed to shearing forces by the movement of oral mucosa or by saliva. In Figure 1, a holder is shown which allows stagnation of the saliva between the vertically positioned test prisms and the buccal surfaces of the first lower premolar teeth. When these films and prisms were cultivated using standard media and techniques, the identified microorganisms were those observed in early phases of plaque formation on teeth and artificial materials.[17,18]

As a further step in the ongoing surface chemical studies in the oral cavity, Glantz, Jendresen and Baier have developed a method to perform intraoral wetting studies and thereby develop an ability to calculate the clinical adhesiveness of tooth surfaces and restorative dental materials.[19] These studies showed that, *in vivo,* the critical

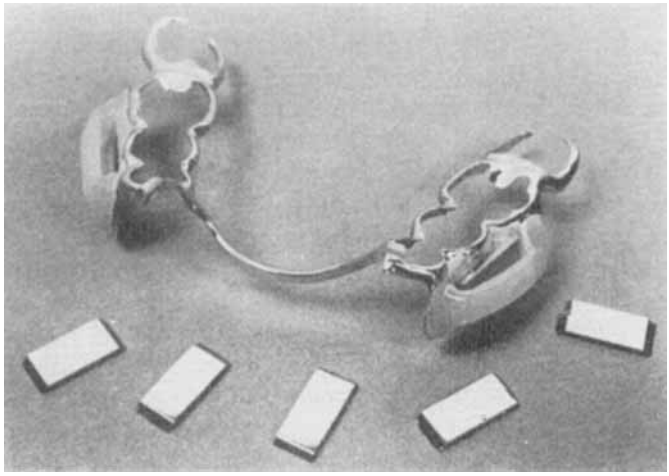

FIGURE 1 Ge-prisms and intraoral holder for such prisms used to collect salivary films in oral areas of moderate stagnation.

surface tensions for all tested surfaces and subjects were in the bio-adhesive range of 32–50 dynes/cm.[20] The formation of organic films on solid surfaces in the oral cavity thus brings these to a common state of bio-adhesiveness.[21] The formation of organic films was also observed to have a masking effect on minor surface irregularities.[22] Generally speaking, these results support the findings from the prism studies, *i.e.*, 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 small variations from one person to another.

B. Probable roles of the adsorbed biofilms

The oral cavity is exposed deliberately or accidently to a wide range of substances, some of which are or may be toxic, abrasive, and/or infected. Yet, expecially the teeth are expected to withstand repeated and sometimes prolonged attacks from such substances without showing mechanical or chemical failure. Tooth enamel and dentine are the two most highly differentiated hard tissues that exist in the human body. In many ways they act as the hard tissue counterparts to the brain and central nervous system, which are our

most highly differentiated soft tissues. Enamel has no, and dentine a very restricted, ability to regenerate after having obtained macroscopic defects. Therefore, it must be of special importance for anyone with teeth to try and prevent these precious tissues from being destroyed. The ability of saliva to form oral films, both quickly and completely, probably provides an important step in the processes of preserving good oral health and, consequently, to a certain extent also good general health.

The previously reported studies on various kinds of inorganic and organic surfaces, including clinical wetting studies, indicate that salivary films form with similar speed on any type of surface. It is therefore likely that any solid entering the oral cavity or being present there is provided with a coating of salivary origin. Examples of such solids are food stuffs, prosthetic appliances, dental instruments, as well as microorganisms. In a series of studies, Olsson, Glantz and Krasse[23,24] and Olson and Glantz[25] have used particle micro-electrophoresis to study oral streptococci. Among other things, they found that these cocci behaved much like any other colloid particle and had zeta potentials which (for their signs and magnitudes of charge) were dependent on the composition and properties of the surrounding media.

Under clinical conditions, in the oral cavity, it is therefore likely that microorganisms are also surrounded by the same or a similar type of biological film as teeth and the artificial materials studied. A so-called fuzzy coat has been reported to surround oral microorganisms when studied in transmission electron microscopes.[26,27] It is a fair suggestion that this coating, at least partially, is the mentioned biofilm.

If so, the first adhesive events that occur when a biological particle such as a microorganism comes close to a solid surface, like a tooth or a restoration , are mainly controlled, again, by the nature of the adsorbed or associated films on both the solid and the microorganism. Initially, there is probably only limited effect of the inherent surface chemical properties of either the naked solid or microorganism.

C. Comparison of *in vitro* and *in vivo* test systems

If oral microorganisms, teeth restorations and the oral mucosa are all covered with salivary films affecting adhesion, it must be

extremely difficult to create *in vitro* adhesive systems simulating intraoral conditions. Even if, for example, microorganisms and tooth materials in such a system are primarily covered with the true salivary films, it takes only a slight alteration in the composition, the pH, or the ionic strength of the liquid phase of an *in vitro* system to alter the adhesive properties of the film or even to desorb it, especially from surfaces like a tooth or any other biological surface for that matter. After desorption or alteration has taken place, adhesive events in the *in vitro* situation may well proceed along routes which have no or very little relevance to the *in vivo* situation.

In an *in vitro* system this has been demonstrated by Larsson and Glantz, who prepared lipid multilayers according to the well-known Langmuir–Blodgett technique.[28] In these experiments, a solid was moved up and down through a condensed monolayer of behenic acid on water, thus transferring the monolayer to the solid in such ways that the carboxyl groups formed the outer surface when the solid exposed its interface towards water, and the methyl end groups formed the surface towards air (Figure 2). When identical amounts of an oral strain of *Streptococcus sanguis* were added to these two types of surfaces, a drastic difference in adhesion of the microorganisms was observed. Methyl group surfaces showed no significant visual difference in bacterial adhesiveness compared to ordinary metal surfaces, whereas no visually noticeable adhesion at all was observed on the carboxyl group surfaces. It was observed that the obstruction of adhesion in the presence of lipid multilayers with carboxyl group surfaces might be due to electrostatic repulsion. The charge density was very high in these experiments, corresponding to up to one electron per unit area of about 23 $Å^2$. These observed effects were not limited only to the strain of *Streptococcus sanguis,* but could also be demonstrated when a gram-positive organism, *Escherichia coli,* was used under the described conditions.

When the effects of ions were tested in these experiments, already at 0.5% (w/w) of sodium chloride a few colonies could be observed on the carboxyl group surfaces. At a concentration of 2% sodium chloride, there were no longer any significant differences in initial attachment on methyl group surfaces as compared to carboxyl group surfaces. These observations are in agreement with expected

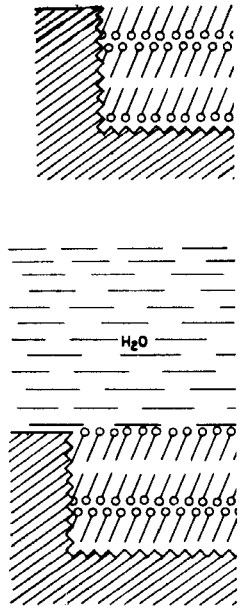

FIGURE 2 Oriented lipid multilayers exposing methyl groups towards air (top) and carboxyl groups towards water (bottom).

effects of salts on the ionic double layer according to the DLVO-theory of colloidal adhesion.[29]

D. Consideration of adhesive specificity for oral microorganisms

In saliva, the number of microorganisms present is generally measured to be about 10^8 per ml. If physical-chemical interactions take place primarily at collision between microorganisms and solid surfaces, the particle mobility is so high that only a few minutes should be needed to cover the surface with a microorganism monolayer in this actual system. Especially as human whole saliva is thixotropic, or shows rheodestruction,[30] moderate shearing of the system should rather promote such reactions. On surfaces exposed to shear in the oral cavity, however, it has been reported to take up to hours for significant numbers of microorganisms to become attached,[4,31] whilst the process seems to be much quicker at

stagnation. This finding indicates that the process of bacterial attachment to oral solid surfaces has a general chemically non-specific background mechanism.

If high concentrations of cocci-shaped and cocci-sized polymeric particles of nonbiological origin are brought close to a tooth surface without direct exposure to saliva, they adhere to it in very much the same fashion as has been described to happen for streptococci. Figure 3 demonstrates the results of a clinical experiment by Glantz, Granath and Holma where a deciduous lower front tooth was first brushed with a toothpaste containing large amounts of polymethylmethacrylate (PMMA) particles (Bofors Tandkram). These particles have a diameter of about 0.7 μm, which is similar to that of most streptococci. After brushing, the tooth was extracted, then rinsed with water and prepared for SEM-study. This study, among others, produced results typified in the given picture (Figure 3) which was taken from the incisal surface of the tooth.

The adsortion pattern for these particles, which by themselves do

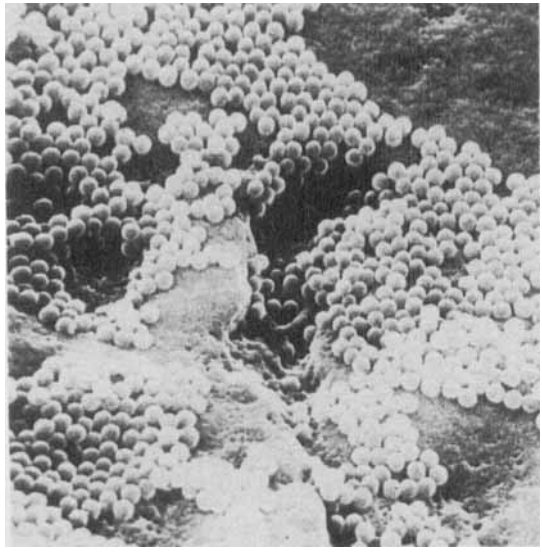

FIGURE 3 SEM-photograph showing incisal surface of deciduous anterior tooth which had been brushed with a toothpaste containing polymethylmethacrylate particles (diameter about 0.7 μm) and then rinsed in running tap water.

not possess any appreciable ability to form so-called specific biochemical bonds with the pellicle-covered tooth, has a striking similarity to that reported for many types of oral streptococci on solid surfaces. The almost monolayer-arrangement is obvious and so is the close packing of the particles. Even after a one-minute alcohol-washing in an ultrasonic bath, followed by a 10-minute treatment with a sodium hypochlorite solution, some of these particles still adhere to the surface (Figure 4), which indicates that they must have comparatively strong attachment to the pellicle-covered enamel.

Another important observation is that, on some clean solid surfaces placed in the oral cavity, the majority of the first adhering microorganisms retained after rinsing are rod-shaped (Figure 5). This might be explained on the basis of surface area considerations, provided that the work of adhesion per unit area of the microorganism/solid interfaces is comparatively low.

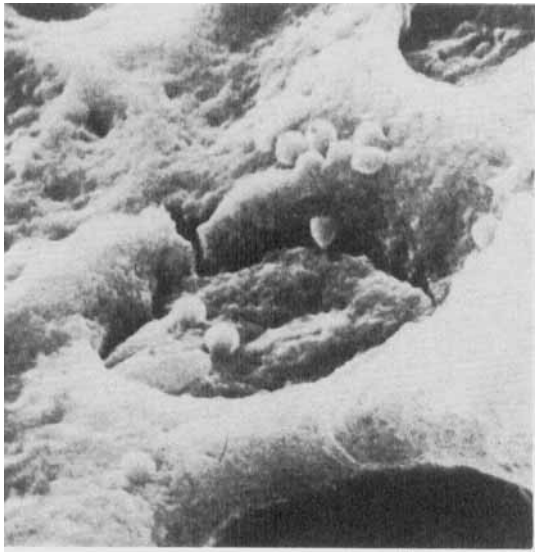

FIGURE 4 SEM-photograph showing polymeric particles from a toothpaste on the incisal surface of a deciduous anterior tooth. After brushing and rinsing in tap water the tooth was sonicated (90 kHz) in ethanol for 60 sec. and then treated with a sodium hypochlorite solution (10–12 percent active chlorine, pH 11–12) for about 10 min.

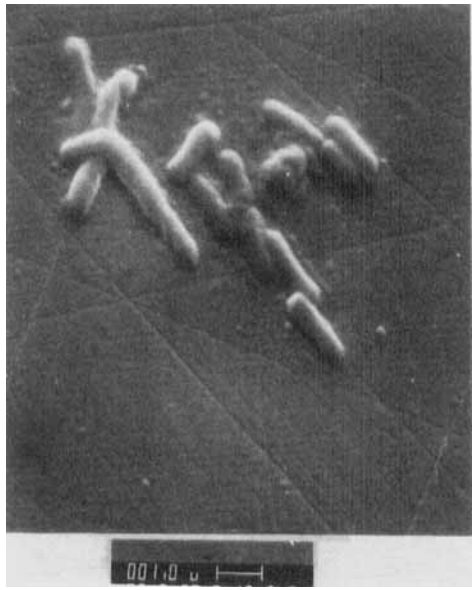

FIGURE 5 SEM-picture (7000 ×) showing adhering mostly rod-shaped microorganisms after two-hour intraoral exposure of originally detergent-washed Ge-prism.

Another reason why certain microorganisms are found more often than others on solid surfaces could be that—out of the random population that first adheres nonspecifically to a clean or protein-coated surface—they are the ones that have an ability to alter the environment in their own favour. For instance, metabolism and growth could form additional bonds over the interface (especially, resulting from extracellular exudates). Variations in the ability to metabolize on film-covered solid surfaces, and not in primary adhesion to such surfaces, could thus be the important etiological factor for common oral diseases. This theory is supported by the findings illustrated in Figures 6, 7, and 8. As can be seen around some of the coccoid particle groups shown in Figure 6, a halo can be observed. This halo suggests that, inside its periphery, a biochemical transformation and/or addition of material might have taken place as a consequence of microbiological metabolism. In higher magnification photos, the halo-surrounded groups of

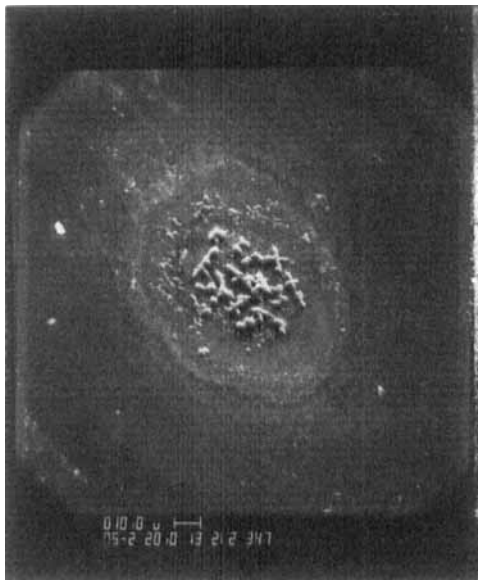

FIGURE 6 SEM-photograph (original magnification 500 ×) showing particles, judged to be microorganisms, adhering to film-covered Ge-prism at the end of a four-hour intraoral exposure. Note the halo surrounding the particles at an approximate distance of 20 μm.

particles were clearly seen to be covered by an extracellular layer (Figure 7). Since, on the very same test plates, other particles of debris of non-microbial origin were found not to have such coverage (Figure 8), it was concluded that this observation was not an experimental artifact, but that the microorganisms in Figure 6 and 7 had surrounded themselves and the area next to them with a film of sufficient cohesive characteristics to resist removal by rinsing with distilled water. Polysaccharides are typical 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 newly arriving microorganisms. It should also be noted that the film shown in Figure 7 was air-dried. In its original oral environment, it is likely that the film material occupied a considerably larger volume. We have regularly observed similar exudates around spontaneously attached marine microor-

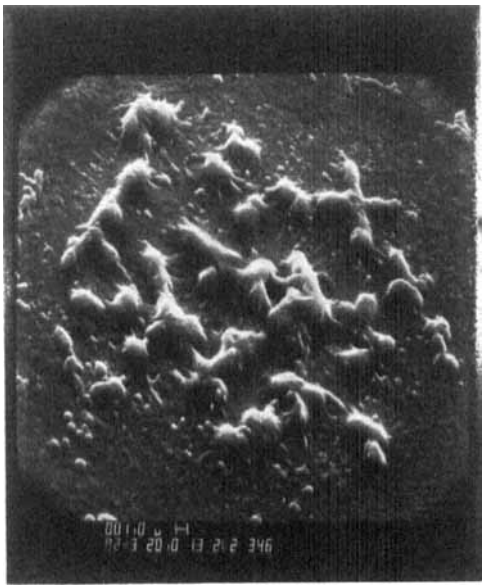

FIGURE 7 SEM-photograph (original magnification 2000×) of same group of particles as in Figure 6. Note the overlying coating on the mostly sphere-shaped particles, judged to be microorganisms. Note also that these particles were observed on the same prism as those shown in Figure 8.

ganisms, in other experiments, and are reasonably confident that the features illustrated in Figures 6, 7, and 8 are not artifacts of dried liquid droplets.

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 or retention of microorganisms on intraoral solid should not be studied on surfaces previously colonized by microorganisms. Furthermore, the surface conditions of dental implants should be given special attention to provide the necessary contacts between the implant material and the special biofilms required for attachment of fibroblasts or osteoblasts.

In this context, it is perhaps of interest to observe that the presence in saliva of high amounts of sucrose, a substance known to promote caries activity, was not found to increase the number of

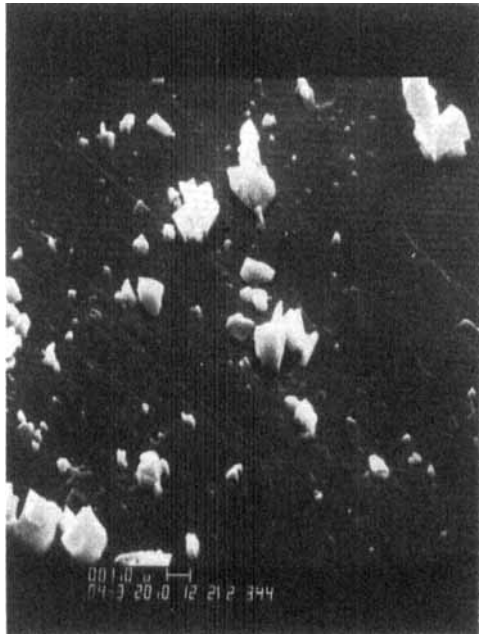

FIGURE 8 SEM-photograph (original magnification 4000×) showing irregularly shaped debris particles adhering to film-covered Ge-prism at the end of a four-hour intraoral exposure. Note also that these particles were observed on the same prism as illustrated in Figures 6 and 7.

adhering microorganisms on test prisms but, rather, to alter the carbohydrate content of the films adsorbed to these prisms.[14]

E. Mechanochemical features of intraoral films

Bearing in mind that teeth perform very special mechanical actions, *e.g.*, during mastication, they must have suitable mechanical properties. This concerns, for example, their moduli of elasticity and surface hardness. These properties are primarily the result of the mineral phase of teeth. It is obvious that adhesion-modifying oral films have to be good wear protectors too, and at least semipermeable to components capable of repairing the tooth mineral (for example, to calcium).

The well-known clinical fact that mildly decalcified enamel

surfaces get remineralized fairly quickly when left exposed to saliva supports this. Based on the observation that oral biological films, oral microorganisms and all living cells studied so far exhibit net negative surface potentials, this barrier function could be based on fairly simple types of electrostatic interactions and screenings. If so, penetration of biological films should also occur when cations other than calcium are studied. When we studied oral film formation on silica and germanium test pieces, energy dispersive x-ray analysis did not show appreciable amounts of calcium to be present in the films on any of these solids after they had been rinsed with distilled water. However, when excess saliva was not rinsed off the test plates, calcium was already found by the same analysis and so were crystals on the dried film surface during the simultaneously performed SEM-studies (Figure 9).

The observation that high amounts of calcium are often found in dental pellicles from teeth could then simply be due to the facts that the pellicle itself is permeable to calcium, and that inside of the pellicle there are high amounts of calcium and that outside it there is a definite calcium content, too. Consequently, intermediate amounts of calcium have to be present in the pellicle, without it

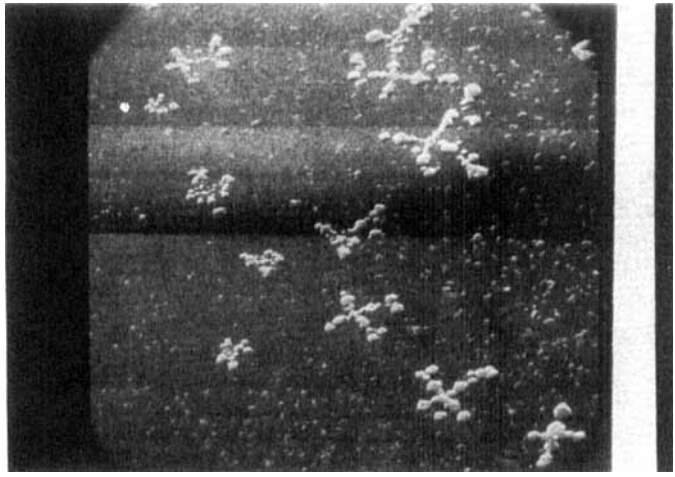

FIGURE 9 SEM-photograph of an originally detergent-washed Ge-prism maintained in the oral cavity for 15 minutes.

necessarily possessing any calcium binding capacity. With this comment we do not claim that calcium cannot participate in the buildup of oral films and other types of oral adhesive events. It certainly can do so whenever there is a possibility, for instance, for the formation of calcium ion bridges across negatively charged sites. In our opinion, however, calcium is neither essential for the formation of biological films nor for other oral adhesive events. Its most important contribution in saliva probably is to provide material for preservation of the tooth mineral and to maintain the exquisite mechanical properties of the precious enamel.

The mechanical, and to a certain extent also the chemical, stability for oral films is very high. As has been reported by Baier and Glantz,[4] biofilm-covered test pieces can be left idle in a laboratory for several weeks without showing any signs of decomposition or secondary contamination. Even the abusive action of transatlantic mail transportation passed unnoticed. The only normally appearing clinical actions so far found to alter the surface chemical characteristics of the films are drops in pH. In ongoing *in vivo* studies, it has even been noticed 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 previously discussed general protective actions of oral films, it is understandable that they are pH-sensitive; if not, they would have protective effects for masticated food stuffs even after passage to the stomach, and thus have delaying influences on digestive processes.

One can, of course, speculate that acid-producing oral microorganisms are able to induce caries—at least in part—because in the oral cavity they provide the conditions for film breakdown that is physiologically not supposed to take place until the films have reached the stomach.

FUTURE STUDIES NEEDED

What kinds of actions or "driving forces" are responsible for the spontaneous formation of the oral biological films? This question cannot be precisely answered at our present state of knowledge. Bearing in mind, however, that in saliva and other biological fluids there are organic macromolecules with hydrophilic, hydrophobic,

and ionically charged groups such as sulphates, phosphates, amines, and carbonyls, it is not impossible that—due to the comparatively high spreading pressures of such macromolecules—they cover the surfaces of all solids present by mechanisms explained thermodynamically on the basis of dehydration and favorable entropy shifts.[12] Much more work is required to elucidate this process.

Also, although we do think that generally nonspecific types of interactions are involved during primary biological adhesion to teeth and oral solid surfaces, we also believe in the later appearance of biologically specific adhesive events (such as lectin-receptor interactions or enzyme-substrate reactions) in the oral cavity. Certain variations in general physico-chemical interactions could thus cause the appearance of dramatic biological alterations,[29] for example, and here again considerably more effort is required to define these conditions.

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