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PHYSICO-CHEMICAL INTERACTIONS IN INITIAL MICROBIAL ADHESION AND RELEVANCE FOR BIOFILM FORMATION

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Abstract—This paper summarizes initial microbial adhesion events in dental plaque formation, including the physico-chemistry of the interaction between micro-organisms and solid substrata, detachment phenomena under the fluctuating shear of the oral cavity, co-adhesion between pairs of microbial strains, and biosurfactant release. A hypothesis is forwarded on how these initial events might influence the final microbial composition and structure of the plaque, although it is simultaneously emphasized that the necessary techniques for verification of the hypothesis have only recently become available, and supporting evidence is still to be collected.

Key words: Microbial adhesion, dental plaque, conditioning film, detachment, biofilm, co-adhesion, biosurfactants.

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All adhesive interactions among macroscopic bodies, colloidal particles—whether micro-organisms or inert synthetic particles—and molecular entities are mediated by physico-chemical interactions. There are only a few basic forces in nature able to exert these interactions (Van Oss, 1995). These include the ever-present Lifshitz-Van der Waals forces, electrostatic forces, hydrogen bonding, and Brownian motion forces. All other forces are direct or indirect corollaries of these basic forces. Consequently, specific recognition between microbial adhesins and, *e.g.*, salivary components in the acquired enamel pellicle also results from these basic forces, although there is a tendency in the literature to discuss microbial adhesion, in terms of specific interaction forces, as being a (non-existing) separate class of forces (Busscher and Weerkamp, 1987; Busscher *et al.*, 1992a). This tendency arises partly from the fact that the presence of specific adhesins on microbial cell surfaces is not always expressed, in physico-chemical cell-surface properties, as charge and hydrophobicity (Busscher *et al.*, 1991; Van Raamsdonk *et al.*, 1995a). Understanding of microbial adhesion in terms of physico-chemical interactions would obviously be easiest when the microbial cell surface would be chemically homogeneous and devoid of structural features. Naturally, the presence of specific adhesins on a significant part of the cell surface will be accompanied by a noticeable expression in microbial cell-surface hydrophobicity and charge (Van der Mei *et al.*, 1987), but the presence of only a few specific adhesins will not be expressed in overall physico-chemical cell properties. Simultaneously, however, this raises the question, unanswered until now, of how many microbial adhesins are actually involved in an effective bond to a substratum surface.

All interaction forces between molecules have their own characteristic decay with distance between the interacting molecules (Van Oss, 1995). This implies that, in a specific approach toward microbial adhesion, only the interaction forces arising from the adhesins are considered, but that in reality they always act in addition to the interaction forces arising from the entire body of the organism (see Fig. 1). Philosophically speaking, in an evolutionary sense, it can therefore be argued that micro-organisms, attempting to colonize a substratum still unknown to them, have to do so using non-specific interaction forces, without the aid of specific adhesins to be developed. Only when non-specific forces have enabled an organism to adhere to a substratum can the evolutionary process of exploring the chemical details of the substratum surface begin, ultimately leading to the development of adhesins specifically required to adhere

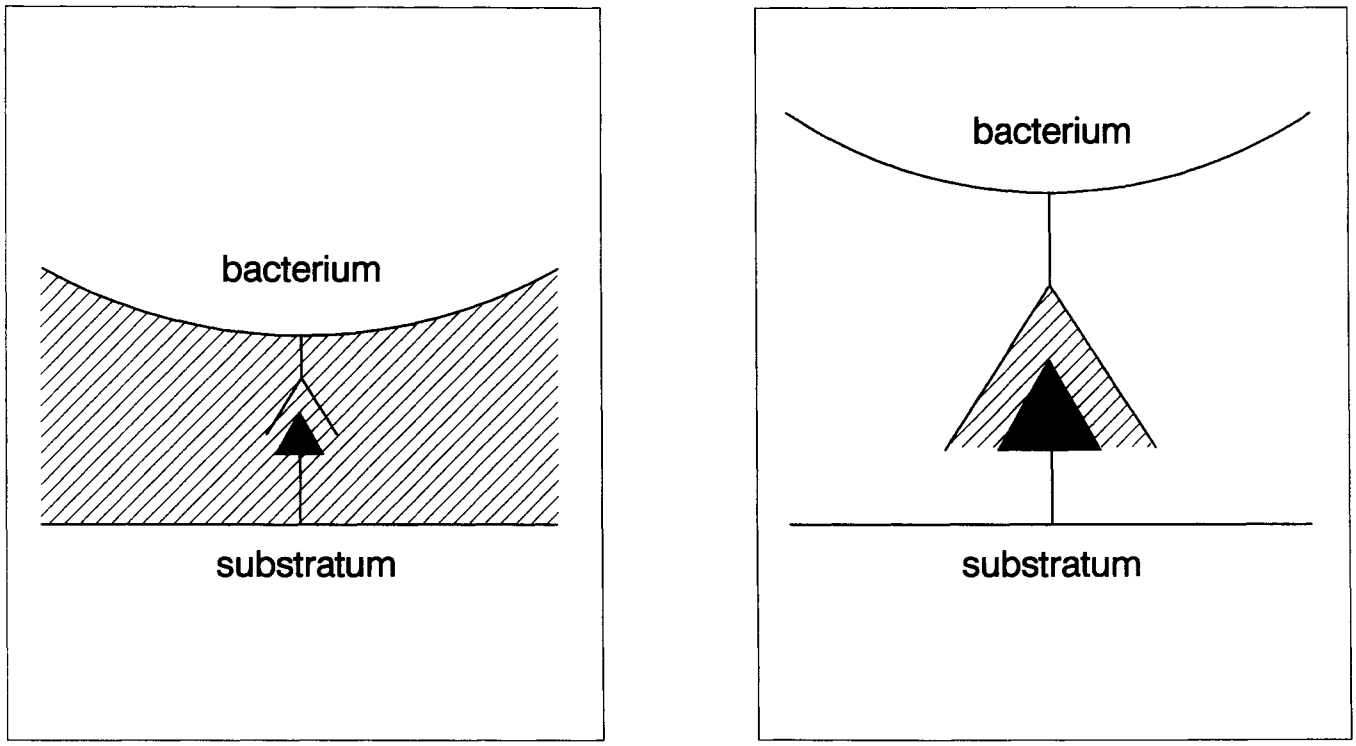

Fig. 1—(left) Van der Waals and electrostatic forces originate from the entire body of a micro-organism and for that reason may not be neglected as compared with the adhesive effects of specific adhesins. (right) A specific bond between stereochemical groups on the microbial cell and substratum surfaces consists of a combination of attractive, Van der Waals, and electrostatic forces and hydrogen bonding, originating from highly localized chemical groups, which together form a stereochemical combination. Adapted from Busscher et al., *1992a.*

more firmly to that material.

Oral streptococci constitute a group of micro-organisms for which adhesion to inert substrata could be almost flawlessly described in physico-chemical terms (Busscher *et al.*, 1984, 1986a,b; Uyen *et al.*, 1985; Van Pelt *et al.*, 1985; Pratt-Terpstra *et al.*, 1988) and whose cell surface hydrophobicity and charge could be explained on the basis of their overall chemical surface composition (Van der Mei *et al.*, 1987, 1988a,b, 1989, 1991a). Unfortunately, groups of micro-organisms also exist with sparsely distributed adhesins (Van der Mei *et al.*, 1991b, 1992; Cowan *et al.*, 1992a,b) for which physicochemistry seems not to work (of course, until the availability of microscopic characterization techniques). Also, most likely due to damage done to the cell surfaces during sample preparation (Marshall *et al.*, 1994), few advances in this field have been achieved for Gram-negative organisms such as *Escherichia coli* (Harkes *et al.*, 1992).

Initial microbial adhesion, as governed by physico-chemical interaction forces, is only a step in the development of a mature biofilm (Van Loosdrecht *et al.*, 1990). After adsorption of conditioning film components (Gristina, 1987) and adhesion of initial colonizers (Kolenbrander and London, 1992), many subsequent events—such as interspecies binding (co-aggregation [Kolenbrander and London, 1992] and co-adhesion [Ellen *et al.*, 1994; Bos *et al.*, 1994, 1996]), biosurfactant release (Rosenberg, 1986), the occurrence of metabolic advantages among organisms, and prevailing nutrient conditions (Fletcher *et al.*, 1991; Bradshaw *et al.*, 1994; Marsh *et al.*, 1995) or sloughing (Rittmann, 1989) during high shear periods—determine the ultimate microbial composition and structure of a mature biofilm.

The importance of initial events in biofilm development is often underestimated (Busscher *et al.*, 1995) due to the multitude of subsequent events taking place on a much longer time scale. Yet, the bond between a biofilm and a substratum is established solely through the link constituted by the conditioning film and the initially adhering micro-organisms.

The aim of the present review is to hypothesize on the importance of physico-chemical interactions in initial microbial adhesion and to present a new, generalized biofilm model, in which more emphasis is given to the importance of initial events for the ultimate microbial composition and structure of a biofilm.

HYDROPHOBICITY AND DENTAL PLAQUE FORMATION

In the early 1970s, Glantz (1971) reported less dental plaque on low-surface, free-energy, hydrophobic substrata inserted into the dentures of human volunteers and analyzed gravimetrically than on hydrophilic substrata. More recently, Quirynen *et al.* (1989) glued polymer strips onto the front incisors of human volunteers, while requesting them to refrain from all oral hygiene for nine days. Using a

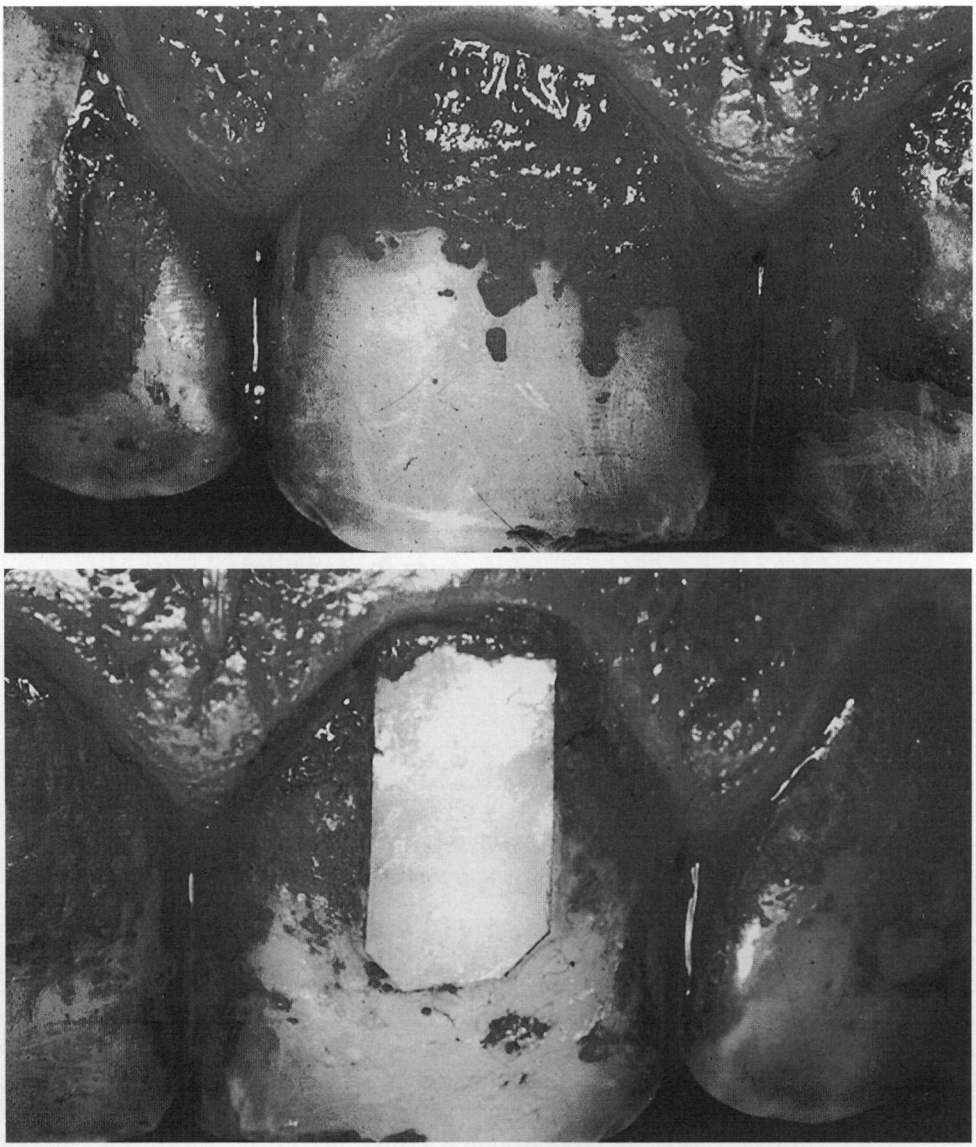

Fig. 2—Planimetrically stained plaque after 9 days of no oral hygiene on a polished tooth and on a low-surface-free-energy Teflon strip, glued onto a front incisor of a human volunteer. See Quirynen et al. *(1989) for details.*

planimetric plaque assay, they demonstrated that significantly less plaque had accumulated on the hydrophobic polymer strips than on the hydrophilic strips (Fig. 2). In addition, microbial analysis showed no noteworthy differences in plaque composition. These *in vivo* observa-tions are in accordance with inter-facial thermodynamics (Busscher *et al.*, 1984) stating that high-surface-free-energy strains, as are the ma-jority of strains found in the oral cavity (Van Pelt *et al.*, 1984), should adhere preferentially to hydrophilic substrata, while low-surface-free-energy strains, which are found only rarely in the oral cavity, would adhere better to hydrophobic substrata. For a given microbial strain, interfacial thermodynamics, for its *in vitro* adhesion to different bare substrata, could indeed be verified, but, due to variabilities among different strains not accounted for in the cell-surface free energy, adhesion of different strains to one given substratum could not be explained (Pratt-Terpstra *et al.*, 1988, 1989a) on the basis of inter-facial thermodynamics (see Fig. 3). Moreover, also *in vitro*, the influence of substratum surface properties was greatly diminished by the adsorption of a salivary conditioning film (Pratt-Terpstra *et al.*, 1989b; Pratt-Terpstra and Busscher, 1991; see also Fig. 3), to the extent that the *in vivo* observations are hard to reconcile with *in vitro* findings.

To reconcile the discrepancy between *in vivo* and *in vitro* work, investigators hypothesized (Busscher *et al.*, 1992a) that, due to the fluctuating shear forces operative in the oral cavity, micro-organisms adhere during low shear periods, while they may detach during high shear periods, presumably as a result of cohesive failure in the conditioning film. This hypothesis is supported by the observations of Quirynen *et al.* (1993) that subgingival plaque formation was significantly less influenced by substratum surface free energies than was supragingival plaque formation, obviously due to the absence of (fluctuating) shear forces in the subgingival environment.

MICROBIAL DETACHMENT, CONDITIONING FILMS, AND DENTAL PLAQUE FORMATION

Oral detachment forces acting on the adhering micro-organisms vary enormously during the day. Detachment forces can be virtually absent, as during sleeping when only viscous forces arising from the salivary flow are operative. Alternatively, during eating, speaking, and drinking, the detachment forces may well exceed the adhesive forces. A simple analysis—*e.g.*, of the interfacial forces acting upon an adhering micro-organism when a liquid-air interface is passed over it (see Fig. 4)—indicates that a liquid-air interface (Leenaars and O'Brien, 1989) yields a detachment force of around 10^{-7} N, which is greater than the most conservative estimates of the forces by which micro-organisms adhere to a substratum (Rutter and Vincent, 1988). To add more relevance to our *in vitro* flow chamber studies on oral microbial adhesion, we commenced to pass an air bubble through the flow chamber after the experiments (Busscher *et al.*, 1992b; Van Raamsdonk *et al.*, 1995b), to mimic the dynamic shear conditions of the oral cavity and

examined the detachment of the adhering organisms. Interestingly, different strains detached in different numbers from bare substrata, indicative for the individual adhesive capacity of each strain, but detachment percentages became independent of the strain involved when the organisms adhered to a salivary conditioning film on the substrata (Busscher *et al.*, 1992b). Also, in general, more detachment of micro-organisms adhering to conditioning films was observed than of micro-organisms adhering to bare substrata. From these observations, we concluded that detachment of micro-organisms adhering to salivary conditioning films occurs through cohesive failure in the conditioning film (Fig. 5). Presumably, since more detachment of micro-organisms adhering to conditioning films seemed to occur on hydrophobic than on hydrophilic substrata, conditioning films on hydrophobic substrata may have a cohesive strength lower than that of those on hydrophilic substrata.

Approaches to decrease the enamel surface-free energy by surfactant adsorption (De Jong *et al.*, 1984; Gaffar *et al.*, 1987; Busscher *et al.*, 1988) on caries-susceptible sites might thus, through this mechanism, be a valid approach to the reduction of plaque formation. Since such a mechanism is based not on microbial adhesion but rather on detachment stimulated by cohesive failure in the pellicle, there is no risk that other, possibly mutated, strains will colonize the oral surfaces.

CO-ADHESION AND DENTAL PLAQUE FORMATION

Co-aggregation between oral microbial pairs, *i.e.*, the interactions between two micro-organisms of different strains or species in suspension (Kolenbrander and London, 1992), has been extensively studied and can be considered as a mechanism by which micro-organisms are cleared from the oral cavity. Unfortunately, co-aggregating pairs usually also have the potential to co-adhere (co-adhesion being defined as the interaction between an adhering, sessile micro-organism and a planktonic organism of a different strain or species [Bos *et al.*, 1994]). The difference between co-aggregation and co-adhesion is diagrammatically illustrated in Fig. 6). Often, there is a metabolic advantage for microbial pairs to co-adhere (Bradshaw *et al.*, 1994). Streptococci co-adhering with actinomyces can be envisaged to create an anaerobic micro-environment needed for the optimal growth of the actinomyces (Marsh *et al.*, 1995). Obviously, when nature has intended microbial pairs to take advantage of each other, the organisms must be equipped with surface components that allow for their co-adhesion. Recently, Bos *et al.* (1996) demonstrated that the interaction forces responsible for the co-adhesion of streptococci with actinomyces are partly electrostatic and acid-base.

Co-adhesion between oral microbial pairs is generally considered as one of the many initial events contributing to the development of dental plaque, although several studies have doubted whether co-adhesion actually influences the microbial composition of dental plaque *in vivo* (Van der Hoeven *et al.*, 1985; Skopek *et al.*, 1993). Co-adhesion may also, however, contribute to dental plaque formation as a determinant of its final structure. This suggestion remains to be confirmed by, *e.g.*, confocal scanning laser microscopy.

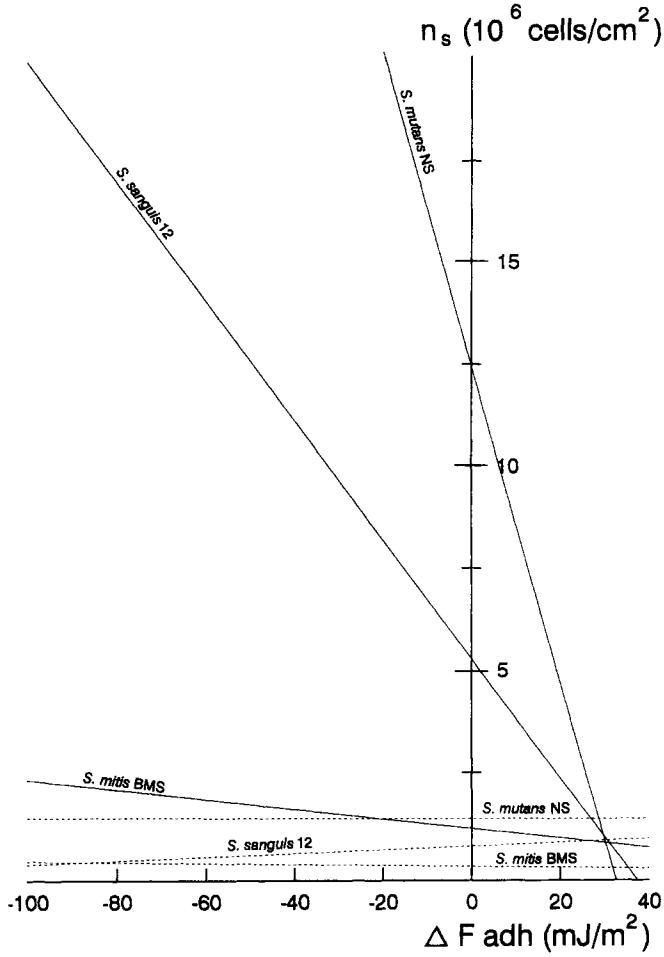

Fig. 3—Adhesion of various strains of oral streptococci to different substrata as a function of the interfacial free energy of adhesion, calculated on the basis of the microbial cell and bare substratum surface-free energies, for adhesion in the absence (drawn lines) and presence (dotted lines) of a salivary conditioning film. Adapted from Pratt-Terpstra et al., *1988.*

BIOSURFACTANTS AND DENTAL PLAQUE FORMATION

Biosurfactant-releasing microbial strains have been described in the literature (Rosenberg, 1986; Desai and Desai, 1993). Often, strains release biosurfactants to make nutrients available through emulsification. Sometimes, micro-organisms release biosurfactants as biological markers or to stimulate their own detachment when conditions have become unfavorable. Upon the first discoveries of biosurfactants, huge industrial advantages of biosurfactants over synthetic surfactants were foreseen. Unfortunately, mass production of microbial surfactants has hitherto been too costly for large-scale industrial applications. Nevertheless, the presence of extremely small amounts of biosurfactants in

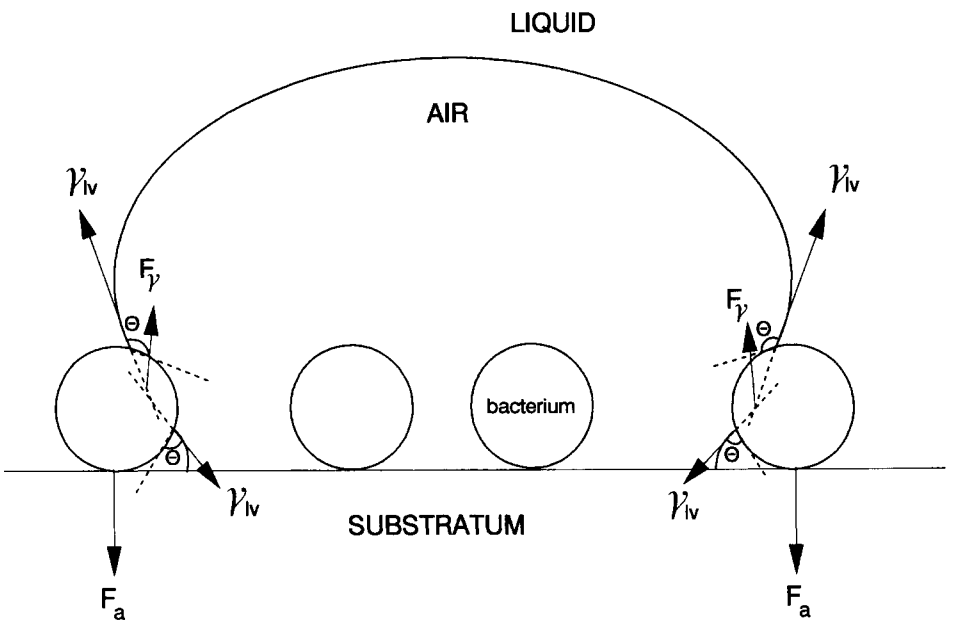

Fig. 4—Interfacial tension forces upon an adhering micro-organism during the passage of an air-liquid interface. Detachment occurs when the resultant of the perpendicular components of the interfacial tension forces, F_γ exceeds the adhesive force, F_a Adapted from Leenaars and O'Brien, 1989.

a bio-film may have tremendous effects on the composition and structure of the biofilm. For instance, 10^{-4} to 10^{-5} mg of a 60-kDa globular biosurfactant can be calculated to cover 1 cm^2 of substratum surface.

Biosurfactant release of oral micro-organisms has been described for *S. mitis* strains (Van der Vegt *et al.*, 1991). Both ad-hering *S. mitis* strains, as well as isolated biosurfactants released by *S. mitis* isolates, greatly dis-couraged adhesion of an *S. mutans* strain (Pratt-Terpstra *et al.*, 1989a), as schematically summa-rized in Fig. 7. Through biosurfactant release, micro-organisms can create an environment devoid of competing organisms, contrary to the metabolic advantages of co-adhesion processes.

SYNTHESIS AND FUTURE CHALLENGES

The influence of the microbial composition of plaque on its cariogenicity and potential to cause periodontal disease is well-known, and the most important causative organisms of these diseases have been identified. However, the structure of the plaque may be equally important with regard to its pathogenicity, especially when it is realized that the biofilm mode of growth protects the organisms against phagocytosis, antibiotics, detergents, and other antimicrobials, such as those used in oral health care products.

Fig. 8 presents an artist's rendering of how initial events in oral microbial adhesion may govern dental plaque formation. Co-adhesion and biosurfactant release govern the microbial composition and structure of the plaque. Substratum properties determine the cohesive strength of the conditioning film, constituting the link attaching the plaque to the enamel. Although it is our present belief that the initial events in microbial adhesion will, at least partly, determine the final

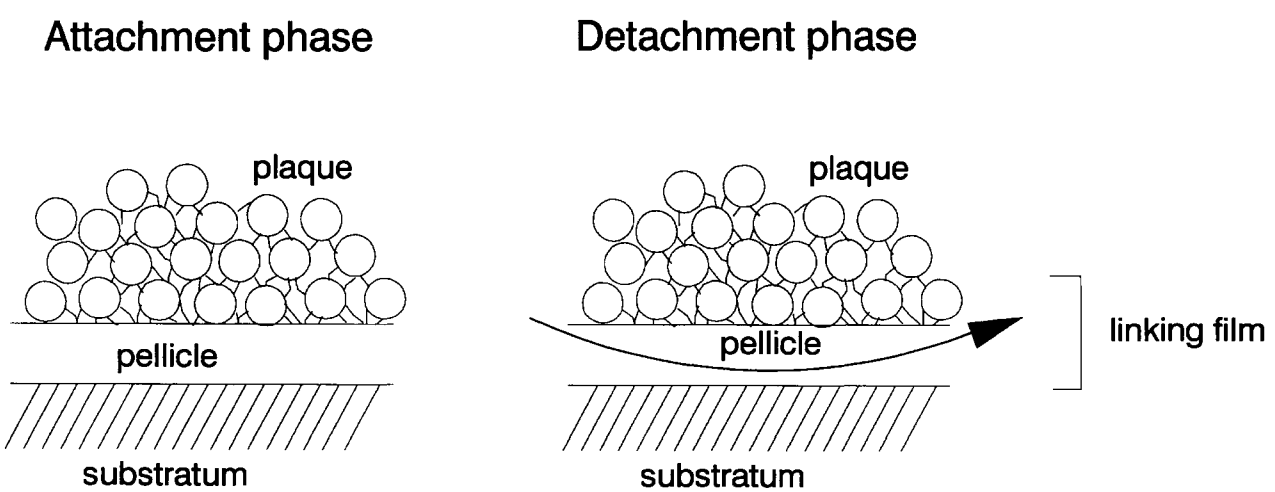

Fig. 5—The oral shear forces may vary over a factor of at least 50. Hence, under in vivo *conditions, so-called attachment and detachment phases must be distinguished. During the detachment phase, high shear forces—such as during eating, speaking, and drinking—may cause detachment of the entire plaque mass by cohesive failure in the linking film.*

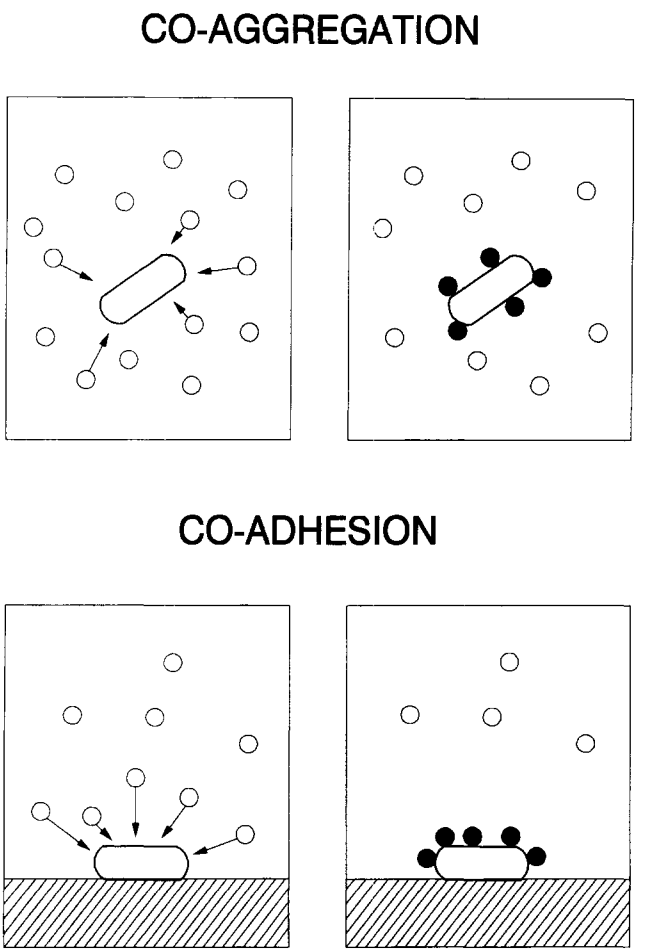

Fig. 6—Co-aggregation is the interaction between planktonic micro-organisms of a different strain or species, while co-adhesion is the interaction between a sessile, already adhering organism and planktonic micro-oganisms of a different strain or species.

composition and structure of a mature biofilm through mechanisms as depicted in Fig. 8, experimental evidence is lacking at present. Until several years ago, the proper *in situ* techniques required for verification of the above hypothesis were non-existent. However, now that we can measure initial microbial adhesion *in situ* under relevant shear conditions

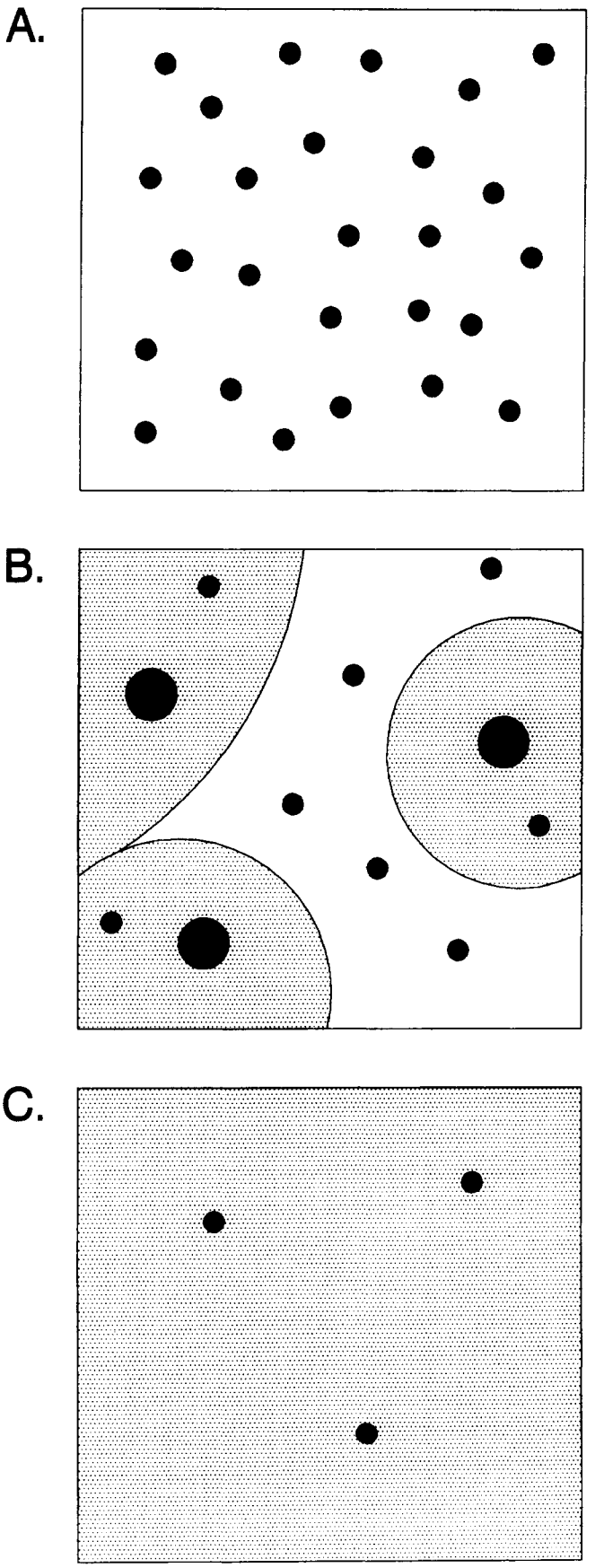

Fig. 7—Schematic presentation of the influence of S. mitis *biosurfactants upon the adhesion of an* S. mutans *strain in which the small dots represent* S. mutans *cells and the larger dots are* S. mitis *cells. The shaded area is substratum surface covered with adsorbed biosurfactants released by the* S. mitis *strains. (A) Stationary-state adhesion of* S. mutans *NS to glass (17.0 $10^6 cm^{-2}$). (B) Biosurfactant releasing* S. mitis *BMS (1.0 10^6 cm^{-2}) reduces the stationary-state adhesion of* S. mutans *NS to 1.5 10^6 cm^{-2}. (C) Stationary-state adhesion of* S. mutans *to glass (2.9 10^6 cm^{-2}), coated with biosurfactants released by* S. mitis *BMS.*

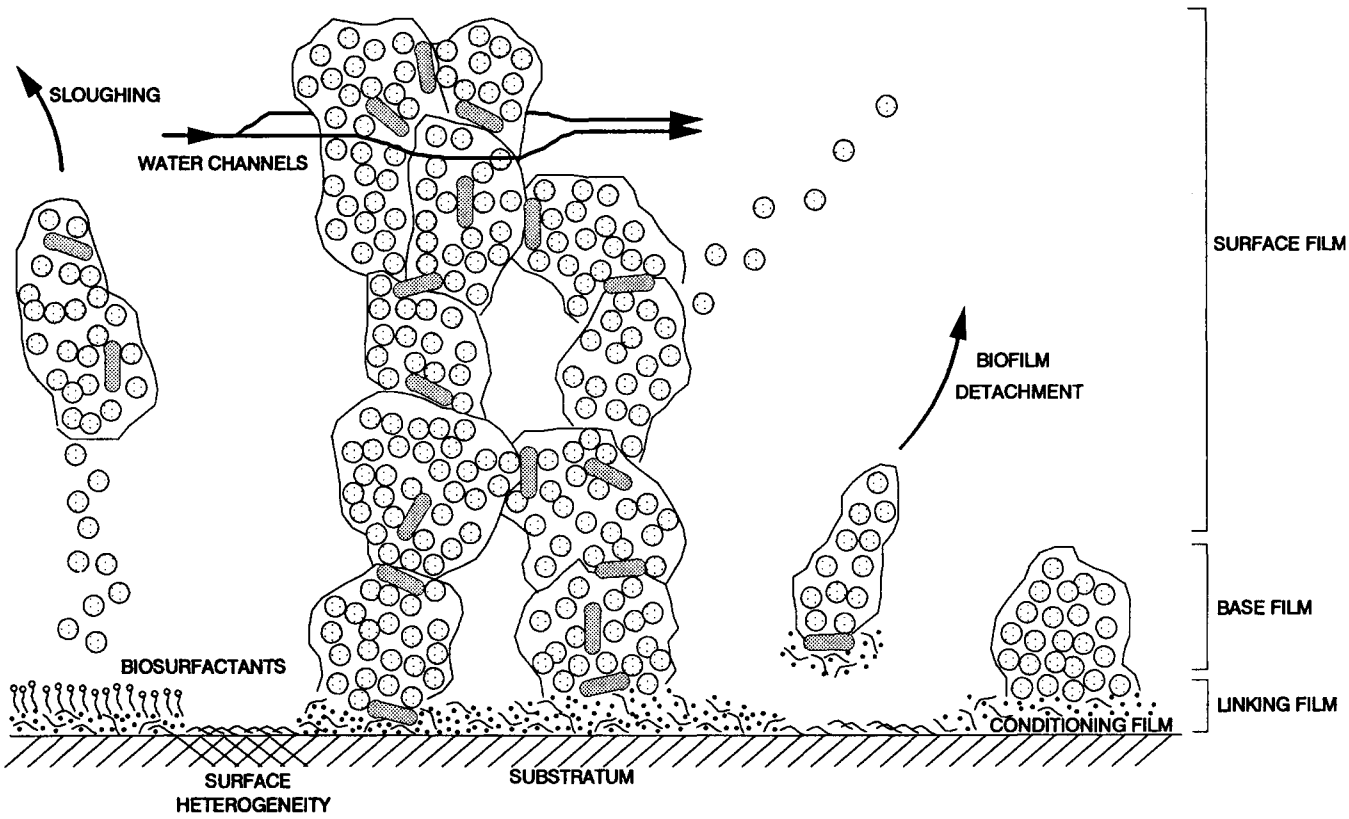

Fig. 8—Hypothetical presentation of how initial events in microbial adhesion may determine the ultimate composition and structure of a biofilm.

(Busscher and Van der Mei, 1995)—even in two strain experiments (Bos *et al.*, 1994) with image analysis—study *in situ* the composition and structure of biofilms through confocal scanning laser microscopy (Lawrence *et al.*, 1991, 1994), and determine *in situ* the penetration of antimicrobials (Suci *et al.*, 1994) by Fourier transform infrared spectroscopy (Nivens *et al.*, 1993), the techniques required to establish the relevance of initial microbial adhesion events for final biofilm, *i.e.*, dental plaque formation, have become available.

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