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The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man

A review of the literature

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Abstract. In the oral cavity, an open growth system, bacterial adhesion to the non-shedding surfaces is for most bacteria the only way to survive. This adhesion occurs in 4 phases: the transport of the bacterium to the surface, the initial adhesion with a reversible and irreversible stage, the attachment by specific interactions, and finally the colonization in order to form a biofilm. Different hard surfaces are available in the oral cavity (teeth, filling materials, dental implants, or prostheses), all with different surface characteristics. In a healthy situation, a dynamic equilibrium exists on these surfaces between the forces of retention and those of removal. However, an increased bacterial accumulation often results in a shift toward disease. 2 mechanisms favour the retention of dental plaque: adhesion and stagnation. The aim of this review is to examine the influence of the surface roughness and the surface free energy in the adhesion process. Both in vitro and in vivo studies underline the importance of both variables in supragingival plaque formation. Rough surfaces will promote plaque formation and maturation, and high-energy surfaces are known to collect more plaque, to bind the plaque more strongly and to select specific bacteria. Although both variables interact with each other, the influence of surface roughness overrules that of the surface free energy. For the subgingival environment, with more facilities for microorganisms to survive, the importance of surface characteristics dramatically decreases. However, the influence of surface roughness and surface-free energy on supragingival plaque justifies the demand for smooth surfaces with a low surfacefree energy in order to minimise plaque formation, thereby reducing the occurrence of caries and periodontitis.

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The oral cavity, as part of the oro-pharynx, should be considered as an open growth system with an entrance to the gastro-intestinal tract. This system is constantly contaminated by a complex diversity of microbial species. Most of these organisms, especially those which are responsible for caries and peri-

odontal infections, can only survive in the oral cavity when they can adhere to non-shedding surfaces.

In dental health, a dynamic equilibrium exists between the retention forces of micro-organisms and the removal forces including swallowing, frictional removal by diet, tongue and oral hygiene implements. An increase in bacterial accumulation is often associated with a shift towards periodontitis (Löe et al. 1965). The principal mechanisms considered as favouring the retention of organisms are selective adhesion and stagnation (Newman 1980). The latter may be associated with soft

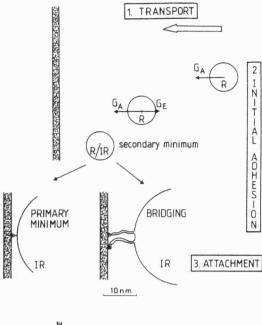




Fig. 1. Schematic representation of the sequencing steps in the colonization of intraoral hard surfaces by microorganisms: 1. Transport of bacterium to the surface, 2. Initial adhesion at secondary (often reversible: R) or primary minimum (irreversible: IR) depending on the resultant of the van der Waals attractive force $(G_{\rm A})$ and the electrostatic repulsive force $(G_{\rm E})$, 3. Attachment of bacterium to the surface by specific interactions, 4. Colonization of the surface and biofilm formation. The size of the bacterium is too small in relation the separation gap. Adapted from Van Loosdrecht et al. (1990), and from Busscher et al. (1990).

diet texture (Newman 1974), inadequate oral hygiene, reduced salivary flow, poorly contoured restorations, anatomical factors, etc.

This article aims to review the literature for the influence of the roughness and the free energy of intra-oral hard surfaces on the initial bacterial adhesion, in vitro but especially in vivo. If these surface characteristics are of clinical importance, a manipulation of these variables might facilitate the prevention of dental disease.

Up to now, no uniform theory has been developed to explain the fundamental mechanisms of cell adhesion. Moreover, it would be impossible and erroneous to conclude that one single mechanism dictates the adhesive tendency of microorganisms because the situation is too complex (Ho 1986). However, the following concept (Figs. 1, 2) probably most approaches reality (Rutter & Vincent 1984, Busscher & Weerkamp 1987, Van Loosdrecht et al.

1989, Krekeler et al. 1989, Van Loosdrecht et al. 1990, Van Loosdrecht & Zehnder 1990, Busscher et al. 1990).

Phase 1. Transport to the surface

The transport of bacteria towards the surface can occur by different modes: diffusion by Brownian motion (average displacement of 40 μ m/h), convective transport due to liquid flow (several orders of magnitude faster than diffusion), and active bacterial movement (chemotactic activity).

Phase 2. Initial adhesion

Initial adhesion is initiated by the fact that a bacterium and a surface interact with each other from a certain distance (50 nm) through long and short-range forces.

Long-range forces

Bacteria may be considered as living colloidal particles, and as such they obey

the laws of physical chemistry. One should however, keep in mind that bacteria are far from "ideal" colloidal particles because they have no sharp surface boundary, simple geometry, or uniform molecular surface composition and because internal chemical reactions can lead to changes in both interior and surface molecular composition (Uyen et al. 1988, Van Loosdrecht & Zehnder 1990).

If a colloidal particle approaches a surface, it interacts with that surface by means of 2 forces: Van der Waals forces (the first force to become active at distances even above 50 nm) and electrostatic forces (at closer approach).

Van der Waals forces (G_A)

3 types of van der Waals forces have been identified (Fig. 2): (i) when 2 atoms approach each other up to a certain separation gap, they will attract each other due to an instantaneous induction of dipoles (relative change in the position of the electrons in relation to the neutron: the London dispersion);

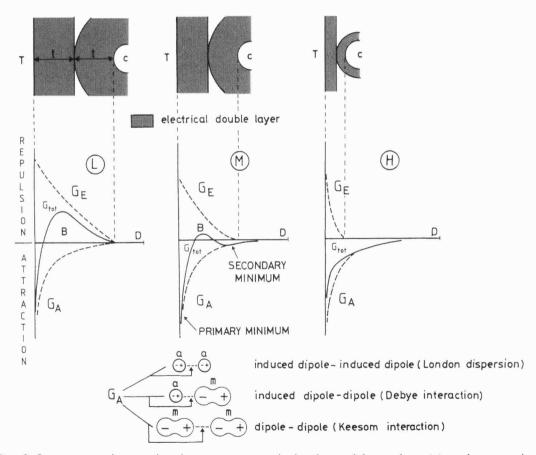


Fig. 2. Long-range interaction between a negatively charged bacterium (c) and a negatively charged surface (τ) according to the DLVO theory (Rutter & Vincent 1984). The Gibbs energy of interaction (G_{tot}) is calculated, in relation to the separation gap (D), as the summation of the van der Waals force (G_A) and the electrostatic interaction (G_E) . 3 different ionic strengths of the suspension medium are considered: low (L), medium (M) and high (H). Electrostatic interactions start when the electrical double layers overlap each other (see upper part of figure with T: solid surface (e.g. tooth), C: bacterial cell, t: thickness of the electrical double layer or Stern layer). The 3 types of van der Waals forces are explained in the lower part of the graphic (a: atom, m: molecule): (i) when two atoms approach each other up to a certain separation gap, they will attract each other due to an instantaneous induction of dipoles; (ii) when a molecule (which normally possesses a dipole) reacts with an atom, a dipole-induced dipole situation is created; (iii) when two molecules approach each other a dipole-dipole interaction appears.

(ii) when a molecule (which normally possesses a dipole) reacts with an atom, a dipole-induced dipole situation is created (Debye forces); (iii) when 2 molecules approach each other a dipole-dipole interaction appears (Kesson forces). The energy of such an interaction between two particles at a given distance is expressed by the Hamaker constant (Hamaker 1937).

Electrostatic forces (G_E)

Charged particles in water will be neutralized by a countercharged layer that is diffusely distributed around the particle (the electrical double layer or Stern layer, Fig. 2). When the double layer of a particle overlaps the double layer of the surface an electrostatic interaction will be created. If both surfaces have the same charge the electrostatic interaction will be of repulsive nature. However, if both structures have an opposite charge an attraction will occur. The energy of this electrostatic interaction is determined by the zeta potential (parameter of electrostatic charge) of the surfaces (Rutter & Vincent 1984). The distance at which this interaction appears, depends on the thickness of the double layers, which themselves depends on the ionic charge of the surface and the ionic concentration of the suspension medium. At high ionic strength, the double layers are small so that both surfaces have to approach each other much closer before an electrostatic interaction can occur (Fig. 2).

Derjaguin, Landau, Verwey, and Overbeek (DLVO) have postulated that, above a separation distance of 1 nm, the summation of the above mentioned 2 forces describes the total long-range interaction (Verwey & Overbeek 1948, Rutter & Vincent 1984). Fig. 2 shows the total interaction energy (also called the total Gibbs energy (G_{tot})), as the result of this summation $(G_{\text{tot}} = G_A + G_E)$, as a function of the separation distance (between a negatively charged particle and a negatively-charged surface), and for different ionic strengths of the suspension medium:

• At low ionic strength $G_{\rm tot}$ consists of a positive maximum (a barrier (B) to adhesion) and a steep minimum (called primary minimum, located at <2 nm away from the surface) where irreversible adhesion takes place. The positive maximum (B) decreases with increasing ionic strength of the medium, due to a reduction in the range within which the repulsive $G_{\rm E}$ forces are active.

- At a medium ionic strength of the suspension medium (e.g., saliva), the positive maximum decreases in size and a secondary minimum is created. The positive maximum is frequently low (the smaller the particle the lower the height of the energy barrier (B) so that a fraction of the particles may contain sufficient thermal energy to pass this barrier in order to reach the primary mini-(irreversible binding). secondary minimum (clearly less important than the primary minimum) exists at a certain distance (±10 nm) from the surface (thus with the interposition of suspension medium). This minimum is greater (deeper) for systems having larger van der Waals attraction and for larger particles (Van Loosdrecht et al. 1989). In the secondary minimum a particle can adhere reversibly (undeep minimum) or irreversibly (deep minimum).
- At high ionic strength, in which G_{tot} is constantly negative, all particles can reach the primary minimum.
- If both surfaces have an opposite charge, $G_{\rm E}$ will become rather attractive than repulsive so that the particle will approach the primary minimum without difficulties.

In nature bacteria and surfaces are predominantly negatively charged and microbes are considered to be large particles. Thus a long-range interaction with a secondary and a primary minimum is frequently encountered. For bacteria the secondary minimum, located at 5-20 nm from the surface (Van Loosdrecht & Zehnder 1990), does not frequently reach large negative values (thus no strong attraction) which implies a reversible adhesion (reversible adhesion defined as a deposition to a surface in which the bacterium continues to exhibit Brownian motion and can readily be removed from the surface by mild shear or the bacterium's own mobility). Such a reversible initial adhesion is characterised by a non-zero separation distance between bacteria and surface (Fletcher 1988). The distance of the separation depends on the ionic strength of the suspension medium as observed by means of interference reflection microscopy (Fletcher 1988).

Short-range interactions

If a particle can reach that primary minimum (<2 nm from the surface) a group of short range forces (e.g., hydrogen bonding, ion pair formation, steric interaction, bridging interaction) domi-

nates the adhesive interaction and determines the strength of adhesion. Therefore, the DLVO theory is only able to predict whether primary minimum adhesion can occur, but it cannot quantify the depth of this minimum.

When a bacterium and a surface make direct contact, provided the water film present between the interacting surfaces can be removed, the interaction energy can be calculated from the assumption that the interfaces between bacterium/liquid (bl) and solid/liquid (sl) are replaced by a solid/bacterium (sb) interface. The change in the interfacial excess Gibbs energy upon adhesion is described by the formula (Absolom et al. 1983, Bellon-Fontaine et al. 1990):

$$\Delta G_{\rm adh} = \gamma_{\rm sb} - \gamma_{\rm sl} - \gamma_{\rm bl}$$

in which the interfacial free energy of adhesion for bacteria ($\Delta G_{\rm adh}$) is correlated with the solid-bacterium interfacial free energy ($\gamma_{\rm sb}$), the solid-liquid interfacial free energy ($\gamma_{\rm sl}$), and the bacterium-liquid interfacial free energy ($\gamma_{\rm bl}$)). This formula assumes that the effect of electric charges as well as specific biochemical interactions may be neglected. If $\Delta G_{\rm adh}$ is negative (nature tends to minimize free energy), adhesion is thermodynamically favoured and will proceed spontaneously.

Bacteria initially adhering in the secondary minimum, may reach the primary minimum by passing the energy barrier (B), if it is not too high, but also by bridging this distance by protruding their fibrils, fimbriae etc. Because fimbriae have considerably smaller radii than the microbe itself, the electrostatic repulsion on these structures (which depends on their radius) will decrease, whereas the attractive van der Waals forces (which do not depend on the radius) remain constant so that for these structures the value of the energy barrier (B) decreases.

For both situations (direct contact or bridging) the water film between the interacting surfaces has to be removed. This dehydrating capacity of bacteria occurs by hydrophobic groups associated with bacteria or their surface appendages. Hypothetically, the removal of interfacial water may be the main mechanism by which "cell surface hydrophobicity" and "substratum surface hydrophobicity" influence bacterial adhesion (Busscher et al. 1986a, Busscher & Weerkamp 1987, Busscher et al. 1992a).

Sometimes, bacteria are forced to

stay at a certain distance from the surface, not because of an energy barrier but because of steric hindrance between the surface coating polymers. Sometimes the electrostatic forces are so important that the thermodynamic concept becomes overruled.

Phase 3. Attachment

After initial adhesion a firm anchorage between bacterium and surface can be established by specific interactions (covalent, ionic, or hydrogen bonding), by direct contact or by bridging true extracellular filamentous appendages (with a length of up to 10 nm). Such bonding is mediated by specific extracellular proteinaceous components of the organism (adhesins) and complementary receptors on the surface (e.g., pellicle mucins), and is species-specific (Gibbons & Van Houte 1971, Van Houte 1983, Gibbons 1980, 1984). The adhesins are often lectins which bind to saccharide receptors, but some adhesins are thought to bind proteinaceous receptors (Ellen 1985, Gibbons 1989). In this way the salivary acidic proline-rich proteins (PRPs), adsorbed onto the tooth surface, may play an important role. Indeed, PRP molecules evidently undergo a conformational change when they adsorb to the tooth surface so that new receptors become available. For example A. viscosus recognizes cryptic segments of the PRPs which are only available in adsorbed molecules (Gibbons & Hay 1988 a,b). This provides a microorganism with a mechanism for efficiently attaching to teeth and also offers a molecular explanation for their sharp tropisms for human teeth. It has been proven convenient to refer to such hidden receptors for bacterial adhesins "cryptitopes" (cryptic=hidden, topo=place). Also collagenous substrata, present on the root surface, seem to attract some bacteria (Naito & Gibbons 1988). In addition, there is evidence which suggests that elevated levels of neuraminidases and proteases associated with gingivitis may generate cryptitopes for Gram-negative organisms and destroy receptors for benign species (Loesche et al. 1987). Bacterial binding consists probably of several interactions which together outweigh shear forces (Gibbons 1984).

Phase 4. Colonization

When the firmly attached micro-organisms start growing and newly formed

cells remain attached, microcolonies or biofilms may develop. From now on, new concepts may be involved because now intra-bacterial connections may occur. Once a monolayer of micro-organisms has been established, a further growth of the plaque mass occurs preferably by the multiplication of already adhering micro-organisms (Brecx et al. 1983), besides the coadhesion between bacterial species. Special examples of inter-microbial co-adhesions are: the corn-cob formation in which, for example, streptococci adhere to filaments of Bacterionema matruchotii (Mouton et al. 1980) or Actinomyces species (Cisar 1982), and the test-tube brush composed of filamentous bacteria to which Gram negative rods adhere (Listgarten

In this concept of bacterial adhesion both, surface roughness and surface free energy of the solid substratum, play an important role. On a rough surface bacteria are more protected against shear forces so that a change from reversible to irreversible bonding occurs more easily and probably more frequently. The substratum surface free energy becomes important when the water film between the interacting surfaces has to be removed before short range forces can be involved.

The complexity of the oral cavity

The oral cavity harbours different surfaces for bacterial adhesion in health: the desquamating epithelium of the gingiva and alveolar mucosa, the dorsum of the tongue roughened by the presence of papillae, the tonsils, the enamel surface, and the gingival crevice. The importance of the latter has often been neglected. The gingival crevice and especially the gingival pocket (in case of periodontal destruction) offer additional niches for adhesion (Fig. 4) and clearly different growth conditions. A gingival pocket should be seen as a solitary swimming-pool, filled with crevicular fluid, where bacteria can survive by swimming, by adhesion to the root cementum (Nyvad & Fejerskov 1987) or its collagen appendages (Naito & Gibbons 1988), or by invading the dentine tubules (Adriaens et al. 1988) or the junctional epithelium (Liakoni et al. 1987) with its large intercellular spaces. These aspects forbid an extrapolation from studies of the supragingival plaque to the subgingival environment.

The intra-oral bacterial adhesion

process is challenged by several physicochemical reactions mediated through intermittent food intake, and by mechanical forces during speech and chewing (Newman 1974). In vivo, the rate of early supragingival plaque formation is time dependent. In general, planimetrically, it follows an exponential curve, with, however, a 50% reduction during the night (Quirvnen & Van Steenberghe 1989). Christersson et al. (1988) observed that bacterial attachment and retention were not influenced by temperature changes within the range of 22-37°C, but that both parameters were clearly dependent on shear forces. A 70 to 80% detachment of bacteria was observed when the shear forces were increased from 0.03 to 1.01 dynes/cm². This corresponds well with clinical observations on the rate of plaque formation (Simonsson et al. 1987). However, it remains difficult to mimic all these parameters in vitro.

Bacteria are far from "ideal" particles (Fletcher 1987). They have no sharp surface boundary, simple geometry, or uniform molecular surface composition. They have a number of different types of polymers (lipopolysaccharides, proteins, and polysaccharides for Gram-negative bacteria; peptidoglycan, secondary wall polymers, proteins, and polysaccharides on Gram-positive bacteria) which can act potentially as adhesives, or combinations of polymers or interaction sites may act in concert (Doyle et al. 1982). This is clearly illustrated by an in vitro study in which the adhesion of polystyrene particles was compared with that of Streptococcus mitis, (both structures with almost identical zeta potentials and surface free energies). The $\Delta G_{\rm adh}$ governed the relative number of adhering particles and bacteria, although microorganisms adhered more frequently (Uyen et al. 1988).

Moreover, salivary bacteria are often coated by a hydration layer and/or organic salivary components: immunoglobulins (IgA: Brandtzaeg et al. 1968), fibronectin (Ericson & Tynelius-Bratthall 1986), mucins (Levine et al. 1978, Gibbons & Qureshi 1978), high-molecular-weight glycoprotein adhesins (Ericson & Rundegren 1983), B 2-microglobulin (Ericson et al. 1979), lysozyme (Douglas & Russell 1984), and alfa-amylase (Douglas 1983, Scannapieco et al. 1989).

Another feature of bacteria which might confuse our understanding of ad-

hesion mechanisms is the fact that they are dynamic, living cells with adhesive properties which may change over time. Some recent studies on streptococcal adhesion have demonstrated how the results of experimental measurements of adhesion can be influenced by the time at which the measurement is taken (Busscher et al. 1986a, Cowan et al. 1986).

Influence of substratum surface free energy (sfe) on bacterial adhesion

The importance of the substratum sfe (γ_{sv}) can be depicted from the formula: $\Delta G_{\text{adh}} = \gamma_{\text{sb}} - \gamma_{\text{sl}} - \gamma_{\text{bl}}$. A theoretical calculation of the change in $\Delta G_{\rm adh}$ for the attachment of bacteria, in suspension, to substrata with different sfe is illustrated in Fig. 3 (Absolom et al. 1983, 1988). The input data required for the development of such a plot are the sfe of the three interacting species, i.e., the sfe of the bacterium γ_{bv} , the sfe of the substratum γ_{sy} and the surface tension of the suspending medium γ_{lv} . When γ_{lv} is greater than the surface free energy of the bacterium (γ_{bv}) then ΔG_{adh} becomes progressively less negative with increasing substratum surface free energy (γ_{sv})

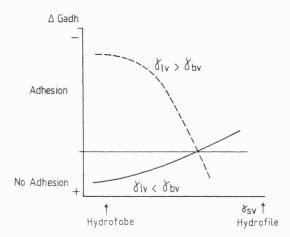


Fig. 3. Theoretical calculation of the free energy of adhesion (ΔG_{adh} , adhesion is favourable if $\Delta G_{adh} < 0$) of a single bacterium suspended in a medium with a surface tension lower than the surface free energy of the bacterium ($\gamma_{lv} < \gamma_{bv}$), or higher ($\gamma_{lv} > \gamma_{bv}$) as a function of the substratum surface free energy γ_{sv} . When $\gamma_{lv} > \gamma_{bv}$ (dotted line) then ΔG_{adh} becomes progressively less negative with increasing substratum surface free energy (γ_{sv}) predicting enhanced adhesion on the low energy (hydrophobic) substrata. On the other hand, when $\gamma_{lv} < \gamma_{bv}$ (continuous line) the opposite pattern of behaviour is predicted, i.e., enhanced adhesion on the high energy (hydrophilic) substrata. For the rare cases in which $\gamma_{lv} = \gamma_{bv}$, ΔG_{adh} becomes equal to zero independently of the value of γ_{sv} Adapted from Absolom et al. 1983, 1988.

predicting enhanced adhesion on the low energy (hydrophobic) substrata. On the other hand, when $\gamma_{lv} < \gamma_{bv}$ the opposite pattern of behaviour is predicted, i.e., enhanced adhesion on the high energy (hydrophilic) substrata. For the rare cases in which $\gamma_{lv} = \gamma_{bv}$, ΔG_{adh} becomes equal to zero independently of the value of γ_{sv} . It is however, important to realize that this model does not predict the number of bacteria that will adhere, but only predicts the relative extents (i.e., greater or lesser) of bacterial adhesion that are likely to be observed (Absolom et al. 1983, 1988).

From this mathematic equation 2 conclusions may be made:

(1) Since most oral bacteria have a high $\gamma_{\rm bv}$ (Van Pelt et al. 1984), and because the saliva has a relative low $\gamma_{\rm lv}$ (Glantz 1970) the situation $\gamma_{\rm lv} < \gamma_{\rm bv}$ will be frequently (for most bacteria) encountered so that one might conclude that

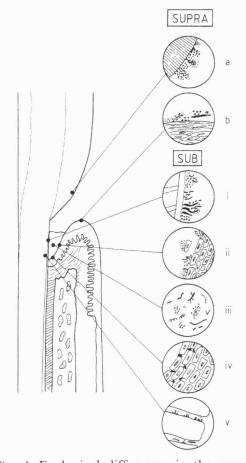


Fig. 4. Ecological differences in the supraand subgingival environment which are of importance when bacterial adhesion is considered. Supragingivally bacteria can adhere to the enamel surface (a) or, to a lower extent, to the desquamating oral epithelium (b). Subgingivally more niches are available for bacterial survival: (i) adhesion to the root cementum; (ii) adhesion to the desquamating pocket epithelium; (iii) swimming in the creviculair fluid; (iv) invasion in the soft tissue; (v) invasion into the hard tissue via the dentine tubules. Adapted from Quirynen et al. (1994a).

the higher the substratum sfe (Table 1) the easier bacterial adhesion occurs (Fig. 3).

(2) Moreover this formula would suggest that bacteria with a low γ_{bv} would preferentially adhere to substrata with a low sfe, whereas bacteria with a high γ_{bv} would prefer high sfe substrata.

The surface tension of a liquid can be directly measured by the so-called ringbalance or tensiometer, whereas the sfe of a solid substratum (γ_{sv}) or of a monolayer of bacteria (γ_{bv}) can only be experimentally (and indirectly) determined by different techniques from which the sessile drop technique (in which the contact angle formed by a series of liquids deposited on the surface are measured) is the most frequently used (Absolom 1988). Several methods of calculation exist to transform these contact angle data to γ_{sv} . Because there is so far no scientific agreement as to which formula is to be preferred, this paper will only use the contact angles (Table 1).

In vitro test to bare solids

Absolom et al. (1983) found that when suspensions of bacteria (108 Staphylococcus aureus, Stephylococcus epidermis, Escherichia coli, and Listeria monocytogenes) were brought into contact with several polymeric surfaces with different γ_{sv} for 30 min, the number of adhering bacteria per unit surface area correlated well with the above mentioned thermodynamic prediction. Minagi et al. (1985) analysed the adhesion of Candida albicans (high γ_{bv}) and Candida tropicalis (low γ_{bv}) to denture base resin plates with different sfe and concluded that their results fitted well with the thermodynamic model of adhesion.

Adhesion experiments (for 1 h in a flow cell system) to inert substrata and ground and polished enamel have shown that low surface free energy strains (e.g., Streptococcus mitis) adhered in higher numbers (Uyen et al. 1985, Sjollema et al. 1988) to hydrophobic substrata than to hydrophilic substrata, while the opposite was true for high surface free energy strains (e.g., S. mutans). Moreover, it was observed that bacteria adhered more reversibly if the ΔG_{adh} was positive (Busscher et al. 1986b), which indicates that the substratum surface free energy is also related to the binding force of bacteria (Van Pelt et al. 1985). Additional studies indicated, however, that the re-

Table 1. Contact angle values for sessile drop technique (S.D.R.: with water and α -bromonaphtalene) or captive bubble method (C.B.M.: in water) of bare and coated substrata. The larger the contact angle θ , the lower the surface free energy

		Contact angle										
			Bare surfa	ce	Coated surface							
		S	.D.R.	C.B.M.	S	C.B.M.						
Substratum		water α-br napht		water	water	α-br napht	water					
PTFE	(1) (2) (3) (4) (5)	110 117 107	65 70 72	95	91 [§] (80)	58 (43)	33					
composite resin (Silar®, Clearfil F3® Microrest®)	(6)	69–64										
amalgam alloy (Valiau®, Flouralloy®)	(7)	73–69										
dentine	(1) (2) (7) (4)	58 57 50	19 17 22		43* 54 [§] (64)*	25 36 (34)						
enamel	(1) (2) (7) (8) (4)	57 50 53 48	15 16 12 26		29* 69 [†] 65 [†] (59)•	21 22 35 (35)						
titanium	(5)			20			20					
gold	(2) (5)	37	spread	18			19					
glass	(1) (4) (5)	24	28	17	65 [†] (53) °	42 (21)	16					
stainless steel	(2)	spread	spread									
enamel+fluoridation untreated NaF 10 min APF 10 min AmF 10 min	(9) (9) (9) (9)	48 55 44 83	26 28 22 4		35□ 51□ 53□ 40□	27 26 26 23						
treated dentine untreated NaF 10 min APF 10 min AmF 10 min	(9) (9) (9) (9)	60 71 58 59	23 24 20 26									

Authors: (1) Van Dijk et al. 1987; (2) Glantz (1969); (3) Quirynen et al. 1989; (4) Van Dijk et al. 1988; (5) Jansen 1984; (6) Satou et al. 1988; (7) Weerkamp et al. 1988; (8) Van Pelt et al. 1983; (9) De Jong 1984.

lationship between the substratum sfe and the number of adhering bacteria, as indicated above, remained but became less distinct with time (Busscher et al. 1986a). Moreover, the relationship between $\Delta G_{\rm adh}$ and the number of adhering cells seemed to be strain dependent, with for some species of the strain even the possibility of adhesion in a situation with a positive $\Delta G_{\rm adh}$ (Pratt-Terpstra et

al. 1988). All these exceptions were explained as accommodations of the bacteria to the surface, enabling a more irreversible bonding through conformational changes in fibrillar surface structures or by the extrusion of an intercellular glue, or both (Busscher et al. 1986a). It is thought that these species might possess surface appendages which are shown to affect both various

physicochemical surface properties and specific molecular interactions (Van der Mei et al. 1987, Weerkamp et al. 1986).

For some strains of oral microorganisms the electrostatic potential of the substratum surface seems, however, to be more important than its surface free energy, indicating that for these organisms electrostatic interactions are not negligible, a factor which is essential for the thermodynamic approach (Satou et al. 1988, Bellon-Fontaine et al. 1990).

In vitro test-solids coated with a protein film

In the oral environment, natural as well as artificial surfaces will instantly become conditioned by a protein-rich film, the acquired pellicle (Meckel 1965). The main contributors to the composition of the acquired enamel pellicle have been proposed to be salivary proteins (Sönju & Rölla 1973, Al-Hashimi & Levine 1989), especially acidic proline-rich proteins (PRPs, Bennick et al. 1983). The amount of pellicle increases during the first 90 min and then levels off to a thickness in the range of 0.1 to 0.7 µm (Sönju & Rölla 1973). In comparison to a "2 hours pellicle", the chemical composition of a "24 hours pellicle" changes after the intake of a normal diet but not in the case of fasting, indicating a dietary contribution to pellicle formation or a bacterial degradation of the pellicle (Rykke & Sönju 1991).

This protein coating has a dramatic effect on the final substratum surface free energy (Table 1). The γ_{sv} for low surface free energy substrata increases, whereas, the γ_{sv} for high surface free energy substrata decreases (Jansen 1984, Van Dijk et al. 1987, Schakenraad et al. 1989). For a tooth surface, a pellicle coating will result in a lowering of the sfe (Van Pelt et al. 1983, Van Dijk et al. 1987). Thus, due to the coating, the substrata free energies slowly converge (Van Dijk et al. 1988).

If the relationship between bacterial adhesion and the substratum surface free energy (based on the $\Delta G_{\rm adh}$) is reconsidered for coated surfaces the following observations may be made.

• A pellicle coating results in a general reduction in number of adhering bacteria, irrespective of the substratum surface free energy (Rölla et al. 1977, Pratt-Terpstra et al. 1989, 1991, Weerkamp et al. 1988, Christersson & Glantz 1992).

[§] in vivo coating with pellicle; * 3 h in vitro incubation; • observation after 2 h and 48 h in vivo incubation;

application of fluoride solution after pellicle formation (1 h underneath tongue); NaF (Sodium fluoride); APF (acidulated phosphate fluoride); AmF (aminefluoride).

- The thermodyamic approach remains of value, but its importance decreases (Pratt-Terpstra et al. 1989, 1991).
- · Only small differences in the adhesion process exist in relation to an "early (5 min)" or a "ripened (2 h)" pellicle (Pratt-Terpstra et al. 1989), which implies that even an "early" pellicle reduces bacterial adhesion to the tooth surface. These differences are strain dependent and are probably caused by changes in the composition of the pellicle during maturation. For example the increased adhesion of S. mutans to a "ripened" pellicle might be due to an increase in the fraction of adhesion-promoting mucins during maturation (Gibbons et al. 1986). Conversely, decrease in S. sanguis adhering during pellicle ripening might be due to a decrease in mucins of lower molecular weight (Loomis et al. 1987).
- If experiments were performed in a flow cell system with controlled shear forces (Christersson et al. 1987), it was observed that the number of retaining cells depended on the initial substratum surface free energy, with most retention on surfaces with a so-called critical surface tension (the surface tension of a saliva coated tooth, see Table 1) but lower bacterial retention on surfaces with a lower or extremely high sfe (Christersson et al. 1989, Christersson & Glantz 1992). Moreover, detachment of bacteria was found to be caused by cohesive failures in the pellicle which naturally is substratum surface dependent (Busscher et al. 1992b, 1992c).
- On coated surfaces a new mechanism called "positive cooperativity" was observed, meaning that the adhesion of one or a few cells enhanced the probability of adhesion of other cells, by initiating additional sites for adhesion (Doyle 1991). It is however difficult to distinguish between this phenomenon and simple bacterial growth because both offer the same microscopical image (Caldwell 1987).

These observations confirm the statement that the physical and chemical nature of solid substrata significantly affects the relevant physico-chemical surface properties, the composition, packing, density, and/or the configuration of the pellicle coating (Lee et al. 1974, Baier & Glantz 1978, Ruan et al. 1986, Fine et al. 1984, Rykke et al. 1991). Absolom et al. (1987) even observed a clear relation between the type of pro-

teins adsorbed and the substratum sfe. This indicates that substrata properties, at least partly, are transferred from the substratum-protein interface to the protein-cell interface (Pratt-Terpstra et al. 1989, 1991) and consequently influence also initial bacterial adhesion. How this occurs is not yet well understood. Also in the extra-oral environment the importance of this thermodynamic concept (and thus of the influence of the substratum surface free energy) is confirmed (for binding strength and facility of adhesion) in in vitro experiments observing: the adhesion of uropathogens to polymer materials (Hawthorn & Reid 1990); the colonization of vascular prostheses or prosthetic materials for abdominal wall reconstructions (Schmitt et al. 1986, Brown et al. 1985); the adhesion of catheter-associated bacteria (Harkes et al. 1992); the attachment of freshwater bacteria to solid surfaces (Dexter et al. 1975, Pringle & Fletcher 1983, Fletcher & Pringle 1985); the adhesion of mussels and barnacles to solid substrata (Crisp et al. 1985); the binding strength of green alga to several surfaces (Fletcher & Baier 1984); the attachment of insect residues to aircraft wings (Siochi et al. 1987); the adhesion of Salmonella typhimurium to soil particles (Stenstrom 1989).

In vivo experiments

Glantz (1969) was the first to recognize and to verify in vivo a positive correlation between substratum sfe and the retention capacity of supragingival plaque. In an experiment, in which undisturbed plaque formation (weight measurement at days 1, 3, and 7) was followed on test pieces with different surface free energies, mounted on a partial fixed bridge, a positive correlation was found between substratum surface free energy and the weight of the accumulated plaque, at least when low (poly-tetra-fluoro-ethylene or teflon) and medium sfe substrata (equal to the sfe of enamel and dentine) were considered (Glantz 1969). However, between substrata with high (gold) and extremely high (stainless steel) sfe no significant differences could be detected (Glantz 1969).

Counting the number of adhering micro-organisms on solid surfaces with different sfe after 2 hours incubation in the oral cavity of beagle dogs (Van Dijk et al. 1987), low sfe surfaces (like teflon and parafilm) were found to collect

slightly fewer microorganisms than medium or high sfe (dentine, enamel, glass). In man also a positive correlation was observed between initial substratum sfe and amount of plaque accumulated over 9 days (Quirynen et al. 1989, 1990). Hydrophobic surfaces (teflon) harboured $10 \times$ less plaque than hydrophilic ones (enamel). Moreover, it was observed that the low sfe substrata possessed a lower plaque retention capacity because plaque mass frequently decreased between days 6 and 9 (Quirynen et al. 1989). Plaque samples collected at day 3 indicated that low sfe substrata were preferably colonized by low surface free energy bacteria, whereas the opposite was observed for surfaces with medium sfe (Weerkamp et al. 1989). Moreover, strains of S. sanguis I isolated from a low energy surface (teflon) were significantly more hydrophobic than those isolated from higher energy surfaces (Weerkamp et al. 1989). Treatment of enamel surfaces with a silicone oil, which lowered the surface free energy, also resulted in vivo in a significant reduction in plaque formation (Rölla et al. 1991). In a recent clinical trial, which compared 3 months old plaque from pure titanium or teflon coated abutments in patients with habitual oral hygiene (Quirynen et al. 1994b), low energy surface harboured a significant less mature plaque characterized by a higher concentration in coccoid cells and a lower concentration in motile organisms and spirochetes. It should be mentioned, however, that subgingivally the differences between both abutment types were clearly reduced.

This in vivo overview indicates that a lowering of the free energy of intra-oral hard surfaces results, supragingivally and to a lower extent subgingivally, in a retardation of plaque formation and maturation through a reduction in initial adhesion and a decrease in retention capacity of the microorganisms.

Influence of surface roughness (sr) on bacterial adhesion

The roughness of intra-oral surfaces influences the initial bacterial adhesion as well as it's stagnation. Scanning electron microscopy clearly revealed that initial colonization of the enamel surface starts from surface irregularities such as cracks, grooves, perikymata, or abrasion defects, and subsequently spreads out from these areas (frequently

along the perikymata) as a relatively even monolayer of cells. With time, plaque areas develop at the irregularities which alternate with less extensively colonized surrounding areas (Lie 1977, 1978, 1979, Lie & Gusberti 1979, Nyvad & Fejerskov 1987). Similar observations were recorded for the colonization of the fitting surface of acrylic dentures (Morris et al. 1987). Colonization of a tooth root surface, with its collagen fibres, was found to be faster and characterized by a haphazard distribution (Nyvad & Fejerskov 1987).

Thus, initial adhesion, especially supragingivally, preferably starts at locations where bacteria are sheltered against shear forces, because the change from reversible to irreversible attachment can be established more easily and thus more frequently in these sites. Since several studies stated that the proliferation of the initial adhering microorganisms accounts for the major part of the microbial mass increase during early plaque formation (Breex et al. 1983), this may explain the importance of surface roughness in this phase of plaque formation.

At surface irregularities and other stagnant sites, bacteria, once attached, can survive longer because they are protected against natural removal forces (Newman 1974) and even against oral hygiene measures (Quirynen 1986). Moreover, a roughening of the surface increases the area available for adhesion by a factor 2 to 3.

In vitro

Few in vitro studies reported on the influence of sr on plaque formation. When teeth were suspended in bacterial cultures, a 10 fold increase in c.f.u. was observed after surface roughening (Swartz & Phillips 1957). Moreover, S. mutans was found to adhere more frequently to rough cements than to filling materials that take a high polish (Einwag et al. 1990). However, when the adhesion of S. sanguis to composite materials with comparable roughness (ranging from 0.8 to 1.4 μ m) was examined, only negligible differences were registered. Thus these studies indicate a positive correlation between significant changes in surface roughness and initial bacterial adhesion.

Yamauchi et al. (1990), who examined the effects of various denture-baseresin surface textures on adhesion of specific micro-organisms stated that the

influence of surface roughness was strain dependent. Some strains (*S. oralis, P. gingivalis* C-101, and *P. intermedia*) were found in higher proportions on rough sites, whereas other strains (*S. mutans, S. sanguis, S. mitis* and *P. gingivalis* ATCC 33277) were found in higher amounts on smooth surfaces.

The effect of surface roughness on the plaque retaining capacity was also tested, but these observations were somewhat confusing. Wise & Dykema (1975) ranked the retention capacity against brushing of the highly polished materials as: acrylic=glazed porcelain<type III gold<ceramco metal. Tullberg (1986) found that polishing increased the adhesion capacity of gold and resin, whereas Yamauchi et al. (1990) observed that C. albicans was better retained by rough resin denture bases. These studies indicate that the influence of sr on retention capacity is not yet well understood.

In vivo studies on supragingival plaque

Numerous in vivo studies examined the effect of surface roughness on supragingival plaque formation and on periodontal health. Table 2 gives an overview of these studies leading to the following general statements:

- Rough surfaces (crowns, implant abutments, and denture bases) accumulate and retain more plaque (thickness, area, and colony forming units). These observations were less obvious in patients with optimal oral hygiene or when plaque was scored with crude indices.
- After several days of undisturbed plaque formation, rough surfaces harbour a more mature plaque characterized by an increased proportion of motile organisms and spirochetes.
- As a consequence of the former, crowns with rough surfaces were more frequently surrounded by an inflamed periodontium, characterized by a higher bleeding index, an increased crevicular fluid production, and/or a histologically inflamed tissue.

Von Mierau and co-workers, who demonstrated intra-subject reproducibility as regards plaque formation pattern, suggested that differences in plaque growth rate between slow and fast plaque formers were caused by clinically detectable (with a probe) differences in enamel surface roughness (Von Mierau & Singer 1978, Von Mierau

1979, Von Mierau et al. 1982). They even stress the importance of surface roughness evaluation, particularly with adolescents, when preventive measures are undertaken, since they believe this factor should help in the individualization of the oral hygiene need.

The importance of supragingival surface roughness justifies the demand for extra caution when performing the following treatments which are known to increase surface roughness: excessive brushing of amalgam, acrylic veneers, composites and gold (Van Dijken & Ruyter 1987, Johannsen et al. 1989, 1992), the use of polishing pastes on enamel at high speed and load (Christensen & Bangerter 1987), the use of polishing or prophylactic pastes (especially those containing pumice) on restorative materials (Roulet & Roulet-Mehrens 1982. Serio et al. 1988), the application of a 1.23% acidulated phosphate fluoride gel or 8% stannous fluoride on dental porcelain (Wunderlich & Yaman 1986), the application to titanium implants of acidulated (pH<5) fluoride gels or gels containing hydrofluoric acid (Pröbster 1992), and the use of air-powder abrasive systems on all materials (Bergendal et al. 1990, Barnes et al. 1991, Eliades et al. 1991).

In vivo studies on subgingival plaque

It is technically difficult to alter the surface roughness of subgingival surfaces (polishing) without surgical intervention. This might explain the low number of publications dealing with this subject.

Waerhaug (1956) observed in dogs and monkeys that roughening of the subgingival enamel, without optimal hygiene, resulted in increased deposition of plaque and calculus, and in pronounced connective tissue inflammation. Khatiblou & Ghodssi (1983) compared, in man, the healing after periodontal treatment between smooth and roughened roots. No differences in pocket reduction or gain of attachment were detected. However, since only teeth with a very advanced periodontitis were roughened (probing pocket depth: 7.4 mm for rough sites versus 5.9 mm for smooth sites) the important initial difference in probing pocket depth might have masked a possible negative effect of the roughening. Indeed, the deeper the pocket, the more gain in attachment one might expect from a periodontal treatment (Badersten et al. 1984). Re-

Table 2. The influence of surface roughening on plaque formation and periodontal health: in vivo

					Plaque (supra)			Gingiva				
Author	Experimental design	Period	Loc	Hygiene	Ind.	Area	C.F.U.	Flora	Gi	BL	Inf	Fluid
1. Turesky et al. '61	Strips (s or r)	1-30d	b±	Ø		7						
2. Sanchez et al. '69	Class V (diff mat) (s or r)	2w	$b\pm$	Ø							7	
3. Larato '72	Class V composite vs tooth	2m	b±	Ø		7						
4. Trivedi & Talim '73	Class V (diff mat) (s or r)	8w	b±	Н							7	
5. Mörmann et al. '74	Approximal inlays (s or r)	12w	b±	Н	=	7						=
6. Weitman & Eames '75	Class V composite (# Ra)	3d	b±	Ø		7						
7. Gildenhuys et al. '75	Gold crowns (# Ra)	1d	S	Ø		7		→ density				
8. Blank et al. '79	Adaptic filling vs tooth	1 y	b±	O	=				=			7
9. Keenan et al. '80	Retainer + gold sets # Ra	3d	S	Ø		1						
10. De Wet '80	Class IV partially glazed	10d	S	Ø	7							
11. Smales et al. '81	Class V # mat in denture	3d	S	Ø		7						
12. Budtz-Jorgensen Full denture (half S/half R) '86		1w	I			7	7	[candida]				
13. Shafagh '86	Crown # Ra	3d	S	Ø	7							
14. Van Dijken et al. '87a	Class III composite (# Ra) exper. ging.	7d	b±	Ø	=				=			=
15. Van Dijken et al. '87b	Class III composite (# Ra)	1–4y	b±	Н	===					7		7
16. Scheutzel '89	Denture: coated half	3m	S	Н	1							
17. Quirynen et al. '90	Strips with # Ra on teeth	3,6,9 d	S	Ø		1		≯ mature				
18. Siegrist et al. '91	Facings (diff mat) (# Ra)	1d	S	Ø			7	_				
19. Quirynen et al. '93	Implant abutments (# Ra)	3m	Sub	Н	-	***		≯ mature				

Experimental design: (s or r)=smooth or rough surfaces; # Ra=different surface roughnesses; Period: time of observation; loc: location of rough part (b=both supra & sub, S=supra, I=internal part of prosthesis, Sub=subgingivally); Hygiene: \emptyset =no special oral hygiene instructions; H=habitual oral hygiene; O=optimal oral hygiene.

cently, subgingival plaque around titanium abutments, of dental implants with different sr, was compared within subjects (Quirynen et al. 1993). When the to the abutment adhering plaque was considered, rough abutments were found to harbour 25× more bacteria, with slightly more non coccoid cells. However, when the swimming flora was considered, the differences became less obvious.

These observations therefore indicate that the importance of the surface roughness is reduced for the subgingival environment. Nevertheless it still justifies extra caution performing the following treatments which are known to increase the subgingival surface roughness: the use of hoes (Green & Ramfjord 1966), rotating diamond, or ultrasonic instruments (Meyer & Lie 1977, Walmsley et al. 1990) during root planing of teeth, the use of metal instruments during subgingival cleaning of titanium abutments (Fox et al. 1990, Speelman et al. 1992), and the use of polishing pastes on dentine at higher speed and load (Christensen & Bangerter (1987).

Interaction between surface roughness and surface free energy

The effect of surface roughening on the contact angles of polymers and thus also on their surface free energy has been studied extensively. Changes in solid surface Ra below 0.1 µm have no effect on contact angle, and above 0.1 μm the effect depends on the initial contact angle as measured on a smooth surface (see Table 1): if the initial contact angle is below 60° (e.g. enamel), surface roughening will further decrease this angle; if the initial contact angle is above 86°, surface roughening will further increase this angle, and for surfaces with initial contact angles between 60° and 86°, surface roughening has no influence (Busscher et al. 1984).

The relative importance of both parameters (sfe and roughness) on the supragingival plaque formation has been examined in vivo by following the undisturbed plaque formation on polymer strips (with low and medium sfe) from which one half was smooth and the other roughened (Quirynen et al. 1990). Surface roughening resulted in a

4 fold increase in plaque formation (extension as well as thickness) for both polymers (Fig. 5). Although roughening should have resulted in a larger difference in sfe between both surfaces, the inter-polymer differences almost disappeared when the rough halfs were considered. These results indicate that the influence of the surface roughness overrules the influence of the surface free energy.

Conclusions

Both the free energy and the roughness of intra-oral hard surfaces have a major impact on the initial adhesion and the retention of oral microorganisms. Especially supragingivally, an increase in surface roughness or surface free energy was found to result in a faster colonization of the surfaces and a faster maturation of the plaque, thereby increasing the risk for periodontal infections. Subgingivally, the influence of both parameters is less dramatic, probably because this environment offers more niches for bacterial adhesion and survival. The dominant effect of the sur-

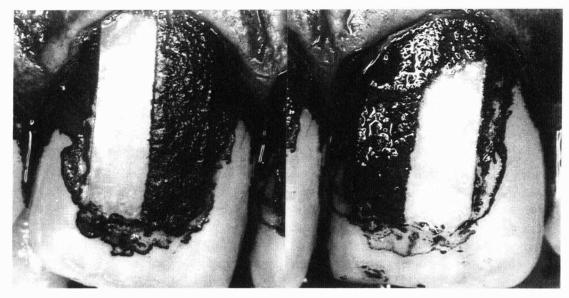


Fig. 5. The clinical importance of surface roughness and surface free energy is illustrated by these photographs, showing 2 strips which were glued to the central incisors of one patient. The 3-days old supragingival plaque (undisturbed formation) was disclosed by means of neutral red 0.5%. Each strip is devided in 2 halfs (a rough part (Ra $2.0~\mu m$) mesially located, and a smooth part (Ra $0.1~\mu m$) distally. The left strip (on the 11) is made out of fluorethylenepropylene (sfe: $20~{\rm erg/cm^2}$), and the right strips (on the 21) is made out of cellulose acetate (sfe: $58~{\rm erg/cm^2}$). The smooth parts represents the influence of the surface free energy, the rough parts demonstrates the predominance of the surface roughness.

face roughness justifies a demand for more clinical attention for this parameter.

Zusammenfassung

Der Einfluß der Oberflächenrauheit und der freien Oberflächenenergie auf die supra- und subgingivale Plaqueanlagerung beim Menschen. Eine Literaturübersicht

Für die meisten Bakterien ist die bakterielle Adhäsion an desquamationsfreie Oberflächen die einzige Möglichkeit, in einem offenen Vegetationssystem, wie der Mundhöhle, zu überleben. Die Adhäsion vollzieht sich in 4 Phasen: dem Transport der Bakterie zur Oberfläche, die initiale Adhäsion mit einer reversiblen und irreversiblen Phase, dem Haftungsvorgang durch spezifische Interaktionen und schließlich der Kolonisation zur Bildung eines Biofilms. In der Mundhöhle bieten sich verschiedene Hartsubstanzoberflächen mit unterschiedlichen Charakteristika an (Zähne, Füllungsmaterialien, dentale Implantate oder Prothesen). Bei gesunden Verhältnissen existiert auf solchen Oberflächen ein dynamisches Gleichgewicht zwischen retinierenden und abstoßenden Kräften. Erhöhte bakterielle Anhäufung in der Mundhöhle hat jedoch oft die Verschiebung in eine krankhafte Situation zur Folge. Die Retention dentaler Plaque wird durch 2 Mechanismen begünstigt: die Adhäsion und die Stagnation. Die vorliegende Arbeit untersucht den Einfluß der Oberflächenrauheit und der freien Oberflächenenergie auf den Adhäsionsprozess. Sowohl in vitro – als auch in vivo Untersuchungen unterstreichen die Bedeutung beider Variablen bei der Anlagerung supragingivaler Plaque. Rauhe Oberflachen begünstigen Plaquebildung und Reife

und es ist bekannt, daß hochenergetische Oberflächen mehr Plaque sammeln, Plaque stärker binden und spezifische Bakterien begünstigen. Obwohl beide Variablen miteinander interagieren, überwiegt die bestimmende Wirkung der Oberflächenrauheit den Einfluß der freien Oberflächenenergie. Im subgingivalen Milieu, mit seinen besseren Voraussetzungen für das Überleben von Mikroorganismen, ist allerdings die Bedeutung der Oberflächenrauheit bedeutend geringer. Der Einfluß der Oberflächenrauheit und der freien Oberflächenenergie rechtfertigt jedoch die Forderung nach glatten Oberflächen mit niedriger freier Oberflächenenergie zur Minimisierung der Plaqueanlagerung. Dadurch wird das Aufkommen von Karies und Parodontitis reduziert

Résumé

Influence de la rugosité de surface et de l'énergie libre de surface sur la formation de la plaque sus- et sous-gingivale chez l'humain. Une revue de la littérature

Dans la cavité buccale, un système de croissance ouvert, l'adhésion bactérienne aux surfaces non-éliminées est, pour la plupart des bactéries, le seul moyen de survivre. Cette adhésion se produit en quatre phases: le transport de la bactérie vers la surface, l'adhésion initiale avec une phase réversible et une non-réversible, l'attache grâce à des interactions spécifiques et, finalement, la colonisation de manière à former un bio-film. Diverses surfaces dures sont disponibles dans la cavité buccale (dents, obturations, implants ou prothèses) ayant toutes des caractéristiques de surface différentes. Dans une situation saine, un équilibre dynamique existe sur ces surfaces entre les forces de rétention

et celles d'enlèvement. Cependant, une accumulation bactérienne accrue entraîne souvent le développement de la maladie. Deux mécanismes favorisent la rétention de la plaque dentaire. l'adhésion et la stagnation. Le but de cette revue est d'examiner l'influence de la rugosité de surface et de l'énergie libre de surface dans le processus d'adhésion. Les études in vitro et in vivo soulignent l'importance des deux variables dans la formation de la plaque sus-gingivale. Des surfaces rugueuses favorisent la formation et la maturation de la plaque dentaire, et les surfaces à haute énergie attirent plus de plaque, pour lier la plaque plus fortement et sélectionner des bactéries spécifiques. Bien que ces 2 variables agissent mutuellement, l'influence de la rugosité de surface domine celle de l'énergie libre de surface. Pour l'environnement sous-gingival, offrant davantage de facilités aux microrganismes pour survivre, l'importance des caractéristiques de surface diminue énormément. Cependant, l'influence de la rugosité de surface et celle de l'énergie libre de surface sur la plaque sus-gingivale justifient la demande de surfaces lisses avec faible énergie libre de surface.

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