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On the relative importance of specific and non-specific approaches to oral microbial adhesion

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1. SUMMARY

In this paper, it is suggested that specificity and non-specificity in (oral) microbial adhesion are different expressions for the same phenomena. It is argued that the same basic, physicochemical forces are responsible for so-called 'non-specific' and 'specific' binding and that from a physico-chemical point of view the distinction between the two is an artificial one. Non-specific interactions arise from Van der Waals and electrostatic forces and hydrogen bonding, and originate from the entire cell. A specific bond consists of a combination of the same type of Van der Waals and electrostatic forces and hydrogen bonding, now originating from highly localized chemical groups, which together form a stereochemical combination. The absence or presence of specific receptor sites on microbial cell surfaces must therefore be reflected in the overall, non-specific surface properties of cells as well. This point is illustrated by showing that glucan-binding lectins on mutans streptococcal strains may determine the pH dependence of the zeta potentials of these cells. When studying microbial adhesion, a non-specific approach may be better suited to explain adhesion to inert substrata, whereas a specific approach may be preferred in case of adhesion to adsorbed protein films. Adhesion is, however, not as important in plaque formation in the human oral cavity as is retention, because low shear force periods, during which adhesion presumably occurs, are followed by high shear force periods, during which adhering cells must withstand these detachment forces. Evidence is provided that such detachment will be through cohesive failure in the pellicle mass, the properties of which are conditioned by the overall, non-specific substratum properties. Therefore, in vivo plaque formation may be more readily explained by a non-specific approach.

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2. INTRODUCTION

The controversies between those favouring a specific approach [1–24] and those favouring a non-specific approach [25–37] of (oral) microbial adhesion has lingered on for several years with little signs of a scientific unification of ideas as required in order to advance our understanding of microbial adhesion.

In the specific approach of (oral) microbial adhesion, it is usually argued that adhesion cannot be understood unless the adhesin, a molecular structure on the cell surface which is stereochemical with a substratum receptor site, has been properly identified [38]. Indeed, many adhesins have been described in the literature and it has been shown that blocking of these adhesins can inhibit adhesion. Well known examples of this specific type of interaction can be found in *Escherichia coli*, a non-oral microorganism. *E. coli* strains can elaborate tip-localized proteins on *pap* fimbriae, which are important in uroepithelial attachment [39]. K88 and K99 are other *E. coli* fimbrial antigens which complex with receptors on intestinal epithelium [40,41]. Oral microorganisms also possess a wide variety of specific adhesin molecules. Table 1 gives a far from complete overview of adhesins and their functions, as described for a variety of oral bacteria. It may be obvious, considering the number of strains and species in the oral cavity, that continuation along this line of research will yield an infinite number of adhesins identified in due time and it is unlikely that specific receptors will ever be found for all the different polymers existing (and to be developed) like Teflon, polymethylmethacrylate or polyvinylchloride. Also, none of the receptors identified seem to be known at a molecular level, and only vague descriptions of the receptors exist in most cases.

In the non-specific approach of oral microbial adhesion, adhesion is described as the combined result of overall, macroscopic surface properties, such as charge, surface free energy and hydrophobicity [28,31,33], being the physico-chemical expression of the chemical composition of the

Table 1

Overview of adhesive interactions in the oral cavity and adhesins identified

Strain	Adhesin	Partner	Adhesin	Reference
Actinomyces viscosus	Type 2 fimbriae	Mammalian cells	Galactose residues	[10]
Actinomyces naeslundii				[14]
Actinomyces viscosus	Type 2 fimbriae	*S. sanguis*	Internal GalNacβ1 → 3Gal	[4]
Actinomyces naeslundii				
Actinomyces viscosus	Type 1 fimbriae	SHA[a]	Proline-rich proteins, statherin	[9] [7]
Bacteroides gingivalis	150 kDa component	Mammalian cells	Fibrinogen	[8]
Bacteroides gingivalis	Galactose-containing carbohydrate	*Fusobacterium nucleatum*	Protein	[5]
Bacteroides gingivalis		Mammalian cells	Arginine residues	[12]
Bacteroides gingivalis		SHA	Proline-rich protein	[21]
Bacteroides intermedius		Mammalian cells	Galactose residues	[11]
Bacteroides loescheii	75 kDa polypeptide 45 kDa polypeptide	*S. sanguis* *A. israelii*		[23]
Eikenella corrodens		Epithelial cells	Galactose residues	[13]
Streptococcus mitis	Sialic acid binding protein	SHA	Sialic acid	[2]
Streptococcus sanguis				[1,22]
Streptococcus salivarius HB	Veillonella binding protein	*Veillonella parvula*		[3]
Streptococcus salivarius HB	Glycoprotein	Buccal epithelial cells		[3]
Streptococcus sanguis 12	Adhesin B	SHA	pH sensitive receptor	[24]
Streptococcus sobrinus		SHA	Glucans, glucosyltransferase	[6]

[a] SHA = saliva-coated hydroxyapatite.

cell surface [42,43]. Glantz, one of the pioneers of physico-chemical approaches of oral microbial adhesion, showed that the amount of plaque accumulated in vivo on intra-oral surfaces decreased greatly with decreasing substratum hydrophobicity, as assessed from water contact angles [44,45]. A similar study by Quirynen et al. [46] demonstrated that planimetric plaque scores on hydrophobic materials glued to the front incisors of human volunteers were much lower than on hydrophilic materials. The non-specific approach has also been successfully applied to show that hydrophobic bacterial strains, as assessed from water contact angles, preferentially adhere to hydrophobic substrata and vice versa [47–49].

So far, little success has been reported in explaining oral microbial adhesion to pellicle-coated materials with different hydrophobicities in vitro on the basis of a non-specific approach, although a minor, sustaining influence of the underlying, overall substratum properties has been described [50,51].

Several years ago, we published a hypothesis [52] in which overall, macroscopic cell surface properties were assumed to be responsible for the initial approach of a cell towards a substratum, whereas specific interactions were thought to commence operating when the distance between cell and substratum had become sufficiently small and interfacial water was removed by hydrophobic moieties. Thus it can be speculated that, although specific interactions may occur as a consequence of close approach to pellicle, non-specific forces are always important in bacterial adhesion phenomena. Also, many cariogenic mutans streptococci have been found to bind at least partially non-specifically to pellicle, i.e. their binding was of low affinity and not saturable or inhibitable with specific macromolecules [53,54]. Thus, the adhesion of these members of the plaque consortium is best described using a non-specific approach. Furthermore, two separate investigations [55,56] of the kinetics of *Streptococcus sanguis* adhesion found two distinct adhesion phases: an initial phase, mediated by non-specific forces and a second, high-affinity phase in which specific interactions have become operative. This argues that even in the absence of specific interactions, adhesion will take place.

It is the aim of this paper to demonstrate that specificity and non-specificity arise from the same basic physico-chemical forces and are thus different expressions for the same phenomena. Furthermore, a hypothesis will be presented on the relative importance of specific and non-specific approaches to oral microbial adhesion.

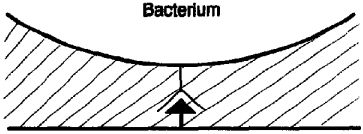
3. BASIC FORCES AND INTERACTIONS

It is presently a modern trend in physico-chemistry to explain poorly understood phenomena in terms of so-called additional forces. Van Oss [57] recently summarized these 'additional forces' of unknown origin and came to no less than 17 types of forces. Van Oss argued that in reality these could all be reduced to three categories: (i) (Lifshitz) Van der Waals forces; (ii) electrostatic forces; (iii) hydrogen bonding.
These are the basic physico-chemical forces reponsible for adhesion. Mathematical expressions for the interaction energies between a cell and a substratum according to the DLVO-theory on the basis of these forces are given elsewhere [32]. Since these forces are relatively long-range and originate from the entire body of the cell and substratum, the interaction energy decreases with distance^{-2} [32]. In the presence of sufficient electrostatic repulsion, a so-called secondary interaction minimum occurs between approximately 10 and 20 nm. A potential energy barrier, that can become as high as several thousand kT units, may exist at smaller distances (< 10 nm) prior to the occurrence of a primary interaction minimum, provided again there is sufficient electrostatic repulsion between the interacting surfaces [32]. It is important to realize that the above forces are always present, regardless of the absence or presence of specific receptor sites (see also Fig. 1), which may (or may not) contribute to adhesion, in addition to the non-specific interactions.

A specific bond can also be envisaged as resulting from the above mentioned basic physico-chemical forces [58], now acting between extremely small, highly localized and spatially well

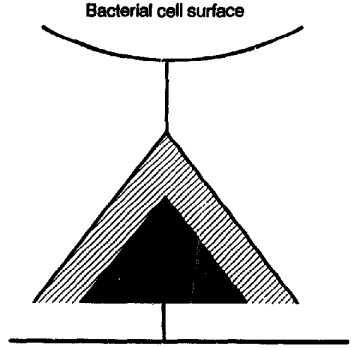
organized, opposing molecular groups on both interacting surfaces (see Fig. 2). For completeness, it is noted that the electrostatic nature of many specific bonds can be demonstrated by showing the dependence of the bonding on ionic strength or pH of the medium in which bonding occurs.

Despite the fact that specificity and non-specificity originate from the same basic, physico-chemical forces, there is one important phenomenological difference between the two. When specific bonds are superimposed on non-specific interactions, the highly localized character of the specific bonds not only causes adhesion, but also immobilization [27]. Non-specific forces are only able to cause adhesion; i.e. to keep adhering cells on a substratum surface, but they allow for sliding [27], an aspect that can be of major medical and ecological importance. Furthermore it has been argued that specific bonds are stronger than non-specific bonding, and that non-specific bonding occurs immediately when the cell comes in the vicinity of a surface, whereas specific bonding may be more time-consuming due to possible necessary rearrangement of stereochemical, molecular groups to interact, or even expression of new macromolecules by a cell in response to a surface [59,60]. However, because specificity arises from the same basic physicochemical forces as usually said to cause non-specificity, the absence or presence of specific receptor sites on microbial cell surfaces should have an effect on the overall, macroscopic cell surface properties as well. Most frequently, investigators do not seek to examine relations between specific and non-specific cell surface properties.




region in which van der Waals and electrostatic forces and hydrogen bonding occur

Fig. 1. Non-specific forces (Van der Waals and electrostatic forces and hydrogen bonding) originate from the entire cell and for that reason may not be neglected as compared to the effect of specific adhesins.




region in which van der Waals and electrostatic forces and hydrogen bonding occur

Fig. 2. A specific bond between stereochemical molecular groups on the 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.

4. GLUCAN-BINDING LECTINS AND ZETA POTENTIALS OF MUTANS STREPTOCOCCI

Recently, we published a detailed study [61] on the hydrophobicity (assessed by water contact angles), zeta potentials and overall elemental surface and molecular composition of four mutans

streptococcal species: *S. sobrinus, S. cricetus, S. rattus* and *S. mutans*. Results showed that *S. rattus* strains were slightly less hydrophobic than the other strains and had elevated amounts of surface phosphate. However, the most noteworthy difference between these species was the different pH dependence of their zeta potentials in 10 mM potassium phosphate buffer (see Table 2).

Taking a specific approach, Drake et al. [16] studied the absence or presence of glucan-binding lectins on mutans streptococcal strains by measuring macroscopic agglutination of cells by glucan T2000 with light scattering. Their data are summarized in Table 2, expressed as an agglutination rate constant. Drake et al. [16] argued that agglutination by glucan T2000 indicates the presence of a glucan-binding lectin (GBL) on the cells.

Although we have no proof that the cells grown by Van der Mei et al. [61] and Drake et al. [16] are identical, it is interesting to compare their data in order to illustrate that specificity and non-specificity can indeed be different expressions for the same phenomenon.

S. sobrinus, S. cricetus and *S. mutans*, all possessing GBLs, show a significant increase in their zeta potentials towards positive values upon lowering the pH. One of the possible explanations of these observations is that GBLs counterbalance the expression of negative charge especially at low pH, and the highly negative zeta potential of *S. rattus* over the entire pH range can be considered as a corollary of the absence of GBLs.

Taking a non-specific approach, we have recently explained the poor adhesion of *S. rattus* to artificial salivary pellicles on glass as a result of strong electrostatic repulsions between the cells and the pellicle. In a specific approach, the lack of GBLs on *S. rattus* strains would probably have been used to explain these observations.

The above example has been included in order to show that specific cell surface properties influence non-specific cell surface properties, as a corollary of the chemical composition of the cell surface. These ideas may not be used for any generalization concerning the pH independence of zeta potentials and the presence of GBLs on cell surfaces. In another situation, as e.g. in the case of *Streptococcus salivarius* HB and a series of fibril-deficient mutants [43], hydrophobicity or any other overall surface characteristic may be the more appropriate characteristic to consider in relation with the absence or presence of specific receptor sites on cells. However, this example does show the great potential of pH-dependent zeta potential measurements, reflecting very sen-

Table 2

Zeta potentials of mutans streptococcal strains in 10 mM potassium phosphate as a function of pH and agglutination rates by glucan T2000

Species and/or original strain number [a]	Zeta potential (mV)			Rate constant (min^{-1})
	pH 2.0	pH 7.0	pH 9.0	
S. sobrinus	+1.0	−11.7	−10.5	$0.7–1.1\times10^{0}$
S. cricetus	0.0	−16.7	−19.3	$3.1–3.2\times10^{-1}$
AHT	−2.5	−16.8	−14.8	3.2×10^{-1}
E49	−1.7	−15.3	−23.8	3.1×10^{-1}
HS-6	+4.1	−18.1	−19.2	3.1×10^{-1}
S. rattus	−24.3	−28.1	−28.1	0.0
FA-1	−26.6	−24.4	−18.6	0.0
BHT	−18.6	−38.4	−37.1	0.0
S. mutans	+8.4	−14.9	−13.2	0.0 [b]

[a] Strain numbers and data are only given when both Van der Mei et al. [61] and Drake et al. [16] included these strains. Otherwise data per species are given.

[b] For several of the *S. mutans* strains, macroscopic agglutination was reported to be not readily measurable by light scattering. After centrifugation, these strains were observed to be agglutinated by glucan T2000.

sitively the effects of (de)protonation of ionic groups on the cell surface.

5. SPECIFICITY vs. NON-SPECIFICITY IN ADHESION

In vitro adhesion experiments with oral streptococci to inert, artificial solid substrata of different surface free energies have yielded a number of important observations: (i) hydrophobic strains, such as *S. mitis* adhere in higher numbers to hydrophobic substrata than to hydrophilic substrata [48]; (ii) hydrophilic strains, like *S. mutans*, adhere in higher numbers to hydrophilic substrata [48]; (iii) adhesion of hydrophobic strains is more reversible on hydrophilic substrata than on hydrophobic substrata, whereas hydrophilic strains adhere more reversibly to hydrophobic substrata [47].

The degree of preference of strains for substrata of similar hydrophobicity turned out to be strain-dependent [62] and influenced by the absence or presence of surface appendages and whether or not the cells were able to produce biosurfactants that could mask the substratum properties [63]. Interestingly, data on ground and polished human enamel fitted exactly within the relations found for the artificial substrata [51]. Thus we can conclude that a non-specific approach is quite adequate in explaining oral streptococcal adhesion to both artificial solid substrata as well as to ground and polished human enamel.

Our observations on oral streptococcal adhesion to protein-coated artificial solid substrata and human enamel could be explained only partly on the basis of a non-specific approach. In the case of an albumin coating and substrata covered with an artificial salivary pellicle, all the above described tendencies were greatly attenuated but still present [51], suggesting that cells could probe the properties of the underlying substratum through the adsorbed protein film.

Contrary to the above, *S. mutans* adhered to mucin-coated hydrophobic substrata in much higher numbers than to mucin-coated hydrophilic substrata [64], at odds with expectations on the basis of observations with bare substrata. It was hypothesized that the degree to which hidden receptor sites of the mucin molecule ('cryptitopes') for *S. mutans* were exposed, was regulated by the hydrophobicity of the substratum to which the mucin was adsorbed [6,21,65]. Thus it is obvious to conclude that, despite a small sustaining influence of non-specific substratum properties, a specific approach is probably better suited to explain oral streptococcal adhesion to protein-coated substrata.

Surprisingly, however, both Glantz [44,45] as well as Quirynen et al. [46] found by independent measures that plaque formed up till 9 days in the human oral cavity accumulated to a much lesser extent on hydrophobic substrata than on hydrophilic substrata. Thus it appears that non-specificity is more important under the dynamic, in vivo conditions of the oral cavity than specificity. Since protein adsorption proceeds on a much faster timescale than microbial adhesion, bacterial adhesion in vivo will always be to an adsorbed protein layer. The differences in amount of plaque accumulation can only be explained if the characteristics of the adsorbed protein layer are influenced by the physico-chemistry of the underlying surface. This observation is difficult to reconcile with the conclusion that specificity prevails in adhesion to adsorbed protein films and actually constitutes a clear contradiction: "When specificity prevails in bacterial adhesion to protein-coated substrata in vitro, then why is not this the case for plaque formation in vivo?"

An explanation for this contradiction can probably be found in the realization that plaque formation equally involves microbial adhesion as well as retention [29,30,66,67] of adhering cells. Shear forces acting in the oral cavity may vary by a factor of 50–100 at rest as compared to during swallowing, eating, drinking and speech [68,69]. It seems reasonable to assume that cells will adhere predominantly during periods of low shear force, whereas detachment of adhering cells will occur more readily during the periods of high shear force (see Fig. 3). Detachment of cells at the adsorbed protein–cell interface is unlikely, because this adhesion is thought to be mediated by strong specific bonds. Therefore detachment must be through cohesive failure in the adsorbed pro-

tein mass or, alternatively, though less likely, at the adsorbed protein–substratum interface (see Fig. 3). This puts major emphasis on the hypothesis that adsorbed protein films are conditioned in various aspects by non-specific substratum properties, a hypothesis for which increasing evidence has become available (see Table 3).

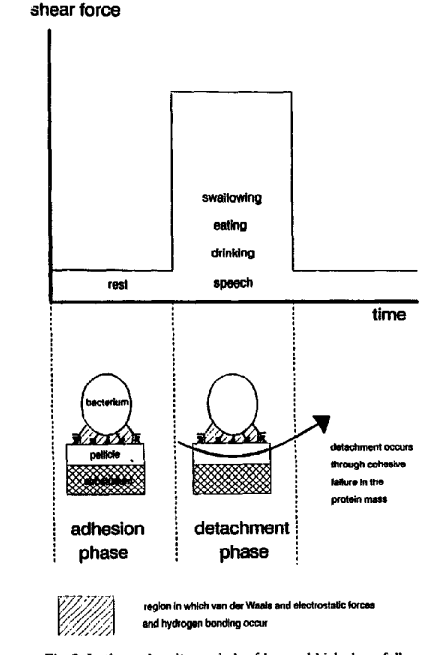


Fig. 3. In the oral cavity, periods of low and high shear follow each other rapidly. Since salivary protein adsorption proceeds at a much faster timescale than microbial adhesion, bacteria adhere predominantly to the pellicle surface during periods of low shear. Specific bonding together with non-specific forces mediates a firm adhesion between cells and the pellicle surface. During periods of high shear, detachment occurs through cohesive failure in the protein mass, the properties of which are determined by non-specific substratum properties.

Table 3

Overview of studies indicating that adsorbed protein films are conditioned according to the overall, macrosopic properties of the substrata

Technique	Summary of conclusions	Reference
Ellipsometry and infrared spectroscopy	Relative film density of adsorbed salivary proteins is higher on hydrophobic than on hydrophilic materials	[37]
Infrared spectroscopy	Amide I and Amide II absorption bands differ on hydrophobic and hydrophilic materials	[70]
Amino acid analysis	Amino acid composition of adsorbed salivary proteins is different on hydrophobic and hydrophilic materials	[71–73]
Transmission electron spectroscopy	Adsorbed proteins form a contiguous film on hydrophilic materials and form island-like structure on hydrophobic materials	[64,75]
Photo acoustic spectroscopy	Photo acoustic response of organic films depends on hydrophobicity of the substrata	[76]
Radio-iodination	Selective adsorption of proteins from plasma depends on hydrophobicity of the substrata	[77,78]

The data in Table 3 suggest that this conditioning mechanism probably involves:
(i) the amount of protein adsorbed;
(ii) the thickness of the adsorbed protein layer;
(iii) the relative density of the adsorbed protein layer;
(iv) the spatial arrangement of the adsorbed proteins;
(v) the configuration of the adsorbed proteins;
(vi) selective adsorption of specific proteins.

6. CONCLUDING REMARKS

This paper attempts to illustrate that specificity and non-specificity originate from the same basic physico-chemical forces. Lifshitz-Van der Waals, electrostatic forces and hydrogen bonding

[57,79,80] are usually summarized as the non-specific forces. Since these types of forces are also basic to specificity, there is no such a thing as a specific force. One can, however, refer to 'specific interactions', which are mediated through 'non-specific' forces, and 'specific approaches' to bacterial adhesion problems.

Specific approaches may be preferred when studying microbial adhesion to adsorbed protein films in vitro and under constant shear forces, whereas a non-specific approach is better suited to explain both microbial adhesion to inert substrata and plaque formation in the oral cavity. This conclusion is likely to hold for every application in which adhering cells are exposed to varying shear forces as e.g. uropathogens on catheter surfaces [81], viridans streptococci adhering to artificial heart valves [82], microorganisms infecting artificial vascular grafts [83] or bacteria in aquatic environments [84].

In the course of our studies we have encountered some bacterial strains with particularly unusual surface architecture, such as tufted *S. sanguis* strains [85] and *Actinobacillus actinomycetemcomitans* strains with very long and sparsely distributed hydrophobic fimbriae [86] that act as exceptions to the principles outlined here. The current state of physico-chemical techniques does not allow examination of surfaces at the level of a molecularly local detail. As these techniques advance to be more sensitive for local architecture of cell surfaces as well, it will be found that these unusual strains also will adhere according to the principles outlined in this paper.

It is often said that 'bacteria stick to any surface'. Whether this is always true or not is beyond the scope of this paper, but it is certainly true that non-specific forces will enable cells to adhere to many surfaces, despite the fact that they may not yet have developed specific receptor sites for that surface. Bacteria may develop new specific receptor sites in order to colonize a new host surface, often first maintaining a non-specific association with the substratum in question.

Thus, while scientists argue about specificity and non-specificity, bacteria make clever use of this controversy to adhere to the surfaces of their choice.

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