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Interfacial re-arrangement in initial microbial adhesion to surfaces

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ABSTRACT

Upon initial microbial adhesion to a surface, multiple events occur that include interfacial re-arrangements in the region between an adhering organism and a surface. Application of physico-chemical mechanisms to explain microbial adhesion to surfaces requires better knowledge of the interfacial re-arrangement occurring immediately after adhesion than hitherto available.

1. Introduction

Biofilms form on virtually all surfaces exposed to natural and industrial environments and their formation commences with adhesion of microorganisms. Surfaces to which microorganisms adhere can be in a pristine state or covered with a conditioning film consisting of adsorbed macromolecular components [1,2]. The presence of a conditioning film greatly complicates the mechanism of microbial adhesion, as microscopic specific ligand–receptor bonds may be involved in microbial adhesion to e.g., protein-coated surfaces [3–5], whereas on a pristine surface adhesion is mainly governed by the macroscopic properties of the interacting surfaces [6•,7••,8].

Microbial adhesion, as the initial step in biofilm formation, excludes metabolic processes such as excretion of extrapolymeric substances [9] and growth, which justifies to treat initial microbial adhesion according to the mechanisms proposed to be valid for inert particle adhesion [10••]. Neglecting the structural complexity and chemical heterogeneity of microbial cell surfaces [11], indeed a search has been initiated many years ago into microbial zeta potentials [12•], contact angles [13•], cell surface hydrophobicities [14–16], surface free energies [17], and other physico-chemical properties of microbial cell surfaces with the aim of applying surface thermodynamics [17,18•] or DLVO theories [6,19••,20] to explain initial microbial adhesion to surfaces. More recently, various groups have been involved in the direct measurement of microbial interaction forces with substratum surfaces, either pristine or conditioning film coated, employing atomic force microscopy (AFM) [21,22] or optical tweezers [23,24].

Microbial adhesion to surfaces is initially reversible, but even in the absence of metabolic processes becomes essentially irreversible shortly after first contact. Experiments in flow displacement systems have indicated that desorption probabilities of both microorganisms as well as of inert polystyrene particles decrease by several orders of magnitude within 1 to 2 min after contact with a substratum surface [25,26]. AFM has confirmed that microbial adhesion forces indeed strengthen exponentially over time by progressively invoking acid–base interaction forces [27••].

Yet, detailed knowledge of the interfacial re-arrangements responsible for bond strengthening is lacking. Few studies have been conducted over the past decades to address these interfacial re-arrangement, but physico-chemical understanding of microbial adhesion within and beyond the initial 1 or 2 min after first contact is essential in order to advance our understanding to a level relevant to practical applications. Quartz crystal microbalance with the ability to measure dissipation (QCM-D) has been applied by several groups [9,28] to assess the physico-chemical nature of the coupling of microorganisms to a surface and the changes occurring over time.

In this review, we briefly summarize the mechanisms of microbial adhesion to surfaces as revealed by application of surface thermodynamics and DLVO theories, AFM and QCM-D. Rather than providing a

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summary of what has been achieved by applying these methods, focus will be on remaining challenges that need to be solved in order to provide a better basis for understanding the mechanism of microbial adhesion to surfaces.

2. Microbial adhesion

Strange as it may seem, the simple expression "microbial adhesion" is used in literature for widely different phenomena. Table 1 summarizes essentially different parameters that are generally used to characterize "microbial adhesion". Yet, these parameters refer to different aspects of the adhesion process.

Mass transport conditions are most important in microbial adhesion, as they not only determine the rate at which organisms arrive at a substratum surface [29,30], but they also dictate the shear off and lift forces during adhesion [31]. Under controlled mass transport conditions, experimental deposition rates can be compared with theoretical upper limits, such as resulting from the Smoluchowski–Levich approach [29]. The ratio between experimental deposition rates and theoretical upper limits is called the "deposition efficiency", and expresses the fraction of organisms arriving at a surface that actually manages to adhere. Deposition rates depend on the microbial concentration in suspension and for that reason a dimensionless deposition rate, the so-called Sherwood number, has been introduced according to [32]

$$Sh = \frac{jl}{D_0 c} \quad (1)$$

where j is the deposition rate, l denotes a characteristic length, like e.g. the depth of the flow channel, D_0 the microbial diffusion coefficient and c the microbial concentration in suspension.

Flow displacement systems offer the best control over experimental mass transport conditions and when equipped with real-time, *in situ* analysis options also enable to directly measure desorption rate coefficients (β) in the various stages of an experiment as a function of the residence time of adhering particles or microorganisms (see example in Fig. 1). In Fig. 1 it can be observed that bacterial desorption rate coefficients decrease exponentially with increasing residence time ($t-\tau$) of the adhering bacteria on the surface from an initial value, β_o to a final value, β_∞ and once adhering for longer than 20 to 25 s desorption becomes highly unlikely. The desorption kinetics can be described assuming one relaxation time constant, $1/\delta$, according to [33••]

$$\beta(t-\tau) = \beta_\infty - (\beta_\infty - \beta_o)e^{-\delta(t-\tau)}. \quad (2)$$

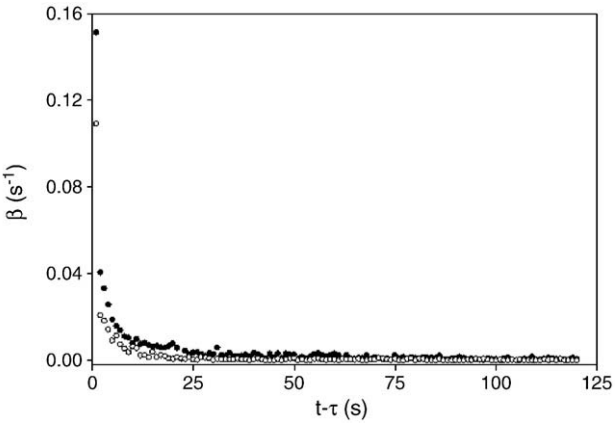

Fig. 1. Residence-time dependent desorption rate coefficients ($\beta(t-\tau)$) for *S. epidermidis* HBH$_2$ 3 from hydrophilic glass (black dots) and hydrophobic, DDS-coated glass (white dots). Figure taken from Boks et al. [26].

The relaxation time constant is the characteristic time of bond maturation between an adhering bacterium and a substratum surface and essentially describes the rate at which the bond matures.

As not only bacteria but also inert polystyrene particles show similar bond-maturation characteristics [34], it is emphasized that this type of bond maturation is purely physico-chemical in nature and has little or nothing to do with metabolic processes inherent to the use of microorganisms. Physico-chemically, bond maturation has been associated with the progressive removal of interfacial water, unfolding of surface structures or rotation of an entire particle to have its most favourable site opposing a substratum surface. Interestingly, increasing the residence time of a BSA-coated microsphere on a surface consistently increased the adhesion force measured during retraction of the sphere from the surface [35,36], demonstrating the important contribution of protein unfolding to bond maturation.

In the absence of *in situ* observation methods of microbial adhesion, substrata with adhering organisms have to be taken out of a microbial suspension, and sometimes rinsed to remove so-called "loosely adhering" organisms prior to actual enumeration. These actions, and particularly traversing of a substratum with adhering micron-sized particles through a liquid–air interface, exert high detachment forces upon adhering particles [37•], causing the removal of an unknown number of adhering organisms from the substratum surface [38,39]. Moreover, under sufficiently vigorous rinsing all adhering organisms may appear as "loosely adhering". Thus the outcome of experiments involving application of detachment forces higher than those prevailing under the conditions of attachment do

Table 1
Various parameters used in the literature referring to different aspects of what is generally called "microbial adhesion".

Parameter	Description	Units
Initial deposition rate	Number of organisms initially arriving and adhering at the surface per unit time and area under the experimental mass transport conditions	$cm^{-2}\,s^{-1}$
Deposition rate	Number of organisms arriving and adhering at the surface per unit time and area under the experimental mass transport conditions	$cm^{-2}\,s^{-1}$
Deposition efficiency	Ratio between experimental deposition rate and a theoretically calculated upper limit	
Initial desorption rate	Number of organisms initially leaving the surface per unit time and area under the experimental mass transport conditions	$cm^{-2}\,s^{-1}$
Final desorption rate	Number of organisms leaving the surface per unit time and area under the experimental mass transport	$cm^{-2}\,s^{-1}$
Adhesion number	Number of organisms adhering to the surface per unit area at time under the experimental mass transport conditions	cm^{-2}
Retention number	Number of organisms adhering to the surface per unit area after application of a detachment force at time t exceeding the one of the experimental mass transport conditions	cm^{-2}