Coupled adhesion of bacteria to surfaces

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CHAPTER 1

General Introduction and Aim of the Thesis

INTRODUCTION

The complexity of microbial adhesion as well as its significance for the surrounding environment has been greatly studied for the past several decades, including the physico-chemical mechanisms that govern it. In the medical field microbial adhesion can lead to infectious biofilm formation on biomedical implants and devices, thereby causing a most severe condition to patients.[1,2] In order to prevent bacterial adhesion in the medical field, but also in many other applications such as in food industry, drinking water systems and in the marine environment, it is crucial to obtain more information on the bond between a bio-colloid, such as a bacterium and a surface as well as of the potential mechanistic differences between colloidal particle adhesion and microbial adhesion. Initially, particle adhesion is reversible and led by a plethora of different forces between a (bio)colloid and a substratum, including attractive Lifshitz-Van der Waals forces, acid-base interactions as well as repulsive or attractive electrostatic forces. The Derjaguin, Landau, Verwey and Overbeek-theory,[3–6] also known as the DLVO theory, for (bio)colloidal interactions explains the sum total of these particular interactions from which it is possible to predict the outcome of particle deposition towards adhesion by analyzing their interaction energy. Note that in case acid-base interactions are included, one speaks about the extended DLVO-theory.

Over time a (bio)colloid reaches the point of adhesion to a substratum surface at which adhesion is no longer considered reversible. Irreversible adhesion of colloids develop through progressive removal of interfacial water, conformational changes in cell surface proteins or re-arrangement of binding tethers[7] while in case of bacteria the production of extracellular polymeric substances, aid further in making an irreversible bond to the substratum surface.

The bond between a (bio)colloid and a substratum surface has long been considered rigid, but it is currently evident that the bond between a bacterium and a substratum surface is viscoelastic.[8,9] The viscous part of the bond encompasses the reversible deformation when stress is applied to the bond, which then returns back to its original state as soon as the stress is relieved under the influence of its elastic part. The viscosity is usually represented in models as a piston, also known as a dashpot. The elastic part of the bond is usually represented as a spring. The spring and dashpot can either be placed in parallel, referred to as the Kelvin-Voigt model,[10] or in series, referred to as the Maxwell model.[10] These models are also known from previous studies where the viscoelasticity of entire biofilms, i.e. not of single bonds, has been studied in relation with their composition and structure.[11] Analysis of the kinetics of (bio)colloidal particle adhesion makes it possible to obtain spring constants, as well as drag coefficients, thereby gaining insight into which factors influences adhesion and how and at which force the colloids can be removed from a surface.

Several instruments thus far have been used to obtain valuable understanding into bond-substratum interaction, including Atomic Force Microscopy (AFM)[12–14]and optical tweezers,[15,16] which

measure the individual bacteria one-by-one. Albeit effective, these methods are time-consuming and handling the instruments is tedious.[17] Moreover, the AFM uses a cantilever to push down a (bio)colloidal particle to a substratum surface which misrepresents naturally occurring adhesion processes (Figure 1a).

Another popular method to study the dynamic process of initial (bio)colloidal particle adhesion is the use of a parallel plate flow chamber offering real-time *in situ* observation of adhesion by means of a CCD camera (Figure 1b). This type of observation can be extended to include the Brownian motion[18] displayed by (bio)colloids, in which the (bio)colloids are suspended in a flowing fluid and exposed to random displacements due to collisions with other molecules in their aqueous environment. Analyzing the vibrations of adhering bacteria led to the conclusion that the viscoelastic properties of bacteria depend on the type of surface appendages they possess. Although these types of study offer insight into the bond properties of adhering (bio)colloids, it lacks the ability to measure these viscoelastic bond properties in three dimensions,[18] as well as quantitatively determination of the drag force.

The study of Brownian motions of adhering (bio)colloidal particles can also be performed by using total internal reflection microscopy (TIRM) (Figure 1c).[19] TIRM employs an evanescent wave that is produced when optical waves, produced by a laser, are incident to a glass-water interface at an angle that is larger than the critical angle ($\theta > \theta_c$) (see Figure 1c).[20] (Bio)colloids scatter light in three dimensions in the evanescent wave when the refractive indices of the colloids differ from that of the suspending fluid. The intensity of the scattered wave light emitted from the colloids decays exponentially from the interface, indicating where the particle is located compared to the planar surface. TIRM has been used in previous studies to measure cell-surface separation distances, as well as reversible and irreversible adhesion properties of microorganisms. [21,22] This way of employing the scattered light intensity to measure or determine the separation distance, including the vibrations of individual (bio)colloids and an interface offers a fast, high throughput and nonintrusive way of obtaining information of the bond between a (bio)colloid and any type of planar substratum, and quantitative determination of the spring constant of the bond.

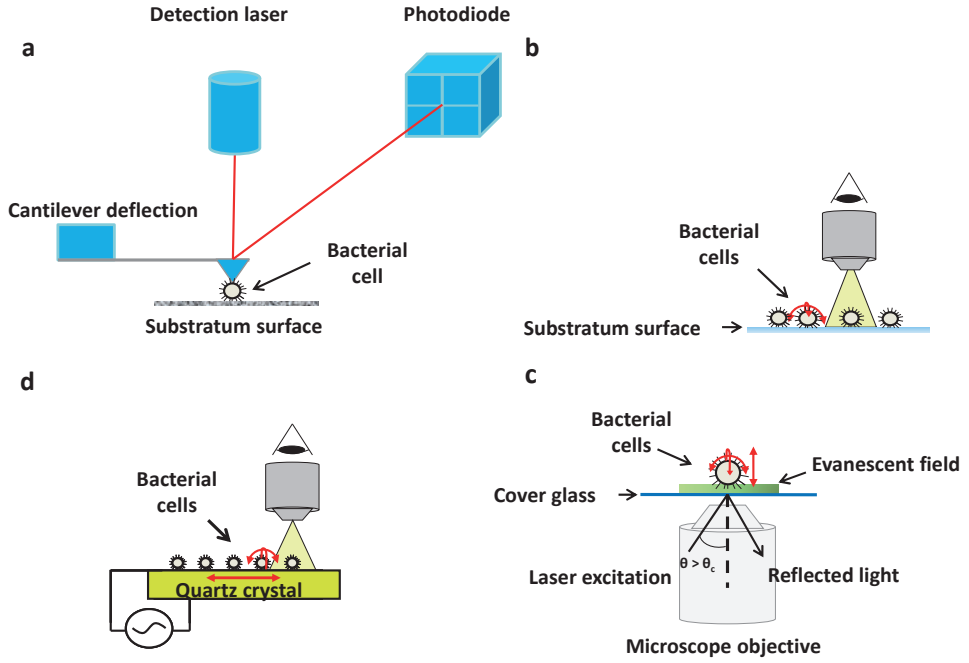

Figure 1. Schematic representation of different techniques available to determine the viscoelastic properties of the bond between (bio)colloids and substratum surfaces. a) Atomic force microscopy (AFM),[23] b) Vibration spectroscopy,[18] c) Total internal reflection microscopy (TIRM), and d) Quartz crystal microbalance with dissipation (QCM-D).

An additional technique that has been utilized in determining the viscoelastic properties between bacteria, or other colloids and different types of substrata is a high frequency sensing device, called the Quartz Crystal Microbalance with Dissipation (QCM-D) (Figure 1d).[24–26] Unlike AFM, QCM-D utilizes the naturally occurring adhesion forces between (bio)colloids and substratum surfaces, but on the other hand operates at non-naturally occurring high frequencies, never encountered by an adhering particle unless purposely imposed.

QCM-D has previously been used in molecular adsorption where according to the conventional mass loading theory,[27] the adsorbed mass couples directly to the sensor surface (an AT-cut quartz crystal) thus increasing its effective mass, and reducing its resonance frequency leading to negative shifts in resonance frequency. Mass loading is commonly observed when molecular layers adhering to the sensor surface are thinner than 250 nm. When the crystal is brought into oscillation at its resonance frequency, changes in frequency as well dissipation are observed upon adhesion of (bio)colloids. In contrast to molecular adsorption, (bio)colloidal particles adhere to the sensor surface via a tethered, non-rigid bond, causing positive frequency shifts,[28–30] Positive frequency shifts can be explained according to what is known as the coupled resonator model.[9,28] In this model/theory, it is postulated

that an adhering (bio)colloidal particle functions as a resonator of its own coupled to the QCM-D crystal surface. The frequency shifts of this particular coupled resonance system is determined by the ratio between the quartz crystal surface's resonance frequency and the colloids resonance frequency, where soft contact points produce positive frequency shifts, and stiff contact points yield negative frequency shifts.

QCM-D is a extensively used technique to explore both mass changes as well as interactions between surfaces coated with specific (bio)materials and ligands, as well as determining the rigidity and softness of surface bound materials, such as polymers, proteins etc.

AIM OF THIS THESIS

The aim of this thesis is to gain insight into how the viscoelasticity of the bond between (bio)colloids and a substratum surface depends on environmental conditions like ionic strength of the surrounding fluid, substratum hydrophobicity or absence or presence of binding tethers on a bacterial cell surface.

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