Coupled adhesion of bacteria to surfaces

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CHAPTER 6

General Discussion

GENERAL DISCUSSION

In this thesis, focus is on the viscoelastic nature of the adhesive bond between (bio)colloids and substratum surfaces, or more specifically how environmental conditions (i.e. ionic strength and surface hydrophobicity) influence the bond characteristics. Several instruments were used to this end. The quartz crystal microbalance (QCM), measures the changes in frequency and dissipation upon (bio)colloidal adhesion to determine the viscoelastic properties of the bond. A vector network analyzer based QCM (VNA), which holds the ability to change the driving voltage of the resonance crystal, was used to examine whether bonds can be ruptured and at what force. Lastly, total internal reflection microscopy (TIRM) was employed for analysis of nano-scopic vibrations exhibited by bacteria with different surface appendages, in which the elasticity of the bond is related to the vibrations.

(Bio)colloidal adhesion is a topic that has gained a large interest over the past decades[1–3] due to the fact that gaining knowledge on the forces responsible for adhesion and even more so for particle detachment from substratum surfaces is crucial in many biomedical, industrial and environmental processes. Several models[4,5] have been developed to determine the bond strength between (bio-)colloids and substratum surfaces. To date, the most common physico-chemical approaches employed to study (bio-)colloidal adhesion to substratum surfaces are surface thermodynamics[6,7] and (extended) Derjaguin, Landau, Verwey and Overbeek (DLVO)-types of analyses.[8,9] Surface thermodynamics comprise the calculation of free energies calculated by means of contact angles of surfaces in order to obtain interfacial free energies. These interfacial energies predict the outcome of adhesion. DLVO analyses include Lifshitz-Van der Waals, electrostatic and acid-base interactions.

(Bio)colloidal adhesion analysis by means of QCM-D was explored in chapter 2. The positive frequency shifts as well as the dissipations shifts retrieved from the experiments were related to the viscoelasticity of the (bio)colloidal-substratum bond according to a coupled resonator model.[10] All quantitative properties of the adhesive bond reported in chapter 2 have been obtained by fitting the spring constant (k), drag-force (ξ), and the mass of the particle (m_p). The coupled resonator model was first explored by Dybwad[10,11] in the 1950s to explain the positive frequency shifts exhibited by colloidal particles, and it was concluded that colloidal adhesion is a coupling between two oscillators through very small contact points, thus the frequency is not only dependent on the degree of adsorbed mass but also on the stiffness of the bond between the adsorbed mass and surface. Chapter 2 focuses mostly on the possibility to obtain the viscoelastic parameters for (bio-)colloidal adhesion, which up until now was not possible by means of QCM-D, and in the past only the spring constant could be determined.[12] Good qualities of the fits were confirmed by the low root mean square deviation (RMSD). Values for k were much higher for the bacteria indicative of much stiffer contact points, which is not unexpected since bacteria, especially those with surface tethers form stiff contact points followed by bond maturation,[13] causing a lesser impact from the drag coefficient. Oppositely it was observed for silica

particles which showed little influence from k but a large influence from ξ. The masses calculated matched very well the expected value for bacteria, albeit in the case of silica particles there was a slight over-estimation. This over-estimation could be explained by reasoning that since it is known that viscoelasticity influences the frequency shifts, this further influences the mass. More specifically, the resonance frequency (f) is given by[14]

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} \qquad [1]$$

in which it k is the spring constant and m is the mass. Furthermore, it can be seen that if the resonance frequency decreases, the mass increases, and depending on how the (bio)colloids adhere and influence the frequency shifts, it shows that the mass is affected simultaneously. Also what should be taken into consideration is the fact that employing the coupled resonator model, the (bio)colloids function as resonators themselves, and perhaps in the case for silica particles they do not always adhere through specific contact points but instead move along with the movement of the crystal, leading to more negative frequency shifts and therefore influence the masses calculated.

Chapter 3 also focused on the quantitative analyses of the physical-chemical bond characteristics obtained by means of the coupled resonator model. In addition a comparison was made based on phenomenological Kelvin-Voigt or Maxwell models and the possible role which polydispersity might have in the analysis of the QCM-D response to adhesion of a fibrillated and non-fibrillated streptococcal strain and abiotic polystyrene particles. It was shown that polydispersity only made a difference for the fitting of a Maxwell model. Moreover, it was not possible at this time to conclude which model gave the best output parameters, instead the study showed that dependent on which model was used, different bond characteristics were obtained. The Maxwell model showed more emphasis on the elastic response than the Kelvin-Voigt model, meaning since spring and dashpot are put in series, the elastic response in the Maxwell model acts independently of damping. Oppositely, in the Kelvin-Voigt model, the spring is placed in parallel with the dashpot and continuously opposes its response through the dashpot. Moreover, it should also be noted that with regards to (bio)colloidal adhesion it perhaps is not sufficient to model the contact points as either one Kelvin-Voigt element or one Maxwell element but instead a series of each of these elements. If a Maxwell element is put into series the first initial spring and dashpot would function as the spring and the remaining springs and dashpots in series would then function as a dashpot as a whole, basically the same principle as in a Kelvin-Voigt model. Compared, if a Kelvin-Voigt model was put into a series the initial spring would be the most dominant one, and remaining springs would then have little or no influence. These are all concepts needed to be explored, albeit this thesis is a first step into quantitatively obtaining physico-

chemical bond parameters from the QCM with regards to (bio)colloidal adhesion, and gaining insight into the factors which influence initial bacterial adhesion. The two first experimental chapters of this thesis (chapter 2 and chapter 3) focused on obtaining bond properties for (bio)colloidal adhesion. Yet a few things have remained unexplored. Adhesion of bacteria to different substratum surfaces was done by employing an adhesion buffer, yet this does not constitute a real environment for bacteria, because the bacteria employed were accordingly not physiologically active in absence of nutrients. In order to have them physiologically active, adhesion should be performed in a growth medium specific for these bacteria. One problem arises in this case, since the nutrients present in the medium would also adsorb to the QCM-D crystal surface thereby giving rise to molecular adsorption rather than colloidal adhesion. In a previous study[15] it was shown that bacteria producing extracellular polymeric substances (EPS) adsorb directly on the crystal, according to the conventional mass-loading theory[16] rather than via the coupled resonance theory as described above for colloids. In order to make up for that Olsson et al.[15] adhered EPS producing bacteria in one experiment and non-EPS producing bacteria in another. From the obtained changes in frequency and dissipation they could subtract QCM-D signals from each other and obtain the correct changes in frequency and dissipation. In a similar manner, to account for physiologically active bacteria in a nutrient-rich solution, it could be envisaged to adhere bacteria from medium by means of QCM-D to obtain the changes in frequency and dissipation, and in a next series of experiments allow bacteria to adhere from a buffer or only adsorption of medium components. By subtracting the changes in frequency and dissipation in different experiments, changes in frequency and dissipation for bacteria adhering in their natural environment, including effects of their physiological activity can be obtained.

In a preceding study it was investigated that for bacteria the vibrational amplitudes or distance variations parallel to a surface decreased with increasing adhesion forces acting perpendicular to the surface.[5] Meaning that by investigating the vibrational amplitudes of (bio)colloids would also create a deeper understanding of the binding mechanisms and how these are influenced. Moreover the study confirmed that for (bio)colloids the adhesion to a substratum surface involved multiple binding tethers which detached and re-attached to a surface, leading to an irreversible adhesion. Inspired by this new knowledge on how bacteria adhere and move parallel to a given substratum surface, knowledge on how they move perpendicular to a surface was pursued. Therefore, in chapter 4, by means of TIRM having a penetration depth of approximately 200 nm, it was studied how two different strains of streptococci interact with a substratum surface or more specifically, how their outermost surface would interact with a substratum surface. In chapter 4 two types of substratum surfaces, hydrophobic and hydrophilic were employed at various ionic strengths to control the level of bacterial adhesion. Bacteria containing fibrillar surface tethers adhered to a substratum surface in an irreversible fashion by tether-coupling to the surface. Bacteria, known as having a more “bald” surface (no demonstrable

fibrillar surface tethers), adhered differently; they abide by DLVO-types of analysis, where the bacteria reside in a secondary energy minimum. Accordingly, a distinction between "tether-coupled" and "floating" adhesion was made, which presents a new way to look at how bacteria adhere to a surface. Although novel insight is obtained on the adhesion mechanisms, several questions arise when analyzing the vibrational amplitudes for the fibrillar and non-fibrillar streptococcal strains perpendicular to the surface. Most notably is the fact that the largest population of the adhering fibrillar bacteria only move around 5-10 nm away from the surface irrespective of ionic strength of the buffer and substratum surface hydrophobicity. This is opposite to what has been found for bacteria through parallel movement, moving around 100-200 nm over a certain time period and what was confirmed by *in silico* modeling.[5,12] Accordingly the question arises, what makes parallel versus perpendicular movement/measurement of adhesion so different. It is not expected that bacteria will move as much in the perpendicular direction especially not when tether-bound as compared to in a parallel direction, since bacteria move over a larger distance parallel to a surface than secondary minimum or tether-extension will allow, till they have found a high-affinity spot where they will permanently attach.[17] It has been postulated that the penetration depth plays a vital role in TIRM monitoring the perpendicular Brownian motion displayed by (bio)colloids, mostly because TIRM has a small penetration depth and therefore when monitoring the adhesion it is no longer long-range Lifshitz-Van der Waals forces that are in play but instead shorter-ranged attractive forces. Thus what is then observed in TIRM are the bacteria that are strongly adhering as compared to monitoring with a bright field camera. Although this is a valid point, it may not be sufficient to explain the smaller vibrational amplitudes obtained in chapter 4. As calculated by Sjollema et al.,[5] one way to more precisely determine how bacteria move according to a surface is by calculating their mean squared *perpendicular* displacement as function of time.

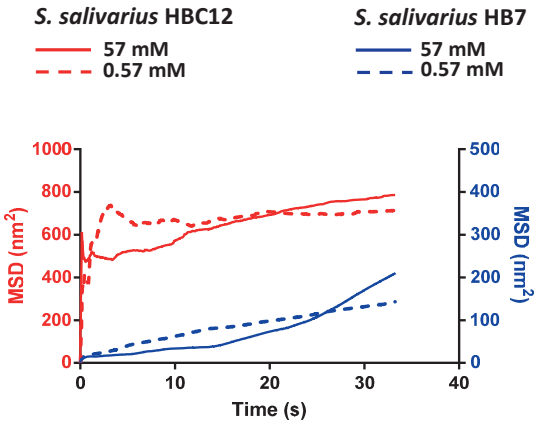

Figure 1. Mean Squared *perpendicular* Displacement (MSD) as a function of time for two different bacterial strains, *S. salivarius* HBC12 and *S. salivarius* HB7. The colored lines are for the adhesion at 57 mM and the dotted lines for the adhesion at 0.57 mM.

In Figure 1 it can be seen that for *S. salivarius* HB7, the variations over time are around 10 nm, confirming that for fibrillar bacteria movement perpendicular to the surface is very limited. Oddly, it seems that for the adhesion in a high ionic buffer solution (57 mM) adhering bacteria keep moving much more, and not really reaching a plateau. This could be due to the compression of fibrils, making them act like more unsteady springs, thereby causing a constant movement of the bacteria. For *S. salivarius* HBC12, the MSD is quite stable, after the first 5 s a plateau is reached. The variations over time are around 30 nm which is also what has been deduced from calculating the vibrational amplitude.

Since chapter 4 gave insight into more detail about the physico-chemical properties of two different strains of bacteria, combining this knowledge with the physico-chemical properties obtained from the QCM-D, exploration into more detail regarding (bio)colloidal binding is pivotal.

In chapter 5 a vector network analyzer-based QCM was employed where it was possible to vary the driving voltages from 0.01 V to 0.4 V. Moreover, it was possible to calculate the oscillation force exerted on the adhering (bio)colloids offering the possibility to measure the force needed to prevent (bio)colloidal adhesion or to cause detachment from different crystal surfaces. Unfortunately within the range of oscillation forces possible with the instrument, it was not feasible to observe any detachment from the surface for the fibrillar streptococcal strain. This was not entirely unexpected since the bacteria are let to adhere for an hour before applying an oscillation force, leading to a prolonged residence time on the surface causing a stronger bond.[18] Although a shorter adhesion time could increase the chances of fibrillar bacteria to detach, it might not be sufficient to cause detachment. Other factors such as crystal resonance frequencies as well as QCM quality factors should

be increased.[19] It was believed, by changing the viscosity of the system, i.e. subjecting the (bio-)colloids to a glycerol solution, causing faster dampening of the oscillation amplitudes, the Q factors might increase, thereby increasing the oscillation force (see Eq. 2)

$$F_{osc-P} = \frac{16\rho R^3 (CQV_d)^2 f_0^2}{5\sqrt{(CQV_d)^2+4R^2}} \quad [2]$$

This was evaluated for the hydrophilic SAM-coated crystals where the glycerol concentration was changed from 10% to 50%, and unfortunately no increase in Q was observed (see Table 1), but instead a systematic decrease. This possibly indicates that the glycerol adjacent to the oscillating crystal moves along with the crystal.

Table 1 Quality factors, Q for the VNA-based QCM for hydrophilic SAM-coated crystals at six different overtones submerged in different glycerol concentrations. Data represent averages ± standard deviations over triplicate experiments with separate crystals.

Overtone (n)	Hydrophilic SAM		
	50% glycerol	20% glycerol	10% glycerol
1	1174 ±10	2416±40	2830±22
3	2051±19	4298±157	4972±13
5	2713± 56	5171±278	6353±32
7	3062± 178	6511±51	7733±17
9	3465±326	7423±17	8686±67
11	3864±273	8547±54	9103±211

Next step would be to change the crystal dimensions such that their resonance frequencies (f_0) would be higher. Another interesting part in regards to preventing adhesion or causing detachment of (bio)colloids from a surface would be to evaluate the size or the size distribution of bacteria. Most bacteria have radii of around 500 nm, but rod-shaped bacteria have different dimensions. This difference in dimension would be interesting to evaluate with the VNA-based QCM, since Eq. 2 also

states that the radius (R) plays a crucial role. Use of rod-shaped coliform bacteria such as *Escherichia coli* would offer insight into the role of shape on adhesion of bacteria to substratum surfaces.

CONCLUSION

This thesis demonstrates that analysis of the bacterium-substratum interface with regards to their viscoelastic properties offers novel and pivotal insight into the mechanisms governing initial bacterial adhesion. Moreover, the use of several types of instruments in this thesis has helped gain insight into the bond properties of (bio)colloids to different types of substratum surfaces, which strengthens the understanding of the adhesive bond stiffness as well as their perseverance of adhesion. Coupled adhesion of (bio)colloids to surfaces is complex and therefore needs to be explored further, preferably assessed with multiple non-invasive techniques.

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SUMMARY

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Bacterial adhesion occurs on essentially all natural, as well man-made surfaces and poses a major concern in engineering and medicine. Controlling initial bacterial adhesion is of key importance to prevent later problems after bacteria have grown into e.g. pathogenic biofilms on biomaterials implants and devices that pose a threat to human health.

Chapter 1 gives a brief overview of bacterial adhesion phenomena, as well as of the methods employed in this thesis to study adhesion. Methods are explained and the significance to study bacterium-substratum bonds are explained, giving rise to the aim of this thesis, which is to gain insight into how the viscoelasticity of the bond between (bio)colloids and a substratum surface depend on environmental conditions, like ionic strength of the surrounding fluid, substratum hydrophobicity and absence or presence of binding tethers on a bacterial cell surface.

The bond between a (bio)colloid and a substratum surface is not rigid as previously thought, but instead, is currently considered to be viscoelastic.

Based on this consideration, **chapter 2** focuses on the quantitative derivation of the viscoelastic bond parameters using QCM-D on bacteria and silica particles adhering to QCM-D crystal surfaces. The quartz crystal microbalance with dissipation (QCM-D) has become a powerful tool for studying the bond viscoelasticity of biotic and abiotic colloidal particles adhering to substratum surfaces. A window-equipped QCM-D allows high-throughput analysis of the average bond viscoelasticity, measuring over 10^6 particles simultaneously in one single experiment. Other techniques require laborious analyses of individual particles. In the protocol described in **chapter 2**, the quantitative derivation of the spring-constant and drag-coefficient of the bond between adhering colloidal particles and substratum surfaces using QCM-D is explained for bacteria and silica particles, using the particle-mass derived for validation. Bond viscoelasticity is calculated using a coupled resonator model, paying special attention to the protocol for mathematical fitting needed to obtain reliable quantitative output. Knowledge of the viscoelasticity of the bond between colloidal particles and substratum surfaces facilitates development of new strategies to detach adhering particles from or retain them on a surface.

Next, in **chapter 3** the bond between two hydrophilic streptococcal strains with 91 nm long and without fibrillar surface appendages and micron-sized hydrophobic polystyrene particles on QCM-D crystal surfaces with different hydrophobicities, were analyzed employing both a Kelvin-Voigt and a Maxwell model. A Poisson distribution was implemented in order to determine possible virtues of including polydispersity when fitting model parameters to the data. Quality of the fits did not indicate whether a Kelvin-Voigt or Maxwell model is preferential and only polydispersity in spring-constants improved the fit for polystyrene particles. Kelvin-Voigt and Maxwell models both yielded higher spring constants for the bald streptococcus than for the fibrillated one. In both models, the drag coefficients

increased for the bald streptococcus with the ratio of electron-donating over electron-accepting parameters of the crystal surface, while for the fibrillated strain the drag coefficient was similar on all crystal surfaces. Combined with the propensity of fibrillated streptococci to bind to the sensor crystal as a coupled resonator above the crystal surface, this suggests that the drag experienced by resonator-coupled, hydrophilic particles is more influenced by the viscosity of the bulk water than by interfacial water adjacent to the crystal surface. Hydrophilic particles that lack surface tethers are mass-coupled just above the crystal surface and accordingly probe a drag due the thin layer of interfacial water that is differently structured on hydrophobic and hydrophilic surfaces. Hydrophobic particles without surface tethers are also mass-coupled, but their drag coefficient decreases when the ratio of electron-donating over electron-accepting parameters increases, suggesting that hydrophobic particles experience less drag by structured water adjacent to a surface.

Chapters 4 and 5 further advance a new model recently proposed (Sjollema J. et al., Detachment and successive re-attachment of multiple, reversibly-binding tethers result in irreversible bacterial adhesion to surfaces. Scientific Reports, 2017, 7:4369) which describes bacterial adhesion as a result of tether-binding to a surface, based on Total Internal Reflection Microscopy (TIRM) and QCM, respectively. Current models for bacterial adhesion to substratum surfaces all include uncertainty with respect to the (ir)reversibility of adhesion. In a model, based on vibrations exhibited by adhering bacteria parallel to a surface, adhesion was described as a result of reversible binding of multiple bacterial tethers that detach from and successively re-attach to a surface, eventually making bacterial adhesion irreversible. Here, we use TIRM to determine whether adhering bacteria also exhibit variations over time in their perpendicular distance above surfaces. Streptococci with fibrillar surface tethers showed perpendicular vibrations with amplitudes of around 5 nm, regardless of surface hydrophobicity. Adhering, non-fibrillated streptococci vibrated with amplitudes around 20 nm above a hydrophobic surface. Amplitudes did not depend on ionic strength for either strain. Calculations of bacterial energies from their distances above the surfaces using the Boltzman equation showed that bacteria with fibrillar tethers vibrated as a harmonic oscillator. The energy of bacteria without fibrillar tethers varied with distance in a comparable fashion as the DLVO (Derjaguin, Landau, Verwey and Overbeek)-interaction energy. Distance variations above the surface over time of bacteria with fibrillar tethers are suggested to be governed by the harmonic oscillations, allowed by elasticity of the tethers, piercing through the potential energy barrier. Bacteria without fibrillar tethers “float” above a surface in the secondary energy minimum, with their perpendicular displacement restricted by their thermal energy and the width of the secondary minimum. The distinction between “tether-coupled” and “floating” adhesion is new, and may have implications for bacterial detachment strategies.

Forces required to detach adhering bacteria from substratum surfaces are not trivial to measure and hence in **chapter 5** it was attempted to use the QCM to this end. Variation of the driving

voltage and crystal oscillation amplitude in QCM has seldom been explored but offers interesting possibilities to measure (bio)colloidal particle adhesion forces and characteristics of the bond, as achieved during different development histories. To this end, we built a modified QCM, referred to as a vector network analyzer (VNA) which allows to vary the QCM driving voltages from 0.01 V to 0.4 V. Quality factors Q of the VNA-based QCM, using the same crystals and chamber as the Q-sense, were found comparable with those of the Q-sense QCM-D and were used to calculate the oscillation forces exerted on the (bio)colloidal particles during adhesion to an oscillating crystal in the VNA-based QCM. Forces to prevent (bio)colloidal particle adhesion ranged from 0.2 pN to 30 pN, while forces required to detach adhering particles were higher and ranged from 2 pN to 30 pN. Although these forces are orders of magnitude smaller than generally derived from atomic force microscopy, they are of the same order of magnitude as obtained using optical tweezers and flow displacement systems. This negates often voiced criticism on QCM data that its high oscillation frequency influences (bio)colloidal particle adhesion. However, bond characteristics, derived in a coupled resonator model based on the routine QCM output and use of a phenomenological Kelvin-Voigt model, varied with the oscillation forces applied during adhesion. Importantly, this chapter adds a simple, and easy to interpret instrument to measure (bio)colloidal adhesion forces, that coincide generally well with force values obtained using optical tweezers and flow displacement systems, but not with forces obtained using atomic force microscopy. Therewith, the chapter helps solve the question whether bacterial adhesion forces are in the nN-range (atomic force microscopy) or pN- range (QCM, optical tweezers and flow displacement systems).

In **chapter 6**, the general discussion of this thesis, critical points are highlighted with respect to the different chapters, including suggestions for future experiments like carrying out QCM experiments with bacteria in growth medium. These critical points raised in combination with the thesis as a whole, may help strengthening current understanding of the adhesive bond between (bio)colloid and substratum surfaces.

SAMENVATTING

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Bacteriële hechting komt veel voor op natuurlijke en artificiële oppervlakken en veroorzaakt een groot probleem binnen talrijke industriële en geneeskundige processen. Het beperken van de initiële hechting van bacteriën is van cruciaal belang in het voorkomen van deze problemen, wanneer bacteriën zijn gegroeid tot een pathogene biofilm, zoals op biomedische implantaten en apparatuur.

Hoofdstuk 1 geeft een kort overzicht over bacteriële hechtingsmechanismen en de methoden die in deze thesis gebruikt zijn om hechting te bestuderen. De methoden worden onderbouwd en het belang van de bacterie-substraat interactie wordt uiteengezet, welke leiden tot de doelstelling van deze thesis, namelijk het verkrijgen van inzicht in de visco-elastische interactie tussen (bio)colloïdale deeltjes en een oppervlak onder verschillende ion sterkten en op materialen met verschillende hydrofobiciteit.

Hoofdstuk 2 richt zich op de kwantificering van visco-elastische bindingen door middel van QCM-D analyse van gehechte bacteriën en silicadeeltjes. Een QCM-D meetkamer met een inkijk venster, maakt een analyse van de gemiddelde visco-elasticiteit van de bindingen met hoge efficiëntie mogelijk, door het meten van meerdere miljoenen deeltjes tegelijkertijd in een enkel experiment. Andere technieken vereisen veel tijd doordat ze individuele deeltjes bemeten. In het protocol beschreven in **hoofdstuk 2** wordt de kwantitatieve afleiding van de veerconstante en de frictiecoëfficiënt gebruikt om de binding tussen gehechte colloïdale deeltjes en oppervlakken, gebruikmakend van de QCM-D, besproken. De afgeleide massa van de deeltjes wordt hierbij gebruikt ter validatie. Berekeningen zijn gebaseerd op het resonantie model voor twee gekoppelde resonatoren.

Vervolgens wordt in **hoofdstuk 3** de binding tussen twee hydrofiele streptokokken met en zonder 91 nm lange fibrillen en hydrofobe polystyreen deeltjes op QCM-D kristaloppervlakken met verschillende hydrofobiciteiten bestudeerd, middels een Kelvin-Voigt en een Maxwell model voor gekoppelde resonatoren. Een Poisson verdeling werd geïmplementeerd om de mogelijke invloed van polydispersiteit te onderzoeken. De kwaliteit van de fit was voor het Kelvin-Voigt en het Maxwell model gelijk, terwijl alleen het toevoegen van polydispersiteit in de veerconstante voor de polystyreen deeltjes tot een beter fit leidde. Zowel in het Kelvin-Voigt model als ook in het Maxwell model, hadden de kale streptokokken een hogere veerconstante dan de gefibrilleerde variant. In beide modellen verhoogde de frictiecoëfficiënt van de kale streptokokken wanneer de ratio van elektron-donerende en elektron-accepterende parameters van het kristaloppervlak hoger was, terwijl bij de gefibrilleerde streptokokken de frictiecoëfficiënt op alle kristaloppervlakken gelijk was. Dit suggereert dat de frictie ervaren door de gefibrilleerde streptokokken meer beïnvloed wordt door de viscositeit van het omgevingswater dan door de gebonden waterlaag aan het kristaloppervlak. Hydrofiele streptokokken, zonder fibrillen worden vlak boven het kristaloppervlak gebonden en voelen daardoor de frictie van

de dunne laag water in het raakvlak, welke een verschillende structuur heeft op hydrofobe en hydrofiele oppervlakken.

In de **hoofdstukken 4 en 5** wordt een nieuw model voor bacteriële hechting verder uitgewerkt (Sjollema J. et al., Detachment and successive re-attachment of multiple, reversibly-binding tethers result in irreversible bacterial adhesion to surfaces. Scientific Reports, 2017, 7:4369) en beschrijft bacteriële hechting aan het oppervlak als gevolg van "ankerverbindingen" zoals fibrillen, gebaseerd op Totale Interne Reflectie Microscopie (TIRM) en QCM. Huidige modellen voor bacteriehechting aan de oppervlakken van substraten bevatten onzekerheid voor wat betreft van de (on)omkeerbaarheid van de hechting. In een model gebaseerd op de vibraties van hechtende bacteriën parallel aan het oppervlak, kan deze hechting omschreven worden als het resultaat van reversibele bindingen van meerdere bacteriële ankerverbindingen, die kunnen losraken en ook succesvol opnieuw hechten maar nooit allemaal tegelijk loslaten van een oppervlak, wat uiteindelijk leidt tot onomkeerbare bacteriële hechting. Hier hebben we TIRM gebruikt om te bepalen of de hechtende bacteriën ook variaties laten zien in hun afstand loodrecht boven een oppervlak. Streptokokken met fibrillen als ankerverbindingen laten loodrechte vibraties zien met amplituden van ongeveer 5 nm, ongeacht de hydrofobiciteit van het oppervlak. Hechtende kale streptokokken vibreerden met amplituden van ongeveer 20 nm boven een hydrofoob oppervlak. De amplituden waren niet afhankelijk van de ion sterkte. Berekeningen van de bacteriële hechtingsenergie gebaseerd op de afstand tot het oppervlak middels de Boltzman vergelijking, toonden aan dat bacteriën met fibrillen als ankerverbinding op een zelfde manier vibreren als een harmonische oscillator. De hechtingsenergie van bacteriën zonder fibrillen varieerde met de afstand op een vergelijkbare manier als beschreven in DLVO (Derjaguin, Landau, Verwey and Overbeek)-interactie energie. Er wordt gesuggereerd dat de verschillen in afstand boven het oppervlak in de tijd voor bacteriën met fibrillen als ankerverbinding worden gereguleerd door de harmonische oscillaties, een eigenschap mogelijk door de elasticiteit van de ankerverbindingen. Bacteriën zonder ankerverbindingen zweven als het ware boven het oppervlak in het secundaire DLVO energie minimum, waarbij hun loodrechte verplaatsing beperkt wordt door de thermische energie van de bacterie en de breedte van het secundaire minimum. Het onderscheid tussen "gekoppelde" en "zwevende" hechting is nieuw en kan belangrijke gevolgen hebben voor bacteriële verwijderingsstrategieën.

De krachten benodigd om gehechte bacteriën te verwijderen van oppervlakken zijn niet gemakkelijk te meten en om die reden werd in **hoofdstuk 5** de QCM gebruikt om dit probleem aan te pakken. De variatie in het aandrijvingsvoltage en de amplitude van de kristaloscillatie in QCM is nog maar zelden onderzocht, maar biedt interessante mogelijkheden voor het meten van de hechtingskrachten van (bio)colloïdale deeltjes en de karakteristieken van de verbindingen. Hiervoor werd er een gemodificeerde QCM gebouwd (de vector netwerk analysator (VNA)), welke het mogelijk

maakt het aandrijvingsvoltage te variëren tussen 0.01 V en 0.4 V. De kwaliteitsparameters Q van de VNA-gebaseerde QCM, gebruikmakend van hetzelfde kristal en meetkamer als in de Q-sense, waren vergelijkbaar met die van de Q-sense QCM-D en werden gebruikt voor de berekening van de oscillatie krachten uitgeoefend op de (bio)colloïdale deeltjes gedurende hechting aan een oscillerend kristal in de VNA-gebaseerde QCM. Krachten benodigd om de hechting van (bio)colloïdale deeltjes te voorkomen varieerde van 0.2 pN tot 30 pN, terwijl krachten benodigd om gehechte deeltjes te verwijderen hoger waren, variërend van 2 pN tot 30 pN. Alhoewel deze krachten orden van grootte lager uitvallen dan gemeten met atomaire krachtmicroscopie, zijn ze in dezelfde orde van grootte als gemeten worden met optische pincetten of stroomkamers voor bacteriële hechting. Dit weerlegt de vaak gehoorde kritiek op QCM gegevens dat de hoge oscillatie frequentie de (bio)colloïdale deeltjeshechting beïnvloedt. De bindingskarakteristieken, afgeleid in een gekoppeld resonantiemodel gebaseerd op de standaard QCM output en het gebruik van een fenomenologisch Kelvin-Voigt model, varieerden met de oscillatiekrachten toegepast gedurende de hechting. Dit hoofdstuk voegt een simpele en gemakkelijk te interpreteren instrument toe aan de methoden die gebruikt worden om de hechtingskrachten van (bio)colloïdale deeltjes aan oppervlakken te meten, en geeft resultaten die goed overeenkomen met de krachten gemeten met optische pincetten en stroomkamers voor bacteriële hechting. Daarmee helpt dit hoofdstuk de vraag te beantwoorden of bacteriële hechtingskrachten zich in het nN-bereik (atomaire krachtmicroscopie) of in het pN-bereik (QCM, optische pincetten en stroomkamers voor bacteriële hechting) bevinden.

In **hoofdstuk 6**, de algemene discussie van deze thesis, worden belangrijke kanttekeningen geplaatst met betrekking tot de verschillende hoofdstukken, waaronder suggesties voor toekomstige experimenten zoals het uitvoeren van QCM proeven met bacteriën in groeimedium.

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We are now venturing towards colder weather, that is right; we are going to Scandinavia…

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