Protocols

Quantification of the viscoelasticity of the bond of biotic and abiotic particles adhering to solid-liquid interfaces using a window-equipped quartz crystal microbalance with dissipation

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ABSTRACT

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 this protocol, 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.

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1. Introduction

Control over the adhesion of biotic (such as bacteria) and abiotic colloidal particles (such as silica, polystyrene or latex particles) is a key concern in engineering and medicine. In particular, adhesion of bacteria to surfaces can form a hazard to human health [1], while adhesion control of abiotic particles is essential in areas such as sensing and data storage [2,3]. The bond between a colloidal particle and a substratum surface is seldom rigid and mostly comprises an elastic and viscous component [4,5]. The viscoelasticity of a bond is not only determinant for particle adhesion, but also for the mechanism of particle detachment [6,7]. For bacteria, the viscoelastic properties of their bond with a substratum surface often allow adhering bacteria to remain adhering under shear conditions through gradual elongation of the bond [8].

Several experimental techniques have been developed to study the adhesive bond between adhering colloidal particles and a substratum surface, such as the use of atomic force microscopy (AFM) [9–11] and optical or magnetic tweezers [12]. In these techniques, a single colloidal particle is forced to contact a substratum surface after which it is pulled off and the force required to break the bond is taken as the adhesive force. Elasticity and viscosity of the bond can be measured using AFM by pressing a particle on a substratum surface under a constant, applied force and measuring deformation or by measuring the force resulting from an applied, constant deformation of the particle. Using this approach, Lu et al. [13] described the bond components of bacterial cell surfaces as a spring, representing the elastic component as recognized in the standard solid model, placed in a series with a combination of a spring and a dashpot in parallel. In order to ensure that such modeling only comprises the bond between an adhering particle and a substratum surface and not the bulk of a particle as in traditional Hertz, Johnson-Kendall-Roberts or Derjaguin-Muller-Toropov models, Chen et al. [14] suggested to consider the bond between an adhering particle and a substratum surface as a cylin-

drical volume that deforms under conditions of constant volume to provide a method allowing to confine traditional analysis to the bond itself. Analysis of the Brownian motion induced nanoscopic vibrations exhibited by biotic [15] and abiotic [7,16] colloidal particles offers a completely different way to obtain the elasticity of the bond, with smaller vibrational amplitudes being indicative of higher elasticity [15]. Apart from the assumptions involved in each of the above-mentioned techniques, they all possess the common limitation that adhering particles must be studied one-by-one on an individual basis, which makes it hard to obtain statistically reliable, quantitative data.

The quartz-crystal-microbalance-with-dissipation (QCM-D) effectively avoids this "one-by-one" drawback, and has been applied to analyze the viscoelasticity of the bond between biotic and abiotic particles adhering to solid-liquid interfaces over large numbers of adhering particles, typically in the order of 10^{10} per m^2 of a sensor surface, approximately equivalent to 10^6 particles on the sensor surface. Nanometer-scale shear oscillations of the sensor cause deformation of the bonds with an adhering particle, with an opposing force arising from the surrounding liquid. The QCM-D registers the shift in resonance frequency of the sensor (Δf) due to particle adhesion as well as the energy loss to the surrounding liquid (change in "dissipation" (ΔD)). Moreover, for studies involving colloidal particles, it is advantageous to use a QCM-D equipped with a window chamber, allowing simultaneous microscopic registration of the number of particles adhering to the sensor surface.

Traditionally, QCM-D has been mostly used to determine adsorption of molecular mass to a sensor surface, assuming the adsorbed mass directly couples to the sensor surface. According to Sauerbrey's relation [17], an adsorbed coupled mass increases the effective sensor mass, yielding a reduction in the sensor resonance frequency, or in QCM-terms a negative (resonance) frequency shift. Since the penetration depth for the shear wave in QCM-D is less than 250 nm (at 5 MHz), this represents the maximum thickness of adsorbed films that can be reliably measured. The sensitivity of the mass detection in QCM is in the nanogram range. Particles however, do not necessarily mass-couple to the sensor surface, but instead may adhere as coupled resonators [18–21]. The sensor resonates at different fixed frequencies. A 5 MHz sensor resonates not only at 5 MHz but also at its overtones (15, 25 up to 65 MHz). Provided not touching each other, all colloidal particles adhering to the sensor surface act as individual, coupled resonators (Fig. 1a) with an impact on the resonance frequency shift (Δf) measured. Energy dissipation change (ΔD) is maximal when the particle resonance frequency (f_p) matches the sensor resonance frequency (f_s). The development of the coupled resonator model has greatly widened the possibilities of QCM-D, which were previously confined to molecular mass adsorption. It is interesting to note that whereas bond viscoelasticities of individual particles of the same kind often show large variations [8,15,22], QCM-D identifies a well-defined zero-value in sensor resonance frequency shift (Δf_s) when particle and sensor resonance frequencies match (zero crossing frequency (f_{ZC}), see Fig. 1b). Zero crossing frequencies are only observed when the adhering colloidal particles oscillate at frequencies within the window of the sensor resonance frequency and its observable overtones, which range from 5 MHz to 65 MHz (see Fig. 1b). Positive frequency shifts observed in the literature, could not be explained prior to the introduction of the coupled resonator model [4,5,19].

In the coupled resonator model, the frequency shifts and dissipation changes derived from the QCM-D can be related in a Kelvin-Voigt model to the viscoelasticity of the bond according to

$$\Delta f + \frac{i \Delta D f_s}{2} = \frac{f_F m_p}{\pi Z_q} \cdot N_p \left[\frac{\omega_s^3 (\omega_p^2 - \gamma^2) - \omega_s \omega_p^4}{(\omega_s^2 - \omega_p^2)^2 + \omega_s^2 \gamma^2} + i \frac{\omega_s^4 \gamma}{(\omega_s^2 - \omega_p^2)^2 + \omega_s^2 \gamma^2} \right] \quad (1)$$

where Δf (Hz) is the shift in QCM-D resonance frequency, ΔD is the change in dissipation, f_F is the fundamental resonance frequency of the sensor (5 MHz), f_s is the QCM-D sensor surface resonance frequency, m_p is the particle mass (kg), ω_s is the sensor resonance angular frequency ($\omega_s = 2\pi f_s$), ω_p is the particle resonance angular frequency ($\omega_p = 2\pi f_p$), Z_q is the acoustic impedance of an AT-cut quartz crystal (8.8×10^6 kg m^{-2} s^{-1}), N_p is the number of adhering particles per unit sensor area (m^2), γ equals ξ/m_p with ξ being the drag coefficient (see Fig. 1a), indicative of the viscous component of the bond. The elastic component of the bond follows from ω_p equaling with $\sqrt{k/m_p}$. Note that the coupled resonator model as described in Eq. (1) assumes complete coupling of adhering particles with the sensor surface, including torsion and shear deformation of the sensor surface due to particle oscillations [23]. Moreover, it is assumed that the spring and dashpot are independent from the frequency according to the coupled resonance model, which means that the frequency should not be causing any perturbations of the adhesive bond. Still it should be noted that bond stiffnesses determined using the QCM-D may be influenced by the experimental condition of having been determined in the MHz range.

In this protocol paper, we explain the use the QCM-D and data analysis to obtain elasticities (the spring constant, k) and viscosities (the drag coefficient, ξ) of the bond of biotic and abiotic particles adhering to sensor surfaces, as calculated with the coupled resonator model. Knowledge of the elasticity and viscosity of the bond through which colloidal particles adhere to substratum surfaces will assist in a better understanding of the mechanisms of particle adhesion and in developing new strategies to detach adhering particles from or retaining them on a surface.

2. Materials and preparatory procedures applied

Reagents used

- 1-octadecanothiol (Sigma-Aldrich, Zwijndrecht, The Netherlands)
- 11-mercapto-1-undecanol (Sigma- Aldrich, Zwijndrecht, The Netherlands)
- Ammonia (NH_3) (Merck, Darmstadt, Germany)
- Calcium chloride ($CaCl_2$) (Merck, Darmstadt, Germany)
- Ethanol 100% (VWR Chemicals, Fontenay-Sous-Bois, France)
- Hydrogen peroxide (H_2O_2) (Merck, Darmstadt, Germany)
- Ultrapure water (>18 MΩ cm)
- Potassium chloride (KCl) (Sigma-Aldrich, Zwijndrecht, The Netherlands)
- Potassium phosphate (KH_2PO_4) (Merck, Darmstadt, Germany)
- Sodium dodecyl sulphate (SDS) 2% (w/v) (Merck, Darmstadt, Germany)
- Silica particles (radius 0.5 μm) (Bangs laboratories, Inc., Fischer, IN, USA)
- Todd Hewitt broth (THB) (Oxoid, Basingstoke, UK).

Main equipment used

- Centrifuge (J-lite, JLA 16.250 Fixed Angle Rotor, Beckman Coulter, CA, USA)
- Sonicator bath (Transsonic TP 640, Elma GmbH & Co Singen, Germany)
- Sonicator (Vibra Cell model 375: Sonica and materials, Danbury CT, USA)
- UV/Ozone (Bioforce Nanosciences, Slough Berkshire, United Kingdom)
- Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) E1 system (Q-Sense AB, Stockholm, Sweden)