Real-time examination of the adhesive behavior of biofilm-forming bacteria on ordered molecular assemblies using quartz crystal microbalance with energy dissipation monitoring

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Abstract
We analyzed *E. coli* adhesion on self-assembled monolayers (SAMs) with various terminal groups using quartz crystal microbalance with energy dissipation (QCM-D) and optical microscopy. We found that bacterial cells densely adhered to hydrophobic and hydrophilic protein-adsorbing SAMs, while they weakly attached to hydrophilic protein-resisting SAMs, forming sparse but dissipative layers. Positive shifts in resonant frequency for hydrophilic protein-resisting SAMs at high overtone numbers indicated bacterial appendages clinging to surfaces. By examining acoustic wave penetration depths, we estimated the distance of bacterial cell bodies from surfaces, which gives insights to bacterial attachment strength. This understanding helps identify surfaces prone to biofilm contamination and guides the design of bacteria-resistant surfaces and coatings.

1. Introduction
Bacterial biofilm formation can hinder the efficiency of industrial and biomedical devices. It begins with the weak, reversible attachment of bacterial cells to surfaces, followed by bond maturation and the secretion of polymeric substances that form stable biofilms. Understanding the initial attachment stage is crucial for developing biofilm prevention technologies. Bacterial attachment varies with surface chemistry, typically favoring hydrophobic surfaces, though some studies report greater adhesion to hydrophilic surfaces. Variations in surface topography, bacterial introduction, and rinsing procedures contribute to these differing results. QCM-D can address these issues by providing ultra-sensitive mass and viscoelasticity measurements, with controlled fluid injection mimicking in vivo conditions and preventing dislodging of adherent cells. Moreover, using SAMs on QCM sensors helps isolate the effects of surface chemistry from topography. In this study, we report the influence of surface chemistry on bacterial adhesion using QCM-D.

2. Experiment
Six types of SAMs (hydrophobic CH_3-terminated, hydrophilic COOH, NH_2 and OH-terminated, nonionic oligo(ethylene glycol) and zwitterionic sulfobetaine-terminated) were fabricated by immersing the QCM sensors in 1 mM precursor thiol solutions. The functionalized sensors were then placed in the flow modules of a QCM-D setup. Frequency (Δf) and dissipation (ΔD) shifts were recorded during three phases: establishing a baseline in a PBS environment, introducing *E. coli* solution until signal stabilization, and rinsing with PBS until stabilization. Changes in Δf (Hz) and ΔD (ppm) were monitored for six overtones (n = 3,

5, 7, 9, 11, and 13). Optical microscopy images of the actual bacterial adhesion were also obtained for each SAM.

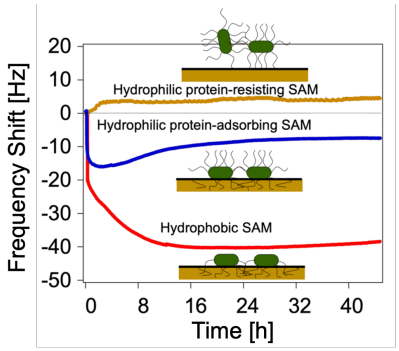

Fig.1 Real-time monitoring of bacterial adhesion onto different SAMs using QCM-D.

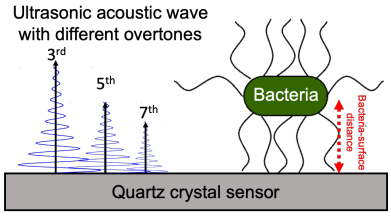

Fig.2 Measurements of the distance between bacteria and surface using ultrasonic waves.

3. Results and discussion

Fig.1 shows the Δf versus time profiles of the QCM sensors functionalized with different SAMs. The hydrophobic SAMs showed a rapid initial drop in Δf after the bacterial suspension injection, followed by a continuous decline indicating rapid attachment and accumulation of bacterial cells. Hydrophilic protein-adsorbing SAMs also exhibited a rapid decrease in Δf post-injection. The increase in Δf after the initial maximum indicates a loss in adhered bacterial mass and offers insights into the stability of biofilm buildup on charged hydrophilic surfaces. Interestingly, hydrophilic protein-resisting SAMs displayed positive Δf which does not follow the conventional mass loading regime. According to the coupled-resonator model [1], bacterial cells attach using their appendages, reinforcing sensor oscillation and resulting in positive Δf. This suggests that bacterial appendages penetrate the layer of structured interfacial water barrier surrounding protein-resisting SAMs, while the bacterial body remains in the bulk liquid, explaining the positive Δf. By analyzing differences in acoustic wave penetration depths at each overtone as shown in Fig.2, we estimated the distances between the clinging bacterial cell body and the surface. We report that protein-resisting SAMs maintain a submicron distance from the bacterial cell which may be due to a layer of interfacial water preventing direct attachment [2]. On the other hand, direct bacterial contact dominates for SAMs following the conventional mass loading, leading to stable biofilms. These estimated distances provide insights into the varying strengths of bacterial attachment on different surfaces.

4. Summary

Our findings showed that hydrophobic and hydrophilic protein-adsorbing SAMs are more prone to bacterial fouling, while hydrophilic protein-resisting SAMs exhibited weaker and less bacterial attachment. We revealed the role of bacterial appendages in initiating contact and forming elastic spring-like connections, leading to positive Δf. By analyzing overtone dependence, we estimated the distances between bacterial cell bodies and different SAM surfaces, correlating these distances to the strength of bacterium-substratum bonds influenced by surface functionality. Understanding bacterial adhesion to various surface chemistries helps identify surfaces susceptible to bacterial fouling and informs the design of biofilm prevention strategies for biomedical and industrial devices.

References

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