Application Note

(Macro)molecular adsorption onto hydrophilic and hydrophobic surfaces probed with QCM

The human body produces a class of glycoproteins, so called mucins, which fulfill critical functions during physiological processes. Mucins can adsorb onto surfaces with different properties, and this ability is critical for mucins to function properly. The Quartz crystal microbalance (QCM) qCell T Q2 is utilized to quantify the adsorption behavior of mucins. Measurements were successfully conducted on different surfaces and in the presence of other potentially interfering proteins as well as formation of a mucin-based multi-layer system.

Summary

The human body produces a class of glycoproteins, so called mucins, which fulfill several critical functions during physiological processes; examples include hydration, lubrication, and wear protection of epithelial surfaces [1,2]. Mucins can adsorb onto surfaces with different properties[3], and this ability is critical for mucins to function properly. As we describe here, quartz crystal microblance (QCM) measurements are well suited to quantify the adsorption behavior of mucins. We summarize example measurements conducted on different surfaces and in the presence of other, potentially interfering proteins. Finally, we show how the successfully formation of a mucin-based multi-layer system can be monitored by QCM. All experiments described here were conducted on a qCell T-Q2 platform.

Method

To investigate the adsorption kinetics of mucin glycoproteins onto surfaces with different wetting behaviors, we developed a process that allows for applying a thin layer of hydrophobic polydimethylsiloxane (PDMS; Sylgard 184, Corning) onto a commercial gold sensor (obtained from 3T-Analytik): First, PDMS was mixed in a prepolymer/cross-linker ratio of 10:1 and diluted to 1 % (v/v) in n-hexane. 100 µL of this solution was applied to the center of a quartz crystal and distributed by spin-coating for 60 s using a WS-400B-6NPP/LITE spin coater (Laurell, North Wales, USA) set to a rotational speed of 3000 rpm. Afterward, the PDMS was cured at 80 °C for 4 h. As previously shown by a profilometric analysis of such PDMS-coated crystals, the thickness of the PDMS layer generated with this approach is ~3 µm. [4]

Results

When a water droplet is placed onto either a steel sensor or a gold sensor, static contact angles < 90° are measured, which corresponds to hydrophilic surface properties. In contrast, for a PDMS coated quartz sensor, the determined contact angle is well above 90° (Figure 1A), which is a clear indication of hydrophobic surface properties. We monitored the adsorption of mucin onto these three types of surfaces by recording the shift in resonance frequency over a time span of 45 min [2]. Such frequency shifts reflect the amount of mucin molecules, which adsorbed onto these surfaces. The amount of adsorbed mass can be estimated using the Sauerbrey equation - even if the adsorbed molecules form viscoelastic layers [5,6], which is the case for mucins. In our study, we obtained the strongest signal for PDMS-coated quartz sensors (Figure 1B). We interpreted this result such that, 2 on those hydrophobic surfaces, the mucins can adsorb via either one or both of their hydrophobic termini to constitute a brush-like surface layer (Figure 1C).

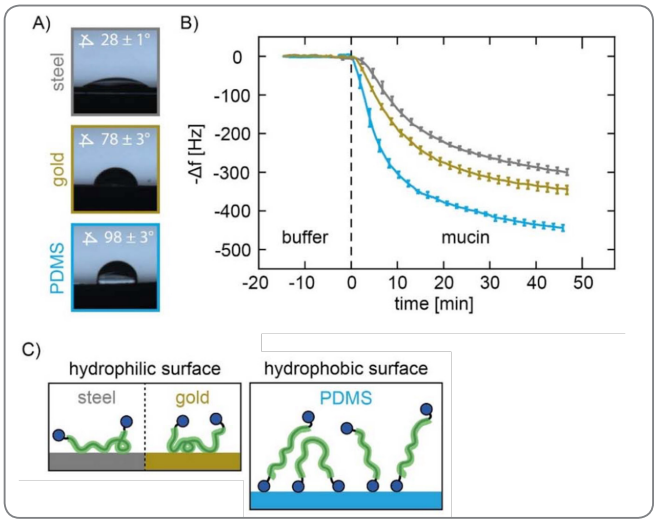

Figure 1. Adsorption of mucin glycoproteins onto steel, gold, and PDMS surfaces, respectively. A) Steel and gold surfaces are hydrophilic as indicated by a contact angle < 90°, whereas PDMS coated surfaces are hydrophobic. B) Mucin adsorption induces the strongest frequency shift on PDMS and the weakest frequency shift on steel surfaces. Error bars denote the standard error of the mean as obtained from at least three independent measurements. C) Mucins likely adsorb onto hydrophilic surfaces via their hydrophilic core region, whereas they are expected to adsorb onto hydrophobic surfaces via their termini.

In contrast, the frequency shifts we obtained when the mucin molecules were allowed to adsorb onto hydrophilic gold or steel surfaces were considerably lower, indicating that fewer molecules adsorbed. We speculated that mucins adsorb onto hydrophilic surfaces via their hydrophilic, elongated core region. In such a scenario, the area occupied by a single mucin molecule can be expected to be larger than when mucins adsorb via their termini – resulting in a lower density of adsorbed macromolecules.

We also employed QCM to study the competition between different molecules adsorbing to the same surface [7]. The combined adsorption of (pre-mixed) mucin and BSA showed almost no difference compared to the adsorption kinetics of a pure BSA solution alone (Figure 2A). This indicates, that BSA blocked the surface for the adsorption of mucin. For other combinations, i.e., mucin/amylase and mucin/lysozyme, we observed a stronger frequency shift compared to the when the corresponding small protein is tested alone. This showed that, different from what we described above for BSA, in the presence of either lysozyme or amylase, mucin adsorption is still possible – either onto free areas not covered by the smaller proteins or onto pre-adsorbed lysozyme/

amylase layers. Since the molecular weight of BSA, amylase, and lysozyme is much smaller than mucin, it appears reasonable that the resulting differences in their diffusion constants allows the small proteins to adsorb faster onto a surface than the large macromolecule mucin. To test this idea, a sequential exposure test was conducted, i.e., the small proteins were allowed to adsorb first followed by mucin adsorption (Figure 2B). In full agreement with our interpretation of the results obtained for competing adsorption, we detected successful adsorption of mucins onto pre-adsorbed protein layers generated by either amylose or lysozyme.

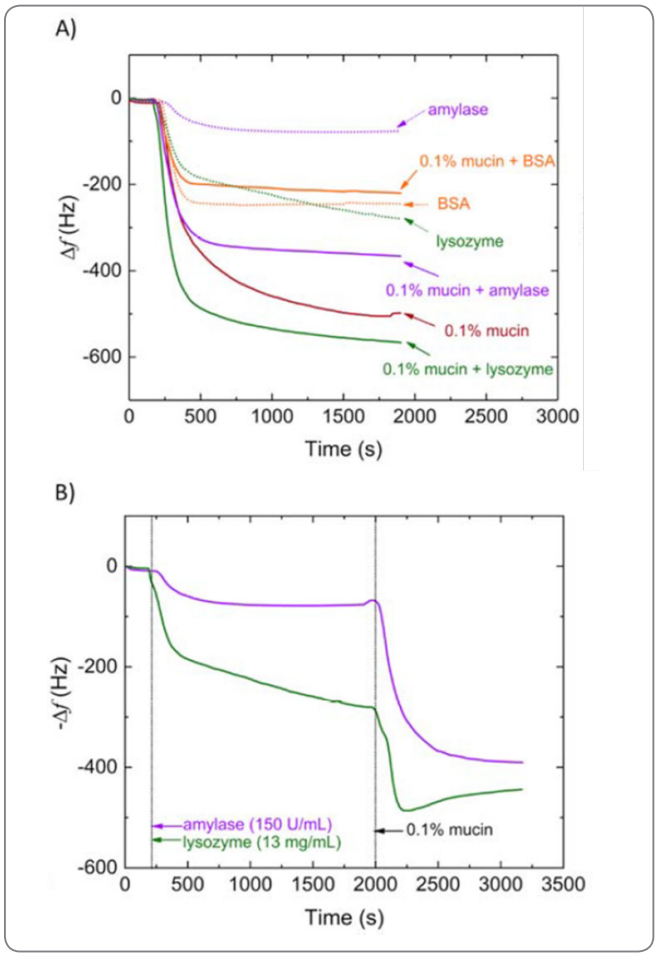

Figure 2. Competitive binding of mucins and different small proteins to PDMS surfaces. A) QCM measurements depict alterations in the frequency shift (Δf) as a function of time when probing the adsorption of BSA, amylase or lysozyme (in 20 mM HEPES buffer, pH 7) onto PDMS-coated Au-chips in the presence (solid line) or absence (dashed line) of 0.1 % (w/v) mucin, respectively. B) Sequential adsorption measurements onto PDMS-coated Au-chips. Here, first the small protein (amylase or lysozyme) is allowed to adsorb. Then, the precoated chip is exposed to a mucin solution.

Since monitoring such sequential adsorption events is possible, QCM can also be a powerful tool to monitor the assembly of molecular multi-layers. Here, the sequential injection of different molecules will entail a stepwise frequency shift indicating successful adsorption events. We attempted the experimental verification of a molecular multi-layer by QCM and obtained the results shown in Figure 3. Indeed, the recorded frequency signal not only showed that mucin-based multilayers can be successfully deposited onto a PDMS surface by employing molecular 'gluing' layers based on either lectin or dopamine [8]. We could also verify that a controlled, partial disassembly of this multi-layer is possible when exposing the system to a 'molecular scissor' (in this case, the small molecule GlcNAc).

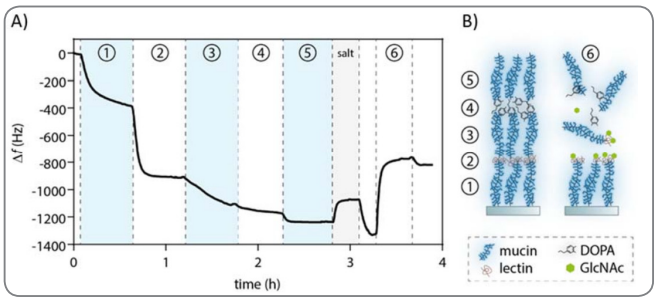

Figure 3: Assembly of a mucin based multi-layer construct verified by QCM. A) The successful layer-by-layer assembly (as well as their partial disassembly in the presence of GlcNAc) of mucin-based multi-layers cross-linked with two types of connector molecules, i.e., lectin and dopamine, is demonstrated by QCM measurements. B) Schematic representation of the generated mucin-based multi-layers.

Conclusion

Quartz crystal microblance (QCM) measurements are well suited to quantify the adsorption behavior of mucins with measurements conducted on different surfaces and in the presence of other, potentially interfering proteins. Successful formation of a mucin-based multi-layer system can be monitored by QCM.

Reference

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