Modulating Mucin Hydration and Lubrication by Deglycosylation and Polyethylene Glycol Binding

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A key property of mucin glycoproteins is their exceptional capacity to hydrate and lubricate surfaces. In vivo, mucins assemble into mucus hydrogels that cover the epithelium and protect it from dehydration and shear stress. A better understanding of the origin of these properties could lead to new treatment strategies for patients with poor mucus coverage, defective mucus production, or glycosylation as caused by Sjögren syndrome, dry eye, or in the case of certain bacterial infections. In this work, mucin coatings are used to show that mucin-associated glycans are essential for the formation of such hydrated and lubricating layers. Native mucins are compared with deglycosylated mucins by analyzing their hydration and it is shown that their lubricative potential in the boundary and mixed lubrication regime is linked to the hydration. The removal of glycans from the mucin results in a 3.5-fold decrease in hydration and an increase in friction by two orders of magnitude. This loss of function is countered by grafting polyethylene glycol (PEG) molecules to defective mucins through lectin–glycan interactions. This lectin-PEG conjugation restores hydration and improves lubrication of the partially deglycosylated mucin coatings. Thus, local complementation of defective mucus layers could prove to be a useful new treatment strategy.

1. Introduction

Mucus is a hydrogel that covers all wet epithelia in our body, including the eyes, lungs, gastrointestinal and urogenital tracts. Its gel-forming building blocks are secreted mucin glycoproteins, a family of high molecular weight and densely glycosylated biopolymers. Those mucins can act as an infection shield by trapping various viruses in the biopolymer matrix[1] and they reduce bacterial adhesion to surfaces.[2,3] Mucins also form highly hydrated and lubricative layers that protect the underlying epithelium from dehydration and shear stress that emerge during eye blinking or when swallowing food. Water typically accounts for up to 95% of the total mass in the mucus gel.[4,5] Shifts in the mucus water content correlate with substantial changes of the mucus barrier function[6–8] and result in important pathological disorders such as dry mouth[9] and dry eye.[10] Mucin-associated glycans contribute up to 80% of the molecular weight of mucins[11] and contain highly hydrated hydroxyl groups. Differences in the amount and composition of glycans from mucins extracted from pig stomachs and mucins from bovine submaxillary glands correlate with their capacity to adsorb water.[12] However, other properties such as protein composition also differ between these two mucin species, precluding the establishment of a definitive link between the presence of mucin glycans and hydration.

Mucins, and other well-hydrated molecules such as zwitterionic polymers, polysaccharides, and polyelectrolytes are generally good boundary lubricants when adsorbed[13,14] or assembled in brush structures[15–18] on surfaces. Hence, mucin-associated glycans could also be essential for the lubricative function of mucus. In a related system, the mucin-like macromolecule lubricin showed a significant decrease in lubricity when its associated glycans were removed.[19] Moreover, the lubricity of salivary films, which contain mucins, has also been correlated with the total amount of glycosylation present in the film. However, also here, the complexity of saliva composition precluded the identification of the specific role of mucin glycosylation.[20] Hence, there is a need for a more defined experimental system to determine if mucin glycosylation is essential to the hydration and lubrication properties of mucins.

This study explores the role of glycosylation in mucin hydration and lubricity, using mucin coatings as a simplified model system. Such mucin coatings can reconstitute levels of surface hydration[21,22] and lubrication[23–25] similar to those found in native mucus. Our data show that the removal of mucin-bound glycans results in a significant reduction in hydration and lubricity of the mucin-coated surface. We show that the hydration and lubricity of deglycosylated mucins can be partially

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restored by sequestering polyethylene glycol (PEG) polymers to the mucins. Together, this work provides a more detailed insight to the origin of mucus-mediated hydration and lubrication. It also shows that the substitution of mucin-bound glycans with synthetic polymers can, to a large extent, restore mucin hydration and lubricity.

2. Results and Discussion

2.1. Native and Deglycosylated Mucins Form Coatings with Different Quartz Crystal Microbalance with Dissipation (QCM-D) Signatures

To investigate the potential of mucin-bound glycans for hydration and lubrication, we used mucin coatings as a simplified model system. Mucin coatings were generated by adsorbing mucins onto gold surfaces. An analysis of the coating by QCM-D showed that the mucins formed voluminous and highly viscoelastic layers on the surface, as suggested by the high frequency shift and dissipation values recorded (**Figure 1**A,D). Moreover, the coatings appeared to be homogeneous at the microscopic scale when inspected by fluorescence microscopy (Figure 1A, inset). To evaluate the contribution of mucin-associated glycans to the properties of mucin coatings, these were chemically removed either completely (ApoMucin) or partially (pApoMucin), and coatings were generated from the deglycosylated mucins (Figure 1E). The reduction in glycan content following deglycosylation was confirmed by a periodic acid-Schiff assay and lectin staining for the mucin solutions and the resulting coatings, respectively (Figure S1, Supporting Information). QCM-D analysis showed that partially deglycosylated mucins formed coatings with lower QCM-D dissipation values than fully glycosylated mucins, suggesting a more collapsed and stiff layer (Figure 1B). To account for slight variations in adsorbed mass between mucin and pApoMucin, we normalized the dissipation (D, related to the mechanical properties) to the frequency shift (Δf, related to the mass adsorbed) (Figure 1C). A coating with native mucin resulted in a higher slope of the $D/\Delta f$ curve than a coating with pApoMucin. This confirmed that pApoMucin formed stiffer coatings per adsorbed unit mass than native mucin coatings.

2.2. Mucin Deglycosylation Results in Dehydrated and Poorly Lubricating Coatings

To analyze the role of mucin-bound glycans for hydration of the mucin coating, we measured the hydration of native and deglycosylated mucin coatings. The hydration of the coatings (**Figure 2**A) was determined by comparing their hydrated mass, obtained via QCM-D, with their dry mass, obtained by drying the same samples then subjecting them to ellipsometry measurements. To best isolate the contribution of mucin glycans and since the hydration of mucin coatings did not seem to be sensitive to changes in NaCl concentrations (Figure S2, Supporting

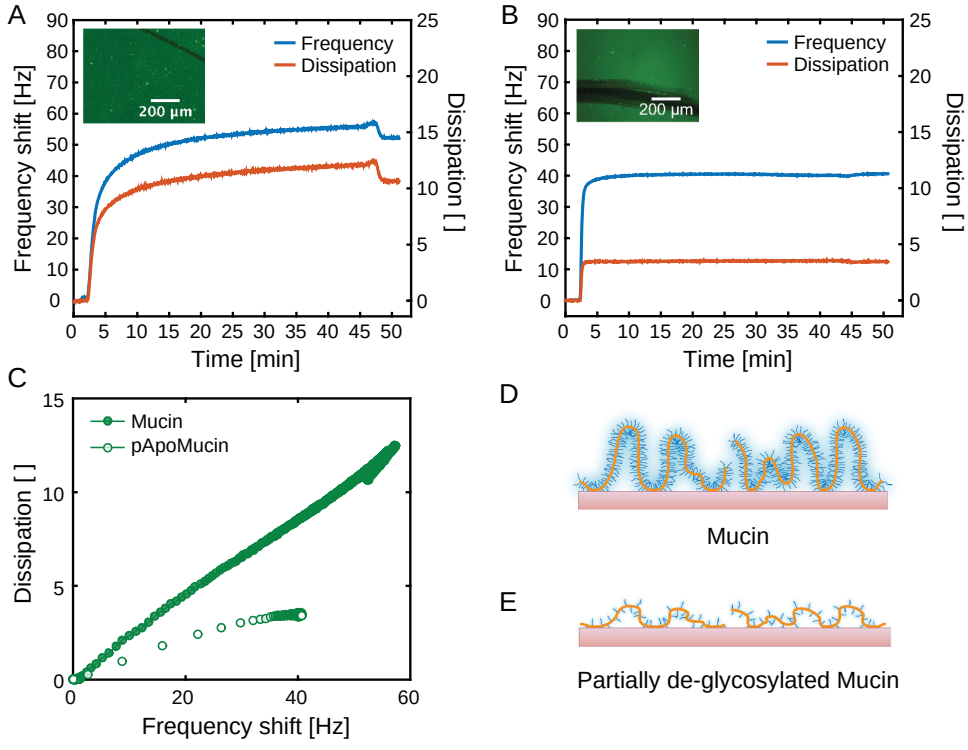

Figure 1. Native and deglycosylated mucin coatings. Quartz crystal microbalance frequency shift and dissipation measurement due to the adsorption of A) native mucin and B) partially deglycosylated mucin to a gold surface. The inset pictures show fluorescence microscopy images of the coatings with a scratch to enhance contrast. C) Changes in dissipation and frequency shift during adsorption. Schematic depiction of D) native mucin coating and E) partially deglycosylated mucin coatings.

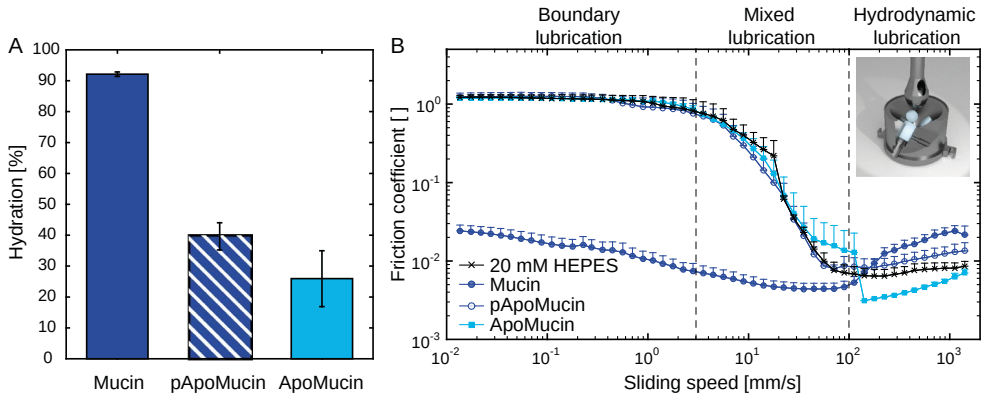

Figure 2. Mucin deglycosylation results in coating dehydration and loss of lubricity. A) Hydration of native mucin, partially deglycosylated (pApoMucin) and fully deglycosylated (ApoMucin) mucin coatings. The error bars denote the standard deviation to the mean of at least seven independent experiments. B) Lubrication of PDMS surfaces by native mucin, pApoMucin, and ApoMucin compared to aqueous buffer (HEPES). The error bars denote the error of the mean as obtained from three independent experiments. The inset depicts a schematic representation of the tribological setup used for friction measurements.

Information), we kept the pH and ionic strength of mucin adsorption and wash buffers constant for our experiments. The removal of mucin glycans resulted in a sharp drop of hydration ($p < 0.001$), from 92 ± 1% to 40 ± 4% and 26 ± 9% for partially and fully deglycosylated mucin, respectively. Similar to native mucins, both partial and fully deglycosylated mucins adsorbed to polydimethylsiloxane (PDMS) surfaces used for tribological measurements and formed coatings of comparable surface density to those on gold as judged by fluorescence measurements (Table S1, Supporting Information).

Since mucus layers experience a variety of different sliding speeds, contact pressures, and diverse lubricant viscosities, it is important to characterize the lubricating potential of mucins over a broad range of velocities. For instance, in the ileum a sliding speed of 2.3 mm s^{-1} was measured whereas in the jejunum the corresponding value is with 20.2 mm s^{-1} one order of magnitude higher.[26] During eye blinking, a friction speed of 150 mm s^{-1} was reported.[27] Our tribological results show that native mucins can effectively lubricate PDMS surfaces over a broad range of sliding speeds (Figure 2B), primarily in the boundary and mixed lubrication regime. Here, the mucin coating reduced the friction coefficient by two orders of magnitude.

Mucins may achieve energy dissipation and thus friction reduction by two independent mechanisms: First, surface bound mucin can provide hydration lubrication which is based on an exchange of trapped water molecules with free water molecules in the fluid.[28] Second, shearing off whole adsorbed mucin macromolecules from either the PDMS or the steel surface may additionally contribute to the observed reduction in friction.[24]

When coatings were built from partially or fully deglycosylated mucins, the friction coefficient was as high as without any mucin coating. This effect can result from a combination of multiple phenomena: The loss of hydration upon mucin deglycosylation can affect the hydration lubrication at the molecular scale. It can also influence the coating structure, which has been shown to impact mucin coating lubricity.[24] In addition, molecular interactions between the deglycosylated mucins and the two surfaces can bind the opposing surfaces together and increase friction. Such bridging effects have indeed been demonstrated with porcine gastric mucins at high contact pressures (several MPa) and at the nanoscale.[29] Finally, also the adsorption efficiency and adsorption strength of the different mucin variants may be affected by altering the density of mucin glycans, and this could influence both the efficiency of mucin desorption from and readsorption to surfaces during a friction measurement.

Obviously, mucin-bound glycans play an important role in mediating hydration and lubrication, and an alteration of mucin glycosylation by pathogens or dysfunctional mucin expression is likely to result in dehydrated and poorly lubricating mucus. A molecular "repair" approach, which compensates the loss of mucin function triggered by deglycosylation, might open new avenues for the treatment of some mucus-related diseases.

2.3. PEG Can be Sequestered to Mucins by Conjugation to a Lectin

The mixing of polymers such as PEG,[30] and other polymers[31–33] to mucins has been used to alter the properties of mucinous systems. We here developed an alternative strategy by substituting the glycans with PEG polymer chains (**Figure 3**A). PEG was chosen for its known biocompatibility and the well-characterized hydration[34,35] and lubrication[36–39] capacity. Deglycosylation can decrease the molecular weight of mucins by up to 80%,[40,41] thus relatively large 40 kDa PEG chains were chosen to compensate for that loss. PEG itself interacts only minimally with mucin coatings (Figure S3, Supporting Information). To facilitate mucin/PEG interaction, PEG was conjugated to wheat germ agglutinin (WGA), a lectin-type protein that binds sugars found in mucins and on cell surfaces.[42,43] The conjugation reaction resulted in high molecular weight macromolecules (>135 kDa) consisting of three or more PEG chains associated per WGA monomer (Figure S4, Supporting Information). The bioconjugate (WGA-PEG) was able to adsorb to both coatings from native mucins and from partially deglycosylated mucins (Figure 3B) in a mucin concentration dependent manner, suggesting that the PEG grafting did not inhibit the sugar binding capacity of the lectin.

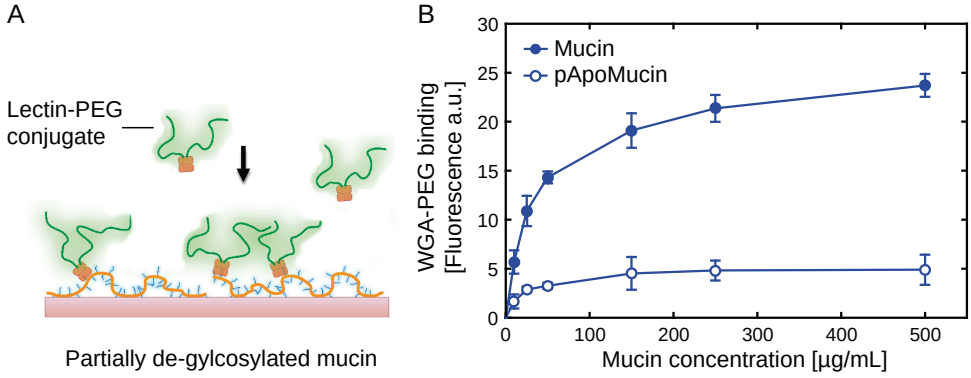

Figure 3. WGA-PEG conjugates can bind both mucins and partially deglycosylated mucins. A) Schematic illustration of our strategy to restore hydration and lubrication of deglycosylated mucin coatings by grafting PEG to mucins through mucin–lectin interactions. B) Fluorescence of FITC labeled WGA-PEG conjugates bound to immobilized native mucin and partially deglycosylated mucins (pApoMucin). The mucin concentration indicated is the concentration used to immobilize mucins to the surface. The error bars denote the standard deviation to the mean of three independent experiments.

2.4. Lectin-PEG Recovers Hydration and Lubrication of Deglycosylated Mucin Coatings

We added the PEG-WGA conjugates to partially deglycosylated mucin coatings and measured the resulting hydration of the coating. **Figure 4**A shows that the addition of PEG-WGA to deglycosylated mucin coatings resulted in a boost of hydration ($p < 0.001$) close to levels found in fully glycosylated mucins. For comparison, neither WGA nor PEG alone significantly ($p = 0.167$ and $p = 0.181$, respectively) impacted the hydration of the coating, which stagnated at around 50% of the hydration of unaltered mucins (Figure S5A, Supporting Information). As a second control, PEG conjugates were generated with Sambucus Nigra (SNA) lectin. SNA binds sialic acid residues,[44] which are rare in the pig gastric mucin used for this study.[45] As a consequence, SNA binding to mucin is weak (Figure S7, Supporting Information), and there was no significant impact of SNA-PEG on hydration ($p = 0.151$), confirming that binding of the PEG to the pApoMucin coating is necessary to restore hydration. If the hydration state of deglycosylated mucins can be brought back to that of intact mucins, then the lubricating abilities of those "repaired mucins" might also approximate those of fully glycosylated mucins. We tested this hypothesis by quantifying the lubricating abilities of partially deglycosylated mucin coatings to which WGA-PEG was bound. Indeed, WGA-PEG binding to pApoMucin improved the lubricity of the coating both in the boundary and mixed lubrication regime (Figure 4B). In the latter, i.e., between friction speeds of 1 and 100 mm s^{-1}, pApoMucin + WGA-PEG layers were even equally efficient in reducing friction as intact mucin. Compared to the other lubricants (pApoMucin and pApoMucin + SNA-PEG), a significantly decreased friction was observed at sliding speeds smaller than 1.78 mm s^{-1}.

In contrast, adding just PEG alone did not alter the lubrication of the coating (Figure S5B, Supporting Information), suggesting that direct binding of the PEG to the mucin is critical to restore its lubricating properties. This was confirmed by a series of control experiments, which showed that the SNA-PEG variant added to pApoMucin coatings had no effect on hydration and lubrication. Similarly, no strong effect was measured

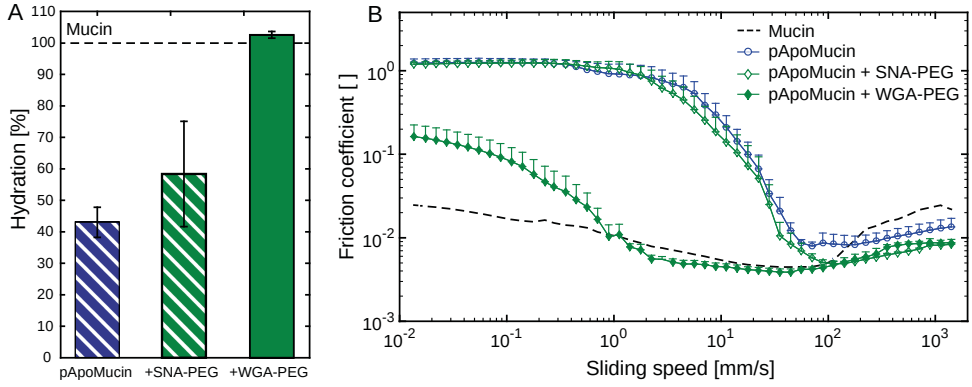

Figure 4. WGA-PEG restores hydration and lubrication of partially deglycosylated mucin coatings. A) Calculated hydration of partially deglycosylated mucin coatings treated with WGA-PEG or SNA-PEG. All values are normalized to the hydration state of native mucin coatings (=100%). The error bars denote the standard deviation to the mean of three independent experiments. B) The lubrication of partially deglycosylated mucin coatings treated with WGA-PEG or SNA-PEG is compared to native mucin coatings. The error bars denote the error of the mean as obtained from three independent experiments.

when WGA-PEG and SNA-PEG were added to ApoMucin coatings, presumably due to ApoMucin preventing nonspecific adsorption on the surface (Figure S5B, Supporting Information) while offering no glycan residues the lectin conjugates could bind to. When combining ApoMucin with either lectin-PEG conjugates, we found that the overall friction curve was shifted to the left (Figure S5B, Supporting Information), suggesting that the mixed and hydrodynamic regime is entered at smaller sliding speeds for those lubricants. Therefore, we speculate that this effect might be due to partial adsorption of lectin-PEG to the steel sphere or the PDMS cylinders. It might be possible that ApoMucin cannot fully block those surfaces thus granting partial access for lectin-PEG binding. Alternatively, differences in the lubricant viscosities could also explain the shifted onset of mixed lubrication. However, the viscosity of all lubricants is similar (Figure S8, Supporting Information) which is also consistent with our observation that no significant alteration of the hydrodynamic lubrication regime occurred for the various lubricants.

Together, these results show that the hydration and lubricative properties of PEG can be transferred to the mucin coating provided that the mucins are targeted by the correct lectin. However, although the improvement brought about by WGA-PEG could be satisfactory in certain cases, native mucin coatings still outperformed the WGA-PEG treated compromised mucin layers at very low friction speeds (Figure 4B). This highlights that the structural and chemical complexity of native mucins cannot be fully recovered by this simple substitute. Moreover, whereas the molecular repair approach demonstrated here can successfully restore the hydration capabilities of compromised mucins, it is unlikely that other mucin properties such as their antibacterial or antiviral activities can be equally rescued by grafting PEG molecules to defective mucins. PEG can only recapitulate some of the properties of the complex mixture of glycans present in native mucins. Future research may build on the strategy demonstrated here and explore other chemical grafting motifs thus addressing a broader range of mucin properties beyond hydration and lubricity. Accordingly, the design and production of fully synthetic mucins which can reproduce the broad spectrum of mucin properties is still an ongoing challenge.

3. Conclusions

In this study, we have shown the importance of mucin glycans in mucin hydration and lubrication. It implies that alterations to the glycans in vivo through dysfunctional post-translational processing or enzymatic digestion of mucin glycans can result in deficient mucus layers. Drugs such as rebamipide are currently investigated for their capacity to boost mucin production and help restore proper hydration of the mucosa,[46] but a topical treatment of the mucus with polymers could be a viable alternative. We show that although the simple addition of PEG to the fluid phase of the lubricant has no effect, immobilizing PEG to the adsorbed mucins greatly enhances hydration and lubrication. Based on these results, one can speculate on the rational for the natural design of mucins. Mucins-associated glycans hold water and confer an elongated structure to the molecule, while the protein backbone provides the macroscopic structure of mucin assemblies and promotes adsorption to surfaces. Our results suggest a correlation between the presence of mucin-associated glycans, the structure of mucin coatings, coating hydration, and the lubricity of these surfaces.

4. Experimental Section

Materials and Reagents: Wheat germ agglutinin lectin (WGA, Vector Labs), fluorescently labeled WGA (FITC-WGA, Vector Labs), Sambucus Nigra lectin (SNA, Vector Labs), fluorescently labeled SNA (FITC-SNA, Vector Labs), and methoxy PEG succinimidyl carboxymethyl ester (40 kDa, mPEG-SCM, JenKem) were purchased. For control experiments using PEG, mPEG-SCM was inactivated by 20×10^{-3} M hydroxylamine (Sigma-Aldrich) at room temperature for 1 h. The hydroxylamine was then removed by centrifugation filtering (Pall, 10 kDa MWCO). Mucins were extracted from pig stomachs (pig gastric mucins, PGM) following a previously published protocol, omitting the cesium gradient density step.[47] Briefly, the mucins were extracted by gently scraping the lumen of pig stomachs and dissolving the mucus in 200×10^{-3} M NaCl adjusted to pH 7.4. The mucins were then purified by a series of centrifugation and size exclusion chromatography separations. Mucin, partially deglycosylated mucins (pApoMucin) and fully deglycosylated mucins (ApoMucins) were fluorescently labeled by mixing a carboxylic acid succinimidyl ester amine-reactive derivative of the Alexa488 dye (Invitrogen) with the protein (at a 1:10 protein to dye ratio) in a carbonate–bicarbonate buffer (0.2 M, pH 8.5) for 1 h at room temperature. The excess dye was eliminated by centrifugation filtering (Pall, 10 kDa MWCO).

Mucin Deglycosylation: Partial deglycosylation (pApoMucins) was obtained by β-elimination of the glycans. In brief, mucins were dissolved in 500×10^{-3} M $NaBH_4$/50×10^{-3} M NaOH solution and incubated at 50 °C for 4 h. The reaction was quenched with ice-cold glacial acetic acid while keeping the sample on ice. The detached glycans were then separated from the protein via centrifugation filtering (Amicon, 30 kDa MWCO). The mucin was fully deglycosylated (ApoMucins) following a previously published protocol consisting of an acidic treatment using trifluoromethanesulfonic acid (TFMS) followed by the oxidation and β-elimination of the residual sugars.[48] The relative glycan content of native mucins, partially deglycosylated mucins, fully deglycosylated mucins, and bovine serum albumin solutions as a negative control were assessed by a periodic acid-Schiff colorimetric assay following a previously published protocol.[49] The decrease in sugar content of the coatings was revealed by an enzyme-linked lectin assay (ELLA) using fluorescently labeled WGA. In short, mucin, deglycosylated mucin, or bovine serum albumin coatings were generated in the wells of 96-well plates. A solution of FITC-WGA (50 μg mL^{-1}, in phosphate-buffered saline, PBS, pH 7.4) was introduced to the wells. After a 30 min incubation time the lectin solution was removed, and the coatings washed three times with 200 μL of PBS before the fluorescence of the wells was measured using a fluorescence plate reader (Spectramax M3, Molecular Device).

Mucin Coatings: Mucin, pApoMucin, or ApoMucin coatings were generated by incubating the surface for 1 h, with a 0.2 mg mL^{-1} solution dissolved in 20×10^{-3} M HEPES, pH 7.4), except for the tribology experiments for which 1 mg mL^{-1} solutions were used. Coatings of fluorescently labeled mucins on gold surfaces were observed using an Observer Z1 inverted fluorescent microscope (Zeiss) and a 10× 0.3 NA objective (Zeiss). To ensure similar coverage between mucin preparations on PDMS surfaces, the surface density of adsorbed protein was quantified. To do so, 96-well plates (351172, Becton Dickinson) were coated with PDMS by introducing 50 μL of PDMS (SYLGARD 184, 10% curing agent, Dow Corning) and rotating the plate to coat the wells. The PDMS was then cured overnight at 60 °C. Alexa488-pApoMucin or Alexa488-ApoMucin was then introduced into the well, incubated for 1 h, and residual mucin was washed off with 20×10^{-3} M HEPES solution,

pH 7.4. The resulting fluorescence was compared to a calibration curve performed in solution.

Lectin-PEG Conjugate Preparation: The lectin-PEG conjugates were synthesized by combining WGA, FITC-labeled WGA, or SNA lectins with 40 kDa mPEG-SCM (1:10 lectin to PEG ratio) in bicarbonate buffer (0.1 M, pH 8). The reaction was left for 2 h at room temperature with moderate shaking. The solution was then purified by multiple centrifugation filtering (100 kDa MWCO) removing free 40 kDa PEG and 36 kDa WGA. The resulting bioconjugate concentration was measured using bicinchoninic acid assays (Invitrogen) using WGA or SNA as standards. Quality control of the bioconjugation was performed by SDS-PAGE, where protein was stained using SimplyBlue stain (Invitrogen) and PEG was stained by soaking the gel in a 5 wt% barium chloride solution (Sigma) followed by a 0.1 M iodine solution (Sigma). The limited binding of SNA to mucins was demonstrated by incubating a polystyrene surface, a mucin coating, or an ApoMucin coating with an FITC-SNA solution (50 μg mL^{-1} FITC-SNA in PBS, pH 7.4). After an incubation time of 30 min, the lectin solution was removed, and the coatings were washed three times with 200 μL of PBS before the fluorescence of surfaces/coatings was measured using a fluorescence plate reader (Spectramax M3, Molecular Device).

Mucin Binding of WGA-PEG: To test the WGA-PEG binding to mucin, WGA was first adsorbed on polystyrene 96-well plate (0.3 mg mL^{-1} in PBS for 30 min). The wells were then blocked with BSA (20 mg mL^{-1} in PBS for 1 h). The wells were coated with mucins or pApoMucin from solutions of various concentrations (0–1 mg mL^{-1}, in 20×10^{-3} M HEPES, pH 7.4, for 30 min) followed by extensive washing to remove unbound mucins. The fluorescently labeled WGA-PEG conjugate was then added (0.15 mg mL^{-1} in PBS for 30 min). Unbound WGA-PEG was removed by washing the wells with PBS. Finally, the fluorescence of the wells was measured using a fluorescence plate reader (Spectramax M3, Molecular Device).

Hydration Measurements: The hydration of the coatings was measured by combining QCM-D monitoring and ellipsometry measurements. A QCM-D instrument equipped with flow cells (E4 system, Q-Sense, Sweden) was used to measure the hydrated mass of the coatings. The crystals used (QSX 301, Q-sense) were purchased, coated with gold, and cleaned by successively treating the surface with a detergent (0.1% sodium dodecyl sulfate, 10 min, 60 °C), acid (1 M HCl, 10 min, 60 °C), and 15 min in an UV–ozone cleaner. The protein solutions were pumped to fill the reservoir above the crystal surface, and then the flow was stopped to allow the coating to form. The crystal vibration was followed at its fundamental frequency (5 MHz) and six overtones (15, 25, 35, 45, 55, and 65 MHz). Changes in the resonance frequencies and in dissipation of the vibration once the excitation was stopped were followed at the seven frequencies. Shifts in frequency are related to changes in adsorbed mass whereas changes in dissipation reflect the mechanical properties of the adsorbed coating. Given the high dissipation values generated by mucin coatings, the Sauerbrey relation that ties frequency and adsorbed mass through a linear relationship did not apply. Thus, a Voigt-based model was used to accurately estimate the hydrated thickness.[50] The density of the mucin coating was fixed at 1050 kg m^{-3} which is between that of pure water (1000 kg m^{-3}) and pure protein (1350 kg m^{-3}).[51]

The dry thickness of mucin coatings was determined by spectroscopic ellipsometry using an XLS-100 ellipsometer (J.A. Woollam Co.). The measurements were performed at an angle of 70° and at wavelengths from 190 to 993 nm with 70 spectroscopic scans per measurements and four measurements per sample. The coatings previously generated on gold covered QCM-D crystals were rinsed with water and dried under nitrogen flow. A multilayer model composed of a silicon substrate (0.2 mm), a gold layer (75 nm), and a Cauchy layer of unknown thickness was used to calculate the mucin coating dry thickness (WVASE32 software, version 3.768). A density of 1200 kg m^{-3} was assumed to calculate the total mass.[52] The level of hydration of the film was deduced from the dry and hydrated mass using the relationship

$$\text{Hydration}\,(\%) = \frac{\text{hydrated mass} - \text{dry mass}}{\text{hydrated mass}} \times 100 \quad (1)$$

Lubrication Measurements: For friction measurements, a commercial shear rheometer (MCR 302, Anton Paar) was equipped with a tribology unit (T-PTD 200, Anton Paar), and the measurements were performed in a ball-on-cylinder geometry as described in ref. [53] and shown as an inset in Figure 2B. As friction partners in the tribology setup, PDMS cylinders and steel spheres with a diameter of 12.7 mm (Kugel Pompel) were chosen. The rationale for this choice was that, with this particular tribo pairing, the dynamic range between lubrication with simple HEPES buffer and lubrication with a solution of intact mucins was largest compared to when glass or Teflon spheres were used as friction probes (Figure S6, Supporting Information). This enabled to better distinguish intermediate levels of lubrication than with the other pairings. The PDMS cylinders were prepared by mixing PDMS (SYLGARD 184, Dow Corning) in a 10:1 ratio with the curing agent and exposing the mixture to vacuum for 1 h to remove air bubbles before curing at 80 °C for 1 h. Before each measurement, the cylinders were cleaned with 80% ethanol and ddH_2O. During the measurements, a normal force of 6 N was applied resulting in a contact pressure of ≈0.1 MPa. This normal force was chosen such that friction in the boundary, mixed, and hydrodynamic regime could be probed. However, here the boundary lubrication regime is focused on as the main aim of this study is to investigate the ability of mucins to act as boundary lubricants. The friction behavior was evaluated by performing a logarithmic speed ramp from 1000 to 0.01 mm s^{-1} and the friction coefficient was measured over 10 s per speed level. Before the first measuring point, the system was allowed to stabilize at the highest rotational speed for 10 s. All measurements were performed at room temperature and the PDMS cylinders were fully covered with lubricant during measurements. When native, partially, or fully deglycosylated mucin solutions (20×10^{-3} M HEPES, pH 7.4, 1 mg mL^{-1}) were used as lubricants, the PDMS cylinders were incubated in the lubricant for 1 h prior to the measurement. When lectin-PEG or PEG was used, the PDMS cylinders were incubated in the mucin solution (20×10^{-3} M HEPES, pH 7.4, 0.85 mg mL^{-1}, final concentration) for 60 min, with the addition of lectin-PEG (20×10^{-3} M HEPES, pH 7.4, 0.15 mg mL^{-1}, final concentration) after the first 30 min. Similarly, the incubation solution was afterward used as lubricant. Using mucin-free buffer as a lubricant after incubating the PDMS and/or steel surface was less efficient in reducing the friction coefficient in the boundary lubrication regime (Figure S9, Supporting Information) compared to when mucin was also present in the lubricating fluid.

Statistical Analysis: The statistical significance of variations in hydration measurements (five independent measurements per condition) was calculated by performing an unpaired *t*-test using SigmaPlot software (v13, Systat Software). The statistical significance of variations in the friction measurements (three independent measurements per condition) was calculated by performing one-way ANOVAs and Tukey post hoc tests. All statistical analyses were performed using R (Foundation for Statistical Computing). A p-value < 0.05 was used as a measure for statistical significance.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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