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Title: Probing the Role of Mucin-Bound Glycans in Bacterial Repulsion by Mucin Coatings

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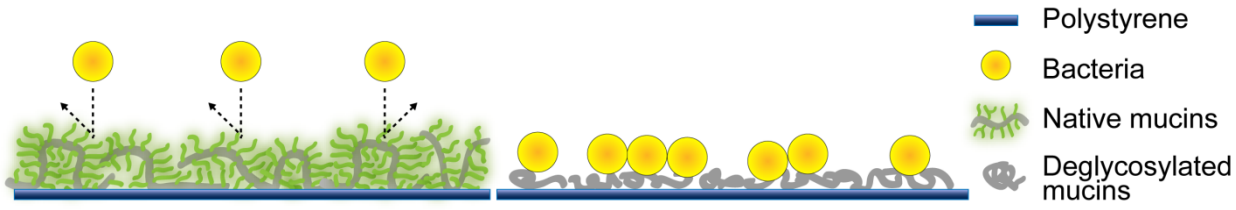
Mucin coatings suppress surface attachment by the bacteria *Streptococcus pneumoniae* and *Staphylococcus aureus*. Bacterial repulsion by mucin coatings is dependent on the presence of mucin-bound glycans. Upon mucin deglycosylation, the coatings display measurable changes in coating thickness and softness, as well as antifouling capacity. This study highlights the structural and functional role of glycans in mucin coatings.

J. Y. Co[†], T. Crouzier[†], and K. Ribbeck*

Probing the Role of Mucin-Bound Glycans in Bacterial Repulsion by Mucin Coatings



Abstract

Microbial colonization of implanted medical devices in humans can lead to device failure and life-threatening infections. One strategy to prevent this unwanted colonization is to coat devices with polymers that reduce bacterial attachment. This study investigates how mucins, a class of biopolymers found in mucus, can be used as surface coatings to prevent attachment of selected respiratory pathogens to polystyrene surfaces. Our data show that coatings of porcine gastric mucins or bovine submaxillary mucins reduce surface attachment by *Streptococcus pneumoniae* and *Staphylococcus aureus*, but not *Pseudomonas aeruginosa*. To elucidate how mucin coatings repel *S. pneumoniae* and *S. aureus*, the molecular components of mucins are examined. Our data suggest that mucin-bound glycans are key structural contributors of mucin coatings and are necessary for the repulsive effects toward *S. pneumoniae* and *S. aureus*.

1. Introduction

Microbial colonization is a leading cause of medical device failure. About 50% of indwelling devices become colonized by microbes,[1] causing a significant fraction of hospital acquired infections.[2] Bacterial colonization begins with cell attachment to the device surface. Attached cells proliferate and mature to form resilient matrix-encased communities called biofilms. Once established, biofilms are difficult to eradicate due to their resistance to antimicrobial treatments. Hence, there is a strong focus on developing new surfaces to prevent bacterial attachment.

This study explores the natural mucus barrier, and specifically its gel-forming mucin polymers, for strategies to prevent surface attachment. Mucus is the hydrated polymer network that lines all wet epithelia in the human body, including the respiratory, digestive, and reproductive tracts. Mucins are highly glycosylated polymers, which exist in secreted and cell-surface forms.[3–6] Both mucin types can protect the underlying mucosal epithelia from microbial infection. For example, the secreted gastric mucin MUC5AC can maintain the bacterium *Pseudomonas aeruginosa*[7] and the yeast *Candida albicans*[8] in a planktonic state and impair biofilm development. A similar effect has been observed for human salivary mucin MUC5B toward the bacterium *Streptococcus mutans.*[9] Moreover, cell-surface mucins can reduce *Staphylococcus aureus* attachment to corneal epithelial cells[10] and can limit *Helicobacter pylori* attachment to gastric epithelial cells[11]

Given their ability to suppress microbial surface attachment in native conditions, mucins have also been studied in the context of microbe-repelling coatings on polyacrylic acid, poly(methyl methacrylate), silicone, polyurethane, and polystyrene surfaces.[12,13] These studies show that it is difficult to predict how mucin coatings perform because their function depends on

the surfaces to which they are adsorbed and on deposition conditions. For example, bovine submaxillary mucin coatings reduce *C. albicans* attachment to silicon surfaces, but increase its attachment to poly(acrylic acid-*b*-methyl methacrylate) (PAA-*b*-PMMA) surfaces.[13] This variability reflects a gap in our understanding of which functional domains and biochemical properties of mucin coatings are important in mucin-microbe interactions.

This study investigates which molecular components of mucins contribute to bacterial repulsion, specifically when coating polystyrene surfaces. The respiratory pathogens *Streptococcus pneumoniae*, *S. aureus*, and *P. aeruginosa* often colonize medical devices to cause infection,[2] and thus were examined here. Our data show that coatings made of gastric or submaxillary mucins efficiently prevented surface attachment of *S. pneumoniae* and *S. aureus*, but not of *P. aeruginosa.* To dissect the role of mucin-bound glycans in bacterial repulsion, properties of native mucin coatings were compared to those of deglycosylated mucin coatings. We found that upon deglycosylation, mucin coatings changed in structure and lost their ability to repel bacteria, indicating that mucin-bound glycans are structural components capable of regulating surface attachment by *S. pneumoniae* and *S. aureus*.

2. Results and Discussion

2.1 Mucin Coatings Reduce Surface Attachment of *S. pneumoniae* and *S. aureus*, but Not of *P. aeruginosa*

Surface attachment of three common respiratory pathogens (*S. pneumoniae, S. aureus,* and *P. aeruginosa*) to mucin coatings was evaluated using in-house purified porcine gastric mucins and commercial bovine submaxillary mucins. First, mucin coatings were created by exposing polystyrene surfaces to mucin solutions. Mucin adsorption to polystyrene, presumably

driven by hydrophobic interactions between the surface and the mucin protein core[14,15] was verified using fluorescence microscopy and quartz crystal microbalance with dissipation monitoring (QCM-D) analysis, which confirmed that the mucins formed relatively homogeneous coatings (Figure S1, Supporting Information). To qualitatively assess the capacity for bacterial repulsion, mucin-coated polystyrene microtiter wells were exposed to bacteria. Attached bacteria were fluorescently stained with SYTO 9 (Life Technologies), then visualized microscopically. **Figure 1A** shows that both gastric and submaxillary mucin coatings reduced *S. pneumoniae* and *S. aureus* surface attachment compared to uncoated polystyrene surfaces, but had no effect on *P. aeruginosa* attachment. To quantify attachment, bacteria bound to the mucin-coated or uncoated polystyrene microtiter wells were evaluated using the CyQuant Assay (Life Technologies). **Figure 1B** reveals that gastric mucin coatings reduced attachment of *S. pneumoniae* by 76.3% ± 8.6% and attachment of *S. aureus* by 81.3% ± 2.0% to underlying polystyrene. Submaxillary mucin coatings were comparably effective, reducing attachment of *S. pneumoniae* by 71.5% ± 3.8% and of *S. aureus* by 81.0% ± 7.5% to the underlying polystyrene. Together, these data suggest mucin MUC5AC isolated from pig stomachs as a potential candidate biopolymer for the engineering of bacteria-repelling coatings. The data also extend our understanding of coatings generated by commercial submaxillary mucins to show that in addition to preventing *S. aureus* surface attachment,[12] they appear to repel *S. pneumoniae*. Importantly, these results highlight the limitations of mucins for universal bacterial-repelling surfaces because they appear ineffective against *P. aeruginosa*, a Gram negative bacterium. This lack of repulsive effect is not generalizable to other Gram-negative bacteria because *Escherichia coli,* for example, can be repelled by mucin coatings.[13] Several mechanisms may contribute to the lack of effect toward *P. aeruginosa*. For example, when adsorbed to a surface, mucins may lose part of the biofilm-

suppressing functionality, which is exhibited in a 3D hydrogel network. Mucin-digesting enzymes secreted by *P. aeruginosa*[16] may also damage coating integrity and hence, its repulsive properties. Moreover, adhesins on bacterial surfaces,[17,18] which bind mucin-associated glycans[19,20] and mucin-peptide moieties,[21] may mediate interactions with the coatings.

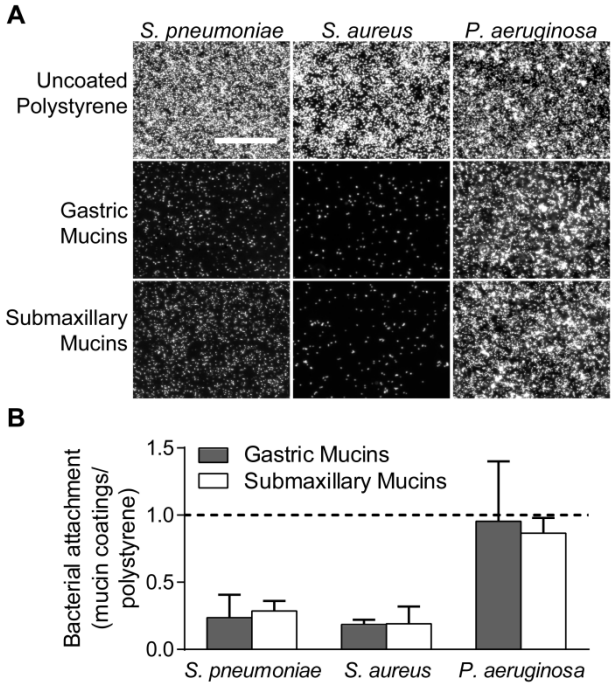


Figure 1. Mucin coatings reduce bacterial attachment. A) *S. pneumoniae*, *S. aureus*, or *P. aeruginosa* attachment to polystyrene microtiter wells coated with in-lab purified porcine gastric mucins or commercially purified bovine submaxillary mucins (Sigma-Aldrich). Attached bacteria were stained with SYTO 9 (Life Technologies) and visualized by fluorescence icroscopy. Scale bar: 100 μm. B) Quantification of bacterial attachment to gastric or submaxillary mucin coatings generated in polystyrene microtiter wells using the CyQuant Assay (Life Technologies). Values represent bacterial attachment to mucin coatings normalized to bacterial attachment to uncoated polystyrene. Error bars represent standard deviation.

2.2 Mucin-Bound Glycans Contribute to Repulsion of *S. pneumoniae* and *S. aureus*

Mucin-bound glycans within mucin coatings can contribute to the repulsion of mammalian cells.[22] To examine if mucin-bound glycans also play a role in bacterial repulsion,

bacterial attachment was tested on coatings made from deglycosylated mucins, which are henceforth referred to as apo-mucins. Apo-mucins were generated from native mucins by chemical removal of mucin-associated glycans. Mucin deglycosylation was verified using the periodic acid-Schiff (PAS) assay (**Figure 2A**). Apo-mucins were adsorbed to polystyrene surfaces to produce coatings, and fluorescence microscopy of Alexa488-labeled apo-mucin coatings confirmed relatively homogeneous surface coverage (Figure S2, Supporting Information).

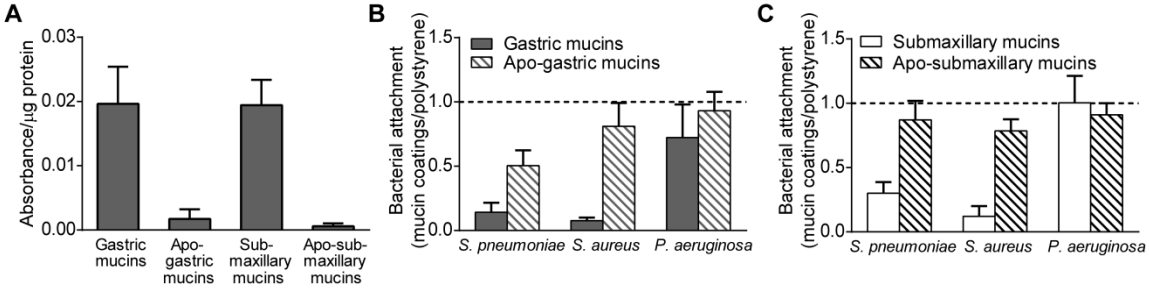


Figure 2. Removal of mucin-bound glycans reduces bacterial repulsion by mucin coatings. A) PAS assay of mucins and apo-mucins verified deglycosylation. B,C) Quantification of bacterial attachment to B) native gastric mucin and apo-gastric mucin coatings or C) native submaxillary mucin and apo-submaxillary mucin coatings generated in polystyrene microtiter wells using the CyQuant Assay (Life Technologies). Values represent bacterial attachment to mucin coatings normalized to bacterial attachment to uncoated polystyrene. Error bars represent standard deviation.

Figure 2B shows that the removal of glycans from mucins reduced the coatings' ability to repel *S. pneumoniae* and *S. aureus*, but not *P. aeruginosa*, whose attachment was comparable on native mucin and apo-mucin coatings. Specifically, apo-gastric mucin coatings exhibited a 4.2-fold increase in *S. pneumoniae* attachment and a 10.8-fold increase in *S. aureus* attachment relative to native gastric mucin coatings (**Figure 2B**). Similarly, apo-submaxillary mucin coatings had 3.1-fold more *S. pneumoniae* and 8.3-fold more *S. aureus* attached compared to their glycosylated counterparts (**Figure 2C**). These data indicate that mucin-bound glycans

contribute to the bacterial repulsion observed with gastric and submaxillary mucin coatings. The glycan compositions of gastric and submaxillary mucins differ considerably,[23,24] suggesting that the repulsive effect of the coatings observed here is dictated not by the specific glycan components, but instead by general physico-chemical properties conserved among the different mucin types.

2.2 Physico-chemical Analysis of Mucin Coatings

Deglycosylation alters the biochemistry and structure of the mucin polymers, which in turn will affect the overall properties of the mucin-coated surfaces. Generally, both the charge and hydrophilic properties of surfaces can affect their interactions with bacteria.[25–29] Therefore, these two properties were evaluated for the bacteria and the mucin-coated surfaces. First, we investigated the role of surface charge using zeta potential measurements. The data indicate that the surfaces of all three bacterial species used in this study had a negative zeta potential (Table S1, Supporting Information). To measure the zeta potential of mucin-coated surfaces, we used polystyrene beads (800 nm diameter) to which native or deglycosylated mucins were adsorbed. As depicted in **Table 1**, uncoated polystyrene beads exhibited a stronger negative charge than beads coated with the fully glycosylated mucins. For comparison, apo-gastric mucins appeared to render the polystyrene surface more negatively charged than fully glycosylated native gastric mucins. Beads coated with apo-submaxillary mucins displayed no substantial change compared to glycosylated mucins (**Table 1**). One limitation of this experiment is that we were not able to identify if the changes in surface charge between native and apo-mucins stem from differences in surface adsorption, or from differences in the biochemical properties between the different mucin species. However, what may be concluded is that there is no measurable correlation between the

zeta potential of the mucin-coated surfaces and their strength of repulsion, suggesting that surface charge is probably not the main parameter in this system to control bacterial surface adhesion.

Surface	Zeta potential [mV]	Contact angle [°]
Polystyrene	-35.0 ± 6.4	65.6 ± 3.3
Gastric mucins	-14.1 ± 1.1	25.5 ± 6.6
Apo-gastric mucins	-29.5 ± 2.8	29.0 ± 8.5
Submaxillary mucins	-21.8 ± 1.7	24.0 ± 6.4
Apo-submaxillary mucins	-16.2 ± 4.1	30.3 ± 8.4

Table 1. Biochemical properties of mucin coatings from glycosylated and deglycosylated mucins. Zeta potential measurements were used to determine relative charge of native mucin and apo-mucin coatings on polystyrene beads. Contact angle measurements were used to determine hydrophobicity of native mucin and apo-mucin coatings. Reported values are mean ± standard deviation.

In the next set of experiments, we used water contact angle measurements to investigate the hydrophilicity of the bacteria and the mucin-coated surfaces. Contact angles of bacteria-coated polystyrene slides revealed that all three species tested in this study were hydrophilic (Table S1, Supporting Information). Using polystyrene slides coated with the different mucin species, we showed that mucin-coated polystyrene surfaces were more hydrophilic than uncoated polystyrene (**Table 1**). This observation is consistent with previous findings that mucins reduce the contact angle of polystyrene surfaces.[12] Our data also show that the deglycosylation of mucins did not substantially alter the contact angle of the mucin-coated surfaces. The lack of correlation between the contact angle of the different surfaces and their ability to prevent bacterial adhesion suggests that the hydrophilicity of the surface may not be the dominant factor

to control microbial adhesion in this system. However, we note that these data need to be interpreted with caution, given their high variability.

As a third line of characterization, QCM-D was used to examine the hydrated thickness and softness of native mucin and apo-mucin coatings. **Figure 3A** shows that gastric mucin coatings were 35.0 ± 9.9 nm thick, while submaxillary mucin coatings were 60.3 ± 3.2 nm thick. In contrast, apo-gastric mucins and apo-submaxillary mucins both formed thinner coatings that were less than 4 nm thick. QCM-D analysis also provided information about the softness of the coatings as a measure of energy dissipation of the acoustic waves. **Figure 3B** shows that the dissipation was greater for coatings of native mucins than for their apo-mucin counterparts. Upon deglycosylation, the dissipation measurements decreased 21-fold for gastric mucin coatings and 2.5-fold for bovine submaxillary mucin coatings, indicating that apo-mucin coatings are stiffer than the native mucin coatings. Together, these data reveal that mucin-bound glycans provide mucin coatings with a certain thickness and softness, which are lacking in coatings generated with the apo-mucins (**Figure 3C**). Thickness and softness can increase bacterial repulsion of coatings made from certain classes of synthetic polymers, such as polyethylene glycol.[30–32] Hence, also in the context of mucins, these parameters are likely important to modulate interactions with the bacteria. How the thickness and softness of coatings contribute to bacterial repulsion, whether by steric repulsion, the degree of hydration, or by other mechanisms, remains to be determined.

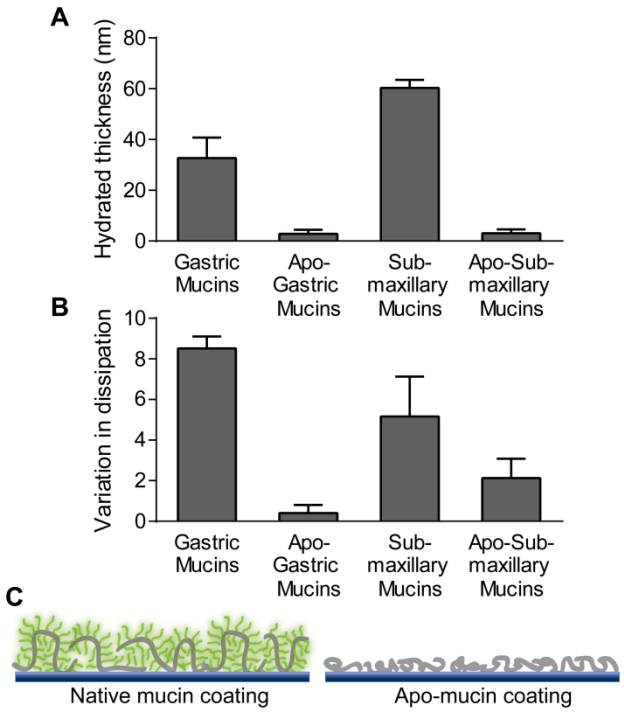


Figure 3. Mucin glycans contribute to the thickness and softness of mucin coatings. A,B) Coatings produced from native mucins or apo-mucinswere analyzed with QCM-D. Native mucins formed A) thicker coatings and showed B) greater dissipation, which is indicative of a softer coating. Error bars represent standard deviation. C) Schematic depiction of glycan contributions to the structure of mucin coatings.

3. Conclusions

In humans, implanted medical devices are frequently a cause of nosocomial infections, which, if left untreated, can lead to high rates of device failure and severe systemic infection. In the body, mucins help protect the wet epithelia from microbial attachment and subsequent infection, and hence, show potential as building blocks for microbe-repelling surface coatings. This study shows that coatings of gastric mucins and submaxillary mucins on polystyrene surfaces prevent attachment of *S. aureus* and *S. pneumoniae*, two common respiratory pathogens. Our work furthermore suggests that mucin-bound glycans are necessary to prevent bacterial attachment, potentially by introducing a critical thickness and softness to the mucin coatings, which in the context of other polymer systems, is associated with enhanced antifouling

properties.[30–32] Together, these findings support a role for mucin-bound glycans in regulating host-microbe interactions. Mucin coatings could also inform the development of new antifouling materials that reduce unwanted microbial colonization of implanted medical devices.

4. Experimental Section

Preparation of Mucins: Bovine submaxillary mucins (Sigma-Aldrich) were dialyzed for 4 d against ultrapure water using a Spectra/Por Float-A-Lyzer G2 dialysis membrane (100 kDa MW cutoff, Spectrum Labs), then lyophilized for storage. Native porcine gastric mucins were purified as previously reported.[33] Briefly, mucus was scraped from fresh pig stomachs and solubilized in saline buffer with protease inhibitors and sodium azide. Insoluble material was pelleted by ultracentrifugation and mucins were purified using size exclusion chromatography on a Sepharose CL-2B column. Mucin fractions were desalted, concentrated, and lyophilized for storage. To produce fluorescent mucins, gastric and submaxillary mucins were labeled with Alexa488 (Life Technologies) following the manufacturer's instructions. Briefly, Alexa488 succinimidyl ester in DMSO (10 μL, 10 mg mL^{-1}) was added to mucins (1 mL, 3 mg mL^{-1}) in bicarbonate buffer (0.2 M, pH 8). After incubation at room temperature for 1 h, free dye was separated from the labeled mucins using a Macrosep centrifugal filter (100 kDa MW cutoff, Pall).

Preparation of Apo-mucins: Mucins were deglycosylated by treatment with trifluoromethanesulfonic acid (TFMS), followed by oxidation and beta-elimination of the residual sugars as previously described.[34] Lyophilized mucins (5 mg) were cooled on ice and mixed with ice-cold TFMS (375 μL) containing anisole (10% v/v). The solution was gently stirred on ice for 2 h then neutralized by the addition of a solution containing 3 parts pyridine, 1

part methanol and 1 part water. Precipitates were dissolved by adding water. The solution was dialyzed for 2 d against ultrapure water using a dialysis membrane (20 kDa MW cutoff, Spectrum Labs). NaCl and acetic acid were then added to the solution (final concentration of 0.33 M and 0.1 M, respectively). The solution was adjusted with NaOH to pH 4.5. For the oxidation step, ice-cold $NaIO_4$ (0.2 M) was added to the mucin solution (final concentration of 0.1 M $NaIO_4$), and incubated at 4 ºC for 5 h in the dark. The unreacted periodate was destroyed by adding ½ volume of neutralizing solution containing $Na_2S_2O_3$ (0.4 M), NaI (0.1 M), and $NaHCO_3$ (0.1 M). For elimination, the mucin solution was adjusted to pH 10.5 using NaOH (1 M) and incubated at 4 ºC for 1 h. The solution was then dialyzed overnight at 4ºC against $NaHCO_3$ buffer (5×10^{-3} M, pH 10.5) and further dialyzed for 2 days against ultrapure water. The resulting apo-mucin solution was then concentrated and dissolved in the appropriate buffer.

Apo-mucins were evaluated for glycan removal using the periodic acid-Schiff assay as previously described.[35] Briefly, in a 96 well plate, a mixture (120 µL) of acetic acid (7%) and periodic acid (0.06%) was added to the sample (20 µL). The solution was incubated for 1.5 h at 37ºC. Schiff reagent (100 µL) was added to the wells, and allowed to react at room temperature for 10 min before measuring absorbance (550 nm) using a SpectraMax M2 Microplate Reader (Molecular Devices). The ratio of absorbance to mucin mass contained in each sample was reported.

Mucin Coatings: Mucin coatings were generated by incubating gastric mucins (200 μg mL^{-1}) or submaxillary mucins (200 μg mL^{-1}) in HEPES (0.02 M, pH 7.4) on the surface of interest. For mucin coatings in 96-well polystyrene microtiter plates, native mucins (200 μg mL^{-1}) were incubated in wells at room temperature for 1 h, followed by 3 washes with dH_2O. For apo-mucin

coatings, deglycosylated mucins (200 μg mL^{-1}) were incubated in wells at room temperature for 1 h, followed by 3 washes with dH_2O.

Microscopy of Mucin Coatings: To verify homogeneity, mucin coatings were visualized in the polystyrene microtiter wells. The wells were coated with gastric mucins, submaxillary mucins, or their apo-mucin counterparts labeled with Alexa488. A scratch was made in the film with a pipette tip for reference. The fluorescent coatings were imaged using an Axio Observer Z1 inverted epifluorescence microscope (Zeiss) and a 40×/0.75 NA objective (Zeiss) and Hamamatsu OrcaR2 camera.

Measuring Bacterial Attachment to Mucin Coatings: Bacterial attachment to mucin coatings and apo-mucin coatings was evaluated for *S. pneumoniae, S. aureus*, and *P. aeruginosa*. Microtiter wells were coated with mucins as described above, or left untreated as a control. *S. pneumoniae* TIGR4 serotype 19F was cultured in Todd Hewitt Broth (Becton Dickinson) supplemented with 0.5% Yeast Extract (Becton Dickinson) in static conditions at 37°C with 5% CO_2. *S. aureus* UAMS-1 was cultured in Brain Heart Infusion (Becton Dickinson). *P. aeruginosa* PAO1 was cultured in Luria Broth (Becton Dickinson). *S. aureus* and *P. aeruginosa* were grown shaking at 37°C. Bacteria were grown to logarithmic phase, centrifuged and resuspended in PBS to OD_{600} 0.4. Bacteria were incubated in uncoated or mucin-coated wells at 37°C for 1.5 h for *S. aureus* and *P. aeruginosa* or 3 h for *S. pneumoniae*, which required a longer incubation to achieve detectable attachment. Unattached bacteria were aspirated, and wells were washed with dH_2O. For visualization, the attached bacteria were stained with SYTO 9 (15×10^{-6} M , Life Technologies) in PBS for 15 min, then imaged using an Axio Observer Z1 inverted

epifluorescence microscope (Zeiss) with a 10×/0.3 NA objective (Zeiss) and Hamamatsu OrcaR2 camera. For quantification, the attached bacteria were evaluated using the CyQuant direct cell proliferation assay (Life Technologies) following the manufacturer's protocol, and measured with a SpectraMax M2 Microplate Reader (Molecular Devices). Fluorescence of bacteria attached to mucin coated wells was normalized to fluorescence of bacteria attached to uncoated control wells. Experiments were performed at least in triplicate.

Zeta Potential: Zeta potential measurements for mucin coatings were obtained by adsorbing native mucins or apo-mucins on the surfaces of polystyrene beads (~800 nm diameter, Sigma). Beads (4 μL of a 10% solids solution) were incubated for 1 h in native mucins (100 μL, 200 μg mL^{-1}) or apo-mucins (100 μL, 200 μg mL^{-1}) in HEPES (0.02 M, pH 7.4), then washed 3 times with PBS. Zeta potential measurements were carried out in PBS using a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments). As a control, zeta potential was measured for uncoated polystyrene beads in PBS. Zeta potentials of bacteria were measured using a solution of bacteria OD_{600} 0.05 in PBS. Measurements were performed in triplicate.

Contact Angle: Contact angle measurements for mucin coatings were obtained using coatings of native mucins or apo-mucins on polystyrene slides (Electron Microscopy Sciences). Native mucins (100 μL, 200 μg mL^{-1}) or apo-mucins (100 μL, 200 μg mL^{-1}) in PBS were incubated on polystyrene surfaces for 1 hour at room temperature. The coatings were washed with PBS 3 times, followed by a dH_2O wash to prevent salt crystal formation. Contact angle measurements for bacterial surfaces were obtained using bacteria adsorbed to polystyrene slides. Bacteria (100 μL) at OD_{600} 0.4 in PBS were incubated on polystyrene slides for 3 h, followed by a wash with

MilliQ water. The mucin or bacteria coatings were then air dried for 1 h, and the contact angle of a MilliQ water drop (~5 μL) was measured using a goniometer (camera-equipped VCA 2000, AST Products). The reported values are averages of triplicate measurements of the advancing angle, meaning the constant angle between the liquid and the surface as the drop increased in volume.

QCM-D: Quartz crystal microbalance with dissipation monitoring (QCM-D, E4 system, Q-Sense) was used to measure the hydrated mass of mucins adsorbed to a polystyrene-coated quartz crystal (QSX305, Q-sense). Solutions of native mucins (200 μg mL^{-1}) or apo-mucins (200 μg mL^{-1}) were adsorbed to the crystal. The crystal vibration was followed at its fundamental frequency (~5 MHz) and five overtones (15, 25, 35, 45, 55 and 65 MHz). Once the excitation was stopped, changes in the resonance frequencies and dissipation of the vibration were followed at the six frequencies. When the adsorbed layers are highly hydrated they usually possess viscoelastic properties requiring the measurement data to be modeled. Hydrated thickness was calculated using the Q-Tools 3.0.12.518 software that includes the Voigt model (i.e. a spring and dashpot in parallel under no slip conditions)[36] assuming a density of 1050 kg m^{-3} (as validated for a related system)[37] and that the coating is homogeneous in thickness and over the crystal's surface. Measurements were performed at least in duplicate.

Supporting Information

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

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Supporting Information

Probing the Role of Mucin-Bound Glycans in Bacterial Repulsion by Mucin Coatings

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Figure S1. Mucin coatings are homogeneous. (A, B) Coatings were produced on polystyrene slides of Alexa488-labeled (A) gastric mucins or (B) submaxillary mucins and visualized microscopically. A scratch was made in the coatings with a pipette tip for visual comparison with uncoated polystyrene regions. Scale bars: 50 μm. (C, D) QCM-D analysis shows saturation of mucins on a polystyrene coated crystal for (C) gastric mucins or (D) submaxillary mucins.

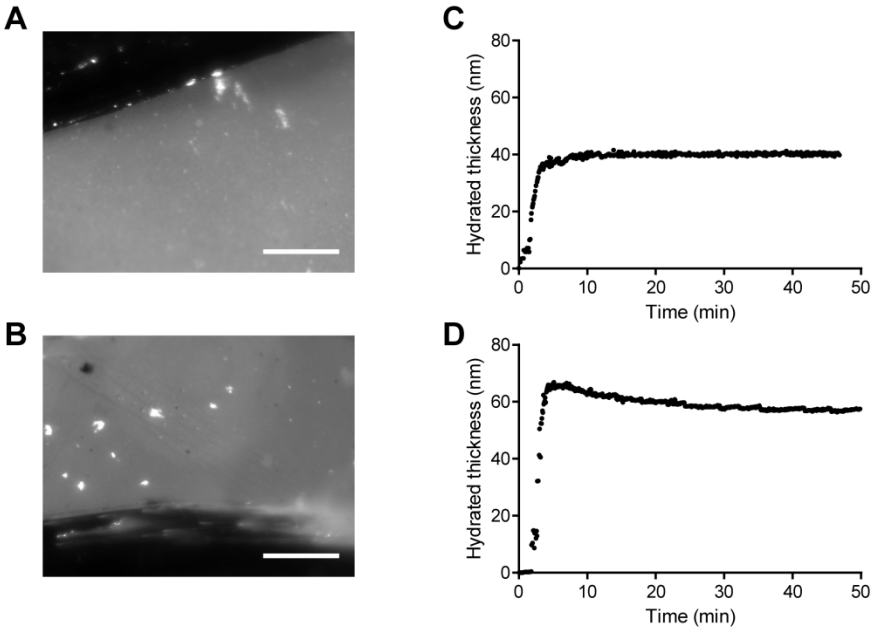

Figure S2. Apo-mucin coatings are homogeneous. (A, B) Coatings were produced on polystyrene slides of fluorescently labeled apo-gastric mucins (A) or apo-submaxillary mucins and visualized microscopically (B). A scratch was made in the coatings with a pipette tip for visual comparison with uncoated polystyrene regions. Scale bars: 50 μm.

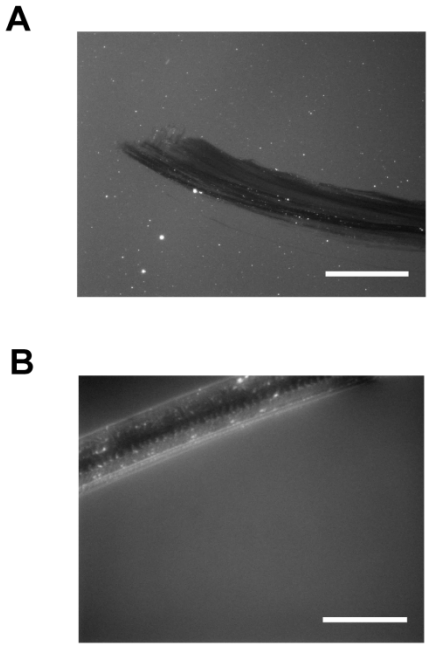

Table S1. Bacteria are negatively charged and hydrophilic. Zeta potential measurements were used to determine relative charge of *S. pneumoniae, S. aureus*, or *P. aeruginosa* cells. Contact angle measurements were used to determine hydrophobicity of *S. pneumoniae*, *S. aureus*, or *P. aeruginosa* coated surfaces. Reported values are mean ± standard deviation.

Organism	Zeta potential [mV]	Contact angle [°]
S. pneumoniae	-24.3 ± 0.8	43.0 ± 2.7
S. aureus	-18.9 ± 2.7	50.6 ± 24.0
P. aeruginosa	-17.5 ± 3.2	40.2 ± 15.8