Mucin-inspired lubrication on hydrophobic surfaces

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ABSTRACT

In the human body, high-molecular-weight glycoproteins called mucins play a key role in protecting epithelial surfaces against pathogenic attack, controlling the passage of molecules towards the tissue and enabling boundary lubrication with very low friction coefficients. However, neither the molecular mechanisms nor the chemical motifs of those biomacromolecules involved in these fundamental processes are fully understood. Thus, identifying the key features that render biomacromolecules such as mucins outstanding boundary lubricants could set the stage for creating versatile artificial superlubricants. We here demonstrate the importance of the hydrophobic terminal peptide domains of porcine gastric mucin (MUC5AC) and human salivary mucin (MUC5B) in the processes of adsorbing to and lubricating a hydrophobic PDMS surface. Tryptic digestion of those mucins results in removal of those terminal domains which is accompanied by a loss of lubricity as well as surface adsorption. We show that this loss can in part be compensated by attaching hydrophobic phenyl groups to the glycosylated central part of the mucin macromolecule. Furthermore, we demonstrate that the simple biopolysaccharide dextran can be functionalized with hydrophobic groups which confers efficient surface adsorption and good lubricity on PDMS to the polysaccharide.

Keywords: Biopolymers, Boundary lubrication, Hydrated layer, Hydration lubrication, Amphiphiles
INTRODUCTION

In the human body, all wet epithelia are covered with mucus, a transparent viscoelastic hydrogel which has two important functions: first, mucus shields the underlying tissue from pathogenic attack, and second, it provides mechanical protection for the tissues when they are exposed to shear forces. The macromolecular key component of mucus is mucin, a complex glycoprotein which can have molecular weights up to several MDa. Those densely glycosylated proteins can be divided in three distinct groups: membrane-bound epithelial mucins, secreted non-gel-forming mucins and secreted gel-forming mucins with the latter being the major constituent of mucus. Reconstituted solutions of manually purified mucins reproduce key properties of native mucus, i.e. they reduce viral activity, limit biofilm formation, and (when used as coatings) reduce cell and bacterial adhesion to surfaces. Moreover, similar to native mucus systems such as saliva and tear fluid, mucin solutions reduce friction both on artificial and biological surfaces: in the boundary lubrication regime, the friction coefficient $\mu$ measured with mucin-based lubricants can be as small as $\mu = 0.01$ or even less.

In this boundary regime, hydrodynamic effects are negligible and two opposing surfaces come into direct contact. The very low friction coefficients observed with mucin-based lubricants are therefore critically related to the ability of the highly glycosylated mucin molecules to strongly adsorb to a broad range of surfaces. Both on hydrophilic and hydrophobic surfaces, mucins assemble into well-hydrated macromolecular layers, which prevent two opposing surfaces from getting into direct contact with each other. This process enables mucin or mucin-like molecules to lubricate numerous tissues in the human body: those tissues often comprise a mixture of
hydrophilic and hydrophobic parts (e.g. the corneal epithelium, the tongue or the surface of articular cartilage) with the latter being rendered hydrophilic by adsorption of e.g. mucins 24-28.

In part, this separation of two counter surfaces during shear is achieved by the macromolecule itself which is adsorbing to the surfaces and forms a hydrated layer. Friction is reduced by shearing off this polymer layer (‘sacrificial layer mechanism’) 28-30. The second mechanism that is involved in reducing friction between opposing surfaces is based on surface-bound water molecules (‘hydration lubrication’) trapped by the various hydrophilic moieties on the mucin molecule 31,32 (Figure 1a). To be efficient, the first mechanism requires the mucin molecule to quickly reattach to a surface after it was detached by shear forces during a friction process. The second mechanism involves the exchange of free water molecules from the lubricant solution with water molecules in the hydration layer of surface-adsorbed mucin molecules. This exchange takes place as a consequence of the shear occurring during the friction process and provides a surface-bound water layer that reduces friction.
Figure 1: Schematic overview of the molecular mechanisms responsible for the lubricity of mucin-based lubricants and mucin structure. a) Both the formation of a sacrificial surface layer and hydration lubrication contribute to the lubricity of mucin solutions (see main text for details). The amino acid sequence of human MUC5AC (b) and human MUC5B (c) is analyzed in terms of polar/nonpolar side chains and net charge (see methods for details). The red marks indicate the fragments of the mucin polypeptide which were detected by mass spectrometry after tryptic digestion (see Table S1 and S2 in the Supporting Information).

Together, sacrificial layer formation and hydration lubrication enable mucin-based lubricants to decrease friction by up to two orders of magnitude compared to lubrication with simple buffer \(^{11,12}\). Mucins carry both charged and polar moieties as well as hydrophobic residues \(^4\). Thus, mucin physisorption to surfaces can be mediated by two different types of physical forces: electrostatic interactions between charged moieties of the glycoprotein and oppositely charged groups on the surface of the material on the one hand, and hydrophobic interactions between the glycoprotein and the surface on the other hand. A mathematical analysis of the amino acid sequence of human MUC5B (salivary mucin) and human MUC5AC (gastric mucin) reveals that both types of interactions are indeed possible for either mucin variant studied here. Both mucins exhibit a mixed...
distribution of charged amino acids throughout the whole sequence, and the terminal domains of both MUC5AC and MUC5B feature an increased density of hydrophobic amino acids compared to the central (glycosylated) region of the glycoprotein (Figure 1b and c). In this central part of the molecule, the high amount of serine and threonine (both hydrophilic amino acids to which glycans are typically attached to via O-glycosidic bonds) is responsible for the comparably strong hydrophilic character of the peptide sequence. Together, this unfolded central hydrophilic part and the globular hydrophobic termini result in a daisy-chain-like configuration with amphiphilic character \(^{34, 35}\). It was already shown that the high glycosylation density on the mucin glycoprotein is crucial for mucin hydration, and that both the hydration state of mucin \(^2\) and the detailed glycosylation pattern \(^3\) are directly related to the lubricity of mucins \(^7\). This particular molecular architecture suggests that the hydrophilic amino acids in the central region of the polypeptide serve as anchor points for the glycosylation of the protein promoting mucin hydration. In contrast, the hydrophobic terminal regions may be responsible for mediating the attachment of the glycoprotein to hydrophobic surfaces.

We here show that the hydrophobic terminal peptide sequences of both human salivary mucin and porcine gastric mucin are crucial for the lubricating abilities of these glycoproteins on hydrophobic surfaces. Enzymatic removal of those peptide sequences not only eliminates mucin lubricity but also reduces the adsorption efficiency of the glycoproteins to PDMS. Vice versa, we demonstrate that the addition of hydrophobic groups to synthetic dextran molecules promotes the dextran adsorption to PDMS and conveys lubricity to the dextran solution if a hydrophobic surface is part of the tribological material pairing. Finally, we present a molecular repair approach that partially restores the lubricating potential of enzymatically treated mucins on hydrophobic surfaces by grafting artificial hydrophobic groups onto the damaged mucins.
MATERIALS & METHODS

Mucin purification

The purification process of mucins was described in detail previously\(^\text{[33]}\). In short, mucus was obtained by manual scraping pig stomachs after rinsing them gently with tap water. The mucus was diluted 5-fold in 10 mM sodium phosphate buffer (pH 7.0) containing 170 mM NaCl and stirred overnight at 4 °C. Cellular debris was removed via several centrifugation steps and a final ultracentrifugation step (150000 \(\times\) g for 1 h at 4 °C). Afterwards, the mucins were separated by size exclusion chromatography using an ÄKTA purifier system (GE Healthcare) and a XK50/100 column packed with Sepharose 6FF. The obtained mucin fractions were pooled, dialyzed against ultrapure water and concentrated by cross-flow dialysis. The concentrate was then lyophilized and stored at -80 °C. For purification of human salivary mucin (MUC5B), unstimulated human whole saliva was collected from healthy, non-smoking, 20-30 year old donors, which refrained from consuming food or beverages other than water for 1 h prior to saliva donation. Saliva samples were stored on ice during collection and purified using the same protocol as used for MUC5AC. Monomeric MUC5AC and MUC5B has a molecular weight of ~ 3 MDa including the glycan motifs attached to the protein backbone. As the formation of oligomers is possible for both mucin types, the molecular weight range for oligomeric MUC5AC and MUC5B is expected to be heterogeneous.

Dextrans

Dextrans with a MW of 150 kDa were obtained from TdB Consultancy (Uppsala, Sweden). The polysaccharides were either unmodified or modified with carboxymethyl (CM), diethylaminoethyl
(DEAE), or phenyl groups - the latter of which were present on the dextrans at densities of either 0.15 phenyl groups/glucose or 0.40 phenyl groups/glucose, respectively. 40 kDa dextran with a phenylation degree of 0.32 to 0.40 phenyl groups/glucose was obtained from Sigma Aldrich (St. Louis, MO).

Enzymatic digestion of mucin

Enzymatic treatment of mucins with trypsin was performed as described in Madsen et al. In brief, mucin was dissolved at 10 mg/mL in a 50 mM ammonium bicarbonate solution, and disulfide bonds were reduced by adding 5 vol% 200 mM DTT (dissolved in 100 mM ammonium bicarbonate) at room temperature for 1 h. 4 vol% of 1 M iodoacetamide dissolved in a 100 mM ammonium bicarbonate solution was added to alkylate the mucin again at room temperature for 1 h. The reaction was quenched by adding 20 vol% of DTT. For proteolytic degradation, 40 vol% of 1 mg/mL trypsin (from bovine pancreas, Sigma Aldrich, St. Louis, MO) dissolved in a 100 mM ammonium bicarbonate solution was added to the mucin solution and incubated at 30 °C for 18 h. Afterwards, the trypsinated mucin was purified and desalted via size exclusion chromatography and cross-flow filtration as described above for native mucin.

Mucin sequence analysis

The mucin peptide sequences used for analysis in this work were obtained from the UniProt protein database (MUC5AC, P98088; MUC5B, Q9HC84). Up to now, no peptide sequence for porcine gastric mucin is available yet. Thus, for our analysis, we used the sequence of human MUC5AC instead. Both the sequences of MUC5B and MUC5AC were first divided into sections of 50 amino acids each. To characterize the hydrophobic character of these sections, the number
of amino acids with nonpolar side chains (i.e., Gly, Ala, Val, Met, Leu, Ile, Pro, Trp, Phe) and polar side chains (i.e., Arg, Lys, Asn, Asp, Gln, Glu, His, Tyr, Ser, Thr, Cys) was counted. To analyze the charge distribution along the amino acid backbone, the number of charged amino acids at neutral pH (negatively charged amino acids: Asp, pKa = 3.9; Glu, pKa = 4.1; positively charged amino acids: Arg, pKa = 12.5; Lys, pKa = 10.5) was counted. Each charged amino acid was assigned one elementary charge, and the net charge of each section of 50 consecutive amino acids is displayed as a bar in Figure 1. The glycosylation pattern was estimated based on the distribution of the amino acids serine and threonine. They serve as an anchor for O-linked glycans with each O-linked oligosaccharide side chain comprising up to 20 sugar units. Each displayed glycan molecule in Figure 1 represents 10 monosaccharides. Cleavage sites of trypsin in the sequences of MUC5AC and MUC5B were analyzed with the tool PeptideCutter from ExPASy Bioinformatics Resources Portal.

Adsorption measurements

Adsorption measurements were performed with an eCell-T quartz crystal micro-balance (3T-analytik, Tuttlingen, Germany) and a Gamry eQCM 10M data acquisition device (Warminster, Pennsylvania, USA). The quartz crystals used for this study have a gold surface, which was spin-coated with a thin layer of polydimethylsiloxane (PDMS, Sylgard 184, DowCorning, Wiesbaden, Germany). Therefore, PDMS was mixed in a prepolymer/cross-linker ratio of 10:1 and diluted to 1 vol% in n-hexane. 100 µL of this solution was applied to the center of the quartz crystal distributed by operating the spin-coater at 3000 rpm for 60 seconds. Afterwards, the PDMS was cured at 80 °C for 4 h. A profilometric analysis of the coated crystals showed that the thickness of the PDMS layer was ~3 µm (see Supporting Information Figure S1).
The concentration of biomolecules used for adsorption measurements was 100 µg/mL, and each biomolecule type was diluted in filtered 20 mM HEPES buffer (filter threshold: 0.22 µm). For each measurement, a quartz crystal with a fresh PDMS coating was used. Prior to each measurement, the setup was equilibrated with 20 mM HEPES buffer until a stable frequency signal was reached. This procedure ensured that the coated PDMS layer (which can absorb small amounts of water \(^{4}\)) has reached an equilibrated state so that water uptake into this PDMS layer does not affect the measurement. At the beginning of each measurement, 2 minutes of HEPES buffer signal was recorded as a baseline. Afterwards, the biomolecules were injected at 100 µL/min for 2 minutes, and the flow rate was set to 10 µL/min.

Tribology

Friction measurements were conducted on a commercial shear rheometer (MCR 302, Anton Paar, Graz, Austria) equipped with a tribology unit (T-PTD 200, Anton Paar). The measurements were performed as described previously \(^{4}\). In brief, the setup used was a ball-on-cylinder geometry. As opposing friction partners, PDMS cylinders (Ø 5.5 mm) and steel spheres (Ø 12.7 mm, Kugel Pompel, Wien, Austria) were used. The PDMS cylinders were prepared by mixing PDMS prepolymer and cross-linker in a 10:1 ratio (Sylgard 184, DowCorning), exposing the mixture to 1 h vacuum and performing a final curing step at 80 °C for 4 h. Before a measurement, three pins were inserted into the sample holder and cleaned with 80 % EtOH and ultrapure H2O. The measurements were performed at room temperature and the PDMS cylinders were fully covered with lubricant. To be consistent with our previous study on the lubricity of mucin solutions \(^{22}\), a normal force of \(F = 6\) N was applied (leading to a contact area of \(\sim 8.1\) mm\(^2\) (Figure S2) and thus a contact pressure of \(\approx 0.35\) MPa), and the friction coefficient was recorded for sliding speeds from
1000 to 0.01 mm/s (logarithmic speed ramp, 10 measuring points per decade) using a measuring
time of 10 s per data point. The PDMS surface topology was analyzed (Figure S3) after lubricating
with unmodified dextrans (0.1 % in 20 mM HEPES) to rule out any artefacts generated by wear
while sliding a steel sphere over the PDMS surface. All lubricants were used at a concentration of
1 mg/mL diluted in 20 mM HEPES, pH 7.4.

Hydration measurements

The hydration of the mucin and dextran coatings was measured by combining two
complementary techniques. First, the hydrated mass of mucin and dextran coatings was assessed
using quartz crystal microbalance with dissipation monitoring (QCM-D, E4 system, Q-Sense)
using gold coated crystals (QSX 301, Q-sense) that were cleaned prior to use with a mixture of
20 % hydrogen peroxide and 80 % ammonium heated at 80 °C for 10 minutes. The mucin and
dextran solutions were prepared at 1 mg/mL in 20 mM HEPES pH 7 buffer and injected into the
instrument at a rate of 200 µL/min. In QCM-D, the changes in dissipation reflect the viscoelastic
properties of the adsorbed coating. The frequency and dissipation shifts were fed into a
Voigt-based model which was used to accurately estimate the hydrated mass (Q-tools software). 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$^3$).

Second, the dry mass was measured using a surface plasmon resonance technique (SPR, Biacore
2000, GE Healthcare). Mucins or dextrans were injected at a concentration of 1 mg/mL, dissolved
in 20 mM HEPES, pH 7. The adsorbed mass density was estimated assuming one converting
response units corresponded to 0.1 ng/mm$^2$. The hydration level of the coatings was deduced from
the dry and hydrated mass using the relationship described in Equation 1.
Repair of trypsinated mucin

Since chemical modification of the termini of trypsin treated mucins is very challenging, phenylation of trypsin treated MUC5B was performed by carbodiimide coupling of phenylethylamine (Sigma-Aldrich) to the carboxylic groups of sialic acid motifs along the central part of the trypsin-treated mucin molecules. Trypsin treated MUC5B was dissolved in a 20 mM MES buffer solution (pH 5.5) together with 58 mM EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, ThermoFisher) and 27 mM NHS (N-hydroxysulfosuccinimide, Sigma-Aldrich). After complete dissolution, 173 mM phenylethylamine dissolved in 20 mM MES buffer solution (pH 5.5) were added to the mixture, and the pH was adjusted to pH 5.5 by addition of HCl. The solution was left to react at room temperature for 2 hours. The mucin was then desalted and purified by chromatography (PD-10 column, GE Healthcare) and lyophilized before further use. The addition of the phenyl functionalities was verified by UV spectra measurement using 1 mg/mL solutions of the mucin variants.
RESULTS AND DISCUSSION

Previous studies revealed that commercially available mucins (e.g. porcine gastric mucin from Sigma Aldrich) lack key properties observed for native mucin systems. Prominent examples are that solutions of industrial gastric mucins lack the ability to form oligomers, they do not exhibit a sol-gel transition at acidic pH, and they are not able to reduce friction in the boundary lubrication regime at neutral pH. As a consequence, we here manually purify porcine gastric mucin following a previously described protocol, which preserves its native functional properties. To investigate the contribution of the terminal hydrophobic peptide domains of mucins to the lubrication potential of mucin solutions, we performed an enzymatic digestion of both MUC5AC and MUC5B using trypsin. This enzyme usually cleaves a broad range of peptide sequences. For this enzyme, an analysis of the sequences of human MUC5AC and MUC5B predicts ~300 cutting sites for either mucin (see Methods), and those cutting sites are distributed equally throughout the peptide sequence. However, both mucins are densely glycosylated, and protein glycosylation is generally known to protect the polypeptide against proteolytic activity. Indeed, it has been demonstrated that porcine gastric mucin is partially resistant to peptidases, and accordingly, we expect only partial degradation of MUC5AC and MUC5B by trypsin. A chromatographic separation after enzymatic treatment of either mucin (Figure 2a and b) indicated that the treated mucins in fact retained a very large molecular weight and that the cleaved groups were much smaller than the remaining glycoprotein.
Figure 2: Lubricity of native and enzymatically treated mucins. Chromatograms of size exclusion chromatography of MUC5AC and MUC5B after tryptic digestion are shown in (a) and (b), respectively. Tribological measurements were performed with a steel/PDMS pairing using 0.1 mg/mL mucin dissolved in HEPES buffer. The measurements show the lubricating abilities of either native or tryptic digested MUC5AC (c) or MUC5B (d), respectively. HEPES buffer is included as a reference. The error bars denote the standard deviation as obtained from three independent measurements.

Mass spectrometry confirmed that the peptide fragments obtained after trypsin treatment of human MUC5B indeed originate from the terminal region of the glycoprotein (Figure 1c and Table S2). We interpret this result such that the central part of human MUC5B, which is densely glycosylated, is shielded against proteolytic degradation. The unprotected terminal parts, however, are accessible for the enzyme and seem to be broken down into small fragments of similar size. For porcine MUC5AC, a detailed peptide sequence is not available yet in the literature. However, the chromatographic profiles obtained for both trypsin treated mucins are very similar and only exhibit one second peak at later fractions in addition to the mucin main peak, which occurs at similar fractions as untreated mucin. Additionally the analysis of the peptide fragments of trypsinated porcine MUC5AC via mass spectroscopy show a coverage with the human MUC5AC sequence (Table S2). The overall coverage is lower than for MUC5B but the pattern is similar, as
fragments only of the terminal mucin domains can be identified. This suggests that, also for the porcine MUC5AC used here, trypsin treatment did mostly generate small peptide fragments which are likely to originate from the terminal, unglycosilated region of the glycoprotein. After separating them from the proteolytic fragments, the remaining mucin glycoproteins were tested for their lubricity. When the treated mucins were used as a 0.1 % (w/v) lubricant in a steel/PDMS tribology setup, we observed an almost complete loss of their lubricating abilities, especially in the boundary lubrication regime (Figure 2c and d).

To efficiently reduce friction by enabling hydration lubrication, macromolecular lubricants such as mucins have to form well-hydrated surface coatings. Thus, the inability of trypsin-digested mucins to lubricate in a hydrophobic PDMS/steel pairing could be due to a reduced hydration of such glycoprotein coatings compared to untreated mucins. Since the strong hydration of mucins is established by the high density of hydrophilic glycans in the central region of the protein, the amount of mucin-bound water should not be strongly influenced by our proteolytic degradation procedure. Indeed, hydration measurements of trypsin-digested MUC5AC and MUC5B showed no significant reduction in the amount of mucin-bound water (Figure 3, for detailed information see Table S3 in the Supporting Information).
**Figure 3:** Hydration measurements of surface adsorbed layers of different macromolecules. The hydration is calculated for surface coatings of native MUC5AC and MUC5B, trypsin treated MUC5AC and MUC5B, unmodified dextrans and phenylated dextrans (phenylation degree 0.15 and 0.40, respectively), as well as trypsin treated MUC5B with attached phenyl groups. The error bars denote the standard deviation as obtained from three independent measurements.

An alternative explanation for the almost complete loss of mucin lubricity in the boundary lubrication regime could be that the adsorption efficiency of enzymatically treated mucins to surfaces is weakened compared to native mucins. It has been put forward that the terminal (hydrophobic) regions of mucins are involved in the adsorption process of the glycoproteins to hydrophobic surfaces whereas the glycosylated (hydrophilic) central region of mucins are relevant for mucin adsorption to hydrophilic surfaces. This model suggests that the hydrophobic moieties of the mucin glycoprotein might also be necessary for conveying lubricity on apolar surfaces. Thus, in a next step, we analyzed the adsorption behavior of native and trypsin treated mucins to a hydrophobic PDMS surface. Indeed, in contrast to native MUC5AC and MUC5B, the trypsin treated mucins showed a strongly reduced adsorption to the hydrophobic PDMS as indicated by the drastically reduced shift in resonance frequency reported by QCM (**Figure 4a and b**).
Figure 4: Adsorption kinetics of different mucin and dextran variants onto PDMS. The adsorption of native and trypsin treated mucins (MUC5AC (a) and MUC5B (b), respectively) and different dextran variants (phenylated (c) and charged (d)) to PDMS-coated QCM sensors is shown. The error bars denote the standard deviation as obtained from three independent measurements.

This result motivates that, whereas mucin hydration is still high after trypsin treatment, the efficiency of hydration lubrication will be drastically reduced as this mechanism requires surface adsorbed mucin molecules to take effect. Also, the formation of a sacrificial layer, i.e. a dynamic shear-off and re-adsorption cycle, will be hampered if mucin adsorption is reduced. Together, these findings explain the observed loss in mucin lubricity very well and suggest that the hydrophobic terminal domains of MUC5AC and MUC5B are crucial for promoting mucin adsorption to PDMS as required for a good boundary lubricant on hydrophobic surfaces.
To verify our hypothesis that hydrophobic groups are required for the adsorption and subsequent lubricity of well-hydrated macromolecules on hydrophobic surfaces, we make use of a bottom up approach. The idea is to test whether a macromolecule that is not an efficient lubricant on hydrophobic PDMS can be turned into a good lubricant when its adsorption to PDMS is improved. Of course, the trypsin treatment performed on the two mucin variants did not only remove hydrophobic amino acids from the terminal region of the polypeptide but also cleaved positively and negatively charged amino acids. As a molecular platform to systematically probe the influence of charged and hydrophobic modifications on the adsorption efficiency and lubricity of the molecule, we chose dextrans. Dextrans are strongly hydrated molecules, and their good hydration is due to the high density of hydrophilic hydroxyl groups along the polysaccharide. However, solutions containing unmodified dextrans hardly adsorb to PDMS surfaces at all (Figure 4c). Consistently, the Stribeck curves obtained with solutions containing such dextrans are very similar to those obtained with buffer lacking any macromolecules (Figure 5a).
**Figure 5: Rotational tribology with dextran solutions as lubricants.** Tribological measurements between a steel/PDMS pairing with 0.1 mg/mL dextran solution. Measured were the lubricating abilities of either hydrophobic (phenyl) dextrans (a) or charged dextrans (b). The dotted line represents the Stribeck curve for HEPES buffer as a reference, the dashed line the Stribeck curve of native MUC5AC. The error bars denote the standard deviation as obtained from three independent measurements.

In a next step, we test a dextran variant which was modified to carry hydrophobic moieties. The idea is that the addition of hydrophobic groups to dextrans should improve the adsorption of the molecules to hydrophobic PDMS and thus provide lubricity to the dextran solution. Indeed, phenylated dextrans with a phenyl content of 0.15 phenyl substituents per glucose molecule adsorb to a hydrophobic PDMS surface, although the recorded shift in crystal resonance frequency is smaller than that observed for adsorption of either MUC5AC or MUC5B (*Figure 4c*). A possible explanation for the comparably lower adsorption efficiency of phenyl-dextran might be that the amount of hydrophobic groups present on the dextran molecule is smaller than the corresponding number of hydrophobic moieties on the mucin glycoprotein. This idea would be consistent with the biochemical structure of the mucins which comprises large areas with numerous hydrophobic amino acids. To test this hypothesis, we repeated the adsorption measurements with dextrans carrying an increased density of phenyl groups (0.40 phenyl groups per glucose molecule), i.e. a higher number of hydrophobic groups per dextran molecule. Indeed, for this dextran variant, we observe a stronger shift in the crystal resonance frequency corresponding to more efficient adsorption than for the 0.15 phenyl-dextran variant. For the 0.40 phenyl-dextran, this frequency shift is now also similar in magnitude as that observed for the adsorption of native mucins (*Figure 4c*). These findings suggest that the increased degree of dextran phenylation leads to stronger interactions with the hydrophobic PDMS surface and therefore should aid in maintaining a hydrated polymer film on the surface – provided that the phenylation procedure did not interfere with dextran hydration. QCM measurements, however, show that the high degree of hydration
observed for native dextrans is maintained after introducing phenyl groups to these polymers (Figure 3). As a consequence, we expect that both phenylated dextrans should show improved lubricity on PDMS compared to unmodified dextrans, but the dextran variant with the higher phenylation degree should exhibit better lubricating abilities than the 0.15 phenyl-dextran.

Tribological measurements with the two phenylated dextran variants on hydrophobic PDMS indeed agree with this expectation: both phenyl-dextran variants significantly reduce the friction coefficient by up to two decades, especially in the mixed lubrication regime, i.e. for sliding speeds between 1 mm/s and 100 mm/s (Figure 5a). In the boundary lubrication regime, i.e. at low sliding speeds below 1 mm/s, the 0.40 phenyl-dextran is more efficient: we here measure constantly low friction coefficients on the order of \( \mu \sim 0.03 \) which is a bit higher than the value obtained for mucins but more than an order of magnitude lower than the friction coefficient measured for the 0.15 phenyl-dextran in this regime. This observation is consistent with our notion that a stronger adsorption of hydrated molecules, i.e. a more stable hydrated polymer film on the PDMS surface, will lead to better lubrication.

At this point, it is important to recall that the enzymatic treatment of the mucin glycoproteins has not only removed hydrophobic groups from the macromolecule, but also positively and negatively charge amino acids. However, we do not expect that those charged groups contribute significantly to the adsorption of mucins to hydrophobic PDMS surfaces. To verify this, we next test two dextran variants which were modified with anionic carboxymethyl (CM) and cationic diethylaminoethyl (DEAE) groups, respectively. For those two charged dextran variants, we observe similarly low adsorption to PDMS surfaces as for the unmodified dextrans (Figure 4d). This underscores our notion that there are no strong binding interactions between the charged CM or DEAE groups and hydrophobic PDMS, and it suggests that solutions containing those
macromolecules should be poor boundary lubricants. Indeed, the lubricity of those macromolecular solutions on PDMS is low: the Stribeck curves measured in lubrication tests performed with these charged dextran variants resemble the results obtained with either simple buffer or unmodified dextrans (Figure 5b). Together, these findings are in agreement with our hypothesis that it is the hydrophobic character of the terminal peptide sequences of mucins and not the charged groups in this region of the glycoproteins that confers adsorption and lubricity on hydrophobic surfaces such as PDMS to mucins.

The results presented so far highlight the importance of hydrophobic moieties for the lubrication process on hydrophobic surfaces. Furthermore the extent of these hydrophobic modifications on dextran molecules seems to be linked to the lubricating potential of those polymers. However, a higher phenylation degree not only increases the number of phenyl groups on the dextran molecule but also the density of those hydrophobic groups. Thus, we now ask, whether the overall number of hydrophobic groups on dextrans is important for conveying lubricity or if a certain density of hydrophobic motifs, i.e. spatial proximity of phenyl groups, is required for dextran molecules to act as an effective boundary lubricant. To tackle this question, we probe the lubricating abilities of another dextran variant having a lower molecular weight (i.e. 40 kDa) than the dextrans tested so far (150 kDa) but a similar phenylation degree of ~0.40 phenyl groups/glucose. Interestingly, for the small dextran molecules, we obtain Stribeck curves which are virtually identical to those obtained with the larger dextrans (Figure 5a). Since the phenylation degree is 0.40 in both cases, this may indicate, that it is indeed the density of hydrophobic groups on a hydrated macromolecule rather than the total number of hydrophobic motifs which is relevant for providing low friction in the boundary lubrication regime.
Together, these results demonstrate that the presence of hydrophobic moieties is crucial for biopolymers such as mucin or dextran to act as a boundary lubricant on hydrophobic surfaces.

Since the trypsin treatment of mucins entailed a nearly complete loss of mucin lubricity on hydrophobic surfaces, we now, in a last step, try to “repair” the enzymatically treated mucin by grafting artificial hydrophobic groups to the “damaged” mucins. For this approach, we choose to covalently add phenyl groups to the carboxylic groups of trypsin treated mucin (Figure 6a).

**Figure 6**: Adsorption properties and lubricity of phenylated trypsin-treated MUC5B. A schematic representation of the “repair approach” (= phenylation) of trypsin treated MUC5B is depicted in (a). (b) Spectroscopic comparison of MUC5B-Trypsin and MUC5B-Trypsin with covalently attached phenyl groups. (c) Adsorption kinetics of native, trypsin treated and “repaired” MUC5B onto PDMS. (d) Lubricity of solutions of phenylated MUC5B as probed on a steel/PDMS pairing. The Strubeck curves obtained for native MUC5B (solid line) and trypsin treated MUC5B (dashed line) are shown for comparison. The error bars denote the standard deviation as obtained from three independent measurements.
Since the terminal peptide domains of the degraded mucin are no longer present and the central part of the polypeptide sequence is shielded by glycans, we aim to graft those phenyl groups to the carboxyl group of sialic acid, a charged monosaccharide, present among the mucin glycans. For optimal efficiency of this grafting process, we perform these experiments with MUC5B which has a higher sialic acid content than MUC5AC. Since UV-absorbance measurements indicated a successful phenylation of “damaged” (= trypsin treated) MUC5B, we next analyze if the addition of those hydrophobic groups would promote the adsorption of the “repaired” mucin onto a hydrophobic PDMS surface. Indeed, we observe a significant increase of adsorbed mucin molecules on a PDMS-coated quartz crystal. However, the amount of adsorbed molecules seems not to be as high as for native MUC5B. Thus, in a next step, we ask if the “repaired” molecule can achieve similarly good lubricity as native mucin.

When performing friction tests with the “repaired” mucins, we observe a strong decrease of friction coefficient in the mixed lubrication regime (i.e. for sliding speeds between 1 mm/s and 100 mm/s) compared to trypsin treated MUC5B. This decrease in the friction coefficient is, however, less pronounced in the boundary lubrication regime where we measure a friction coefficient of ~0.2 - 0.3. This value is still ~3fold lower than the corresponding value obtained for “damaged” mucin but more than an order of magnitude larger than the friction coefficient obtained for native mucins in this regime. It is likely that the incomplete recovery of both the adsorption kinetics as well as the lubricating abilities of this phenylated mucin compared to native mucin can be attributed to the limited amount of phenyl groups grafted onto the damaged mucin molecules. As discussed before, the density of phenyl groups on dextran seems to be directly linked to both the adsorption kinetics of the macromolecule and its lubricating ability. Therefore, the amount of sialic acid groups which can be targeted with our phenylation procedure...
might be too low to achieve a phenylation density that is high enough to achieve adsorption kinetics and lubricity on a level comparable to that of native mucin. This assumption is in good agreement with previous work where it was shown that the adsorption strength and lubricating potential of boundary lubricants are linked. However, those findings indicate that MUC5B digested with trypsin can indeed be ‘repaired’: covalently attaching phenyl groups seems to compensate for the lost hydrophobic peptide termini, at least to a certain degree.

CONCLUSION

We here demonstrated, that the hydrophobic domains of mucins are crucial to adsorb and further lubricate hydrophobic surfaces such as PDMS. Since several tissue surfaces in the human body exhibit a hydrophobic character, the interaction of mucins with these surfaces by means of hydrophobic interactions is essential to provide boundary lubrication in vivo. When the mucin molecules are deprived of their hydrophobic domains, this lubrication on hydrophobic surfaces cannot take place anymore. However, we were able to compensate for the loss of these hydrophobic domains by grafting phenyl groups onto the damaged mucins, which in part recovered their ability to adsorb and lubricate hydrophobic surfaces. This approach can also be transferred to other biomolecules: Dextrans, highly hydrophilic molecules, can function as good boundary lubricants on hydrophobic PDMS when equipped with phenyl groups. The density of attached hydrophobic groups during this bottom-up approach determines the adsorption kinetics and lubricity of this mucin-inspired macromolecule. Of course, we here study macromolecular lubricity on simple PDMS surfaces which are hydrophobic but uncharged. On more complex surfaces which combine hydrophobic and charged characteristics, a phenyl-modified polyelectrolytic dextran variant might provide even better lubricity than the uncharged phenylated dextran molecules presented here.
The results presented here may pave the way towards the rational design of macromolecular superlubricants which provide ultra-low friction on a broad range of biological and technical surfaces. A suitable polymer for such an artificial lubricant could be polyethylene glycol (PEG) which is non-toxic, water soluble, and available with different molecular weights. PEG is already used in numerous fields ranging from industrial to pharmaceutical applications. PEG is highly hydrated and a good lubricant when utilized as surface attached polymer brushes. Moreover, it has been demonstrated that linking PEG polymer chains to deglycosylated mucin can serve as a replacement for the hydrated glycans and restore lubricity. Combining those existing strategies with the results shown here could lead to an artificial brush-like macromolecule with great potential for providing ultra-low friction on various surfaces.
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BK, OL and TC proposed the experiments. BK, FW, GP and VS performed the experiments and analyzed the data. The manuscript was written by BK and OL.

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SUPPORTING INFORMATION

- Analysis of PDMS-layer thickness on QCM-chips
- Detailed information on the mass spectrometry analysis of trypsin digested MUC5AC and MUC5B.
- Detailed information on the hydration measurements
- Surface topology analysis of the PDMS pin surface


23. Feiler, A. A.; Sahlholm, A.; Sandberg, T.; Caldwell, K. D., Adsorption and viscoelastic properties of fractionated mucin (BSM) and bovine serum albumin (BSA) studied with quartz crystal microbalance (QCM-D). *J. Colloid Interface Sci.* 2007, 315, (2), 475-481.


TABLE OF CONTENTS GRAPHIC

hydrophobic surface  hydrated polymer  hydrophobic motifs

lubrication  no lubrication  lubrication

enzymatic treatment  phenylation