The lubricity of mucin solutions is robust against changes in physiological conditions

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Abstract:

Solutions of manually purified gastric mucins have been shown to be promising lubricants for biomedical purposes, where they can efficiently reduce friction and wear. However, so far, such mucin solutions have been mostly tested in specific settings, and variations in the composition of the lubricating fluid have not been systematically explored. We here fill this gap and determine the viscosity, adsorption behavior, and lubricity of porcine gastric mucin solutions on hydrophobic surfaces at different pH levels, mucin and salt concentrations and in the presence of other proteins. We demonstrate that mucin solutions provide excellent lubricity even at very low concentrations of 0.01 % (w/v), over a broad range of pH levels and even at elevated ionic strength. Furthermore, we provide mechanistic insights into mucin lubricity, which help explain how certain variations in physiologically relevant parameters can limit the lubricating potential of mucin solutions. Our results motivate that solutions of manually purified mucin solutions can be powerful biomedical lubricants, e.g. serving as eye drops, mouth sprays or as a personal lubricant for intercourse.

Keywords: lubricity; mucin; purification; pH; salt; protein
1 Introduction

Body fluids, a kind of aqueous lubricants, play essential roles to assist the rubbing contacts in the human body. Lacking sufficient biolubrication can result in some severe clinical problems, such as dry irritated eyes, vaginal dryness, dryness of the mouth impeding proper speech and mastication, and excessive friction and wear of articulating cartilage surfaces, especially for the elderly and patients with Sjogren’s syndrome. Moreover, prolonged contact between an artificial, hard material and soft tissue surfaces in the human body may cause inflammation and tissue damage and thus significant patient discomfort.

To prevent those issues, using some artificial biolubricants to reduce friction and wear would be a promising strategy. Mucus is an adhesive substance that is widely secreted in living organisms, which can protect the lung airways, eye, gastrointestinal (GI) tract, vagina, and other mucosal surfaces. It is a hydrogel containing water (> 90 wt.%), inorganic salts, mucins, and some minor components depending on the source. The main functional component of mucus is mucin, a group of high molecular weight (0.5–20 MDa) and densely glycosylated biopolymers (glycoproteins). More specifically, the mucins can be roughly classified into three groups: membrane-bound epithelial mucins, secreted non-gel-forming mucins, and secreted gel-forming mucins with the latter being the major component of mucus. One of the key characteristics of mucins is their ability to form viscoelastic solutions or gels. Those mucin gels act as a chemical and biological barrier towards pathogens, dust particles and toxins and provide lubrication and hydration to protect tissues from dehydration, shear stress and wear damage.

Due to their outstanding performance as biolubricants, the tribological properties of mucin solutions have been widely studied during the past years. For instance, Pult et al. investigated friction between the cornea and the eyelid as well as between the contact lens surface and the eyelid, and the authors indicated that the tribological properties of the eyes were significantly correlated to the quantity and quality of the mucins in the tear film. Winkeljann et al. suggested that purified gastric mucins can reduce the formation of tissue damage on porcine cornea as it can be induced by contact lenses and serve as a powerful tool in fighting ocular dryness. Mucin-based lubricants typically promote boundary lubrication both on artificial and biological surfaces, i.e. they provide low friction coefficients $\mu$ ranging from 0.2 to less than 0.01 with different material pairings. This is attributed to the mucins and mucinous glycoproteins, i.e. highly surface-adhesive macromolecules, which can adsorb to a wide variety of surfaces. Owing to the absorption of mucins,
hydrophobic (artificial and biological) surfaces are rendered hydrophilic, which facilitates the formation of lubrication water film on those surfaces which, in turn, leads to the separation of the load-bearing surfaces under shear force. This hydrated boundary layer further improves lubrication via the hydration lubrication mechanism. In addition, shearing off the adsorbed mucin macromolecules from the substrates can further result in the reduction in friction.

Although the studies mentioned above give good examples of how recent research provided new insights into the lubricating potential of mucin solutions, some of them have been conducted by employing commercially available mucin specimens. However, solutions containing such industrial mucins are poor lubricants, and they also lack the gel-forming abilities and antiviral properties observed for manually purified mucin glycoproteins. Hence, to develop highly functional mucin-based solutions, it is crucial to use manually purified mucins which have maintained their native properties. Meanwhile, the human body is a complex system, which demonstrates different pH, proteins as well as salts in different organs and body fluids. It deserves to study the tribological performances of the purified mucins in physiologically relevant conditions to extend their potential use in biomedical applications, such as artificial joint fluid, eye drops, personal lubricant, and mouth spray (Fig. 1).

![Fig. 1](image-url) Potential use of mucin-based solutions in biomedical applications: (a) artificial joint fluid for viscosupplementation, (b) eye drops, (c) personal lubricant, and (d) mouth spray.

This work is motivated by such considerations. The lubricity of manually purified gastric
mucin on hydrophobic surfaces with different pH, proteins, mucin and salt concentrations is investigated and the potential use of mucin-based solutions in biomedical applications is evaluated. A better understanding of the regulation mechanisms of pH, protein, mucin and salt concentrations will be achieved.
2 Materials and methods

2.1 Mucin purification

Mucin purification was conducted as described earlier in detail. In brief, fresh porcine stomachs were opened, and food debris washed off with tap water. Then, crude mucus was collected by scraping the mucosal surface of the tissue. The obtained mucus was pooled and diluted in PBS buffer (10 mM, pH = 7.4) for overnight solubilization. Cellular debris and lipid contaminants were removed from this solubilized mucus via two centrifugation steps (first at 8300 x g at 4 °C for 30 min, then at 15000 x g at 4 °C for 45 min) and a final ultracentrifugation step (150000 x g at 4 °C for 1 h). Afterwards, the mucins were separated from other macromolecules by size exclusion chromatography using an Äkta purifier system (GE Healthcare, Chicago, IL, USA) and an XK50/100 column packed with Sepharose 6FF. Then, the mucin fractions were pooled, dialyzed against ultrapure water, and concentrated by cross-flow filtration. Finally, the concentrated mucins lyophilized and stored at -80 °C. All purified mucins were exposed to UV-light for 1 h for sterilization before use.

2.2 Buffer solutions

To avoid introducing artefacts associated with buffering substances, four kinds of buffer solutions with different components were tested. Phosphate buffer was prepared by dissolving selected amounts of sodium dihydrogen phosphate monohydrate (Sigma, St. Louis, USA) in ultrapure water. The pH was adjusted to 7 by adding 32 % NaOH dropwise. HEPES buffer was prepared by dissolving 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Roth, Karlsruhe, Germany) in Millipore water and pH was adjusted to 7 by adding 0.1 M NaOH. Universal buffer (UB, pH 2.0-8.2) and Britton-Robinson buffer (BRB, pH 1.9-11) were prepared as described in the literature. In detail, UB was prepared by combining 20 mM HEPES, 20 mM 2-(N-morpholino)ethanesulfonic acid (MES, Sigma, St. Louis, USA) and 20 mM sodium acetate (Roth, Karlsruhe, Germany) in ultrapure water, and pH was adjusted with 10 M NaOH or 5 M HCl as needed. BRB was obtained by mixing 40 mM acetic acid (Roth, Karlsruhe, Germany), orthophosphoric acid (Roth, Karlsruhe, Germany) and boric acid (Roth, Karlsruhe, Germany) into ultrapure water, and the pH was adjusted with 0.2 M NaOH.
2.3 Lubricant solutions

Solutions with different mucin concentrations were generated in 20 mM HEPES, pH 7. Unless stated otherwise, a mucin concentration of 0.1\% (w/v) (≈ 1 mg/mL) was chosen to ensure comparability to earlier studies\(^\text{14, 19}\). To investigate the performance of mucin solutions at different pH, the mucins were dissolved in UB at pH 2, 4, 6 and 8, respectively. BSA (FraktionV T844.1, Roth), amylase (10065, Sigma), and lysozyme (L6876, Sigma) were dissolved in 20 mM HEPES buffer (pH 7) at a concentration of 80 mg/L, 300 U/mL and 26 mg/mL, respectively. Then, the protein solutions were mixed in a ratio of 1:1 with a 0.2\% (w/v) mucin solution, which was prepared as described above. Thus, final protein concentrations of 40 mg/mL BSA, 150 U/mL amylase, and 13 mg/mL lysozyme were achieved, which is similar to the concentration of those proteins in body fluids\(^\text{20, 21}\). To study the influence of the buffer ionic strength, different concentrations of NaCl were added to the mucin solution (in 20 mM HEPES buffer, pH 7) when preparing the test fluid.

2.4 Viscosity measurements

The viscosities of the mucin-based lubricant solutions used in this study were determined on a commercial shear rheometer (MCR 302, Anton Paar, Graz, Austria) using a cone-plate geometry (CP50-1, Anton Paar). For each measurement, 570 µL of the test solution were pipetted onto the stationary plate to fully fill the space between the measuring head and the sample plate. Measurements were conducted at 21 °C, and the shear rate was varied from 10 to 1000 s\(^{-1}\). The viscosity values shown in table 1 for each solution represent averaged measurement results acquired at a shear rate of 100 s\(^{-1}\) from three independent samples.

2.5 Tribological tests

A steel-on-PDMS tribo-pairing was chosen to evaluate the lubricity of mucin solutions. Steel spheres with a diameter of 12.7 mm (Kugel Pompel, Vienna, Austria) were used as received. PDMS pins were prepared as cylinders with a diameter of 6.1 mm. In detail, PDMS prepolymer and cross-linker (Sylgard 184, Dow Corning, Wiesbaden, Germany) were mixed in a ratio of 10:1. Then, the mixture was placed into a vacuum chamber for 1 hour to remove air bubbles. Afterwards, the solution was poured into a steel mold and cured at 80 °C for 4 h. Both, the steel spheres and PDMS pins were used without further polishing as they showed low roughness (\(S_q\text{steel} < 200 \text{ nm, } S_q\text{PDMS} < 50 \text{ nm}\)) when investigated with a laser scanning microscope (VK-X1100, Keyence, Osaka,
The tribological experiments were performed at 21 °C using the tribology unit (T-PTD 200, Anton Paar) of a commercial shear rheometer (MCR 302, Anton Paar) as described before. In brief, three PDMS pins were mounted into a pin holder and washed with ethanol and ultrapure water. Then, 600 µL of a lubricant solution were applied to ensure full coverage of the pins. The normal load was chosen to be 6 N, resulting in an average contact pressure of ~0.3 MPa. The sliding velocity was varied from $10^{-5}$ to $10^{0}$ m/s to probe as many lubrication regimes as possible. For each condition, three independent experiments were carried out using a fresh set of PDMS pins for each measurement.

### 2.6 Adsorption measurements

The adsorption properties of mucins on hydrophobic PDMS surfaces were studied by quartz crystal microbalance with dissipation monitoring (QCM-D) using a qcell T-Q2 platform (3T-Analytik, Tuttlingen, Germany). Gold sensor chips were coated with a thin PDMS film to obtain similar hydrophobic surfaces as used in the tribological tests. To obtain this coating, PDMS prepolymer and cross-linker (Sylgard 184, Dow Corning, Wiesbaden, Germany) were mixed in a ratio of 10:1, and were further diluted with n-hexane to obtain a 1% (v/v) polymer solution. Then, a bare gold sensor chip was placed into the center of a spin coater (WS-400B-6NPP/LITE, Laurell, North Wales, USA), and 100 µL of the prepared PDMS mixture was pipetted onto the gold chip. To distribute the PDMS solution, the spin-coater was set into rotation - first at 1500 rpm for 20 s and then at 3000 rpm for 60 s. Afterwards, the coated sensor chip was cured at 80 °C for 4 h.

At the beginning of each adsorption test, a pure buffer solution (without any proteins, mucins or salts) was injected at a flow rate of 100 µL/min until a stable baseline was obtained. Afterwards, a mucin-based solution was injected at 100 µL/min for ~30 min to obtain an adsorption curve. The resulting frequency shift ($\Delta f$, Hz) and dissipation shift ($\Delta D$) were automatically calculated by the software “qGraph” (3T-Analytik, Tuttlingen, Germany).
3 Results and discussion

To evaluate the potential of mucin solutions as biolubricants for human use, several physiological parameters need to be considered, which might affect mucin lubricity. We here focus on variations in pH and ionic strength as those two parameters can be quite different on different body sites. Moreover, we aim at identifying the minimal mucin concentration that still conveys good lubricity, and we ask if prominent examples of proteins from body fluids interfere with the lubricating properties of mucin. As a material pairing for the tribological experiments, we choose a steel-on-PDMS setup. This particular material pairing is selected, since both, steel and PDMS-based materials, are commonly used in various medical devices \(^{23, 24}\). Moreover, a hard-on-soft pairing such as this involving both a hydrophilic and a hydrophobic surface is also frequently employed in biotribological studies to mimic, e.g., the tongue-palate interface. Furthermore, with the steel-PDMS paring, all three lubrication regimes are clearly visible which makes it easier to analyze the tribological performance of different lubricants.

3.1 Choosing the right buffer system

When working with solutions of biological molecules such as mucins, using buffers to control pH and ionic strength is critical, especially if the influence of either parameter is on properties of the solution is investigated. Although phosphate based buffers are frequently used in mucin tribology \(^{25-27}\), one needs to be aware, that – depending on the material pairing studied – buffer substances may have a significant influence on the experimental outcome. For a steel/PDMS pairing, which is not only used in this study but is regularly employed in the field of biotribology \(^{6, 14, 28-31}\), this can be indeed an issue: As shown in Fig. 2(a), the friction coefficient in the boundary lubrication regime drops by almost one order of magnitude when the phosphate concentration in a standard PBS buffer is increased from 10 mM to 500 mM. This can be attributed to the ability of phosphate ions to readily react with the steel surface – this is a frequently reported mechanism to modify the surface of steel components for industrial applications \(^{32, 33}\). Although such a phosphate buffered tribology system would still allow for direct comparisons between buffered biopolymer solutions and buffer only, it still disturbs the measurement outcome in two ways: First, one main advantage of a steel/PDMS pairing is the fact, that all three lubrication regimes are clearly distinguishable; thus, the influence of different (macro)molecular ingredients can be easily identified. However,
when using a phosphate-based buffer, the Stribeck curve is less pronounced and the maximal ambitus between ‘poor lubricity’ and ‘great lubricity’ is reduced, which makes it harder to detect gradual improvements in lubricity provided by lubricating molecules. Second, a reaction of the phosphate ions with the steel surface might cause secondary effects arising from a changed surface structure or surface chemistry, and this can complicate the interpretation of the results.

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**Fig. 2** Stribeck curves as a function of the sliding velocity. The data shown was obtained with different buffer solutions on a steel-PDMS material pairing: (a) 10 mM phosphate, 25 mM phosphate, 50 mM phosphate, 100 mM phosphate, 250 mM phosphate, 500 mM phosphate and 20 mM HEPES. (b) UB and BRB at different pH levels (from pH 2 to 8).

A material incompatibility between the friction partners and buffer solution is not the only condition that needs to be considered when selecting a buffer system. Proteins, such as mucins, might be able to operate in a wide range of pH settings as they also occur in the human body. In potential applications as a biolubricant, the relevant pH range a mucin solution might encounter can span from slightly alkaline (pH ~ 8 in the oral cavity) to considerably acidic (pH ~ 4 in the vaginal tract). Hence, conducting measurements at different pH levels is important to assess the working range of mucin solutions. However, only few buffers are efficient over such a wide pH range, and using different buffer substances at different pH levels may lead to artefacts that again complicate the interpretation of the results. We here compare two established buffer systems which have a broad operating range, i.e., a universal buffer (UB, pH range: 2.0 - 8.2) and a Britton-Robinson buffer...
As presented in Fig. 2(b), the Striebeck curves obtained with either buffer show a slight pH dependence, and this effect is more pronounced for BRB. Moreover, in the boundary lubrication regime, the friction coefficients measured with BRB are almost one order of magnitude lower than those determined with UB. At this point, it is important to realize that one of the components of BRB is phosphate – which explains the lower friction coefficients compared to UB. Thus, although BRB can operate in a broad pH range, it is not an ideal buffer system for our tribological experiments.

Based on those results, we conclude that phosphate-free buffers are better suited for tribological investigations employing a steel-PDMS paring. Accordingly, for our study, we choose HEPES buffer for experiments at neutral pH, and we use UB in those experiments, where the pH is varied.

3.2 Viscosities of the different mucin-based lubricants

Before we study the tribological performance of mucin solutions at different conditions, we first assess the viscosities of the different mucin-based mixtures. These values are summarized in Table 1 and sorted in groups according to the different parameters (mucin concentration, buffer pH, buffer ionic strength, influence of other proteins) whose influence on mucin lubricity is assessed later. As can be expected, increasing the mucin concentration increases the viscosity of the solution, with the 1% (w/v) mucin solution showing the highest viscosity of 8.3 mPa·s. The viscosities of mucin/protein mixtures are about 2-fold compared to those of solutions containing mucin only – owing to the increased total protein concentration. All other solutions have viscosities around 1 mPa·s, and thus have values similar to that of pure water. Thus, with the exception of the 1% (w/v) mucin solution, we do not expect that those minor changes in lubricant viscosity will have perceivable impact on the results obtained with tribology: This is why we do not recalculate the obtained data into representations using the Sommerfeld number as a variable, but show the dependencies of the friction factor on the sliding speed in all diagrams.

Table 1. Viscosities of different mucin-based solutions. The error values shown depict the standard deviation as obtained from \( n = 3 \) independent measurements.
| No. | Buffer | pH  | Components                        | Viscosity (mPa·s) |
|-----|--------|-----|-----------------------------------|------------------|
| A1  | HEPES  | 7.0 | 0.005 % mucin                     | 0.99 ± 0.01      |
| A2  | HEPES  | 7.0 | 0.01 % mucin                      | 0.99 ± 0.01      |
| A3  | HEPES  | 7.0 | 0.05 % mucin                      | 1.07 ± 0.01      |
| A4  | HEPES  | 7.0 | 0.1 % mucin                       | 1.24 ± 0.18      |
| A5  | HEPES  | 7.0 | 1.0 % mucin                       | 8.30 ± 0.05      |
| B1  | UB     | 2.0 | 0.1 % mucin                       | 1.04 ± 0.10      |
| B2  | UB     | 4.0 | 0.1 % mucin                       | 0.92 ± 0.11      |
| B3  | UB     | 6.0 | 0.1 % mucin                       | 1.13 ± 0.42      |
| B4  | UB     | 8.0 | 0.1 % mucin                       | 1.23 ± 0.05      |
| C1  | HEPES  | 7.0 | 0.1 % mucin + 20 mM NaCl          | 1.22 ± 0.05      |
| C2  | HEPES  | 7.0 | 0.1 % mucin + 50 mM NaCl          | 1.38 ± 0.11      |
| C3  | HEPES  | 7.0 | 0.1 % mucin + 150 mM NaCl         | 1.23 ± 0.07      |
| C4  | HEPES  | 7.0 | 0.1 % mucin + 500 mM NaCl         | 1.19 ± 0.01      |
| D1  | HEPES  | 7.0 | 0.1 % mucin + BSA (40 mg/mL)      | 2.41 ± 0.49      |
| D2  | HEPES  | 7.0 | 0.1 % mucin + Amylase (150 U/mL)  | 1.89 ± 0.55      |
| D3  | HEPES  | 7.0 | 0.1% mucin + Lysozyme (13 mg/mL)  | 1.84 ± 0.28      |

### 3.3 Influence of the mucin concentration

Owing to the complex purification procedures and low production volume, the manually purified mucins are quite expensive to some extent. It would be nice if the effective lubrication range of those mucins can be figured out. The obtained friction coefficients of the buffer solution go down by tuning the mucin concentration from 0.005% to 1.0%, suggesting the improvement of lubricity. As displayed in Fig. 3(a), the mucin solution shows superior lubrication properties, with friction coefficients < 0.02 in all the three lubrication regimes, even diluted in a concentration of 0.01%, suggesting that the superlubricity status of purified mucin is easy and quick to achieve. However, when the mucin was further diluted to 0.005%, the lubricity was getting worse, which was just the same as the pure HEPES buffer. It is noting that there is no obvious difference between the friction coefficient curves of 0.1% and 1.0% mucin solutions. Thus, in this study, adding too much mucin will not be of great help to the lubricity.

In the next step, we investigated the adsorption properties of different mucin concentrations. According to the results displayed in Fig. 3(b), higher mucin concentration leads to quicker
adsorption speed. Indeed, with the increasing mucin concentration, strongly increased adsorption to PDMS can be found, as indicated by the drastically increased frequency shifts shown in Fig. 3(b). Therefore, one can conclude that the sufficient adsorbed mucins onto PDMS surface are needed to provide excellent lubricity. That is the reason why lubricant solution with a mucin concentration of 0.005% failed. Moreover, owing to the steric effect, once sufficient mucin molecules are offered, the addition of mucins will not help to improve the lubricity a lot. Thus, the lower limit for the mucin to offer the lubricity is a concentration of 0.01%. As for the upper limit, it depends on different situations, and 0.1% is recommended for normal conditions.

![Fig. 3 Lubricity and adsorption properties of manually purified mucins with different concentrations.](image)

3.4 Influence of pH

Due to the broad working pH range in potential applications, the lubrication and adsorption properties of the manually purified mucin at different pH were evaluated in this study. As can be seen in Fig. 4(a), with the increasing pH value from 2 to 8, the friction coefficients decrease first and then increase. Mucin solution at pH 4 demonstrates the lowest friction coefficients (< 0.01 over the whole speed range), corresponding to the highest frequency shift shown in Fig. 4(b), indicating pH 4 is the optimum value for the lubrication of purified mucin. Indeed, pH 4 is a key point for the lubricity of mucin. Once the pH value departure from 4, the mucin will perform relatively higher friction coefficients, suggesting the lubricity is getting worse. Also, similar situations can also be
observed in the results of adsorption measurement displayed in Fig. 4(b). Therefore, the lubrication properties of purified mucin at the sliding interface of steel and PDMS in aqueous condition can be attributed to its adsorption behavior, which is highly dependent upon the pH value of lubricant solutions.

It is suggested that the conformation of gastric mucin changed from a random coil to an anisotropic with the decreasing pH (at pH ≥ 4) and further extended at pH < 4. Cell et al. also suggested that pH 4 is the turning point of the mucin properties from the rheological point of view. At pH 4, the lubricant offers the most adsorbed mucin molecules on PDMS surfaces, leading to the lowest friction among all the tested solutions. It is worth noting that the pH of vagina fluid in the human body is ~4 in normal condition, indicating that the mucin solution can offer superior lubricity when being applied as a personal lubricant. In terms of the mucin solution at other pH values, although their friction coefficients are higher than the one at pH 4, the friction coefficients obtain are all < 0.1, which are still excellent for biomedical applications.

![Fig. 4 Lubricity and adsorption properties of 0.1% manually purified mucins in universal buffer (UB, a combination of 20 mM HEPES, 20 mM MES and 20 mM CH3COONa) at different pH values. (a) Tribological measurements were conducted with a steel-PDMS paring tuning pH value from 2 to 8. (b) Adsorption properties of 0.1% mucins in UB at pH 2, 4, 6 and 8 onto PDMS surfaces.](image)

**3.5 Influence of salt concentration**

In the next step, the lubricity and adsorption properties of the mucin solution with different salt concentrations were tested in this study. The idea is that salt ions, especially NaCl, play a vital role in the regulation of many body functions and is also an important part of the body’s fluid balance.
control system. Sometime, the salt concentration in body fluids will change with different health conditions. In order to understand the lubricity of mucin under different salt concentrations, we developed a series of mucin/NaCl solutions, and their tribological behavior as well as adsorption properties were evaluated in this study.

According to the results obtained in Fig. 5(a), the manually purified mucin is more sensitive to salt ions, which is different from the commercial one. By adding NaCl into mucin solution, a noticeable rise of friction coefficients can be found, suggesting the introduced salt ions make mucin lubrication properties worse. Moreover, dissolving NaCl in mucin solution does not change the shape and trend of the friction coefficient curves, and just leads to the curve shifts. Surprisingly, even in a high salt concentration (500 mM NaCl), purified mucin performs an excellent lubricity with the friction coefficients < 0.1 under various sliding velocities, indicating that purified mucin can still work well in the salt condition of body fluids.

The adsorption properties of mucin solution with different salt concentrations were carried out. Fig. 5(b) presents their frequency shifts as a function of time. In order to better understand the adsorption kinetics, the dissipation shifts are also provided in these tests. It is indicated that the frequency drops while the dissipation increases with increasing the salt concentration from 20 mM to 500 mM. In order to further study the interaction between mucin and salt ions, additional two-step QCM measurements were conducted, which was first evaluated with 0.1\% mucin solution and then followed by 20 mM or 500 mM NaCl in 20 mM HEPES buffer. As displayed in Fig. 5(c), there is no obvious influence on the adsorption performance of mucin solution upon injecting 20 mM NaCl. However, a significant decline of the frequency and raise of dissipation can be found after injecting 500 mM NaCl. High ionic strength influences charge shielding and further changes the available number of charged groups for ionic paring. It has been also suggested that the range and magnitude of the steric forces encountered between mucin layers decrease with increasing NaCl concentration, which can be attributed to the decrease in Debye-length. Mucin undergoes a conformational change from a fully extended state to a collapsed state in salt solutions, due to the electrostatic screening effect and osmotic pressure in chains. It is proposed that the activity of water molecules is limited under the influence of salts and a lot of electric charges on the protein surface are neutralized. Thus, adsorbed mucin molecules are decreased and some of the hydration layers are destroyed. As a consequence, higher friction coefficients are observed.
Fig. 5 Lubricity and adsorption properties of 0.1% manually purified mucins with different salt concentration in 20 mM HEPES buffer at pH 7. (a) Tribological measurements were conducted with a steel-PDMS paring in 0.1% mucin solution with a NaCl concentration of 20 mM, 50 mM, 150 mM and 500 mM, respectively. (b) Changes in frequency (solid line) and dissipation (dashed line) for PDMS-coated chips in contact with 0.1% mucin solution interacted with 20 mM, 50 mM, 150 mM and 500 mM NaCl in 20 mM HEPES buffer. (c) Changes in frequency (solid line) and damping (dashed line) for PDMS-coated chips in contact with 0.1% mucin solution followed by 20 mM or 500 mM NaCl in 20 mM HEPES buffer.

3.6 Influence of proteins

It is reported that lysozyme, amylase, and serum albumin are the main proteins in natural and artificial body fluids, such as tear, salivary, synovial fluid and vaginal fluid. For the purpose of investigating the interaction of mucins and three proteins in body fluids, they were chosen in this study. The friction coefficients versus sliding speed plots of mucin solution with three different proteins (BSA, amylase, and lysozyme) are shown in Fig. 6(a). For the purpose of comparison, the protein-free mucin solution and pure HEPES buffer are also presented. It reveals that the protein-
free mucin solution shows excellent lubricity, giving a relative stable friction coefficient around 0.01 at various sliding speed. Higher friction coefficients are obtained when proteins are introduced. More specifically, by adding BSA or amylase into mucin solution, the friction coefficients, which are all higher than the one of pure mucin solution, still display a noticeable reduction in the mixed lubrication regime in comparison with the pure HEPES buffer solution, while increase gradually towards pure buffer in the boundary lubrication regime. Different from the above two mucin/protein solutions, the friction coefficients of mucin/lysozyme solution decrease when lubrication regime change from mixed lubrication to boundary lubrication, remaining excellent lubricity of this solution.

To figure out the relationship between tribology and adsorption, QCM-D measurements were conducted with the three mucin/protein lubricants for ~30 min to evaluate how the mucin adsorption behavior would be like with the existed protein. For the adsorption kinetics of mucin/protein solutions displayed in Fig. 6(b), the mucin/BSA solution demonstrated the least adsorption on PDMS while mucin/lysozyme gave the highest adsorption. As indicated by the frequency shifts, the mucin/BSA and mucin/amylase solutions adsorbed onto PDMS quickly and maintained relatively stable status with the increasing measurement time. Regarding the mucin/lysozyme solution, though a drastically frequency drop was observed at the beginning, the frequency shift still decreased versus time, which is similar to the trend of protein-free mucin solution shown in Fig. 3(b), indicating the continuous adsorption on PMDS and resulting in the optimum lubricity among the three mucin/protein lubricants. For neutral pH, i.e. at pH 7, mucin, BSA and amylase are negatively charged while lysozyme is positively charged 51, 52. The molecular weight of BSA, amylase, and lysozyme are ~66 kDa, ~51 kDa and ~14 kDa, which are all much smaller than mucin (0.5-20 MDa). The smaller protein molecules can diffuse to the substratum easier and adsorb onto PDMS surfaces faster. It can be assumed that the proteins used in this study adsorbed onto PDMS first, and then influenced the mucin adsorption with their different charges and molecular properties. For the negatively charged proteins, i.e. BSA and amylase, they adsorbed onto PDMS and blocked surface, preventing the negatively charged mucin adsorption altogether under electrostatic interaction, which is similar to the adsorption behavior of bovine submaxillary gland mucin (BSM) with BSA reported in a previous study 53. Compared with pure mucin solution, higher frequency shift is found when introduced positively charged lysozyme in mucin solution. Whereas, the effective space for mucin adsorption is decreased owing to the anchored protein molecules on PDMS. In addition, the obtained results also indicate that through the protein adsorbed on the PDMS surface hydrophobic interactions between mucin and PDMS are weakened which are still stronger than the electrostatic
ones. This might be the reason why mucin alone shows better lubrication than combined with positively charged lysozyme. Consequently, higher friction coefficients are obtained in comparison with the protein-free mucin solution.

![Fig. 6 Lubrication and adsorption properties of mucin/protein solutions. (a) Tribological tests were conducted with a PDMS/steel setup with 0.1% mucin solution with and without proteins (BSA, amylase and lysozyme). Protein-free mucin solution and pure HEPES buffer were used for reference. (b) QCM-D measurements of change in frequency (Δf) versus time upon adsorption of 0.1% mucin solution (in 20 mM HEPES buffer, pH 7) with BSA (40 mg/mL), amylase (150 U/mL) and lysozyme (13 mg/mL) onto PDMS. Protein-free mucin solution was used for reference.](image)

Indeed, mucin should encounter different proteins for potential biomedical applications. From the tribological points of view, some of them will not help improve the lubricity of mucin. But noticeable reduction of friction coefficients of mucin-based protein solutions can still be observed compared to pure buffer solution. In this presented study, only the lubricity and adsorption properties of mucin with single protein fraction were evaluated. Considering the complex components of human body fluids, further studies will be conducted to investigate the tribological properties of mucin with multiple proteins in one solution.
4 Conclusions

In this work, we have shown the viscosity, adsorption, and lubricity of manually purified porcine gastric mucin on hydrophobic surfaces. Different pH, proteins, mucin and salt concentrations were investigated to mimic different physiologically relevant conditions. It implies that the mucin can offer excellent lubricity even with ultralow concentration. The lower limit of the mucin concentration in aqueous solution to offer the lubricity is 0.01%. As for the upper limit, it depends on different situations, and 0.1% is recommended for normal conditions. It is indicated that pH 4 is the turning point for the adsorption and lubricity of mucin owing to the conformational change, which provides the optimum friction coefficients and adsorption kinetics. Because of the electrostatic screening effect and osmotic pressure, the introduced salt ions in mucin solution make side effects on the adsorption and lubricity of purified mucin. The addition of proteins in mucin solutions increase the friction coefficients. The purified mucin demonstrates optimum lubrication behavior with positively charged proteins rather than the negatively charged ones. In summary, the results presented here can provide some theoretical evidence for extensive uses of manually purified mucin solutions in biomedical applications, such as artificial joint fluid for viscosupplementation, eye drops, mouth spray and personal lubricant personal lubricant for intercourse.
Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgments

We thank Matthias Marczynski and Christine Braig for assistance with the mucin purification. This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 754462.

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