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Gastric Mucus and Mucus-like Hydrogels: Thin Film Lubricating Properties at Soft Interfaces

Running title: Mucus-like Hydrogels at Rubbing Interfaces

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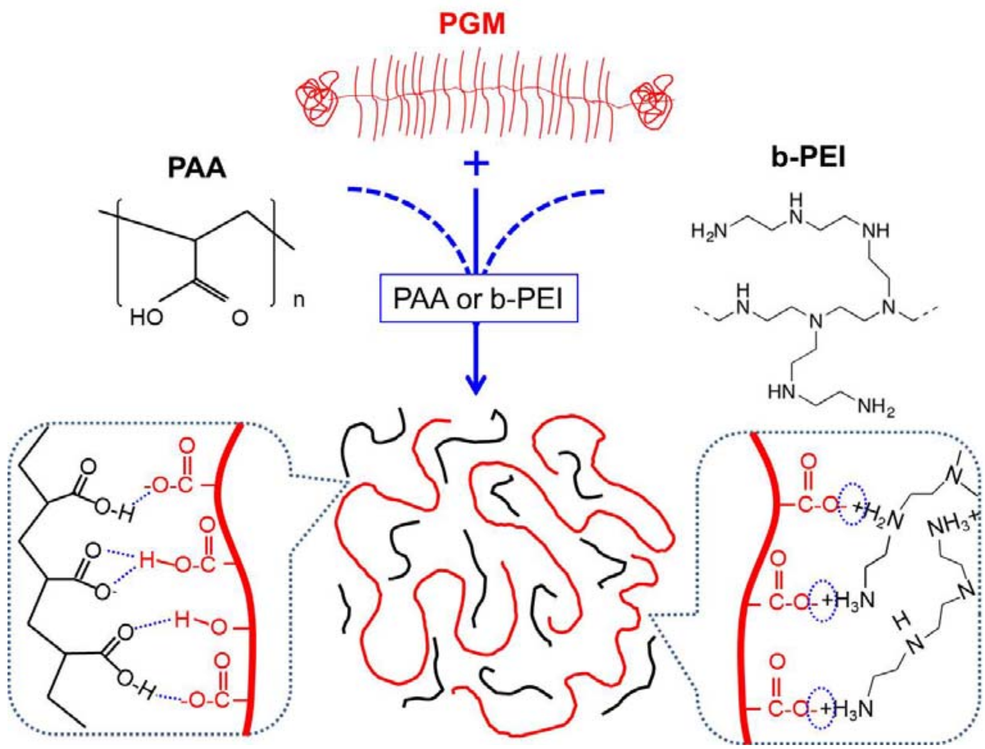
Mucus is a viscous slime that plays a vital role in protecting and lubricating biological tissues, in particular soft epithelium interfaces such as in the stomach, intestines and esophagus. Previous attempts to generate mucus models that mimick or simulate its characteristics have been predominantly focused on rheological properties. This study investigates both rheological and tribological shear properties of thin films of gastric mucus from a porcine source and its mimics at compliant soft interfaces. The lubricating efficacy of biological mucus and its mimics was observed to be superior at hydrophilic tribological interfaces compared to hydrophobic ones. Facile spreading of all mucus samples at hydrophilic steel-polydimethylsiloxane (PDMS) interfaces allowed for the retainment of the lubricating films over a wide range of speed, slide/roll ratio, and external load. In contrast, poor wetting at hydrophobic PDMS-PDMS interfaces led to depletion of the mucus samples from the interface with increasing speed. Among the different mucus models investigated in this study, fluid mixtures of commercially available porcine gastric mucin (PGM) and polyacrylic acid (PAA) displayed the most persistent lubricating effects under various tribological experimental conditions. A mixture of PGM and PAA holds a high potential as mucus mimic, not only for its rheological similarity, but for its excellent lubricity in soft compliant and hydrophilic contacts.

I. INTRODUCTION

Mucus is a biological hydrogel (ca. 95 wt.% water content) where amphiphilic and high molecular weight glycoproteins (MDa range) called mucins make up scaffold.[1]

The physiological function of mucus in the digestive system of the stomach and intestines is known to protect the underlying tissues from pathogens and mechanical stress as well as lubricate the tissue surfaces.[1,2] However, native mucus is difficult to obtain and its rheological properties vary according to individual batches and preparation details, and degrade with time,[3] which is a barrier for extensive research on mucus and its potential applications. Hence, developing mucus-like hydrogels with properties similar to native mucus is a compelling idea, and in fact various attempts to develop mucus models or mimics have been reported in literature.[3-7] Yet, these studies have predominantly focused on characterizing and mimicking the rheological properties of mucus,[4-7] whereas similar studies on the lubricating properties are very rare to date.[8] Even though rheological properties are one of the key parameters affecting the lubricating properties of a fluid, the latter are further dependent on many other interfacial parameters such as wetting, morphological, chemical, and mechanical properties of the shearing surfaces. It should be noted that while the lubricating properties of dilute mucin solutions at various interfaces and contact scales have been studied,[9-14] they do not represent the lubricity of mucus hydrogels. For instance, the lubricity of dilute mucin solutions are achieved mainly by the activity of mucins as boundary lubricant additives, i.e. adsorption onto moving surfaces from base stock (aqueous) solution and modification of the interface shear strength. Thus, its application as lubricant is limited to enclosed systems, e.g. bearings, in which fluid lubricants can be sealed.[11,15] Meanwhile, an outstanding feature of mucus as a lubricant is, similar to grease lubricants, its ability to stay at the tribological interface as spread thin films, and it does not necessitate flooded immersion of the tribopair.

The objective of this study is to generate various mucus models and characterize their lubricating properties, and compare them with native mucus, as thin films (thickness ≤ 1 mm prior to tribological experiments) at compliant contacts. Because of their wide availability and low cost, commercially available mucins are first candidates to construct mucus models in large volume. However, commercial mucins, e.g. porcine gastric mucin (PGM), have long been known to fail in replicating the rheological properties of native mucus by simply dissolving in aqueous solvents at the physiological or even higher concentrations.[16] This is ascribed to the fact that irreversible changes occur in the process of isolation and purification from native mucus and deteriorate the self-aggregating properties of mucins, which is essential to form mucus gels.[16] In order to overcome this drawback, we have employed mucoadhesive polymers, either poly(acrylic acid) (PAA) or branched poly(ethyleneimine) (b-PEI), as a "crosslinker". PAA is a representative mucoadhesive polymer that displays associative interaction with PGM mainly via hydrogen bonding as reported in previous studies.[17-20] In addition, recent studies on the interaction between PGM and b-PEI have also shown associative interaction mainly via electrostatic attraction and improved lubricating properties in the dilute concentration regime (≤ 1 mg/mL in total concentration).[21,22] An illustration of the chemical structure of PAA and b-PEI, as well as the interaction mechanism with PGM, is presented in Schematic 1.



Schematic 1. Chemical structures of PAA (top-left) and b-PEI (top-right) and an illustration of the associative interaction with PGM.

It is hypothesized that the associative interaction between PGM and the two polyelectrolytes may be extended to the high concentration regime (ca. 50 – 100 mg/mL in concentration). Ultimately, mucoadhesive polymers may assist recovering the rheological as well as tribological properties of mucus generated from commercially available mucins. High concentration solutions of neat (polymer-free) PGM or the polymer itself were also employed as references. In the characterization of lubricating properties of various mucus models, the correlation with their rheological properties was first investigated. Moreover, a number of interfacial and measurement properties, including wettability of the fluids on tribopairs, speed, load, and slide-to-role ratio, were varied in order to fully characterize and understand the lubricating properties of mucus models.

II. EXPERIMENTAL

A. Mucin, Polymers and Model Mucus

PGM, b-PEI (Mw 25 000 Da), PAA (Mv 1 250 000 Da) were purchased from Sigma Aldrich (Brøndby, Denmark) and used as received from the manufacturer. Basic information on the structural and compositional characteristics of PGM can be found in literature.[23,24] The model mucus samples were prepared by dissolving PGM and b-PEI or PAA in non-saline phosphate buffer (denoted as "PBS-0", where NaCl is excluded from standard PBS, pH 7.4) at a concentration of 100 mg/mL (wt. ratio 1:1 of PGM:b-PEI) or 59 mg/mL (wt. ratio 50:9 of PGM:PAA as in "biosimilar" mucus,[7] except that BSA and lipids were excluded), and they were denoted as **PGM:b-PEI** and **PGM:PAA**, respectively. The mixing ratios for the two PGM:polyelectrolyte mixtures were determined based on the optimization in previous studies for PGM:b-PEI[21,22] and PGM:PAA,[7] respectively. Neat (unmixed) PGM and b-PEI (100 mg/mL) solutions were also employed for comparison, and denoted as **PGM** or **b-PEI**, respectively.

B. Preparation of Biological Porcine Gastric Mucus (PGMS)

A batch of crude mucus scrapes was obtained from a porcine stomach about an hour after slaughter. The stomach was dissected and rinsed with PBS containing 0.02% w/v sodium azide and 5 mM ethylene diamine tetra-acetic acid (EDTA) as the chelating agent, to remove any extraneous debris. Mucus was scraped from the epithelial surface of the stomach with plastic cell scrapers (Corning® Small Cell Scraper, NY). Care was taken to extract only mucus and avoid any contamination from the bile and/or excess food content. The crude mucus extracts were aliquoted on 50 mL centrifuge tubes and stored in a laboratory freezer (-26 °C) until further treatment. To this end, the frozen mucus samples were thawed on ice and mixed with equal volume of PBS (pH 7.4) containing 0.02% w/v sodium azide and 5 mM EDTA. The samples were gently stirred for an hour to obtain a homogenous mixture. Samples were then centrifuged at 10,000 rpm for 15 min.[25] The supernatants were discarded and the steps were repeated twice. Finally, the insoluble mucus gels were re-suspended in PBS to the initial volume of mucus. Biological porcine gastric mucus samples are denoted as **PGMS**.

C. Shear and Oscillation Rheometry

Rheological properties of mucus samples were characterized with a controlled stress HAAKE™ MARS™ rheometer (Thermo Scientific Inc., Germany) using a serrated bottom and a serrated upper parallel-plate (diameter of 60 mm) and a gap of 1.0 mm. To avoid evaporation during the experiment, the sample edges were covered with silicone oil. The samples were loaded onto the rheometer sample plate at 37 °C and the frequency sweep in the linear viscoelastic region (0.1 to 150 rad/s) was carried out, keeping the temperature constant. Flow measurements were performed right after frequency sweeps also at 37 °C.

D. Tribopairs

Two different types of tribopairs with ball-disc geometry were employed: poly(dimethylsiloxane)-poly(dimethylsiloxane) (PDMS-PDMS) to represent soft and hydrophobic interfaces and steel-PDMS to represent soft and (half) hydrophilic interfaces. The steel balls were bearing balls (AISI 52100, ¾ inch diameter, 19.05 mm) commercially available from PCS Instruments (London, UK). Steel balls were hydrophilized by air plasma treatment with a plasma cleaner/sterilizer (Harrick Plasma, model PDC-002, New York, NY) for 2 min at 30 W prior to tribological experiments. PDMS balls and discs were fabricated from a two-component kit (SYLGARD® 184, Dow Corning, Midland, MI) consisting of base PDMS and cross-linker according to a standard procedure.[26] For PDMS discs, the mixture fluid of base PDMS and cross-linker was poured on top of a polytetrafluoroethylene (PTFE) disc in a plastic mould with nearly the same diameter and cured overnight at 70 °C. The PDMS thickness on top of the PTFE was 2 mm. PDMS balls with ¾ inch (19.05 mm) diameter were cast in an epoxy half-sphere mould with a home-machine aluminum mold. A Ramé-hart (model 200 F1) goniometer analyzed water contact angles of untreated and plasma-treated steel substrates provided in Table 1.

Surface roughness of the discs and balls was characterized by acquiring root-mean-square roughness (R_q) from the tapping-mode AFM topographic images (100 μm × 100 μm area). Three different spots were characterized for statistical evaluation. The full list of the surface roughness of the tribopairs is shown in Table 1.

Table 1.

Substrate	Young's Modulus (MPa)	Contact Pressure (MPa)[b] at 5, 10, 20 N load	Poisson's Ratio	Roughness, R_q (nm)		Static contact angle (°) by MilliQ water (5 μL)
				Disc	Ball	
PDMS	2.0[27] (6.9)[a,28]	0.24, 0.35, 0.44	0.5[27]	1.6 ± 0.3	16.2 ± 1.3	105.6 ± 2.2

Steel (AISI E 52100)	210000[29]	0.65, 0.81, 1.02	0.3[29]	-	19.0 ± 0.7	untreated: 35.8 ± 3.8 plasma treated: < 10

[a]6.9 MPa represents the effective Young's modulus of a 2 mm PDMS layer on PFTE. [b]Contact pressures in MTM experiments for PDMS-PDMS and steel-PDMS contacts. Hertzian hemisphere on plane contact mechanics was applied in calculations. For steel-PDMS effective modulus (E´) was used.

E. Mini-traction Machine (MTM)

A mini-traction machine (MTM) (MTM2, PCS Instruments, London, UK) with software version 3.2.3.0 was employed to characterize the lubricating properties of mucus samples. The MTM consists of an independently rotating ball and disc with the possibility to control speed, load, slide/roll ratio, and temperature. The friction forces are measured with a strain gauge connected to the ball shaft arm. The coefficient of friction, μ, is defined from the relationship, $\mu = F_{\text{friction}}/F_{\text{load}}$ where F_{friction} and F_{load} are the friction and load, respectively. The independent control of disc and ball speeds allows for the variation of the degree of slide/roll ratio. The mean speed is defined as $|\text{speed}_{\text{ball}} + \text{speed}_{\text{disc}}|/2$. The slide/roll ratio is defined as $\text{SRR}(\%) = 100\% \times |\text{speed}_{\text{ball}} - \text{speed}_{\text{disc}}|/[(\text{speed}_{\text{ball}} + \text{speed}_{\text{disc}})/2]$, where 0% SRR represents pure rolling and 200% SRR represents pure sliding contact. The applied loads were 5, 10 and 20 N. Apparent Hertzian contact pressures for each tribopair are shown in Table 1. The temperature was set to 37 ± 1 °C for all the experiments using a built-in thermistor in the MTM pot. For thin film lubrication experiments, 200 µL of mucus model lubricant was placed along the sliding track on the disc, resulting in ca. 1 mm thick circular puddle. For flooded lubrication experiments, 15 mL of fluid was transferred into the pot, leading to full immersion of the ball-on-disc tribopair. For speed dependence experiments, two consecutive sweeps were measured to obtain the average μ and standard deviation. For the cases where the μ values of the second sweep were much higher due to centrifugation out of the samples from the contact, only the result from the first sweep is shown.

III. RESULTS AND DISCUSSION

A. Rheological Properties

FIG. 1 shows small amplitude oscillatory shear measurements on the mucus samples.

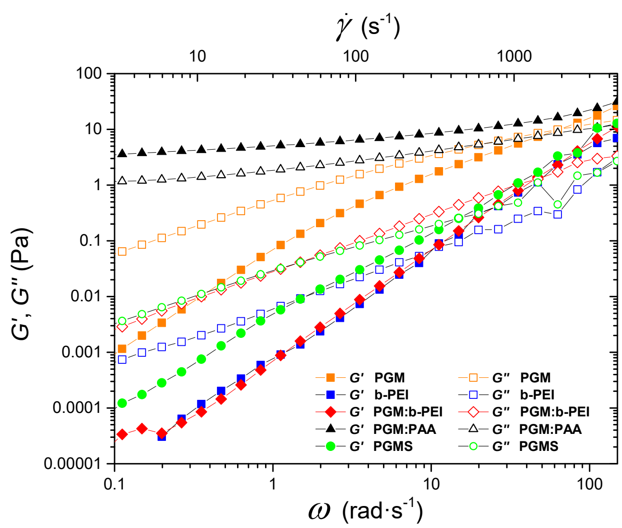


FIG. 1 Data shows results of small amplitude oscillating shear rheological measurements of different mucus models. Elastic modulus *G´* (filled symbols) and loss modulus *G´´* (open symbols) vs. angular velocity (*ω*) and shear rate (*γ*) in oscillating mode for the mucus samples.

For most mucus samples, the loss moduli (*G´´*) are higher than the elastic moduli (*G´*), indicating that viscous behavior is dominant over elastic behavior, except for **PGM:PAA**, where *G´* dominates *G´´*. For all samples, both moduli increase with increasing angular velocity (ω) (or shear rate ($\dot{\gamma}$)). The *G´* and *G´´* of **PGM:PAA** are similar to those previously reported by Boegh et al.[7] It is most noticeable that *G´* and *G´´* of **PGMS** are very low and show a strong viscous character (*G´* < *G´´*). The elastic and viscous moduli of **PGMS** in this study are 3-4 orders of magnitude smaller than those reported by Kocevar-Nared et al[16] or Talyor et al,[30] but are fairly consistent with those reported by Celli et al. for porcine gastric mucus prepared by rehydration of purified mucins.[31] While most of the other studies in literature were conducted at room temperature (20 – 25 °C),[16,30,31] all the rheological measurements of the present study were conducted at 37 °C, and it may partly account for the relatively lower G' and G'' values in FIG. 1. Moreover, the discrepancy in the reported rheological properties of native mucus samples could be due to intrinsic batch differences related to age, species, and healthy status of slaughtered animals, as well as from fairly different preparation procedures across studies. Overall, it might be more difficult to compare the studies involving native mucus samples. In contrast, the excellent agreement of the rheological properties of **PGM:PAA** with those reported in literature[7] highlights its reliability and merit as a mucus mimic.

Shear rates in biological gastric conditions are estimated to be of the order of $\dot{\gamma} \approx u/h = 0.4 - 7.2\ s^{-1}$ with flow velocities $u = 0.08 - 0.72\ mm \cdot s^{-1}$ in the jejunum and ileum,[32] and mammal mucus thickness $h = 100 - 200\ \mu m$ in the jejunum.[33] Thus, the largest shear rates applied by rheometry in this study are probably much higher than in biological systems.

The rheological behavior of the mucus samples under shear flow is displayed in FIG. 2. While the shear stress (τ) and viscosity (η) were measured during both increasing and decreasing shear rate, the data acquired during increasing shear rate only are presented for clarity. The data during decreasing shear rate are presented in FIG. S1(a-b). in the Supplementary Materials.

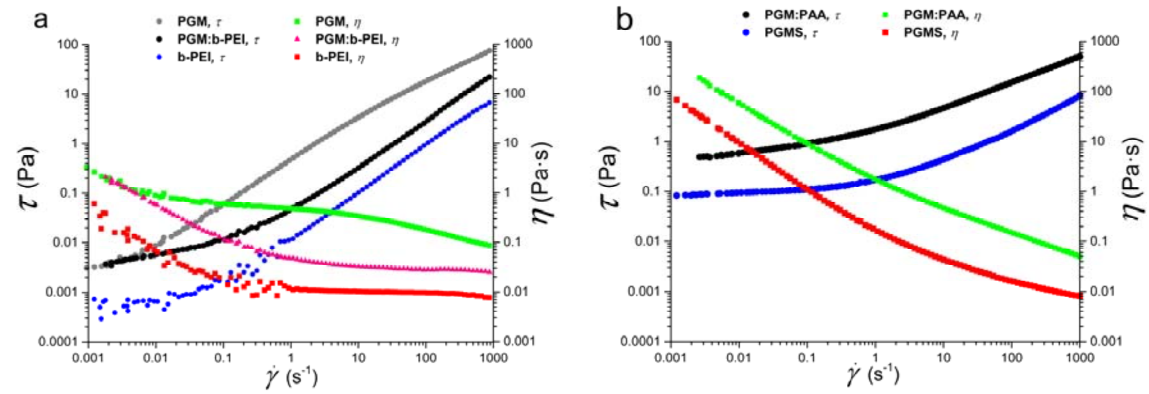


FIG. 2. Measurements of shear flow rheological measurements of the different mucus models. Shear stress (τ) and viscosity (η) for (a) **PGM**, **b-PEI** and **PGM:b-PEI** and for (b) **PGM:PAA** and **PGMS**, acquired during increasing shear rates.

Here the shear stress and viscosity are characterized under continuous flow, thereby affording the apparent viscosity: $\eta = \tau/\dot{\gamma}$. Overall, the viscosities of all the mucus samples decreased with increasing shear rate, i.e. shear thinning was observed. **b-PEI** has a substantially lower molecular weight than the mucins and no particular self-association is expected. This can explain the lowest viscosity of 0.01 Pa·s for all the samples. PGM showed a low-to-medium viscosity of ca. 0.5 - 0.1 Pa·s, from highest to lowest shear rates. **PGM:b-PEI** showed intermediate rheological properties between PGM and b-PEI. The decreased viscosity of **PGM:b-PEI** compared to **PGM** may be explained by molecular-level electrostatic screening of the repulsion between negatively charged PGM molecules and positively charged b-PEIs, with consequent contraction of the PGM chain persistence length and gyration radius.[21] The pervaded volume, chain overlap, and entanglement of PGM molecules decreased accordingly, eventually lowering the viscosity. This is in a strong contrast with **PGM:PAA**, for which the viscosity is somewhat higher (ca. 500 Pa·s, at lowest shear rates) than **PGM** even though the concentration of PGM is still dominant in the total concentration. This behavior can be ascribed to the well-known mucoadhesive interaction between PGM and PAA[17-20] and the strong interpenetrative interaction between them. For all the mucus samples in FIG. 2a, the viscosities were very similar in their increasing and decreasing shear rate sweeps without visible hysteresis, indicating homogeneous fluids with fast relaxation times. In FIG. 2b, **PGMS** showed medium viscosity of 40 - 50 Pa·s in the initial increasing sweep at the lowest shear rates, and continued to show shear-thinning behavior down to 0.008 Pa·s at the maximum shear rate. For decreasing shear rates (FIG. S1(a-b)), the viscosities of **PGMS** and **PGM:PAA** also did not show much hysteresis compared to the data during increasing shear rates (FIG. 2), except for a small degree of hysteresis for **PGM:PAA** from ca. 1 s^{-1} that may indicate fast recovery or relaxation times. **PGM:PAA** generally displayed ten times higher viscosity than **PGMS** at all shear rates, which is in good agreement with a previous study on "biosimilar" mucus composed of PGM, PAA, albumin, and lipids.[7] **PGM** displayed a viscosity ca. 2 orders of magnitude lower than **PGMS** at low shear rate despite the fact that the PGM sample contained twice as much PGM protein as **PGM:PAA**.

B. Lubricating Properties

B-1. Modelled Lubrication Thickness and Friction Coefficients

As shown in Section A, mucus models of **PGM:b-PEI**, **PGM:PAA** and mucus **PGMS** showed viscosities of ≥ 0.01 Pa·s even at the highest shear rates of 10^3 s^{-1}. Since the normal load in tribological contacts for the effective shear rates are usually substantially larger, up to 10^6 s^{-1}, it causes significantly smaller viscosities at severe shear thinning.[34] In order to establish a theoretical prediction on whether the observed viscosities in Section A would be realized in the tribological contact as well, calculations on the lubricating film thicknesses solely due to entrainment forces, and also the effective coefficient of friction, were carried out according to the soft elastohydrodynamic lubrication (EHL) model:[35]

$$h = 3.28\cdot(w^{-0.21}E'^{-0.45}\eta_0^{0.66}u^{0.66}R_x^{0.76}) \quad (1)$$

where h is the lubricating film thickness, η_0 lubricant's viscosity at atmospheric pressure, u mean speed, E' reduced Young's modulus, w applied load, and R_x radius in the x direction of motion (equal to R_y in circular contact). For this calculation, experimental data for u, E', w, and R_x were used. For η_0, we arbitrarily chose three different viscosities of 0.001, 0.010 and 0.100 Pa·s, i.e. two orders of magnitude difference, in order to calculate the onset of the EHL regime. These viscosities are in the range of the samples at high shear rates in FIG 2. For PDMS-PDMS contact, the Stribeck parameter ($\lambda = h/\sigma$, film thickness over roughness ratio, see also below) is larger than 10 for 0.100 and 0.010 Pa·s for the entire speed range 10-1000 mm/s, thus indicating fully separated contact in the EHL regime. At 0.001 Pa·s, full separation EHL regime is calculated to onset at 60 mm/s. Calculations of steel-PDMS contact afforded $\lambda > 10$ at all speeds for 0.100 Pa·s, at 35 mm/s for 0.010 Pa·s, and 360 mm/s for 0.001 Pa·s. Further details on the lubricating film thickness calculation according to this model are provided in the Supplementary Materials (FIG. S2 and S3). This model was originally proposed and validated for fully flooded lubrication. We assume that the model can be extended to thin film lubrication, as long as the inlet of the contact area is flooded with the lubricants. MTM friction experiments employed only a small amount of mucus samples to generate ca. 1 mm thickness (under atmospheric pressure prior to the tribological contacts). Under this condition, insufficient inflow of lubricant to the tribological contacts can lead to lubricant starvation and a smaller lubricating film thicknesses h than calculated from Equation 1. Whether or not lubricant starvation occurs can be evaluated by a formula proposed by Brewe and Hamrock:[36] $H_{\text{inlet}} = 4.11 \cdot H^{0.36}$, where H_{inlet} is the dimensionless inlet thickness and H is the dimensionless minimum film thickness in the contact zone ($H_x = h/R_x$, for x direction). When the applied lubricant film thickness is larger than H_{inlet}, there is enough lubricant to avoid starved lubrication. We can calculate the required lubricant inlet thickness in dimensional terms for a calculated film thickness of, for example, h = 163 nm to meet the condition of Stribeck (or lambda, λ) value being > 10 for soft PDMS-PDMS contact[37] ($\lambda = h/(\sigma_{\text{ball}}^2 + \sigma_{\text{disc}}^2)^{½}$, σ_x is the RMS roughness of the tribopair surfaces, see Table 1), then $h_{\text{inlet}} = 4.11 \cdot (H)^{0.36} \cdot R_x = 4.11 \cdot (163\ \text{nm}/(9.5\ \text{mm}))^{0.36} \cdot 9.5\ \text{mm} = 0.75\ \text{mm}$). Hence applying ca. 1.0 mm thickness of mucus sample should be sufficient to validate the application of Equation 1. In practice, however, two potential changes are envisioned. Firstly, upon initiation of circular rotation of the disc, the film thickness h_{inlet} is expected to be reduced due to spin-off of the lubricants. Secondly, shear thinning of the mucus samples, which is even more significant under tribological contact due to extremely high shear rates, renders the viscosity lower than the values measured in rheology tests (FIG. 2).

While the lubricating film thickness can be readily modeled according to soft EHL theory as described above, it is not straightforward to verify the applicability to the present case because it is very challenging to determine the lubricating film thickness experimentally. Meanwhile, friction forces can be easily measured and the application of the soft EHL model to the contacts in this study can be indirectly verified based on the characteristic behavior of friction forces. In other words, if hydrodynamic lubrication indeed takes place, i.e. the two surfaces in sliding contact are completely separated by the lubricant film and Equation 1 is valid, the friction forces are dominated by the shear stress (τ) associated with shearing of the lubricant: $\tau = \dot{\gamma} \cdot \eta$. This behavior, observed by rheometry (FIG. 2(a)), is also expected for friction experiments with MTM if the viscosities at the tribological interface are high enough to induce hydrodynamic lubrication. A model incorporating both Couette and Poiseuille flow by de Vicente et al. predicts the μ associated with the hydrodynamic shearing of the (Newtonian) lubricant:[37]

$$\mu_{\text{total}} = \tau/w = 1.46 \cdot \bar{U}^{0.65} \cdot \bar{W}^{-0.70} + \text{SRR} \cdot (3.8 \cdot \bar{U}^{0.71} \cdot \bar{W}^{-0.76} + 0.96 \cdot \bar{U}^{0.36} \cdot \bar{W}^{-0.11}) \quad (2)$$

Here $\bar{U} = u\eta/(E'R')$ and $\bar{W} = w/(E'R'^2)$ are the dimensionless speed and load parameters, respectively, and SRR is the slide-to-roll ratio. The μ calculated from Eq. 2 as function of mean speed (u), assuming Newtonian (constant) viscosity, are shown in a semi-log plot in FIG. 3, where μ is seen to increase exponentially with u. This model will be used to assess the frictional properties of the mucus samples in the following sections.

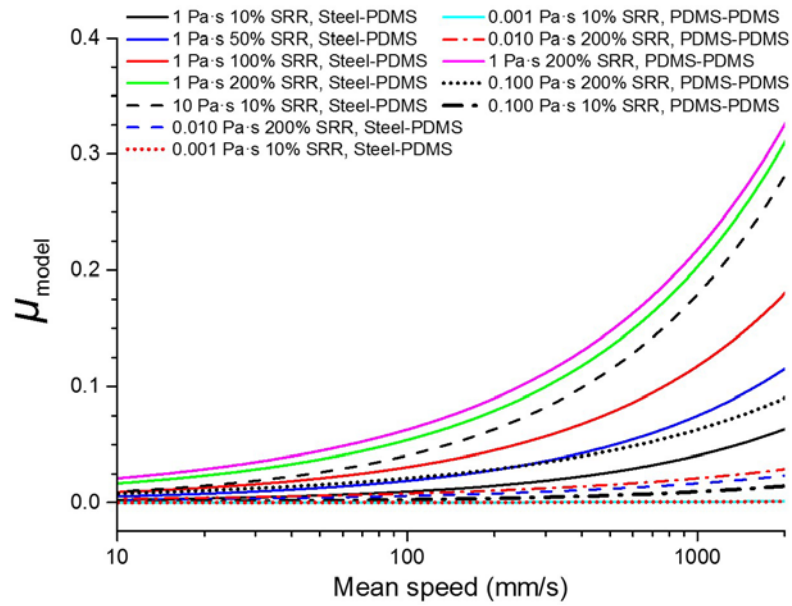


FIG. 3. Friction coefficients (μ) calculated from Equation 2 as a function of the mean speed (u), viscosity (in Pa·s) and SRR ratios for steel-PDMS and PDMS-PDMS contacts (5 N load) lubricated by generic Newtonian fluids. The model assumes constant viscosity as in a previous study by de Vicente et al.[37]

B-2. Wettability of the Tribopair Surfaces with Mucus Models

The degree of wetting is proportional to the affinity and bonding between the surface and lubricant. Good wetting favors no-slip condition, which is a requirement of Reynold's equation (from which Equation 1 is derived), and provides boundary lubrication due to the bonding forces.[38] The static contact angles of the different mucus samples on the tribopair substrate surfaces are shown in FIG. 4.

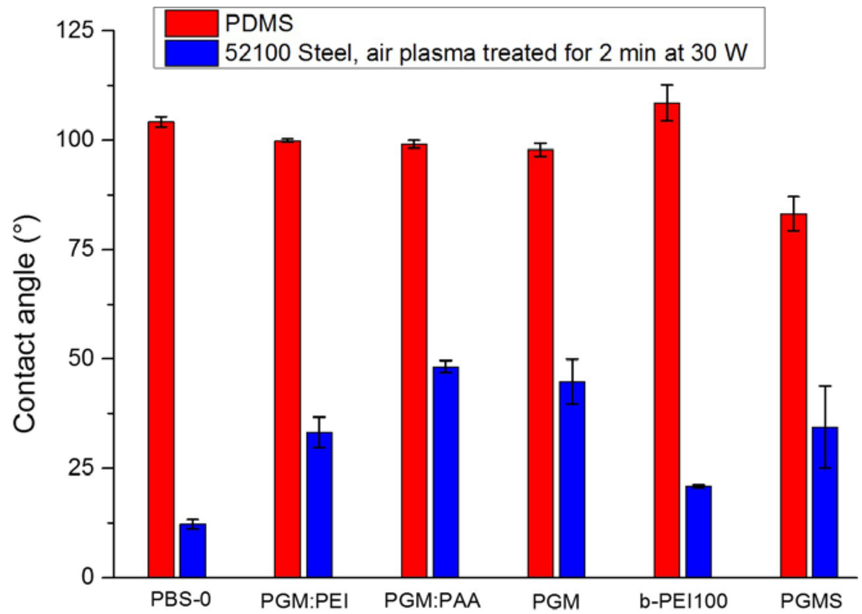


FIG. 4. Static contact angle of 10 μL applied samples on PDMS and steel surfaces.

For all mucus samples, the contact angles on PDMS (hydrophobic with 20 mJ/m^2 surface tension)[39] were high (ca. 100°) and similar to that of PBS-0, with the exception of **PGMS** showing a somewhat lower contact angle of 83.2 ± 3.9°. These results imply that the mucus samples' surface tension is not lowered compared to neat water by the mucin or polymer content in the mucus models. This is similar to the behavior of some amphiphilic macromolecules in dilute solutions that do not lower the surface tension in contrast with small surfactant molecules.[40,41] Mucin molecules form aggregates in the mucus samples, and thus their movement at the interface is not as feasible as in dilute solutions. Moreover, the rigidity generated for hydrogel-like mucus samples counteracts the spreading of the fluids.

The contact angles of the mucus samples on the 52100 steel surface were much lower (< 50°) than those on PDMS, which is attributed to its high surface energy after plasma cleaning. The contact angles of the mucus samples on the 52100 steel surface were in the order: **b-PEI** (20.8 ± 0.4°) < **PGM:b-PEI** (33.2 ± 3.5°) ≅ **PGMS** (33.4 ± 9.4°) < **PGM** (44.8 ± 5.1°) ≤ **PGM:PAA** (48.2 ± 1.4°). The fact that contact angles on the 52100 steel surface by the mucus samples were somewhat higher than those by PBS-0 (12.2 ± 1.1°) can be ascribed to the resistance to flow of hydrogel and higher viscosities of the samples, i.e. counteracting the spreading. Nevertheless, the overall trend of **PGMS** and the other mucus samples were similar and showed more enhanced wetting on hydrophilic surfaces than on hydrophobic surfaces.

B-3. Experimental Friction Measurements; Surface Hydrophilicity of the Tribological Interface and Shear Thinning of Mucus Samples

As mentioned in the previous section, if the viscosities of the mucus at the tribological contacts were similar to those measured in rheological tests with low shear rates (1 - 10 Pa·s, FIG. 2), μ should increase exponentially in a semi-log plot as a function of the speed, as in FIG. 3. Namely, Equation 2 gives μ = 0.05 and 0.20 for 1 Pa·s and 10 Pa·s, respectively, at the highest mean shearing speed, i.e. 1,200 mm/s and 10% SRR conditions. These values can be compared with the friction coefficients experimentally determined with MTM. For steel-PDMS contact (FIG. 5a), the μ values were very low, ≤ 0.02, over the entire speed range, indicating that all model mucus were effective lubricants for this tribopair under 5 N load (Hertzian contact pressure, 0.65 MPa) and near-rolling contact (SRR, 10%).

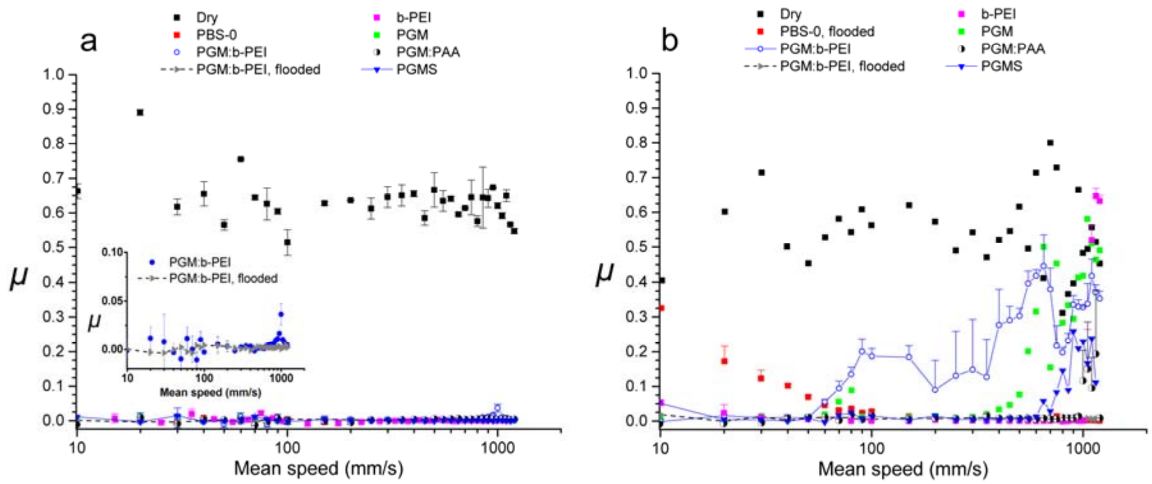


FIG. 5. μ vs speed measured with MTM for (a) steel-PDMS and (b) PDMS-PDMS interface lubricated with the mucus samples (load = 5 N, SRR = 10%) For PGM and PGMS, only data points from the first speed sweep are shown due to much higher friction in the second sweep caused by centrifugation depletion. Mucus samples were applied as thin film lubricants, unless 'flooded' is stated. Some negative error bars were removed for clarity. The lines connecting the data points are a guide to the eye.

A more direct comparison between thin film vs. flooded lubrication can be made for **PGM:b-PEI** as both cases were tested as shown in FIG 5 (see the inset). Thin film lubrication is generally expected to be inferior to flooded lubrication especially at higher mean speeds, however friction forces displayed by the thin film **PGM:b-PEI** were indistinguishable from those obtained from flooded **PGM:b-PEI**; the spike at 1000 mm/s for **PGM:b-PEI** is ascribed to observed resonance wobbling during the measurement. This observation supports the validity of the equation by Brewe and Hamrock in estimation of the minimum thin film thickness to invoke hydrodynamic lubrication without starvation. However, the absence of a characteristic increase of μ with increasing speed, as displayed in FIG. 3, indicates that the predicted hydrodynamic lubrication may not be activated in practice. Nevertheless, it should be emphasized that this was observed for both thin films as well as flooded lubrication in the case of **PGM:b-PEI**.

Several differences were observed for the PDMS-PDMS interface compared to the steel-PDMS interface. Firstly, for the PDMS-PDMS interface, μ was high at low speeds but was decreasing with increasing speed for the flooded lubrication with PBS-0, which is typical behavior of aqueous lubrication for hydrophobic interfaces without surface-adsorbing additives, highlighting the importance of surface hydrophilization for effective lubrication with water.[42,43] Secondly, flooded lubrication of the PDMS-PDMS interface with **PGM:b-PEI** displayed $\mu \leq 0.02$ throughout the entire speed range (similar to the steel-PDMS contact). On the other hand, thin film lubrication by mucus model fluids showed distinctly higher friction coefficients and μ started to increase from ca. 70, 400, 700, 1000 and 1000 mm/s for **PGM:b-PEI**, **PGM**, **PGMS**, **b-PEI**, and **PGM:PAA**, respectively. Generally, the increase in μ values was observed in the high-speed regime and the values were substantially higher than the values predicted from hydrodynamic lubrication (Equation 2). Thus, this behavior is not an indication of the onset of hydrodynamic lubrication but depletion of lubricants from the contact due to centrifugation.

Given that the mechanical properties (contact pressure) of the steel-PDMS and PDMS-PDMS tribopairs are similar (Table 1), drastically different lubricating properties of the model mucus fluids for the two interfaces can be attributed to the different surface hydrophilicity of steel vs PDMS pins. This has been well described in a number of previous tribological studies involving aqueous solutions,[43] and is also clearly manifested in much more effective lubrication of PBS-0 at steel-PDMS (FIG. 5a) than at PDMS-

PDMS interfaces (FIG. 5b). Relatively less recognized to date is that thin film lubrication with hydrogels, such as mucus or mucus mimics considered in this study, is also influenced by surface hydrophilicity of the tribological interface in a similar manner.[38] Even in the absence of a hydrodynamic lubrication mechanism, it is remarkable that only a thin layer of model mucus films of **PGM:b-PEI** on the surface (disc) provides equally effective lubrication as the flooded lubrication case for steel-PDMS interfaces (FIG. 5a). Firstly, this can be ascribed to the hydrophilicity of steel balls, which enables high affinity with hydrogels, and more effective retainment of the lubricating films at the tribological interface as mentioned above. But, it must also be noted that the difference in wettability by various mucus samples on the steel surface (FIG. 4) did not have a significant impact, presumably because the highest contact angle observed from **PGM:PAA** ($48.2 \pm 1.4°$) is sufficiently low for effective lubrication. Secondly, the fluids' high viscosity property may retard the depletion of lubricants from the tribological interface, which is exposed to the risk of spin-off and starvation under influencing forces of centrifugation or gravity. Previous studies on molecularly thin perfluorocarbon lubricants on hard drive discs showed that the magnitude of lubricant film depletion rate from spin-off is proportional to the square root of the rotational speed (ω) and inversely proportional to the viscosity (η), namely $-\mathrm{d}h/\mathrm{d}t \propto \omega^2 \cdot \eta^{-1}$.[44] This relationship is also applicable to spin coating of a thin film.[46] The relationship between rheological properties of the mucus samples and film thickness (assuming that threshold depletion speed is a reflection of the film thickness) is, however, not straightforward in the present case, mainly due to strong shear-thinning effects of all mucus samples (FIG. 2). This may explain why the deviation from the effective lubrication in the high-speed regime at

PDMS-PDMS interfaces, i.e. spin-off and depletion of lubricants, is not simply proportional to inverse viscosity.

Despite the relatively greater hydrophilic characteristics and generally effective lubrication, the hydrodynamic lubrication mechanism was not activated even at steel-PDMS interfaces, either thin film or flooded lubrication, as mentioned above. This is most probably because shear rates in tribological contacts were several orders of magnitude higher than those from rheological interfaces,[47,48] due to a much smaller lubricating film thickness (h) (corresponding to 'gap' in rheometer), $\dot{\gamma} = (dx/dt)/dh \approx u/h$ (Couette flow speed)/(gap height). Moreover, as all the fluids in this study show shear-thinning properties, apparent viscosities at the tribological interfaces are likely to be much lower than observed in the rheological experiments, which retards the onset of hydrodynamic lubrication. Alternatively, very low viscosities in the hydrodynamic regime may afford so small viscous shear forces that they cannot be detected by the MTM instrument ($\mu < 0.008$) and no apparent speed dependence is observed.

B-4. Experimentally Determined Friction Coefficients: Influence of Load and SRR

The lubricating properties of the model mucus fluids were further investigated under harsher tribological conditions, either by increasing load or SRR. Firstly, the load was increased from 5 N to 10 N, and then to 20 N, while SRR was maintained at 10%. As some of the model mucus fluids were centrifuged out from the PDMS-PDMS interface at high speeds (FIG. 5b), the speed was reduced to 20 mm/s. The average μ values were fairly constant over the contact time of 600 s for most cases (FIG. 6).

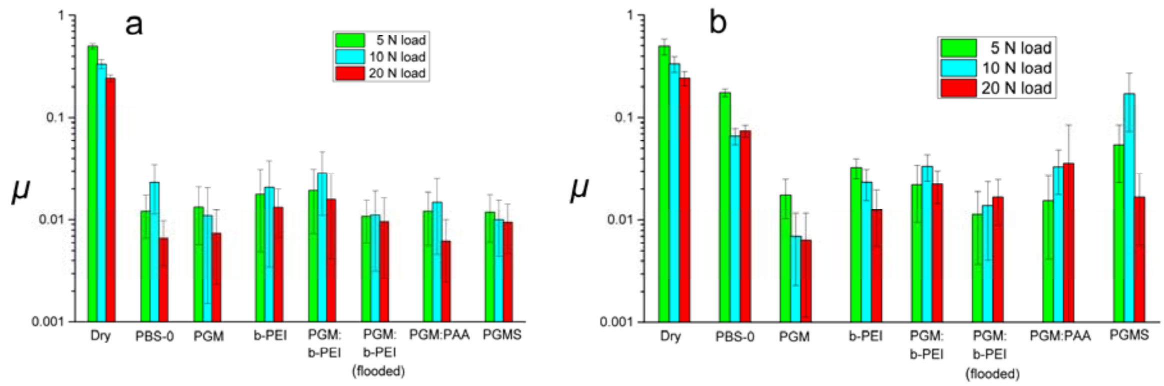


FIG 6. μ values at 5, 10, and 20 N (a) steel-PDMS and (b) PDMS-PDMS interfaces lubricated with the mucus samples as characterized with MTM (speed = 20 mm/s, SRR = 10%).

As shown in FIG. 6a, effective lubrication of steel-PDMS contacts by all the model mucus fluids employed in this study at 20 mm/s and 10% SRR were consistent up to 20 N and the μ values were still within the range of 0.01 - 0.001. No particular load dependence was observed for all cases in PDMS-PDMS contacts either (FIG. 6b), with the notable exception of PGMS showing an increase in average μ values under the contact at 10 N. This is considered as a random outlier because lower μ values were observed again at a higher load, 20 N. Distinctively inferior boundary lubricating properties of thin film flooded PBS-0 compared to the other samples were also persistent up to 20 N.

Lastly, SRR was increased from 0% (pure rolling contact) to 200% (pure sliding contact). The speed and load were fixed at 20 mm/s and 5 N, respectively. For steel-PDMS interfaces (FIG. 7a), as expected, friction coefficients of dry contact gradually increased with increasing SRR, due to increasing sliding characteristics at the tribological interface.

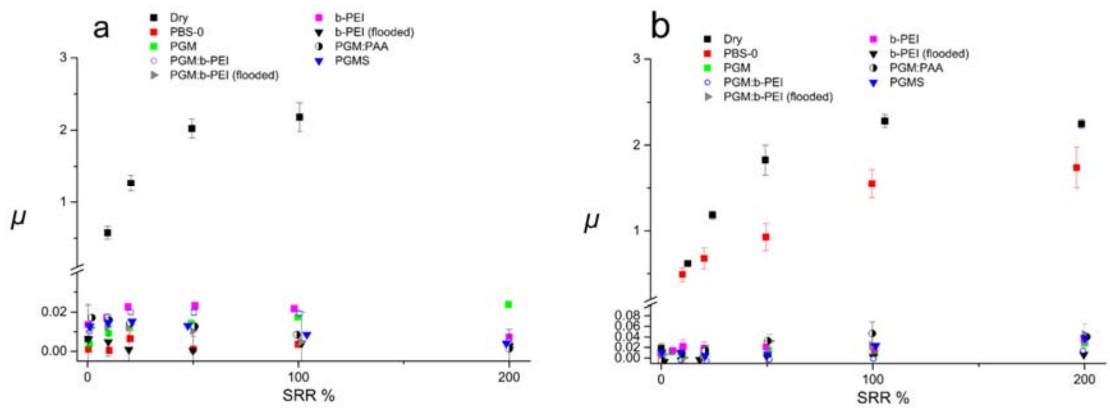


FIG. 7. μ vs SRR for (a) steel-PDMS interface and (b) PDMS-PDMS interface lubricated with thin films of mucus samples as characterized with MTM (speed = 20 mm/s, load = 5 N). Friction was too high in the dry contact for the measurements to commence at 200% SRR for steel-PDMS.

Meanwhile, all the model mucus fluids and flooded PBS-0 buffer were consistently effective in lubrication of the steel-PDMS interface and μ = 0.001 - 0.02. As discussed above, the hydrophilic property of the plasma treated steel ball is an important driving force to retain viscous and hydrophilic fluids at the tribological interface in the boundary lubrication regime. For PDMS-PDMS interfaces (FIG. 7b), distinctly inferior lubricating properties of PBS-0 buffer (FIG. 5b and 6b) were persistent as the variation of μ with the increase of SRR were similar with those of dry contacts. Meanwhile, for all the other model mucus samples, the μ values remained in the range 0.05 - 0.005 despite the increase of SRR from 0% to 200%. This means that as long as spin-off or starvation can be suppressed by keeping the speed low, thin mucus films can effectively lubricate even sliding contacts of hydrophobic PDMS-PDMS interfaces in the boundary lubrication regime. As the tenacity of the mucus samples onto PDMS surface is fairly weak as shown by poor wettability (FIG. 4) and they gradually spin-off from the surface with increasing speed (FIG. 5), this lubricating efficacy can be largely attributed to the low speed in the experiments. This minimizes the reduction in apparent viscosity from shear thinning and further assists the retainment of the fluids at the interface despite the hydrophobic nature of PDMS-PDMS interfaces.

IV. SUMMARY AND CONCLUSIONS

In this study, we have prepared model gastric mucus samples based on commercially available PGM and characterized their lubricating properties, along with biological porcine gastric mucus (**PGMS**), as thin films at elastomeric tribological interfaces. In order to enhance the gel-like structure of the mucus samples, two types of mucoadhesive polymers were employed as crosslinkers, namely PAA and b-PEI, yielding **PGM:PAA** (50 mg/mL:9 mg/mL) and **PGM:b-PEI** (50 mg/mL:50 mg/mL), respectively. The rheological studies have shown that only **PGM:PAA** revealed a gel-like behavior at 37 °C, presumably due to an associative complexation between PGM and PAA, whereas all the other model mucus samples, including **PGM:b-PEI**, **PGM** (PGM at 100 mg/mL), and even **PGMS**, showed fluid-like characteristics. However, **PGM:PAA** exhibited shear properties with closest resemblance to **PGMS,** with high viscosity at low shear rate.

All the model mucus samples showed excellent boundary lubricating properties as thin films at the steel-PDMS interface, with coefficients of friction $\mu \leq 0.01$ over a large speed range (from 10 mm/s to 1200 mm/s), when slide/roll ratio was very low (i.e. near rolling contacts). In particular, **PGM:b-PEI** showed indistinguishably effective lubricity as a thin film or in flooded lubrication of the tribopair, which indicates that a thin layer of **PGM:b-PEI** is sufficient to lubricate the steel-PDMS interface under this condition. The dominance of boundary lubrication mechanisms by the mucus samples even at the highest speed in this study (1200 mm/s) and lack of transition to full fluid lubrication were ascribed to severe shear thinning of the fluids at the tribological interface and consequently much lower apparent viscosities of the fluids. The replacement of hydrophilic steel balls with hydrophobic PDMS balls, however, led to the depletion of the model mucus samples from the tribological interface as the speed was increased and consequently much higher friction shear forces. Given that PDMS-PDMS and steel-PDMS interfaces have nearly identical pressure characteristics, this observation highlights the importance of surface hydrophilicity of the tribopair to retain model mucus samples – hydrogels in a broad sense – at the tribological interface. Nevertheless, as long as the speed was kept low (e.g. 20 mm/s), all mucus samples showed persistently effective lubricating properties ($\mu \leq$ ca. 0.02) at both steel-PDMS and PDMS-PDMS interfaces against even harsher tribological stresses, such as under higher loads (up to 20 N) and under pure sliding contacts. Additionally, much weaker shear thinning and relatively high viscosities at low speeds may further assist the retainment of the fluids at the tribological interface even for hydrophobic PDMS-PDMS interfaces. The particularly effective lubricating effect of **PGM:PAA** under various tribological conditions even as a thin film highlights its potential as an effective mucus mimic, not only for its well-known rheological similarities to **PGMS** but also for its lubricity.

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