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Interaction of Mucins with Proteins and Polymers and Its Influence on the Interfacial Lubricity

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Abstract **-**In this study, we present the interaction of mucins with proteins or polymers and its impact on the conformation, surface adsorption, and lubrication properties at a self-mated sliding contact of poly(dimethylsiloxane) (PDMS) in aqueous solutions. Due to its unique amphiphilicity, mucins are known to spontaneously adsorb onto hydrophobic surfaces and possibly lubricate them in aqueous environment. This study demonstrates that the lubricity of mucins can be substantially altered following the interaction with proteins and polymers, which implies that interaction of mucins with proteins and polymers can be employed to engineer biointerfaces and tailor the lubricity.

Keywords**:** mucin, purification, proteins, mucoadhesion, polycation, polyanion

1. Introduction

Mucins are a major macromolecular component of mucus gels that are known to protect underlying epithelial surfaces against pathogen and mechanical insult (Hattrup et al., 2008). Mucins/ mucus are renowned for their unique lubricity not only for biological tissues, but also for engineering materials in aqueous environment (Cassin et al., 2001, Lee et al., 2005, Bongaerts et al., 2007, Nikogeorgos, et al. 2015). While previous studies have mainly focused on elucidating the generic lubricating properties of mucin itself, in the present study, we present that the lubricity of mucins can be substantially modified by interaction with other macromolecules, namely proteins or polymers. The proteins in this study are selected based on the likelihood that mucins can interact in biological environment, for example, in building up mucus gels (lysozyme and lactoferrin) or during mucin sample collection (scraping mucus) from animal tissues (albumin). Polymers are selected based on their known mucoadhesive interaction (chitosan) or potential adhesive interaction through electrostatic attraction (polyallyl amine (PAAm), polyethyleneimine (PEI)).

2. Materials and Methods

2. 1. Mucins, Proteins, And Polymers

Bovine submaxillary mucin (BSM) and porcine gastric mucin (PGM) were obtained from Sigma Aldrich. Impurities, as represented by bovine serum albumin (BSA), in the samples as received from the manufacture ("ar-BSM"), were minimized by an additional step of anion exchange chromatographic purification (Madsen et al., 2015). Details on BSM purification are available in a previous publication (Madsen, et al., 2015). The fractions containing BSM were pooled, dialyzed against milliQ-grade water (400:1 volume ratio) and freeze-dried to give purified BSM (denoted as "ae-BSM"). The ae-BSM was stored at -20 °C and desiccated prior to use. PGM was also purified according to a slightly modified protocol. As the impact of purification on the tribological properties was of minor importance, a majority of experiments in this study with PGM was carried out after dialysis of the sample. Dialyzed PGM (designated "d-PGM") was freeze-dried, stored at -20 °C, and desiccated prior to use. All proteins (bovine

serum albumin (BSA), lysozyme, and lactoferrin) and polymers (PEI and PAAm) were purchased from Sigma Aldrich.

2. 2. Analytical Techniques

Dynamic light scattering (DLS) was employed to characterize hydrodynamic size distribution and zeta potential of mucins, proteins, polymers, and their mixtures with a Zetasizer Nano ZS instrument (Malvern Instruments Ltd, Worcestershire, UK). Dispersions of 1 mL samples (0.1 to 1 mg/mL) were examined with a 10 mm path–length disposable polystyrene cuvette at 25 °C. The zeta potentials were characterized with a laser Doppler electrophoresis by employing disposable cuvettes (model DTS 1070).

Optical waveguide lightmode spectroscopy (OWLS, Microvacuum, and Hungary) was employed to characterize the surface adsorption properties of samples. OWLS is an optical, non-labelling technique to monitor the adsorption characteristics of macromolecules from liquid to interfacing solid surfaces based on the in-coupling of incident linear polarized laser light with diffraction grating waveguides. In order to emulate the tribopair surface (see the section 2.3), the waveguides for OWLS adsorption experiments were coated with a thin layer of poly(dimethylsiloxane) (PDMS). To this end, waveguides were ultrasonicated in EtOH for 10 minutes and spin-coated with a Sylgard® 184 PDMS kit mixture (base component and crosslinker 3:1 wt. ratio dissolved in heptane to give a spin coating solution of 0.5 wt. %) at 2 000 rpm for 60 s. After spin coating, the waveguides were cured overnight at 70 °C. The reference thickness of the spin-coated PDMS layer as measured on silicon wafers by ellipsometry was 16.4 ± 0.17 nm.

Tapping-mode atomic force microscopy (AFM, Dimension, Brucker) was used to reveal surface morphological features of substrates covered with mucins, proteins, polymers, or mixture of them. Hydrophobized silicon wafer was used as substrate. After the substrates were incubated in the solution for 1 hr, rinsed with PBS buffer, and dried with nitrogen blow. The AFM morphology was acquired in ambient condition.

2. 3. Pin-On-Disk Tribometry

The lubricating properties of mucin, mucin-proteins or mucin-polymer solutions were characterized by acquiring the coefficient of friction, μ (= friction/load), with a pin-on-disk tribometer (CSM Instruments, software version 4.4 M, Switzerland). In this approach, a loaded pin is placed on disk surface, and the disk was allowed to rotate over a defined sliding track (from 0.25 mm/s to 100 mm/s) using a motor underneath the disk. Dead weights (1 or 2 N) were employed to apply external load. The friction forces were detected by strain gauge on the arm holding the pin. PDMS disks and pins were prepared with the PDMS kit mentioned above. Base and crosslinker were mixed at 10:1 wt. ratio. The mixture was then poured into moulds and cured overnight at 70 °C. Home-machined aluminium was used for disc mould (diameter; 30 mm, thickness; 5 mm), and Nunc™ U96 MicroWell™ plates (Thermo Scientific, Denmark) were used for pin (radius; 3.0 mm) mould. The roughness of the PDMS disks and pins was measured by AFM tapping mode. Water contact angle on PDMS surfaces were 105.6 ± 2.2° (tested with Millipore water, standard deviation from 5 measurements).

3. Results and Discussion

3. 1. BSM and PGM

BSM and PGM represent slippery and non-slippery mucins in neutral aqueous solution (e.g. PBS) at PDMS/PDMS sliding contact, respectively. Despite feasible adsorption of both mucins onto hydrophobic surfaces (OWLS experiments, data not shown), PGM displays much inferior lubricity to BSM at neutral pH condition. Thus, BSM in this study is employed when degrading lubricating effect is shown upon interaction with proteins or polymers, whereas PGM is employed when improving lubricating effect is shown.

3. 2. Interaction With Proteins

BSA represents the major impurity in ar-BSM. The comparison of ar-BSM, d-BSM, and ae-BSM showed the friction coefficient in the order of μ (ar-BSM) > μ (d-BSM) ≥ μ (ae-BSM) (Nikogeorgos, et al. 2014), i.e. BSM displays more slippery characteristics with increasing purity. To explore the influence of BSA more directly, purified BSM was mixed with BSA, and substantially higher μ values were observed. This is interpreted as that BSA may dominate the surface adsorption due to its substantially smaller molecular weight and faster convection to PDMS surface under tribological stress. Extreme difficulty of removing BSA from ar-BSM (Madsen et al., 2015) suggests a strong interaction between them. Ironically though, mixing purified BSM (i.e., ae-BSM) and BSA does not necessarily reproduce a strong interaction between them. This is partly due to that both BSM and BSA are negatively charged at neutral pH condition. Meanwhile, both lysozyme and lactoferrin have isoelectric point higher than 7 that strong electrostatic attraction between them is expected. Indeed, tribological studies have shown synergetic lubricating effects between PGM and these proteins to a certain extent.

3. 3. Influence Of Mucoadhesive Polymers

Mucoadhesive polymers are known to interact with mucin or mucus gels. Thus, they are often employed as surface coating materials for drug carrier. Interestingly, strong interaction between mucin and mucoadhesive polymers can be exploited for improving lubricating properties as well, for instance, between porcine gastric mucin (PGM) and chitosan (Nikolaos et al., 2015) for the sliding contacts between PDMS surfaces. In acidic solution (pH 3.2), and even at a very low concentration (0.1 mg mL^{-1}), the interaction of PGM with chitosan led to surface recharge (zeta potential) and size shrinkage (DLS) of their aggregates. This resulted in higher mass adsorption on the PDMS surface with an increasing weight ratio of (chitosan)/(PGM + chitosan) up to 0.50 (OWLS). While neither PGM nor chitosan alone exhibited slippery characteristics, their mixture improved considerably the lubricating efficiency (Fig. 1) and wear resistance of the adsorbed layers. These findings are explained by the role of chitosan as a physical crosslinker within the adsorbed PGM layers, resulting in higher cohesion and lower interlayer chain interpenetration and bridging. Enhanced aggregation between PGM and chitosan in the mixture is also observed in the AFM morphological images (Fig.1).

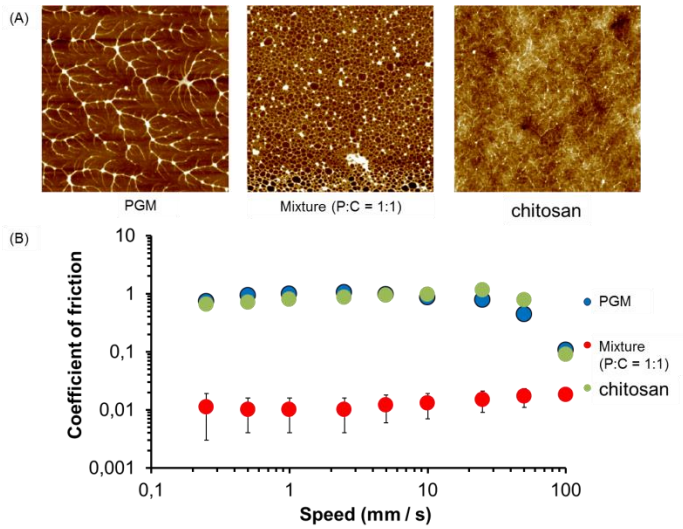

Fig. 1. (A) Morphology of PGM (left), chitosan (right), and the mixture of PGM and chitosan (in the middle) as characterized by tapping-mode AFM (10 × 10 μm^2; substrate, hydrophobized silicone; imaged in ambient). (b) μ vs speed plots of PGM, chitosan, and the mixture solutions in PBS as characterized by pin-on-disk tribometry (load = 1 N, PDMS-PDMS sliding contacts).

3. 3. Influence Of Complexation With Polycations

One of the major contributing factors for the synergetic lubricating effects between PGM and chitosan is electrostatic attraction. As mucins are negatively charged at neutral pH condition, oppositely charged macromolecules, namely polycations, are expected to form aggregates, neutralize the charges, and may display similar synergetic effects. To this end, two polycations, poly(allylamine) (PAAm) and polyethyleneimmine (PEI) with varying molecular weights, have been tested. Interestingly, PEI showed an immediate and substantial reduction in friction coefficient upon mixing with PGM, whereas PAAm did not show as extensive synergetic lubricating effects. Both PEI and PAAm carry a large number of amine moieties, but the interaction characteristics with PGM might be different and lead to different magnitude of synergy. Presently, detailed interaction mechanisms between PGM-PEI and PGM-PAAm are under study.

4. Conclusion

In this study, we have demonstrated that the lubricating properties of mucins can be substantially altered following the interaction with proteins or polymers. On one hand, this is a clear indication that any experimental determination of lubricity of mucins should be conducted with a careful control of purity of mucins. On the other hand, this also implies that the lubricating properties of mucins can be readily tailored by controlled interaction with proteins or polymers, and can lead to the formation of biomimetic mucus-like fluids with more tuneable properties and useful bioengineering applications.

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