Mucin Layers as Biomimetic Coatings for Polymeric Biomaterials

Seunghwan Lee, Jan Busk Madsen, Kirsi I. Pakkanen
Department of Mechanical Engineering, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark

Statement of Purpose: Mucus gel layers are known to protect the epithelial surfaces of organs against foreign bodies or external stimuli by providing hydration, physical barrier, and lubrication. Mucins, high molecular weight glycoproteins secreted by submucosal glands, are the main component of mucus gel and responsible for its viscoelastic gel properties. Recently, mucin has been proposed as potent biomedical coating materials; since mucin/mucus represent outermost layer of epithelium, biomaterials coated with mucins may be recognized as "self" rather than "foreign" bodies by human immune system. The purpose of this work is to generate and characterize mucin layers on polymeric surfaces in order to test and develop mucin-based biomimetic coatings for biomaterials. Among many properties required for biomedical coatings, this work focuses on anti-biofouling properties, i.e. resistance to non-specific adsorption of proteins, and lubricating properties.

Methods: Commercially available bovine submaxillary mucins (BSM, Sigma-Aldrich, both Type I and Type I-S), as well as purified BSM by anion exchange chromatography have been employed as mucin coating materials. BSMs samples are denoted as "as-received BSM (Type I)", "as-received BSM (Type I-S)", and "purified BSM" (purified from Type I-S), depending on the type and purity. Poly(dimethylsiloxane) (PDMS) and Polystyrene (PS) were employed as substrates for model polymeric biomaterials. BSM coatings on the biomaterials surfaces were generated by spontaneous adsorption of BSM molecules from aqueous solution in variation of BSM concentration and immersion duration. The amount and conformation of the BSM coatings generated on the surfaces have been characterized by water wettability, AFM, and Optical Waveguide Light-mode Spectroscopy (OWLS). To understand the conformation of BSM on the surface with that in bulk solution, circular dichroism spectroscopy (CD) studies were carried out over a wide range of BSM concentration (from 0.25 mg/ml to 2 mg/ml). OWLS and Fluorescence microscopy were further used to characterize the interaction of the BSM coatings with blood-borne proteins, including albumin, Immunoglobulin G (IgG), fibrinogen, and serum. Finally, Pin-on-disk tribometer was used to characterize the lubricating efficacy of BSM coatings by employing PDMS as tribopair.

Results: The adsorption profiles of BSM onto PDMS surfaces, as studied by OWLS, were significantly dependent upon the purity of BSM (Figure 1). While both "as-received BSMs" rapidly reached saturation, e.g. within 10 minutes, with equilibrium adsorbed mass of ca. 100 ng/cm^2, that of "purified BSM" showed continuous increase of adsorbed mass up to 24 hours without reaching a saturated value. The adsorbed mass for purified BSM after the first 10 minutes was in the range of 20 to 40 ng/cm^2, and that after 24 hour was ca. 175 ng/cm^2. This difference is mainly attributed to that non-mucinous components from "as-received BSM" samples were removed via chromatographic purification, and thus, polyanionic character of BSM became more pronounced; since PDMS surface is non-polar, electrostatic repulsion between BSM prevents facile adsorption onto the surface. Exposure of the generated BSM coatings to the solutions of blood-borne proteins has shown, however, that the "purified BSM" do not effectively suppress non-specific adsorption of proteins onto PDMS and PS surfaces, as studied by both OWLS and fluorescence microscopy. On the other hand, "as-received BSM Type I", which is essentially a composite of purified BSM and other non-mucinous components of mucus, showed near-perfect anti-biofouling properties. As an example, OWLS data to show the adsorption profile "as-received BSM (Type I)" and "purified BSM" onto PDMS surfaces as well as subsequent exposure to serum is shown in Figure 1.

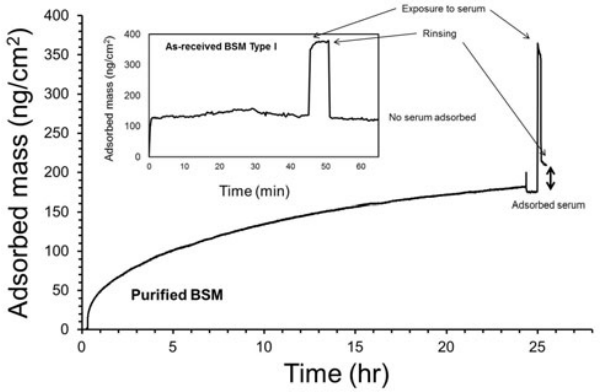

Figure 1. Adsorption profiles of "purified BSM (main)" and "as-received BSM Type I" onto PDMS surface, and subsequent exposure to serum.

Lastly, all BSM solutions (0.5 to 1 mg/ml) showed extremely effective lubricating properties at PDMS surfaces, but the lubricating efficacy was only transient when BSM is employed as coatings, presumably due to weak binding nature of BSM onto PDMS surfaces, under repeated tribostress.

Conclusions: BSM showed a high potential to be developed as biomimetic coatings for polymeric biomaterials for its anti-biofouling properties and lubricity. Interestingly, superior performance as biomedical coatings was observed when a composite of BSM and other integral, non-mucinous components in mucus was employed than purified BSM alone. In turn, the results in this work suggest that even more improved properties can be expected by tailoring the composition of "mucus-like" layers and grafting approaches onto surfaces, which are currently under investigation.