Surface Microphase Structures of Poly(urethane urea) Biomaterials and Protein Adhesion

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Statement of Purpose: Polyurethane block copolymers are widely used in blood-contacting applications as a result of their acceptable blood compatibility and excellent physical characteristics. These properties are believed to be a result of nanoscale chemical heterogeneities arising from the microphase separated structures of the polymers. The microphase structure of polyurethane materials has been extensively studied; yet direct relationships between the phase separated structure and protein adsorption at the molecular scale remain poorly understood. In this study we use atomic force microscopy (AFM) to probe the properties of polyurethane materials and adhesion of proteins with a goal of understanding the relationship between the chemically heterogeneous structure of the polymer and protein-surface interactions in order to better understand the how spatially dispersed chemical functionalities contribute to the success of this material in biomedical applications.

Methods: Poly(urethane urea) (PUU) films prepared from 2000 MW PTMO, MDI and EDA with 22% hard segment weight fraction were prepared by solution casting onto glass cover slips. AFM was used to image the microphase structure of polymers by tapping mode in air and under PBS solution (pH 7.4). Lift mode AFM was also used to measure the locations of the polar hard domains. To measure the adhesion forces of proteins, AFM tips were modified with either BSA or fibrinogen. Force imaging was used to collect 32 × 32 arrays of force curves at a scan size of 500×500 nm. Adhesion forces were measured from the maximum deflection in retraction force curves. To observe single molecule protein adsorption on the films, proteins were conjugated to 1.4 nm diam. gold beads and the location of the gold bead markers was observed by TM AFM.

Results / Discussion:

Microphase structures of polyurethane in ambient and aqueous environments. The separated microphase structures were observed from phase image (Fig. 1b). The lift mode phase image better illustrates the locations of the polar hard domains (Fig. 1c). Phase images under aqueous buffer were different from those obtained in ambient conditions. Sequential images show that hard domains reorient/rearrange to increase their interactions with aqueous buffer resulting in increased polar hard segment content at the hydrated interface (Fig. 2).

Protein adhesion on PUU surface. The PUU surface wettability measured by sessile drop water contact angle was observed to increase with hydration time (within 24 hrs) (Fig. 3a), most likely arising from enrichment of hydrophilic hard domains on surface. Adhesion force measurements showed that the forces between proteins (either BSA or fibrinogen) and PUU surfaces decreased significantly at the initial hydration (within 24 hrs, $p<0.001$) (Fig. 3b, 3c). With increasing hydration time, adhesion forces continue to decrease for BSA, but not as much for fibrinogen. The decrease in adhesion forces is likely due to the enrichment of hydrophilic hard domains on surface.

The nanogold conjugated proteins adsorbed on PUU were predominantly found on soft segment, consistent with the increased adhesion forces on these regions (Fig. 4)

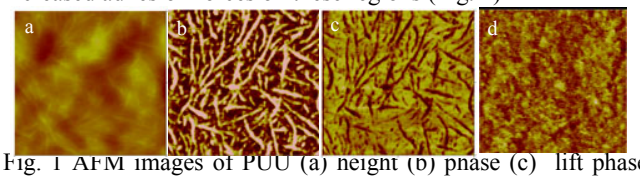

Fig. 1 AFM images of PUU (a) height (b) phase (c) lift phase (lift height = 3 nm) in air , and (d) phase image in PBS buffer after hydration for 20 hrs (scan size: 500×500 nm).

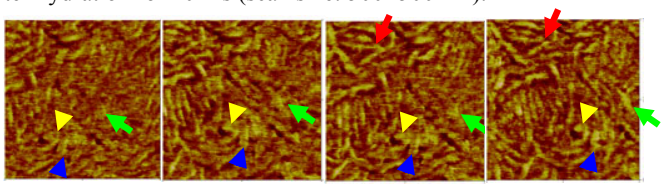

Fig. 2 Sequential AFM phase images of PUU in PBS. The arrows indicate the hard domains reorientation, rearrangement or breakdown induced by water (scan size: 500×500 nm).

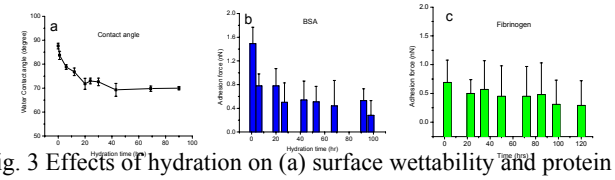

Fig. 3 Effects of hydration on (a) surface wettability and protein adhesion forces (b) BSA and (c) fibrinogen.

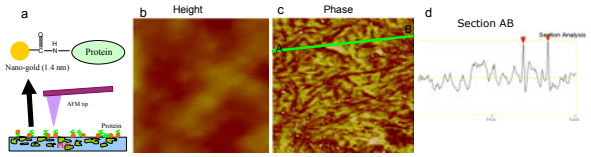

Fig. 4 Single molecule protein (IgG) adsorption on PUU surface, (a) nanogold conjugated protein, (b) height and (c) phase images of PUU with protein, (d) phase section AB.

Conclusions: The separated microphase structure of PUU was observed in air and aqueous environments. Hard domains were enriched on surface during hydration due to structural reorientation. Protein adhesion forces decreased with hydration as the surface becomes enriched in polar hard domain. The microphase structure is related to surface chemistry and the proteins are preferentially adsorbed on soft segment, consistent with measures of protein adhesion showing increased forces on the less polar soft segment.

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