Protein Adsorption and Affects on Platelet Adhesion and Bacterial Adhesion on Submicron Textured Biomaterial Surfaces

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Statement of Purpose: The long term use of synthetic polymeric biomaterials in blood-contacting devices is complicated by the potential for thromboembolic events and microbial infection. Recently, the use of topographical changes for controlling these biological responses is becoming more prevalent. Our previous data have shown that submicron textured polyurethane biomaterial surfaces could reduce both platelet adhesion and bacterial adhesion, thereby inhibiting thrombosis and microbial infection[1,2]. To better understand the role of topography in response to blood, in this study, we further studied the protein adsorption and its effect on platelet and bacterial adhesion on textured surfaces since protein adsorption is a critical early event during the interaction of blood with implanted biomaterials.

Methods: Poly(urethane urea) (PUU) surfaces were textured with ordered arrays of pillars using a soft lithography technique[1]. Two submicron textured patterns with round pillars of diameter and separation of 400 nm and 400 nm (400/400), 500 nm and 500nm (500/500) were used. Protein adsorption was examined by ELISA to quantify overall protein adsorption, and further examined by carbon nanotube atomic force microscopy (CNT-AFM) using antibodies to detect the molecular scale protein adsorption on textured surfaces. The effect of protein adsorption on platelet adhesion and bacterial adhesion was examined in a microwell plate under static condition. Proteins (albumin, fibrinogen, fibronectin, and PPP) were pre-adsorbed on polymer surfaces for 1 hr, and then polymer films were used for human platelet and bacterial adhesion (*S. epidermidis* RP62A) for 1 hr at 37°C. Platelets and bacteria adhered were fixed and labeled with appropriate fluorescence, and examined by optical fluorescence microscopy.

Results / Discussion:

Protein adsorption on textured PUU surfaces: Protein adsorption on textured surface was measured by AFM CNT probes modified with polyclonal antibodies. Result shows that fibrinogen is detected around all areas of textured surface, but the distribution of proteins depends on surface feature, where less protein appears on the top area than on the edge areas, and proteins in both areas appear fewer than that on bottom (Fig. 1). The amount of protein adsorption on textured surface was further measured by ELISA, and results show that more proteins were adsorbed on smooth surface than on textured surface despite surface area differences, and that fibrinogen is 2-3 times higher than that on textured surface. This may also help explain the lower adhesion of platelets on textured surfaces.

Platelet adhesion/activation and bacterial adhesion on surfaces with pre-adsorbed protein: Platelets adhered on surfaces were counted under microscopy and results showed that both textured surfaces significantly reduced platelet adhesion compared to the corresponding smooth surfaces (Fig. 2a). Activation of adherent platelets was estimated from the circularity of platelet shapes. Generally, the circularity of platelets was lower on smooth surface than on textured surfaces, suggesting textured surface also inhibits platelet activation (Fig. 2b).

The similar experiment was carried out for bacterial adhesion. Results showed that protein adsorption (except for albumin) increased bacterial adhesion on smooth surfaces. However, both textured surfaces with protein pre-adsorbed significantly reduced bacterial adhesion, compared to the corresponding smooth surfaces, suggesting textured surface inhibit bacterial adhesion (data not shown) as we have seen in previous studies.

In summary, the submicron textured surfaces reduced protein adsorption on top of pillars and decreased platelet adhesion and activation as well as bacterial adhesion.

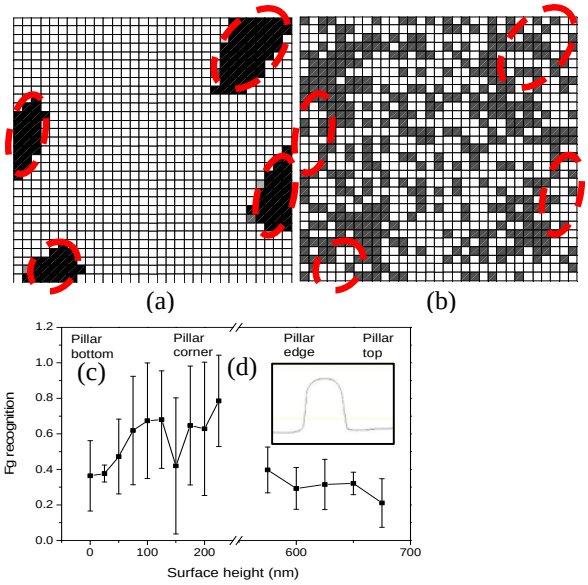

Fig. 1 Fibrinogen adsorption on 500/500 nm PUU surface measured by CNT AFM probe, (a) height map , (b)protein recognition map, (c) quantification of protein recognition as function of topography height (d) section line of a pillar showing features of pillar top, edge, corner, and bottom. Size: 2×2μm^2. Red circles indicate the location of pillars.

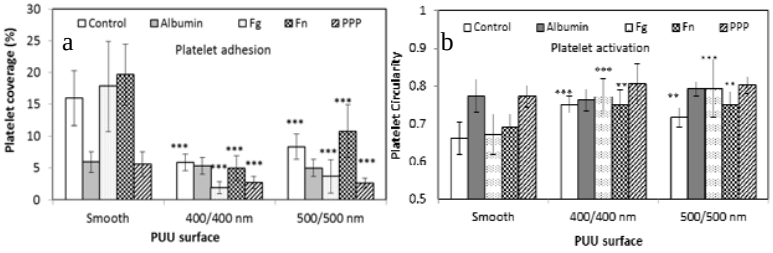

Fig. 2 (a) Platelet adhesion and (b) activation on PUU surfaces with pre-adsorbed with plasma proteins. The statistical analysis is performed between textured and smooth PUU samples.

Reference:

1. Xu, LC and Siedlecki, CA, *Acta Biomateriialia*, 2012, 8, 72.
2. Milner, KR, et al. J. Biomed. Mat. Res. 76A, 2006, 561.