Effect of Protein Adsorption on *Staphylococcus Epidemidis* RP62A Adhesion on Polyurethane Biomaterials Surfaces

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Statement of Purpose: Infection due to bacterial adhesion and biofilm build up is one of the main problems associated with the use of blood-contacting devices. It is well accepted that the initial bacterial adhesion and colonization on an implanted biomaterial surface is mediated by the surface physico-chemical properties and adsorbed proteins. Here we studied the effect of protein adsorption (albumin, fibrinogen, and fibronectin) on adhesion of *Staphylococcus Epidemidis* RP62A on microphase-separated polyurethane biomaterial surfaces. Atomic force microscopy (AFM) was used to measure the interaction forces of proteins and bacterial cell surfaces. The correlation between molecular scale results and macroscale bacterial adhesion may yield important information for understanding the mechanisms of bacterial adhesion and biological responses to materials.

Methods: Polyurethane (PU) films were prepared from Biospan MS/.04 (18% solid weight fraction in DMAC) solution by casting onto glass cover slips and drying in a vacuum oven at 65°C overnight. Human serum albumin (HSA), human fibrinogen (Fg) and human fibronectin (Fn) were selected for protein adsorption. Strain *S. Epidemidis* RP62A was cultured in tryptic soy broth for 24 hrs and collected by centrifuge at 1360 for 10 min. The pellet was resuspended in PBS and the concentration of bacteria was measured by a spectrophotometer at 600 nm. PU films hydrated in H_2O for 24 hrs were pre-adsorbed with proteins for 15 min and incubated in bacterial solution at a concentration of 10^8 cfu/ml for 1 hr. Bacteria attached on PU surfaces were fixed in 2.5% glutaradlehyde and labeled with Hoechst. The bacterial adhesion was counted under a fluorescent optical microscope. To measure the interaction forces between proteins and bacterial surfaces, bacteria were electrostatically bonded onto the glass coverslip coated with poly-L-lysine. AFM probes were modified with fibrinogen or fibronectin. An array of 32×32 or 16×16 force curves were collected by AFM adhesion mode. The maximum deflection of the cantilever from each retracting curve was used in calculating adhesion force.

Results / Discussion:

Bacterial adhesion on PU surfaces with pre-adsorption of proteins: Bacterial adhesion on PU surfaces varied largely with the pre-adsorption of proteins (Fig. 1). Surfaces with pre-adsorption of fibronectin had the largest bacterial adhesion compared with fibrinogen and albumin samples, suggesting the importance of fibronectin in adhesion. Albumin showed lower adhesion than the control surface without protein adsorption. It is interesting to note that the presence of fibrinogen had no significant effect on bacterial adhesion, although a small increase of adhesion was observed on surface with pre-adsorption of 0.3 mg/ml Fg. The surfaces pre-adsorbed with dual component protein solutions (Fg+Fn) had greater bacterial adhesion compared with control or Fn+HSA samples, but lower than those with pre-adsorption from pure fibronectin solution. Results suggest that bacterial adhesion is related to the amount of fibronectin adsorbed on surface.

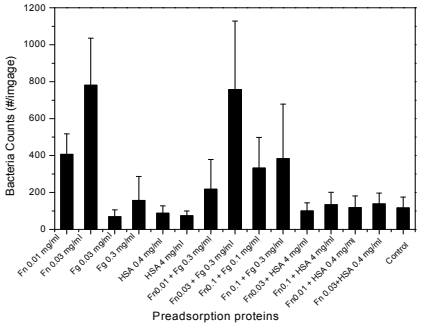

Fig. 1 *S. Epidemidis* RP62A adhesion on PU surfaces with pre-adsorption of proteins.

Adhesion forces of proteins and bacterial surfaces: A protein-modified AFM probe was used to measure the protein-bacteria interaction forces and the distribution of protein receptors on bacterial cell surfaces. The force maps showed a heterogeneous distribution of protein-bacteria interactions. Approximately 32% of measurements showed no interactions between bacterial cell surfaces and the Fg-probe, while only 22% of measurements showed no interaction with the Fn-probes (forces <0.2 nN), suggesting more Fn-receptors than Fg-receptors on cell surfaces. Distribution of interaction forces showed peaks at 0.45 and 1.40 nN, for Fn-bacteria, and a single broad peak around 0.76 nN for Fg-bacteria (Fig. 2). The measured adhesion forces and distribution of protein receptor on bacterial cell surfaces are consistent with the macroscale bacterial adhesion (Fig. 1). Microphase separated polyurethane biomaterials contain hard and soft segments and create a distribution of different physico-chemical properties at interfacial surfaces that influences protein adsorption. The study of protein adsorption at molecular scale level on material surface is necessary for understanding mechanisms of bacterial adhesion.

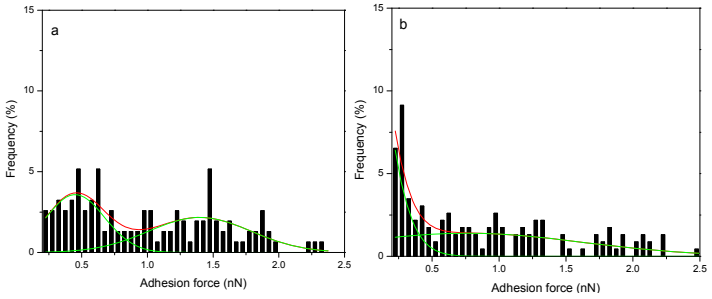

Fig. 2 Adhesion force distributions of (a) Fn-probe and (b) Fg-probe interaction with *S. Epidemidis* RP62A (forces >0.2 nN)

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