# Adsorption and Activity of Fibrinogen on Polyurethane Biomaterials Surfaces in the Presence of Other Proteins

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**Statement of Purpose:** It is well accepted that protein adsorption onto biomaterial surfaces is a critical early event during the interactions of blood with implanted biomaterials and mediates a variety of biological responses including blood platelet adhesion and thrombus formation. Fibrinogen is known to play a prominent role in development of surface-induced thrombosis, but the adsorption behavior in the presence of other proteins is less known, particularly at the molecular level. In this study, we used immuno-AFM techniques to identify fibrinogen adsorption and functional activity at the near-molecular scale and in the presence of competing proteins on microphase-separated polyurethane surfaces. Results show correlation between fibrinogen activity at molecular scale and platelet adhesion, adding to our understanding of biomaterial-induced thrombosis.

**Methods:** Polyurethane (PU) films were prepared from Biospan MS/.04 (18% solid weight fraction in DMAC) by casting onto glass cover slips and drying in a vacuum oven at 65°C overnight. Human serum albumin (HSA), human IgG, and human fibrinogen (Fg) were selected for protein adsorption. Proteins were dissolved in tris buffer (pH7.4) at different molar ratios with a total concentration of 1 μM (except as noted) and adsorbed onto PU surface by flowing at 0.1 ml/min for 15 min in an AFM fluid cell. After adsorption, the protein solution was replaced by buffer at the same flow rate for 5 min. The recognition and distribution of fibrinogen on polymers was assessed by polyclonal antibodies against fibrinogen and by monoclonal antibodies recognizing γ392-411 of Fg (indicating protein activity). An array of 32×32 force curves over a 2×2μm$^2$ scanning area was collected. The retraction force curves were analyzed to distinguish specific and non-specific interactions between the Ab probe and proteins as described previously.[1] Human platelet adhesion was measured by microscope on the PU surfaces with pre-adsorption of HSA and Fg for 15 min.

**Results / Discussion:**

**Fibrinogen adsorption on PU surfaces:** Fibrinogen on PU surfaces in the presence of other proteins was measured by a pAb modified AFM probes (Fig. 1). Results showed that there appears to be a dependence in the amount of Fg on surfaces from Fg+HSA protein solutions, with a large decrease as the molar fraction of HSA goes beyond 68%. There was no significant difference in Fg recognition when the total protein concentration increased to ~600 μM, similar to whole plasma. The presence of IgG was found to generally decrease the recognition of Fg on PU surfaces (Fig. 1b). In the three component protein solutions (Fg+HSA+IgG), a decrease in Fg pAb recognition was only observed for solution of Fg:IgG:HSA=25:25:50.

**Fibrinogen Functional Activity on PU surfaces**: The functional activity of Fg adsorbed on surfaces was measured by a mAb that recognizes the platelet-binding region in Fg, and was found to be lower in pure Fg solution compared to solutions having small fraction of HSA, IgG or HSA+IgG (Fig. 2). Results suggest that the presence of albumin or IgG increases availability of platelet-binding sites in Fg. This was further evidenced by the higher ratio of Fg activity (mAb recognition) to Fg amount (pAb recognition) in the presence of HSA (Fig. 3).

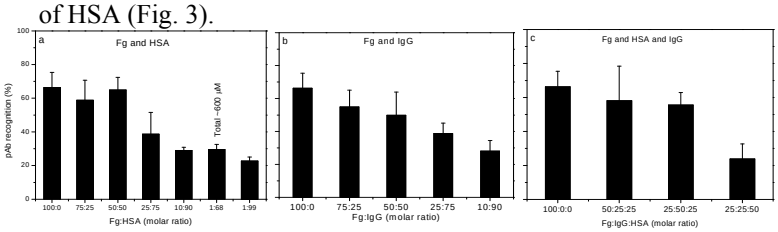


Fig. 1 Fibrinogen adsorption on PU surfaces in the presence of (a) HSA, (b) IgG, and (c) HSA + IgG, recognized by pAb probes.

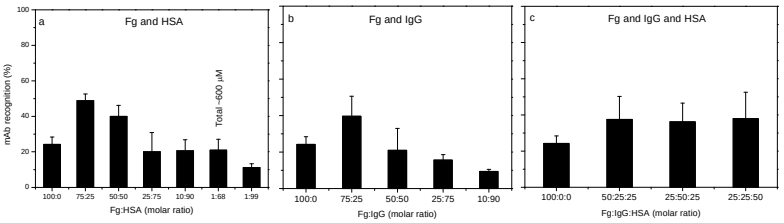


Fig. 2 Fibrinogen functional activity on PU surfaces in the presence of (a) HSA, (b) IgG, and (c) HSA + IgG, recognized by mAb probes.

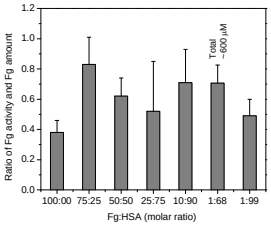


Fig. 3 Ratio of Fg activity to Fg amount in proteins adsorbed on PU surfaces.

**Platelet adhesion on PU surfaces:** Platelet adhesion was measured on the PU surfaces following preadsorption from Fg and HSA protein solutions. Platelet adhesion was found to correlate well with the activity of Fg on the surfaces (Fig. 4). These results suggest that the platelet adhesion is more correlated with availability of the platelet binding sites in the Fg γ-chain dodecapeptide than amount of Fg alone, and that other proteins can affect the activity of fibrinogen on surfaces.

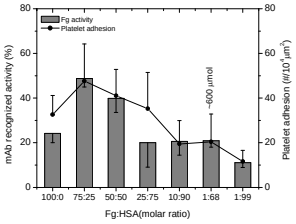


Fig. 4 Correlation of Fg activity and platelet adhesion on PU surfaces.

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**Reference:**

1. Soman et al. Langmuir, 2008, 24, 8801-8806