von Willebrand Function and Structure Is Different When Adsorbed to Different Synthetic Materials

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Statement of Purpose: In regions of high shear in blood, von Willebrand Factor (VWF) is essential for platelet tethering and clot formation (Lenting PJ. Thromb Haemos. 2010;104:449-455.). Therefore von Willebrand Factor function could be an important consideration for blood-contacting implants that experience high shear. VWF binds platelets after undergoing a conformational change when bound to subendothelial collagen during vascular injury or to a synthetic surface. Since VWF-platelet binding is dependent on VWF conformation, the level of platelet binding could change with VWF conformation. Previous AFM studies have shown that VWF adopts a different topography on a hydrophobic versus hydrophilic surface (Raghavachari M. Colloids Surf B. 2000; 19:315-324.). However previous studies of VWF on surfaces have not attempted to relate surface conformation to VWF function. In the studies presented here, we investigate the adsorption behavior and function of VWF adsorbed to glass, tissue culture polystyrene, and polystyrene to determine how the surface influences VWF conformation and platelet binding.

Methods: Surfaces investigated were glass, tissue culture polystyrene, and polystyrene. The A1 domain of VWF contains the binding site for the glycoprotein 1bα (GP1bα) platelet surface receptor. Isolated A1 domain produced in E. coli was used for these studies (Cruz MA. J Biol Chem. 1993;268:21238-21245.). Surfaces were incubated with A1 for 2h at 37°C. For flow chamber and ELISA studies, surfaces were subsequently blocked with BSA overnight. Platelet function was tested using a parallel plate flow chamber. Washed platelets were pushed through the chamber to achieved the desired shears and platelet velocity was measured. XPS was used to determine protein coverage, using atomic percentage nitrogen as a marker of protein. ELISA using three monoclonal antibodies (mAb) identified VWF conformational differences (de Luca. Blood. 2000;95:164-172.). mAB 6G1 binds to a linear region Glu700-Asp709 at the C terminus of A1. mAb CR1 and 5D2 bind to undefined nonlinear epitopes within A1. Following A1 adsorption, surfaces were incubated with primary antibodies for 1h at 37°C, then secondary antibodies conjugated with HRP for 1h at 37°C. ToF-SIMS was used to identify differences in solvent exposure of specific amino acids of VWF on surfaces. A1 contains one Trp residue and two Cys residues, which were used as markers of protein conformation.

Results: When the A1 domain of VWF was adsorbed onto polystyrene, platelets rolled slower than when A1 was adsorbed onto TCPS or glass (Fig. 1). This indicates that platelets exhibit the most binding when A1 is adsorbed onto polystyrene. XPS results showed that there is a comparable amount of protein on each surface. ELISA studies showed that antibody 6G1, with a linear epitope on A1, showed the same amount of binding to A1 on all three surfaces. However antibodies CR1 and 5D2, with nonlinear epitopes on A1, showed lower binding when A1 was adsorbed onto glass compared to the other two surfaces (Fig. 2). ToF-SIMS showed that Trp residues were less solvent-exposed when A1 was adsorbed to glass than to the other two surfaces. Since Trp is close to the GP1ba binding site, this suggests that the binding site is less accessible when A1 is adsorbed to glass than to the other two surfaces. ToF-SIMS showed that Cys residues were less solvent-exposed when A1 was adsorbed onto polystyrene than on glass and TCPS. Within A1, the Cys residues are further from the GP1ba binding site than Trp. Since Cys residues are less exposed when A1 is adsorbed onto polystyrene than the other two surfaces, this could suggest that the GP1ba binding site is more exposed when A1 is adsorbed onto polystyrene.

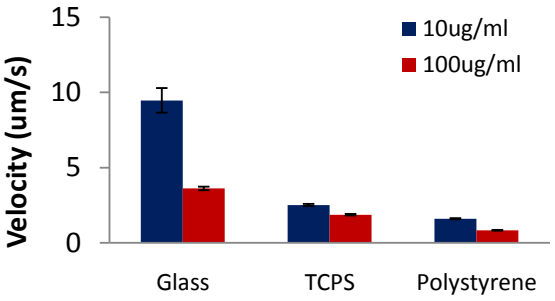

Figure 1. Platelet rolling velocity on adsorbed VWFA1. A1 adsorbed at 10μg/ml or 100μg/ml. Platelet shear stress = 10dyne/cm^2.

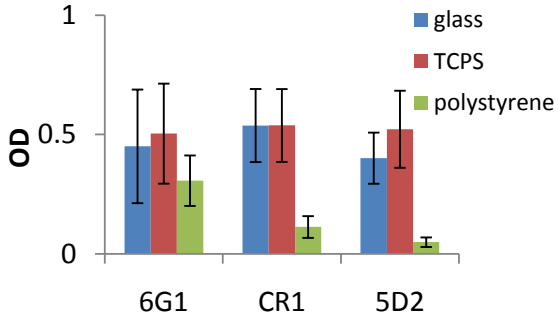

Fig. 2 ELISA results for adsorbed VWF A1 suggest A1 is in a different conformation when adsorbed onto polystyrene.

Conclusions: The function of the A1 domain of VWF which binds to GP1ba on platelets is different when A1 is adsorbed onto different surfaces. Platelets show the most binding when A1 is adsorbed onto polystyrene. Platelets show intermediate binding when A1 is adsorbed onto TCPS, and the least binding when A1 is adsorbed onto glass. These differences are not due to differing amounts of A1 in the three surfaces. Instead, the differences in function are due to A1 adopting different conformations on the three surfaces.