von Willebrand's Factor Has a Major Role in Mediating Platelet Adhesion to Biomaterials at Higher Shear Rates

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Introduction

von Willebrand's factor **(**vWf) does not affect platelet adhesion to biomaterials under static conditions [1]. However, vWf bound to the subendothelium of blood vessels plays a major role in platelet adhesion by rapidly capturing platelets from flowing blood onto injured sites to form hemostatic plugs [2]. In recent work from our lab, a similar major role for vWf on platelet adhesion to polyethylene [3] and glass [4] at high shear rates was shown. Here, further studies of the role of vWf on platelet adhesion to polystyrene at higher shear rates are reported.

Materials and Methods

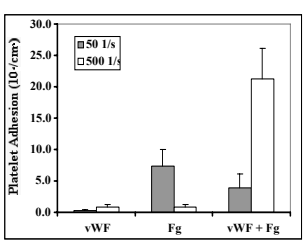
Corning untreated polystyrene (PS) culture dishes (35 mm) were used. vWf and fibrinogen (Fg), human plasma and vWf deficient (vWf-d) plasma were obtained as described previously [3] Mouse anti-human platelet GPIb antibody (AK2) was from Research Diagnostics.

A GlycoTech rectangular, thin channel flow cell (cat. # 31-001) was used. PS dishes were preadsorbed with pure proteins (vWf: 50 µg/ml; Fg: 3 mg/ml) or 10% plasma. Perfusion was carried out at 37°C for 8 min with platelet/red cell suspensions [3]. In antibody experiments, the platelets were pre-incubated with AK2 (5 µg/ml) for 1 hour at room temperature before mixing with red cells. Adherent platelets were quantified with an LDH assay kit (Roche), and also examined with SEM.

Results and Discussion

Fig. 1 shows the effect of pre-adsorption of pure vWf or Fg to PS on platelet adhesion at low and high shear rates. The concentrations of vWf and Fg used were the same as in normal plasma. Platelet adhesion to Fg treated dishes was less at the higher shear rate than at lower shear despite the enhanced transport of platelets to the surface at higher shear rate, indicating that adsorbed Fg on PS cannot effectively capture platelets moving at higher velocity. However, the vWf treated samples showed an increase in platelet adhesion with increasing shear rate. When both vWf and Fg were present during preadsorption, adhesion was markedly increased at the higher shear in comparison to adhesion at the lower shear. These results clearly show that adsorption of purified vWf has a major effect on platelet adhesion to PS at higher shear rates.

To confirm the role of vWf at higher shear rates under more physiologically relevant conditions, 10% normal plasma or vWf-d plasma replenished with various concentration of vWf were used to preadsorb PS. As expected, PS preadsorbed with normal plasma had a significant increase in platelet adhesion at higher shear in comparison to low shear (Fig. 2). In contrast, PS preadsorbed with vWf-d plasma had less than half the platelet adhesion at 500 sec^{-1} than at 50 sec^{-1}. When exogenous vWf was added to vWf-d plasma, platelet adhesion at the higher shear was higher than that at the lower shear, and was very similar to adhesion to PS preadsorbed with normal plasma. Direct observation of platelet adhesion with SEM also showed that platelet adhesion at the higher shear to PS preadsorbed with vWf-d plasma was reduced in comparison to PS preadsorbed with normal plasma, and that the platelets on vWf-d preadsorbed PS were less spread (Fig. 3).



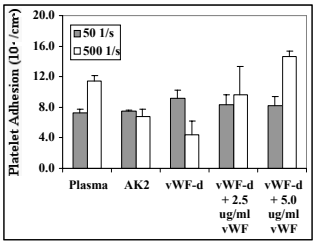
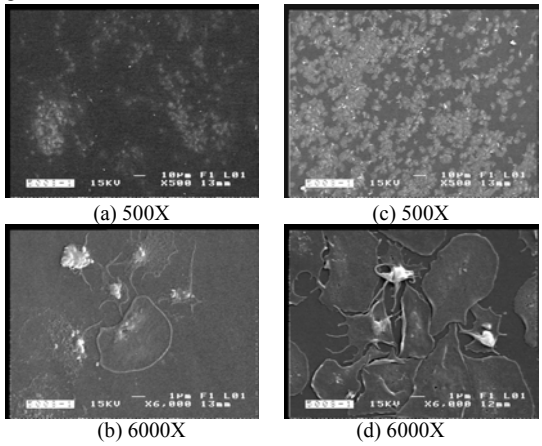


Fig. 1 (left) Platelet adhesion to PS preadsorbed with pure proteins.
Fig. 2 (right) Platelet adhesion to PS preadsorbed with various types of blood plasma.



(a) 500X (c) 500X

(b) 6000X (d) 6000X

Fig. 3 SEM images of platelets adhering on surfaces preadsorbed with (a,b) vWf-d plasma, (c,d) normal plasma at 500 sec^{-1} for 8 min.

AK2 is an antibody that blocks vWf binding to the GPIb receptors of platelets. The use of AK2 significantly reduced platelet adhesion at the higher shear (Fig. 2) although the adhesion was not significantly lower than that at the low shear. A higher antibody concentration may be needed to sufficiently block the interaction between vWf and platelets.

Conclusions

vWf was shown to be very important in mediating platelet adhesion to PS preadsorbed with plasma under higher shear rates. This conclusion also applies to several other biomaterials we have studied but the data was not shown here due to space limitations. The removal of vWf from plasma or blockade of vWf-platelet interaction with an antibody effectively reduced platelet adhesion at higher shear rates. While we previously emphasized the importance of Fg adsorption in platelet adhesion, our new studies show that the reduction of vWf adsorption is also a key design criteria for improving the hemocompatibility of biomaterials.

Acknowledgement
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References

[1] Tsai WB et al., J Biomed Mat Res 2002; 60: 348-359.
[2] Shen Y et al., Blood 2000; 95:903-910.
[3] Kwak D et al., J Biomed Mat Res 2005; 74A: 69-83.
[4] Wu Y et al., SFB 2004.