

# PLATELET BINDING TO BOTH HYDROPHILIC AND HYDROPHOBIC SURFACES IS MEDIATED BY GPIIB/IIIA

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**INTRODUCTION:** When a biomaterial is introduced into the human body a coagulation process immediately starts at the material surface. Hydrophobic and hydrophilic surfaces are known to accumulate different proteins. Platelets are the first cells present on the surface and thus starts the initial process leading to acceptance or rejection of a biomaterial. The binding of fibrinogen to biomaterials has long been known to increase platelet binding, [1] but platelets have a number of different receptors present on the surface providing adhesion to other proteins. We have investigated the adhesion of platelets from whole coagulating blood to hydrophobic and hydrophilic glass surfaces by using antibodies against receptors and integrins on the platelet surface.

**METHODS:** The surface of microscopy slides were cleaned for 30 minutes in 70% ethanol containing 0.35 M HCl generating a hydrophilic surface. After washing some slides were incubated in 0.1% 1,1,1,3,3,3-hexamethylsilazane to generate a hydrophobic surface

Murine monoclonal antibodies against different surface antigens on human platelets were mixed with venous blood from healthy human donors. The blood mixture was incubated on pre heated (37°C) hydrophilic and hydrophobic slides for 2 minutes. The samples were then washed before antibody staining. After staining the number and spreading of platelets adhered to the surface were calculated. Control experiments of the inhibitory effect of the antibodies on receptor adhesion to corresponding surface adsorbed proteins were performed using isolated platelets.

**RESULTS:** The inhibitory effect of antibodies on platelet adhesion to hydrophobic and hydrophilic glass from whole blood is shown in Figure 1. On the hydrophobic glass a significant inhibition of platelet adhesion was seen for antibodies M148 and C17. The reduction in platelet adhesion was highest (96%) for clone M148. For the hydrophilic glass, a similar pattern was seen but the only significant decrease in adhesion was seen for M148 (95%). Significant increases in adhesion on the hydrophilic surface were however seen for AT10 and AN51. The spreading of platelets after whole blood incubation with antibodies was lower on the hydrophilic glass surface than on the

hydrophobic. The only antibody showing significant inhibition of platelet spreading compared to control was M148

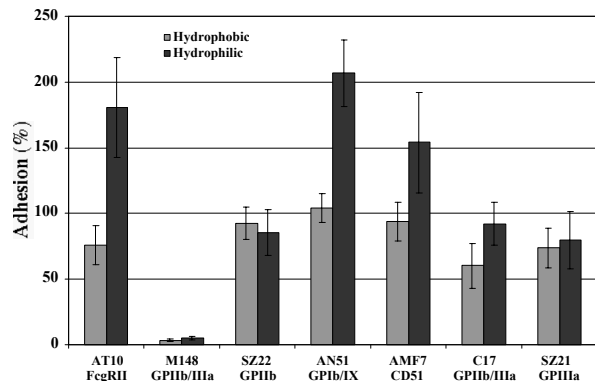


Fig. 1: Platelet adhesion compared to control after incubation of whole blood for 2 minutes on hydrophilic and hydrophobic glass. Mean ± Sem is given.

**DISCUSSION & CONCLUSIONS:** Our results clearly show that the initial binding of platelets to both hydrophobic and hydrophilic biomaterial surfaces, is mediated by GPIIb/IIIa, integrin  $\alpha_{IIb}\beta_{III}$ , and can be inhibited by antibody M148.

The use of antibodies to inhibit platelet integrins is rather controversial as many antibodies are known to activate platelets. The incubation time for whole blood was accordingly kept short to minimise activation effects from the antibodies.

Both antibody SZ21 and C17 has been shown to inhibit induced platelet aggregation and secretion, corresponding to inhibition of the second of the two states of GPIIb/IIIa suggested by Ginsberg et al [2], but none of these antibodies could prevent binding of platelets to the model surfaces. This indicates that the initial adhesion of platelets to biomaterial surfaces does not require any prior receptor activation, which is necessary for binding of the RGD sequence in fibrinogen [3].

**REFERENCES:** <sup>1</sup> M. Zucker, L. Vroman (1969) *Proc Soc Exp Biol Med* **131**:318-20. <sup>2</sup> M. Ginsberg, D. Xiaoping, T. O'Toole, et al (1993) *Thromb Haemost* **70**:87-93. <sup>3</sup> B. Savage, Z. Ruggeri (1991) *J Biol Chem* **266**:11227-33.

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