Platelet deposition in a capillary perfusion model: quantitative and morphological aspects

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The capillary perfusion model according to Cazenave and co-workers was characterized by investigating the effects of protein precoating, perfusion time and shear rate on platelet deposition using ^{111}Indium labelling of human platelets and scanning electron microscopy (SEM). Compared with uncoated polyethylene, platelet deposition was increased after precoating with purified human von Willebrand factor, fibrinogen or fibronectin, and decreased by preadsorbed immunoglobulin G, albumin or whole plasma. Platelet aggregates were observed on immunoglobulin G-coated polyethylene, whereas all other surfaces showed single adherent platelets. Complete platelet spreading was only observed after precoating with fibronectin. The quantitative data concerning platelet deposition were evaluated by using the convective-diffusion theory. Our results indicate the applicability of this perfusion model for the *in vitro* testing of biomaterials.

Keywords: Blood, platelet deposition, protein adsorption, perfusion model, washed platelets

When a foreign surface is exposed to blood, a series of events is initiated which may result in thrombus formation[1]. Adsorption of plasma proteins on the foreign surface is one of the earliest events, and is determined by properties of the material e.g. surface-free energy, crystallinity and surface charge. The intrinsic system of coagulation is initiated by adsorption and activation of coagulation factor XII (Hageman factor). Deposition of platelets, and to a lesser extent leucocytes, invariably occurs on foreign surfaces. The process of cell deposition is largely determined by the nature and conformation of the proteins in the protein layer. The composition of the thrombus is determined by rheological conditions; in low flow areas a fibrin clot with interspersed red cells may develop, whereas in high flow areas platelet deposition is more prominent.

Various *in vitro* methods have been used for studying the biocompatibility of polymer surfaces. In order to evaluate the usefulness of the *in vitro* capillary perfusion system according to Cazenave *et al.*[2] for studying surface thrombogenicity and more specifically the process of platelet deposition on biomaterial surfaces, we have studied the effect of surface protein precoating, perfustion time and shear rate on the deposition of washed ^{111}Indium-labelled platelets. The quantitative data thus obtained were compared with scanning electron microscopical observations.

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MATERIALS AND METHODS

Plasma and purified plasma proteins

Plasma. Blood from 15 healthy volunteers was anticoagulated with 130 mM trisodium citrate; the anticoagulant to blood ratio was 1:9 (v/v). Plasma was prepared by centrifugation (15 min at 1570**g** followed by 15 min at 3000**g**), pooled and stored in liquid nitrogen.

Human fibrinogen. This was obtained from Kabi, Stockholm, Sweden, (Grade L) and further purified by chromatography on aminohexyl-Sepharose 4B, according to Hamer[3]. One g of fibrinogen was dissolved in 50 ml acetate-barbital buffer (28 mM sodium acetate, 28 mM sodium barbital, 150 mM NaCl, 10 mM lysine-HCl) to which the following protease inhibitors were added: 10 mM trisodium citrate, 1 mM benzamidine, 10 mM ε-aminocaproic acid, 1 U/ml trasylol, and 0.1 mM phenylmethylsulphonylfluoride, pH 6.8. After dialysis overnight at 4°C, the protein solution was diluted to 5 mg/ml with acetate-barbital buffer, and applied to a 50 ml aminohexyl-Sepharose 4B column (Pharmacia Fine Chemicals, Uppsala, Sweden) at room temperature. Under

these conditions, fibronectin and von Willebrand factor will bind, while fibrinogen passes through the column. Elution was carried out with acetate-barbital buffer, while the absorption of the eluate was monitored at 280 nm. The fibrinogen containing fractions were pooled.

Intact fibrinogen and fibrinogen lacking parts of the carboxy-terminal end of the A α chain, were separated by 16% ammonium sulphate precipitation at pH 6.8 and 4°C[4]. The precipitate was dissolved in and extensively dialysed against Ca^{2+}/Mg^{2+}-free Tyrode solution (136.9 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 5 mM Hepes, pH 7.3). Fibrinogen concentration was determined using A 280 nm, 1 cm, 1% = 15.5[5]. This purified fibrinogen preparation (4.5 mg/ml) was stored in aliquots at −30°C.

Electrophoresis of fibrinogen in 4–30% SDS-polyacrylamide gel (Pharmacia) after reduction with 2-mercaptoethanol showed that a protein band corresponding to a molecular weight (M_r) of 220 kdaltons, was no longer present after chromatography of the starting material on aminohexyl-Sepharose 4B. This band can originate either from fibronectin or from von Willebrand factor. Electrophoresis of fibrinogen which precipitated after 16% ammonium sulphate saturation, showed 3 protein bands with a M_r of 67, 56 and 47 kdaltons, corresponding respectively to the A α, B β and γ chain.

Fibronectin. This was isolated from human plasma by affinity chromatography on gelatin-Sepharose at room temperature[6]. Phenylmethylsulphonylfluoride (0.1 mM) was added as protease inhibitor to the plasma as well as to all buffers. CPD-A_1 plasma (250 ml, Bloodbank Twente en Achterhoek, Enschede, The Netherlands) was centrifuged at 3000 **g** for 30 min. The plasma was first passed through a 500 ml column of Sepharose 4B, and subsequently applied to a 125 ml column of gelatin-Sepharose 4B (both from Pharmacia). The latter column was washed with several volumes of PBS, 2 vol. of 1 M NaCl, and again with PBS. Elution of fibronectin was carried out with 4 M urea in 50 mM Tris-HCl, pH 7.5. The absorption of the eluate was monitored at 280 nm, and the fibronectin containing fractions were pooled. Fibronectin concentration was determined[7] using A 280 nm, 1 cm, 1% = 12.8. Aliquots (1.7 mg/ml) were stored in the urea solution at −30°C. Before use, the urea was removed by passage through a Sephadex G-25 column (PD-10, Pharmacia), which was equilibrated with Ca^{2+}/Mg^{2+}-free Tyrode solution.

Electrophoresis of the purified fibronectin in 4–30% SDS polyacrylamide gel after reduction with 2-mercaptoethanol, showed a protein band corresponding to a M_r of 220 kdaltons, originating from the fibronectin subunit. No other bands were visible.

Human serum albumin. A 20% solution [Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam] contained less than 5% α- and β-globulins. Albumin was diluted in Ca^{2+}/Mg^{2+}-free Tyrode solution.

Human immunoglobulin G. This was obtained lyophilized (Serva, Heidelberg, FRG) and was more than 97% pure. Immunoglobulin G was dissolved in Ca^{2+}/Mg^{2+}-free Tyrode solution.

Human von Willebrand factor (0.6 mg/ml). This was kindly provided by Dr Ph.G. de Groot (University Hospital, Utrecht, The Netherlands). The purified von Willebrand factor preparation has been extensively characterized[8].

Preparation of perfusates

Blood from healthy volunteers who did not take acetylsalicylic acid during 10 d prior to blood collection, was anticoagulated with ACD [66.6 mM citric acid, 85.0 mM trisodium citrate, 111.0 mM D(+)-glucose, pH 4.5]; the ACD to blood ratio was 1:6 (v/v). The final pH was 6.5 and the final citrate concentration amounted to 21.7 mM.

Platelets were washed according to Cazenave *et al.*[9]. The entire procedure was carried out at 37°C. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 175**g** for 15 min. Platelets were allowed to rest for 30 min and were subsequently pelleted by centrifugation at 1570**g** for 13 min. The pellet was resuspended in Tyrode-albumin solution (TA, 136.9 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 1.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 5.6 mM D(+)-glucose, 0.35% human serum albumin, 5.0 mM Hepes, pH 7.3), containing 10 U/ml of heparin (Hoffman-La Roche, Basel, Switzerland) and 0.5 μM of PGI_2 (Sigma Chemical Co., St Louis, USA). Platelets were allowed to rest for 15 min, then 0.5 μM of PGI_2 was added and the cells were pelleted by centrifugation at 1100**g** for 10 min. The pellet was resuspended in TA solution containing 0.5 μM of PGI_2. This washing step was repeated once, after which the pellet was resuspended in TA solution containing apyrase (grade V, Sigma) at a concentration of 0.05 U ADP-ase activity per ml. The platelet count was adjusted to 300 000/μl using TA solution containing apyrase and finally the suspension was stored for 30 min at 37°C.

Labelling of platelets was performed by incubation for 15 min with 10 μCi of ^{111}Indium oxine (Amersham International, Amersham, UK) per ml of platelet suspension during the first step of the washing procedure. The final oxine concentration was 0.5 mg/l. The labelling efficiency ranged from 80–90%.

Red blood cells were packed at room temperature by centrifugation at 1570**g** for 10 min. The cells were washed 3 times with Ca^{2+}/Mg^{2+}-free Tyrode solution containing 5.6 mM of D(+)-glucose, and finally with TA solution containing 0.05 U/ml of apyrase. Perfusates were prepared by combining the washed platelets and washed red cells (hematocrit 0.4, final platelet count 180 000/μl).

Perfusion experiments

Perfusion experiments were carried out using the capillary flow system according to Cazenave *et al.*[2] which was slightly modified (*Figure 1*). This system consists of a capillary tube connected to a syringe. Liquid flow, which is controlled by a syringe pump, is in principle non-pulsatile. In this way it is possible to study protein adsorption and/or platelet adhesion and secretion under well defined hydrodynamic conditions (Poiseuille laminar flow), at wall shear rates up to several thousands per second.

In this study we used medical grade high pressure polyethylene tubes (Talas, Ommen, The Netherlands) 0.75 mm ID and 30 cm long. To avoid bending, the polyethylene tubes were led through 25 cm long glass tubes which were positioned at 2.5 cm from the entrance of the capillary tube. Polypropylene syringes were purchased from Asik (type Once, 20 and 50 ml, Rodby, Denmark). Two syringe pumps (model 355, Sage Instruments, New York, USA) were used; on each pump 2 syringes could be mounted. One pump was used either to introduce protein solutions or to carry out the flow experiments with perfusates. The other pump was used to rinse the capillary tubes, *via*

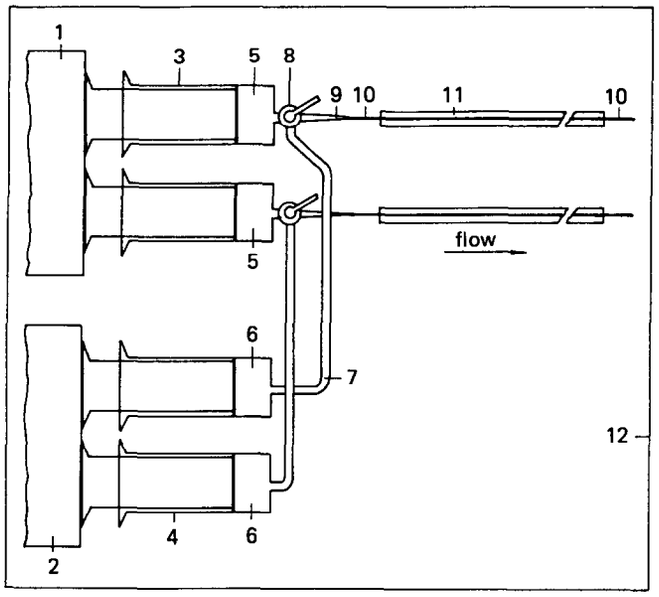

Figure 1 Schematic diagram of the in vitro perfusion system according to Cazenave et al.[2]. 1 and 2, Drive carriages of the two syringe pumps; 3 and 4, polypropylene syringes (20 or 50 ml); 5, protein solution or platelet suspension; 6, rinsing buffer; 7, PVC tube (3 mm ID, 25 cm long); 8, polycarbonate 3-way stopcock; 9, polypropylene pipette tip; 10, polyethylene capillary tube (0.75 mm ID, 30 cm long); 11, glass tube (1.5 mm ID, 25 cm long); 12, incubator.

three-way stopcocks, immediately after perfusion. In the original system according to Cazenave *et al.*[2] a roller pump was used to rinse the tubes. The whole perfusion system was kept at 37° ± 0.5°C, in a homemade incubator.

The capillary tubes were rinsed with ethanol and distilled water respectively, and filled with Ca^{2+}/Mg^{2+}-free Tyrode solution. Protein solutions used to precoat the tubes were always introduced in the absence of an air–liquid–solid interface. At least 4 capillary volumes of protein solution were passed through at a shear rate of 500 s^{-1}. After incubation during 1 h at 37°C, the tubes were rinsed with Ca^{2+}/Mg^{2+}-free Tyrode solution for 5 min at a shear rate of 1000 s^{-1}. Protein concentration was 0.5 mg/ml for the fibrinogen, fibronectin and von Willebrand factor solutions and 4 mg/ml for the serum albumin and immunoglobulin G solutions. Surface protein concentration was measured semi-quantitatively using a two-step enzyme immunoassay[10]. It was found that at the protein precoating conditions mentioned, surface concentration had reached a plateau value for each protein.

Perfusates were prepared shortly before the start of an experiment, and were prewarmed for 5 min at 37°C. Care was taken to avoid the formation of an air–liquid–solid interface at the start of a perfusion experiment. Perfusion was carried out for 2–15 min at wall shear rates of 150–1500 s^{-1}. A wall shear rate of 1500 s^{-1} corresponds to a flow rate of 3.75 ml/min. After perfusion, the tubes were rinsed with Ca^{2+}/Mg^{2+}-free Tyrode solution for 5 min at 1000 s^{-1}.

In order to determine platelet deposition the part of the capillary tube which was led through the glass tube (25 cm) was cut into 6 segments of 4 cm length, the first cm, at the side of the entrance, was discarded. The radioactivity associated with each segment was determined in an LKB-Wallac 1219 Spectral β-counter (LKB, Stockholm, Sweden). The scintillator used was a 1 : 1 mixture of Lumagel (Lumac, Schaesberg, The Netherlands) and 2.9 M $ZnCl_2$.

Platelet aggregation

Platelet aggregation was performed in a dual channel aggregation module (model 600, Payton, Scarborough, Canada), at 37°C and 900 rpm. Aggregation was induced by ADP (Sigma) or thrombin (Hoffman La-Roche). When ADP was used, purified fibrinogen at a final concentration of 0.8 mg/ml was added to the platelet suspension.

Scanning electron microscopy (SEM)

Immediately after a perfusion experiment, the capillary tubes were fixed with Ca^{2+}/Mg^{2+}-free Tyrode solution containing 2% glutaraldehyde. After standing overnight at 4°C, the tubes were dehydrated through a graded series of ethanol solutions to absolute ethanol and dried. Samples were sputter-coated with approx. 10 nm of gold (cathode sputtering unit 07.120, Balzers Union Ltd, Liechtenstein), and examined on a JSM-35 CF scanning electron microscope (Japan Electron Optics Laboratory) at 15 kV accelerating voltage.

RESULTS

Effect of washing procedure on platelet function

Platelet viability after the washing procedure was examined by performing ADP- and thrombin-induced platelet aggregation. Platelets underwent shape change followed by reversible primary aggregation in response to ADP (*Figure 2 a*). A concentration as low as 1 μM induced aggregation, which was completely reversible after 1 min. Aggregation in response to 0.1 U/ml thrombin was irreversible (*Figure* 2 b). Labelling of platelets with ^{111}Indium did not affect aggregation in response to ADP or thrombin.

Effect of protein precoating on platelet deposition

Precoating polyethylene tubes with von Willebrand factor, fibrinogen or fibronectin resulted in an increase of platelet deposition, whereas platelet accumulation on IgG-coated polyethylene was slightly lower compared to uncoated surfaces (*Figure 3*). Platelet deposition on polyethylene precoated with albumin or plasma was almost completely absent. Platelet deposition decreased exponentially as a function of axial distance from the tube inlet on surfaces precoated with von Willebrand factor, fibrinogen or fibronectin. The following equation can be used:

$$\text{Platelet deposition} = A.\ (\text{axial distance})^m \qquad (1)$$

where A is a constant and m the power law exponent. For

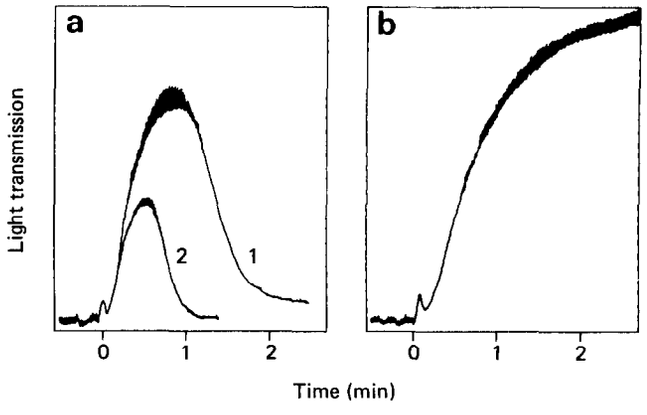

Figure 2 Aggregation of washed platelets after stimulation with: (a) 10 μM ADP (curve 1) and 1 μM ADP (curve 2); (b) 0.1 U/ml thrombin.

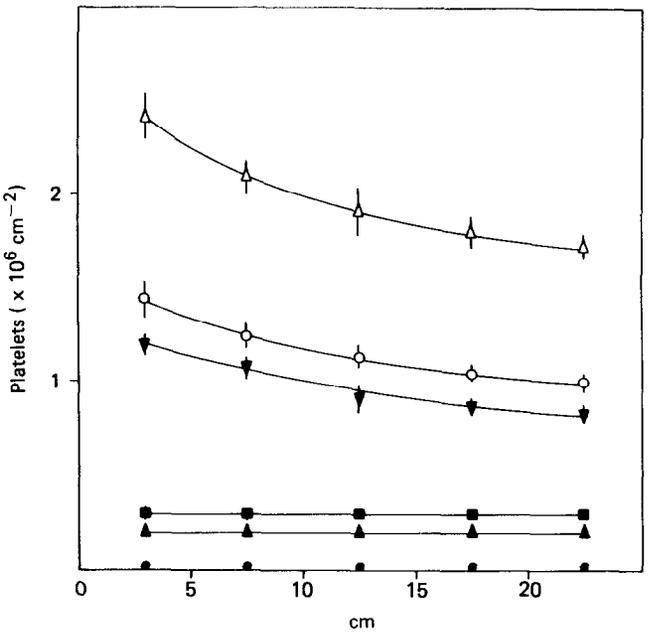

Figure 3 Effect of protein precoating on platelet deposition. Polyethylene tubes were precoated with: △, von Willebrand factor (vWF); ○, fibrinogen (Fb); ▼, fibronectin (Fn); ▲, immunoglobulin G (IgG); ●, albumin (Alb) and plasma; ■, uncoated. Perfusion was carried out for 5 min at a shear rate of 500 s^{-1}. The number of deposited platelets is shown as a function of the axial distance from the tube inlet. All values are mean ± SD (n = 2).

preadsorbed von Willebrand factor, fibrinogen and fibronectin the power law exponent m and correlation coefficient r were: -0.17 ± 0.03 (r = 0.91); -0.19 ± 0.03 (r = 0.92) and -0.20 ± 0.04 (r = 0.89) respectively (± SD, n = 10).

Effect of protein precoating on platelet morphology

The very few platelets adhering to polyethylene precoated with albumin or plasma had a discoid form without pseudopods (*Figure 4 a*). In contrast, platelet-surface interaction in the case of uncoated polyethylene resulted in the formation of pseudopods (*Figure 4 b*). A few platelet aggregates were seen on IgG-coated polyethylene (*Figure 4 c*), but large surface areas did not show platelet deposition. Surfaces precoated with fibrinogen showed coverage with single platelets (*Figure 4 d*). These adherent platelets had developed pseudopods and some had spread on the surface. More extensive platelet spreading was observed on fibronectin-coated surfaces (*Figure 4 e*). Platelets interacting with polyethylene precoated with von Willebrand factor developed long pseudopods but platelet spreading was not observed (*Figure 4 f*). Occasionally, a single red cell was seen on all surfaces.

Effect of perfusion time on platelet adhesion

Platelet adhesion on fibrinogen-coated polyethylene increased when the perfusion time was increased from 2 to 15 min; surface saturation was not observed (*Figure 5 a*). Platelet adhesion on uncoated polyethylene increased during the first few minutes of perfusion but stabilized at 5 min (*Figure 5 b*).

Effect of shear rate on platelet adhesion

Platelet adhesion on fibrinogen-coated polyethylene increased when the wall shear rate was increased from 150 to 1500 s^{-1} (*Figure 6 a*). In contrast, platelet adhesion on uncoated polyethylene decreased with increasing shear rate (*Figure 6 b*).

DISCUSSION

A variety of perfusion techniques has been used to study *in vitro* platelet deposition on surfaces. The technique described by Cazenave *et al.*[2] allows the quantification of platelet accumulation on native and modified artificial surfaces at conditions of laminar flow and physiological wall shear rates. The perfusate, of which only a limited volume is required, is not exposed to large surface areas (e.g. feeder tubing) other than the test surface. We have used ^{111}Indium-labelled platelets to quantify platelet accumulation while the morphology of deposited platelets was examined by SEM. For the study of the interaction of human platelets with surfaces under well characterized conditions, a perfusate consisting of washed platelets (radiolabelled or native) and washed erythrocytes in a Ca^{2+}/Mg^{2+}-containing buffer was used. Polyethylene surfaces were tested both uncoated and after precoating with purified plasma proteins or plasma.

The method used to prepare a suspension of washed platelets should not lead to platelet activation and provide functionally active platelets. Washed platelets were sensitive to stimulation with 1 μM ADP and 0.1 U/ml thrombin. In contrast to thrombin, stimulation with ADP at physiological concentrations of ionized calcium does not lead to the formation of thromboxane A_2 or the release of granule contents[11]. This explains why aggregation in response to thrombin was irreversible whereas ADP-induced aggregation was reversible.

Precoating polyethylene tubes with purified von Willebrand factor, fibrinogen or fibronectin stimulated platelet deposition relative to the uncoated surface. Von Willebrand factor was the most active protein which is in agreement with recent results of Lambrecht *et al.*[12] who used an *ex vivo* canine model. It is very likely that the adhesion promoting effect of fibrinogen, fibronectin and von Willebrand factor is due to the interaction of these proteins with specific binding sites on the platelet membrane which are exposed after platelet activation[13-15]. Platelet deposition decreased exponentially as a function of axial distance from the tube inlet. This can be explained by assuming that depletion of platelets of the near surface layer occurred at a higher rate than delivery of platelets by diffusion through the suspending medium[16].

The measurement of the deposition of radiolabelled platelets does not permit a distinction between adherent *versus* aggregated platelets; therefore platelet morphology was studied by SEM. The single platelets adhering to polyethylene coated with fibrinogen, fibronectin or von Willebrand factor had all developed pseudopods, but complete spreading was only observed after precoating with fibronectin. It has been reported that fibronectin either present in the suspending medium[17] or on the surface[18], promotes the spreading of platelets on surfaces coated with fibrinogen or collagen. Our results show that fibrinogen adsorbed to a surface in the absence of other proteins, also acts as a spreading-factor for platelets.

A few large platelet aggregates were observed on IgG-coated polyethylene while the number of deposited ^{111}Indium-labelled platelets per surface area was lower compared with uncoated surfaces. It has been demonstrated that when platelets make contact with an IgG-coated surface the release of platelet constituents is much higher compared with a fibrinogen-coated surface[19,20]. As a result platelet

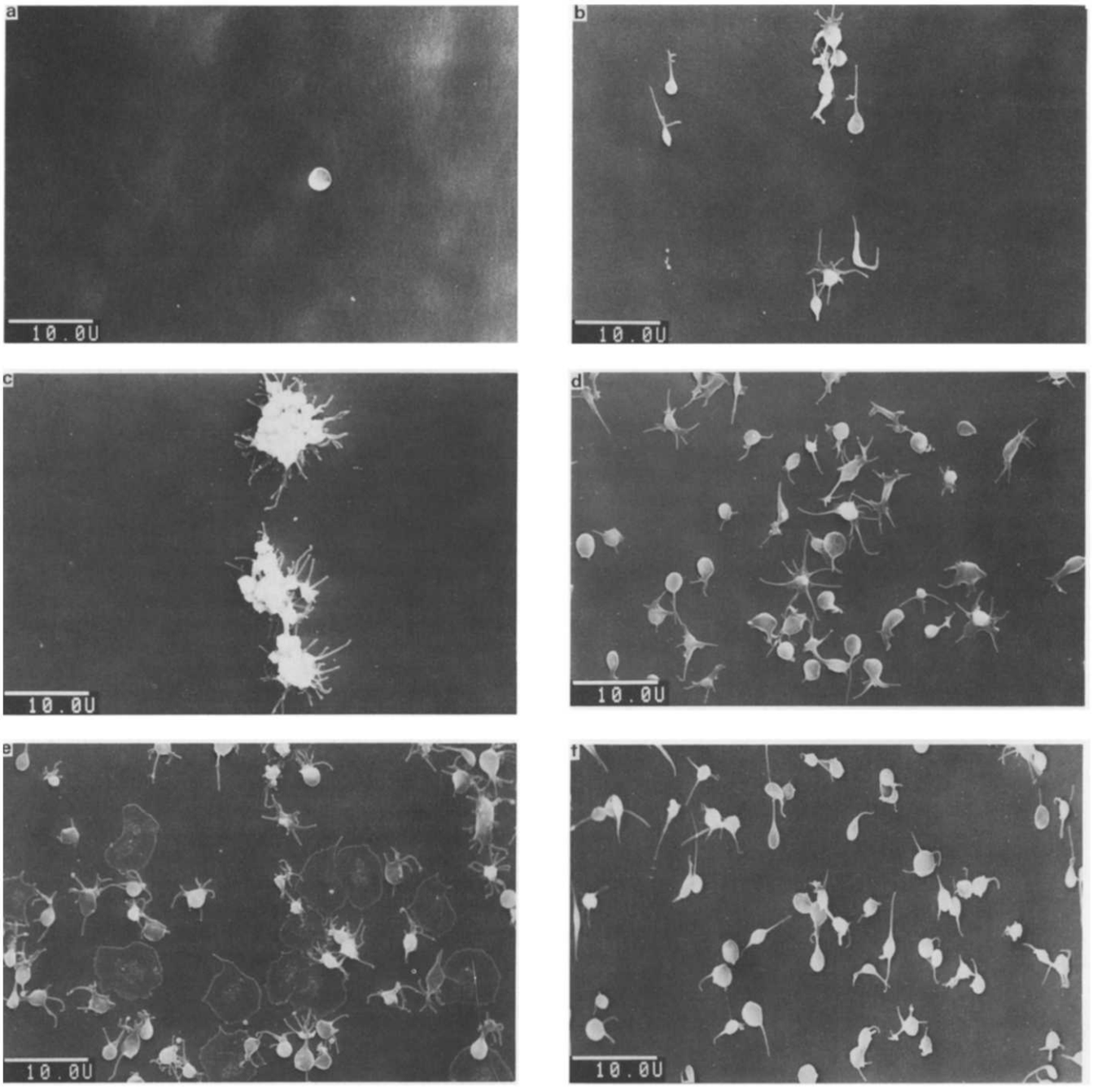

Figure 4 Effect of protein precoating on platelet morphology. Polyethylene tubes were precoated with albumin or plasma (a); uncoated (b); precoated with IgG (c); Fb (d); Fn (e); and vWF (f). Perfusion was carried out for 5 min at a shear rate of 500 s^{-1}.

aggregates are formed which may detach from the surface due to shear forces[20]. This could explain the relatively low platelet numbers per surface area on IgG-coated polyethylene.

Platelet adhesion was almost completely absent on polyethylene precoated with albumin or plasma. Surfaces precoated with albumin show a diminished adhesion[20, 21] and release[20] of platelets. This inhibitory effect of albumin has been attributed to its high degree of hydrophilicity. Young *et al.*[21] have suggested that in contrast with other proteins, adsorbed albumin does not expose nonpolar residues at the aqueous interface. Precoating polyethylene with plasma for 1 hour at 37°C generated a protein layer which proved to be unfavourable for platelet adhesion. Apparently, adhesion promoting proteins were not present at the surface. This might be due to the so-called Vroman effect[22], which implies that fibrinogen initially adsorbed out of plasma is in time displaced from the surface possibly by high molecular weight kininogen. Adsorption experiments by Uniyal and Brash[23] showed that fibrinogen adsorption out of plasma onto polyethylene passes through a maximum in less than 2 min, and then decreases to near zero.

When the perfusion time was increased up to 15 min at a shear rate of 500 s^{-1}, the adsorption of platelets on fibrinogen-coated polyethylene increased without surface saturation. Platelet adhesion on uncoated polyethylene did not increase further after 5 min of perfusion, probably because the surface was passivated by albumin adsorbed from the perfusate.

According to Turitto *et al.*[24], the platelet flux to the surface (and thus platelet deposition) can be expressed in terms of two processes: transport of platelets to the surface by convective diffusion and attachment of platelets at the surface which can be seen as a chemical reaction. If the rate of attachment is very high relative to the rate of transport, the

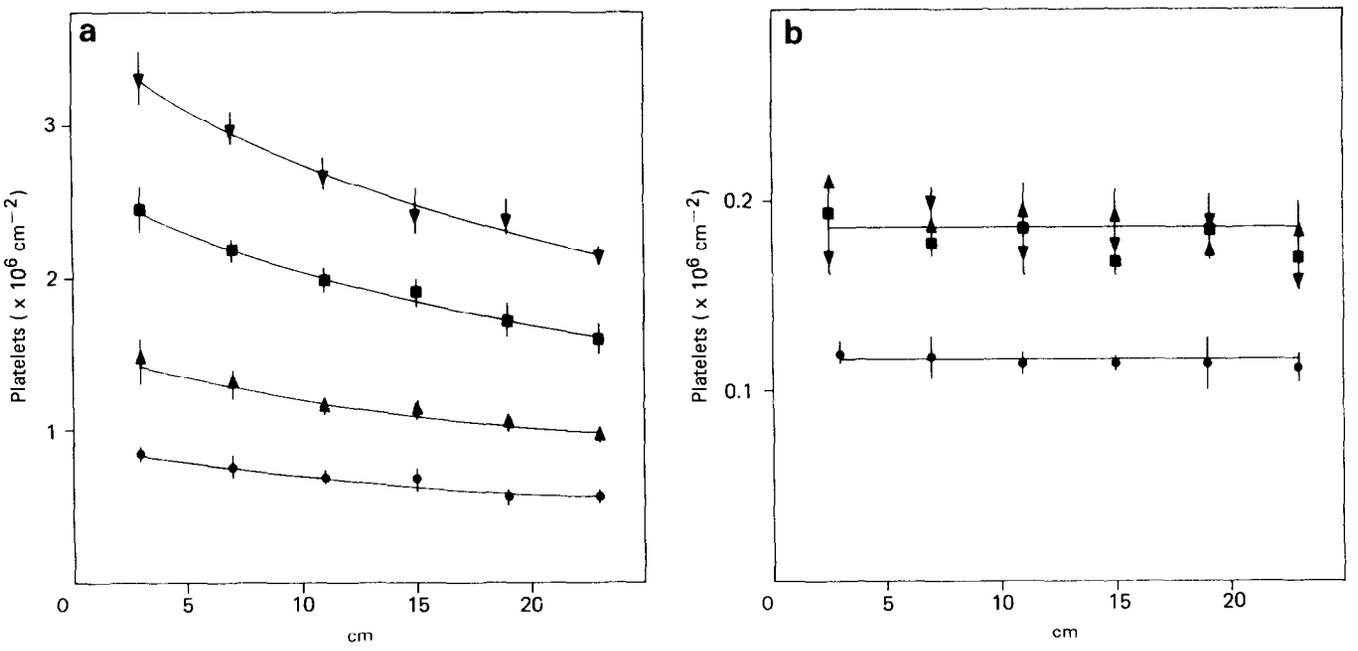

Figure 5 Effect of perfusion time on platelet adhesion to: (a) fibrinogen-coated and (b) uncoated polyethylene tubes at a shear rate of 500 s^{-1}. ▼, 15 min; ■, 10 min; ▲, 5 min; ●, 2 min. All values are mean ± SD (n = 2).

overall process of platelet deposition will be transport-controlled. On the other hand, platelet deposition will be reaction-controlled if the rate of transport is very high relative to the rate of attachment. Intermediate kinetics are possible when the rate of platelet transport and attachment are comparable in magnitude. We observed that platelet adhesion on polyethylene precoated with fibrinogen, fibronectin or von Willebrand factor decreased exponentially as a function of axial distance from the tube inlet. Using Equation (1), the power law exponent m will be −0.33 if initial platelet adhesion is transport-controlled, between −0.33 and 0 in the case of intermediate kinetics, and 0 if initial platelet adhesion is reaction-controlled[25].

For fibrinogen-coated polyethylene the power law exponent m amounted to -0.19 ± 0.03, after 5 min of perfusion at a shear rate of 500 s^{-1}. From the experiments in which the perfusion time was varied, it was learned that under these conditions platelet-surface interaction was not influenced by surface saturation, and thus initial platelet adhesion was measured. From these data it can be concluded that platelet adhesion on fibrinogen-coated polyethylene is dependent on both platelet transport and surface reactivity (intermediate kinetics). Platelet adhesion on polyethylene precoated with von Willebrand factor or fibronectin is probably also determined by intermediate kinetics because the power law exponent m amounted to -0.17 ± 0.03 and -0.20 ± 0.04 respectively, after 5 min at 500 s^{-1}. However, it was not proven that under these conditions initial platelet adhesion was measured.

Platelet adhesion on fibrinogen-coated polyethylene increased with increasing shear rate which can be explained by an increase in platelet transport to the surface. Platelet

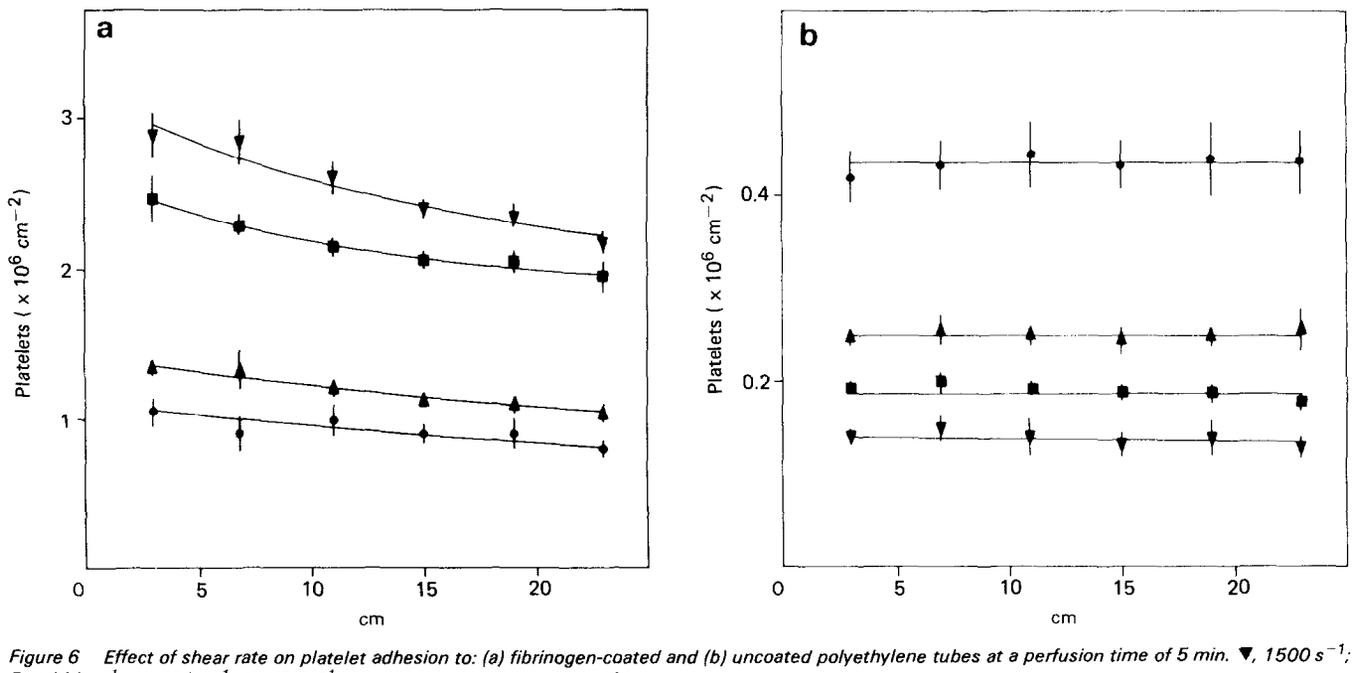

Figure 6 Effect of shear rate on platelet adhesion to: (a) fibrinogen-coated and (b) uncoated polyethylene tubes at a perfusion time of 5 min. ▼, 1500 s^{-1}; ■, 1000 s^{-1}, ▲, 500 s^{-1}; ●, 150 s^{-1}. All values are mean ± SD (n = 2).

transport is thought to be augmented by red cell rotation and translational motion, which increase with increasing shear rate[26-28]. Platelet adhesion on uncoated polyethylene was independent of the axial distance from the tube inlet, and decreased with increasing shear rate. In other words, the power law exponent m was 0 and therefore platelet adhesion must have been reaction-controlled. The observation that platelet adhesion decreased with increasing shear rate, can be explained by assuming that loosely-bound platelets detached from the surface as a result of the increasing shear forces. Moreover, at higher shear rates the residence time for platelets arriving in the vicinity of the surface may be too short to allow irreversible adhesion[24].

This study shows that platelet adhesion is promoted by adsorbed von Willebrand factor, fibrinogen and fibronectin. Von Willebrand factor is the most active protein, platelet spreading is most extensive on fibronectin-coated surfaces. Adsorption of IgG results in platelet aggregation at the surface, platelet adhesion is inhibited by adsorbed albumin. Moreover, our results indicate the applicability of the perfusion system according to Cazenave *et al.*[2] for the *in vitro* testing of biomaterials.

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