Direct Assessment of Platelet Adhesion to Glass: A Study of the Forces of Interaction and the Effects of Plasma and Serum Factors, Platelet Function, and Modification of the Glass Surface

By James N. George

Platelet adhesion to glass has been directly determined with a cover slip chamber. These studies have separated the functions of platelet adhesion to a foreign surface from platelet cohesion (aggregation). The forces of the platelet-glass interaction have been studied, and the qualitative variables affecting this interaction have been defined. Fibrinogen and calcium or magnesium are required for adhesion of either unwashed or washed platelets in plasma. Platelet adhesion in fresh serum requires thrombin and probably the fibrinogen of the platelet surface, since washed platelets are not adherent in fresh serum. Platelets incubated at 37°C for 48 hr can neither spread nor adhere to glass, a defect that may reflect decreased platelet surface area and membrane deformability. Platelets are equally as adherent to siliconized glass as to untreated glass surfaces. However, platelets cannot adhere to glass treated with the hydrogen-bonding polymer, poly(ethylene oxide). In contrast to silicone, poly(ethylene oxide)-treated glass surfaces do not impede blood coagulation. These surface properties further distinguish the two major factors involved in thrombogenesis: platelet adhesion and plasma coagulation.

The adhesive property of platelets to foreign surfaces has been studied in relation to hemorrhagic and thrombotic phenomena for many years, but only since the introduction of a glass bead column technique by Hellem in 1960 have detailed, quantitative measurements been available. The simplified modification of this method developed by Salzman in 1963 and its immediate clinical relevance in the diagnosis of von Willebrand’s disease popularized the assessment of platelet adhesiveness. Both of these techniques required the use of whole blood and were sensitive to the red cell concentration. Platelets suspended in plasma alone were not adherent to the glass beads, but when the red cell effect was defined as adenosine diphosphate (ADP) release, Hellem’s original method was modified to use platelet-rich plasma plus ADP. Since ADP is the primary agent for platelet aggregation, the platelet property assessed by these methods is uncertain: it may be adhesiveness (platelet-glass interaction) and/or cohesiveness (platelet-platelet interaction). Salzman confirmed the presence of platelet aggregation within the
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glass bead column by photomicrographs. Thus, methods using glass bead columns have been termed “platelet retention tests.”

Other methods of examining the platelet interaction with glass have been more direct. Packham et al. used the measure of residual radioactivity in variously treated glass tubes after exposure to a suspension of platelets labeled with 14C-serotonin. Mason and Gilkey and Zucker and Vroman examined cover slips that had been immersed in whole blood or a platelet suspension and then rinsed. These methods allow direct observation of adherent platelets and some variation of the suspending medium, but the forces of the platelet-surface interaction cannot be well studied.

Adhesion to glass surfaces has been studied most frequently, but more recently, adhesion to collagen-coated surfaces has also been quantified, based on the assumption that in vitro study of the platelet-collagen interaction would be more physiologic. This assumption may not be valid, as suggested by the study of platelet adhesion to subendothelial microfibrils in the absence of collagen. This adhesive process of platelets to microfibrils in vitro has qualitative features, such as the influence of divalent cations, that resemble more the platelet-glass interaction than platelet-collagen interaction.

Regardless of the surface used, previous methods for studying platelet-surface interactions have not allowed direct examination of a wide variety of conditions affecting platelet adhesiveness. We have utilized the method of Häyry to study the in vitro adhesiveness of platelets to glass, a cover slip chamber technique that has been used to study the adhesive properties of red cells and leukocytes. Its advantages are that: (1) The physical forces of interaction can be studied by varying the centrifugal attaching and detaching forces. (2) Effects of alteration of platelets (washing, incubation, etc.) and their suspending media can be studied. (3) The surface that platelets contact can be altered by treating the glass cover slip or substituting other materials. (4) The morphology of the adherent and nonadherent platelets can be directly observed. (5) The platelet-foreign surface interaction is assessed in suspensions dilute enough to eliminate platelet cohesion.

MATERIALS AND METHODS

Platelets and plasma were obtained from normal laboratory personnel, except where indicated. Platelet-rich plasma (PRP) was separated from blood anticoagulated with 0.1 vol of 0.13 M sodium citrate by centrifugation at 150 g for 10 min at 20°C. In some experiments, platelets from 1 ml of PRP were washed three times in 35 ml of acid-citrate-dextrose (ACD) with 1% bovine albumin, pH 6.5, centrifuging for 20 min at 1400 g, 20°C. This washing procedure should remove 90% of the surface, exchangeable fibrinogen. Platelets were kept at room temperature and used within 1 hr of preparation.

Platelet-poor plasma was obtained from blood anticoagulated with 0.1 vol of 0.13 M sodium citrate, 0.1 vol of 0.10 M sodium oxalate, 0.1 vol of 0.10 M disodium EDTA, or heparin, 2 U/ml. Fresh serum was obtained from whole blood defibrinated with glass beads. Incubated serum was obtained from whole blood clotted with 0.03 vol of rabbit brain thromboplastin (“activated thromboplastin,” Dade) for 1 hr at 37°C and then separated and kept at 37°C overnight. Bovine thrombin was obtained from Parke-Davis.

Coagulation factors II, VII, IX, and X were adsorbed from oxalate plasma with BaSO4 (Baker), 100 mg/ml, for 20 min at room temperature. Coagulation factors XI and XII were adsorbed from oxalate plasma with celite (“analytical filter aid,” Johns-Manville),
100 mg/ml, for 2 hr at room temperature;\textsuperscript{15} this adsorbed plasma was further incubated at 37°C for 18 hr to inactivate factor V. Plasma deficient in factor VIII activity was obtained from two patients with classical hemophilia. Plasma was also used from two patients with von Willebrand's disease who had Ivy bleeding times of over 20 min and factor VIII coagulation assays of 0.01 U/ml and 0.08 U/ml and immunoreactive factor VIII levels of 0.01 U/ml and 0.03 U/ml. The immunoassays were performed by Dr. R. Breckenridge and Dr. L. Hoyer, using the method of Zimmerman et al.\textsuperscript{16}

Experiments testing the effects of polyvalent cations used oxalate plasma that was adsorbed with BaSO\textsubscript{4} and then dialyzed overnight at 4°C against 300 vol of 0.128 M sodium chloride and 0.025 M sodium citrate.

Clot retraction was evaluated by incubation of recalcified PRP at 37°C in a 10 \times 75 mm glass tube. Platelet aggregation by adenosine diphosphate (ADP) was estimated visually and microscopically after adding 0.1 ml of a 10^{-5} M ADP solution to 0.2 ml of PRP in a 10 \times 75 mm glass tube. Platelet factor 3 release was assayed by the method of Spaet and Clintron.\textsuperscript{17} Whole blood clotting times were done by the standard Lee-White method, using 1 ml of blood in each of three 13 \times 100 mm tubes at 37°C.

Fraction 1 was precipitated from citrate plasma with 0.16 vol of 50% ethanol at -3°C for 30 min. The fraction 1 supernates were dialyzed against a balanced salt solution, pH 7.4 (136 mM NaCl, 2.7 mM KCl, 0.5 mM MgCl\textsubscript{2}, 1.0 mM CaCl\textsubscript{2}, 8.1 mM Na\textsubscript{2}HPO\textsubscript{4}, 1.5 mM KH\textsubscript{2}PO\textsubscript{4}, and 11.1 mM glucose) for 18 hr at 4°C.

Class cover slips (Exax No. 1, Kimble Glass) were prepared in a standard manner by washing with detergent, acid, and ethanol, and drying with acetone as previously described.\textsuperscript{11} These clean cover slips were siliconized for some experiments by immersing them in a 1% silicone solution (Clay-Adams, Siliclad), rinsing with water, and allowing to air-dry at room temperature. Poly(ethylene oxide) was kindly supplied by Dr. Casper W. Hiatt. Clean coverslips were immersed in a 0.4% aqueous solution, rinsed vigorously in distilled water, and allowed to air-dry at room temperature. Tubes for clotting times were prepared similarly.

The cover slip chamber and technique for measuring platelet adhesiveness have been previously described in detail with diagrammatic illustrations of the chamber.\textsuperscript{11,12} All procedures were carried out at room temperature. PRP or a suspension of washed platelets in buffer were diluted 200-fold with the appropriate suspending medium. In most experiments this was plasma or serum from the same donor. Platelets were not centrifuged and resuspended, except when they were washed with ACD-albumin. The platelet suspension was injected into the chamber, which was then sealed with wax. Our standard procedure was to centrifuge these chambers to a maximal force of 862 g (designated as "attaching force") and then immediately to decelerate (the entire centrifugation took 80-100 sec). With this force, all platelets were seen on the bottom cover slip. After approximately 5 min (designated "contact time"), the chamber was inverted and again centrifuged ("detaching force") in an identical fashion. When platelets were allowed to settle by gravity alone, approximately 2 hr were required for all platelets to be seen on the bottom cover slip (the chamber thickness was 0.4 mm). The chambers were then examined by high-power (450X) phase microscopy, counting the adherent platelets on the top (originally the bottom) cover slip and then changing the focus and counting the nonadherent platelets on the bottom (originally the top) cover slip in the same high-power field. The average number of platelets per field (top + bottom cover slips) was 75. Five microscopic fields, or over 300 platelets, were counted in each chamber. The per cent adhesion was calculated by dividing the adherent platelets on the top (originally the bottom) cover slip by the total number of platelets counted on both top and bottom cover slips.

Using this 200-fold dilution of PRP, individual platelets were widely separated on the glass surface and were easy to count; the minor variations of platelet concentration had no effect on the consistency of the data. When a 20-fold dilution of PRP was used, adherent platelets were contiguous and difficult to count, but there was no qualitative difference in the pattern of adhesion. Most platelets were adherent in saline, citrate plasma, or fresh serum.

In our data, each chamber is recorded as a single experiment. All data are recorded as
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the mean value and the standard deviation, and statistical evaluation by the Student's

t test is included where significant differences are relevant to the discussion.

RESULTS

Effects of Attaching Force, Contact Time, and Detaching Force (Table 1)

Platelets were brought into contact with the bottom cover slip of the
adhesion chamber either by allowing them to settle by gravity or by centri-
fuging the chamber up to a force of 862 g. Centrifugation to greater forces
damaged the chamber. Detaching force similarly was either 1 g or 862 g. The
data in Table 1 indicate that platelet adhesion was independent of the contact
time or detaching force. Mean adhesion was less with an attaching force of
1 g than with 862 g. These data were not normally distributed (ten chambers
had an adhesion of 67 ± 10%, similar to the other conditions, while seven
chambers had an adhesion of only 24 ± 11) but were significantly different
from platelet adhesion using the attaching force of 862 g. For all subsequent
experiments, the conditions were an attaching and detaching force of 862 g
and a contact time of approximately 5 min.

The adherent platelets on the top cover slip could be examined by oil
immersion, phase microscopy (1000X). Their morphology was constant: a
dark central region was surrounded by a continuous “skirt” of light, thinly
spread cytoplasm. Nonadherent platelets on the bottom cover slip could be
examined only by focusing through the thickness of the chamber with the
450X high-power magnification. These platelets were rarely spread but
appeared more spherical.

Effects of Plasma Anticoagulants, Platelet-Plasma ABO Blood Group
Incompatibility, and Use of Washed Platelets and Platelets
Suspected in Saline (Table 2)

Using our standard attaching and detaching centrifugal force, most platelets
were adherent in citrate plasma. Platelets were also adherent with oxalate or
heparinized plasma but were not adherent with EDTA plasma. There was

<table>
<thead>
<tr>
<th>Attaching Force (g)</th>
<th>Contact Time (min)</th>
<th>Detaching Force (g)</th>
<th>Per Cent Platelet Adhesion (mean ± SD)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>862</td>
<td>1</td>
<td>862</td>
<td>67 ± 17 (10)</td>
<td></td>
</tr>
<tr>
<td>862</td>
<td>240</td>
<td>862</td>
<td>66 ± 17 (10)</td>
<td></td>
</tr>
<tr>
<td>862</td>
<td>1</td>
<td>1</td>
<td>58 ± 17 (15)</td>
<td></td>
</tr>
<tr>
<td>1 up to 240†</td>
<td></td>
<td>862</td>
<td>42 ± 25 (24)</td>
<td></td>
</tr>
</tbody>
</table>

* Unwashed platelets were suspended in fresh citrate plasma.
† Contact time depended on the time required for platelets to settle onto bottom cover
slip. Data in line 4 represents significantly less adhesion than data in lines 1 (p <0.01), 2
(p <0.02) and 3 (p <0.05). Data in the top 3 lines are not significantly different from each
other.
Table 2. Effects of Plasma Anticoagulants on Platelet Adhesion

<table>
<thead>
<tr>
<th>Plasma Anticoagulant</th>
<th>Per Cent Platelet Adhesion (mean ± SD)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate</td>
<td>65 ± 16</td>
<td>42</td>
</tr>
<tr>
<td>Oxalate</td>
<td>78 ± 8</td>
<td>11</td>
</tr>
<tr>
<td>EDTA</td>
<td>5 ± 6</td>
<td>10</td>
</tr>
<tr>
<td>Heparin</td>
<td>80 ± 7</td>
<td>10</td>
</tr>
<tr>
<td>Citrate (with washed platelets)</td>
<td>51 ± 20</td>
<td>18</td>
</tr>
</tbody>
</table>

* Range of values for these 42 chambers was 34%–95%. In our experiments, groups of three to five chambers were used, and range of mean values for these groups was 44%–85%.

no difference between platelets suspended in their own plasma or plasma from another donor, and specific experiments with group A platelets suspended in citrate plasma from a blood group O donor demonstrated no effect of the anti-A isoantibodies (per cent adhesion = 77 ± 8, n = 10).

Platelets washed three times in ACD-albumin were as adherent as unwashed platelets in citrate plasma. The morphology of the adherent, washed platelets was the same as unwashed platelets. Washed platelets were completely adhesive to glass when suspended in only 0.154 M NaCl (Table 8). The morphology of these adherent platelets was also the same as unwashed platelets in plasma.

Effects of Plasma Coagulation Factors (Table 3)

Removal of factors II, VII, IX, and X, and removal or inactivation of factors V, XI, and XII had no effect on platelet adhesiveness. Platelet adhesion was also normal using plasma deficient in factor VIII activity from two patients with classic hemophilia. Plasma from two patients with von Willebrand's disease was also studied, and platelet adhesiveness was the same as with normal plasma, whether the patient's platelets (62 ± 6%, n = 4) or platelets from a normal donor were used (64 ± 10%, n = 8). Washed, normal platelets were also adherent in von Willebrand's plasma (61 ± 12%, n = 10).

Platelets were not adherent in plasma that had been heated to 56°C for 30 min. Adhesion was increased by the addition of fraction 1 from an equal volume of fresh plasma. Platelet adhesion was also low in the dialyzed
Table 4. Effect of Polyvalent Cations on Platelet Adhesion

<table>
<thead>
<tr>
<th>Suspending Medium</th>
<th>Per Cent Platelet Adhesion (mean ± SD) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dialyzed plasma</td>
<td>11 ± 7 (17)</td>
</tr>
<tr>
<td>Dialyzed plasma + 5 mM CaCl₂</td>
<td>51 ± 14 (12)</td>
</tr>
<tr>
<td>Dialyzed plasma + 5 mM MgCl₂</td>
<td>77 ± 9 (13)</td>
</tr>
<tr>
<td>Dialyzed plasma + 5 mM BaCl₂</td>
<td>22 ± 19 (15)</td>
</tr>
<tr>
<td>Dialyzed plasma + 5 mM SrCl₂</td>
<td>13 ± 7 (12)</td>
</tr>
<tr>
<td>Dialyzed plasma + 5 mM LaCl₃</td>
<td>17 ± 9 (12)</td>
</tr>
</tbody>
</table>

Platelet adhesion in dialyzed plasma + CaCl₂ and in dialyzed plasma + MgCl₂ were different from each other and from all other experiments (p < 0.001). Platelet adhesion in dialyzed plasma + BaCl₂ and in dialyzed plasma + LaCl₃ were each slightly different from plain dialyzed plasma (0.025 < p < 0.05). Samples with BaCl₂, LaCl₃, and SrCl₂ were not different from each other (p > 0.10).

supernate from the ethanol precipitation but was increased by re-adding the precipitate.

**Effect of Polyvalent Cations (Table 4)**
A requirement for divalent cations to support platelet adhesion was suggested by the inhibitory effect of EDTA. Platelet adhesiveness was also decreased in dialyzed plasma, and it was restored by the addition of Ca²⁺ and Mg²⁺. Other cations (Ba²⁺, Sr²⁺, and La³⁺) were less effective. Prothrombin is completely adsorbed by this method with BaSO₄,¹⁴ and no thrombin was formed by the calcium in this procedure.

**Effect of Adenosine Diphosphate and Aspirin (Table 5)**
Platelet adhesiveness was increased by the addition of 10⁻⁵ M ADP to heated plasma. This effect was not inhibited by prior incubation of the platelet-heated plasma suspension with an equimolar concentration of adenosine for 5 min at room temperature, in contrast to the inhibition of ADP-induced platelet aggregation by adenosine.¹⁸

Small doses of aspirin profoundly affect the platelet surface, as demonstrated by inhibition of collagen-induced platelet aggregation beginning 15 min after a single oral dose of 300 mg (one tablet).¹⁹ We tested platelets and plasma from normal subjects who had ingested 600 mg of aspirin 1 hr previously and found no effect on platelet adhesion to glass.

Table 5. Effect of Adenosine Diphosphate and Aspirin on Platelet Adhesion

<table>
<thead>
<tr>
<th>Suspending Medium</th>
<th>Per Cent Platelet Adhesion (mean ± SD) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, heated to 56°C × 30 min*</td>
<td>8 ± 8 (34)</td>
</tr>
<tr>
<td>Plasma (heated) + 10⁻⁵ M ADP</td>
<td>55 ± 23 (10)</td>
</tr>
<tr>
<td>Plasma (heated) + 10⁻⁵ M adenosine, then 10⁻⁵ M ADP</td>
<td>52 ± 18 (11)</td>
</tr>
<tr>
<td>Plasma and platelets after aspirin ingestion</td>
<td>79 ± 11 (11)</td>
</tr>
</tbody>
</table>

* Data are the same as in Table 3.
Table 6. Effect of Serum on Platelet Adhesion*

<table>
<thead>
<tr>
<th>Suspending Medium</th>
<th>Per Cent Platelet Adhesion (mean ± SD) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh serum</td>
<td>83 ± 16 (21)</td>
</tr>
<tr>
<td>Incubated serum</td>
<td>6 ± 11 (25)</td>
</tr>
<tr>
<td>Incubated serum + thrombin (0.1 U/ml)</td>
<td>10 ± 10 (19)</td>
</tr>
<tr>
<td>Incubated serum + thrombin (1.0 U/ml)</td>
<td></td>
</tr>
<tr>
<td>7 donors</td>
<td>82 ± 18 (28)</td>
</tr>
<tr>
<td>1 donor</td>
<td>8 ± 7 (13)</td>
</tr>
<tr>
<td>Fresh serum + heparin</td>
<td>19 ± 21 (10)</td>
</tr>
<tr>
<td>Fresh serum + citrate</td>
<td>4 ± 7 (10)</td>
</tr>
<tr>
<td>Fresh serum + oxalate</td>
<td>6 ± 4 (10)</td>
</tr>
<tr>
<td>Fresh serum with washed platelets</td>
<td>4 ± 4 (10)</td>
</tr>
</tbody>
</table>

* All experiments used unwashed platelets except where indicated.

Data for fresh serum (top line) represents significantly greater platelet adhesion than with citrate plasma (Table 2, top line), \( p < 0.001 \).

**Effect of Serum (Table 6)**

Platelet adhesion was greater in fresh serum than in plasma, but in contrast to plasma, there was no adhesion in serum incubated at 37°C for 18 hr. The adhesion in incubated serum was restored by adding thrombin in a concentration of 1 U/ml but not 0.1 U/ml. Higher concentrations of thrombin caused platelet aggregation, and only amorphous clumps and fragments were visible in the chamber. The incubated serum of one of our normal donors was different from seven others. With added thrombin this serum demonstrated low adhesion at 1.0 U/ml but nearly complete adhesion of intact platelets at 2.0 U/ml. Although this was presumed to result from a difference in serum antithrombin activity, no difference could be demonstrated by the progressive antithrombin assay of Abildgaard et al.20

Table 7. Effect of 37°C Incubation on Platelet Function and Platelet Adhesion*

<table>
<thead>
<tr>
<th>Platelet Factor 3 Released Stypven Time (sec) After 0 and 30 Min Incubation With Kaolin</th>
<th>Clot Retraction</th>
<th>ADP-Induced Aggregation</th>
<th>Per Cent Platelet Adhesion (mean ± SD) (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fresh platelets</td>
<td>303 (0 min), 13.9 (30 min)</td>
<td>4† 4†</td>
<td>65 ± 16</td>
</tr>
<tr>
<td>2. 24-Hr incubation</td>
<td>26.4 (0 min), 22.3 (30 min)</td>
<td>± Poor</td>
<td>40 ± 19</td>
</tr>
<tr>
<td>3. 48-Hr incubation</td>
<td>21.1 (0 min), 20.7 (30 min)</td>
<td>0 0</td>
<td>13 ± 11</td>
</tr>
</tbody>
</table>

* For adhesion experiments, platelets were suspended in fresh citrate plasma. Data on fresh platelets are the same as that in Table 2.
† Mean values for fresh platelets from ten normal individuals and for incubated platelets from four normal individuals.

Platelet adhesion in each group is significantly different from both other groups, \( p < 0.001 \).
Platelet adhesion in fresh serum was inhibited by adding one-sixth volume of 1.3 M sodium citrate, one-sixth volume of 1.0 M sodium oxalate (to achieve concentrations similar to those in plasma from anticoagulated whole blood), or heparin, 2 U/ml. Platelets washed in ACD-albumin were not adherent in fresh serum.

The morphology of adherent platelets in fresh serum and incubated serum plus thrombin was distinct from platelets in plasma: their centers were more dense and refractile and they had long, radial projections. There was no spreading of platelet cytoplasm. In fresh serum, or in the presence of thrombin, adherent platelets often appeared as part of a fibrous network that was on or close to the glass surface. This fibrous network was not seen when citrate, oxalate, or heparin were added to fresh serum or when washed platelets were studied.

**Effect of Platelet Incubation (Table 7)**

Platelet-rich plasma was incubated steriley at 37°C for 48 hr. After 24 hr, platelet factor 3 activity was decreased, clot retraction was absent in three of four samples (and minimal in the fourth), and only rare microscopic platelet aggregates were seen after addition of ADP. Platelet adhesion was studied using a 1:200 dilution of the incubated PRP in fresh citrate plasma. Adhesion was moderately decreased, and most adherent platelets were round, not spread. After 48 hr, platelet factor 3 activity, clot retraction, and ADP aggregation were absent, and adhesion in fresh plasma was minimal with no platelet spreading.

**Effect of Alteration of the Glass Surface (Tables 8 and 9)**

With our standard procedure, platelets in plasma were as adherent to siliconized surfaces as to glass. Even with an attaching force of 1 g (allowing platelets to settle for 4 hr), adhesion was consistently high (79 ± 6%, n = 10). Platelets were also adherent to siliconized surfaces when suspended in serum or saline.

Platelets did not adhere to glass cover slips treated with poly(ethylene oxide) when suspended in plasma or serum. Washed platelets in saline were minimally adherent. Even when the contact time after the 862 g attaching centrifugation was increased to 4 hr and the detaching force was decreased to 1 g, only rare platelets in plasma adhered to this surface (5 ± 2%, n = 10).

Clotting times done with siliconized glass tubes were prolonged compared to untreated glass, but clotting times in poly(ethylene oxide)-coated tubes were no different from those in glass tubes.

**DISCUSSION**

The direct assessment of platelet adhesion to a foreign surface has allowed the study of the forces of interaction, the critical variables in plasma and serum, the effect of platelet incubation, and the effects of different surfaces.

Our studies suggest that platelet interaction with the glass surface, within the limits of the experimental forces applied, is an all-or-none phenomenon, with firm adhesion occurring immediately. Variation of the time allowed for
Table 8. Effect of Alteration of the Glass Surface on Platelet Adhesion

<table>
<thead>
<tr>
<th>Surface</th>
<th>Suspending Medium*</th>
<th>Platelets</th>
<th>Per Cent Platelet Adhesion ± SD</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>Plasma Unwashed</td>
<td>65±</td>
<td>16</td>
<td>(42)</td>
</tr>
<tr>
<td>Glass</td>
<td>Serum Unwashed</td>
<td>83±</td>
<td>16</td>
<td>(21)</td>
</tr>
<tr>
<td>Glass</td>
<td>Saline Washed</td>
<td>97±</td>
<td>2</td>
<td>(10)</td>
</tr>
<tr>
<td>Silicone</td>
<td>Plasma Unwashed</td>
<td>70±</td>
<td>17</td>
<td>(14)</td>
</tr>
<tr>
<td>Silicone</td>
<td>Serum Unwashed</td>
<td>80±</td>
<td>18</td>
<td>(14)</td>
</tr>
<tr>
<td>Silicone</td>
<td>Saline Washed</td>
<td>97±</td>
<td>2</td>
<td>(10)</td>
</tr>
<tr>
<td>Poly(ethylene oxide)</td>
<td>Plasma Unwashed</td>
<td>1±</td>
<td>1</td>
<td>(10)</td>
</tr>
<tr>
<td>Poly(ethylene oxide)</td>
<td>Serum Unwashed</td>
<td>4±</td>
<td>4</td>
<td>(11)</td>
</tr>
<tr>
<td>Poly(ethylene oxide)</td>
<td>Saline Washed</td>
<td>17±</td>
<td>14</td>
<td>(12)</td>
</tr>
</tbody>
</table>

* Fresh citrate plasma and fresh defibrinated serum were used. Saline was 0.9\% NaCl.  
† Same data as in Table 2.  
‡ Same data as in Table 6.

Table 9. Whole Blood Clotting Times in Glass, Siliconized Glass, and Poly(ethylene Oxide)-Treated Glass

<table>
<thead>
<tr>
<th>Surface</th>
<th>Clotting Time (mean ± SD) (min)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass</td>
<td>14 ± 1</td>
<td>(10)</td>
</tr>
<tr>
<td>Siliconized glass</td>
<td>28 ± 6</td>
<td>(10)</td>
</tr>
<tr>
<td>Polyethylene oxide-treated glass</td>
<td>15 ± 2</td>
<td>(10)</td>
</tr>
</tbody>
</table>

Clotting time in siliconized glass is significantly greater than in either untreated or poly (ethylene oxide)-treated glass, p <0.001.

Contact with the glass surface or the detaching force after contact did not alter the fraction of adherent platelets. However, platelet adhesion was not consistent with an attaching force of 1 g and was significantly less than with an attaching force of 862 g. This suggests that some force greater than gravity alone is required to bring the platelets close enough to the glass surface to allow adhesion to occur. However, gravity alone was sufficient for adhesion to siliconized surfaces.

Weiss has studied the interaction forces between tissue culture cells and glass surfaces and determined values in dynes/cell corresponding to the theoretical zones of attraction: the weak attachment of the secondary minimum of adhesion and the short-range firm attachment of the primary minimum of adhesion that develops after the cell overcomes the repulsive barrier of opposing negative charge. Firmly adherent cells were not detached by shearing pressures of \(27 \times 10^{-5}\) dynes/cell, a force approximately 50-fold greater than the maximum energy level for the secondary attractive minimum of \(0.6 \times 10^{-5}\) dynes/cell. Our studies of human red cells, using the same cover slip chamber as with our present experiments, demonstrated that adhesion to glass was likely within the primary minimum since the red cells were resistant to detaching forces of \(4.8 \times 10^{-5}\) dynes/cell, sixfold greater than the energy level of the secondary attractive minimum.\(^{11}\)
However, because of the low effective cell mass of platelets (which equals: 
\[ \text{density}_{\text{cell}} - \text{density}_{\text{medium}} \times \text{cell volume} \]), the detaching force is low even at 862 g, the maximum force obtainable with our technique. Assuming the platelet density to be 1.050 (equivalent to tissue culture cells), the platelet volume to be 7.5 cu μ and plasma density to be 1.026, the effective cell mass (m) equals 1.8 × 10^{-13} g. Centrifugal detaching force (F) = \( mw^2r \), where w is the angular velocity (2 × rpm/60) and r is the centrifuge radius in centimeters. Therefore, at 862 g, F = 1.5 × 10^{-7} dynes/platelet, a value far less than even the energy level of the secondary minimum. Therefore, we cannot determine by our centrifugal force experiments that platelet adhesion to glass is firmly within the primary attractive minimum but we suspect firm attachment by the morphologic appearance of the adherent platelets.

Platelets adherent to glass in saline or plasma had the consistent appearance of a thinly spread “skirt” of cytoplasm to a diameter approximately threefold that of the nonadherent, nonspread platelets. However, the ability of the cytoplasm to spread onto the glass surface is not necessary for adhesion under all conditions. Platelets suspended in fresh serum or incubated serum plus thrombin were adherent by long filaments radiating out from a dense central body. Although this suggests that the mechanism of platelet interaction with the glass surface in serum is different from that in plasma or saline, both situations allow the platelet to develop projections of cytoplasm. Such a “probe” with a lower radius of curvature would favor cell attachment. Decreased surface deformability would decrease adhesiveness by limiting the ability of the platelet to spread on contact with a foreign surface. This may be the acquired defect of older, circulating platelets, which have been demonstrated to be less adherent to collagen. Our experiments with platelets incubated at 37°C were an attempt to study an in vitro model of cell aging. In studies of human red cells, Weed et al. have demonstrated red cell fragmentation with 37°C incubation, resulting in decreased surface area and decreased membrane deformability. If similar changes occur in the incubated platelet, spreading ability would be decreased. This is what we observed. After 24-hr incubation, adhesion was moderately decreased and most adherent platelets were round, not spread. After 48-hr incubation, adhesion was minimal and no spreading was seen. That platelet fragmentation may occur in vivo is suggested by Karpatkin’s studies demonstrating that younger platelets are more than twofold larger than older platelets.

Fibrinogen is certainly the major, if not the only, plasma protein required for platelet adhesion to glass. This is similar to the fibrinogen effect demonstrated by Packham et al. for glass surfaces and by Karpatkin et al. for collagen surfaces. Zucker and Vroman and Mason et al. more specifically defined the requirement for fibrinogen by testing platelet adhesion in congenitally afibrinogenemic plasma.

The deficiency of other coagulation factors did not affect platelet adhesive-ness. This included experiments with plasma and platelets from patients with von Willebrand’s disease who were severely deficient in factor VIII, as determined by coagulation and immunoassays. Using a direct measure of platelet interaction with glass, without the aggregation associated with the
glass bead columns, Mason and Gilkey also demonstrated normal platelet adhesiveness in five of six patients with von Willebrand's disease. These data suggest that the demonstration of platelet abnormality in von Willebrand's disease may specifically require the time-consuming reaction occurring during the flow of whole blood through a glass bead column.

Polyvalent cations are other major determinants of platelet adhesiveness. The lack of adhesion in plasma anticoagulated with EDTA and the decreased adhesion in plasma dialyzed against saline-citrate suggested a requirement for divalent cations. The role of calcium and magnesium was demonstrated by their ability to correct platelet adhesiveness in the dialyzed plasma. Barium and lanthanum were much less effective, and strontium was ineffective in increasing platelet adhesion in dialyzed plasma.

Platelet adhesion in fresh serum is greater but morphologically distinct from adhesion in plasma. The identity of the adhesion-promoting factor of serum as thrombin is suggested by its lability at 37°C, the restoration of adhesion by addition of small concentrations of thrombin, and the inhibition of adhesion by small concentrations of heparin. Decreased adhesion in serum with added citrate or oxalate suggests a requirement for polyvalent cations that is even greater than with plasma. Dialysis experiments were not performed with serum because of the lability of the platelet adhesion-promoting factor(s). Packham et al. have demonstrated increased platelet adhesion to glass surfaces previously coated with thrombin. The observation that washed platelets were not adherent in fresh serum suggests that the thrombin action requires the fibrinogen of the platelet surface.

Therefore, the factors promoting platelet adhesion to glass surfaces (fibrinogen, calcium, and thrombin) are the same as the major factors required for platelet cohesion (aggregation). This is different from the effect of fibrinogen on red blood cells. Our experiments have demonstrated that although fibrinogen promotes red cell cohesion (rouleaux formation), it specifically inhibits red cell adhesion to glass. Adenosine diphosphate (ADP) is the major agent causing platelet aggregation and is critical in platelet retention by glass bead columns. ADP is postulated to cause increased platelet retention in glass bead columns by causing aggregates that are trapped within the column. Our experiments demonstrate that ADP has a direct effect of increasing platelet adhesion to glass surfaces. This effect is qualitatively different from ADP-induced aggregation in that it is not inhibited by preincubation of the platelets with adenosine. Aspirin, which blocks platelet aggregation by collagen, had no effect on platelet adhesion to glass in our experiments.

Reports on the adhesiveness of platelets to siliconized glass surfaces have been conflicting. Hellem found platelet adhesiveness to siliconized glass beads to be only half that of adhesion to untreated glass. However, Packham et al. demonstrated greater adhesion of washed platelets to siliconized glass surfaces. With our technique, using an attaching force of 862 g, platelet adhesion was the same with glass and siliconized-glass surfaces.

Poly(ethylene oxide) completely prevented platelet adhesion. This compound, a polyether of molecular weight about 100,000, has been used to prevent adsorption of rabies virus to porous glass columns. Its postulated mechanism
of action is based on its strong affinity for complex formation by hydrogen bonding, producing multiple bonds with the electronegative oxygen atoms in the —Si— repeating structure of glass. This compound must then block the active sites on glass to which platelets adhere. Of interest is the contrast between the platelet-surface interaction and the surface activation of plasma coagulation. Nossel et al.\textsuperscript{3} have previously demonstrated a difference in the polar group requirement for the collagen activation of Hageman factor and collagen aggregation of platelets. In our experiments, a siliconized glass surface significantly retarded plasma coagulation but did not inhibit platelet adhesion. The reverse was true of glass surfaces treated with poly(ethylene oxide): platelet adhesion is inhibited while plasma coagulation is unaffected. This again demonstrates the independence of these two phenomena that are both critical in thrombogenesis.

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REFERENCES


Direct Assessment of Platelet Adhesion to Glass: A Study of the Forces of Interaction and the Effects of Plasma and Serum Factors, Platelet Function, and Modification of the Glass Surface

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