Fibronectin in Platelet Adhesion to Human Collagen Types I and III

Use of Nonfibrillar and Fibrillar Collagen in Flowing Blood Studies

Patricia F.E.M. Nievelstein, Patrizia A. D’Alessio, and Jan J. Sixma

Platelet deposition at high wall shear rates on collagen type I and type III purified from human umbilical arteries is dependent on the presence of fibronectin and von Willebrand factor (VWF). The role of fibronectin at low wall shear rates (≤ 500 s⁻¹), where platelet deposition was independent of VWF, was studied with purified collagen I and III. Platelet deposition on nonfibrillar collagen I was fibronectin-dependent at all wall shear rates. Platelet deposition on nonfibrillar collagen type III was fibronectin-dependent at 300 s⁻¹ and higher shear rates. By using a mixture of nonfibrillar type I and III, platelet deposition was found to be fibronectin-dependent at the tested wall shear rates (20, 100, and 300 s⁻¹). This dependency was less than with nonfibrillar type I only, but more than with nonfibrillar type III. For platelet deposition on reconstituted type I or type III collagen fibrils, no fibronectin dependency was observed up to the highest wall shear rate tested (1 800 s⁻¹). The same results were obtained with a mixture of native type I and III fibrils. Thus, the dependence of platelet deposition on fibronectin is determined by the collagen type and the wall shear rate. The dependence on the fibronectin concentration was tested with nonfibrillar collagen type I at a wall shear rate of 300 s⁻¹. Platelet deposition increased with increased fibronectin concentration up to a level of 700 µg/ml and leveled off above this concentration.

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One of the first steps in hemostasis and thrombosis is adhesion of blood platelets to perivascular collagen.1 At least two proteins are involved in this process of initial platelet adhesion, von Willebrand factor (VWF) and fibronectin.

The role of VWF in platelet adhesion was substantiated in perfusion studies with blood deficient in VWF which showed abnormal adhesion of platelets to subendothelium of rabbit aorta, human renal and umbilical arteries, or to collagen types I and III purified from human umbilical arteries.2–7 VWF in plasma, in vessel wall, and in endothelial cell matrix mediates platelet adhesion.8,9 The dependence on VWF, however, was present only at wall shear rates above 500 s⁻¹.

In perfusion studies with fibronectin-depleted plasma, platelet adhesion to purified human collagen was inhibited at all wall shear rates tested (≤ 300 s⁻¹).7 This dependence on plasma fibronectin was not seen in platelet adhesion to human umbilical arteries, but blocking studies with antibodies showed that this was because fibronectin was present in the vessel wall.10 In addition, fibronectin in endothelial cell matrix contributed to platelet adhesion.9

In this study, we investigated the role of plasma fibronectin in the adhesion of blood platelets to collagen I and III isolated from human umbilical arteries and we particularly emphasized the adhesion at low shear rates.

Methods

Collagen

Isolation of collagen types I and III from human umbilical arteries and preparation of collagen fibrils was carried out by pepsin digestion as previously reported.7 Native collagen fibrils (polymeric collagen) were isolated according to Stevens’s method12 in which no pepsin is used. Polymeric collagen from human skin consisted of 64% type I collagen, 27% type III, and 9% noncollagenous proteins. Native fibrils of bovine uterus origin consisted of 50% type I, 43% type III, and 7% noncollagenous proteins. As previously described,11 collagen was sprayed on glass coverslips to a surface density at which optimal platelet adhesion was obtained (30 µg/cm² for nonfibrillar collagen and 20 µg/cm² for fibrillar collagen). Before exposure to collagen, the glass coverslips were kept at least 2 hours in chromic acid. They were rinsed 10 times with 80% alcohol and kept in this solution overnight at room temperature. They were then washed 10 times with de-ionized distilled water and dried before exposure to collagen.

Preparation of Fibronectin-Free Plasma and Purification of Fibronectin

Fibronectin was isolated from normal human plasma by affinity chromatography on gelatin-Sepharose.13 Gelatin-Sepharose 4B was prepared by coupling gelatin (E. Merck,
FIBRONECTIN AND PLATELET ADHESION TO COLLAGEN

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AG, Darmstadt, West Germany) to CNBr-activated Sepharose 4B, according to the description of the manufacturer (Pharmacia Fine Chemicals, Uppsala, Sweden). Plasma (300 ml) was passed through the gelatin-Sepharose column (5 × 6.3 cm, equilibrated with 0.05 M Tris HCl, pH 7.4) and was collected. The first and last fractions were discarded to minimize dilution. This "fibronecin-depleted" plasma contained less than 1.5 μg/ml fibronecin as detected in an electroimmunossay. The level of VWF:Ag was unchanged and ristocetin cofactor activity was in the range of 0.70 to 0.85 U/ml. This fibronecin-free plasma was stored at −70°C.

For the purification of fibronecin, the gelatin column was washed with 1 M NaCl in 0.05 M Tris/HCl buffer (pH 7.4, room temperature), followed by 0.2 M arginine in the same buffer.14 Fibronecin was eluted with 1 M arginine in the same buffer.14 The buffers contained 0.1 mM phenylmethylsulfonylfluoride, 1 mM benzamidine, 5 mM e-aminocaproic acid, 10 mM EDTA, and 0.02% (wt/vol) sodium azide. The pooled fractions were dialyzed at 4°C against 50 mM Tris/HCl (pH 7.4) for at least 24 hours. They were then concentrated by dialysis at 4°C against 30% saturated ammonium sulfate for 24 hours. The precipitate was centrifuged for 10 minutes (at 3000 g, room temperature) and dissolved in a small volume of 50 mM Tris, 100 mM sodium chloride (pH 7.4). This was then dialyzed at 4°C for at least 24 hours against this buffer. Fibronecin was stored at 4°C and used for experiments within 1 week.

Electrophoresis on 5% SDS-polyacrylamide gel after reduction with 2-mercaptoethanol showed >95% homogeneity. This fibronecin preparation contained <1.0 mU (10 ng) VWF antigen per milligram protein as determined with an ELISA.15 The fibronecin preparations were also analyzed on 5% nonreduced gels. Zone I (450 kD dimers) and zone II (190 to 235 kD) fibronecin bands, as well as high molecular weight multimers, were present.16 Densitometric scanning showed 86% zone I, 9% zone II, and 5% multimers. The concentration of fibronecin was determined17 by measuring the absorbance at 280 nm (absorbance coefficient:

\[ A_{280}^{1\% \text{ on}} = 12.8 \]

Perfusion Chamber

Perfusions were carried out in a flat perfusion chamber as described by Sakariassen et al.11 with some modifications (Figure 1). The perfusion chambers were longer (14 cm) than the original one used and the central knob was replaced by two knobs. The knobs were equal distances from the center of the chamber (2.25 cm measured from the middle of the knob). The slit heights were: 1, 2, 3, or 6 mm.

Measurements with Laser-Doppler velocimetry11 showed parabolic velocity profiles at both knobs, indicating that the flow was laminar at the region of the coverslips (data not published). There was no systematic difference between platelet deposition on the first knob compared with deposition on the second knob.

Wall shear rates were calculated with the formula used by Muggli et al.18

\[ \text{Shear rate} = \frac{Q}{4\pi R L} \]

Figure 1. Photograph of the perfusion chamber. A glass coverslip containing the sprayed collagen can be placed on one of the knobs. The knob is inserted in the perfusion chamber, and thus the purified collagen can be exposed to flowing blood. For technical details see Reference 11.

Perfusate Components

Blood from normal donors was anticoagulated with 1/10 vol 110 mM trisodium citrate. Platelet-rich plasma (PRP) was obtained by centrifugation (10 minutes, 200 g, 20°C). Platelets were washed in Krebs-Ringer as described previously4 with some modifications. Platelets were washed twice instead of three times in Krebs-Ringer. Aspirin (10 μM) was added to PRP and mixed with 1 volume Krebs-Ringer (pH 5) before labelling with 111In and centrifugation to prevent aggregate formation during perfusion. Aspirin treatment has no effect on platelet adherence to collagen.5 After the first washing, the platelets (resuspended in Krebs-Ringer, pH 6.0) were labelled with 5 μCi/ml 111In-oxine dissolved in 0.3% NaCl (Byk-Mallinckrodt, C.I., B.V., Petten, The Netherlands). Before adding the In-oxine to the platelet suspension, one volume of 111In-oxine was buffered with 1/10 volume of 0.25 M Tris. To enhance the labelling efficiency, 6.5 μl oxine dissolved in 95% ethanol (1 mg/ml) was added to each milliliter of the platelet suspension. After 10 minutes of incubation at room temperature, the platelets were washed to remove free 111Indium and were resuspended in plasma or plasma substitute to a platelet count of 190 000 per microliter plasma. Plasma substitutes were fibronecin-depleted plasma or fibronecin-depleted plasma with added purified fibronecin (300 μl per milliliter plasma). In several experiments, antifibronecin F(ab')2 fragments (Cappel Laboratories, Cochranville, Pennsylvania, USA) were added to fibronecin-depleted plasma in a concentration of 500 μg/ml (the same antibody was used for the experiments in references 9 and 10). For studies on the very reactive polymeric collagens, perfusions were performed with unlabelled platelets, which were washed twice in Krebs-Ringer and were resuspended in plasma or plasma substitute as described above.

Red blood cells were washed three times by centrifugation in isotonic saline containing 5 mM glucose (2 × 5 minutes, 1 × 15 minutes, 3000 g). Before each perfusion experiment, the perfusate was reconstituted by adding 40% washed and packed red cells to the resuspended platelets in plasma.
**Perfusion Conditions**

Perfusions were performed at 37°C in flat perfusion chambers (described above). A constant flow rate was obtained by gravity. The wall shear rates used during the experiments, the corresponding flow rates, the chamber used, and the perfusion times are summarized in Table I. The perfusion times chosen were those at which optimal platelet adhesion, but no aggregate formation, was observed. Within this period, platelet deposition was linear with time. To be able to compare results at different shear rates, the results were expressed as rates of platelet deposition. The perfusates (20 ml in the chamber, with a slit height of 6 mm and 15 ml in the other chambers) were incubated for 10 minutes in a water bath (37°C) before starting the perfusions. Before and immediately after perfusion, the system was rinsed with 20 ml of 10 mM HEPES, 0.154 M NaCl buffer (pH 7.35).

**Evaluation**

Platelet deposition on nonfibrillar collagen and on reconstituted fibrils was determined by 111In-radioactivity in a gamma counter. Platelet deposition on native fibrils was evaluated morphologically with a light microscope connected to an image analyzer (Quantimet 720, Imago, Royston, U.K.), as described before.

**Statistical Analysis**

Significance of differences between means was calculated with the Peritz’ F test.

**Results**

**Concentration Dependence of Fibronectin-Stimulated Platelet Adhesion**

The dependence of platelet adhesion to nonfibrillar collagen and on reconstituted fibrils was determined by 111In-radioactivity in a gamma counter. Platelet deposition on native fibrils was evaluated morphologically with a light microscope connected to an image analyzer (Quantimet 720, Imago, Royston, U.K.), as described before.

**Table 1. Flow Conditions during Perfusion**

<table>
<thead>
<tr>
<th>Wall shear rate (sec⁻¹)</th>
<th>Flow rate (ml/min)</th>
<th>Slit height (mm)</th>
<th>Perfusion time (min)</th>
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<tbody>
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<td>10</td>
<td>35</td>
<td>6</td>
<td>40</td>
</tr>
<tr>
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<td>70</td>
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<tr>
<td>800</td>
<td>82</td>
<td>1</td>
<td>3</td>
</tr>
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</table>

An increase in platelet deposition was shown with an increase in plasma fibronectin concentration up to 700 μg/ml of fibronectin (Figure 2). Above this concentration, platelet deposition remained constant up to the highest concentration measured (2800 μg/ml) (Figure 1).

The fibronectin concentration in plasma of normal healthy individuals varies between 200 μg/ml and 700 μg/ml. The results obtained here indicate that this natural variation in plasma concentration may influence platelet deposition on collagen. Therefore, we determined the fibronectin concentration in normal plasma before each perfusion experiment. In perfusions with fibronectin-depleted plasma plus purified fibronectin, the amount of purified fibronectin was adjusted to the level in normal plasma.

**Role of Fibronectin on Platelet Deposition on Nonfibrillar and Fibrillar Collagen Types I and III at Different Wall Shear Rates**

The effect of fibronectin on platelet deposition on purified human umbilical nonfibrillar and fibrillar collagen types I and III was studied in perfusions with normal plasma and with fibronectin-depleted plasma.

Platelet deposition on nonfibrillar collagen type I was fibronectin-dependent at all wall shear rates (10, 50, 100, 300, and 800 s⁻¹, Figure 3A). Platelet deposition on nonfibrillar collagen type III was fibronectin-dependent at a shear rate of 300 s⁻¹ and higher. This dependence was more pronounced at the highest wall shear rate (Figure 3B). In all cases where fibronectin dependency was observed, platelet deposition could be restored by adding purified fibronectin to fibronectin-depleted plasma to the same concentration as in normal plasma (Figure 3).

For platelet deposition on fibrillar collagen type I or III, no fibronectin dependency was observed even when wall
shear rates higher than those of nonfibrillar collagen (up to 1800 s\(^{-1}\)) were tested (Figure 4). There was no difference in platelet deposition after perfusion with normal plasma or with fibronectin-depleted plasma. Furthermore, there was no further increase of platelet deposition after addition of purified fibronectin to fibronectin-depleted plasma, which should happen if the system were fibronectin-dependent.

Platelet deposition on a mixture of different collagen types was studied as well. Nonfibrillar collagen type I and III were mixed in an equal ratio and sprayed on glass coverslips at a concentration of 30 µg/cm\(^2\). Perforusions were performed with normal plasma or fibronectin-depleted plasma. After perfusion of this mixture of nonfibrillar collagen type I and collagen type III, platelet deposition was significantly lower after perfusion with fibronectin-depleted plasma compared to perfusions with normal plasma. For wall shear rates of 20 s\(^{-1}\), 100 s\(^{-1}\), and 300 s\(^{-1}\), platelet deposition was 42%, 45%, and 56%, respectively, lower after perfusions with fibronectin-depleted plasma than with normal plasma (see Table 2). As a control, a perfusion run with nonfibrillar collagen type I was performed. As expected, platelet deposition after perfusion with fibronectin-depleted plasma was only 5% of the deposition obtained after perfusion with normal plasma.

![Figure 3](image1.png)  
**Figure 3.** Rate of platelet deposition on nonfibrillar collagen type I (A) and nonfibrillar collagen type III (B) (30 µg/cm\(^2\)) purified from human umbilical arteries. Perfusion were performed with \(^{111}\)In-labelled platelets in reconstituted blood (114,000/µl final concentration). Plasma was normal plasma (solid bars), fibronectin-free plasma (open bars), or fibronectin-free plasma with addition of purified fibronectin in the same concentration as in normal plasma controls (shaded bars). Perfusion times were as illustrated in Table 1. Values are mean ± SEM, n = 24. Error bars at 10 sec\(^{-1}\) and 20 sec\(^{-1}\) are invisible because they fall within the thickness of the line.

![Figure 4](image2.png)  
**Figure 4.** Rate of platelet deposition on fibrillar collagen type I (A) and fibrillar collagen type III (B) (20 µg/cm\(^2\)) purified from human umbilical arteries. Perfusion were performed with \(^{111}\)In-labelled platelets in reconstituted blood (114,000/µl final concentration). Plasma was normal plasma (solid bars), fibronectin-free plasma (open bars), or fibronectin-free plasma with addition of purified fibronectin in the same concentration as in normal plasma controls (shaded bars). Perfusion times were as illustrated in Table 1. Values are mean ± SEM, n = 16.
**Table 2. Platelet Deposition on a Mixture of Nonfibrillar Collagen Type I and Nonfibrillar Collagen Type III (30 μg/cm²)**

<table>
<thead>
<tr>
<th>Wall shear rate (sec⁻¹)</th>
<th>Normal plasma</th>
<th>Fibronectin-depleted plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>13.17 ± 2.22</td>
<td>7.69*</td>
</tr>
<tr>
<td>100</td>
<td>57.97 ± 2.18</td>
<td>31.78 ± 4.37</td>
</tr>
<tr>
<td>300</td>
<td>68.93 ± 5.17</td>
<td>29.38 ± 3.19</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of four determinations except for starred values, which are two determinations.

Perfusions were performed at wall shear rates of 20, 100, and 300 sec⁻¹ for 10, 10, and 5 minutes, respectively. As control, a perfusion with nonfibrillar collagen type I was performed.

**Platelet Deposition on Fibrillar Collagen Type I after Addition of Antifibronectin F(αβ)₂ Fragment to Fibronectin-Depleted Plasma**

For fibrillar collagen (Figure 4), no dependence on fibronectin was observed in the range of wall shear rates tested (10 to 1800 s⁻¹). This could be due to a possible release of fibronectin from the α-granules. Therefore, we added antifibronectin F(αβ)₂ fragments (500 μg/ml) to fibronectin-depleted plasma. Compared to perfusions with normal plasma and fibronectin-depleted plasma, there was a small, but insignificant, decrease in platelet deposition (see Table 3).

**Role of Fibronectin on Platelet Deposition on Native Collagen Fibrils**

Purification of collagen by pepsin digestion results in removal of the telopeptides. Because this may have an influence on collagen organization and on fibronectin dependence, we also investigated native collagen fibrils which were purified in such a way that the telopeptides remained associated with the collagen. On both available collagen types, purified from human skin or bovine uterus, platelet deposition was independent of fibronectin at the examined wall shear rates (300 s⁻¹, 800 s⁻¹, and 1300 s⁻¹) (see Table 4).

**Discussion**

In the present study, we demonstrate that dependence of platelet deposition on fibronectin is determined by the nature of the collagen and the wall shear rate.

Collagen was purified from human umbilical arteries by pepsin digestion. Collagen types I and III were used because they promote adhesion in the perfusion system, whereas adhesion to types IV and V is negligible. Nonfibrillar collagen was prepared by dissolving lyophilized collagen I or III in 50 mM acetic acid. To minimize possible fibril formation, it was dissolved just before spraying. Collagen fibrils were prepared at 4°C by dialysis against 20 mM phosphate buffer (pH 9) to obtain relatively stable and well-ordered fibrils. These were separated from the remaining nonfibrillar collagen by centrifugation. The collagen concentration in the fibrillar suspension was determined by hydroxyproline analysis.

On nonfibrillar human umbilical collagen type I, platelet deposition was dependent on fibronectin at all wall shear rates tested (10 to 800 s⁻¹). On nonfibrillar type III, dependence on fibronectin was observed at wall shear rates above 300 s⁻¹, with a more pronounced effect at higher wall shear rates.

By using a mixture of nonfibrillar type I and III, platelet deposition after perfusion with fibronectin-depleted plasma was less than after perfusion with normal plasma. At wall shear rates of 20 s⁻¹, 100 s⁻¹, and 300 s⁻¹, platelet deposition was 42%, 45%, and 56%, respectively, lower after perfusion with fibronectin-depleted plasma than after perfusion with normal plasma (see Table 2). The control with nonfibrillar type I alone gave a decrease of 95% at 300 s⁻¹ (see Table 2). An intermediate situation was thus obtained by using a mixture of nonfibrillar type I and III, compared to nonfibrillar type I or III alone.

For fibrillar collagen types I and III, no dependence on fibronectin was observed in the range of wall shear rates tested (10 to 1800 s⁻¹). These results seemed to be in contradiction with a recent study in which fibronectin was necessary for optimal platelet deposition on fibrillar collagen type III. While we performed our studies under conditions where only primary platelet adhesion took place, Bastida et al. looked at thrombus formation by taking longer perfusion times. Their loss in adhesion after perfusion with fibronectin-depleted plasma was due to decrease in thrombus formation, whereas the primary adhesion was not influenced.

On a mixture of native collagen types I and III fibrils from human or bovine origin, the same results were obtained as with the reconstituted collagen fibrils. This is not surprising because most active fibronectin-binding sites are localized in the α-chains and not in the telopeptides.

We previously reported that nonfibrillar collagen is less reactive in promoting platelet adhesion under flow conditions than the fibrillar form and that type I is less reactive than type III. The requirement of platelet deposition for fibronectin is most pronounced in the less reactive collagen types.

A possible explanation may be that platelets are activated by fibrillar collagen and release fibronectin from the α-granules. To test this possibility, we added antifibronectin F(ab)₂ fragments to perfusates with fibronectin-depleted plasma and perfused coverslips coated with fibrillar collagen type I. There was no significant difference compared to perfusion without the addition of antibody. The possibility of release is not completely excluded; however, because binding of released fibronectin to the platelet membrane occurs with high affinity. Subsequent binding of the platelet through fibronectin to collagen may occur before fibronectin molecules on the platelet membrane are blocked by antifibronectin. For fibrinogen, for instance, it has been demonstrated that antifibrinogen Fab fragments which could block binding of ¹²⁵I-fibrinogen to platelets could not inhibit surface expression of platelet fibrinogen.

The question can be studied further by the use of platelets of patients with gray platelet syndrome which lack α-granules or with degranulated platelets. On the other hand,
our present results are in agreement with observations in a static system, where platelets are not exposed to shear stress. In that system, enhancement of platelet adhesion induced by fibronectin was highest to the collagen type with the lowest affinity for platelets.  

The conclusion from this study is that dependence on fibronectin for platelet deposition on collagen is not only influenced by the shear rate, but is also strongly associated with the collagen type.

Fibronectin was required for adhesion on the nonfibrillar form of collagen types I and III, but not on the fibrillar form. In vivo, collagen I and III are mainly available as fibrils, although in some cases transition to nonfibrillar collagen can occur. This would suggest that fibronectin plays a minor role in adhesion to the vessel wall. However, previous studies from our laboratory have demonstrated that fibronectin is required for adhesion to the subendothelium of human umbilical arteries and the matrix of cultured human vein endothelial cells. The discrepancy may be due to a changed conformation or a partial denaturation of collagen on a glass surface. Another likely explanation is that collagen present in the complex organization of the vessel wall or matrix behaves differently because it is associated with other components. There is an important observation pointing in this direction: denuded subendothelium has a relatively low thrombogenicity but this increases sharply when various proteins are removed by chymotrypsin treatment and collagen fibrils are exposed. Therefore, fibrillar collagen in the vessel wall may react as nonfibrillar collagen in an isolated form because it is shielded by other proteins and fibronectin may then be required as cofactor.

### Table 3. Platelet Deposition on Fibrillar Collagen Type I (20 μg/cm²)

<table>
<thead>
<tr>
<th>Perfusate</th>
<th>100 sec⁻¹</th>
<th>300 sec⁻¹</th>
<th>800 sec⁻¹</th>
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<tbody>
<tr>
<td>NP + buffer</td>
<td>48.2±3.8</td>
<td>51.2±2.7</td>
<td>58.4±7.3</td>
</tr>
<tr>
<td>- FN plasma + buffer</td>
<td>33.0±8.2</td>
<td>46.6±7.3</td>
<td>43.2±3.3</td>
</tr>
<tr>
<td>- FN plasma + antifibronectin</td>
<td>31.8±7.6</td>
<td>41.6±10.5</td>
<td>46.8±4.6</td>
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</tbody>
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Values are mean ± SEM. The number of determinations is given in parentheses.

Perfusates were performed with 111In-labelled platelets at wall shear rates of 100, 300, and 800 sec⁻¹ for 10, 5, and 5 minutes, respectively. Perfusates contained normal plasma (NP) with buffer (360 μl), fibronectin-depleted plasma (−FN plasma) with buffer (360 μl), or fibronectin-depleted plasma with antifibronectin F(ab')₂ fragments (360 μl, 500 μg/ml plasma).

### Table 4. Platelet Deposition on Native Collagen Fibris

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Perfusate</th>
<th>Platelet adhesion (% surface coverage)</th>
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<tbody>
<tr>
<td>Human skin</td>
<td>NP</td>
<td>15.4±1.1 (12)</td>
</tr>
<tr>
<td></td>
<td>- FN</td>
<td>13.8±1.1 (11)</td>
</tr>
<tr>
<td></td>
<td>- FN + fn</td>
<td>16.1±1.4 (12)</td>
</tr>
<tr>
<td>Bovine uterus</td>
<td>NP</td>
<td>16.2±1.8 (13)</td>
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<tr>
<td></td>
<td>- FN</td>
<td>13.6±1.3 (14)</td>
</tr>
<tr>
<td></td>
<td>- FN + fn</td>
<td>13.7±1.5 (12)</td>
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Values are expressed as means ± SEM. The numbers of determinations are given in parentheses.

Perfusates were performed for 5 minutes at wall shear rates of 100, 300, and 800 sec⁻¹ for 10, 5, and 1300 sec⁻¹ with normal plasma (NP), fibronectin-free plasma (−FN), or fibronectin-free plasma with addition of 300 μg purified fibronectin per milliliter plasma (−FN + fn).

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