Blood Platelets Are Concentrated near the Wall and Red Blood Cells, in the Center in Flowing Blood

Piet A.M.M. Aarts, Sjaak A.T. van den Broek, Gerrit W. Prins, Gerard D.C. Kuiken, Jan J. Sixma, and Robert M. Heethaar

Hematocrit and vessel wall shear rate are important factors in the transport and subsequent adherence of platelets to vessel wall subendothelium. When mass transport theory is applied to platelets in flowing blood, the blood is usually considered to be a fluid with platelet and red cell wall concentrations similar to the average tube concentration. With the laser-Doppler technique, we found how red blood cell ghosts and platelets were distributed radially for various hematocrits and wall shear rates. Red cell ghosts are crowded near the axis of the tube, with a local hematocrit higher than the average tube hematocrit, and they decrease steadily toward the wall. In the absence of ghosts, platelets exhibit the 'tubular pinch' effect (rigid particles crowding at 0.6×tube radius). In the presence of ghosts, the platelets are expelled toward the wall region. This high concentration at the wall increases with higher average tube hematocrit and wall shear rates. Increasing the average tube platelet concentration 10 times causes the wall concentration to increase only three times. The increase in platelet adherence observed with increasing hematocrit and increasing wall shear rate can be partially ascribed to increased platelet concentration near the wall. The observation that the increased platelet concentration does not fully explain the platelet adherence data suggests that platelet transport may also be enhanced by a shear rate-dependent rotary motion. (Arteriosclerosis 8:819–824, November/December 1988)

Adherence of blood platelets to the subendothelium of the damaged vessel wall during blood flow is a fundamental event in the formation of a hemostatic plug or thrombus. This process has been studied thoroughly in the last decade with the in vitro perfusion chamber developed by Baumgartner and Haudenschild. Before platelets can adhere, they have to be transported toward the vessel wall. This process is mediated by hemodynamic and rheological factors. Platelet adherence is strongly dependent on vessel wall shear rate and hematocrit. A hypothesis concerning these phenomena has been formulated by Keller. According to Keller, red blood cells that rotate in the shear-field of flow cause local stirring and enhance the platelet diffusion. On the other hand, it has been reported that red blood cells migrate toward the axis of the stream, leaving a red cell-poor plasma region near the vessel wall. By this mechanism, red blood cells can expell platelets from the core of the stream toward the red cell-poor layer near the vessel wall (platelet skimming). Until now, these phenomena have been studied only in capillary vessels or tubes under low shear conditions.

Our objective was to determine the local concentration of human red cells and platelets under high shear conditions (100 to 1000 s⁻¹) as found in small arteries and to find results meaningful for the study of platelet adherence and thrombus formation. The laser-Doppler technique, which was originally developed for measuring local flow velocity with high spatial resolution, is a suitable technique. Because in larger tubes (>100 μm internal diameter), intact red cells absorb too much light, they were replaced by red cell ghosts. A laser-Doppler velocity meter that operates by the “forward light scatter mode” determines the local velocity from Doppler signals that are scattered by the individual particles. This method, combined with the number of Doppler signals per unit of time, gives information about the local particle concentration. Fixed platelets were stained with OsO₄ to enhance their scattered signals, enabling us to differentiate them from the signals of the ghosts. With this design, the radial distribution of ghosts and platelets in a model glass tube of 3 mm internal diameter was studied.

Methods

A schematic representation of the laser-Doppler equipment and the signal processing for velocity and concentration measurements is presented in Figure 1. The laser light (Laser: Stabilito, model 120, Spectra Physics Incorporated, Mountain View, CA) is split by a rotating frame. These beams were made parallel by lens 1 (focusing distance (f)=200 mm). The beams were focused by lens 2 (f=50 mm). This created an interference section (fringe) of the laser beams which acted as a noninvasive measuring probe. After passing the fluid sample, the beams were made parallel again by lens 3 (f=80 mm). These parallel

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Received April 20, 1987; revision accepted May 13, 1988.
An appropriate height difference. The measuring section difference between two containers filled with perfusate at Santa Clara, CA). Relative local particle concentration was determined from the number of passing panicles and the local velocity ($V$); $N_{obs}/V$.

Steady flow was maintained by a gravitational pressure pump through a flow device similar to the perfusion system. Thus, only the light scattered by the flowing particles passed the diaphragm and was focused on the photomultiplier (home built) by lens 4 ($f=90$ mm). The typical scatter signal, the Doppler burst, is also indicated. Lenses 1, 3, and 4 were obtained from Spindler and Hoyer (Göttingen, FRG) and lens 2 was a television camera lens (Canon, type 103555). The Doppler signals were fed into the electronic equipment.

After the high pass filter (Krohnhite, 3203R, Cambridge MA), the signal was split and processed parallel through the frequency tracker (Type 1032, T.N.O., Delft, The Netherlands) to yield the Doppler frequency and the particle counting device. The particle counter was subsequently equipped with a combined amplifier (mBH × 100) and rectifier (Analag Devices, Cambridge, MA), a low pass filter (Krohnhite, 3303R), an amplifier (Krohnhite 3322 R), a discriminator (type cmp 01: PMI), and a counter (Hewlett-Packard, type 5304 A, Hewlett-Packard, Santa Clara, CA). Relative local particle concentration was calculated for the highest shear rate ($1260$ s$^{-1}$); for the lower shear rates ($760$ s$^{-1}$ and $240$ s$^{-1}$), the entrance lengths were shorter (2.4 cm and 0.76 cm, respectively). The measurements of velocity and concentration were fully developed at 1 cm from the entrance. This may be ascribed to the effect of the silastic tubing (30 cm long) that connected the upper container with the measuring section and the connection of this tubing with the glass tube, which introduced a small flow disturbance. Equation (1) is based on fully disturbed turbulent flow at the entrance of the cylindrical tube.

Preparation of ghost suspensions for laser-Doppler velocimetry has been previously described in detail.$^6$ Platelets were fixed in paraformaldehyde according to the method of Allain et al.$^{12}$ and were then stained with OsO$_4$. Perfusates were prepared by mixing packed ghosts and platelets suspended in $10$ mM Hepes-buffered saline (pH 7.4) to the desired hematocrit and platelet count. Experiments were performed at $37^\circ$C. The size of the fringe in radial direction for velocity measurements was determined by the disappearance of the signal when the fringe was moved into the wall and was approximately $50 \mu m$. For concentration measurements, however, the effective size of the fringe was smaller. The intensity distribution of a laser beam is Gaussian so that light intensity is largest in the center of the fringe. Particles passing through the center, therefore, generate Doppler bursts with higher amplitudes than do particles passing along the edges of the fringe. For this reason, even at low discriminator levels, only particles passing through the center of the fringe were determined. The minimum level of the discriminator was chosen so that the output signal of the discriminators is

$$Le=\frac{3.5Vd^2}{\nu}$$

where $Le$=entrance length (cm), $d$=internal diameter of the tube (cm), $V$=average velocity cm·s$^{-1}$, and $\nu$ is the kinematic viscosity (cSt). In our case the theoretically maximum entrance length (with $d=0.3$ cm, $\nu=0.38$ cSt, and $V=50$ cm s$^{-1}$) was approximately 4 cm. This value was calculated for the highest shear rate ($1260$ s$^{-1}$); for the lower shear rates ($760$ s$^{-1}$ and $240$ s$^{-1}$), the entrance lengths were shorter (2.4 cm and 0.76 cm, respectively).
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Figure 2. Radial concentration distributions in the absence of ghosts at wall shear rates of 1200 s\(^{-1}\) (\(\bullet\)), 760 s\(^{-1}\) (○) and 240 s\(^{-1}\) (△). Measurements were performed by traversing the tube with the fringe in steps of 50 \(\mu\)m, yielding separate measuring points. A. Native platelets. B. Fixed platelets.

Calibrator was not saturated and the counter responded linearly to concentration variations. Crossing the tube with the fringe yielded the local measurements. Concentration profiles of ghosts (without platelets) were obtained at low discriminator levels. Concentration profiles of the platelets were obtained by increasing the discriminator level until no ghost could be detected and by adding the stained platelets.

Calibration to absolute concentration was performed by numerical integration. The curves of the profiles were divided into 60 small segments. By application of Simpson's rule, the area of each segment was determined and summarized to yield the total area under the curve. For both types of concentration profiles (ghosts and platelets), the areas were interrelated to check whether they corresponded to the ratios of average tube concentrations. The average value of the segment areas (total area/number of segments) corresponded to the average tube concentration of the suspension. With this procedure, relative local concentration can be converted to absolute hematocrit or platelet concentration.

Results

The behavior of platelets and ghosts in flow was found to be in agreement with the behavior typical of rigid and deformable particles, respectively, as described by Goldsmith.\(^5\)

In the absence of ghosts, platelets demonstrated a distribution of rigid particles in flow, as shown in Figure 2A and 2B. Rigid particles migrated to a stationary position of 0.6 \(R\) (\(R=\)tube radius), and maximal particle concentration was found at this position; at the axis the concentration was lower, and near the wall it was zero. This behavior of rigid model particles, which is called the 'tubular pinch' effect, was originally described by Segré and Silberberg.\(^13\) The behavior of fixed platelets (Figure 2A) was comparable to the behavior of native platelets (Figure 2B) measured in platelet-rich plasma within 3 hours after blood collection. This result indicates that the
use of OsO₄-stained platelets gives a good approximation of the behavior of native platelets.

The concentration distribution of the ghost cells was typical for deformable particles. Their concentration was high at the axis of the tube, which caused a local hematocrit of about zero near the wall, while the hematocrit steadily increased toward the axis to values above the average tube hematocrit. Distribution profiles for ghost cells, at average tube hematocrits of 0.2, 0.4, and 0.6 are shown in Figure 3A, 3B, and 3C for three wall shear rates (240 s⁻¹, 760 s⁻¹, and 1260 s⁻¹). From these distributions, the following becomes clear: in regard to the local hematocrit at the axis, the elevation (about twice the average tube hematocrit) was more pronounced at the lower average tube hematocrit of 0.2; the elevation decreased for the higher average tube hematocrits of 0.4 (about factor 1.6) and 0.6 (1.35). The effect of the hematocrit increased slightly with increasing shear rates.

The distribution of the platelets changed drastically in the presence of ghosts. The effects of the hematocrit and the shear rate on platelet distribution are presented in Figure 4. The data indicate that the platelet concentration was higher near the wall and lower in the core. As the shear rate increased, the platelets were increasingly expelled from the core region (defined by 0<r<0.10 cm) toward the wall region (defined by 0.10 cm<r<0.15 cm), which further enhanced wall concentration and decreased core concentration. This effect also increased with increasing hematocrit. The completed data given in Table 1 show a platelet shift from the core region to the wall region with increasing shear rate and average tube hematocrit.

The effect of variation in platelet concentration is shown in Figure 4. The areas under the platelet concentration profiles were proportional to the average tube platelet concentration ratios. This indicated that the experimental setup responds linearly to variations in platelet concentration. The actual wall concentration of platelets, however, did not demonstrate a linear increase with average tube concentration. At an average tube concentration of 50 000 platelets/μl, the wall concentration was 450 000 platelets/μl (nine times greater), whereas at a average tube concentration of 500 000 platelets/μl, the wall concentration was elevated to 1 500 000 platelets/μl (three times greater).

Discussion

The considerable effect of the ghost cells on the distribution of platelets may be explained by the fact that the concentration maxima of the platelets at about 0.10 cm from the wall in ghost-free suspensions were shifted toward the wall by the introduction of ghost cells, which occupied the core of the tube. The increasing effect of higher average tube hematocrits may be seen in the fact that at a low hematocrit of 0.2, ghost cells were relatively more concentrated in the core than at a high hematocrit of 0.6. The effect of shear rate may be explained by the phenomenon that ghosts are more concentrated in the core at increasing shear rate, with a concomitant decrease of platelet concentration in the core and a further enhancement of platelet shift toward the wall region.

Nonhomogeneous distribution of red cells and platelets across blood vessels has often been discussed in the literature. Until now, however, a direct explanation concerning this phenomenon, especially for vessels larger than capillaries, has not been presented.

Table 1. Distribution of Platelets in Wall Region (0.10<r<0.15 cm) at Various Hematocrits and Shear Rates

<table>
<thead>
<tr>
<th>Shear rate (s⁻¹)</th>
<th>0</th>
<th>0.20</th>
<th>0.40</th>
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<tr>
<td>240</td>
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<td>59</td>
<td>72</td>
<td>71</td>
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<tr>
<td>760</td>
<td>34</td>
<td>66</td>
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<td>1260</td>
<td>38</td>
<td>73</td>
<td>85</td>
<td>90</td>
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Table 2. Platelet Adherence Calculations

<table>
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<th>Hematocrit (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>10</td>
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<tr>
<td>0.2</td>
<td>30</td>
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<tr>
<td>0.4</td>
<td>50</td>
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<td>0.6</td>
<td>80</td>
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Platelet adherence was calculated from a theory assuming that red cells did not induce enhanced platelet diffusion, but that this was caused by enhanced wall concentrations of platelets. These calculations were compared with experimental data (wall shear rate = 800 s⁻¹).

The formula used here was derived in previous study:²¹

\[ \text{Adh} = 0.83 \times 10^{-6} \times \text{Co} \times (0.15) \times L^{−1/3} \times \gamma^{3/2} \times 0.86 \times h^{0.6} \]

where \( \text{Adh} \) = platelet adherence (platelets/cm²), \( \text{Co} \) = platelet average tube concentration (platelets/cm³), \( t \) = exposure time (s), \( L \) = length of the vessel wall segment (cm), \( \gamma \) = vessel wall shear rate (s⁻¹), and \( h \) = hematocrit (½).

Figure 4. Platelets (-) in the presence of ghosts (—) at a hematocrit of 0.4 and a wall shear rate of 1260 s⁻¹ for various platelet average tube concentrations. 1) 50 000/μl, 2) 120 000/μl, 250 000/μl, and 4) 500 000/μl. The ratios (in arbitrary units) of concentration profile area and average tube platelet concentration were 633, 619, 636, and 614, respectively. For clarity, the concentration distributions are indicated as lines through the separate measuring points.

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platelet concentration near the vessel wall was previously reported by Tangelder et al. However, these studies were performed in small capillary tubes (<100 μm) at low shear rates (<20 s⁻¹) and did not clarify whether axial migration of the red cells and platelet skimming occurred simultaneously. Blasberg et al. found elevation of platelet concentrations near the tube wall in larger tubes and at wall shear rates comparable to those in this study, although studies on axial migration of red cells were not performed.

In this study, red cell ghosts were used instead of red blood cells because intact red blood cells absorb too much light. The question may arise as to how far the present results with ghosts can be extrapolated to the in vivo situation. The similar results obtained by Tangelder et al. in small vessels by direct observation suggest that this can be done. More firm support has been given by Goldsmith and Marlow’s extensive study which concluded that ghosts provided a good physical model for red blood cells and by previous results from our laboratory which indicated that red cell ghosts produced enhancement of platelet adhesion to collagen fibers identical to that produced by intact red blood cells.

The outcome of our study is important for platelet adherence studies with the Baumgartner chamber, where platelet adherence in flowing blood increases strongly with increasing hematocrits and shear rates. Until now, these phenomena were mainly ascribed to the rotational mixing concept of Keller. According to this theory, the red cell rotating in the shear field of flow enhances the diffusion of platelets, and the effect increases with increasing hematocrits and shear rates. Red cell size and deformability are also important in this concept, as confirmed by Wang and Keller’s study on electrolyte transport and by our previous studies on platelet transport. The results of the present study, however, indicate that the enhancement of platelet concentration near the wall, by the crowding of ghost cells at the center of the tube, may also be responsible for enhanced platelet adherence. An increased platelet concentration by itself, however, is not likely to be completely responsible for enhanced platelet adherence, since Brownian diffusion of platelets is very low.

Turitto used the convection diffusion theory to describe the dependence of platelet adherence. This theory, in which blood is considered as a homogenous fluid, describes the influence of parameters that are important for platelet adherence, such as shear rate, platelet concentration, and platelet diffusion. Application of this theory, using the increased platelet concentration near the wall instead of the average tube concentration and Brownian platelet diffusivity, indicates that enhanced platelet concentration near the wall is not sufficient to explain the effect of hematocrit and shear rate on platelet adherence (Table 2). A formula to calculate this was presented in a previous work. A shear rate-dependent red cell motion, which enhances platelet diffusivity, must also be present as a driving force to utilize the concentration gradient.

The effect of nonuniform blood cell distribution as an additional contribution on platelet deposition was also predicted on theoretical considerations by Eckstein who called this rheophoretic platelet dispersivity. Although the ghost concentration near the wall was low, it was not absolutely zero, and it was dependent on the bulk hematocrit. Diluted suspensions of ghosts in these regions may exhibit fully free rotational motion dependent on shear rate, which enhances platelet diffusion and which makes the platelet concentration gradient more effective. This explanation also counters the objections against the Keller concept that free red cell rotation is not likely to occur at hematocrits above 0.10 because of frequent collisions of the red cells.

We conclude that the effect of increased shear rates and hematocrits on increased platelet adherence to subendothelium can, to a large extent, be ascribed to the increase of platelet concentration near the vessel wall. The enhancement of platelet transport and subsequent adherence, however, must also be supported by a shear-dependent red cell motion, which enhances platelet diffusion to utilize the concentration gradient.

References


Index Terms: blood platelets • hematocrit • flow • platelet adherence.
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Arterioscler Thromb Vasc Biol. 1988;8:819-824
doi: 10.1161/01.ATV.8.6.819

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