Flow Effects on Coagulation and Thrombosis

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Abstract—Thrombosis occurs in a dynamic rheological field that constantly changes as the thrombus grows to occlusive dimensions. In the initiation of thrombosis, flow conditions near the vessel wall regulate how quickly reactive components are delivered to the injured site and how rapidly the reaction products are disseminated. Whereas the delivery and removal of soluble coagulation factors to the vessel is thought to occur via classic convection–diffusion phenomena, the movement of cells and platelets to the injured wall is strongly augmented by flow-dependent cell–cell collisions that enhance their ability to interact with the wall. In addition, increased shear conditions have been shown to activate platelets, alter the cellular localization of proteins such as tissue factor (TF) and TF pathway inhibitor, and regulate gene production. In the absence of high shearing forces, red cells, leukocytes, and platelets can form stable aggregates with each other or cells lining the vessel wall, which, in addition to altering the biochemical makeup of the aggregate or vessel wall, effectively increases the local blood viscosity. Thus, hemodynamic forces not only regulate the predilection of specific anatomic sites to thrombosis, but they strongly influence the biochemical makeup of thrombi and the reaction pathways involved in thrombus formation. (Arterioscler Thromb Vasc Biol. 2006;26:1729-1737.)

Thrombosis is thought to begin with an event such as plaque rupture, vessel damage, or dysfunctioning endothelium, resulting in the exposure of active tissue factor (TF) on the vessel wall. The exposed TF binds circulating factor VII/VIIa (fVII/fVIIa) with high-affinity (Kd < 10 pM), forming a reactive vessel surface that proteolytically cleaves fIX and fX. The rate of this surface-bound reaction depends not only on biochemical factors (number and surface density of TF:VIIa complexes, intrinsic kinetic activity, and local phospholipid composition) but on the rate at which the substrates fIX and fX are transported by the flowing medium to the reactive surface and the rate at which product is removed. Moreover, the local accumulation of reaction product may be critical in overpowering endogenous inhibitors and successfully initiating coagulation.

Although convective flow forces dominate the axial movement of blood components (coagulation factors, platelets, red cells, leukocytes, and inhibitors) throughout most of the body, in the few microns approaching the vessel wall where thrombosis is thought to initiate, the frictional drag of the vessel wall causes the axial flow velocity to slow to 0, and the convective and diffusive motion of blood components becomes comparable. In this initial coagulation reaction, blood flowing parallel to the wall convectively transports coagulation factors (fIX and fX) from upstream to a region close to the reactive site, and Brownian motion mediates the radial movement toward the reactive wall. In the case of platelets adhering to the wall, a similarly defined adhesion rate is often inferred. The bound proteins fIX and fX become proteolytically activated by TF:VIIa at a rate typically described using...
a Michaelis–Menten model. In cases in which the enzymatic reaction rate on the wall is slow compared with the rate at which molecules reach the wall, then the enzyme surface concentration and activity determine the overall reaction rate. However, if the flow-mediated transport of these substrates (fIX and fX) to the wall is slow compared with the enzymatic reaction rate at the wall, then the overall rate of product formation becomes a function of how quickly these substrates reach the wall.\textsuperscript{3} Results from model systems have shown that the convective–diffusive delivery of both fX to a wall containing TF:VIIa,\textsuperscript{3} and platelets to a subendothelial surface\textsuperscript{6} can be regulated strongly by local flow conditions.

Local flow conditions near the wall can significantly enhance or impede the transport of molecules to (and away from) a reactive surface and are typically characterized by the wall shear rate ($\gamma_s$), which is the rate at which the axial flow velocity increases as one moves directly away from the vessel wall toward the center of the vessel. It basically describes how rapidly substrates, agonists, or inhibitors are delivered within a short, radial distance of the wall and conversely, how rapidly a generated product will be carried downstream as it diffuses away from the wall. For a typical venous shear rate of 100/s, the axial flow velocity at a radial distance of 8 $\mu$m from the wall (the diameter of a red cell) can be approximated as 800 $\mu$m/s or 100 red cell diameters/s. Because the axial velocity profile across the vessel is roughly parabolic, the $\gamma_s$ approximates the flow velocities only near the wall. For fully developed, laminar flow through a rigid tube, the $\gamma_s$ can be calculated by knowing the volumetric flow rate ($Q$) and the vessel radius ($r$) as shown:

$$\gamma_s = 4 \cdot \frac{Q}{\pi \cdot r^2}.\,$$

Because the axial flow velocity at the vessel wall is 0, the diffusive motion of proteins and cells in this region becomes significant. Diffusion coefficients ($D$) characterize the mean square displacement a molecule, macromolecule, or cell that moves in a given amount of time because of Brownian motion, and its magnitude can easily be estimated based on the molecule size, shape, and the kinematic viscosity ($\mu$) of the medium using the Stokes–Einstein relationship.\textsuperscript{5} Proteins the size of fIX and fX typically have a $D$ on the order of $5 \times 10^{-7}$ cm$^2$/s, indicating it takes $\approx 1$ s to diffuse via a random walk, a short, 1-way distance ($\delta$) of 10 $\mu$m away from a wall.\textsuperscript{5}

$$t = \delta^2 / 2D, \text{ where } t=\text{time}.\,$$

However, because the time required for diffusion is related to the square of the distance, the time to diffuse a distance of 1 mm would be 2.8 hours. Thus, diffusion is only an effective means of transport over short distances and not an effective means of transport across distances such as the diameter of a coronary vessel. Although the example above focuses on fIX and fX, the same convective and diffusive forces govern the transport of other coagulation factors, inhibitors, pharmaceutical agents, and cells to and away from the vessel wall.

Under steady laminar flow conditions, the rate at which substrate molecules (or cells) reach the vessel wall ($J$) can be estimated based on the $\gamma_s$, the substrate (or cell) D, bulk substrate (or cell) concentration ($S_{\text{bulk}}$), and the length of the reactive tube ($L$).\textsuperscript{7} The number of collisions between the substrate (or cell) and wall increases with a one-third power dependence on the $\gamma_s$ and a two-thirds power dependence on the mass diffusivity. Thus, molecular fluxes to and from the vessel surface can easily vary by an order of magnitude under physiological conditions and more so in pathologic situations:

$$J = 0.81 \cdot S_{\text{bulk}} \cdot (\gamma_s \cdot D^3 / L)^{1/3}.\,$$

### Enzymes on Surfaces

Enzymatic coagulation reactions such as TF:VIIa and prothrombinase have frequently been described using Michaelis–Menten kinetics, in which the relative velocity of the reaction depends hyperbolically on the available substrate concentration. In the vessel wall, bound reactions where flow augments the delivery of substrate to the surface, the local available substrate concentrations can be much lower than that in the bulk medium. Ladner et al investigated the role of enzyme kinetics on surfaces under steady, laminar flow conditions to show that the relative “steady-state” velocity of product formation on a surface ($v/V_{\text{max}}$) can be expressed in classic Michaelis–Menten form as a hyperbolic function of the readily known bulk substrate concentration ($[S]_{\text{bulk}}$) and a flow-adjusted $K_M$ ($K_{M,\text{flow}}$), the bulk substrate concentration yielding half maximal reaction velocity:

$$v / V_{\text{max}} = [S]_{\text{local}} / K_{M,\text{flow}} + [S]_{\text{local}} / [S]_{\text{bulk}}.\,$$

The maximum reaction velocity ($V_{\text{max}}$), which is approached when substrate is in great excess, remains independent of flow-mediated substrate delivery to the surface. Transport resistances between the bulk flow and the wall are reflected by an increase in apparent $K_M$ ($K_{M,\text{flow}}$), the parameter relating to enzyme-substrate collisional efficiency, and these resistances are most pronounced in conditions with low $\gamma_s$ and high surface reactivity, that is, catalytic rate constant ($k_c'$), enzyme surface concentration ([E]), and length of the reactive patch ($L$). $K_{M,\text{flow}}$ represents the true Michaelis–Menten $K_M$:

$$K_{M,\text{flow}} = K_{M,\text{flow}}' + 0.62 \cdot k_c' \cdot [E] \cdot (L / D^2) \cdot (\gamma_s)^{1/3}.\,$$

These expressions indicate that catalytic efficiencies ($k_c' / K_{M,\text{flow}}$) of surface-bound enzyme vary by an order of magnitude over physiological shear conditions and also with the spatial distribution of enzyme on the surface. In cases in which the reaction product quickly adheres and functions on a nearby surface or high-affinity receptor, then dissemination of the product may be of little consequence, and the overall rate of product generation, described above, is a key determinant in triggering coagulation and thrombosis.

Because the coagulation system is balanced by numerous inhibitory and positive-feedback mechanisms, a single molecule of fXa generated on the vessel wall is likely insufficient to trigger thrombosis. Using numerical techniques, Beltrami and Jesty investigated the role of local flow conditions on the positive feedback loops and activation thresholds associated with zymogen activations in the early stages of coagulation\textsuperscript{8} and found that lower flow rates and larger patches of active membrane facilitate the local accumulation of product and the likelihood of an activation threshold being exceeded. Thus, although increased flow rates and consequently $\gamma_s$ may serve to enhance the overall rates of substrate activation, it
also dilutes the generated product, transporting it away from the reactive site. Geometries associated with prolonged fluid residence times near an injured vessel wall, such as recirculation zones distal to stenotic lesions or anastamotic junctions, would appear to favor the local accumulation of coagulation products, thus providing a potential explanation for the high levels of fibrin accretion observed in such zones.

**Physiologic and Pathophysiologic Flow Conditions**

Movement of blood throughout the vasculature is pressure driven across a branched network of vessels of varying contours, diameters, and lengths, some with varying mechanical properties and others with partial obstructions. Arterial flow is pulsatile with flows for coronary-sized arteries ranging from 0, or even reversed flow, to more than twice the time-averaged flow velocity during each cyclic period; the amplitude of pulsatility decreases with distance from the heart. Waveforms vary somewhat among arteries and may be strongly affected by vessel obstructions and large downstream resistances. In general, time-averaged mean $\gamma_w$ s are used to characterize local flow conditions in different vessels because this parameter relates to both mass transport features and local fluid shear forces. Typical time average values for healthy vessels range from 300/s in the femoral artery, 1500/s in small arteries (300 $\mu$m diameter), and may easily reach 2800/s as blood approaches the capillaries (Table).

Flow throughout most of the vasculature is laminar, which can be thought of as adjacent fluid layers or laminae sliding smoothly over one another. In laminar flow, the viscous forces damp out relative momentum differences between adjacent fluid layers, preventing disturbances between the layers and creating a smooth velocity profile across the vessel, approximating a parabola (Figure). Blood flowing over a stationary vessel surface exerts a frictional drag force per unit area along the wall referred to as the wall shear stress ($\tau_w$; units of dyne/cm$^2$), which is related to the $\gamma_w$ through the fluid viscosity ($\mu$):

$$\tau_w = \gamma_w \cdot \mu.$$

For most physiological shear rate conditions (> 50 to 100/s), the viscosity of whole blood is $\sim$0.035 P (g/cm-s). Hence, physiological wall shear stress values typically range from 1 to 10 dyne/cm$^2$ for venous and 10 to 50 dyne/cm$^2$ for arterial flow. Erythrocyte concentration constitutes the primary determinant of blood viscosity because the complex deformation and movement of these densely packed, viscoelastic bodies serve to dissipate fluid momentum. Plasma viscosity (0.011 P) serves as a minor determinant and has been correlated with fibrinogen and von Willebrand factor (vWF) content; for comparison, the viscosity of water at 37°C is 0.007 P.

Because blood viscosity is a rheological parameter that depends on complex interactions and deformations of suspended cells, physical effects that alter the local hematocrit vary somewhat among arteries and may be strongly affected by vessel obstructions and large downstream resistances. In general, time-averaged mean $\gamma_w$ s are used to characterize local flow conditions in different vessels because this parameter relates to both mass transport features and local fluid shear forces. Typical time average values for healthy vessels range from 300/s in the femoral artery, 1500/s in small arteries (300 $\mu$m diameter), and may easily reach 2800/s as blood approaches the capillaries (Table).

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**Characteristic Values of Flow Parameters Within the Human Vasculature**

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Diameter (mm)</th>
<th>$\gamma_w$ (s)</th>
<th>$\tau_w$ (dyne/cm$^2$)</th>
<th>Re</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascending aorta</td>
<td>23–45</td>
<td>50–300</td>
<td>2–10 (+45/–2)</td>
<td>800–1600</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>5.0</td>
<td>300</td>
<td>11</td>
<td>280</td>
</tr>
<tr>
<td>Common carotid</td>
<td>5.9</td>
<td>250</td>
<td>8.9</td>
<td>330</td>
</tr>
<tr>
<td>Internal carotid</td>
<td>6.1</td>
<td>220</td>
<td>8</td>
<td>220</td>
</tr>
<tr>
<td>Left main coronary</td>
<td>4.0</td>
<td>460</td>
<td>16</td>
<td>240</td>
</tr>
<tr>
<td>Right coronary</td>
<td>3.4</td>
<td>440</td>
<td>15</td>
<td>150</td>
</tr>
<tr>
<td>Small arteries</td>
<td>0.3</td>
<td>1500</td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td>Arterioles</td>
<td>0.03</td>
<td>1900</td>
<td>60</td>
<td>0.04</td>
</tr>
<tr>
<td>Large veins</td>
<td>5–10</td>
<td>200</td>
<td>7</td>
<td>200–600</td>
</tr>
<tr>
<td>Inferior vena cava</td>
<td>20</td>
<td>40–60</td>
<td>1.5–2.1</td>
<td>300–500</td>
</tr>
</tbody>
</table>

These are approximate, time-averaged values compiled in part from material by Goldsmith and Wootton. Re describes the ratio of inertial to viscous forces in the fluid.

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Flow and transport in a vessel. Top, Blood with a parabolic velocity profile accelerates as it passes through a narrowing in the channel, resulting in much higher $\gamma_w$ values near the apex of the stenosis. After the stenosis, fluid layers may separate from one another as it decelerates, forming stagnation zones and areas of recirculation. Bottom, Whereas convective forces transport cells, coagulation factors and inhibitors from an upstream source, diffusive forces mediate the radial motion to and from the wall. The scaling of the velocity profile shown represents a very low $\gamma_w$. Note the dimensions of the individual components of the thrombus are trivial compared with the overall dimension of a typical coronary vessel.
and radial distribution of cells across a vessel affect blood viscosity in a non-Newtonian manner. In 1931, Fahraeus and Lindquist described a decrease in the apparent viscosity of whole blood when flowed through capillaries of decreasing diameter (<0.8 mm);12 this effect is most acute as the reduction from 100 to 10 μm. Several physical factors are thought responsible for this effect on apparent viscosity. Erythrocytes flowing through small vessels have been shown to concentrate toward the high-velocity region near the center of the vessel, leaving a thin, slower-moving, cell-poor layer near the vessel wall. The high-velocity movement of erythrocytes through small vessels relative to that of plasma is associated with depressed local hematocrit values in such vessels (fahraeus effect), thereby partially explaining the local decrease in blood viscosity. In addition, the radial distribution of erythrocytes leaves a thin plasma layer occupying the high fluid shear region near the vessel wall, thereby reducing viscous resistance at the blood–wall interface.

A second non-Newtonian feature relates to the thixotropic increase in blood viscosity as the shear rate decreases <50 to 100/s. At very low shear conditions, such as those associated with stagnant zones, complex flow regimes, postcapillary venules, and transient flow stoppages, the fluid shearing forces of blood are not sufficient to overwhelm the forces associated with cell–cell interactions. Stable interactions between leukocytes, erythrocytes, and platelets can be observed in these low shear environments and are thought to increase the effective blood viscosity by as much as an order of magnitude,13,14 resulting in increased flow resistance. Computational models of complex arterial flow patterns based on non-Newtonian approximations of blood viscosity have shown relatively minor effects on the dominant arterial flow patterns.15–17 However, non-Newtonian considerations do impact the size and fluid residence time of low shear regions.18

Pathologic Flow Conditions

In vessels containing atherosclerotic lesions, prosthetic devices, or anastomotic graft junctures, irregular vessel geometries may result in the development of complex flow patterns that are thought to favor thrombosis. Of particular interest in atherosclerosis are stenotic regions of a vessel, typically on the order of 1 cm in length, where there is a large reduction in the luminal flow area. Clinically described by the percent reduction in vessel diameter,9 a 75% stenosis results in a 94% decrease in the luminal cross-sectional area and an ≈64-fold increase in wall shear conditions, assuming the flow remains laminar. Pathological γs can exceed 10 000/s,19,20 and some have suggested they may reach 100 000/s for severe stenosis.9 Computational simulations of stenotic flow have indicated that the γs peak sharply just upstream of the throat of the stenosis,5 with sustained high shear conditions within the stenosis. Depending on the vessel geometry and fluid acceleration within the stenosis, the increased fluid momentum may create a jetting effect as the fluid exits the stenosis into an expansion, whereby the streamlines do not follow the contour of the vessel (flow separation). This may simply result in low shear regions near the vessel wall and away from the jet or more complex 3D flow regimes with recirculation vortices that substantially increase the residence time of fluid components (Figure).6 The ratio of fluid momentum forces to viscous forces, or Reynolds number (Re), is typically used to characterize the extent to which fluid inertia affects flow (some typical values are shown in the Table). Young et al21 have shown that for a 70% stenosis, clearly discernable shear layers can develop distal to the stenosis if the upstream Re, proximal to the stenosis, exceeds 10. When the Re upstream of a 70% stenosis exceeds 300, fluid momentum forces within the stenosis may overwhelm the viscous dampening forces, resulting in a transition to turbulent flow,5 which is often sustained for 1 to 6 diameters downstream.22 The transition to turbulence occurs when the actual Re within the stenosis (not upstream Re) exceeds an approximate value of 2300, resulting in an unorganized, chaotic flow regime that is thought to influence cell behavior and favor thrombosis. Although turbulent flow conditions may manifest at sites of stenosis and prosthetic heart valve replacements, flow throughout most of the vasculature is laminar.

Platelets and High Shear Environments

Platelets play a multifaceted role in thrombosis in their ability to adhere to injured surfaces (adhesion), aggregate with other platelets (aggregation), adhere to vessel wall-bound platelets (cohesion), and support thrombin generation on their surface. In 1975, Brown used a cone and plate viscometer to demonstrate that exposing platelet-rich plasma to fluid shear stresses >50 dyne/cm² resulted in changes in platelet morphology, along with secretion (ADP, ATP, serotonin) and aggregation.23 Higher levels of shear stress were thought to induce platelet lysis (100 dyne/cm²) and fragmentation (>250 dyne/cm²). Shortly thereafter, Weiss et al, who were studying platelet interactions with the vessel wall, discovered that bleeding complications associated with von Willebrand disease (deficient in vWF) and Bernard–Soulier syndrome (platelets deficient in platelet glycoprotein Ib [GP Ib])24 were related specifically to the inability of platelets to adhere to endothelium at elevated shear conditions (>50 dyne/cm²), in these patients, platelet adhesion at shear stresses <50 dyne/cm² appeared normal. Subsequent investigations demonstrated that the shear-induced aggregation was specifically mediated by interactions of vWF with GP Ib/IX/IV and GP IIb/IIIa25,26 and that these interactions were essential for both platelet aggregation and adhesion under high shear conditions.24,25,28,33

It is now thought that under high shear conditions, multi-meric vWF transiently bridges the platelet GP Ib/IX/V receptor with either other platelet GP Ib/IX/V receptors or vessel wall constituents (collagen I, III, V), thereby tethering the platelet to the vessel surface.29 Through a mechanism that is not yet understood, the engagement of vWF with GP Ib/IX/V specifically under high shear (>60 to 80 dyne/cm²) initiates platelet signaling mechanisms as evidenced by an influx of calcium ions, the secretion of granule contents including ADP,30,31 and the formation of active IIb-IIIa complexes.32,33 Interestingly, this signaling pathway is somewhat different from chemically induced activation pathways,34,35 which may explain why aspirin has little effect on shear-induced platelet activation.36 The shear-mediated re-
lease of ADP potentiates the shear-induced platelet response, but blocking of ADP released from stimulated platelets has been shown to inhibit shear-induced platelet aggregation without affecting the intracellular calcium increase. Platelet–platelet aggregation then proceeds via vWF-mediated bridging of active IIb-IIIa complexes on adjacent platelets.

Early studies of shear-induced platelet activation in rotational viscometers focused on relative long exposure times to elevated shear stresses (>50 to 100 dyne/cm²). However, shorter exposure times (30 s) to more intense shear conditions could also elicit platelet activation. Experiments by others using flow through small diameter tubing, or capillary viscometers, found that platelets became activated when exposed to much higher shearing forces (300 to 1000 dyne/cm²) but for only very brief durations (0.02 to 2 s). Hellemans later consolidated the findings from several groups using different flow devices to show that shear-induced platelet activation, as measured by serotonin release, was a function of both the shear stress magnitude and exposure time. Boreddu later derived a platelet stimulation function such that platelet stimulation function = τ × e^(-κt), where the activation state was linearly dependent on the magnitude of shear stress exposure (τ) and somewhat less dependent on duration (t). Exposing platelets to increased levels of shear stress in small diameter tubing has also been shown to elicit procoagulant activity on the platelet surface, thereby linking shear-mediated platelet responses to coagulation reactions. Thus, shear-induced platelet secretion and expression of procoagulant activity depend on the shear stress history of the platelet. Using these relationships, Einav and Bluestein used computational techniques to investigate the trajectories and shear histories of platelets as they pass through an 84% eccentric stenosis and poststenotic vortex and found that most of the platelets would experience shear stress-time histories that exceed the threshold for activation.

Shear stress–mediated activation of platelets depends on a complex interaction of physical shear stress, receptor-ligand interactions, and chemically modulated positive feedback signaling (ie, ADP). For example, using an ex vivo parallel plate, stenosis model Holmes observed significant platelet activation when the wall shear stress was 315 dyne/cm²; however, much more platelet activation was observed in the same model when a collagen surface was present. Thus, in cases in which the shear stress history does not exceed the theoretical activation threshold, it likely enhances the activation state of the platelet, making it more sensitive to other agonists such as ADP, thrombin, and collagen.

Red Cells and Platelets

Erythrocytes constitute nearly half the blood volume and account for most of the viscous and elastic properties of the fluid. Their unusual biconcave shape allows them to readily deform without significant strains to membrane surface area or changes in the cytoplasmic volume as the concentrated suspension of cells roll over one another. The parabolic velocity profile of flowing blood, along with the unusual red cell geometry and dense concentration, cause erythrocytes to move over one another with a shear dependent rotational motion that likely serves as micro mixers within the blood, radially displacing proteins and small cells considerable distances compared with those expected from Brownian motion alone. As early as 1910, Duke reported that red cell deficiencies were associated with prolonged bleeding times. Later studies of platelet adhesion to denuded endothelium found red cells enhanced the delivery of platelets to the vessel wall. Whereas classical convection–diffusion theory indicates the flux of platelets to the vessel wall should depend on the shear rate to the one-third power, studies by Turitto demonstrated that in the presence of red cells (40% hematocrit), the flux of platelets to the vessel wall depended much more strongly on the γ_s (two-thirds power). This and similar studies indicated that collisions with flowing red cells serve to enhance the molecular motion of platelets by 2 to 3 orders of magnitude over that from Brownian motion alone, thereby enhancing the ability of platelets to interact with the vessel wall.

In addition to the shear-mediated enhancement on platelet dispersion, Tangelger used intravital fluorescence microscopy techniques to show that in mesenteric arterioles (21 to 35 μm diameter), platelets concentrations near the vessel wall were 2 to 3× higher than the center of the vessel. Eckstein later used platelet-sized latex beads to demonstrate that in small channels (50 to 200 μm diameter), flowing erythrocytes enhance the near wall concentrations of platelet-sized particles by >3× that of the tube center. This enhancement depended on red cell deformability, shear rate (>430/s), and fluid viscosity. Interestingly, the peak platelet concentration was not at the wall, but rather a short 5- to 10-μm distance away from the vessel wall, an effect attributed to hydrodynamic lift or repulsion forces between the platelets and the wall. Aarts investigated the radial concentration profiles of platelets and ghosted red cells in much larger tubes (3 mm) using laser Doppler techniques and showed that at 50 cm downstream of the tube entrance, the near-wall concentrations of platelets were much greater than in the tube center. Platelet margination near the wall increased dramatically with both hematocrit and shear rate, whereas red cells showed the opposite effect, concentrating toward the center of the tube. Recently, Xu and Wootton performed similar studies using a specially constructed 3-mm diameter tube with a sample port built onto the tube wall to extract small fluid volumes from the near wall region. Coupled with computational simulations, they found that under steady flow conditions, the near-wall concentration of platelets at 50 cm downstream of the tube entrance was 1.6 to 2.0× that of the tube inlet concentration. Under pulsatile flow conditions, they found margination occurred more readily with a 2.2 to 2.4 margination ratio at a distance of only 10 cm downstream of the tube entrance. Thus, under most arterial flow conditions, platelets appear to marginate toward the vessel wall in a shear- and hematocrit-dependent manner, enhancing the near-wall platelet concentration by a factor of 2 to 3.

Erythrocytes also appear to play a role in platelet activation under high shear stress conditions via their release or leakage of the platelet agonist ADP. Both erythrocytes and platelets contain internal stores of ADP that on release serve to enhance the activation state of platelets, and this release
may partially explain enhanced thrombus formation observed at high shear rates and high hematocrits. Clinically, patients with a delta storage pool deficiency that leaves their platelets deficient in dense granules, a chief source of platelet ADP, experience a platelet adhesion defect at high shear rates that can be corrected by the addition of red blood cells. Hence, through a variety of shear-dependent mechanisms, red cells are able to both chemically enhance the activation of platelets and physically increase the rate at which they arrive at the vessel wall. Together, these phenomena are thought to explain the mechanisms relating to enhanced platelet adhesion under high shear conditions.

Cell–Cell Interactions Under Low Shear Conditions

At very low shear conditions, such as those associated with postcapillary venules, various plasma proteins, in particular fibrinogen, facilitate the formation of cell–cell bridges, which augment the motion and velocity of these cells. One of the most well-known examples is rouleaux formation, whereby erythrocytes aggregate similar to a 2D stacking of coins. However, in clinical conditions, erythrocytes may form more complex 3D clumps, which are difficult to disperse and effectively increase the local blood viscosity. In severe cases, the increased vascular resistance attributable to aggregation can result in decreased blood flow and consequently lower shear rates, thus potentiating further aggregate formation and inducing a viscous cycle of blood sludging. As discussed previously, the apparent viscosity of blood increases in a non-Newtonian manner as the shear rate decreases <100/s, thereby increasing vascular resistance; in fact, some studies have indicated that ≥50% of postcapillary vascular resistance is attributable to erythrocyte aggregation. Factors that influence these reversible interaction between erythrocytes include red cell concentration, the concentration and bridging force of the linking macromolecule, fluid shear forces, and such erythrocyte properties as deformability, surface charge, and geometry. Clinically, red cell aggregation is gauged by erythrocyte sedimentation, in which higher levels of fibrinogen or globulins are typically associated with more rapid sedimentation. However, new in vitro techniques are emerging that assay the shear rate at which aggregates dissociate. Enhanced red cell aggregation has been associated with multiple risk factors for cardiovascular disease, including diabetes, obesity, and hypertension.

In addition to red cell–red cell interactions, low shear conditions also favor a number of other cell–cell interactions involving platelets, leukocytes, and red cells. It is well known that red cells associate with thrombi that form under low shear conditions (red thrombi), yet the precise mechanism by which they associate has not been well understood. Goel and Diamond demonstrated that at shear rates <100/s, specific receptor–ligand interactions mediate the association of red cells (intercellular adhesion molecule-4) with platelets (CD36, GPIb) and neutrophils (CD11b) via tethers extending from the red cell.

Erythrocyte interactions have also been shown to increase the margination of leukocytes in postcapillary venules, thus favoring collisions between leukocytes and the vessel wall. Leukocyte adhesion to the wall during inflammation as well as leukocyte–red cell interactions under such conditions can serve to further increase flow resistance. Low shear conditions that favor these types of cell–cell interactions may occur in coincidence with erythrocyte aggregation in the postcapillary venules and complex flow regimes or in transient stasis conditions such as those associated with airplane economy class syndrome joint replacement surgery.

Cells Lining the Vessel Wall

Healthy endothelium exposed to normal physiological shear stresses serves as a nonthrombogenic, atheroprotective surface that actively responds to local physical forces to regulate functions such as vasomotor tone and long-term vessel remodeling. Under healthy arterial wall shear conditions, endothelial cells align and elongate in the direction of flow and stimulate increased levels of cyclooxygenase-2 (and prostaglandin E2), NO, and antioxidants. However, very low arterial wall shear stresses (<4 dyne/cm²) and in particular oscillatory or reversed shear stress regions, such as those incurred at bifurcations or distal to stenotic lesions, are associated with sustained expression of nuclear factor κB–regulated genes leading to an inflammatory state with leukocyte recruitment and extravasation and the development of atherosclerotic lesions. High throughput studies using gene chip arrays have facilitated the identification of endothelial gene clusters differentially regulated by well-defined flow conditions. The mechanism(s) by which endothelial cells transduce mechanical stimuli remain a focus of intense study and have been reviewed by Li.

Although healthy vascular endothelial cells do not possess TF, they can generate active TF in response to agonists typically associated with inflammation (interleukin-1). Grabowski found the application of steady fluid shear stress (0 to 13 dyne/cm²) to interleukin-1–treated endothelial cells triggered a 5-fold increase in the appearance of TF antigen on the cell surface. However, the actual TF activity of these cells was suppressed by concomitant secretion of TF pathway inhibitor. In similar studies using fibroblasts, exposure to shear (0 to 2.7 dyne/cm²) resulted in a 200-fold increase in the level of fX activation by the cells, a much larger enhancement than would be expected by the flow-enhanced delivery of substrate to the cell surface. Hence, shear stress dynamically augments the presentation of TF on the surface of vascular cells, and the resulting procoagulant activity may be regulated by endogenous TF pathway inhibitor.

Temporal and Spatial Considerations in Thrombus Growth

During thrombus formation, multiple components of the blood and vessel wall come into contact with each other and interact to form occlusive thrombi that can grow to span a radial distance on the order of 1500 platelet diameters. Moreover, this process can occur very rapidly because human coronary thrombi have been observed to grow to occlusive sizes within minutes. As the initial few layers of platelets and thrombus material accrete on the vessel surface, the injured vessel wall and the TF:VIIa associated with it quickly become physically isolated from the reactions occurring at
the front of the growing thrombus.\textsuperscript{2,80} Because thrombin generation is essential for thrombus stabilization,\textsuperscript{41} the source of fXa and fX generation that drive thrombin generation and thrombus propagation beyond the few tens of microns from the vessel wall remains unclear.\textsuperscript{80}

In 2000, Giesen et al reported that TF, from an as yet unknown source, is localized to growing platelet thrombi,\textsuperscript{82} and since then, other studies have confirmed these findings\textsuperscript{83,84} and shown that TF in thrombus is detectable within the first few minutes of formation.\textsuperscript{85} The incorporation of TF into platelets and thrombi has been shown to depend on specific receptor-ligand interactions, including CD15, P-selectin, and P-selectin glycoprotein ligand-1.\textsuperscript{86,87} Moreover, 2 separate in vivo models have confirmed that circulating TF plays a functional role in promoting thrombus growth.\textsuperscript{88,89} It has been suggested and demonstrated that the incorporation of TF into thrombi is shear dependent,\textsuperscript{86,90,91} and most studies supporting a functional role for circulating TF have used models involving flow.\textsuperscript{82,85,86,89,90} Although a functional role for circulating TF in vivo models have confirmed that circulating TF plays a functional role in promoting thrombus growth,\textsuperscript{88,89} it has been suggested and demonstrated that the incorporation of TF into thrombi is shear dependent,\textsuperscript{86,90,91} and most studies supporting a functional role for circulating TF have used models involving flow.\textsuperscript{82,85,86,89,90} Although a functional role for circulating TF, which becomes active on platelet activation, has been strongly supported and garnered mainstream acceptance over recent years, some studies have argued against a functional role for circulating TF.\textsuperscript{92,93} However, the merits and scientific rationale used by these dissenting studies are suspect.\textsuperscript{94–96} The source of circulating TF is thought to be P-selectin glycoprotein ligand-1–containing microparticles, which bind to P-selectin on the activated platelet surface,\textsuperscript{87} thus providing an explanation for the flow dependence of TF incorporation. Flow effects on such microparticles have not been investigated extensively, and it is unknown whether red cell–drift forces enhance their near-wall concentration. Using porcine models of thrombus deposition onto excised vessel segments, Karnicki et al found that thrombus deposition varied strongly with individual blood donors and relatively little with the source of the aortic segment.\textsuperscript{97} Together, these studies suggest that in contrast to thrombus initiation, the primary regulators of thrombus growth may come from the blood and not the vessel wall, and that these regulators may vary significantly among individuals.

The ability of flow to convectively deliver TF, the primary initiator of coagulation, to the surface of a growing thrombus, where its activity becomes manifest, dramatically alters previous notions and paradigms regarding how thrombi assemble both temporally and spatially. Identifying which components of thrombus formation are convectively delivered from blood-borne sources and which come from the vessel wall is a fundamental step in developing a comprehensive description of how thrombi form under physiological flow conditions. Also, many parameters such as those describing biophysical interactions of coagulation proteins with lipid surfaces differ in the literature by several orders of magnitude.\textsuperscript{4} Currently, there are no comprehensive “flow” models of thrombosis that adequately and fully describe all of the following: (1) coagulation reactions as “surface”–dependent reactions, (2) the temporal and spatial transport of coagulation products (eg, fXa and fXa) from their site of activation to the site of their intended function, and (3) the propagation of thrombi from a few microns in thickness to occlusive diameters. Investigating how these processes come together in time and space in a dynamic flowing environment remains a continuing challenge in thrombosis research.

**Disclosures**

None.

**References**


Flow Effects on Coagulation and Thrombosis

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