

Scientific and Standardization Committee Communication

Definitions in Biorheology: Cell Aggregation and Cell Adhesion in Flow

Recommendation of the Scientific Subcommittee on Biorheology of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis

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Use of Rheological Devices in Clinical Testing

The biology of blood cell adhesion and aggregation manifests itself under flow conditions. Functional information about a patient's blood can be assessed by biorheological testing. Fluid flow dictates the cell-cell collision frequency, collision duration, and hydrodynamic forces during collision, while the probabilities of adhesion and disaggregation are strongly influenced by the abundance and function of surface molecules (or captured ligands) presented on platelets and other blood cells. Similarly, during cell deposition on a surface, flow dictates the rate of cell arrival at the surface and the probability of adhesion or detachment. The choice of flow devices and defined chemistries (surface density of active molecules) on flat surfaces exposed to flow or on particles in suspension are critical for the evaluation of: specific bonding dynamics; induction of intracellular signaling; and pharmacological antagonism of adhesion. In particular, clinical assays that evaluate blood performance under flow are finding increasing use. However, testing at several shear rates is recommended for full functional phenotyping of blood.

For clear interpretation of an assay involving flow, the following recommendations are given:

- 1) Flow conditions must be clearly defined in standard geometries (circular or rectangular capillary; cone-and-plate, concentric cylinder) and the relevant shear rates or shear stresses calculated for the device. Aggregometry is not considered a standardized technique with respect to the flow dependency of aggregate formation since both very high (near the stir bar) and very low shear rates ($< 100 \text{ s}^{-1}$) elsewhere are present in the assay.
- 2) Whole blood assays under flow are subject to red cell influences, some of which are physiological (increased platelet flux to the wall; rouleaux formation at shear rates below 100 s^{-1} ; hematocrit-dependent viscosity) while other effects may be artifact-prone (ADP release).
- 3) Bulk aggregation assays and deposition assays can both be used to test the performance of cell-cell adhesion interactions under a defined regimen of hydrodynamic loading.
- 4) Shear rates above 6000 s^{-1} (shear stresses of ~ 60 to 200 dyne/cm^2) are pathological (severe stenosis; artificial valves) and cause shear induced platelet activation (SIPA). Blood performance at venous shear stresses (~ 1 to 5 dyne/cm^2) and arterial shear stresses ($\sim 20 \text{ dyne/cm}^2$ on average) is physiologically relevant. Turbulence is extremely rare in the human circulation, possibly present in arteriovenous fistulas. Time dependent, complex secondary flows (vortexes, recirculation zones) are not turbulent.
- 5) Potential influences in assay design include the possibility of secondary flows and unintended biomaterial interactions. In preparing isolated cells, unintended activation is best detected by P-selectin display on platelets and β_2 -integrin upregulation or L-selectin shedding by neutrophils. Even in buffer, washed platelets after activation can display significant amounts of adhesive ligands such as fibrinogen, vWF, fibronectin, and thrombospondin.
- 6) Purified receptors will continue to play an important role in evaluating cell aggregation and adhesion under flow conditions. However, recombinantly expressed receptors require validation to assure that performance under fluid mechanical assay is similar to that of endogenous receptors in platelets or neutrophils. A comparison of the collision efficiency in bulk aggregation assays or rolling velocity or pause time distribution in surface adhesion assays is useful to validate recombinant receptors and ligands against wildtype.
- 7) Molecular mechanisms of bond formation and bond rupture should account for the flow sensitivity of various bonding pairs and delineation of receptors responsible for mediating transient capture or rolling as opposed to those mediating firm arrest.
- 8) Above a shear rate of 100 s^{-1} and in geometries with length scales exceeding 50 microns, blood behaves essentially as a Newtonian fluid. Gelation of blood or its components can yield a material whose dynamic biorheology is a sensitive indicator of fibrin polymerization and fibrin structure.

In general, rapid on-rates and off-rates of molecules such as P-selectin, GPIIb/IIIa, vWF, and thrombospondin on platelets, and L-selectin and PSGL on neutrophils, mediate initial cell-cell aggregation or surface capture under high flow, but may be subject to force-induced bond breakage. In contrast, integrin bonding (β_2 and β_3 integrins) is slower but leads to firmer adhesion and stable aggregates. Cell-based assays under flow can provide a precise understanding of receptor function in a given hemodynamic context. At present, it is unclear if a single mathematical kinetic expression for bond formation and bond rupture under loading is generally assay-independent or cell type independent although such flow assays are currently sensitive to detect changes in adhesion due to mutation.

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