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VWF self-association requires tensile force

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In this issue of *Blood*, Fu et al[1] measure von Willebrand factor (VWF) self-association with single-molecule fluorescence microscopy. Real-time, high-resolution imaging of VWF concatemers demonstrate that self-association requires high tensile force for binding between tethered and free VWF concatemers.

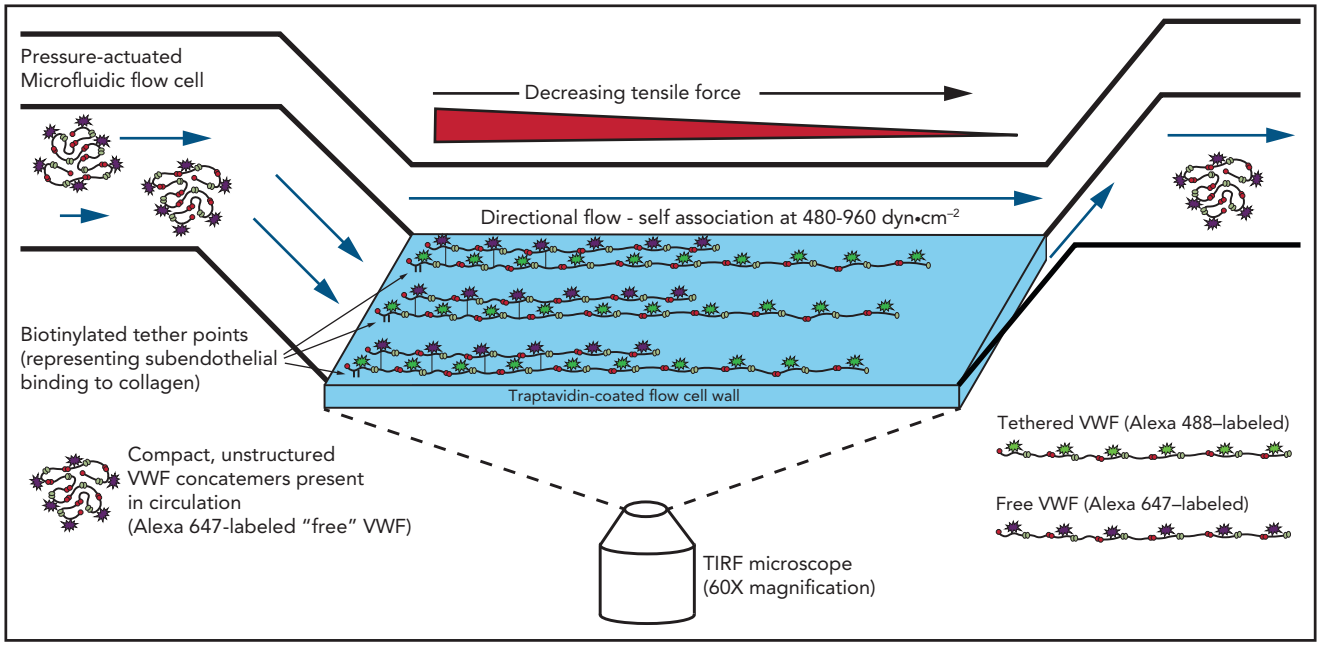
At sites of vascular rupture or scission, blood flow characteristics change dramatically to rapidly address the damage and repair it through platelet adhesion and activation in concert with the blood coagulation cascade, to form the insoluble fibrin mesh. At the center of these processes is VWF, a large plasma glycoprotein that promotes platelet adhesion and aggregation and serves as the circulatory partner for blood coagulation factor VIII.[2]

The monomeric structure of VWF contains a sequence of domains denoted D1-D2-D′-D3-A1-A2-A3-D4-(C1-C6)-CK.[3] This species of VWF dimerizes through disulfide bonds between CK domains. After cleavage of the propeptide between D2 and D′, the VWF dimer then forms head-to-head disulfides between D3 domains, resulting in large concatemers with successive head-to-head and tail-to-tail connections. Upon secretion, tethered VWF concatemers unfurl under neutral pH and shear flow. The size of these concatemers is controlled through proteolysis of the nascently unfolded A2 domain by ADAMTS13.[4]

Disruptions to VWF structure, function, and regulation result in various hematologic and thrombotic disease states.[5] von Willebrand disease is caused by decreased levels of or dysfunctional VWF multimers. By contrast, congenital or autoimmune-induced deficiencies of ADAMTS13 result in the unregulated presence of ultralarge VWF concatemers, producing the disorder thrombocytopenic thrombotic purpura.[4]

The dynamics of VWF size and shape in circulation play a critical role in its function.[3] Untethered, VWF concatemers exist in a more compact, largely unstructured state in static or unimpeded shear flow conditions.[6] Upon exposure of subendothelial collagen at the site of vascular injury, VWF becomes tethered through collagen-binding sites in the A1 and A3 domains. Tethered VWF concatemers unfurl and elongate under higher levels of sheer stress, which localizes this topological state largely to sites of vascular damage. Several previous studies have shown that, under this tethered, elongated state, VWF binds with high affinity to platelet glycoprotein GPIbα through the A1 domain in a partially unfolded state. A recent study related to Fu et al characterizes the interaction between tethered VWF and GPIbα, demonstrating the tensile force requirements for association.[7]

In this study, Fu et al focus on the real-time, direct observation of VWF self-association to characterize the tensile force requirements and kinetic behavior



Free VWF association and dissociation from tethered VWF was measured in real time at the single-molecule level with a pressure-actuated microfluidic flow cell attached to a TIRF microscope. VWF concatemers were initially labeled with biotin and Alexa 488 (green) and immobilized on the traptavidin-coated flow cell wall (tethered VWF). A second fraction of VWF, labeled with Alexa 647 (purple), flowed through the microfluidic cell at different rates. High-flow rates resulted in high tensile forces proximal to the tether points, which are necessary for VWF self-association.

for the binding and dissociation of this interaction under differing levels of shear stress. To visualize this process, recombinant, concatemeric VWF was double labeled with a fluorophore and biotin, which was subsequently immobilized under static conditions in a microfluidic flow channel that was mounted with a total internal reflection microscope (TIRF). For flow cell measurements of binding, a second pool of VWF was labeled with a different fluorophore. These proteins were introduced to the microfluidic flow cell through nitrogen-pressurized vials at one end of the flow chamber that can rapidly switch to different flow rates to measure fluorescence microscopic images in real time (see figure).

Initial measurements demonstrated that tethered VWF elongates as a function of shear stress, reaching a maximal longitudinal distance at 960 dyn·cm^{-2}, which rapidly relaxes to a more compressed state once flow rates have returned to the basal level of 80 dyn·cm^{-2}. When free VWF was introduced to the flow cell, however, little self-association was detected at low shear stress levels, only to increase significantly at flow rates of 480 and 960 dyn·cm^{-2}. Interestingly, the association of free VWF concentrated toward the attachment point for tethered VWF, and binding migrated down the longitudinal spine as a function of increased flow rates. Once the flow rate was returned to the basal level of 80 dyn·cm^{-2}, free VWF rapidly dissociated from tethered VWF, which returned to its more compressed state.

Tensile force within a tethered species under shear stress is proportional to the length downstream on which the hydrodynamic drag force is exerted. Thus, the localized tensile force at any point along VWF from the tether point, can be calculated based on the number of VWF monomers that are downstream along the direction of shear flow. Fu et al binned different ranges of tensile force along the VWF spine to show that self-association in this context requires high levels of tensile force. The amount of binding can also be robust, as increasing the concentration of free VWF resulted in binding ~1.5-fold more mass than the overall mass of the tethered VWF.

Because the A1 domain has been shown to bind GPIbα in a tension-dependent manner,[7] and the A domains have also been implicated in VWF self-association,[8] Fu et al also measured VWF self-association in the presence of GPIbα. GPIbα nominally decreased VWF self-association, as did the presence of an anti-A1 domain aptamer. These data suggest that the major determinant for VWF self-association occurs in an adjacent domain, possibly the A2 domain as it unfolds under tension.[8]

The binding of GPIbα to tethered VWF requires approximately twice the amount of tensile force as VWF self-association,[7] which is driven by electrostatic steering.[9] A fundamental difference between these 2 studies, however, is the potential for avidity effects of VWF self-association. Fu et al expected that free VWF would associate with tethered VWF at multiple points, which would minimize the total tensile force exerted on the associated VWF concatemers, thereby limiting the total amount of platelets that may bind through GPIbα. It would be interesting to probe the nature of the GPIbα/VWF interaction by generating a protein-based nanoparticle that displays multiple copies of GPIbα to assess the role of avidity in platelet/VWF binding.

This study is an elegant demonstration of how emergent technologies can illuminate new insights into previously unclear biological processes in real time and at the molecular level. As the dynamic properties of VWF concatemers are further studied with high-resolution techniques, such as cryogenic electron microscopy and single molecule fluorescence microscopy, more details about VWF self-association and platelet recruitment will come to light.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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