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Shear-mediated platelet activation in the free flow: Perspectives on the emerging spectrum of cell mechanobiological mechanisms mediating cardiovascular implant thrombosis

Marvin J. Slepian [a,b,*], Jawaad Sheriff [b], Marcus Hutchinson [a], Phat Tran [a], Naing Bajaj [a], Joe G.N. Garcia [a], S. Scott Saavedra [c], Danny Bluestein [b]

[a] Departments of Medicine and Biomedical Engineering, University of Arizona, Tucson, AZ 85721, United States
[b] Department of Biomedical Engineering, Stony Brook University, NY 11794, United States
[c] Departments of Chemistry and Biochemistry, University of Arizona, Tucson, AZ 85721, United States

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ABSTRACT

Shear-mediated platelet activation (SMPA) is central in thrombosis of implantable cardiovascular therapeutic devices. Despite the morbidity and mortality associated with thrombosis of these devices, our understanding of mechanisms operative in SMPA, particularly in free flowing blood, remains limited. Herein we present and discuss a range of emerging mechanisms for consideration for "free flow" activation under supraphysiologic shear. Further definition and manipulation of these mechanisms will afford opportunities for novel pharmacologic and mechanical strategies to limit SMPA and enhance overall implant device safety.

1. Introduction

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in the western world (Writing Group et al., 2016). While great advances have been made in the pharmacologic management of cardiovascular disease, for many CVD conditions, implanted medical devices, rather than drugs have emerged as the mainstay of therapy. In advanced atherosclerotic coronary artery disease, stents and endovascular scaffolds are the present standard-of-care for high-grade obstruction (Iqbal et al., 2013; Task Force et al., 2013). In patients with critical aortic stenosis, percutaneous heart valves in the form of transcatheter aortic valve replacement (TAVR) are being implanted at an increasing rate (Vahl et al., 2016). For patients with advanced and end-stage congestive heart failure, mechanical circulatory support devices in the form of ventricular assist devices (VADs) and the total artificial heart (TAH) are in widespread and increasing use (Kirklin et al., 2015). Despite the efficacy of these implants, they remain hampered and limited by a common adverse event – that of device-related thrombosis. Further, recent studies have demonstrated that present anti-coagulant and notably anti-platelet agents have limited efficacy as a means of limiting thrombosis (Sheriff et al., 2014; Valerio et al., 2016). The severity of this limitation covers a spectrum ranging from reduction of flow due to space occupation of the flow path, to possible thromboembolic events including stroke and most severely, complete cessation of flow leading to device failure with accompanying ischemia, infarction and possible death.

Central to device-related thrombosis is the initiation and propagation of thrombus formation as a result of platelet activation. When one considers mechanisms of platelet activation, prime drivers which always come to mind first are the numerous, redundant biochemical pathways involved, i.e. ADP, and collagen (Jennings, 2009). Additionally, thrombosis is often viewed from the perspective of Virchow's triad – with contributions and a delicate balance between endothelial dysfunction or surface interactions, "inflammatory blood" (biochemical mediators), and altered flow (historically stagnation) (Kroll et al., 1996). However, in the realm of cardiovascular implant devices (CVIDs), and in the case of MCS devices in particular, many of these parameters are largely absent or minimally contributory. For example, VADs are devoid of endothelial cells in their endoluminal flow path and propel blood at high flows without large regions of stagnation. As such, for most CVIDs it is *flow* – notably in the form of high flow with accompanying elevated shear stress – that is the dominant activating element driving platelet activation and subsequent thrombosis. To provide perspective, while shear stresses in most arterial flows are in the range of 0–30 dynes/cm^2 (Kroll et al., 1996), within present day continuous flow VADs, shear may exceed 1000 dynes/cm^2 (Girdhar et al., 2012; Pirbodaghi et al., 2014).

* Correspondence to: Departments of Medicine and Biomedical Engineering, Sarver Heart Center, 1501 North Campbell Avenue, Bldg 201E, Rm 5146, P.O. Box 24-5037, Tucson, AZ 85724, United States. Fax: +520 626 7625.
E-mail address: chairman.syns@gmail.com (M.J. Slepian).

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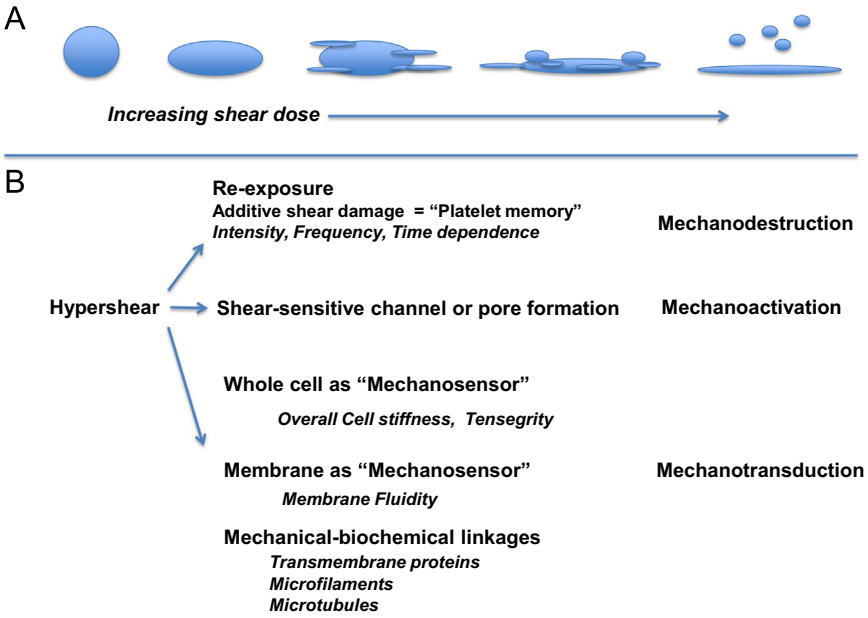

Fig. 1. Proposed additional mechanisms of hypershear-mediated platelet activation. (A.) With increasing shear dose=intensity × time, platelets undergo shape change, pseudopod extension, progressive additive damage with membrane rents and pore formation, fragmentation, membrane eversion, and ultimately microparticle generation. (B.) Three mechanistic pathways are outlined: a mechanodestructive pathway in which repetitive shear damage accumulates, platelets being incapable of repair, eventually sustain irreversible damage – as illustrated in (A) above; a mechanoactivation pathway wherein shear-sensitive channels, pore and gates may open; and a mechanotransductive pathway in which the whole cell (based on stiffness), the cell membrane (based on fluidity), and mechanic-biochemical linkage pathways capture and convert shear to internal activating signals.

In this paper, we highlight the increasingly expanding range of mechanisms and properties that appear to contribute to platelet activation under "hypershear" conditions, i.e. shear stress >300 dynes/cm^2, commonly operative in CVIDs. We outline these largely mechanical, less conventional mechanisms, as they represent opportunities for novel pharmacologic and alternative therapeutic development to limit platelet activation.

2. Shear-mediated platelet activation – the traditional view

Shear-mediated platelet activation (SMPA), traditionally called shear-induced platelet activation (SIPA), has been studied for many years, with early work suggesting that a defined threshold exists for activation – below which platelets remain intact and above which they become activated (Hellums, 1994). Accompanying this concept has been the phenomenon of von Willebrand factor (vWF) – GPIb interaction leading to high shear flow platelet tethering, partitioning and loose adhesion to the vessel wall, allowing further integrin-mediated high affinity adhesion and biochemical mediator-facilitated activation (Chow et al., 1992; Moake et al., 1986). In recent years, our thinking as to SMPA has expanded. Specifically, it has become recognized that activation may occur directly in the "free flow" – within regions of a flowing blood column imparting high levels of intermittent or sustained shear exposure, without wall or conduit contact. Supporting this shift in perspective, are the fact that in many CVIDs the endothelium is absent, and as a result vWF tethering is non-operative, yet thrombosis occurs. Further, it has come to be recognized recently that under "hypershear" conditions, large molecular weight multimers of vWF are cleaved, rendering remnant vWF incapable of interacting effectively with platelets to initiate thrombosis (Meyer et al., 2010).

3. Potential mechanisms of hypershear mediated platelet activation

How then can platelets be activated, independent of a GP1b–vWF mechanism, while rapidly flowing, rotating and spinning in free flowing blood under supraphyisologic shear (Soares et al., 2013)? Herein we outline a range of potential mechanisms (Fig. 1) including: 1) "mechano-destruction" – i.e. additive platelet (membrane) damage leading to a progressive increase in porogenisity and/or leakiness of the platelet with resultant influx of activating mediators with shape change and membrane fragmentation and inversion; 2) "mechano-activation" – i.e. shear-mediated activation of shear-sensitive channels and pores allowing influx of *specific* activators; and 3) "mechano-transduction" – that of "outside-in" signaling via a range of transducers – beyond previously described GP1b or platelet integrin (GPIIb/IIIa) pathways. These pathways include but are not limited to the a) cell membrane and b) defined biochemical-mechanical transmembrane and intracellular linkage elements leading to activation. Further, these mechanisms may be modulated via the intrinsic nature, or the modification thereof, of the material properties of the platelet – specifically overall *platelet stiffness* or *platelet membrane fluidity*. Below we provide evidence in support of these mechanisms.

4. Additive platelet damage as a mechanism of activation – the mechano-destructive pathway

Early studies of SMPA largely utilized only constant shear stress exposures, with no analysis of subsequent platelet behavior or response during both low shear stress conditions (i.e. normal circulation) or repeated high shear stress exposure (i.e. recirculation in a CVID). The need to examine platelet behavior during and after dynamic, device-related flow conditions was highlighted by the observation of chronic platelet activation and thromboembolic events in mechanical heart valve patients despite antiplatelet therapy (Butchart et al., 2003). These complications still remain a