Biomaterials Science: Processing, Properties and Applications III.
Edited by Susmita Bose, Amit Bandyopadhyay and Roger Narayan.
© 2013 The American Ceramic Society. Published 2013 by John Wiley & Sons, Inc.

MECHANISMS OF PLATELET ACTIVATION BY BIOMATERIALS AND FLUID SHEAR FLOW

Sri R. Madabhushi and Sriram Neelamegham*

Chemical and Biological Engineering, State University of New York, Buffalo, NY 14260
* Correspondence Author: neel@buffalo.edu

ABSTRACT

Severe defects in the heart or blood vessels leads to various cardiovascular diseases (CVD) and patients sometimes require biomaterial based implants to replace/overcome these defects. However, introduction of biomaterials into the patient's anatomy leads to bleeding and thrombosis complications. To date, the search for a completely non-thrombogenic surface is not complete. Various factors account for the challenges found in this regard: i. design factors of mechanical devices, especially at the sharp edges and connections, introduce non-physiological flow patterns, ii. blood protein responses to the biomaterial vary based on the specific biomaterial used in the vascular grafts; iii. multiple pathways are stimulated due to biomaterial-blood interactions which include interactions both at the protein and the cellular levels; iv. the pathways are intricate and inter-linked due to which perturbing a single reaction does not usually eliminate the problem; and v. the exact molecular mechanisms that trigger these thrombogenic processes is still not fully understood. Platelets are the key cellular players in both the hemostatic and thrombotic processes in blood. In this review, we describe an overall view of some of the thrombogenic processes initiated due to biomaterial-blood interactions, with focus on the role of von Willebrand factor in shear induced platelet activation and aggregation processes.

INTRODUCTION

Disorders of heart and blood vessels result in cardiovascular diseases (CVD) which are the leading cause of death in the world[1]. Based on the cause and specific location of the defects, CVDs can be classified into various types. These include the defects in different blood vessels that supply blood to: the heart muscle (coronary heart disease), brain (cerebrovascular disease), arms and legs (peripheral arterial disease); or the damage of the heart muscle and valves from rheumatic fever (rheumatic heart disease); or birth defects of heart structure (congenital heart disease); or blood clots in legs and veins that can dislodge and move to heart and lungs (deep-vein thrombosis and pulmonary embolism. Various factors lead to the damage of the vascular system including inflammation, diabetes, high blood pressure, and unhealthy diet. In order to treat these deadly diseases, a variety of cardiovascular devices have been developed to replace or overcome these defects[2].

Implantable devices (both mechanical and bioprosthetic) have improved the treatments for various cardiovascular diseases. As required by the type of the disease, different kinds of devices are used. For example, in case of atherosclerosis which causes narrowing of the blood vessel wall, vascular bypass grafts are implanted[3]. Synthetic grafts, stents as well as heart valves (mechanical and bioprosthetic valves) are also widely used to treat various valve disorders. While these surgical implants have immensely improved the treatment of various CVDs, these are often associated with thromboembolic complications[4; 5]. Often, patients receive anticoagulant medication which might induce vulnerability to hemorrhage [6]. Efforts in this regard, are being made towards developing non-thrombogenic biomaterial surfaces which might significantly improve the success rate of these vascular devices [3].

Thrombogenecity of implantable vascular devices are determined by the material and blood properties that come in contact with each other, as well as the non-physiological flow conditions introduced in the body by these devices [5; 7]. Platelets are the primary blood cells involved in physiological and pathological thrombus formation in circulation [8]. A better understanding of the molecular mechanisms that lead to platelet thrombus formation are essential to improve the performance of these devices in vivo. In this review, we describe the various mechanisms involved in thrombus formation in the context of biomaterials with major focus on the shear induced platelet activation and adhesion mediated by the blood protein von Willebrand factor (VWF). An overview of the concepts discussed in this article is provided in Figure 1.

NON-PHYSIOLOGICAL HYDRODYNAMIC CONDITIONS CAUSE PLATELET ACTIVATION

Platelets are anuclear, discoid shaped cell fragments derived from megakaryocytes in the bone marrow. They have an average diameter of 2-4 μm and circulatory concentrations of 150 - 400 x 10^6/ml. Their primary role in circulation is to survey and maintain the integrity of vasculature [9]. This is facilitated by various ligand-receptor interactions which allow the platelets to sense and respond to various extracellular stimuli. Some of these include the collagen receptors GpIa/IIa and GpVI; von Willebrand factor receptors GpIbα and GpIIbIIIa, Thrombin receptor, ADP receptor as well as the Thromboxane A_2 receptor [5]. In response to the stimuli, platelets get activated, adhere to the surface, and aggregate, release various compounds and proteins that initiate and amplify the coagulation process, which subsequently leads to thrombus formation.

To a large extent, fluid dynamics plays a significant role in determining the key players in thrombus formation [10-12]. In contrast to red blood cells, platelets have a relatively rigid membrane and therefore experience higher shear stresses [13]. Venous thrombi contain greater concentrations of fibrin along with trapped red blood cells and few platelets and are called "red clots". In arterial circulation with comparatively higher shear regimes, the clots contain higher platelet number and lower fibrin content and are therefore called "white clots". Under physiological conditions, blood more or less follows a laminar flow regime. However, under pathological conditions of stenosis or in the presence of prosthetic devices, non-physiological flow patterns are introduced in the circulation[7]. These include regions of recirculation, shed vortices, turbulence as well as flow separation[14; 15].

Steps, sharp edges as well as gaps in the biomaterial are the usual design parameters that cause non-physiological flow conditions [16]. Following an artificial heart valve implantation, hemolysis and platelet activation are usually observed in these regions. Irregular flow patterns have been observed to cause platelet activation leading to thromboembolic complications [17; 18]. For example, in case of mechanical heart valves, the connections between different components of the device as well as between the device and the normal vasculature contain various unavoidable irregularities. In the proximity of these step regions, Corbett et al. observed that larger steps, especially the negative step regions, support larger thrombus deposits [19].

Platelets have a circulatory lifetime of 7 days during which they pass through the vascular grafts multiple times. Accumulation of damage on platelets over time due to the history of exposure to irregular flow conditions has been reported [20]. Using a combination of experimental measurements and computation fluid dynamics, Alemu et al. described a cumulative study of the flow-induced platelet activation and damage accumulation in mechanical heart valves [18]. Hellums et al. and Shankaran et al. studied the time dependent shear stress mediated activation of platelets and described a threshold value of 35-50 dynes/cm^2 which results in detectable levels of platelet activation [10; 20]. In this regard, bioprosthetic valves perform better by resulting in accumulated shear stress values 10% of that seen using mechanical heart valves [21]. While it is

not certain that the same platelets follow the same path during their multiple cycles through vascular grafts and whether damage recovery happens during their flow in normal circulation, multiple passages through the non-physiological flow regimes might result in damage accumulation of platelets.

The exact mechanism of how shear induces platelet activation is not fully understood. The next section of this review looks at the role of a blood glycoprotein, von Willebrand factor (VWF) in shear induced platelet activation, adhesion and aggregation.

VON WILLEBRAND FACTOR

von Willebrand factor (VWF) is the largest blood glycoprotein that plays an important role in shear induced platelet activation and aggregation [10; 22; 23]. It is synthesized by endothelial cells and megakaryocytes and is constitutively present in circulation with an average concentration of 10 μg/mL [24]. During its biosynthesis, the multi-domain VWF molecule forms 500 kDa C-terminal dimeric units in the endoplasmic reticulum. These dimeric units then undergo linear polymerization via inter-N-terminal disulfide bond formations to yield higher multimers with molecular weights ranging up to 20,000-40,000 kDa [25]. Multimer size distribution of VWF is, in fact, crucial for facilitating effective shear induced platelet activation by the protein. Higher multimers have higher hemostatic potential. Freshly secreted VWF from endothelial cells have ultra large multimers (ULVWF) that can spontaneously bind to platelets [26]. Presence of ULVWF leads to clot formations and faster clearance of platelets from circulation as seen in the case of Thrombotic Thrombocytopenic Purpura (TTP) patients [27]. A VWF specific metalloprotease, called ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), cleaves it and regulates the VWF size in circulation thus preventing spontaneous clot formation and platelet clearance [28-30].

VWF binds to platelets in a shear dependent manner. While VWF and platelets are present together in circulation, no detectable interaction is observed between the two molecules unless at the sites of vascular injury or under high shear stress conditions. In solution, VWF assumes a ball-and-stick structure with the N-terminal domains (D'D3, A1, A2, A3) forming the globular ball region and the C-terminal domains forming the stick region, the D4 domain being positioned at the linker between the two regions [23]. The arrangement of the domains in the globular section is thought to conceal the platelet receptor's binding epitope in the A1 domain [31-33] and the ADAMTS13 cleavage site in the A2 domain [34]. At the sites of vascular injury, collagen of the sub-endothelial matrix gets exposed. VWF-A3 domain recognizes the exposed collagen and causes the immobilization of the protein at the injured blood vessel walls. The VWF multimers are then subjected to wall shear stress which probably unveils the A1 domain which then facilitates platelet adhesion at vascular injury site.

Multiple studies have looked at the role of shear stress in inducing conformational changes in VWF [35-38]. In this regard, using atomic force microscopy, Siedlecki et al. observed a transition from a globular structure to an extended chain form when VWF was subjected to shear stress > 35 dyn/cm^2 on a hydrophobic surface [35]. In our laboratory, multiple approaches have been used to gain insight into the aspects of fluid shear on VWF structure. Singh et al. employed small angle neutron scattering (SANS) to measure protein conformational changes in response to shear rates < 3000 s^{-1} [37]. SANS was employed in these studies since it provides structural information over wide length scales starting from domain-level features of ~ 2 nm to higher structural features of ~ 100 nm. Quantitative modeling of SANS data showed that domain level rearrangements were induced by application of shear stress on VWF. Using the fluorescent probe, bis-ANS (4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid), that binds to hydrophobic regions of a protein, Themistou et al. observed enhanced binding of bis-ANS to VWF subjected to fluid shear [38]. The shear rate for exposure of hydrophobic domains was higher (>45 dyn/cm^2)

and this suggests that multiple structural changes may occur when VWF is subjected to hydrodynamic shear. Together, these studies highlight the role of fluid shear in inducing structural changes in VWF.

That the binding of VWF to platelet receptor GpIbα under hydrodynamic shear causes shear induced platelet activation and aggregation was further examined in our laboratory. To this end, Shankaran et al. employed a cone-plate viscometer to subject a mixture of VWF and platelets to defined shear stress conditions and quantified Annexin V binding to measure platelet activation levels [10]. It was noted that within 10 seconds of shear application, detectable Annexin V binding to platelets was observed. As mentioned previously, VWF undergoes shear mediated conformational changes which facilitates its binding to platelets. Using static light scattering, an increase in the hydrodynamic radius of VWF was observed from a value of ~ 68 nm in the absence of shear to ~466 nm upon application of 6000 s^{-1} shear for 120 s. It was hypothesized that structural changes in VWF leading to exposure of hydrophobic domains might also cause the self-association of VWF molecules increasing the size of VWF in solution. In this regard, Dayananda et al. used a recombinant VWF molecule with a deletion of the platelet binding A1 domain (ΔA1-VWF) [22]. Studies using this molecule demonstrated that ΔA1-VWF bound to platelets via self-association with exogenously added full-length VWF molecule. This indicates that application of fluid shear on VWF not only helps in exposure of the VWF-A1 domain, but also increases effective size of VWF via self-association. Presence of these large VWF molecules bound to GpIbα enhances the amount of hydrodynamic force applied on the GpIbα molecule (~ 10pN) which might then trigger mechanotransduction and cell activation [10; 11].

The physiological significance of VWF in mediating platelet adhesion, activation and aggregation might also be of importance in the context of biomaterials. In this regard, studies using monoclonal antibody (6D1) against the platelet receptor GpIbα showed significant inhibition of platelet retention on polyethylene (PE) surfaces [39]. In vitro studies using artificial microvascular grafts made of polytetrafluroethylene (PTFE) showed 82% reduction in platelet retention in the presence of 6D1 [40]. In vivo studies in guinea pigs using a $F(ab')_2$ fragment of anti-GpIbα monoclonal antibody, PG-1, showed significant prolongation of artificial microvascular graft patency (AMG) [41]. Additionally, longest prolongation of AMG patency was observed using a combination of GpIbα and thrombin inhibition.

It is, therefore, likely that von Willebrand factor mediated platelet activation and aggregation not only plays a critical role in physiological hemostasis, but also might be important in the context of the thromboembolic complications seen in case of non-physiological shear regimes in cardiovascular devices. In addition to its effect on platelet activation in solution, von Willebrand factor can also facilitate platelet adhesion on artificial surfaces by adsorbing onto these surfaces. The high on- and off-rates of VWF-A1 domain and GpIbα interaction makes it a suitable candidate in enabling platelet adhesion on surfaces [26].

In the next section of this review, we will briefly describe other mechanisms that can lead to thrombus formation on biomaterial surfaces.

PLASMA PROTEIN ADSORPTION ON BIOMATERIALS

Shortly after the biomaterials come in contact with blood, plasma proteins get adsorbed onto the surface based on both the protein as well as the surface properties [5]. Additionally, through interlinked series of events, the devices trigger activation of the coagulation cascade as well as activation and adhesion of different blood cells like the platelets and leukocytes.

Blood plasma contains about 300 proteins that make up 7% of its volume [42]. Concentrations of the constituent proteins vary over a dynamic range of 10 orders of magnitude, serum albumin being the most abundant protein. Within 5 seconds of exposure of the biomaterial surface to blood, the adsorption of plasma proteins onto surfaces is observed based on their

hydrophobic or hydrophilic properties. Thermodynamics of the protein-solvent-surface system greatly define the amount and type of protein adsorbed onto these surfaces [43]. Surface immobilization results in about 1000 fold increase of protein concentrations in comparison to their concentration in solution. Additionally, the surface composition of adsorbed proteins is highly dynamic in nature and is described as "Vronman effect" [44]. Interactions of blood cells with the biomaterial surface is mediated by this adsorbed protein layer. Protein adsorption in the context of thrombus formation will be briefly discussed here due to its relevance in cardiovascular device functionality.

The intrinsic coagulation pathway of the coagulation cascade begins with the contact activation of Factor XII [45]. The biomaterial surface, in some cases, is thought to provide the required anionic surface for Factor XII activation. The intrinsic pathway consists of a series of steps wherein the inactive zymogen is activated. The starting reaction in this cascade involves the conversion of Factor XII to Factor XIIa, and this signal is amplified in the subsequent series of steps which results in the conversion of prothrombin to thrombin. Thrombin, then, facilitates the conversion of fibrinogen to fibrin which polymerizes and forms the mesh that constitutes a clot. Factor XII has been thought to play a minor role in hemostasis due to the lack of any phenotypic effects of its deficiency [46; 47]. While the physiological blood vessel wall might not support contact activation of Factor XII, vascular grafts were found to have moderate levels of Factor XII adsorbed on them [48]. However, the relative significance of the stimulation of the intrinsic coagulation pathway in the complications of vascular grafts is still unclear.

Tissue factor expression of activated monocytes initiates the extrinsic coagulation pathway [49]. In vivo activation of monocytes was noted due to cardiopulmonary bypass. Factor VII, circulating in the blood, binds to the tissue factor expressed on these activated cells and this complex leads to the activation of Factor X and Factor IX [5]. These zymogens subsequently lead to the formation of thrombin. The ultimate product of both the intrinsic and extrinsic coagulation pathways is the generation of active thrombin. While the two pathways are not mutually exclusive, the time scales of their initiation are different. Since the intrinsic pathway only requires surface activation, it might proceed shortly after the blood-biomaterial contact happens. However, the extrinsic pathway might kick in at a later time since it relies on tissue factor production after the activation of the monocytes. The relative importance of these two processes on thrombosis might differ.

Among the other relevant proteins that adsorb onto vascular grafts and contribute to thrombosis are fibrinogen and von Willebrand factor. Fibrinogen is a plasma glycoprotein that circulates at a high concentration of 1.5-3 mg/mL [50]. In addition to its contribution to thrombus formation by converting to fibrin via the action of thrombin, fibrinogen also facilitates platelet adhesion. Fibrinogen binds to the platelet integrin GpIIbIIIa [51]. Other glycoproteins that can bind to the platelet receptor, GpIIbIIIa include von Willebrand factor, fibronection, thrombospondin and vitronectin. These proteins contain the RGD sequence required to bind to integrins. About 40-80,000 GpIIbIIIa receptors are present on a resting platelet surface [52]. However, these receptors are in the inactive state. Binding of the platelet receptor to the adhesive glycoproteins adsorbed on the biomaterial surface requires the activation of the integrins. Thrombin, formed by the intrinsic or extrinsic coagulation pathways, is an agonist for platelet activation. This might facilitate the conversion of the integrin to an active state. Additionally, surface immobilization of the fibrinogen is thought to induce in a conformational change in it allowing it to bind to the low-affinity platelet receptor [53].

von Willebrand factor can facilitate platelet adhesion on surfaces without the prerequisite of platelet activation. The VWF receptor on platelets, GpIbα, is another abundant receptor with about 25,000 copies present on the platelet surface [54]. The VWF-A1 and GpIbα interaction is characterized by a fast ON and a fast OFF rate. This allows platelet recruitment on surfaces

especially in the presence of high shear conditions. Once the platelets are slowed down on surfaces, be the injured vasculature of probably even on biomaterial surfaces, firm adhesion happens through other ligand-receptor interactions. von Willebrand factor and fibrinogen also act as molecular bridges between two platelets causing platelet aggregation. In this regard, monoclonal antibodies against GpIIb-IIIa as well as GpIbα were observed to be potent inhibitors of platelet deposition on artificial surfaces [39]. Although bleeding and thrombocytopenic complications were observed, clinical trials demonstrated an improvement in the graft performance. Further understanding with respect to identifying the case/device specific problems and developing strategies to make the surfaces non-thrombogenic is required.

EFFORTS TO PRODUCE NON-THROMBOGENIC CARDIOVASCULAR DEVICES

Non-thrombogenecity of biomaterial surfaces, to some extent, has been achieved using few strategies. In this regard, surfaces coated with anticoagulants like heparin, hirudin, thrombin inhibitors and tissue factor pathway inhibitors have been explored [3; 55-58]. However, these have met with limited success due to their shortcomings. Heparin is an anticoagulant which binds to antithrombin III and causes conformational changes in the protein which then facilitates the inactivation of various coagulation factors including thrombin [59]. Clinical studies showed significant improvements in graft patency using heparin coated vascular grafts [55]. However, heparin targets only one of the thrombin formation mechanisms and also binds to various growth factors and matrix proteins leading to some side effects. Hirudin, a protein derived from leech, is a natural inhibitor of thrombin. Hirudin coated surfaces showed thrombus reduction in in vitro as well as a few in vivo studies [56; 60]. While in vivo effects of other thrombin inhibitors is still under investigation, currently all the trials have been unsuccessful in completely eliminating the thromboembolic risks due to the multi-pathway intricate system that maintains hemostasis in the body.

Endothelial cells are the best non-thrombogenic surfaces known in nature [61]. They i. synthesize and secrete nitric oxide and prostaglandin I_2 that inhibit platelet adhesion; ii. produce tissue-type plasminogen activator (t-PA) that degrades fibrin; iii. bind ectonucleotidases that hydrolyze ADP; iv. contain a glycocalyx layer which consists of heparin sulfate proteoglycan that serves as a co-factor of antithrombin III; and v. synthesize tissue factor pathway inhibitor (TFPI). Due to its success as the natural non-thrombogenic material, attempts have been made towards manufacturing tissue engineered cardiovascular grafts which have an endothelial monolayer covering them. Collagen and synthetic matrix have been used as the underlying structural base for these grafts [3]. However, in vivo success of these implants has been limited. Some of the problems include loss of the seeded endothelial cells within 24 h and limited patency [62]. This could probably be addressed by the recent attempts ensued by various groups in incorporating biophysical and biochemical modifications in the material to improve endothelial cell retention, and or achieving in situ endothelialization of vascular implants [63; 64]. Additional studies towards this aspect might reveal new insights towards addressing with the thrombogenesis issues of biomaterials.

CONCLUSION

Hemostasis is in itself a complex process which involves a fine balance between the pro- and anti-coagulation factors in circulation. These factors also play an important role in the thrombogenesis of biomaterials using in cardiovascular implants. Introduction of biomaterials in the body results in the generation of complex non-physiological flow patterns as well as adsorption of proteins on the surfaces that cause platelet activation, adhesion and aggregation, as well as thrombus formation. von Willebrand factor is a key player in mediating both shear

induced platelet activation in solution as well as on surfaces. However, this is just a small part of the entire complex mechanism. In order to succeed in making a truly non-thrombogenic surface, multiple mechanisms must be utilized similar to how the endothelial cells achieve this phenomenon.

FOOTNOTES
Supported by N.I.H. grants HL77258 and HL103411

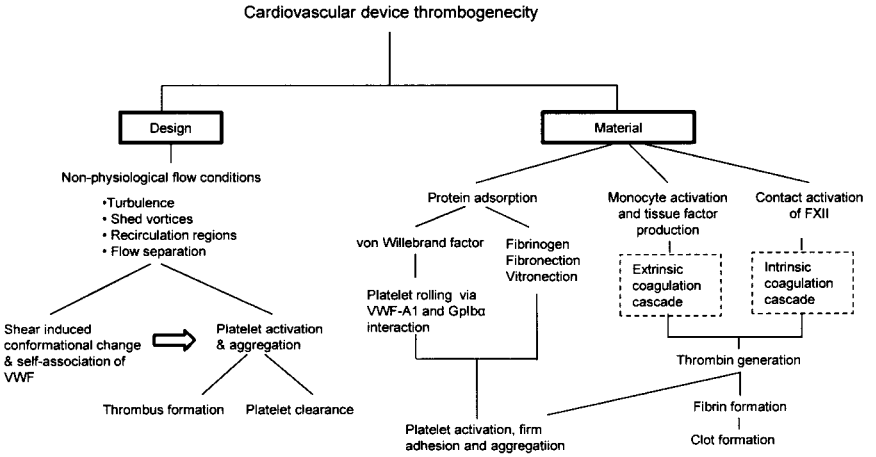

Figure 1. Overview of the some of the major factors that lead to thromogenecity of cardiovascular devices

REFERENCES

[1]World Health Organization (2012) Cardiovascular diseases - Fact Sheet.
[2]M.H. Yacoub and J.J. Takkenberg Will heart valve tissue engineering change the world?, *Nat Clin Pract Cardiovasc Med*, **2**, 60-61 (2005).
[3]S. Li and J.J. Henry Nonthrombogenic approaches to cardiovascular bioengineering, *Annu Rev Biomed Eng*, **13**, 451-475 (2011).
[4]L.H. Edmunds, Jr. Thrombotic and bleeding complications of prosthetic heart valves, *Ann Thorac Surg*, **44**, 430-445 (1987).
[5]M.B. Gorbet and M.V. Sefton Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes, *Biomaterials*, **25**, 5681-5703 (2004).
[6]L.H. Edmunds, Jr., S. McKinlay, J.M. Anderson, T.H. Callahan, J.H. Chesebro, E.A. Geiser, D.M. Makanani, L.V. McIntire, W.Q. Meeker, G.K. Naughton, J.A. Panza, F.J. Schoen and P.

Didisheim Directions for improvement of substitute heart valves: National Heart, Lung, and Blood Institute's Working Group report on heart valves, *J Biomed Mater Res*, **38**, 263-266 (1997).
[7]L.P. Dasi, H.A. Simon, P. Sucosky and A.P. Yoganathan Fluid mechanics of artificial heart valves, *Clin Exp Pharmacol Physiol*, **36**, 225-237 (2009).
[8]D.D. Wagner and P.C. Burger Platelets in inflammation and thrombosis, *Arterioscler Thromb Vasc Biol*, **23**, 2131-2137 (2003).
[9]D.J. Angiolillo, M. Ueno and S. Goto Basic principles of platelet biology and clinical implications, *Circ J*, **74**, 597-607 (2010).
[10]H. Shankaran, P. Alexandridis and S. Neelamegham Aspects of hydrodynamic shear regulating shear-induced platelet activation and self-association of von Willebrand factor in suspension, *Blood*, **101**, 2637-2645 (2003).
[11]H. Shankaran and S. Neelamegham Hydrodynamic forces applied on intercellular bonds, soluble molecules, and cell-surface receptors, *Biophysical journal*, **86**, 576-588 (2004).
[12]H.R. Baumgartner The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi, *Microvasc Res*, **5**, 167-179 (1973).
[13]P.A. Aarts, R.M. Heethaar and J.J. Sixma Red blood cell deformability influences platelets--vessel wall interaction in flowing blood, *Blood*, **64**, 1228-1233 (1984).
[14]A.P. Yoganathan, Y.R. Woo and H.W. Sung Turbulent shear stress measurements in the vicinity of aortic heart valve prostheses, *J Biomech*, **19**, 433-442 (1986).
[15]J.F. LaDisa, Jr., I. Guler, L.E. Olson, D.A. Hettrick, J.R. Kersten, D.C. Warltier and P.S. Pagel Three-dimensional computational fluid dynamics modeling of alterations in coronary wall shear stress produced by stent implantation, *Annals of biomedical engineering*, **31**, 972-980 (2003).
[16]G. Girdhar and D. Bluestein Biological effects of dynamic shear stress in cardiovascular pathologies and devices, *Expert Rev Med Devices*, **5**, 167-181 (2008).
[17]D. Bluestein, W. Yin, K. Affeld and J. Jesty Flow-induced platelet activation in mechanical heart valves, *J Heart Valve Dis*, **13**, 501-508 (2004).
[18]Y. Alemu and D. Bluestein Flow-induced platelet activation and damage accumulation in a mechanical heart valve: numerical studies, *Artif Organs*, **31**, 677-688 (2007).
[19]S.C. Corbett, A. Ajdari, A.U. Coskun and N. Hashemi In vitro and computational thrombosis on artificial surfaces with shear stress, *Artif Organs*, **34**, 561-569 (2010).
[20]J.D. Hellums 1993 Whitaker Lecture: biorheology in thrombosis research, *Annals of biomedical engineering*, **22**, 445-455 (1994).
[21]E. Sirois and W. Sun Computational evaluation of platelet activation induced by a bioprosthetic heart valve, *Artif Organs*, **35**, 157-165 (2011).
[22]K.M. Dayananda, I. Singh, N. Mondal and S. Neelamegham von Willebrand factor self-association on platelet GpIbalpha under hydrodynamic shear: effect on shear-induced platelet activation, *Blood*, **116**, 3990-3998 (2010).
[23]I. Singh, H. Shankaran, M.E. Beauharnois, Z. Xiao, P. Alexandridis and S. Neelamegham Solution structure of human von Willebrand factor studied using small angle neutron scattering, *The Journal of biological chemistry*, **281**, 38266-38275 (2006).
[24]J.E. Sadler Biochemistry and genetics of von Willebrand factor, *Annu Rev Biochem*, **67**, 395-424 (1998).
[25]S.R. Madabhushi, C. Shang, K.M. Dayananda, K. Rittenhouse-Olson, M. Murphy, T.E. Ryan, R.R. Montgomery and S. Neelamegham von Willebrand factor (VWF) propeptide binding to VWF D'D3 domain attenuates platelet activation and adhesion, *Blood*, **119**, 4769-4778 (2012).
[26]G.L. Mendolicchio and Z.M. Ruggeri New perspectives on von Willebrand factor functions in hemostasis and thrombosis, *Semin Hematol*, **42**, 5-14 (2005).

[27]Z.M. Ruggeri, P.M. Mannucci, R. Lombardi, A.B. Federici and T.S. Zimmerman Multimeric composition of factor VIII/von Willebrand factor following administration of DDAVP: implications for pathophysiology and therapy of von Willebrand's disease subtypes, *Blood*, **59**, 1272-1278 (1982).
[28]J.F. Dong, J.L. Moake, L. Nolasco, A. Bernardo, W. Arceneaux, C.N. Shrimpton, A.J. Schade, L.V. McIntire, K. Fujikawa and J.A. Lopez ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions, *Blood*, **100**, 4033-4039 (2002).
[29]G.G. Levy, W.C. Nichols, E.C. Lian, T. Foroud, J.N. McClintick, B.M. McGee, A.Y. Yang, D.R. Siemieniak, K.R. Stark, R. Gruppo, R. Sarode, S.B. Shurin, V. Chandrasekaran, S.P. Stabler, H. Sabio, E.E. Bouhassira, J.D. Upshaw, Jr., D. Ginsburg and H.M. Tsai Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura, *Nature*, **413**, 488-494 (2001).
[30]K.M. Dayananda, S. Gogia and S. Neelamegham Escherichia coli-derived von Willebrand factor-A2 domain fluorescence/Forster resonance energy transfer proteins that quantify ADAMTS13 activity, *Analytical biochemistry*, **410**, 206-213 (2011).
[31]H. Ulrichts, M. Udvardy, P.J. Lenting, I. Pareyn, N. Vandeputte, K. Vanhoorelbeke and H. Deckmyn Shielding of the A1 domain by the D'D3 domains of von Willebrand factor modulates its interaction with platelet glycoprotein Ib-IX-V, *The Journal of biological chemistry*, **281**, 4699-4707 (2006).
[32]C. Martin, L.D. Morales and M.A. Cruz Purified A2 domain of von Willebrand factor binds to the active conformation of von Willebrand factor and blocks the interaction with platelet glycoprotein Ibalpha, *J Thromb Haemost*, **5**, 1363-1370 (2007).
[33]A. Bonnefoy, H. Yamamoto, C. Thys, M. Kito, J. Vermylen and M.F. Hoylaerts Shielding the front-strand beta 3 of the von Willebrand factor A1 domain inhibits its binding to platelet glycoprotein Ibalpha, *Blood*, **101**, 1375-1383 (2003).
[34]J.T. Crawley, R. de Groot, Y. Xiang, B.M. Luken and D.A. Lane Unraveling the scissile bond: how ADAMTS13 recognizes and cleaves von Willebrand factor, *Blood*, **118**, 3212-3221 (2011).
[35]S.W. Schneider, S. Nuschele, A. Wixforth, C. Gorzelanny, A. Alexander-Katz, R.R. Netz and M.F. Schneider Shear-induced unfolding triggers adhesion of von Willebrand factor fibers, *Proc Natl Acad Sci U S A*, **104**, 7899-7903 (2007).
[36]C.A. Siedlecki, B.J. Lestini, K.K. Kottke-Marchant, S.J. Eppell, D.L. Wilson and R.E. Marchant Shear-dependent changes in the three-dimensional structure of human von Willebrand factor, *Blood*, **88**, 2939-2950 (1996).
[37]I. Singh, E. Themistou, L. Porcar and S. Neelamegham Fluid shear induces conformation change in human blood protein von Willebrand factor in solution, *Biophysical journal*, **96**, 2313-2320 (2009).
[38]E. Themistou, I. Singh, C. Shang, S.V. Balu-Iyer, P. Alexandridis and S. Neelamegham Application of fluorescence spectroscopy to quantify shear-induced protein conformation change, *Biophysical journal*, **97**, 2567-2576 (2009).
[39]R.A. Sheppeck, M. Bentz, C. Dickson, S. Hribar, J. White, J. Janosky, S.A. Berceli, H.S. Borovetz and P.C. Johnson Examination of the roles of glycoprotein Ib and glycoprotein IIb/IIIa in platelet deposition on an artificial surface using clinical antiplatelet agents and monoclonal antibody blockade, *Blood*, **78**, 673-680 (1991).
[40]P.C. Johnson, R.A. Sheppeck, S.R. Hribar, M.L. Bentz, J. Janosky and C.S. Dickson Inhibition of platelet retention on artificial microvascular grafts with monoclonal antibodies and a high-affinity peptide directed against platelet membrane glycoproteins, *Arterioscler Thromb*, **11**, 552-560 (1991).

[41]W.H. Dascombe, C. Hong, K.O. Garrett, J.G. White, V.A. Lyle, J.L. Miller and P.C. Johnson Artificial microvascular graft thrombosis: the consequences of platelet membrane glycoprotein Ib inhibition and thrombin inhibition, *Blood*, **82**, 126-134 (1993).
[42]N.L. Anderson and N.G. Anderson The human plasma proteome: history, character, and diagnostic prospects, *Mol Cell Proteomics*, **1**, 845-867 (2002).
[43]J. Satulovsky, M.A. Carignano and I. Szleifer Kinetic and thermodynamic control of protein adsorption, *Proc Natl Acad Sci U S A*, **97**, 9037-9041 (2000).
[44]H. Noh and E.A. Vogler Volumetric interpretation of protein adsorption: competition from mixtures and the Vroman effect, *Biomaterials*, **28**, 405-422 (2007).
[45]A.H. Schmaier Contact activation: a revision, *Thromb Haemost*, **78**, 101-107 (1997).
[46]B. Lammle, W.A. Wuillemin, I. Huber, M. Krauskopf, C. Zurcher, R. Pflugshaupt and M. Furlan Thromboembolism and bleeding tendency in congenital factor XII deficiency--a study on 74 subjects from 14 Swiss families, *Thromb Haemost*, **65**, 117-121 (1991).
[47]D. Gailani and T. Renne Intrinsic pathway of coagulation and arterial thrombosis, *Arterioscler Thromb Vasc Biol*, **27**, 2507-2513 (2007).
[48]N.P. Ziats, D.A. Pankowsky, B.P. Tierney, O.D. Ratnoff and J.M. Anderson Adsorption of Hageman factor (factor XII) and other human plasma proteins to biomedical polymers, *J Lab Clin Med*, **116**, 687-696 (1990).
[49]M. Ernofsson, S. Thelin and A. Siegbahn Monocyte tissue factor expression, cell activation, and thrombin formation during cardiopulmonary bypass: a clinical study, *J Thorac Cardiovasc Surg*, **113**, 576-584 (1997).
[50]D. Siegismund, T.F. Keller, K.D. Jandt and M. Rettenmayr Fibrinogen adsorption on biomaterials--a numerical study, *Macromol Biosci*, **10**, 1216-1223 (2010).
[51]J.J. Calvete Clues for understanding the structure and function of a prototypic human integrin: the platelet glycoprotein IIb/IIIa complex, *Thromb Haemost*, **72**, 1-15 (1994).
[52]C.L. Wagner, M.A. Mascelli, D.S. Neblock, H.F. Weisman, B.S. Coller and R.E. Jordan Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets, *Blood*, **88**, 907-914 (1996).
[53]K.A. Moskowitz, B. Kudryk and B.S. Coller Fibrinogen coating density affects the conformation of immobilized fibrinogen: implications for platelet adhesion and spreading, *Thromb Haemost*, **79**, 824-831 (1998).
[54]T.J. Kovacsovics and J.H. Hartwig Thrombin-induced GPIb-IX centralization on the platelet surface requires actin assembly and myosin II activation, *Blood*, **87**, 618-629 (1996).
[55]B.H. Walpoth, R. Rogulenko, E. Tikhvinskaia, S. Gogolewski, T. Schaffner, O.M. Hess and U. Althaus Improvement of patency rate in heparin-coated small synthetic vascular grafts, *Circulation*, **98**, II319-323; discussion II324 (1998).
[56]B. Seifert, P. Romaniuk and T. Groth Covalent immobilization of hirudin improves the haemocompatibility of polylactide-polyglycolide in vitro, *Biomaterials*, **18**, 1495-1502 (1997).
[57]J.M. Maraganore, P. Bourdon, J. Jablonski, K.L. Ramachandran and J.W. Fenton, 2nd Design and characterization of hirulogs: a novel class of bivalent peptide inhibitors of thrombin, *Biochemistry*, **29**, 7095-7101 (1990).
[58]W.X. Li, A.V. Kaplan, G.W. Grant, J.J. Toole and L.L. Leung A novel nucleotide-based thrombin inhibitor inhibits clot-bound thrombin and reduces arterial platelet thrombus formation, *Blood*, **83**, 677-682 (1994).
[59]E. Ersdal-Badju, A. Lu, Y. Zuo, V. Picard and S.C. Bock Identification of the antithrombin III heparin binding site, *The Journal of biological chemistry*, **272**, 19393-19400 (1997).
[60]E. Alt, I. Haehnel, C. Beilharz, K. Prietzel, D. Preter, A. Stemberger, T. Fliedner, W. Erhardt and A. Schomig Inhibition of neointima formation after experimental coronary artery stenting: a

new biodegradable stent coating releasing hirudin and the prostacyclin analogue iloprost, *Circulation*, **101**, 1453-1458 (2000).
[61]Z.M. Ruggeri Von Willebrand factor, platelets and endothelial cell interactions, *J Thromb Haemost*, **1**, 1335-1342 (2003).
[62]K.P. Walluscheck, G. Steinhoff, S. Kelm and A. Haverich Improved endothelial cell attachment on ePTFE vascular grafts pretreated with synthetic RGD-containing peptides, *Eur J Vasc Endovasc Surg*, **12**, 321-330 (1996).
[63]S.P. Massia and J.A. Hubbell Vascular endothelial cell adhesion and spreading promoted by the peptide REDV of the IIICS region of plasma fibronectin is mediated by integrin alpha 4 beta 1, *The Journal of biological chemistry*, **267**, 14019-14026 (1992).
[64]J.G. Meinhart, J.C. Schense, H. Schima, M. Gorlitzer, J.A. Hubbell, M. Deutsch and P. Zilla Enhanced endothelial cell retention on shear-stressed synthetic vascular grafts precoated with RGD-cross-linked fibrin, *Tissue Eng*, **11**, 887-895 (2005).