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Letter to the Editor:

Thrombogenicity and hemocompatibility of biomaterials

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The success of cardiovascular implants – particularly of small vascular prostheses – depends on the biological reactions occurring at the blood-material interface. Immediately after implantation proteins can adhere to the implant surface, thereafter platelets and leukocytes. The cells become activated during the adhesion process, which is associated with the secretion of various mediators. Some of the mediators induce the adherence and aggregation of further platelets so that thrombi can grow; others trigger the plasmatic coagulation and immunological responses. The protein adsorption on polymer-based biomaterials depends on the characteristics of the surface and the fluid shear. By investigating changes in plasma protein adsorption onto these surfaces, three aspects of protein adsorption were identified as crucial for activation processes: protein quantity [10, 16], protein layer composition [2, 15] and conformational changes of the protein [30, 36, 37]. In addition, the local shear rates of the blood flow determine whether passing blood cells adhere to a surface or not. Depending on the shear rates, different plasma proteins mediate the adhesion of the blood cells [38] via platelet membrane receptors [44], while the aggregation of further cells can also be triggered receptor-independent [42]. As a typical example, von Willebrand factor circulates in a globular form, so that binding sites for blood cells are hidden. However, as soon as the shear rates are high enough (> 1000 1/s [14, 1]), the von Willebrand protein becomes unfolded and in this filamentous form the binding sites are accessible for receptor binding so that adherence to surfaces or binding to blood cells can be mediated [41]. In addition, protein surface adsorption is a reversible process, and the composition of absorbed proteins can change over time. This dynamic change in protein monolayer composition is particularly evident on

negatively charged hydrophilic surfaces [43] and appears to be independent of flow [29]. Protein adsorption is the initiating event in thrombus formation because the protein layer modulates the subsequent reactions. Key elements of the formation of thrombi are platelets [23, 32]. Therefore, the interaction of platelets with the surface of blood contacting biomaterials is the key for the understanding of material *thrombogenicity* [31]. The latter is still not clearly defined, but at least comprises the activation and adherence of platelets and – from a clinical point of view – the generation of thrombi. It is dominated by a lot of factors [22]: Surface properties such as availability of certain functional groups (surfaces functionalized with $–CH_3$ or –COOH groups induced an increased platelet adhesion, while on –OH terminated SAMs nearly no platelets adhered), domain structure, electrical charge, hydrophilicity/hydrophobicity, linker molecules, interfacial adaptability or surface roughness are considered to determine the adherence of platelets [7, 9, 36, 39]. The binding of ligands to platelet receptors initiates a rapid activation process associated with a shape change and the degranulation of α and dense granula [34] so that the aggregation of further platelets occurs, then including also other blood cells ending up in thrombus growth. In arteries, the bulk of a thrombus often seems to be a mass of platelets, the so called white clot [33, 40]. Thrombus formation is considered to be the earliest of all possible complications of polymer-blood interactions and the most frequent unwanted major cardiovascular event [4]. Thrombi are able to occlude vessels or they detach forming emboli, possibly leading to life-threatening events. *Thrombogenicity* represents the most common cause of graft failure and of acute or late stent thrombosis [8, 20]. This characteristic of materials is usually assessed using platelet rich plasma. While the adsorption of proteins as well as the activation of platelets is a very rapid process (~180 ms) the stable adherence of platelets on implanted materials is in the order of seconds, the activation of the coagulation cascade with fibrin polymerization then takes at least 30 seconds and the formation of a stable clot 3 to 5 minutes [3, 6, 13, 26].

The thrombus growth can also be affected and be strengthened through a material-induced activation of the contact activation and the complement system involving further blood cells, particularly leukocytes [19, 25]. In a very short period of time platelet activation and the so called “contact activation” occur where high molecular weight kininogen (HMWK) and the activated factor XII interact and generate bradykinin. This will further enhance the activation of the coagulation cascade also implying the activation of the complement system. A more or less generalized complement activation can have serious consequences due to the reduced removal of antibody complexes, which generally need complement decoration for their elimination. And there are more mechanisms to burden the immune system beside the lack of

complement. Degradation particles from polymers can become coupled to haptens and lead to immune reactions. This very complex process of different interactions and feedback loops depends not only on platelets and can be defined as *hemocompatibility* of biomaterials, which has to be assessed using whole blood.

The current control of thrombus formation in patients with cardiovascular implants generally requires a systemic anticoagulation or dual anti-platelet therapy. Unfortunately, both therapies can be associated with severe complications, such as thrombocytopenia, neutropenia and hemorrhage [35]. Therefore, the development of a biomaterial that offers improved - if not perfect - thrombogenicity and hemocompatibility is still a clinical need.

Though the principles of thrombogenicity / hemocompatibility testing have been established in ISO 10993-4, they are generally considered as minimum requirements, and most groups perform supplementary tests. Moreover, the thrombogenicity or hemocompatibility of a material is not a defined physical quantity with an absolute value. Both characteristics can only be evaluated or classified with reference to known thrombogenic or hemocompatible surfaces (negative and positive control). Using such an approach, the materials studied can be categorized between these two extremes [21]. Reference materials may also be useful in identifying test procedures, which are most sensitive to describe thrombogenic or hemocompatible materials. The National Heart, Lung, and Blood Institute called for more reliable approaches in studying biomaterials and their interactions with blood in 1977. Three primary reference materials were selected for immediate testing: low-density polyethylene (LDPE), silica-free polydimethylsiloxane (PDMS), and fluorinated ethylene propylene (FEP). By 1980, recommendations for standardized methods and materials were published [17, 18]. In the early 1990s, the International Union for Pure and Applied Chemistry (IUPAC) started a second inititative “Interactions of Polymers with Living Systems.” The main topics were: (1) the interactions of materials with blood; (2) biocompatibility and inflammation; and (3) *in vivo* studies. Unfortunately, the results of these studies were never published. In 1992, the book “The reference materials of the European communities: results hemocompatibility tests” continued a number of publications released by the European Concerted Action „EUROBIOMAT – Hemocompatibility“ (Medical Research Program, Project: II.1.2/2, supported from 1988-1992) [24].

Though some uncertainties – which are still under discussion in the scientific community [7] – there are standardized procedures, which allow to categorize the thrombogenicity – using platelet rich plasma – or the hemocompatibility of biomaterials – using whole blood. However, the right choice of the anticoagulant with the appropriate dosage needs further

studies – although functional aspects of various anticoagulants have been reviewed extensively [5, 7, 12, 22, 27, 28]. Anticoagulation of blood samples is mandatory in order to avoid spontaneous coagulation processes, which are not induced by the implant material in the *in vitro* test system but already during the pre-processing of the blood sample. Commonly used anticoagulants are heparin for *in vitro* whole blood studies, sodium citrate for studies focussing on platelet-biomaterial interactions, or hirudin for complement studies [5, 11, 12, 21, 31].

Concluding it can be stated that thrombogenicity and hemocompatibility are two sides of the same coin but not identical characteristics of a material: on the one hand thrombi can grow without involving the contact or complement system and also without leukocytes. On the other hand, certain materials were reported to have a strong influence on contact activation and in consequence on the activation of complement and coagulation also ending up in thrombus growth. Future studies have to show which *in vitro* characteristic has a higher predictive value for thrombotic processes at the implant surface in vivo or if both characteristics have to be assessed.

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