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BloodSurf 2017: News from the Blood-Biomaterial Frontier.

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Abstract

From stents and large-diameter vascular grafts, to mechanical heart valves and blood pumps, blood-contacting devices are enjoying significant clinical success owing to the application of systemic antiplatelet and anticoagulation therapies. On the contrary, research into material and device hemocompatibility aimed at alleviating the need for systemic therapies has suffered a decline. This research area is undergoing a renaissance fueled by recent fundamental insights into coagulation and inflammation that are offering new avenues of investigation, the growing

recognition of the limitations facing existing therapeutic approaches, and the severity of the cardiovascular disorders epidemic. This Opinion article discusses clinical needs for hemocompatible materials and the emerging research directions for fulfilling those needs. Based on the 2017 *BloodSurf* conference that brought together clinicians, scientists, and engineers from academia, industry, and regulatory bodies, its purpose is to draw the attention of the wider clinical and scientific community to stimulate further growth.

Statement of Significance: The article highlights recent fundamental insights into coagulation, inflammation, and blood-biomaterial interactions that are fueling a renaissance in the field of material hemocompatibility. It will be useful for clinicians, scientists, engineers, representatives of industry and regulatory bodies working on the problem of developing hemocompatible materials and devices for treating cardiovascular disorders,

Keywords: Blood-biomaterial interactions, Hemocompatibility, Vascular implants, Platelets

1. Blood-Biomaterial Interactions: State of the Art and Clinical Needs

Existing biomaterials have failed to meet clinical needs in blood-contacting applications. After over 70 years of research, there is no formula for material design to ensure blood compatibility and many questions on how synthetic materials interact with blood remain unanswered. Although functional mechanical heart valves, large- diameter vascular prostheses, vascular stents, blood oxygenators, and blood pumps are now widely used clinically, the interactions between the synthetic materials used in these devices and blood invariably result in adverse, and often disastrous effects, including, perhaps most significantly, thrombotic and thrombo-embolic events.

Recently, it has become clear that elements of the inflammatory system are also involved, and that they impact the thrombotic process that has been the main focus of blood compatibility research for several decades. Research in the fields of biomaterials and surface science aimed at addressing thrombosis, thrombo-embolization, and the impact of inflammation on these phenomena has suffered a decline over the past several years and progress has slowed accordingly as discussed recently[1-4]. The perceived intractability of the blood compatibility problem may have contributed to this decline, thus motivating investigators to shift their efforts to other areas of biomaterials.

Currently, thrombotic complications from the use of artificial cardiovascular implants are managed pharmacologically using systemic antiplatelet and/or anticoagulation therapies (APT and ACT) [5-8]. The development of these therapies has allowed device-based cardiovascular interventions to become common, relatively safe treatment that are saving and improving the quality of millions of lives worldwide. Recent statistics on these medical successes were compiled in a previous review[4].

Systemic APT/ACT therapeutic approaches are widely used, and new opinions are continuously being developed[9,10]. They do, however, have well-recognized limitations such as risk of hemorrhage, costly long-term patient management, contraindications for many patients, as well as the persistence of thrombotic events in significant numbers of patients treated with ATP and/or ACT[2, 4, 6-8]. Furthermore, current pharmacological strategies have been ineffective in preventing the thrombotic occlusion of synthetic vascular grafts with diameters smaller than 6 mm[11], and this "barrier" remains to be broken. Despite recent developments with decellularized vascular matrices[12,13], the lack of synthetic small-diameter grafts constitutes a serious limitation on the treatment of small vessel occlusion, the current gold standard for the treatment of which remains autologous vein grafting-not an option for many patients.

Clearly, then, there is an urgent clinical need for blood-contacting devices that do not provoke thrombo-embolic events, and that function without the need for long-term systemic pharmacologic intervention. It follows that the essential element required to fill this need is the availability of non-thrombogenic biomaterials. Given the global prevalence of cardiovascular diseases, much of which can be alleviated using blood contacting devices[14], the resolution of the material hemo*in*compatibility problem should be seen as a high priority for the biomaterials research community. The challenge is great and the stakes are high, and it seems opportune at this time to call for a renaissance of research activity.

Recent years have witnessed significant advances in the understanding of hemostasis and thrombosis, platelet physiology, and the interactions between thrombotic and inflammatory responses, affording a fresh look at the problem of blood-biomaterial interactions. To catalyze these efforts, and to facilitate the integration of the new knowledge into materials research and development, a series of meetings, termed "BloodSurf," were initiated that connected clinicians, scientists, engineers, and representatives of industry and regulatory bodies—i.e. all of the partners necessary for effectively tackling the multifaceted problem of hemocompatibility. The first of these was held in Frejus, France in October of 2014, and the second took place at Clemson University (Clemson, SC, USA) in September of 2017[15], with some 20 invited speakers addressing the critical issues related to the design and testing of hemocompatible biomaterials (Figure 1). The highlights of this meeting are presented herein.

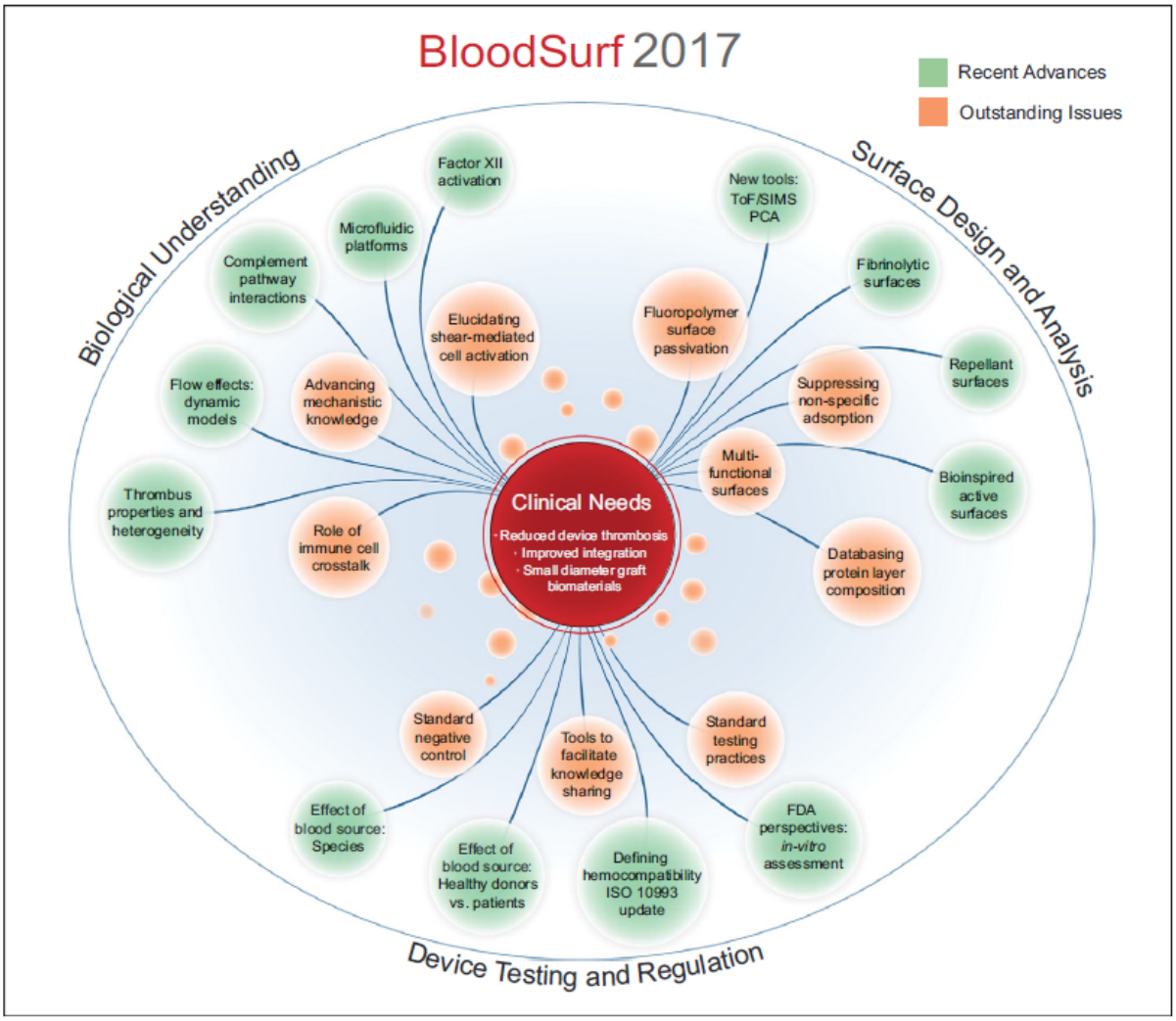

Figure 1: Key topics shaping blood-biomaterial interaction research, from recent advances to outstanding issues centered on the clinical needs.

2. Recent Advances

2.1 Probing the fundamentals of hemocompatibility

A major impediment to designing bloos-contacting devices that meet clinical needs has been the lack of a mechanistic understanding of blood-material interactions. Blood contains sensitive defense systems—hemostatic and inflammatory cascades—that react readily, and often catastrophically, to contact with "foreign" materials. A key recent realization is the significant degree to which hemostatic and inflammatory cascades are interconnected[1, 16-21]. Examples of their

interconnected nature include the contribution of surface-adhering leukocytes to material-induced thrombosis, and surface-activated platelets—to the material-induced inflammatory response,[22-26] as well as interactions between complement, platelets, and coagulation at the blood-biomaterial surface[17, 27-30]. Several studies furthermore point to the importance of platelet-monocyte interactions in connecting the inflammatory and hemostatic pathways[23, 31-35], suggesting that the role of these interactions in the response of blood to biomaterials should be examined in more detail. Thus, while platelets do occupy a central position at the intersection between hemostatic and inflammatory cascades, making them excellent targets for systemic therapies, materials or devices with the sole property of avoiding platelet activation are not likely to be fully blood compatible.

Another recent advance is the elucidation of the role of the contact (intrinsic) pathway of the coagulation cascade in thrombosis. Here, factor XII (FXII, Hageman factor) is activated through contact with surfaces, leading to thrombin generation, fibrin clot formation, and proinflammatory signaling. Activation of the contact pathway by negatively charged surfaces is the basis for the activated partial thromboplastin time (aPTT) test widely used in the clinic to evaluate coagulation[86]. Yet, the role of the contact pathway in physiological hemostasis is not significant. It turned out, however, to play a key role in pathological thrombosis[36,37], making FXIIa, as well as another factor of the contact pathway, FXIa, attractive targets for developing new anticoagulants[38,39]. Indeed, there is evidence of reduced bleeding risk when using FXIIa function-neutralizing antibodies in rabbits on cardiopulmonary bypass[40], and clinical trials are under way evaluating several different FXI-targeting anticoagulants in humans[39]; some are showing promising results (superior efficacy without increased bleeding risk), bit not yet in areas related to cardiovascular implants.

In summary, understanding in greater detail how the various elements of the hemostatic and inflammatory cascades fit together appears to be a major requirement for further progress in the development of clinically relevant biomaterials.

Fresh insight into blood-biomaterial interaction mechanisms is also expected from recent studies of the formation, organization, and dynamics of thrombi *in-vivo* using combined intravital microscopy/computational fluid dynamics techniques and systems approaches41-47. Studies of this kind highlight the crucial role of the damaged endothelium as the locus of thrombin generation, and show that platelet aggregation in primary hemostasis results in clots with layered structures consisting of a dense platelet "core" and a more loosely packed "shell". The higher platelet density near the core functions to restrict platelet agonist diffusion to the bulk blood flow. In the design of blood-contacting devices, the pertinent questions are not only why current biomaterial surfaces activate these innate systems, but how the mechanisms of activation differ in the presence of synthetic surfaces and what can be done to control or prevent these effects.

Also of fundamental importance in blood-biomaterial interactions are the flow conditions, and particularly the shear stresses to which the system is exposed. Shear stresses in blood circulation range from zero (stagnant blood) to low (venous flows) to high (arterials flows). There is evidence that the intrinsic clotting system is activated at low shear stress conditions while platelets are activated at high shear stress[48]. Thus the designation of a material as “blood compatible” should specify flow conditions, with some materials being relatively compatible in venous flows and others in arterial flows. These ideas fit with the recently articulated notion that biocompatibility is a function of the device and its biological environment, rather than of the device alone[49].

2.2 Assessing Material Hemocompatibility

Decades of research have failed to yield a specific, universally accepted, set of metrics for quantifying the extent to which a material is prothrombotic or proinflammatory in the clinical setting. The lack of such metrics severely limits design and development of new biomaterials. Recent studies have, however, led to some progress in this regard. The emerging consensus is that for material assessment, fresh whole human blood is preferable to platelet-rich plasma, other blood fractions, or animal blood[50]; that whole blood assays must be performed under flow (or at least agitation) to limit RBC sedimentation and to take account of the augmentation effect of red cell motions on platelet adhesion[51]; that EDTA or citrate anticoagulation can substantially suppress blood-material interactions due to their strong calcium chelating ability, and may not be suitable for material assessment in many cases; and that both surface-and fluid-phase components of the response need to be measured. Furthermore, using blood from apparently healthy subjects versus donors with coronary artery disease, who more closely reflect the clinical situation, may lead to an underestimation of material thrombogenicity[53,54].

With the multitude of factors at play, there is an acute need for systematic inter-laboratory comparisons aimed at standardizing experimental procedures for assessing material hemocompatibility. In this context, a recent randomized, double-blind multicenter round-robin study demonstrated that inter-laboratory consistency can be achieved in the *in-vitro* thrombogenicity testing of biomaterials when critical steps of the test protocol are stringently standardized, underscoring the need for more detailed characterization of blood before it is used in biomaterial assessment[52]. Harmonization of the donor group (apparently healthy adult human donors), the test setup, pre-analytical characterization and handling of the blood, blood preparation (e.g. centrifugation) and processing (e.g. storage time and total duration of the test), material

exposure time as well as selection of a specific anticoagulant and its dosage, are key protocol parameters.

Another pressing issue is the lack of an agreed-upon standard negative (minimally thrombogenic) control for assessing hemocompatibility. Currently, PTFE[55] is often used as a standard to compare materials, but its use as a negative control is not without controversy, and both clinicians and researchers agree that a better standard is needed for material comparison. Its design remains an open challenge.

It is important to note that development and standardization of the material assessment assays are relied upon by regulatory agencies, such as the Food and Drug Administration (FDA). Therefore, challenges facing biomaterial and material device thrombogenicity evaluation translate into a discord between the regulatory agencies and research. For example, ISO-10993-4[56] contains a wider selection of tests for blood-device interactions than are fully recognized by the FDA as of 2017.

2.3 Emerging Trends

Based on recent advances in the understanding of thrombosis, hemostasis, inflammation, and their interactions as referred to above, several key research directions were identified by the *BloodSurf* 2017 meeting. The aim is to provide clinicians with blood-contacting materials that manage material-induced adverse effects locally as they occur at/on the device instead of relying on systemic APT/ACT. This approach implies that the materials should be intrinsically non-thrombogenic and non-inflammatory.

First, investigations of blood-biomaterial interactions and the evaluation of material hemocompatibility *in-vitro* and *in-vivo* should exploit multiparametric assay[57] and systems

approaches[47] that are uniquely suitable for grasping the interconnected nature of the coagulation and inflammatory cascades.

In-vitro, there are examples of studies doing just that: Engberg et al. examined correlations between adsorption of proteins from plasma on polymeric materials and proinflammatory cytokine levels in whole blood exposed to the same materials. They found a correlation between the ratio of C4 to C4BP (C4 binding protein) on material surfaces and levels of several proinflammatory cytokines in whole blood exposed to these materials58; Donati et al., in the work presented at the meeting, examined correlations between hemostatic and inflammatory activation markers in whole human blood interacting with metallic biomaterials, and found a correlation between activation of platelets and leukocytes (monocytes and neutrophils) in the platelet-leukocyte aggregates and thrombin generation[59].

In-vivo, the development of assays targeting biomarkers for monitoring disease progression or predicting device-associated adverse effects is an area of research that is beyond the scope of this article. Some highlights include the early realization that in the case of bare metal stents, baseline or post-implantation levels of an inflammatory marker, C-reactive protein (CRP), and post-implantation levels of platelet activation, as measured by the expression of CD62P (P-selectin), were predictive of restenosis and stent thrombosis, respectively60-62. More recently, it was shown in heart failure patients on ventricular assist devices (VAD) that the assessment of inflammatory biomarkers (CRP, cytokines, leukocytes, and their microparticles) could allow disease progression and the effects of VAD therapy on the failing heart to be monitored, highlighting the need for evaluating inflammatory markers during VAD design in vitro[63]. Blood cell microparticles were reported as potentially relevant for predicting adverse events in VAD patients[64], with the caution that their analysis by flow cytometry presents certain challenges[65]. Last but not least, analysis of

microRNAs (miRNAs), which are emerging as a source of diagnostic and prognostic information in cardiovascular disorders[66], appears to also enable monitoring of the normalization of heart function in left ventricular assist device patients[67].

Second, research on so-called protein resistant surfaces (also referred to as antifouling, barrier, or passivating surfaces) suggests that protein-surface interactions are an inescapable reality, and cannot be completely suppressed[3, 68, 69]. Adsorption of proteins is the first significant event that occurs when blood contacts a foreign surface and the layer generally contains proteins which initiate the coagulation and thrombotic responses. Therefore, surface design must include the control of protein-surface interactions, with the aim of suppressing non-specific adsorption (including that of prothrombotic proteins such as von Willebrand factor and fibrinogen) as much as possible and promoting the adsorption of antithrombotic or "friendly" proteins such as albumin, antithrombin, plasminogen and tissue plasminogen activator. To this end, the interactions of the blood proteins at and with surfaces should be understood in much more detail, and sophisticated proteomics methods specific to this task should be developed. In this context, there is a need to compile an extensive database on the composition of the protein layers absorbed from blood for a broad range of materials and material types. It is also important to know how the identities and relative quantities of the adsorbed proteins vary over time, how the various proteins compete for different surface sites, whether proteins in the fluid phase can displace those on the surface, and whether adsorbed proteins have altered bioactivity. Some of these questions are currently under investigation in studies of nanoparticle-blood interactions, where a protein layer, called the "protein corona" forms rapidly on the surface of the particle[70-72].

Third, in contrast to most previous designs based on emulating a single biological function multi-functional surfaces, taking cues from the vascular endothelium, should be developed. It seems

unlikely that surfaces having only one kind of function or activity will be sufficiently blood compatible under all conditions and over long times as was already discussed above in the context of platelet-, leukocyte-, and complement-surface interactions. Vascular endothelium relies on multiple mechanisms to maintain blood fluidity: antifouling, anticoagulant, antiplatelet (nitric-oxide releasing), and pro-fibrinolytic (clot-destroying). Several such biomimetic strategies are under development, including super-slippery liquid perfluorocarbons[73], thrombin-responsive fibrinolytic hydrogels[74], and nitric-oxide-releasing coatings[75]. The challenge is to combine these and other functions in a single material. Much work remains to be done before these ideas can be translated into a successful clinical device.

2.4 New Tools for Studying Blood and Blood-biomaterial Interactions

Part of the *BloodSurf* 2017 meeting was dedicated to new and emerging methods for studying blood in the context of blood-biomaterial interactions and the interactions themselves. Highlights include *in vitro* analysis of coagulation at surfaces by videoimaging[76,77] and by fluorescence microscopy and image analysis in cuvettes and flow chambers[78]. The latter technique provides extremely detailed quantitative information on platelet localization and movement, clot contractility, and density within the thrombus in real time[79]. The adoption of this technique more broadly is likely to give new insights and to have a strong impact on our understanding of blood-biomaterial interactions. Another highlight is Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). An ultra-high vacuum surface analytical technique, ToF-SIMS has been used in the past to study blood proteins adsorbed on biomaterial surfaces[80, 81]. More recent biomedical application include analysis of biopsies and cancer cells[82, 83]. Finally, emerging microfluidics testing platforms to study blood-biomaterial interactions allow the exposure of blood to complex flow geometries, controlled shear stresses, different surface chemistries, and different

anticoagulation conditions, providing new opportunities for multiplexed analysis of the intricate interactions between blood and materials[84,85].

3. Summary and Outlook

Blood-contacting materials and devices currently used in patients with cardiovascular disorders rely on systemic anticoagulation and antiplatelet therapy to function and avoid thromboembolic problems. However, there is a recognized clinical need for materials and devices that manage adverse thrombotic and inflammatory effects locally at the device/blood interface, thereby minimizing the need for systemic therapy. Recent progress in the understanding of coagulation and thrombosis, and of the cross-talk between the hemostatic and inflammatory cascades, opens new avenues of investigation for studying blood-biomaterial interactions to address this challenge. The *BloodSurf* meetings were organized to catalyze interactions and collaborations among clinicians, scientists, and engineers from academia, industry, and regulatory agencies working towards this goal. In this contribution, we reflect on the state-of-the-art of the field and offer suggestions for future directions that emerged from the discussions. We invite the wider clinical and scientific community, industrial partners, and regulatory and funding agencies to join these efforts to solve the blood compatibility problem. The stakes in terms of human health, healthcare, and quality of life are too high for this problem to be ignored, as has largely been the case in recent years. A renewed assault armed with new knowledge and tools, some presently available, some yet to be discovered, is overdue.

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References:

1. M.B. Gorbet, M.V. Sefton, Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials* **25** (26) (2004) 5681-5703.

2. B.D. Ratner, The catastrophe revisited: Blood compatibility in the 21st century. *Biomaterials* **28** (34) (2007) 5144-5147.

3. X. Liu, L. Yuan, D. Li, Z. Tang, Y. Wang, G. Chen, H. Chen, J.L Brash, Blood compatible materials: state of the art. *J. Mater. Chem. B* Mater. Biol. Med. **2** (35) (2014) 5718-5738.

4. I. Reviakine, F. Jung, S. Braune, J.L. Brash, R. Latour, M. Gorbet, W. van Oeveren, Stirred, shaken, or stagnant: What goes on at the blood-biomaterial interface. *Blood Rev.* **31** (2017) 11 - 21.

5. J. Dunning, M. Versteegh, A. Fabbri, A. Pavie, P. Kolh, U. Lockowandt, S.A.M. Nashef, E.A.G. Comm, Guideline on antiplatelet and anticoagulation management in cardiac surgery. *Eur. J. Cardiothorac. Surg.* **34** (1) (2008) 73-92.

6. J. Papp, P. Kenyeres, K. Toth, Clinical importance of antiplatelet drugs in cardiovascular diseases. *Clin. Hemorheol. Microcirc.* **53** (1-2) (2013) 81-96.

7. C. Patrono, B. Coller, G.A. FitzGerald, J. Hirsh, G. Roth, Platelet-Active Drugs: The Relationships Among Dose, Effectiveness, and Side Effects: The Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* **126** (3 Supplement) (2004) 234S-264S.

8. J.-F. Tanguay, A.D. Bell, M.L. Ackman, R.D.C. Bauer, R. Cartier, W.-S. Chan, J. Douketis, A. Roussin, G. Schnell, S. Verma, G. Wong, S.R. Mehta, Focused 2012 Update of the Canadian Cardiovascular Society Guidelines for the Use of Antiplatelet Therapy. *Can. J. Cardiol.* **29** (11) (2013) 1334-1345.

9. M. Laine, F. Paganelli, L. Bonello, P2Y12-ADP receptor antagonists: days of future and past, World J. Cardiol. **8** (5) (2016) 327-332.

10. K.A. Packard, J.A. Campbell, J.T. Knezevich, E.M. Davis, Emerging antiplatelet therapy for coronary artery disease and acute coronary syndrome, Pharmacother. J. Human Pharmacol. Drug Ther. **32** (3) (2012) 244-273.

11. B.H. Walpoth, G.L. Bowlin, The daunting quest for a small diameter vascular graft. *Expert Rev Med Devices* **2** (6) (2005) 647-651.

12. A. Mahara, S. Somekawa, N. Kobayashi, Y. Hirano, Y. Kimura, T. Fujisato, T. Yamaoka, Tissue-engineered acellular small diameter long-bypass grafts with neointima-inducing activity, Biomaterials 58 (2015) 54-62.

13. K.H. Schneider, M. Enayati, C. Grasl, I. Walter, L. Budinsky, G. Zebic, C. Kaun, A.Wagner, K. Kratochwill, H. Redl, A.H. Teuschl. B.K. Podesser, H. Bergmeister, Acellular vascular matrix grafts from human placenta chorion: impact of ECM preservation on graft characteristics, protein composition and in vivo performance, Biomaterials 177 (2018) 14-26.

14. C.J. McAloon,L.M. Boylan, T. Hamborg, N. Stallard, F. Osman, P.B. Lim, S.A. Hayat, The changing face of cardiovascular disease 2000 - 2012: An analysis of the world health organisation global health estimates data. *Int. J. Cardiol.* **224** (2016) 256-264.

15. www.ireviakine.net/bloodsurf

16. L.L. Swystun, P.C. Liaw, Ther role of leykocytes in thrombosis, Blood **128** (6) (2016) 753-762

17. K.N. Ekdahl, Y. Teramura, O.A. Hamad, S. Asif, C. Duehrkop, K. Fromell, E. Gustafson, J. Hong, H. Kozarcanin, P.U. Magnusson, M. Huber-Lang, P. Garred, B. Nilsson, Dangerous liaisons: complement, coagulation, and kallikrein/kinin cross-talk act as a linchpin in the events leading to thromboinflammation. *Immunol. Rev.* **274** (1) (2016) 245-269.

18. A. Vieira-de-Abreu, R.A. Campbell, A.S. Weyrich, G.A. Zimmerman, Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin Immunopathol* **34** (1) (2012) 5-30.

19. B. Dahlback, Coagulation and inflammation--close allies in health and disease. *Semin Immunopathol* **34** (1) (2012) 1-3.

20. A.T. Nurden, Platelets, inflammation and tissue regeneration. *Thromb. Haemost.* **105** (Suppl 1) (2011) S13-S33.

21. E.I. Peerschke, W. Yin, B. Ghebrehiwet, Complement activation on platelets: implications for vascular inflammation and thrombosis. *Mol. Immunol.* **47** (13) (2010) 2170-2175.

22. M.B. Gorbet, M.V. Sefton, Leukocyte activation and leukocyte procoagulant activities after blood contact with polystyrene and polyethylene glycol–immobilized polystyrene beads. *J. Lab. Clin. Med.* **137** (5) (2001) 345-355.

23. M.B. Gorbet, M.V. Sefton, Material-induced tissue factor expression but not CD11b upregulation depends on the presence of platelets. *J. of Biomed. Mater. Res. A* **67A** (3) (2003) 792-800.

24. M. Fischer, C. Sperling, P. Tengvall, C. Werner, The ability of surface characteristics of materials to trigger leukocyte tissue factor expression. *Biomaterials* **31** (9) (2010) 2498-2507.

25. X.J. Chang, M. Gorbet, The effect of shear on invitro platelet and leukocyte material-induced activation. *J. Biomater. Appl.* **28** (3) (2013) 407-415.

26. V. Sreeramkumar, J.M. Adrover, I. Ballesteros, M.J. Cuartero, J. Rossaint, I. Bilbao, M. Nacher, C. Pitaval, I. Radovanovic, Y. Fukui, R.P. McEver, M.D. Filippi, I. Lzasoain, J. Ruiz-Cabello, A. Zarbock, M.A. Hidalgo, Neutrophils scan for activated platelets to initiate inflammation. *Science* **346** (6214) (2014) 1234-1238.

27. S. Huang, A.E. Engberg, N. Jonsson, K. Sandholm, J.A. Nicholls, T.E. Molines, K. Fromell, B. Nilsson, K.N. Ekdahl, Reciprocal relationship between contact and complement system activation on artificial polymers exposed to whole human blood. *Biomaterials* **77** (2016) 111-119.

28. K.N. Ekdahl, S. Huang, B. Nilsson, Y. Teramura, Complement inhibition in biomaterial- and biosurface-induced thromboinflammation. *Semin. Immunol.* **28** (3) (2016) 268-277.

29. B. Nilsson, K.N. Ekdahl, T.E. Mollnes, J.D. Lambris, The role of complement in biomaterial-induced inflammation. *Mol. Immunol.* **44** (1-3) (2007) 82-94.

30. M.M. Markiewski, B. Nilsson, K.N. Ekdahl, T.E. Mollnes, J.D. Lambris, Complement and coagulation: strangers or partners in crime? *Trends Immunol.* **28** (4) (2007) 184-192.

31. J.M. van Gils, J.J. Zwaginga, P.L. Hordijk, Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. *J. Leukocyte Biol.* **85** (2) (2009) 195-204.

32. J. Sarma, C.A. Laan, S. Alam, A. Jha, K.A. Fox, I. Dransfield, Increased platelet binding to circulating monocytes in acute coronary syndromes. *Circulation* **105** (18) (2002) 2166-2171.

33. J.E. Freedman, J. Loscalzo, Platelet–Monocyte Aggregates: Bridging Thrombosis and Inflammation. *Circulation* **105** (18) (2002) 2130-2132.

34. A.D. Michelson, M.R. Barnard, L.A. Krueger, C.R. Valeri, M.I. Furman, Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation

than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* **104** (13) (2001) 1533-1537.

35. M.I. Furman, S.E. Benoit, M.R. Barnard, C.R. Valeri, M.L. Borbone, R.C. Becker, H. B. Hechtman, A.D. Michelson, Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *J. Am. Coll. Cardiol.* **31** (2) (1998) 352-358.

36. T. Renné, A.H. Schmaier, K.F. Nickel, M. Blombäck, C. Maas, In vivo roles of factor XII. *Blood* **120** (22) (2012) 4296-4303.

37. A. Matafonov, P.Y. Leung, A.E. Gailani, S.I. Grach, C. Puy, Q. Cheng, M.-F. Sun, O.J.T. McCarty, E.I. Tucker, H. Kataoka, T. Renné, J.H. Morrissey, A. Gruber, D. Cailani, Factor XII inhibition reduces thrombus formation in a primate thrombosis model, *Blood* **123** (11) (2014) 1739-1746.

38. J.C. Fredenburgh, P.L. Gross, J.I. Weitz, Emerging anticoagulant strategies, *Blood* **129** (2) (2017) 147-154.

39. J.I. Weitz, N.C. Chan, Advances in Antithrombotic Therapy, *Ateriоscler. Thromb. Vasc. Biol.* **39** (1) (2019) 7-12.

40. M. Larsson, V. Rayzman, M.W. Nolte, K.F. Nickel, J. Björkqvist, A. Jämsä, M.P. Hardy, M. Fries, S. Schmidbauer, P. Hedenqvist, M. Broomé, I. Pragst, G. Dickneite, M.J. Wilson, A.D. Nash, C. Panousis, T. Renné, A Factor XIIa inhibitory antibody provides thromboprotection in extracorporal circulation without increasing bleeding risk, *Sci. Transl. Med.* **6** (222) (2014) 222ra17.

41. J.D. Welsh, R.W. Muthard, T.J. Stalker, J.P. Taliaferro, S.I. Diamond, I.F. Brass, A systems approach to hemostasis: 4. How hemostatic thrombi limit the loss of plasma-borne molecules from the microvasculature. *Blood* **127** (12) (2016) 1598-1605.

42. T.J. Stalker, J.D. Welsh, M. Tomaiuolo, J. Wu, T.V. Colace, S.I. Diamond, I.F. Brass, A systems approach to hemostasis: 3. Thrombus consolidation regulates intrathrombus solute transport and local thrombin activity. *Blood* **124** (11) (2014) 1824-1831.

43. M. Tomaiuolo, T.J. Stalker, J.D. Welsh, S.I. Diamond, T. Sinno, L.F. Brass, A systems approach to hemostasis: 2. Computational analysis of molecular transport in the thrombus microenvironment. *Blood* **124** (11) (2014) 1816-1823.

44. J.D. Welsh, T.J. Stalker, R. Voronov, R.W. Mithard, M. Tomaiuolo, S.L. Diamond, L.F. Brass, A systems approach to hemostasis: 1. The interdependence of thrombus architecture and agonist movements in the gaps between platelets. *Blood* **124** (11) (2014) 1808-1815.

45. T.J. Stalker, E.A. Traxler, J. Wu, K.M. Wannemacher, S.I. Cermignano, R. Voronov, S.L. Diamond, L.F. Brass, Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. *Blood* **121** (10) (2013) 1875-1885.

46. M. Mirramezani, B.A. Herbig, T.J. Stalker, I. Nettey, M. Cooper, J.W. Weisel, S.L. Diamond, T. Sinno, L.F. Brass, S.C. Shadden, M. Tomaiuolo, Platelet packing density is

an independent regulator of the hemostatic response to injury. *J Thromb Haemost* **16** (2018) 973-983.

47. S.L. Diamond, Systems biology of coagulation. *J Thromb Haemost* **11** (2013) 224-232.

48. L.A. Harker, S.J. Slichter, Platelet and fibrinogen consumption in man. *N. Engl. J. Med.* **287** (20) (1972) 999-1005.

49. D.F. Williams, There is no such thing as a biocompatible material. *Biomaterials* **35** (38) (2014) 10009-10014.

50. Q. Lu, B.V. Hofferbert, G. Koo, R.A. Malinauskas, In vitro shear stress-induced platelet activation: sensitivity of human and bovine blood. *Artif. Organs* **37** (10) (2013) 894-903.

51. K.H. Keller, Effect of fluid shear on mass transport in flowing blood, *Fed. Proc.* **30** (5) (1971) 1591-1599.

52. S. Braune, C. Sperling, M.F. Maitz, U. Steinseifer, J. Clauser, B. Hiebl, S. Krajewski, H.P. Wendel, F. Jung, Evaluation of platelet adhesion and activation on polymers: Round-robin study to assess inter-center variability. *Colloids Surf. B. Biointerfaces* **158** (Supplement C) (2017) 416-422.

53. S. Braune, M. Gross, M. Walter, S. Zhou, S. Dietze, S. Rutschow, A. Lendlein, C. Tschope, F. Jung, Adhesion and activation of platelets from subjects with coronary artery disease and apparently healthy individuals on biomaterials. *J Biomed Mater Res B Appl Biomater* **104** (1) (2016) 210-217.

54. K. Vanschoonbeek, M.A. Feijge, R.J. Van Kampen, H. Kenis, H.C. Hemker, P.L. Giesen, J.W. Heemskerk, Initiating and potentiating role of platelets in tissue factor-induced thrombin generation in the presence of plasma: subject-dependent variation in thrombogram characteristics. *J Thromb Haemost* **2** (3) (2004) 476-484.

55. L.M. Szott, C.A. Irvin, M. Trollsas, S. Hossainy, B.D. Ratner, Blood compatebility assessment of polymers used in drug eluting stent coatings, *Biointerphases* **11** (2) (2016) 029806.

56. ISO 10993-4 DIS, future replacement for ISO 10993-4 (2002; Amended 2006), Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood. Association for the Advancement of Medical Instrumentation. (2015).

57. M. Weber, H Steinie, S. Golombek, I. Hann, C. Schlensak, H.P. Wendel, M. Avci-Adali, Blood-contacting biomaterials. In vitro evaluation of the hemocompatibility, *Front. Bioeng. Biotechnol.* **6** (2018).

58. A.E. Engberg, P.H. Nilsson, S. Huang, K. Fromell, O.A. Hamad, T.E. Mollnes, J.P. Rosengren-Holmberg, K. Sandholm, Y. Teramura, I.A. Nicholls, B. Nilsson, K.N. Ekdahl, Prediction of inflammatory responses induced by biomaterials in contact with human blood using protein fingerprint from plasma. *Biomaterials* **36** (2015) 55-65.

59. A. Donati, A multilevel study of platelet activation at biomaterial surface: from evaluating to controlling hemocompatibility Ph.D. Thesis, Faculty of Chemistry and Biosciences, Karlsruhe Institute of Technology, Karlsruhe, 2016.

60. A. Gaspardone, F. Crea, F. Versaci, F. Tomai, A. Pellegrino, L. Chiariello, P.A. Gioffre, Predictive value of C-reactive protein after successful coronary-artery stenting in patients with stable angina, *Am. J. Cardiol.* **82** (4) (1998) 515-518.

61. G. Niccoli, R. Montone, G. Ferrante, F. Crea, The evolving role of inflammatory biomarkers in risk assessment after stent implantation, *J. Am. Coll. Cardiol.* **56** (22) (2010) 1783-1793.

62. M. Gawaz, F.J. Neumann, I. Ott, A. May, S. Rudiger, A. Schomig, Role ofactivation-dependent platelet membrane glycoproteins in development of subacute occlusive stent thrombosis, *Coron. Artery Dis.* **8** (3-4) (1997) 121-128.

63. G. Radley, I.L. Pieper, S. Ali, F. Bhatti, C.A. Thornton, The inflammatory response to ventricular assist devices, *Front. Immunol.* **9** (2018) 2651.

64. W.P. Jeske, J.M. Walenga, B. Menapace, J. Schwartz, M. Bakhos, Blood cell microparticles as biomarkers of hemostatic abnormalities in patients with implanted cardiac assist devices, *Biomark. Med.* **10** (10) (2016) 1095-1104.

65. E. Shantsila, S. Montoro-Garcia, P. Gallego, G.Y. Lip, Circulating microparticles: challenges and perspectives of flow cytometric assessment, *Thromb. Haemost.* **111** (6) (2014) 1009-1014.

66. C. Schulte, M. Karakas, T. Zeller, MicroRNAs in cardiovascular disease - clinical application, *Clin. Chem. Lab. Med.* **55** (5) (2017) 687-704.

67. S.J. Matkovich, D.J. Van Booven, K.A. Youker, G. Torre-Amione, A. Diwan, W.H. Eschenbacher, L.E. Dorn, M.A. Watson, K.B. Margulies, G.W. Dorn, Reciprocal regulation of myocardial micrornas and messenger RNA in human cardiomyopathy and reversal of the microrna signature by biomechanical support, *Circulation* **119** (9) (2009) 1263-1271.

68. P.W. Serruys, M.J. Kutryk, A.T. Ong, Coronary-artery stents. *N. Engl. J. Med.* **354** (5) (2006) 483-495.

69. T. Riedel, Z. Riedelova-Reicheltova, P. Majek, C. Rodriguez-Emmenegger, M. Houska, J.E. Dyr, E. Brynda, Complete identification of proteins responsible for human blood plasma fouling on poly(ethylene glycol)-based surfaces. *Langmuir* **29** (10) (2013) 3388-3397.

70. C. Corbo, R. Molinaro, A. Parodi, N.E.T. Furman, F. Salvatore, E. Tasciotti, The impact of nanoparticle protein corona on cytotoxicity, immunotoxicity and target drug delivery. *Nanomedicine* **11** (1) (2016) 81-100.

71. C.C. Fleischer, C.K. Payne, Nanoparticle-Cell Interactions: Molecular Structure of the Protein Corona and Cellular Outcomes. *Acc. Chem. Res.* **47**(8) (2014) 2651-2659.

72. T. Cedervall, I. Lynch, S. Lindman, T. Berggard, E. Thulin, H. Nilsson, K.A. Dawson, S. Linse, Understanding the nanoparticle-protein corona using methods to quantify exchange

rates and affinities of proteins for nanoparticles, Proc. Natl.Acad. Sci. U.S.A. 104 (7) (2007) 2050-2055.

73. D.C. Leslie, A. Waterhouse, J.B. Berthet, T.M. Valentin, A.L. Watters, A. Jain, P. Kim, B.D. Hatton, A. Nedder, K. Donovan, E.H. Super, C. Howell, C.P. Johnson, T.L. Vu, D.E. Bolgen, S. Rifai, A.R. Hansen, M. Alzenberg, M. Super, J. Alzenberg, D.E. Ingber, A bioinspired omniphobic surface coating on medical devices prevents thrombosis and biofouling. *Nat Biotech* **32** (11) (2014) 1134-1140.

74. W.J. Zhan, X.J. Shi, Q. Yu, Z.I. Lyu, L.M. Cao, H. Du, Q. Liu, X. Wang, G.J. Chen, D. Li, J.L. Brash, H. Chen, Bioinspired Blood Compatible Surface Having Combined Fibrinolytic and Vascular Endothelium-Like Properties via a Sequential Coimmobilization Strategy. *Adv. Funct. Mater.* **25** (32) (2015) 5206-5213.

75. E.J. Brisbois, T.C. Major, M.J. Goudie, R.H. Bartlett, M.E. Meyerhoff, H. Handa, Improved hemocompatibility of silicone rubber extracorporeal tubing via solvent swelling-impregnation of S-nitroso-N-acetylpenicillamine (SNAP) and evaluation in rabbit thrombogenicity model. *Acta Biomater.* **37** (2016) 111-119.

76. H. Fink, I. Faxalv, G.F. Molnar, K. Drotz, B. Risberg, T.L. Lindahl, A. Sellborn, Real-time measurements of coagulation on bacterial cellulose and conventional vascular graft materials, *Acta Biomater.* **6** (3) (2010) 1125-1130.

77. L. Faxalv, T. Ekblad, B. Liedberg, T.L. Lindahl, Blood compatebility of photografted hydrogel coatings, *Acta Biomater.* **6** (7) (2010) 2599-2608.

78. K. Tunstomer, L. Faxalv, N. Boknas, T.L. Lindahl, Quantification of platelet contractile movements during thrombus formation, *Thromb. Haemost.* **118** (9) (2018) 1600-1611.

79. K.Claesson, T.L. Lindahl, L. Faxalv, Counting the platelets: a robust and sensitive quantification method for thrombus formation. *Thromb. Haemost.* **115** (6) (2016) 1178-1190.

80. M.S. Wagner, D.G. Castner, Characterization of adsorbed protein films by time-of-flight secondary ion mass spectrometry with principal component analysis. *Langmuir* **17** (15) (2001) 4649-4660.

81. R. Tejero, P. Rossbach, B. Keller, E. Anitua, I. Reviakine, Implant surfaces activated with plasma rich in growth factors: time of flight secondary ion mass spectrometry and principal component analysis study. *Langmuir* **29** (2013) 902 - 912.

82. M.A.Robinson, D.J. Graham, F. Morrish, D. Hockenbery, L.J. Gamble, Lipid analysis of eight human breast cancer cell lines with ToF-SIMS. *Biointerphases* **11** (2) (2015) 02A303.

83. L.J. Gamble, D.J. Graham, B. Bluestein, N.P. Whitehead, D. Hockenbery, F. Morrish, P. Porter, ToF-SIMS of tissues: "Lessons learned" from mice and women. *Biointerphases* **10** (1) (2015) 1-9.

84. S. Zhu, B.A. Herbig, R. Li, T.V. Colace, R.W. Mithard, K.B. Neeves, S.L. Diamond, In microfluidico: Recreating in vivo hemodynamics using miniaturized devices. *Biorheology* **52** (5-6) (2015) 303-318.

85. T.V. Colace, G.W. Tormoen, O.J.T. McCarty, S.L. Diamond, Microfluidics and Coagulation Biology. *Annu. Rev. Biomed. Eng.* **15** (2013) 283-303.

86. R.D. Langdell, R.H. Wagner, K.M. Brinkhous, Effect of antihemophilic factor on one-stage clotting tests: a presumptive test for hemophilia and a simple one-stage antihemophilic factor assay procedure, J. Lab Clin. Med 41 (4) (1953) 637-647.