Hemocompatibility of Stents

W. VAN OEVEREN
Blood Interaction Research, University of Groningen, The Netherlands

P. SCHÖN, C.A. MAIJERS
HaemoProbe BV, Groningen, The Netherlands

S.H. MONNINK, A.J. VAN BOVEN
Department of Cardiology, Academic Hospital Groningen, The Netherlands

Summary
There are many challenges in the design of urinary, tracheal and vascular stents. The tissue-biomaterial and blood-material interfaces are particularly important for optimization of vascular stents. To gain insight into the hemocompatibility of vascular implants, the International Organisation for Standardization (ISO) is preparing a new standard test format according to which vascular devices must be tested. One of the main requisites for a biocompatibility test is that it should be able to predict the biological response for a time span often exceeding the test period. Therefore, the new standards to obtain FDA and/or CE approval will be based on up-to-date knowledge of the systems involved in blood activation. The most important issues are complement activation, interaction with platelets, leukocyte activation and thrombosis. The impact of these issues is examined here. From what is known to date about blood activation by biomaterials it can be concluded that strategies to improve the quality of stents should be directed at the reduction of platelet deposition and (shear-stress-induced) activation, the reduction of leukocyte activation and the reduction of growth factor release.

Key Words
Biomaterials, hemocompatibility, certification, blood activation, coronary stenting

Introduction
Today, biomaterials have become indispensable in modern medicine and state-of-the-art patient care. World-wide, over 500 million medical devices are used yearly with estimated costs over 90 billion US dollars. Permanent implantation of biomaterials is becoming increasingly common in modern medical practice. Urinary, tracheal and arterial blood vessel stents have become of particular therapeutic importance. The tissue-biomaterial and blood-material interfaces must be understood to ensure a benevolent interaction between the implant and the patient. These interactions are of critical importance with vascular stents.

Biocompatibility
Five aspects of the response of host tissue (including blood) to biomaterials deserve attention: carcinogenicity, sensitization, cytotoxicity, inflammatory reaction and coagulation (Figure 1). Since after implantation a biomaterial remains in permanent contact with host tissue, implant devices must satisfy most of the criteria for biocompatibility, including in vitro testing and animal implantation tests. Therefore, the International Organization for Standardization (ISO) is preparing a standard test format according to which devices must be tested. One of the main challenges facing any biocompatibility test is that it should be able to predict the biological response for a time span often exceeding the test period.

Hemocompatibility
To understand the meaning and possible impact of laboratory test results for short and long term effects
after implantation a brief introduction to the systems involved in blood activation is useful. First, it must be noted that hemostasis after implantation is a complex system. Not only is the biocompatibility of the material important, but also its geometry and other factors, such as the patient-specific blood composition (variable concentrations of prothrombotic or pro-inflammatory mediators), the condition of the vessel wall, and the blood flow through the implant. Of particular importance is to understand the role of endothelial cells in controlling hemostasis. Prior to activation or injury, endothelial cells have a number of antithrombotic functions which are not provided by standard biomaterials (Figure 2). Endothelial cells inhibit platelet aggregation by means of prostaglandin and nitric oxide release; they indirectly activate fibrinolysis by binding of plasminogen activator inhibitor (PAI), an inhibitor of the fibrinolytic system, and they have anticoagulant properties which are mediated by thrombomodulin, protein C and heparan sulphate on the cell surface.

After intravascular implantation of a device endothelial cells are damaged or removed, which results in loss of platelet-inhibitor release, inhibition of fibrinolysis and exposure of tissue factor, the most vigorous activator of the coagulation system. Moreover, the subendothelial matrix may be exposed, which contains collagen, vitronectin and other platelet-binding proteins. Clearly, even the most inert biomaterial implant may induce a thrombogenic response, solely by this traumatic mechanical effect on the blood vessel wall. If, in addition, a biomaterial implant provides a matrix which supports thrombus formation and adhesion on its surface, a rapid thrombotic reaction will occur. Thus, today all stent implantations are performed in the presence of inhibitors of the clotting system and of platelets.

Thrombus formation on the biomaterial at the injured site is an acute event. Long-term stent patency also involves the response of the intima of the adjacent vessel area. This response is in part influenced by an inflammatory reaction to the biomaterial. Activated neutrophils can markedly reduce the cell proliferation-controlling capacity of endothelial cells. Therefore, the inflammatory reaction to the biomaterial, as estimated by the extent of complement activation and the levels of neutrophil release products, is an important determinant for the success of an implant.

**In vitro and ex vivo Hemocompatibility Tests**

Since the introduction of hemocompatibility testing only two types of tests have been required for obtaining FDA or CE registration: the hemolysis and plasma-clotting tests. Hemolysis of red blood cells is a measure of the cytotoxicity of biomaterial towards these cells. Cytotoxicity results in rupture of red-blood-cell membranes and release of hemoglobin. Photospectrometry of free hemoglobin allows estimation of the extent of cytotoxicity. Unfortunately, this test has little to do with thrombosis or inflammation and a failure to comply to this test can only be found
under extreme conditions or after the use of notably toxic materials, such as copper and remnants of acids, softeners, glues and detergents used during the production of the material.

The second obligatory test is based on in vitro activation of the clotting system in plasma. Most often the material is incubated for 30 - 60 minutes in citrated plasma. After the addition of Ca\(^{2+}\)-ions and components that mimic activated platelet membranes, a reduced clotting time is a measure for thrombogenicity of the test material. In this test, glass is most often used as positive reference, since it markedly activates the clotting system. While this test gives some information about the activation of the coagulation cascade under static conditions, the results obtained do not accurately reflect the dynamic conditions present in vivo during the interplay between the biomaterial, the coagulation pathway, the blood cells and the vessel wall. Therefore, taking the above described systems involved in blood activation into account, assessment of complement activation and the extent of platelet interaction, leukocyte activation and thrombosis will be part of the new standards to obtain FDA and/or CE approval for intravascular implants.

Complement activation is most often characterized by generation of the complement byproduct C3a. This test is unreliable because of the extensive spontaneous hydrolysis of C3 under the test conditions in vitro in the absence of inhibitors. Recently, new tests based on the formation of C3- and C5-convertase on the biomaterial have been developed.

Platelet interaction with the biomaterial may be considered of more clinical relevance than activation of the clotting system under conditions of arterial blood flow. Platelets are capable of interacting during split-second contact, whereas clotting requires 10 seconds or more. Platelet tests include adhesion, aggregation, release reaction and whole blood function tests. Although sometimes difficult to achieve, platelet tests have to be performed with fresh intact platelets. Consideration should thus be given to donor-dependent differences in responsiveness. Only results within one experiment allow comparisons between different materials.

The release of leukocyte-specific enzymes is a good measure for leukocyte activation. However, the low number of leukocytes in blood renders this method less sensitive for samples of blood which have been in contact with the biomaterial. An alternative, more sensitive way to determine the interaction of leukocytes with biomaterials is to perform measurements of immunologic markers expressed on the leukocytes in circulation and on those bound to the biomaterial.

Thrombosis is considered as an overall test to predict the outcome of an implant after a period of exposure to circulating blood. Initially "thrombosis" was tested in animal models; however, recently, in vitro circulation models were developed that can be used as long as the physiological conditions (temperature, flow) are comparable to the anticipated environment of the implant. If human blood is used in such an in vitro circulation model, more details of blood activation can be obtained due to the availability of human-specific immunoassays. Moreover, the test conditions can be kept constant, which allows comparison between different implants within one series of experiments.

While the clotting test mentioned above is poorly reproducible, the new category of tests, which must be applied in the near future in order to obtain FDA or CE approval, are as yet even more difficult to standardize. Only specialized laboratories will be able to perform these tests with satisfying reproducibility. However, it is clear that more extensive testing is needed to gain a useful insight into the hemocompatibility of biomaterials.

Effects of Stent Implantation

To provide a picture of the complexity of hemostasis in the setting of stent implantation, the individual subprocesses are examined below. These include: 1) biocompatibility of stents; 2) the role of thrombin; 3) platelet response and 4) intimal proliferation.

1) Biocompatibility of Stents

Stents were initially made from stainless steel, which is known to be a thrombogenic material. More recently, some stents are made from tantalum, which has the advantage of a higher radio-opacity than stainless steel. Moreover, tantalum was initially reported as being less thrombogenic than stainless steel [1]. Other studies, however, showed a high incidence of thrombotic stent occlusion [2]. In baboons, no difference was found between stainless steel and tantalum with regard to platelet and fibrin deposition [3]. We found, after in vitro circulation of human blood through stainless steel and tantalum stents, a higher deposition of platelets and
leukocytes on the tantalum surface. Further development of stent material resulted in construction of stents from nickel-titanium alloys (nitinol) and the coating of stents with heparin or silicon-carbide. Nitinol has been applied for blood-clot filters in the caval vein. Its hemocompatibility was reported to be similar to that of stainless steel [4]. However, since some nickel is exposed to blood, concerns exist with regard to corrosion of nitinol [5].

Heparin-coated stents are not commonly employed despite the potential inhibitory effect of the heparin on the coagulation system. As discussed above, initial platelet activation occurs quickly at high flow conditions in arteries, whereas coagulation is a secondary factor contributing to thrombosis. Therefore, heparin coating appears to be less effective for this application. Platelet binding to a heparinized surface might be increased [6]. Surface-released heparin might become available in the circulation. Taking the pharmacologic effects of heparin into account, heparin-coated devices would have to be considered as drug/biomaterial combinations and not as biomaterial alone for the purpose of FDA and/or CE approval. However, coating stents with heparin-like molecules could be beneficial, since these molecules may adsorb growth factors [7], thus reducing the proliferative response of the vessel wall and enhancing the long term patency.

Coating stents with Silicon Carbide seems to be a promising alternative to improve the biocompatibility of metal stents. Silicon Carbide's primary effect is a reduction of platelet and leukocyte binding, paralleled by a reduction in activation of these cells [8][21]. Furthermore additional binding of heparin or other drugs on the silicon carbide is possible, which is thought to reduce the restenosis.

2) The role of Thrombin

Once thrombin is formed in the coagulation cascade, either on the damaged vessel wall after tissue factor exposure, on the biomaterial surface or on an activated-platelet surface, it has a great impact on the subsequent outcome of stent implantation. Thrombin can bind to the material surface or to surface-bound fibrin [9]. Thus, thrombin may further promote coagulation, activate platelets and serve as a binding ligand for platelets on the biomaterial surface or on endothelial cells [10]. Thrombin also enhances the proliferation of vascular smooth muscle cells and stimulates the release of platelet-derived growth factor and fibroblast growth factor [11]. Neutrophil recruitment by thrombin [12] may have consequences for endothelial cell-mediated down-regulation of proliferation and may thus result in further growth of vascular smooth muscle cells. Today, new thrombin generation tests are being developed to determine the levels of this pivotal enzyme.

3) Platelet Response

Platelets possess a number of receptors for adhesive proteins, such as fibrinogen, von Willebrand factor (vWF), fibronectin, vitronectin, laminin and collagen [13]. It appears that platelets from patients with arteriosclerotic diseases possess a higher number of exposed adhesive receptors [14] and also have increased receptors for platelet activators such as thromboxane A2 [15]. Recent studies in our laboratory indicated a reduced effect of acetylsalicylic acid (ASA) on the inhibition of platelet functions in arteriosclerotic patients as compared to healthy individuals. Moreover, in patients, a treatment with ASA may have an increasing effect on platelet adhesion to endothelial cells by the inhibition of prostacyclin production [16]. Thus, it seems that patients requiring a stent are also at higher risk to adverse platelet responses at the implant site.

On the surfaces of subendothelium and stent, platelets adhere via their receptors, an event which is often measured by photographic or immunologic methods. Platelet activation and the release of platelet products in the circulation may also be considerable by shear stresses imposed on the blood. At a shear stress of 120 dyne/cm² vWF multimers are released and the adhesive GpIIb/IIIa receptors are expressed on the platelet membrane [17]. In the presence of ADP, which is released from damaged erythrocytes, the vWF multimers bind immediately to the GpIIb/IIIa receptors, thus ensuring irreversible platelet adhesion after contact. It is conceivable that these events take place on the damaged inner site of a stented vessel, resulting in deposition of platelets distal to the stent. Stent geometry might be expected to play an important role in this respect and attention must be given to flow dynamics in stented vessels in relation to stent design. The pivotal role of platelets in the development of smooth muscle cell proliferation was established long ago in experimental studies in rabbits [18].
4) Intimal Proliferation

Intimal hyperplasia is considered to be the leading cause of late occlusion after stenting [19]. Various studies have shown the role of growth factors in the onset of proliferation, as being part of the normal response to repair injury of the vessel wall [20]. An excess of growth factor as well as a sustained effect and/or an impaired control of this event may lead to hyperplasia and its deleterious consequences.

As discussed above all these conditions exist to some extent in patients undergoing stent implantation. The processes that eventually lead to intimal hyperplasia primarily take place in the first few days after implantation. This indicates that the first reactions to the stent as a foreign body are certainly of importance for the late outcome, even if a rapid thrombotic reaction is prevented.

Conclusion

Hemocompatibility of stents after implantation is a complex cascade of events, depicted in Figure 3, some of which can be controlled by the manufacturer, such as the surface characteristics and geometry of the stent, while some can only be manipulated medically, such as the constitution of the patients blood. Clearly, the existing tests of hemocompatibility, the hemolysis test and the clotting test, are not sufficient to anticipate the outcome after stent implantation in patients.

Strategies to improve the quality of stents should be directed at the reduction of platelet deposition and (shear-stress-induced) activation, the reduction of leukocyte activation (by determining complement activation) and the reduction of growth factor release in order to prevent excessive proliferation of smooth muscle cells.

References


[17] Moake JL; Turner NA, Strahopoulos NA, Nolasco L, Hellums JD. Shear-induced platelet aggregation can be mediated by vWF release from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. Blood 1988; 1366-74.


