The Interactions of blood with polymeric materials
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In modern medicine devices or systems for transportation of large quantities of blood are frequently used. Examples are heart/lung machines to be used during complicated heart surgery, dialysis systems to detoxify blood from patients suffering from kidney problems, vessel prostheses for replacement of diseased blood vessels, etc. These devices have in common that a large surface area of material interacts with blood. However, the blood material interaction is not fully understood. This blood material interaction is called the compatibility of blood with materials. To improve the knowledge of blood compatibility, it is necessary that more integration should take place between the materials science, biomedical technology and hematology. Laboratory evaluation of proposed blood compatible materials should take place with the use of a series of standardized tests. These standardized tests should comprise: protein adsorption, contact activation, activation of the intrinsic coagulation, and adhesion and activation of thrombocytes. Preferably the in vitro tests should have a predictive value for the in vivo behaviour of the materials.

In this dissertation the development and application of tests to be used for the assessment of blood material interaction are described. When in one test system different blood components can be measured, it is possible to establish relations between the test results obtained under the same conditions. Before going into subjects regarding newly developed tests and the application of these test systems, various aspects of blood material interactions are reviewed in chapter 1. These interactions include blood protein adsorption, contact activation, intrinsic coagulation and fibrinolysis, platelet adhesion and - activation, and the role of natural inhibitors in blood. This knowledge serves as a background for the development of in vitro test methods to study the blood material interaction and for the evaluation of heparin coated materials in vivo.

In the chapters 2 till 5, two novel in vitro test methods are described to examine the blood compatibility of various polymeric materials. The in vitro experiments are carried out with standardized test plates, incubation time and temperature, and a multi protein system of 25% citrated plasma obtained from healthy donors. The interaction of plasma with three commercially available polymeric materials and glass (chosen as reference material) was measured. These materials are, high density polyethylene (HDPE), polytetrafluoroethylene (PTFE) and polydimethylsiloxane (PDMS) which are widely used in medicine.

In chapters 2 and 3, the first part of the intrinsic coagulation pathway, also known as the contact system, is described. This pathway involves the generation of factor XII fragments and kallikrein activity which can be measured by means of a simple chromogenic assay. The assay is based on the conversion of the substrate Z-Lys-Phe-Arg-pNA-2HCl by human factor XII fragments and kallikrein. Due to the activity of factor XII fragments and kallikrein, the -pNA part is cleaved from the Z-Lys-Phe-Arg, which results in a yellow colour measurable by optical instruments. To discriminate factor XII from kallikrein, aprotinin was added to one of two complementary plasma samples to selectively inhibit the kallikrein activity. It was concluded that the polymeric materials, which were all characterized as hydrophobic materials, induce...
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For the assessment of blood components obtained under the intrinsic coagulation system and the assessment of the capability of blood with protein adsorption, inhibition and activation of the kallikrein/kinin system, the contact system, and the adhesion of platelets to materials, the use of this new assay at the department of Chemical Engineering and Center for Bioengineering of the University of Washington, Seattle, WA is described in chapter 3. At that laboratory this assay was used to evaluate the blood compatibility of different types polyurethanes and glass (reference). Three custom synthesized available polyurethanes, a biomer-like 2000 M_w polytetramethyleneoxide (PTMO) containing polyurethane, an octadecyl extended biomer-like 2000 M_w PTMO containing polyurethane and a hard segment polyurethane were included in this study. Also two commercially available polyurethanes (Biomer® and Pellenthane®) were screened. The commercially available polyurethanes activate the contact system significantly more than the custom synthesized polyurethanes, although all five polyurethanes appeared to be mild activators of the contact system compared with the glass.

In chapter 4, a new method to measure the adhesion of platelets onto materials is described. Gel filtrated platelets were labeled with europium ions by exposing platelets to hypotonic shock. The hypotonic shock treated and labeled platelets were resuspended in autologous platelet poor plasma. The adherence of the labeled platelets onto the polymeric materials; HDPE, PDMS and PTFE, and glass, was determined by means of time resolved fluorometry on the europium ions. Through this method numbers of adhering platelets as low as 10^6 per square centimeter could be detected. The aggregation capacity of labeled platelets using the stimulating agents polybrene and equine collagen was not affected which confirms that the platelet function was well preserved. Moreover, after aggregation no release of label in the supernatant was observed, suggesting that the label was firmly attached to the platelets after the hypotonic shock treatment. The whole procedure of platelet isolation and labeling could be performed within four hours and required no treatment of platelets with radiolabeled components. It is concluded that using this labeling method platelet adhesion studies can be carried out in a reproducible way.

Finally, in chapter 5, a number of test methods is described to assess the blood compatibility of the polymeric materials; HDPE, PDMS and PTFE, and glass. The test methods included the contact activation test (chapters 2 and 3), the newly developed quantitative platelet adhesion method (chapter 4), an iodine radio-labeled fibrinogen adsorption assay, and two commercially available enzyme immuno-assays. The latter assays were used to determine the release of thrombin/antithrombin III complexes, and the release of thromboxane B2 from adhered and activated platelets. The most important finding of this study was that a higher platelet adhesion and -activation was found using the polymeric materials in comparison with glass. The polymeric materials also adsorbed a higher amount of fibrinogen than glass. In contrast, these polymeric materials induce a lower level of activation of the contact and clotting system than glass. The thrombogenicity of polymeric materials was mainly exerted by platelet binding and

primarily activation of the kallikrein/kinin system, whereas glass mainly promotes the intrinsic clotting system. Within the polymeric material group, our data show that PDMS is the most potent activator, PTFE an intermediate, and HDPE the weakest activator of the contact system.

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activation and therefore localized on the material surface, whereas the thrombogenicity of glass was exerted by activation of the clotting systems.

The next part of this dissertation consists of two chapters, in which blood material interaction is studied from a clinical point of view. In chapter 6, a series of seven animal (calve) experiments are described, in which the effects of the use of a polyurethane ventricular assist device (HIA-VAD's) on hemostatic parameters were measured. The circulatory support with the HIA-VAD's was carried out for one week. Also three Bioline heparin coated HIA-VAD's (a covalent binding type of heparin) were tested. All animals were anticoagulated with heparin prior to implantation of the HIA-VAD's and systemically during the circulatory support period. During this period no substantial red blood cell trauma could be determined in both groups. This indicates good mechanical properties of the HIA-VAD's. However, in the coated HIA-VAD group thrombi were located in the mid-section of the inflow cannula and between the connection of the inflow cannula and the pump housing. These thrombi might be induced by traumatized coating areas caused by the surgical procedure. Additionally, already after 24 hours of circulatory support a decrease in platelet numbers was measured. The number of platelets in this group recovered after 72 hours. The platelet consumption correlated with a lower degree of platelet aggregation by equine adenosine diphosphate. Also the fibrin(ogen) degradation products (FDP) increased immediately after implantation. The substantial increase of FDP was not measured in the control group. The FDP levels remained higher in the heparin coated HIA-VAD group suggesting a preceding activation of the intrinsic coagulation pathway, which is further supported by more heparin consumption. In the non-coated HIA-VAD group, after six days of circulatory support the platelet function also diminished, heparin levels dropped, and FDP levels increased as well. However, these animals were clinically healthy after seven days of circulatory support. The study using calves showed that hemostasis was impaired in both groups after six days of circulatory support, whereas the heparin coated HIA-VAD's in calves did not improve the blood compatibility in this period of circulatory support.

The last chapter, chapter 7, describes the blood compatibility of Duraflo II blood circulatory support systems (an ionic binding type of heparin, coated on the surface of extracorporeal circuits) during cardiopulmonary bypass compared with the blood compatibility of non-coated systems. This study was carried out with humans. The contact activation measured by factor XIIIf activity in plasma tended to be lower with the use of the Duraflo II tubing, whereas on this tubing a higher binding of antibodies against adsorbed factor XII was measured than on the control tubing. However, factor XIIIf/C1 inhibitor (C1INH) complex level increased to the same extent in both groups. The ex vivo measured thrombin binding capacity of the tubing remained high on the Duraflo II tubing whereas the thrombin/antithrombin III (T/AT) complex levels in the Duraflo II group increased slower during the first phase of cardiopulmonary bypass than in the control group. However, the fibrinopeptide A levels increased to the same extent in both groups during this period. This indicates that no loss of thrombin activity could be obtained
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The biocompatibility of glass material interaction is assessed in vascular assist device experiments with both coated and non-coated HIA-VAD's (a device coated with heparin). Platelet interactions were studied in both groups. In the control group, thrombin activity was enhanced after release of the aortic crossclamp which was measured in both groups. Platelet numbers tended to increase more in the control group, likely by the interaction of platelet GpIb receptors to the Duraflo II tubing. This was indicated by the higher amount of antibody binding against adsorbed platelet receptors GpIb. However, the platelet activation assessed by the release of β-thromboglobulin was similar in both groups. In conclusion, the advantages of Duraflo II heparin coating in this study were mainly reduced by material independent stimuli and the return of shed blood.