TITLE: THROMBUS FORMATION ON ARTIFICIAL SURFACES

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Introduction. The interaction of blood with non-endothelial surfaces is responsible for the majority of the thrombotic problems encountered in the implantation and use of intravascular devices. Attempts to describe the thrombogenicity of materials in terms of surface charge, critical surface tension, disturbance of flow, and other surface science parameters have lead to crude correlations that are valid for certain subsets of materials, but there is little or no general correlation between surface properties and blood compatibility. In this investigation the idea of blood compatibility is taken from a point of view that encompasses all interactions resulting from the introduction of an artifical surface into the cardiovascular system. Simple, well characterized materials surfaces have been studied to develop information about the sequence of events and the constituents involved during the development of a thrombotic deposit beginning with the initial protein adsorption and proceeding to the subsequent development of a macroscopic thrombus. The purpose of this study was to investigate the morphological and histological character of developing surface-induced thrombi. However, the course of events that resulted in the delineation of the sequence of surface-blood interactions culminated with the development of a surface preparation that is resistant to thrombic development.

Methods. The initial materials to be studied were selected to provide a cross-section of thrombogenic character and still maintain relatively simple surfaces which were easily prepared. These included mica, platinum, gold, aluminum oxide, amorphous carbon, and pyrolytic carbon. These materials were exposed to human blood and individual blood constituents to ex vivo experiments, and to canine blood under in vivo implantation conditions. The materials surfaces and thrombotic deposits were examined using transmission electron microscopy and scanning electron microscopy. Also qualitative immunoassays were performed to identify the various constituents of the developing thrombi as a function of time of exposure and material type. Fibrinogen was cross-linked with dimethyl subermidate in an effort to maintain the native conformation of the molecule during adsorption onto the surface of the test materials. Cross-linking efficency of the agent was tested by comparative SDS-Polyacrylamide Gel Electrophoresis of native fibrinogen, cross-linked reduced fibrinogen, and reduced native fibrinogen.

Results. The individual materials exhibited distinct surface dependent characteristics in the initial layer of thrombus formation. However, all materials developed on initial proteinacious layer upon exposure to blood or plasma. In gold and platinum, this layer appeared to occur predominantly in a clumped, denatured form; in contrast, the initial protein layer of aluminum and carbon was formed in dense fibrin mats of a homogeneous nature. The immunochemical assays illustrated in a qualitative manner the composition of the adsorption protein layer as a function of time and materials. The assays for Factor XII & XI, fibrinogen, albumin, globulin, ceruloplasmin, IgA-IgE-IgM-IgG & serum showed Factor XII and fibrinogen heavily adsorbed on aluminum, platinum, and gold, while only fibrinogen was heavily adsorbed on Carbon. Essentially no albumin or globulin was involved in the surface layer. These results indicated that fibrinogen was the most surface active protein. Surfaces coated with fibrinogen cross-linked into its native structure showed significantly increased antithrombogenicity and maintained a uniform protein layer without developing into a fibrin layer or promoting platelet aggregation as seen with native fibrinogen coated surfaces. Furthermore, platelets on the prepared fibrinogen surface did not form pseudopoda and appeared not to spill granules. Therefore, the dimethyl suberimidate cross-linked fibrinogen coated surface demonstrated uniquely superior antithrombogenicity over all other test surfaces. The cross-linked effect of the prepared fibrinogen, demonstrated by immunoassay, showed that even after molecular reduction by peptide cleavage, the cross-linked fibrinogen maintained its tertiary structure.

Discussion. The mechanism of thrombus formation involves the sequential adsorption and transformation of blood constituents onto a non-endothelial surface. The morphology and histology of the deposit is material dependent with this material specificity occurring at all stages of thrombotic deposition. The nature and physical state of the initially adsorbed protein layer dictates the subsequent behavior of further thrombic development. It has been demonstrated that native protein does not adsorb onto native protein. This suggests that, in thrombic formation, the initially deposited protein must change to a denatured form that promotes further protein adsorption. This initial layer is shown to be predominantly fibrinogen. It followed from these observations that the thromboresistance of a surface may be improved by coating the surface with a protein layer of "native fibrinogen". This layer would be "seen" by blood as a natural interface that would not promote further deposition of protein or other blood constituents. The results of exposure of the dimethyl suberimidate cross-linked fibrinogen coated surface to blood shows a significant reduction in thrombogenicity, especially on the less thrombogenic surfaces such as carbons.