PROTEIN BONDED POLYMERS: AN APPROACH TO ANTI-THROMBOGENIC SURFACES

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ABSTRACT

Covalent bonding of plasma proteins to suitably derivatized polymer surfaces appears to be a promising method of preparing anti-thrombogenic materials. Plasma protein human serum albumin has been incorporated in derivatized commercial polymers and their blood compatibility evaluated.

PROGRESS in the design and construction of various prosthetic devices, artificial internal organs and heart-lung bypass systems has been severely hampered by the lack of availability of suitable blood compatible materials. All known surfaces other than the vascular interface give rise to blood coagulation on contact with blood. It has been long recognised that the primary event in the clotting process is the denaturation of blood platelets by a foreign surface releasing certain clotting factors[1]. The interaction between the surface of the material and the platelets in the blood is sought to be minimised by modifying the surface using principally three methods : physical, physico-chemical and chemical.

In the purely physical methods of surface modification surface properties of materials like wettability[2] and zeta potential[3] have been investigated in relation to their clotting properties. Gott[4] has tried a novel approach to improve the clotting properties of colloidal graphite by cross-linking the anticoagulant heparin on the surface through a cationic surface active agent. The modified graphite surface obtained by the physico-chimecal method has come to be known as the graphite-benzalkonium-heparin (GBH) surface. The GBH surface has been found to be clot free for 10 days in blood compatibility tests. Later, this technique crosslinking heparin *in situ* has been extended to polymer surfaces[5].

Gott's[4] success with the incorporation of a plasma component on material surfaces clearly shows that the plasma component, being native to blood, would not cause platelet damage when coated on a foreign surface and thus would prevent the initiation of the clotting process. Physical and physico-chemical methods of protein incorporation on a given surface suffer from the disadvantage of weak bonding between the plasma component layer and the polymer molecules. This drawback could be eliminated by covalently linking the plasma components to suitably derivatised polymer and other surfaces. Covalent bonding offers the unique method of preparing water insoluble derivatives which will not go into solution when assayed for activity. Insolubilised proteins find several important applications in biochemical reactions. In the present investigation, however, an attempt has been made to produce thrombo-resistant materials by the incorporation of plasma protein albumin on polymeric materials. The modified polymeric materials have been tested for their blood-compatibility.

MATERIALS AND METHODS

Human serum albumin (HSA) in lyophilized form has been incorporated on polymeric materials like cellulose, polystyrene 6–6 nylon, Dow–Corning silicone elastomer and a commercially available epoxy polymer. In all, six modified surfaces have been prepared using these materials. Four modified surfaces have been prepared by covalently bonding HSA to cellulose, polystyrene and 6–6 nylon. Two modified surfaces have been prepared by physically mixing HSA with Dow–Corning silicone elastomer and epoxy resin. Each of these polymeric materials has been cast into a sheet for evaluating its blood compatibility.

Cellulose—HSA :

Covalent bonding of HSA to *p*-amino-benzyl cellulose was achieved by first preparing the diazonium salt and then adding this to an ice-cooled 1% solution of the plasma protein in borate buffer according to the method of Campbell *et al.*[6].

Polystyrene—HSA :

Polystyrene was first converted to polyaminostyrene by nitration followed by reduction of the surface. The polyaminostyrene surface was then diazotized and HSA coupling was carried out in borate buffer according to the method of Hornby *et al.*[7].

Nylon—HSA :

The technique of Allison *et al.*[8] has been adopted for coupling HSA to 6, 6-nylon. Nylon surface was first hydrolysed by treating with strong (4·5 M) hydrochloric acid. The hydrolysed surface was then treated with glutaraldehyde and HSA in phosphate buffer.

The surfaces have been prepared by mixing HSA with silicone elastomer and epoxy resin and casting the material into sheets,

TABLE I

Control surface	Time for 100% clot formation min.	Protein bonded surface	Time for 100% clot formation min.
6, 6-Nylon ..	7·8	6, 6-Nylon + HSA	16·0
Polystyrene ..	9·5	Polystyrene + HSA	23·0
Epoxy polymer ..	16·0	Epoxy polymer + HSA	29·5
Silicone elastomer ..	31·0	Silicone elastomer + cellulose HSA (32 : 1)	59·0
Silicone elastomer ..	do.	Silicone elastomer + cellulose HSA (24 : 1)	61·0
Silicone elastomer ..	do.	Silicone elastomer + HSA polymer	74·0

Folin's test[9] and the method of acid hydrolysis[9] have been used to identify the protein in the cellulose HSA conjugate. In the case of polystyrene and nylon protein conjugates infrared spectra have been obtained before and after chemical bonding. A comparison of amide absorption bands at 1650 cm^{-1} to 1550 cm^{-1} showed qualitatively the presence of bonded protein.

The blood compatibility of the protein bonded surfaces was evaluated by the kinetic method of Imai *et al.*[10] using citrated goat blood plasma. Briefly, this method consisted of recalcifying a known volume of plasma on a given surface to initiate the clotting process and of determining the weight of the clot formed after various intervals of time. At the end of a known time interval the clotting process was stopped by diluting the mass with water. The thrombus formed was washed, fixed with formaldehyde, dried and weighed. For each surface a plot of per cent clot formed *versus* time was prepared. Per cent weight of clot is based on the weight of clot formed at infinite time on the same surface. The time taken for 100% clot formation was read from the plot in each case. These times of clotting are compared with values obtained on control surfaces in Table I.

RESULTS AND DISCUSSION

Protein bonded surfaces have shown definite improvement over the control surfaces in their blood compatibility. The highest clotting time obtained was of the order of one hour and the lowest about 15 minutes. Silicone elastomer with HAS polymer and celulose–HSA conjugate gave better results than the other protein bonded polymers. Silicone elastomer is known to be a good blood compatible material and it gave the highest clotting time among the control surfaces. With the modified surfaces clotting times of the order of days have been expected. The low values obtained could be attributed to the possibility of the surface not being completely saturated with protein. This could not be quantitatively checked in the absence of a reliable method for the estimation of the bonded protein. One possible approach to this end would be radio-labelling of the protein.

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