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Biomaterials: Role of surface modifications

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Abstract. The fundamental concepts related to biomaterials and blood/tissue–material interactions at the interface have been reviewed. The relevance of surface modification to enhance blood and/or tissue compatibility of materials has been discussed and its role in selected prosthetic applications described.

Keywords. Biomaterial; surface modification; protein adsorption; blood compatibility; tissue compatibility; calcification; hemoperfusion; artificial skin.

1. Introduction

When one looks at the intima of a blood vessel, it appears hydrated, gelatinous, flexible and multiphase to prevent protein and platelet reactions with its carbohydrate-rich outer coat (Rigby 1961). Further, on closer investigation, one will find a definite arrangement of endothelial cells having specific physiological responses, even secreting pharmacologically active substances such as prostaglandin PGI_2 possessing antiplatelet activity by stimulating membrane-bound adenyl cyclase and thereby raising the intracellular levels of cyclic AMP (cAMP) within platelets (Moncada and Vane 1979; Bonne *et al* 1981). Thus the intima's complex role of keeping the blood flowing in living blood vessels makes it impossible to duplicate it by any artificial means. Attempts have been made by grafting hydrogels, developing textures or microdomain structures, or hydrophobic–hydrophilic optimization on polyurethane substrates (Sharma 1991) to enhance blood compatibility. Negative surface charge (Sawyer 1985) and even electrical conduction and semiconduction properties based on specific structural regularity of various proteins and polymers also seem to play a role in enhancing blood compatibility (Bruck 1979).

Scientific efforts have been made to immobilize covalently (or facilitate controlled release of) the selected bioactive molecules such as PGI_2, PGE_1, phosphorylcholine, heparin, hirudin, urokinase or streptokinase, antithrombin III, cAMP collagen, etc and their complexes with albumin onto the substrates along with oral antiplatelet drugs like aspirin; vitamins C, E, B_6; antibiotics; etc (Sharma 1991). It seems that antiplatelet biomolecules preferentially promote albumin adsorption. Efforts have also been made to graft heparinoid polyelectrolyte, synthesized in our laboratory from natural rubber on polyetherurethane urea (PEUU) surfaces. These surfaces have a marked effect in decreasing platelet adhesion with minimal protein interaction, and can act as a passive layer enhancing blood compatibility (Sharma and Nair 1984). It has also been found that surfaces adsorbing more high-density lipoprotein (HDL) compared to low-density lipoprotein (LDL) are relatively more blood-compatible (Chandy and Sharma 1991). Endothelialization of porous substrates via cell culture technique with coimmobilization of cell-growth and cell-adhesive factors has also been tried (Ito 1992). However, the problem of immune response and steady supply of cells is not fully solved. Genetically engineered endothelial cells have been

seeded on fibronectin-coated vascular grafts to secrete increased amounts of tissue plasminogen activator enhancing thromboresistance. The production of plasminogen activator has been found to be higher on collagen-impregnated Dacron than on expanded poly(tetrafluoroethylene) (PTFE) or polyurethane/Dacron composite (Shayani *et al* 1992). Pentapeptides have been used to form an elastic matrix via crosslinking by gamma irradiation and addition of fibronectin for cell attachment. Fibroblasts, endothelial cells and other cells have been used for such studies (Urry *et al* 1992). Even a composite graft made of polyurethane-coated pericardium has been developed (Tolia and Sharma 1986), with all these efforts there is still no small-diameter vascular graft (< 5 mm diameter) available today except sephanous vein to replace coronary artery without thrombus formation. Attempts have been made to develop small-diameter vessels from PEUU. However, in *in vivo* trials in mongrel dogs at iliac artery, the graft showed thrombus formation initiated from the suture site at the anastomosis within 20 days (Sharma 1983). Therefore the core of our discussion here is related to the surface energy parameters and the necessity of optimizing them with biophysicochemical aspects comprehensively for understanding the blood/tissue–material interactions at the interface.

2. Background

Any nonliving materials used in a medical device intended to interact with biological systems is called a biomaterial (Williams 1987). Obviously it should be nontoxic, fabricable, sterilizable, and stable during implantation and as required by application. It should not corrode, degrade, or be carcinogenic. Further the ability of a material to perform with an appropriate host response in a specific application is termed biocompatibility. This is controlled at tissue–material interfaces by pore size and density (soft tissue $\approx 50\,\mu$, hard tissue (bone) $> 100\,\mu$), geometrical nature, controlled chemical breakdown by the resorption of material where material may be replaced by regenerating tissue, and controlled surface reactivity. If the surface reactivity is high, usually healing is delayed by formation of giant cells, and the fibrous tissue layer is much thicker causing tissue instability. Some of the tissue-compatible materials (materials used in contact with tissue) are titanium, cobalt–chromium alloy, bioglass, aluminium oxide, hydroxylapatite, chitosan, etc.

Ideal blood-compatible materials (materials used in contact with blood) do not activate the intrinsic blood coagulation system or to attract or alter platelets or leucocytes (Forbes and Prentice 1978). Blood compatibility of any material is dependent on surface charge, surface free energy, chemical group distribution, heterogenicity, surface texture, porosity, smoothness and flow conditions. International efforts continue towards production of materials that prevent thrombosis, surface modification to enhance blood compatibility, and use of therapeutic substances that prevent deposition of fibrin. Some of the commonly used blood-compatible materials are polydimethylsiloxane, cellulose acetate, polyacrylonitrile, PTFE, nylon, polycarbonate, polyurethane, poly(methyl methacrylate) (PMMA), pyrolytic and low-temperature isotropic (LTI) carbons, etc.

3. Theory and experimental

When any material comes in contact with blood instant adsorption of proteins takes place with a certain selectivity, rate and concentration. The interaction involves

intermolecular forces which develop at the interface, e.g. London dispersion forces, hydrogen bonds, dipole–dipole interactions, donor–acceptor bonds, electrostatic interactions, acid–base interactions, etc. Albumin-coated surfaces do not seem to attract platelets (Lyman *et al* 1968; Kim and Lyman 1973), whereas γ-globulin and fibrinogen coatings cause not only platelet adhesion but also aggregation and release of platelet constituents (Vroman and Adams 1971). The processing parameters also can change the nature of the adsorbed proteins due to variations in microstructure; for example, the glass side and air side of cast polyurethane urea with polypropylene glycol (MW 1025) are found to be different as the air-side domain size is 80–120 Å and the glass-side size 30–50 Å (Sharma 1980).

When blood comes in contact with any material it probably involves the following steps (Vroman 1984–85) in the intrinsic system as follows:

(i) The factor XII protein (Hageman factor) adsorbed onto the surface becomes activated and this (XIIa) activates other molecules of XII.
(ii) The molecules of high-molecular-weight kininogen (HMWK) carrying factor XI are adsorbed near factor XIIa, along with other molecules of HMWK carrying prekallikrein.
(iii) Factor XIIa activates factor XI to factor XIa and converts prekallikrein to kallikrein.
(iv) Kallikrein can activate more factor XII.
(v) With the activation of factor XIa the fluid phase of clotting with the requirement of calcium ion begins with further activation of factor IX to IXa.
(vi) The γ-carboxyglutamic acid groups and the negatively charged groups on the phospholipid droplets with calcium ions as bridges, factor IXa comes closer to factor VIII anchored, in the uncharged group of the phospholipid droplet, factor VIII is converted to VIIIa.
(vii) Similar reactions progress and convert factor X to Xa, V to Va; factor Xa and Va convert prothrombin to thrombin with phospholipid and calcium ion. Thrombin converts fibrinogen to fibrin and factor XIII to XIIIa which in presence of calcium ion stabilizes the fibrin into crosslinked fibrin.

In case of extrinsic system factor VII converts to VIIa with tissue factor and calcium ions. The factor VIIa converts factor X to Xa with similar interactions as described above and further reactions follow as in the intrinsic cascade system. The nature of the protein adsorbed may also affect the activation of blood coagulation, complement and fibrinolytic system. It is also now known that receptors of fibrinogen exist on the platelet surface. Therefore platelets adhere where they find fibrinogen (Marguerie *et al* 1979); these receptors are available to fibrinogen when ADP is added to a platelet suspension. Therefore much attention has been paid in our laboratory to understand fibrinogen adsorption onto various substrates in comparison to albumin.

The complexity of blood–material interactions at the interface is depicted in figures 1 and 2. Table 1 lists the blood coagulation factors.

Lyman *et al* (1965) studied several polymer surfaces and evaluated their critical surface tension (γ_c) and surface free energy (γ_s) using contact angle data determined by the method of Zisman (1964). While the initial studies showed a relationship between the rate of clotting and γ_s, later studies indicated that this relationship does not hold rigorously, probably because of the heterogenicity of the surface.

Andrade *et al* (1973) postulated that material surfaces which tend to have an interfacial energy of zero will be highly thromboresistant. The fact that materials

Table 1. Coagulation factors.

Factor	Name
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium ion
V	Proaccelerin
VI	Not assigned
VII	Proconvertin
VIII	Antihaemophilic factor
IX	Christmas factor
X	Stuart factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor
XIII	Fibrin stabilizing factor

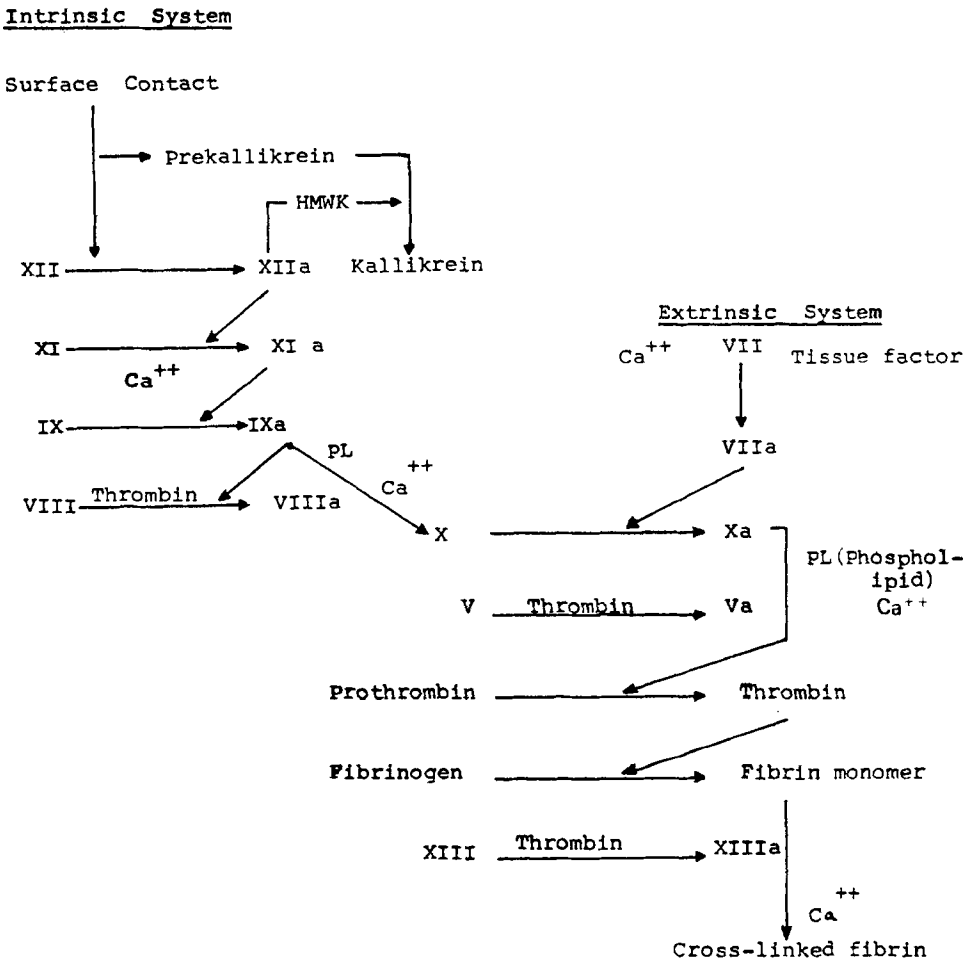

Figure 1. Blood coagulation cascade.

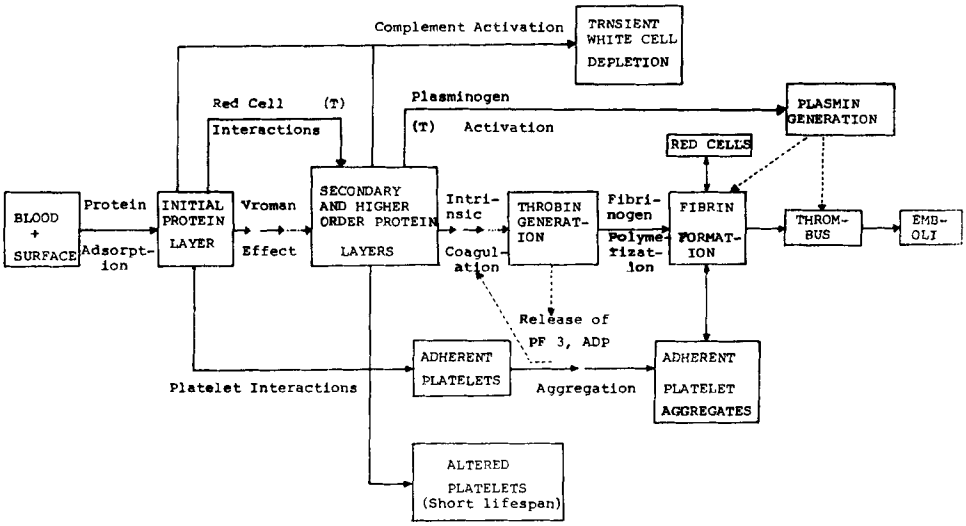

Figure 2. Blood-material interaction sequence.

with a minimal interfacial energy like hydrogels do possess a relatively low thrombus adherence enhances the validity of this hypothesis. Similarly the surface free energies of many polymers have been calculated by contact angle measurement. It was observed that a polymer having a critical surface tension of around 20–30 dynes/cm is highly blood-compatible (Baier *et al* 1970), but exceptions have been reported as in LTI carbons ($\gamma_c \simeq 50$ dynes/cm). Further it has been indicated that dispersion $\alpha_s = \sqrt{\gamma_s^d}$ and polar $\beta_s = \sqrt{\gamma_s^p}$ components of polymer surfaces play an important role in interfacial interactions. It was found that low-dispersion, high-polarity surfaces, typified by surface-treated stillite 21 with $\alpha_s = 5{\cdot}0(\text{dynes/cm})^{1/2}$ and $\beta_s \geqslant 5{\cdot}0(\text{dynes/cm})^{1/2}$, provide surface energies that appear to favour weak adsorption and retention of plasma proteins, i.e. a poor surface from the compatibility point of view. Again, high-dispersion, low-polarity implants, e.g. LTI carbon with $\alpha_s \geqslant 6{\cdot}0(\text{dynes/cm})^{1/2}$ and $\beta_s \leqslant 2{\cdot}0(\text{dynes/cm})^{1/2}$, provide surface energetics favouring stable plasma protein retention, i.e. an excellent surface for blood compatibility (Kaelble and Moacanin 1977). For LTI carbons α_s and β_s values are $7{\cdot}38(\text{dynes/cm})^{1/2}$ and $2{\cdot}04(\text{dynes/cm})^{1/2}$. Sharma (1980) suggested an optimum α_s of $4{\cdot}7(\text{dynes/cm})^{1/2}$ and β_s of $3{\cdot}0(\text{dynes/cm})^{1/2}$ for a possible blood-compatible surface based on experimental observations and theoretical calculations as depicted in table 2. Such surfaces seem to adsorb albumin preferentially on the basis of the dispersion and polar components of the protein interacting with the surface.

The dispersion α_s and polar β_s components can be determined for any substrate as suggested by Kaelble and Moacanin (1977) as follows:

$$\gamma_{SV} = \gamma_{SV}^a + \gamma_{SV}^p = \alpha_S^2 + \beta_S^2,$$
$$\gamma_{LV} = \gamma_{LV}^d + \gamma_{LV}^p = \alpha_L^2 + \beta_L^2.$$

Adhesion energy, which is basically a measure of interface bonding, is given by

$$W_{SL} = \gamma_{LV}(1 + \cos\theta_L) \leqslant 2\gamma_{LV},$$

Table 2. Surface free energy components for various polymers.

Polymer	α_s	β_s	γ_s	γ_c	γ_{ij}
Silastic rubber (SR)	4·4	3·0	28·4	25·5	17·2
SR (Cast) – air	4·6	2·1	25·6	24·0	25·4
SR (Cast) – FEP	4·6	2·1	25·6	24·0	25·4
Polyethylene	5·15	2·0	30·5	33·0	26·7
Polypropylene	5·1	1·75	29·0	30·0	29·3
TPX	5·1	2·0	30·0	25·0	26·6
2025 – air	5·2	2·3	32·3	31·5	23·7
2025 – glass	5·15	2·8	34·3	31·5	19·0
1025 – air	4·9	3·3	34·9	31·5	14·8
1025 – glass	4·9	3·5	36·3	31·5	13·4
710 – air	5·15	2·85	34·6	31·5	18·7
710 – glass	5·15	2·75	34·1	31·5	19·5
Biomer – air	4·8	3·2	33·3	25·0	15·5
Biomer – glass	4·8	3·2	33·3	25·0	15·5

TPX, 4-methyl poly – pentene – 1
Block copolymer urethane ureas were based on polypropylene glycols of MW 2025, 1025, 710.

where θ_L is the contact angle of various organic solvents on the substrates.

$$W_{SL} = 2[\alpha_S \alpha_L + \beta_S \beta_L],$$

$$\frac{W_{SL}}{2\alpha_L} = \alpha_S + \beta_S \left[\frac{\beta_L}{\alpha_L}\right].$$

Thus $\left[\frac{W_{SL}}{2\alpha_L}\right]$ vs $\left[\frac{\beta_L}{\alpha_L}\right]$ is plotted as a straight line with intercept α_S and slope β_S. Since α_S and β_S are square roots of dispersion γ_{SV}^d and polar γ_{SV}^p parts of γ_{SV}, these components can be evaluated.

Kaelble (1976) further summarized the general concept for regular adsorption bonding in interfaces in the following relation for interfacial tension:

$$\gamma_{ij} = (\alpha_i - \alpha_j)^2 + (\beta_i - \beta_j)^2 + \Delta_{ij}.$$

Subscripts denote interactions from phase i and j interfaces dominated by van der Waals interactions which are termed as regular interfaces and the values of the term Δ_{ij} describe interdiffusion or ionic covalent interactions, which can be considered negligible. Thus from known values of α and β, γ_{ij} values for substrate – protein system can be evaluated as given in table 3.

From table 3 it seems that the interfacial tension is lowest with albumin in both the cases, viz model surface and LTI carbon, compared to fibrinogen and γ-globulin. However, LTI carbon with high dispersion and low polar components may interact with all proteins under consideration quite intensely, unlike the case of the model surface, where both the dispersion and polar components appear to be taking an active part in the interaction process for albumin. On the other hand polar components are playing a predominant role in case of fibrinogen and γ-globulin. So a

Table 3. Interfacial energy parameters for different proteins and for platelets.

Protein	Polar non-polar ratio	α_i [(dynes/cm)$^{1/2}$]	β_i [(dynes/cm)$^{1/2}$]	Model substrate[a,c] γ_{ij} (dynes/cm)	Model substrate[a,c] $\frac{(\beta_i-\beta_j)}{(\alpha_i-\alpha_j)^2}$ (dynes/cm)	LTI carbon[b,c] γ_{ij} (dynes/cm)	LTI carbon[b,c] $\frac{(\beta_i-\beta_j)^2}{(\alpha_i-\alpha_j)^2}$ (dynes/cm)
Fibrinogen	1·626	4·97	6·35	11·27	151·3	24·39	3·5
γ-Globulin	1·205	5·43	5·96	9·30	16·8	19·20	4·4
Albumin	1·072	5·60	5·79	8·64	9·6	17·30	4·4
Platelets	2·604	4·40	7·10	16·90	186·8	34·48	2·0

[a]For values of $\alpha_j = 4{\cdot}7$ (dynes/cm)$^{1/2}$ and $\beta_j = 3{\cdot}0$ (dynes/cm)$^{1/2}$

[b]For values of $\alpha_j \simeq 7{\cdot}38$ (dynes/cm)$^{1/2}$ and $\beta_j = 2{\cdot}04$ (dynes/cm)$^{1/2}$

[c]The ratio indicates relative contribution of polar to dispersion components toward interfacial energy for the various proteins and for platelets compared for the same surface.

possibility of weak adsorption of these proteins is suggested on this surface relatively (Sharma 1984). In case of LTI carbons, which may interact with all the proteins under discussion equally intensely, taking dispersion and polar components of the proteins into account, no preferential adsorption may be possible. Therefore availability of a particular protein may also be an important parameter in this case. Since albumin content is much higher in blood compared to other proteins, an increased adsorption of albumin on LTI carbons is suggested. Further, albuminated surfaces, as discussed earlier, usually reduce the adhesion of platelets and enhance the blood compatibility. So this may certainly be one of the reasons why LTI carbons are also blood-compatible. Similarly the cell or microbial adhesion onto non-proteinated model surface and LTI carbon can be explained relatively. For example, the dispersion α_p and polar β_p components for platelets has been evaluated (Sharma 1991) to be 4·4 (dynes/cm)$^{1/2}$ and 7·1 (dynes/cm)$^{1/2}$ as shown in table 3. Obviously, for LTI carbons, there is no preferential adhesion of proteins of platelets (cells), while on model surface, albumin adsorption is preferential even in comparison to platelets.

However, the adhesion of platelets onto various substrates is very complex and certainly not governed by a single specific parameter. It is observed with ^{125}I-labelled human fibrinogen or immunoglobulin G (IgG) that the adsorption of fibrinogen and IgG were greater on polyacrylonitrile than on cuprophane. However, fibrinogenated polyacrylonitrile does not promote the adhesion of ^{51}Cr-labelled platelets or polymorphonuclear leucocytes. This may be due to either the direct involvement of fibrinogen-active sites in the interaction of the protein molecule with polyacrylonitrile or a conformational change in the fibrinogen molecule upon adsorption with respect to the surface energy components of the polymer (Chuang *et al* 1982). Further, fibrinogen has platelet adhering sites and these sites may have different energy parameters, compared to the rest of the segment, suitable to encourage the adhesion of platelets. The human fibrinogen molecule consists of three pairs of peptide chains joined by disulphide bridges. It is observed that platelet adhesion to polycarbonate (PC) substrates is inhibited by addition of fibrinogen factor 2b, $(A\alpha, B\beta)_2\gamma\gamma^1$, with no significant difference between the effects of fraction Ia and Ib, i.e. $(A\alpha, B\beta, \gamma)_2$ chain composition. ADP-induced platelet binding to PC substrate also

decreases in presence of fraction 2b (Chandy and Sharma 1987). This may be due to the nonavailability of binding sites on this molecule or because a conformational change in the COOH terminus of the γ chain or γ^1 chain causes a change in fibrinogen conformation itself. On many materials, when blood comes in contact, instant adsorption of fibrinogen takes place, along with activatable clotting factors. Mainly HMWK will displace fibrinogen film within a few minutes. This is difficult in narrow spaces and if dilution is affecting concentration of HMWK or surface-activated blood with insufficient intact HMWK to remove the fibrinogen deposited. In such cases platelet adhesion is promoted. Perhaps while platelets race with fibrinogen/HMWK interactions, granulocytes race with γ-globulin plasma protein interaction of a similar nature (Vroman 1984–85). It is also observed that the incubation of pure fibrinogen in presence of a trace of plasminogen or plasma considerably enhances the plasmin-induced degradation of adsorbed fibrinogen (Brash *et al* 1985). Further, platelets, cells and proteins tend to have a net negative zeta potential of -8 to -13 mV (Sawyer and Pate 1953). If the surface is not highly charged, certain proteins, preferably albumin, may be adsorbed and form a passivating layer, which may ultimately make the material less adverse toward blood components and hence relatively more blood-compatible. For highly charged surfaces, even if protein adsorption does not take place, blood components may still suffer damage. For example, activated platelets may aggregate as microemboli and deposit in various organs causing the implant unsuccessful in the long run (Sharma 1991). Further, there also appears to be a relationship between complement activation and leucopenia occurring in haemodialysis with cellulose membranes. It seems that leucocyte adhesion is mediated by complement components. Complement activation via the alternative pathway, when blood comes in contact with implant with activation of C_3 and C_5 to C_{3a} and C_{5a} respectively, which induce mast cell degranulation resulting in histamine release, also stimulates cytokine (interleukin 1) release from monocytes. Along with cytokine leukotriene B_4 (LTB_4) and platelet activation factor (PAF) enhanced cell adhesion is brought about (including even neutrophil endothelial cell adhesivity). This encourages the formation of superoxide anion, H_2O_2, OH etc; along with collagenase, elastase, gelatinase etc. These products may inhibit the reendothelialization of blood-contacting surfaces. It is believed that tissue necrosis factor (TNF) from activated neutrophils also plays an important role in the above process. The understanding of the above concepts is still not complete.

An attempt was made to further understand blood–material interaction by preparing surfaces having similar surface energy by grafting various hydrogels onto Angioflex via ^{60}Co irradiation (Hari and Sharma 1991) as indicated in table 4. It is obvious from the results that the polyethylene glycol-grafted substrate demonstrated not only adsorption of albumin but also less adhesion of platelets. This reflects the relevance of chemical nature of the substrate besides surface free energy parameters. Further, blood-compatible surfaces do not induce morphological changes in platelets (pseudopods), release of serotonin, ADP and platelet factor (PF_4), etc. Platelets may lose their shape slightly, but come back to original shape if reflected from the substrates (Hari and Sharma 1993).

A similar phenomenon is obvious on biologically modified surfaces as shown in table 5. While octane contact angles are similar, platelet adhesion is low on certain substrates depending upon biological activity.

From the above discussion one can conclude that both physicochemical and

Table 4. Platelet adhesion, amount of proteins adsorbed after 3 h exposure, and surface free energy (γ_{sv}) of Angioflex surfaces grafted with various hydrogels.

Surface	γ_{sv}	No. of platelets adhered/mm^2	Surface conc. of (μg/cm^2) ± S.D.	
			Albumin	Fibrinogen
Bare Angioflex	30·98	12·3 ± 2·3	1·209 ± 0·17	0·662 ± 0·11
Gamma irradiated*	32·10	10·0 ± 2·8	1·046 ± 2·17	0·697 ± 0·11
PHEMA grafted*	35·64	4·5 ± 2·0	1·522 ± 0·15	0·718 ± 0·12
Polyacrylonitrile grafted*	35·47	5·0 ± 1·8	1·543 ± 0·14	0·800 ± 0·12
Polyacrylamide grafted*	35·05	5·3 ± 2·0	1·330 ± 0·11	0·646 ± 0·12
PEG grafted*	35·50	2·8 ± 1·8	1·270 ± 0·32	0·568 ± 0·06
Bare PEUU	—	6·2 ± 2·0	0·280	0·240
PE grafted**	—	1·4 ± 1·0	0·290	0·250

*On Angioflex surface

**Polyelectrolyte (PE) with sulphamate and carboxylate groups like heparin developed from natural rubber (on PEUU surface)

Table 5. Octane contact angle and platelet adhesion to various modified chitosan membranes.

Membrane	Octane contact angle (degrees)	Adhered platelets per mm^2 ± S.D.
Albumin-blended chitosan membrane (ACM)	138·2 ± 2·44	17·7 ± 2·00
ACM + liposome (+ ve)	134·1 ± 2·70	25·7 ± 2·60
ACM + lip. (+ ve) + hirudin	141·3 ± 1·60	12·5 + 2·00
ACM + lip. (+ ve) + PGE_1	122·3 ± 3·17	8·3 ± 1·80
ACM + lip. (+ ve) + AT-III	138·5 ± 1·44	11·7 ± 1·90
ACM + lip. (+ ve) + heparin	137·1 ± 1·81	10·5 ± 2·10
ACM + liposome (− ve)	141·4 ± 2·30	15·0 ± 2·3
ACM + lip. (− ve) + hirudin	143·7 ± 2·10	10·2 ± 1·98
ACM + lip. (− ve) + PGE_1	133·4 ± 2·07	5·9 ± 1·95
ACM + lip. (− ve) + AT-III	143·6 ± 2·06	9·8 ± 1·93
ACM + lip. (− ve) + heparin	144·2 ± 1·35	9·3 ± 1·91

AT-III, antithrombin III.

biological aspects are important while considering blood–material interaction with an objective of developing blood-compatible material surfaces. Surface modification by immobilizing antithrombin III, PGE_1 and methyldopa complex on albuminated substrates has also been attempted, which demonstrated enhanced albumin adsorption compared to bare PC surface (Chandy and Sharma 1989). Similarly aspirin, vitamins, antibiotics, anaesthetics, antihypertensive drugs, etc seem to encourage albumin adsorption in varied degree and also reduce platelet adhesion. This could

be important information from the clinical point of view for patients having an artificial internal organ (Sharma 1991).

Usually in case of polymers the denatured protein–polymer–water system represents a lower energy system than the original state. In case of metals, whether or not an oxide layer is present, there is a large electric field. Small ions accumulate, lowering the strength of the field, but there is a great advantage in replacing these with protein molecules. The dipole moment of a protein is very much greater than that of a small ion and there is a gain in entropy on replacing several small ions by one large molecule. However, denaturation of a protein is not normally experienced in electric fields as the possible loss of free energy is increasing further the dipole moment is rarely greater than the gain due to exposure of the hydrophobic core. Electrostatic and hydrogen bond interactions are other major forces that bind proteins depending upon microenvironment.

Protein adsorption studies on various metal surfaces such as Ti, Al and Ta have been carried out as shown in table 6. It is interesting to note that after a certain optimum thickness Al (500 Å) and Ti (400 Å) become more blood-compatible, while Ta (200 Å) becomes tissue-compatible based on Fib/Alb mole ratio (table 6).

Table 6. Protein adsorption onto titanium, tantalum and aluminium with oxide layer of varied thickness.

Surface	Water contact angle (degrees)	Protein adsorbed in 3 h ($\mu g/cm^2$)		Mole ratio Fib/Alb
		Albumin	Fibrinogen	
I. Titanium[1]				
(a) Cleaned and glow discharged	21·4 + 1·4	0·037	1·086	5·64
(b) With 266 Å oxide layer	109·3 ±·1·8	0·215	1·629	1·44
(c) "408 Å"	117·0 ± 3·2	0·180	1·080	1·14
(d) "1268 Å"	136·3 ± 6·1	0·174	0·876	0·69
II. Tantalum[2]				
(a) Cleaned and glow discharged	Spreading	0·224	0·228	0·186
(b) With 200 Å oxide layer	60·1 ± 2·9	0·127	0·272	0·391
(c) "500 Å"	50·4 ± 2·9	0·193	0·295	0·279
(d) "960 Å"	40·2 ± 5·7	0·248	0·388	0·286
III. Aluminium[3]				
(a) Cleaned and glow discharged	Spreading	0·041	0·060	0·268
(b) With 50 Å oxide layer	26·2 ± 7·8	0·065	0·120	0·338
(c) "350 Å"	33·1 ± 2·2	0·072	0·130	0·331
(d) "500 Å"	34·2 ± 6·6	0·089	0·140	0·288
(e) "840 Å"	31·7 ± 4·7	0·070	0·130	0·340

1. Sunny and Sharma (1991)
2. Sharma and Paul (1992)
3. Sunny and Sharma (1990); Sharma and Sunny (1990)

Problems related to T-cell defects cause autoimmune disease due to overproduction of antibodies by B-cells (up to two to three times more than normal). To help such patients, usually the blood is fractionated via plasmapheresis and plasma is passed through a perfusion column where selected adsorption of pathogenic immunoglobulins occurs. After passing through this column the plasma along with other blood components is passed into the patient. An attempt was made utilizing polyvinyl alcohol (PVA), chitosan, polyacrylamide (PAA) and hydroxyapatite (HA) (200–400 μ) beads modified with amino acids such as tryptophan and phenylalanine for selective adsorption of immunoglobulins from whole blood if possible. The results appear to be encouraging as shown in table 7.

Since bone consists of collagen and HA (60–70%), the latter, $Ca_{10}(PO_4)_6(OH)_2$, was synthesized from $Ca(OH)_2$ and orthophosphoric acid H_3PO_4 as basic component. The microspheres of HA have also been developed by the simple procedure of mixing HA with chitosan in acetic acid and dropping this solution slowly into a polymer solution in toluene. By washing the spheres with toluene, acetone and distilled water and heating at 400°C, the coated chitosen was removed. Such microspheres, of varied sizes and shapes, under pressure with sintering for required porosity may be useful in various dental, orthopaedic and drug-delivery applications. HA–aluminachitosan microspheres have also been developed for similar applications.

Various materials such as PEUU, PVA, chitosan, etc have been used towards artificial-skin applications with and without collagen. *In vivo* experiments on rabbits (dorsal region) have been performed and relative healing has been evaluated as indicated in table 8. It is obvious that chitosan can be a very good substitute in artificial-skin application.

The basic problem with bioprosthetic heart valves such as porcine valve (Carpentier Edwards) and bovine pericardium valve (Ionescu–Shiley) has been calcification. However, by treating with Fe^{3+} or Al^{3+} solutions it seems that the process of calcification can be inhibited. Even cross-linking agents such as carbodiimide or glycerol etc instead of glutaraldehyde, reduce calcification. Prevention of calcification through $FeCl_3$ release from chitosan beads, coimplanted on subdermal pouches of rats, have been studied in our laboratory. It seems that an optimum combination of ferric and magnesium ions also reduces calcification. A beginning has also been made towards the use of chitosan along with liposome encapsulation systems for significant reduction of calcification in bioprosthetic valves.

Table 7. Immunoprotein adsorption from human plasma (GBS) on modified chitosan (CB), polyvinyl alcohol (PVA) and acrylamide (PAA) microspheres.

Protein	Pre-perfusion levels (mg/dL)	Post-perfusion level (mg/dL)			Percentage adsorbed		
		CB	PVA	PAA	CB	PVA	PAA
IgG	763·81	589·59	679·11	666·55	22·8	11·1	12·7
IgM	90·88	81·74	83·57	79·00	10·1	8·1	13·1
IgA	147·27	70·81	95·95	98·20	51·9	34·8	33·3
C_3	200·06	162·86	139·04	176·30	18·6	30·5	11·9

Adsorption was for 3 h on 3 g of microspheres (bead size CB 400–600 μm). GBS, Guillain Barre syndrome (PAA and PVA 600–800 μm).

Table 8. Wound healing pattern in rabbits of different polymeric samples with various modifications.

Sample	Sterilization	No. of days taken for complete wound healing*
PEUU	Chemical	36·38 ± 3·90
PEUU + collagen + albumin	Chemical	25·15 ± 4·32
Albumin + epidermal growth factor	Chemical	24·39 ± 3·83
PVA	Chemical	34·87 ± 4·12
PVA + collagen + albumin	Chemical	38·10 ± 1·27
Chitosan	Steam	32·74 ± 5·23
Chitosan + collagen	Steam	24·93 ± 4·30
Chitosan + fibrin gel	Steam	28·81 ± 8·39

*Each value expressed as mean ± S.D. of three animals (6 wounds, $3 \times 1{\cdot}5\,cm^2$)

4. Conclusion

Surface modification of implants plays an important role in enhancing blood and tissue compatibility. However, many surfaces may change after sterilization so emphasis should also be given to understanding sterilization-induced changes in the functioning of any prosthetic device, *in vitro* and *in vivo*, before review by an ethics committee for selection for limited clinical trials on humans.

Further, controlling the surface properties at the manufacturing stage alone is not enough because changes may come even at stages like handling, packaging and storage, besides sterilization, which can significantly modify the surface properties and ultimately the success of the implant.

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References

Andrade J D, Lee H B, John M S, Kim S W and Hibbs J B 1973 *Trans. Am. Soc. Artif. Organs* **19** 1
Baier R E, Gott V L and Feruse A 1970 *Trans. Am. Soc. Artif. Organs* **16** 50
Bonne C, Martin B, Watada M and Regnault F 1981 *Thromb. Res.* **21** 13
Brash J L, Chan B M C, Szota P and Thibodeau J A 1985 *J. Biomed. Mater. Res.* **19** 1017

Chandy T and Sharma C P 1987 *Polym. Plast. Technol. Engg.* **26** 143
Chandy T and Sharma C P 1989 *Artif. Organs* **13** 229
Chandy T and Sharma C P 1991 *J. Biomed Mater. Res.* **25** 1085
Chuang H Y K, Sharpton T R, Mohammed S F, Sharma N C and Mason R G 1982 *VIII Int. Congress on Thrombosis and Haemostasis THHADO* **46** 304
Forbes C D and Prentice C R M 1978 *Brit. Med. Bull.* **34** 201
Hari P R and Sharma C P 1991 *J. Biomater. Appl.* **6** 170
Hari P R, Ajith Kumar B and Sharma C P 1993 *J. Biomater. Appl.* **8** 174
Ito Y 1992 in *Synthesis of biocomposite materials* (ed.) Y Emanishi (Bola Raton: CRC Press) pp. 285–305
Kaelble D H 1976 *SAMPE Q.* **7** 30
Kaelble D H and Moacanin 1977 *J. Polym.* **18** 475
Kim S W and Lyman D J 1973 *Polym. Symp.* 22 (New York: John Wiley & Sons) pp. 289–297
Lyman D J, Brash J L, Chalkin Klein K G and Carini M 1968 *Trans. Am. Soc. Artif. Int. Organs* **14** 250
Lyman D J, Muir W M and Lee I J 1965 *Trans. Am. Soc. Artif. Organs* **11** 301
Marguerie G A, Plow E F and Edington T S 1979 *J. Polym. Chem.* **254** 53
Moncada S and Vane J R 1979 *Fed. Proc.* **38** 66
Rigby B J 1961 *Biochim. Biophys. Acta* **113** 472
Rusk S D 1979 *J. Polym. Sci., Polym. Symp.* **66** 283
Sawyer P N 1984–85 *Biomater. Med. Dev. Artif. Organs* special issue on blood compatibility **12** 161–196
Sawyer P N and Pate J W 1953 *Surgery* **34** 491
Sharma C P 1980 *J. Sci. Ind. Res.* **39** 453
Sharma C P 1983 *Curr. Sci.* **52** 1057
Sharma C P 1984 *J. Coll. Interface Sci.* **97** 585
Sharma C P 1991 in *Blood compatible materials and devices* (ed.) C P Sharma and M Szycher (Massachusetts: Technomic Pub. Co) pp. 25–31
Sharma C P and Nair A K 1984 *Trans. Biomater.* April 27th–May 1 p. 100
Sharma C P and Paul W 1992 *J. Biomed. Mater. Res.* **26** 1179
Sharma C P and Sunny M C 1990 *Biomaterials* **11** 255
Shayani V, Newman K and Dichek D 1992 *Proc. Cardiovascular Science and Technology Conf. AAMI/NHLBI* p. 53
Sunny M C and Sharma C P 1990 *J. Biomed. Mater. Res.* **24** 455
Sunny M C and Sharma C P 1991 *J. Biomater. Appl.* **6** 89
Tolia J T and Sharma C P 1986 *Trends Biomater. Artif. Organs* **1** 7
Urry D W, Nicol A and Hoban I D 1992 *Proc. Cardiovascular Science and Technology Conf. AAMI/NHLBI* p. 155
Vroman L 1984–85 *Biomater. Med. Devices Artif. Organs* **121** 307
Vroman L and Adams A L 1971 *J. Polym. Sci.* **34** 159
Williams D F (ed.) 1987 *Definitions in biomaterials* (New York: Elsevier)
Zisman W A 1964 American Chemical Soc. Series 43, chapter I