Effects of the Chemical Structure and the Surface Properties of Polymeric Biomaterials on Their Biocompatibility

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Received September 1, 2003; accepted April 16, 2004

Polymeric biomaterials have extensively been used in medicinal applications. However, factors that determine their biocompatibility are still not very clear. This article reviews various effects of the chemical structure and the surface properties of polymeric biomaterials on their biocompatibility, including protein adsorption, cell adhesion, cytotoxicity, blood compatibility, and tissue compatibility. Understanding these aspects of biocompatibility is important to the improvement of the biocompatibility of existing polymers and the design of new biocompatible polymers.

KEY WORDS: biocompatibility; biomaterial; blood compatibility; cell adhesion; cytotoxicity; polymer; protein adsorption; surface property; tissue compatibility.

INTRODUCTION

Synthetic polymers have long played an important role in medical therapy, finding uses in areas such as modulation of wound healing, implantable medical devices and artificial organs, prostheses, ophthalmology, dentistry, bone repair, and drug delivery systems (1–4). Polymeric biomaterials are relatively easy to manufacture into products with various shapes, at reasonable cost, and with desirable mechanical and physical properties. However, one of the major factors limiting the use of these materials is their biocompatibility (defined below). A challenge is thus to enhance their biocompatibility, at least at the interface with host tissues and fluids.

Depending on the intended medical application, all biomaterials are evaluated in terms of biocompatibility (5). Biocompatibility can be defined as the acceptance (or rejection) of an artificial material by the surrounding tissues and by the body as a whole (1). It is generally accepted that this term means not only absence of cytotoxic effect but also positive effects in the sense of biofunctionality (i.e., promotion of biological processes that further the intended aim of the application of the material) (6). The term "biocompatibility" encompasses many different properties of the materials, including toxicity, tissue compatibility, and blood compatibility (hemocompatibility). Two important aspects of biomaterial screening refer to their *in vitro* cytotoxicity and hemocompatibility behavior (7).

The basic factors that govern compatibility of biomaterials are incompletely understood (8). In particular, the design of biocompatible synthetic surfaces that are able to control the interaction between a living system and an implanted material remains a major theme for biomaterial applications in medicine. In this review, we will summarize the effects of the chemical structure and the surface properties of polymer biomaterials that influence their biocompatibility. The effects include (i) the interfacial free energy, (ii) balance between the hydrophilicity and the hydrophobicity on the surface, (iii) the chemical structure and functional groups, (iv) the type and the density of surface charges, (v) the molecular weight of the polymer, (vi) conformational flexibility of the polymer, and (vii) surface topography and roughness. We believe that an understanding of these effects will result in the predictable improvement of existing biopolymers and the design of new biocompatible polymers.

INTERFACIAL FREE ENERGY

The introduction of a biomaterial surface in blood creates a new interface between cellular and fluid components of blood and the material. This results in a thermodynamic driving force that acts to reduce the solid-liquid interfacial free energy at this interface. Ignoring interactions with blood cellular components, the blood plasma-biomaterial interfacial free energy is a thermodynamic quantity that incorporates the surface free energy contributions of both solid and liquid phases and provides a measure of the driving force for the adsorption of blood components on solid surfaces. The configuration of the initially adsorbed proteins on the solid surface may be determined by the magnitude of the blood plasma-biomaterial interfacial free energy (9). Based on this parameter, Andrade proposed the minimum interfacial free energy hypothesis of biocompatibility (10).

Assuming that solids interact with liquids largely by dispersion and polar forces, one can obtain the following expression for the solid-liquid interfacial free energy (11)

$$\gamma_{SL} = \{(\gamma_L^p)^{1/2} - (\gamma_S^p)^{1/2}\}^2 + \{(\gamma_L^d)^{1/2} - (\gamma_S^d)^{1/2}\}^2,$$

where γ_S is the surface free energy of solid, γ_L is the surface tension of the liquid, γ_{SL} is the interfacial free energy at the solid-liquid interface, and the superscripts p, d denote the polar and dispersion contributions, respectively.

In the case of the blood plasma-biomaterial interface, the surface free energy characteristics of a synthetic polymer must be very close to those of water (γ_L^d = 21.8 dyne/cm, γ_L^p = 50.8 dyne/cm) to obtain minimum interfacial free energy, as blood plasma is largely aqueous in nature. It has been suggested that the interfacial free energy region should be at 1~3 dyne/cm to match in long-term compatibility with blood (9). The requirement on the polar surface free energy component (close to 50.8 dyne/cm) is virtually impossible to realize for existing polymeric materials, though many of them come close to satisfying the dispersion surface free energy component requirement. The surface free energy of a material should also be able to provide the interaction energy required for the modulation of protein or cell adhesion (12–14).

To create a desirable increase in the polar surface free energy of polymeric solids for potential biological applica-

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0724-8741/04/0800-1362/0 © 2004 Plenum Publishing Corporation

tions, a variety of techniques, including radiation grafting, plasma discharge, and chemical treatments, have been used to modify the surface of polymeric materials (15–16). Esumi *et al.* (17) investigated the effect of ultraviolet radiation on the surface free energy components of polymers. They selected six hydrophobic polymers, including polystyrene, poly(methyl vinyl ketone), poly(diphenyl siloxane), and copolymers of dimethyl siloxane. The results of their studies show that exposure to ultraviolet radiation caused an increase in their polymeric component surface free energies.

Another technique used to increase the polar surface free energy of polymeric solids is chemical etching. Benderley (18) reviewed several methods of treating fluoropolymer surfaces to promote bond ability, including treatment with solutions of sodium in either naphthalene/tetrahydrofuran or liquid ammonia. Polymeric surfaces have effectively been modified through the application of plasma in recent years. NH_3 or H_2/N_2 plasma has been used to introduce amino groups for the immobilization of heparin on a variety of surfaces (19). Argon plasma-induced poly(2-hydroxyethyl methacrylate) (PHEMA) engraftment onto silicon rubber or poly (4-methyl-1-pentene) was shown to reduce the cells attachment and growth (20).

Water plays an important role in determining the biocompatibility of synthetic materials. Ratner *et al.* (21) have recognized that high water levels within the surface of materials will help provide a low interfacial free energy with blood and will reduce both protein adsorption and cell adhesion on the polymeric surface. Therefore, a surface with a hydrated polymer (hydrogel) coating (prepared by radiation grafting of water-soluble or high polar polymers onto nonpolar polymeric supports) would be expected to be more compatible with body fluids than a nonpolar or less hydrated type of surface. Proof of this concept has been demonstrated by platelet adhesion studies (22) using a graft copolymer of *N,N*-dimethylacrylamide (DMAA) with polyethylene (PE), polytetrafluoroethylene (PTFE), and of acrylic acid (AA) with polyvinyl chloride (PVC) (Table I).

Puleo *et al.* (23) have reviewed the state of current knowledge of the bone-biomaterial interface. Once again, modulation of surface energy is one of the physicochemical characteristics that have been altered to improve the bone-implant interface. Glow discharge has been used to increase surface free energy in order to increase tissue adhesion (24).

BALANCE BETWEEN HYDROPHILICITY AND HYDROPHOBICITY

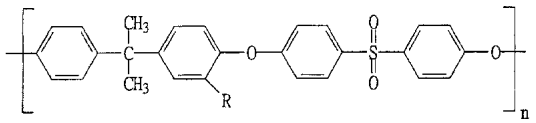
When a foreign material comes into contact with blood, initially there is a rapid adsorption of plasma proteins onto its surface followed by platelet adhesion and activation. Platelet activation initiates the coagulation process, resulting in the formation of clots (25–26). In general, hydrophobic surfaces tend to adsorb larger amounts of proteins than hydrophilic ones (27). Therefore, some investigators have proposed that to increase blood compatibility, one should attempt to incorporate hydrophilic surfaces (28). Many biologically compatible polymers such as poly(ethylene glycol) (PEG), poly(hydroxyethylmethacrylate) (PHEMA), poly(acrylamide) (PAA), and poly(*N*-vinyl-2-pyrrolidone) (PVP) are hydrophilic polymers. Higuchi *et al.* (29) have studied the chemical modification of polysulfone (PSF) hollow fibers with PVP. The structures of PSF and its surface-modified PSF are shown in Fig. 1. The immobilized amount of VP on the PVP-PSF membranes can be controlled by the amount of VP monomer in the reaction solution and the reaction time. Selected results are summarized in Table II. We can see from Table II that the PVP-PSF membranes are the most hydrophilic examples (lowest water contact angle) between the PSF and the other surface-modified membranes in their study, which is explained by the long hydrophilic side chain of PVP on the hydrophobic PSF membrane. PVP-PSF membranes had lower plasma protein adsorption than polysulfone and other surface-modified membranes. It is suggested that the hydrophilic surface of PVP-PSF membranes without ionic groups causes the suppression of platelet adhesion (29).

An alternate method for immobilizing hydrophilic polymers onto a surface involves the preparation of interpenetrating polymer networks (IPNs). In recent years, an increasing number of publications have reported the preparation of thermoplastic apparent IPNs to improve the surface properties of polymeric materials for blood-contacting devices (30–31). Roman and his colleagues have synthesized several IPNs by use of a segmented polyurethane urea, Biospan (BS), and vinylpyrrolidone-dimethyacrylamide copolymer (VP-DMAm) (32). They demonstrated that the VP content of this IPN was an important factor for controlling protein adsorption. Decreased fibrinogen and γ-globulin adsorption, and increased adsorption of albumin for these IPNs, was consistently demonstrated with increased VP content.

Protein adsorption is the first step that occurs when a foreign surface is placed in contact with blood. Therefore,

Table I. Biological Properties of the Grafted and Ungrafted Polymeric Films (22)

Sample	Swelling degree (%)	Platelet adhesion (number)
PTFE	0	20 ± 5
PTFE-g-DMAA	9.2	10 ± 3
PE	0	Clot
PE-g-DMAA	15.2	10 ± 3
PVC	4.7	Clot
PVC-g-AA	30.4	40 ± 3

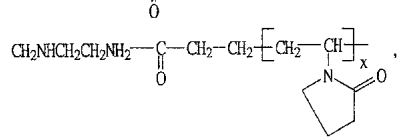


R = H, Polysulfone (PSF);

CH_2Cl, Chloromethylated PSF (Cl-PSF);

$CH_2NHCH_2CH_2NH_2$, Ethylenediamination PSF (EDA-PSF);

$CH_2NHCH_2CH_2NH-\overset{\overset{O}{\|}}{C}-CH=CH_2$, N-succinimidylacrylated PSF (NSA-PSF);



PVP modified polysulfone (PVP-PSF).

Fig. 1. Structures of polysulfone and its modified polymers.

Table II. Water Contact Angles and Protein Adsorption of PSF Membranes

Membranes	Contact angle (°)*	Amount PVP (μmol/cm²)	Adsorbed amount of proteins (μg/cm²)			
			Plasma	BSA	γ-globulin	Fibrinogen
PSF	90 ± 3	—	5.95 ± 1.2	2.14 ± 0.4	1.89 ± 0.4	1.38 ± 0.3
Cl-PSF	90 ± 3	—	7.75 ± 1.6	3.58 ± 0.7	2.14 ± 0.4	1.13 ± 0.2
EDA-PSF	89 ± 3	—	7.63 ± 1.5	2.80 ± 0.6	2.82 ± 0.6	1.51 ± 0.3
NSA-PSF	89 ± 3	—	6.02 ± 1.2	1.72 ± 0.3	2.16 ± 0.4	1.95 ± 0.0
PVP-PSF-1	—	1.9 ± 0.3	2.74 ± 0.6	2.77 ± 0.6	1.41 ± 0.3	1.10 ± 0.2
PVP-PSF-2	—	6.3 ± 1	1.02 ± 0.2	1.95 ± 0.4	1.51 ± 0.3	1.08 ± 0.2
PVP-PSF-3	54 ± 2	11 ± 2	2.69 ± 0.5	1.19 ± 0.2	0.79 ± 0.2	1.04 ± 0.2
PVP-PSF-4	—	16 ± 3	1.90 ± 0.4	0.44 ± 0.1	0.83 ± 0.2	1.15 ± 0.2
PVP-PSF-5	—	20 ± 3	3.02 ± 0.6	0.20 ± 0.1	0.70 ± 0.2	0.73 ± 0.2

* The contact angle is a very useful inverse measure of wettability, as a smaller contact angle implies smaller surface tension, but higher surface wettability.

understanding the mechanism of protein adsorption is very important for the surface design of biomaterials. In particular, conformational change in an adsorption protein is considered to be one of the important aspects affecting blood compatibility (33). Radke and his co-workers have intensively studied the protein/polymer adsorption dynamics at fluid/fluid and fluid/solid interfaces (34–36). They found a commonality in that proteins change conformation from their native structure in order to adsorb and that protein adsorption become progressively more irreversible upon exposure to an interface. Atomic force microscopy (AFM) was used to obtain direct observation of protein adsorption on solid interfaces (36). Tanaka *et al.* (37) have investigated the conformation of protein, which adsorbed onto polyacrylate surfaces, by circular dichroism (CD) spectroscopy with attention to the α-helix content. When a protein adsorbs to the surface of a polymer, its secondary structure changes. A decrease of the α-helix content and an increase of the random fraction and/or β-helix occur. Experimental data proved that different polymers induced different degrees of conformational change of the adsorbed protein. The amount of protein adsorbed onto poly(2-methoxyethylacrylate) (PMEA) was very low, and the conformation of the proteins adsorbed onto PMEA differed only a little from the native one (37). It is proposed that the low platelet adhesion and spreading observed on PMEA may be due to the low degree of the conformational change of the adsorbed bovine serum albumin.

It is well-known that platelet adhesion is inhibited by prior surface adsorption of albumin and promoted when IgG or fibrinogen is preferentially adsorbed to synthetic surfaces (38–39). Platelet adhesion and thrombus formation for BS/VP-DMAm IPN surfaces decrease with increasing amounts of VP are contained in the VP-DMAm added to Biospan matrix. The relationship between thrombus formation and VP content on the BS/VP-DMAm IPNs surfaces are shown in Fig. 2 (32).

Similarly, a hydrophilic polymer coated surface for polyurethane catheters has been developed by the reaction of an epoxy containing PVP (40). In studies of this material, it was found that noncoated polyurethane catheters were covered with thrombi, whereas the hydrophilic polymer coated surface showed no thrombus formation.

Lee *et al.* (41) prepared a wettability gradient polyethylene surface to investigate the adhesion behavior of platelets in terms of the surface hydrophilicity/hydrophobicity of polymeric materials. They observed that the platelet adhesion in the absence of plasma proteins increased gradually as the surface wettability increased along the sample length, whereas in the presence of plasma proteins the platelet adhesion decreased gradually with the increasing surface wettability.

In order to achieve enhanced biocompatibility, some investigators are focusing on the induction of balance between the hydrophilic and hydrophobic properties at the surface. Surface coating of a triblock copolymer, composed of PEG as a hydrophilic segment and poly(propylene glycol) (PPG) as a

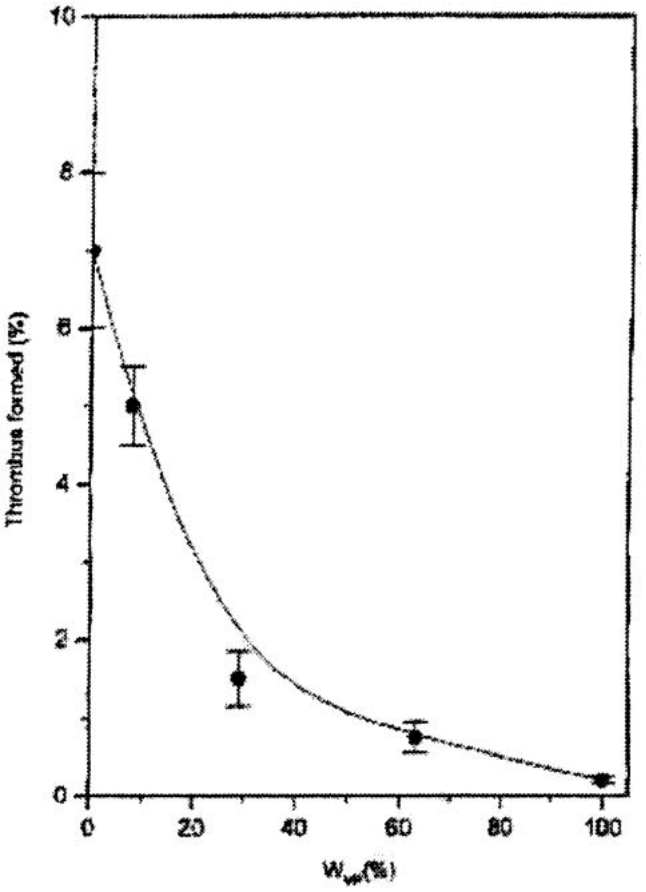


Fig. 2. Thrombus formation on BS/VP–DMAm IPNs of different VP contents (reference: glass, 100% of thrombus formed) (32).

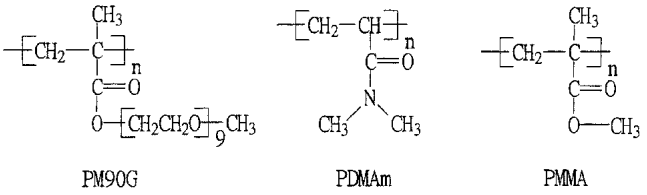


Fig. 3. Molecular structures of surface modifiers (43).

hydrophobic segment, has been reported to reduce protein adsorption, a crucial initiating step in thrombus formation (42). This can be explained by the interposition of the interfacial structure—a hydrophilic segment is oriented toward water (plasma and proteins), whereas the hydrophobic segment is anchored on the surface. The protein-resistant characteristics of the surfaces have been attributed to the hydrophilic PEG segments (42). Matsuda and Ito (43) developed a coating technique using hydrophilic-hydrophobic block copolymers on a hydrophobic poly(acryonitrile) (PAN) hemodialyzer. The hydrophilic block of copolymer was composed of either poly(methoxy polyethylene glycol methacrylate) (PM90G) or poly(dimethyl acryamide) (PDMAm) and the hydrophobic block was poly(methyl methacryate) (PMMA). The molecular structures of surface modifiers are shown in Fig. 3.

Diblock copolymers composed of hydrophilic and hydrophobic segments have also been studied in sustained compound release systems, as alternative drug carriers, as they are known to form a micellar structure (44–47). Hydrophilic-hydrophobic diblock copolymers exhibit amphiphilic behavior and form micelles with core-shell architecture. In one study, amphiphilic diblock polymeric nanosheres, composed of methoxy poly(ethylene glycol) (MePEG) and poly(ε-caprolactone) (PCL), were suggested as a novel injectable drug carrier for hydrophobic drugs such as indomethacin and paclitaxel (42). No significant histopathologic changes were observed in MePEG/PCL nanosphere-treated mice compared with normal mice in various organs such as heart, lung, liver, and kidney. The results indicated that the outer shell composed of hydrophilic MePEG block could reduce the interaction between nanospheres and cells by forming a "stealth" surface for facilitating drug delivery and thereby reducing potential drug toxicity (48).

Stratford *et al.* have reported on a family of bipolar materials based on the copolymers of 2-methacryloyloxyethylphosphorylcholine (MPC) and lauryl methacrylate (LMC) (49–51). These materials are exceptionally amphiphilic, due to the combination of the highly hydrophilic MPC and hydrophobic alkyl chains of the LMA. They demonstrated that coatings of these polymers extracted from a water:alcohol mixture exhibited excellent resistance to adhesion of blood components (51). Block copolymers with hydrophilic poly(2-hydroxylethyl methacrylate) (PHEMA) and hydrophobic chains of polystyrene (PSt) or poly(dimethylsiloxane) (PDMS) have also been reported to show a hydrophilic-hydrophobic microphase separated structure. These two polymers showed promising antithrombogetic properties, and were able to function without occlusion for 20 days for HEMA-St block polymer and for 12 days for HEMA-DMS block copolymer (52).

Finally, the natural substance chitosan was partially N-acylated (less than 50%) with various carboxylic anhydrides for prevention of gelation. *N*-acyl chitosan, especially *N*-hexanony chitosan, showed the best blood compatibility due to the induction of balance between the hydrophilic and hydrophobic properties on the surface (53). Clearly, modification of surface hydrophilicity is important in design of biocompatible polymers.

CHEMICAL STRUCTURE AND FUNCTIONAL GROUPS

Functional groups on polymers may play an important role in determining therapeutic and/or toxic characteristics (3). Ratner *et al.* (54) investigated the effect of various functional groups on biological activity using self-assembled monolayers. Gold alkanethiolates, X-$(CH_2)_{15}$-SH, where X = C(O)OH, $C(O)OCH_3$, CH_2OH, or CH_3, were used to compare the effect of these groups on cell growth and protein adsorption. The results indicated that the growth of endothelial cells was influenced by functional group substitution and increased in the following order: $CH_2OH < C(O))OCH_3 < CH_3 \ll C(O)OH$ (54). A similar approach has been used to study effect of functional groups on apatite formation for self-assembled monolayers (55). In this study, alkanethiols with terminal groups, such as H_2PO_4, C(O)OH, $C(O)NH_2$, OH, NH_2, and CH_3, were used. The following trend was obtained with various functional groups: $H_2PO_4 > C(O)OH \gg C(O)NH_2 \approx OH \approx NH_2 \ll CH_3$.

Cellulose polymers, commonly used in hemodialysis, were modified by introduction of a hydroxyl group or diethylamino-ethyl group to improve blood compatibility (8). Figure 4 shows the surface of the poly(aryl ether ether ketone) (PEEK) that was reduced by sodium borohydride in DMSO to introduce hydroxyl groups, resulting in a moderate improvement in the biocompatibility of this polymer (56).

Anionic groups have a noticeable effect on blood platelet adhesion and thrombogenesis (57–59). Sulfonated polyurethane-polyethyleneglycol surfaces (PU-PEG) exhibited a low degree of platelet adhesion and sharp change of platelets. The introduction of sulfonate groups at the end of the PEG chain grafted onto a PU surface markedly enhanced antithrombogenicity. This can be attributed to the synergistic effect of hydrophilic PEG and negatively charged SO_3 groups (59).

An inhibitory effect of negatively charged carboxylate

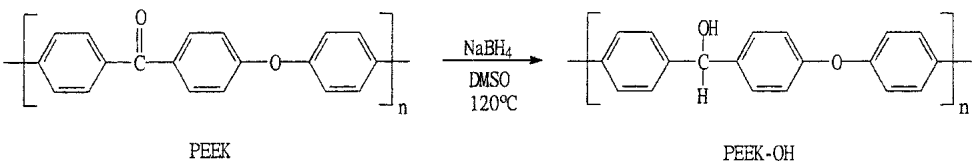


Fig. 4. Introduction of hydroxyl group on PEEK.

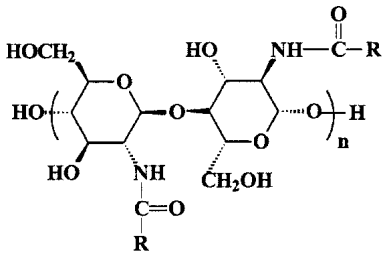
Table III. Adhesion of Macrophages (MΦ) *in Vitro* and Their Spreading and Fusion *in Vivo* on the Surface (61)

Polymer (% of NaMA)	MΦ adhesion serum free (%)	MΦ spreading (μm^2)	Fusion index
Poly HEMA (0)	18.40	419.00 ± 191.25	0.51 ± 0.21
Copolymer HEMA-NaMA (1%)	19.12	270.00 ± 85.00	0.32 ± 0.15
Copolymer HEMA-NaMA (2%)	10.56	223.50 ± 96.00	0.08 ± 0.22
Copolymer HEMA-NaMA (3%)	8.62	200.50 ± 117.50	No fusion

groups on complement activation has also been observed (60). Smetana *et al.* (61) have investigated the effect of carboxylate groups in a copolymer of 2-hydroxyethyl methacrylate (HEMA) with sodium methacrylate (NaMA) on serum albumin adsorption and macrophage adhesion. They found that an increase in the concentration of the anionic group in the polymers decreased the passive adsorption of human serum albumin as well as the *in vivo* spreading of macrophages and their subsequent fusion in foreign-body giant multinucleate cells. Table III shows the modulation of macrophage adhesion with several polymers and demonstrates the negative effect of carboxylate groups on their adhesion. It is thought that the fusion of macrophages into giant foreign-body multicleate cells is related to their ability to spread and aggregate on polymer surfaces (61).

Chitosan (discussed above) and its derivatives have also been found to help repair bone defects and regenerated bone tissue due to their osteoinductive effects (3). Chitosan was partially N-acylated (less than 50%) with various carboxylic anhydrides for prevention of gelation. *N*-acyl chitosan, especially *N*-hexanony chitosan (R = C_5H_{11}), showed the best blood compatibility due to the induction of balance between the hydrophilic and hydrophobic properties on the surface (62). Clearly, the variation in functional substitutions is important in influencing the hemocompatibility of chitosan derivatives. The results of anticoagulability of n-acylated chitosans are shown in Table IV, in which the parameter t_1 is the time corresponding to the initiation of coagulation, t_2 is the time corresponding to the termination of coagulation, and $(t_1 + t_2)/2$ is defined as the rate of coagulation (63).

Table IV. Initiation Time (t_1) and Termination Time (t_2) and Rate ($t_1 + t_2/2$) of Coagulation of Human Plasma Protein in the Measuring Cell Coated with *N*-Acyl Chitosans



Sample code	N-Acyl group (R)	Degree of N-acylation (%)	t_1 (min)	t_2 (min)	$(t_1 + t_2)/2$ (min)
Blank*	—	—	2.9	7.6	5.3
A-1	CH_3	17.5	7.5	9.5	8.5
A-2	CH_3	47.3	9.2	10.9	10.1
B	C_2H_5	41.7	12.6	15.9	14.3
C	C_3H_7	40.4	11.6	15.0	13.3
D	C_4H_9	42.0	12.6	18.4	15.5
E	C_5H_{11}	40.8	15.3	19.0	17.2

* It is a stainless steel measuring cell without polymer coating.

In recent years, biomaterial scientists (mainly in the Chapman and Nakabayashi groups) have attempted to incorporate cell membrane constituents such as phosphorylcholine or phospholipid analogs into polymers (49–51,64–81). They found that 2-methacryloxyethyl phosphorylcholine (MPC) polymers and copolymers demonstrate enhanced hemocompatibility (49–51,69,79–81). Suppression of clot formation following platelet adhesion and activation was observed even when the MPC polymer came in contact with whole blood without anticoagulants. This was due to the reduced protein adsorption on the MPC polymer surface. The hemocompatibility of a polymer containing a phospholipid functional group, poly(MPC-*co*-*n*-butyl methacrylate) (BMA), with human whole blood was evaluated (69). When human whole blood, without an anticoagulant, was placed in contact with the polymer, blood cell adhesion and aggregation on the polymeric surface was extensive. However, this phenomenon was suppressed by increasing the MPC composition in the copolymer (Table V) (69). Nakabayashi *et al.* (79) concluded that high free water fraction of phospholipids polymers having PMC moiety results in a reduction of protein adsorption. Again, the free water fraction must be one of the more important factors that determine hemocompatibility, as we pointed out in the section "Balance Between Hydrophilicity and Hydrophobicity" above. In order to improve biocompatibility, segmented polyurethanes have been modified with various MPC polymers by coating (74,80}, grafting (75), or blending (76–77,81). The structure of MPC is shown in Fig. 5.

Several authors have discussed the role of amine substitution on polymers in influencing toxicity. Dekie *et al.* (82) noted that the presence of primary amines in poly(L-glutamic acid) (PGA) derivatives has a significant toxic effect on red blood cells. Based on studies with modified poly(L-lysine) (PLL), Ferruti *et al.* (83) conclude that polymers with tertiary amine groups exhibit a lower toxicity than those with primary and secondary residues. They have also synthesized tertiary amine group containing poly(amidoamine)s (PAAs); these substituted polymers have good biocompatibility and can form complexes with heparin (84–86). One of the water-

Table V. Whole Blood Coagulation Time (69)

Sample	MPC mole fraction	Coagulation time (min)
Glass	—	8.4 ± 0.46
Poly (BMA)	—	9.6 ± 1.3
Poly(MPC-co-MMA)[a]	0.18	21 ± 0.58
Poly(MPC-co-BMA)	0.26	28 ± 2.6

[a] MMA: methyl methacrylate.

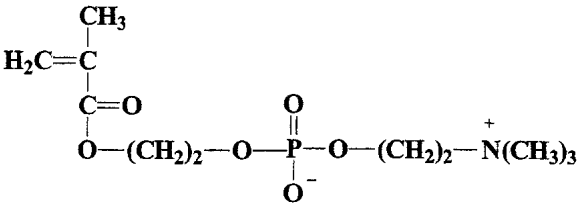


Fig. 5. Chemical structure of 2-methacryloxyethyl phosphorylcholine (MPC).

soluble forms of PAA, obtained from reaction of 1,4-bis-acryloylpiperazine with 2-methylpiperrazine, is shown in Fig. 6. Fischer *et al.* (87) confirmed these observations for PLL and PEI, but argued that cationized human serum albumin and Starburst dendrimer, which also contain primary amino groups, showed only moderate cytotoxic effects. They conclude that not only the type of amino function but also the charge density and arrangement is an important factor for determining cytotoxicity and hence biocompatibility (87).

Hydrogels have been of great interest to biomaterial scientists since the pioneering work of Wichterle and Lim (88) in 1960 on cross-linked hydroxylethyl methacrylate (HEMA) polymer. Information about hydrogels and their biomedical applications can be found in several review papers (89–92).

Smetana *et al.* investigated the influence of hydrogel functional groups on cell adhesion and on the function of macrophages (93–94). The highest level of monocyte adhesion was observed on a surface copolymer of 2-hydroxyethyl methacrylate (HEME) with dimethyl aminoethyl methacrylate (DMAEMA) compared to that of poly(HEME) and the copolymer of HEME with the sodium salt of methacrylic acid (NaMA) (93). This phenomenon shows the stimulatory effect of DMAEMA in the copolymer related to NaMA. They have also found that hydrogel containing –OH, –C(O)NH, and $(CH_3)_2N$– groups induced a spreading of macrophages on polymeric implants, whereas materials containing $–SO_3H$ groups slightly, and materials containing –COOH groups more intensively, inhibited spreading of the macrophages (94). The trend of the fusion of macrophages into multinucleate cells is in the following order: –COOH < $–SO_3H$ < –C(O)NH ~ –OH < $(CH_3)_2N$–, which can be seen in Table VI. The correlation between the macrophages spreading and fusion and surface charge of the hydrogel implant can hypothetically be explained by electrostatic interaction between macrophages cell membrane and implant (94). Secondarily, activation of macrophage metabolism and response to physical parameters of the biopolymer may play roles in determining adhesion and fusion.

The percentage of surface oxygen might be an important determinant of biocompatibility (95–96). Studies were conducted with membranes studied formed from polypropylene (PP, 1.9%), polyacrylonitrile (PAN, 10.2%), polysulfone (PS-F, 14.2%), polymer alloy of polysulfone (PS-K, 16.2%), hemophan (HP, 23.6%), ethylene vinyl alcohol (EVAL, 25.3%), polyvinyl alcohol (PVA, 27.3%), and cuprophan (CP, 37.4%). Generally, high surface oxygen percentages were associated with low protein adsorption and marked suppression of cell transformation (96). The positive effect of oxygen content on biocompatibility is demonstrated with oxygen-contained polymers, such as polyglycolic acid (PGA) (97), polyanhydrides (98), poly(ethylene glycol) (PEG), and their copolymers. PGA is a well-known biodegradable polymer (commonly used in surgical sutures), whereas polyhydrides have been investigated as drug carriers for more than two decades. Polyhydrides do not induce an inflammatory reaction and they are noncytotoxic during their biodegradation. More interesting is that some hydrophobic polyanhydrides display zero-order kinetics of drug release (98). PEG is a typical hydrophilic polymer with high chain mobility and low interfacial free energy with water (42). The nonimmunogenic and nontoxic properties of these materials have been applied to modify proteins, which have longer circulating lives (99–100). PEG coating of nanospheres provides protection against deleterious interactions with blood components, and coated nanospheres may function as repository depots of drugs (101–102). Micelles, based on the biocompatible copolymer of PEG with poly(L-lactic acid) (PLLA) (Fig. 7), have been synthesized (103). Aldehyde groups on the surface of the micelles may react with the lysine residues of cell's proteins. Nanospheres composed of these materials were tested as vehicles for delivery of anti-inflammatory and anti tumor drugs (104).

Table VI. Fusion Indices and Relative Frequency of Multinucleate Foreign Body Giant Cells on the Surface of Hydrogel Implants

Hydrogel	Functional groups	Fusion index	Relative frequency of cells
polyHEMA	–OH	0.39 ± 0.18	0.07 ± 0.04
HEMA-DEGMA	–OH	0.31 ± 0.12	0.07 ± 0.04
polyDEGMA	–OH	0.45 ± 0.19	0.13 ± 0.08
HEMA-PEA	–C(O)NH 5 mol%	0.26 ± 0.02	0.04 ± 0.01
	30 mol%	0.44 ± 0.16	0.10 ± 0.05
	50 mol%	0.34 ± 0.08	0.07 ± 0.02
HEMA-DMAEMA	$(CH_3)_2N$– 10 mol%	0.48 ± 0.25	0.11 ± 0.05
	30 mol%	0.74 ± 0.06	0.19 ± 0.11
Partially hydrolyzed polyHEMA	–COOH	0.00 ± 0.00	0.00 ± 0.00
polyAAMPS	$–SO_3H$ 10 mol%	0.14 ± 0.13	0.03 ± 0.03
	20 mol%	0.14 ± 0.14	0.03 ± 0.03

HEMA, 2-hydroxyethyl methacrylate; DEGMA, diethylglycol methacrylate; PEA, *N*-[1-(2-pyrrolidonyl)ethyl]acrylamide; DMAEMA, dimethyl aminoethyl methacrylate; AAMPS, 2-acrylamido-2-methyl propane sulfate.

Targeting ligands onto the polymeric materials surface also play an important role in determining the biocompatibility. Biochemical interactions at cell surfaces have led to structure elucidation of ligand molecules that bind to cell surface receptors and influence cell behavior (105–107). Theoretical

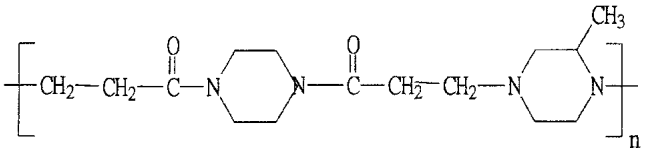


Fig. 6. Structure of polyamidoamine (PAA).

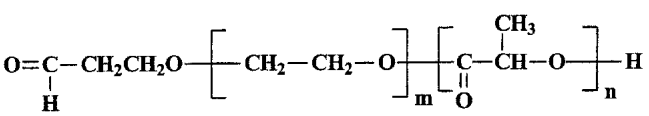


Fig. 7. Structure of copolymer of PEG-PLLA.

and experimental studies were conducted to elucidate the structure and properties of amphiphilic comb polymer thin films presenting nanoscale clusters of Arg-Gly-Asp (RGD) peptides for control of cell adhesion on biomaterials (108–110). Griffith and his co-workers have synthesized this kind of comb polymer [p(MMA-r-pOEM)], which is composed of a poly(methyl methacrylate) backbone and short poly(ethylene oxide) side chain (108). These polymers provide a means for control of cell adhesion through the tailored presentation of nanoclustered RGD peptides to cell surface integrin receptors (109). Biophysical cues such as ligand spatial arrangement and extracellular matrix rigidity are central to the governance of cell responses to the external environment (110). Whitesides' group has developed surfaces that promote the ligand-directed binding of cells and resist the cellular deposition of adhesive proteins based on self-assembled monolayers (SAMs) of alkanethiolates on gold (111). These surface present mixtures of RGD, a tripeptide that promotes cell adhesion, and oligo(ethyleneglycol) moieties, groups that resist nonbiospecific adsorption of protein and cells. Cannizzaro *et al.* (112) have reported the use of biotinylated degradable polymer PLA-PEG-biotin-avidin $(G)_{11}$ with cell adhesion motif GRGDS peptide as the ligand to facilitate cell-surface interaction. The polymer is a block copolymer of biotinyted poly(ethylene glycol) (PEG) with poly(lactic acid) (PLA). They used the high-affinity coupling of the biotin-avidin system to undergo postfabrication surface engineering and demonstrated that the materials promote interaction between cell and the linked biopolymer. Gordon and his co-workers (113) have created a new class of multivalent ligands, "neoglycopolymer," which structure is shown in Fig. 8. These synthetic ligands induce the release of the extracellular portion of L-selectin by appropriating an endogenous protease. Such activities suggest new strategies to generate anti-flammatory agents and regulate the cell surface (113).

TYPES AND DENSITY OF CHARGES IN POLYMERS

In general, neutral polymers and polyanions show less cytotoxicity than polycations. This is understandable because anionic surfaces of macromolecules tend to adsorb less protein than cationic surfaces and because most proteins bear a net negative charge. Polycations such as protamine and poly(L-lysine) have been shown to induce cellular damage in a variety of cultured cells (114–117). Choksakulnimitr *et al.* (118) investigated the cytotoxic effect of various macromolecules in different cell culture systems. They observed that polycations, such as protamine, poly(L-lysine), and histone, caused a high percentage of LDH release and significant morphological changes in all cultured cells, whereas cationized bovine serum albumin (BSA) and DEAE-dextran showed little cytotoxicity. No significant cytotoxic effects were observed when cells were incubated with neutral dextran or polyanions involving BSA, its derivatives and dextran sulfate (118).

Fischer *et al.* (87) performed a comparative *in vitro* cytotoxicity study of different cationic macromolecules using a combination of measurements from the MTT assay, the release of LDH, and microscopic observations. All assays yield comparable results for the ranking of the polymers with regard to cytotoxicity: PEI = PLL > poly(diallyldimethyl ammonium chloride) (PDDA) > DEAE-dextran > poly(vinyl pyridinium bromide) (PVPBr) > Starburst dendrimer (PAMAM) > cationized albumin (cHSA) > native albumin (nHSA).

These assays have shown that the molecular weights, as well as the cationic charge density of the polycation, are key determinants for the interaction with cell membranes and consequently cell damage. High cationic charge density polymers cause higher cytotoxicity than those with low charge densities. PEI was found to be the polymer with the highest cytotoxity in Fischer's study, which is correlated with the large molecular size as well as the high number of charges of PEI. As a trend, an increase of the charge/monomer ratio, determined as the number of cationic charges per monomer unit, is correlated with an increase in the cytotoxic effects (87). Table VII showed the IC_{50} values of various polycations tested at 3, 12, and 24 h with L929 mouse fibroblasts.

The complement system consists of multiple serum components and regulatory plasma proteins that may be activated after contact with foreign materials in contact with the circulation. Complement is activated in several ways called the classical and alternative pathways. Activation of the classical pathway is often initiated by the antigen-antibody complexes but can also be initiated in the absence of specific antibodies by bacterial or viral surfaces and by the contact of blood with certain polymeric materials (119–120). Cationic polymers, such as polybrene, protamine, and poly-L-ornithine, are known to cause activation of the complement system. Polyanions such as dextran sulfate, polyvinyl sulfate, chonddrotin sulfate, and poly(inosinic acid) inactivate C1 or C2 components of the classical pathway of complement activation and can inhibit complement activation by this means (121–123). The negatively charged pyran copolymer was reported to in-

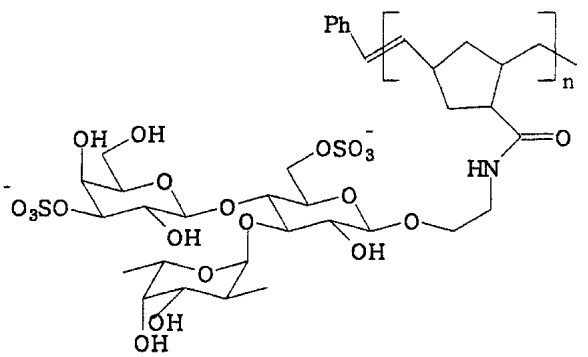


Fig. 8. Structure of neoglycopolymer.

Table VII. IC_{50} of Different Polycationic Macromolecules

Polymer	Charge/monomer ratio	IC_{50} 3 h	IC_{50} 12 h	IC_{50} 24 h
nHSA	n.d.	>10	>10	>10
cHSA	n.d.	>10	>10	9.28
PAMAM	0.0088	>10	>10	>10
PVPBr	0.0054	1.45	0.492	0.246
DEAE-dextran	0.00278	>2	0.011	0.011
PDDA	0.00619	0.096	0.048	0.034
PLL	0.00685	0.032	0.040	0.038
PEI	0.0233	0.031	0.022	0.009

L929 mouse fibroblasts were incubated for 3, 12, and 24 h with polymer solution. Cell viability was quantified by MTT assay (n = 8).

hibit the alternative pathway in a dose-dependent manner, probably by the alteration of factor B (124).

Anderson *et al.* (125) proposed that the induction of apoptosis in cells adherent to biomaterials can be influenced by the chemical properties of the surface of adhesion. A variety of polymeric surfaces were evaluated. The results of these studies indicate that surfaces displaying hydrophilic and anionic properties induce apoptosis of adherent macrophages more readily than hydrophobic or cationic surfaces.

Surface electrical charge may also produce a significant effect on biocompatibility. Tamaqua *et al.* (126) found that cationic macromolecules and their drug conjugates were rapidly eliminated from plasma, whereas weakly anionic macromolecules had a long circulation life. Charged surfaces have been proposed as being conductive to tissue integration. Both positive (127) and negative (128) charged surfaces were observed to promote bone formation.

MOLECULAR WEIGHT OF THE POLYMER

Biocompatibility may be also influenced by various properties of the polymers, including molecular weight, types and density of charges, structure and sequence (block, random, linear, branched, cross-linked), and conformational flexibility. In this section, we will restrict our discussion to the effect of polymer molecular weight.

Nagaoka *et al.* have shown that increasing the molecular weight of PEG grafted at a surface drastically reduced protein adsorption as well as adhesion and spreading of platelets (53). They found that surfaces coated with PEG, with a molecular weight of 5000, exhibited minimal protein adsorption and platelet adhesion.

Fischer *et al.* (87) have observed an increase in cytotoxicity as a function of the molecular weight for diethylaminoethyl dextran (DEAE-dextran). Commercially available 500 DEAE-dextran was hydrolyzed to products with lower molecular weights. The influence of molecular weight on the metabolic activity of mouse fibroblasts in tissue culture is summarized in Fig. 9. Incubation with high-molecular-weight polymers for 12 and 24 h produced a marked cytotoxic effect. In comparison, low-molecular-weight dextrans significantly affected cell viability only after a 24-h exposure time, reducing the number of degenerate cells to 14.0% (5.647) and 32.3% (4.199) (87).

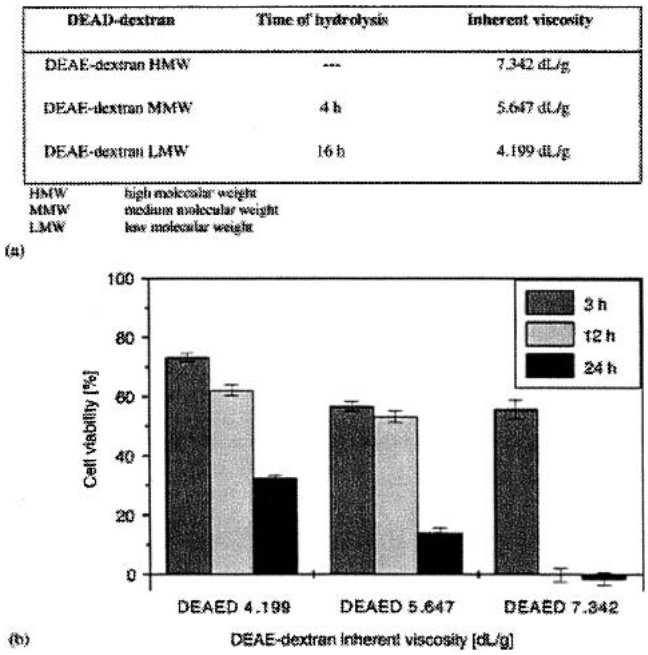


Fig. 9. Decrease of cell viability as a function of molecular weight (MW) (87).

The effect of biopolymer molecular weight on cytotoxicity has also been reported for other polycations, including poly(L-lysine) (PLL) (118,129–130), dendrimers (131), and poly(ethylenimine) (PEI) (132–133). Poly-L-lysine, with low molecular weight (MW 8000), showed less lactate dehydrogenase (LDH) release than poly-L-lysine synthesized with a higher molecular weight (MW 39,800) in cultured cell systems (118). Low-molecular-weight PEI (LMW-PEI, MW 11,900 Da) was less cytotoxic in a broad range of concentrations than the high molecular one (HMW-PEI, 1,616,000 Da). As demonstrated by transmission electron microscopy, LMW-PEI formed only small aggregates, which were efficiently taken up by different cells in the presence of serum. It should be pointed out that the effect of molecular weight applies only for polymers with the same structure.

CONFORMATIONAL FLEXIBILITY OF THE POLYMER

From their studies of various polycations, Fischer *et al.* (87) have proposed that the three-dimensional arrangement of the cationic residues is one of the important factors that modulate cytotoxicity. The arrangement of cationic charges depends on the three-dimensional structure and flexibility of biomolecules (118,134). Rigid molecules have more difficulties attaching to the membranes of cells than flexible molecules (134–135). Polycations with a globular structure (cationized human serum albumin, starburst dendrimer) were found to have good biocompatibility, whereas polymers with a more linear or branched and flexible structure (PDDA, poly-L-lysine, PEI) showed higher cell damaging effects (87). Branched cationic molecules have been found to be more efficient in neutralizing the cell surface charge than polymers with linear or globular structure (118). Protamine and poly-L-lysine have linear chain types and flexible macromolecules; they are more effective to neutralize the cell surface charges than molecules with rigid structures, such as DEAE-dextran (135).

SURFACE TOPOGRAPHY AND ROUGHNESS

Surface topography and roughness are important factors in determing the response of cells to a foreign material (23). Surface with grooves can induce "contact guidance," whereby the direction of cell movement is affected by the morphology of the substrate (136). This phenomenon has been applied to prevent epithelial downgrowth on dental implants and to direct bone formation along particular regions of an implant. *In vitro* studies demonstrated that grooves as small as 0.5 μm in depth were found to align and direct the migration of both fibroblasts and epithelial cells, and tightly spaced grooves (pitch < 30 μm) were more effective than widely spaced grooves in orienting cells (137). Von Recum *et al.* have found that the topography of an implant material (in the size range

1–3 μm) could radically alter the cellular response *in vivo* (138) and *in vitro* (139). Sheppard *et al.* (140) proposed the surface roughness influences thrombogenicity more than the other surface properties by studying the results of in *vitro* protein adsorption and total blood clotting tests. In recent years, substrate topography has been shown to be a key factor to determine the morphology and functional induction of cultured cell *in vitro* (141–143). Moghe *et al.* (144) found that the poly(glycolic-*co*-lactic)acid (PGLA) substrate microtopography can enhance cell adhesive and migratory responsiveness into matrix ligand density. They suggested that the actual nature of topographic regulation would depend on the size scale and geometric configuration, as well as the local density of adsorbed ligands.

Surface patterns, topographically and chemically, on different length scales generate different responses of the biological system. In order to obtain a positive and selective response, the molecular architecture should in general match some recognition site of biomolecules on the biological side of the hybrid interface (145). Whitesides and his co-workers have developed the methods of fabrication of nanostructures and 3-D microstructures to fabricate well-defined patterning organic surfaces (especially using self-assembled monolayers) for the study of biocompatibility (146–149). The soft lithographic techniques are a powerful set of tools for controlling the cell-material interface. They can be used to pattern nonplanar substrates and to make three-dimensional microstructures. They also allow the patterning of delicate ligands on a variety of substrates, including biocompatible substrate. Microcontact printing is a possible technique for controlling the chemistry of the surface at a molecular level, whereas microfluidic channels are well suited for patterning proteins and cells on variety of substrates (147). Whitesides and his coworkers have fabricated a variety of surface patterns using the cell adhesion peptides in combination with hexa-ethylene glycol thiolate which resist nonspecific adsorption of protein and cell (149). The resulting patterns consist are capable of aligning cells in a well-defined manner, leading to specific cell array and pattern formations. This simple and versatile biological surface engineering system may open new research opportunities including the further study of cell-material interactions, cell migration, cell-cell communication, and cell behavior.

Surface porosity also affects the biocompatibility of polymeric biomaterials. Korbelar *et al.* (150) found that the biocompatibility of PHEMA hydrogels increases in proportion to increasing porosity. Porous chitosan scaffolds were implanted in mice, and ELISA assays conducted that showed a very low incidence of chitosan-specific reaction in both lymphocyte proliferation assays and antibody responses (151).

SUMMARY

Biocompatibility is the central theme for the design and application of polymeric biomaterials. This article has discussed the various factors that affect the biocompatibility of materials. In summary, (i) to achieve minimum interfacial free energy, one might increase the polar surface free energy of polymers for potential medical applications; (ii) induction of balance between the hydrophilicity and the hydrophobicity on the surface is a way to enhance biocompatibility, and wettability is one of the most important parameters in the design of biomaterials or implant devices; (iii) the chemical structure and functional groups play a very important role in biocompatibility, and the structure-property relationship of biopolymers is a research topic to be further explored; (iv) surface electric charge may also have a significant effect on biocompatibility—the trend is that neutral polymers and polyanions show less cytotoxicity than polycations; (v) biocompatibility may be also influenced by the molecular weight (MW) of the polymer—low-MW polymer has less protein adsorption and platelet adhesion than a higher one; (6) conformational flexibility of the polymer and surface topography and roughness are important factors in determining the response of proteins and cells to a foreign material, in particular, the surface patterns may open new research opportunities including the further study of cell-material interactions and cell behavior. Though a comprehensive view of all factors affecting biocompatibility is not yet formulated, continued research may provide this understanding within a short time with consideration of chemical, physical, and biological contributions to bioreactivity and biotolerance.

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