4 Surface Engineering of Blood Contacting Polymeric Biomaterials

4.1 Introduction

Whereas ‘biocompatibility’ is usually considered to be the tolerance of liquid or solid body elements to the implant (foreign body), ‘blood compatibility’ is the tolerance of blood to the implant (foreign body) [1-8]. Blood is the first body fluid contacting with implants and many other biomedical and bio-analytical devices, so blood compatibility is of great importance in many clinical procedures, such as blood vessel repair, use of vascular grafts, stents, heart valves and so on. The application of biomaterials in blood contacting biomedical and bioanalytical devices is increasing and because of their exposure to a challenging biological environment during the work, they ultimately need surface engineering for an improvement of the blood compatibility.

The interaction of blood with artificial materials is very complicated. Adsorption of plasma proteins is the first event that occurs on their surface when they come in contact with blood. The platelet adhesion, which, follows contributes to a surface-induced thrombosis. If the adsorbed protein is denatured, the coagulation factors are activated, that causes a series of cascade reactions leading to blood coagulation. The blood - biomaterial interactions are multi-step and interlinked processes, to which much research activity is devoted [1–8]. The main events occurring within some minutes of contact, are protein adsorption, cell adhesion and inflammation leading to thrombi formation and fibrinolysis. Longer exposure of a biomaterial to blood could lead to embolisation, calcification and changes in the biomaterial properties. These interactions strongly affect the short-term and long-term thrombotic response induced by the material [2]. Some blood proteins, and more especially the clotting enzymes and fibrinogen, play an important role in the material associated clotting [7]. Therefore, to reduce clotting, surfaces and materials with highly protein-resistant properties have been extensively investigated [9]. Historically, in the development of blood-contacting materials, hundreds of polymers and other surfaces were evaluated for one or more aspect of their interaction with single proteins, for example, fibrinogen, but overall blood compatibility is assessed less often. Several types of functional groups appear to be highly resistant to fibrinogen adsorption, such as polyethylene glycol (PEG) [10], zwitterionic groups including phosphorylcholine (PC), sulfobetaine (SB), carboxybetaine (CB) [11–15] and polycarboxybetaine
methacrylate (PCBMA) [16, 17] as well as mixed positively and negatively charged self-assembled monolayers (SAM) with balanced charges [18, 19]. Complex blood-biomaterial interactions [20-23] include many influencing factors, such as surface chemical composition, charge, flexibility, wettability and blood flow conditions on which a lot of early work was focused [24-26]. Sperling and co-workers [27] found that leucocytes do not adsorb onto –CH₃-terminated alkane thiol SAM, but their adhesion is greatly enhanced on –OH groups. A strong correlation is observed between the activation of the complement system and the leucocytes adhesion to the –OH groups. The complement system activation is also related to the amount of –COOH groups on the surface. Rodrigues and co-workers [28] showed that fibrinogen adsorption decreases linearly with the number of –OH groups on a SAM surface. The platelet adhesion and complement system activation decreases with increasing surface hydrophilicity. Plasma albumin adsorption passivates the surfaces and lowers the platelet adhesion.

The protein adsorption from plasma is much more complicated than that from a single protein solution. Since the plasma is a concentrated, multi-protein solution and the plasma protein adsorption is a selective and competitive process [29], the ‘non-fouling surfaces’ that are highly resistant to a single protein adsorption do not demonstrate comparable efficiency in resisting plasma protein adsorption. For example, PEG SAM demonstrate very low fibrinogen adsorption from buffer solutions of this single protein, but they are not so resistant to protein adsorption from serum and blood plasma [30–34].

Platelet adhesion is mediated by integrins on the platelet surface, which bond to adsorbed adhesion proteins, especially fibrinogen, and cause platelet activation. The activated platelets then accelerate thrombosis, promoting the formation of thrombin and aggregation [31–34]. Fibrinogen in the blood plasma is particularly important for platelet adhesion, because it can bind to the platelet GP IIb/IIIa receptor [35]. Thus, the very low fibrinogen adsorption and low platelet adhesion are considered an essential biomaterial surface prerequisite to achieve improved blood compatibility [35, 36]. Shen and co-workers [37] find that less than 5-10 ng/cm² fibrinogen adsorption is necessary to prevent the attachment of platelets. Other researchers [38–40] find later that platelet adhesion is also determined by the molecular potency of the adsorbed fibrinogen and sometimes just a few activated platelets are enough to trigger a thrombotic response. Surfaces incorporating zwitterionic moieties, such as phosphobetaines, SB and carboxybetaines, reduce platelet adhesion and blood clotting (when compared to the corresponding surface without zwitterionic moieties), even if they do not adsorb fibrinogen below the threshold of 5-10 ng/cm² [41]. Llanos and Sefton [42] demonstrated that on some PEG-immobilised materials, platelet reactivity in vivo is still high although protein adsorption in vitro is low. Thus, surfaces that are ‘non-fouling’ to fibrinogen
adsorption and reduce platelet adhesion may be not good enough in terms of blood compatibility in vivo, and, thus, some active inhibition of clotting may be needed. In this regard, it is well known that some heparinised and sulfonated materials have anticoagulant activity, resulting in prolongation of the blood clotting time, when they are either dissolved in solution or coated on the blood containers [43, 44]. However, there is a significant lack of information regarding these surfaces for their ‘stealth’, i.e., grafted polymers such as PEG-like surfaces [45] do not alter blood clotting time by themselves and have no anticoagulant properties, whereas other grafted polymers can prolong blood clotting time by themselves, such as heparinised surfaces. Various reviews have been published that are devoted to a comprehensive discussion of different aspects of blood-compatible biomaterials [46–49]. A recent review by Chen and co-workers [50] highlights a new, although not yet mature idea, namely to improve the current biomaterials’ blood compatibility by introducing bioinspired surface micro- or nano-scale topographies, focusing mainly on their anti-platelet effects. The surface design of biomaterials for blood-contacting devices is of particular interest, and different approaches to the creation of biomaterials with improved thromboresistance are described in a number of reviews and books [51–56]. Because the blood is in contact with the material surface, surface coating and surface modification are the most general approaches to improving blood compatibility. Examples include minimisation of blood-biomaterial interactions, chemical modification with drugs and endothelial cell seeding, but these methods are still far from perfect. Surface chemical modification requires rather complex experimental procedures and it is expensive; long-term stability and potential downstream complications are additional aspects requiring improvement [47]. Endothelial cell seeding has the potential to provide effective surface modification because the outer surface of the material, covered by endothelial cells (EC), may function in the same mode as the endothelial surface itself. However, the adhesion and proliferation of EC on artificial surfaces are very complex phenomena [57]; moreover, the formation of these cell layers is a slow process so that this method could not be used in an emergency situation [58]. According to Ratner, we still do not have truly blood compatible surfaces after more than 60 years of serious study of blood compatibility and after numerous approaches have been tried [59]. Different concepts are currently employed to create biomaterials with improved blood-contacting properties:

- Physicochemical (zero critical surface tension or interfacial free energy);
- Microheterogeneous surfaces [polymers with microphase separated structure and segmented polyurethanes (SPU)];
- Simulation of blood vessel properties (surfaces with hydrophilic nature and high mobility, negatively charged surfaces);
• Utilisation of biologically active molecules (sustained release of heparin; heparinised surfaces); and
• Biomembrane-like surfaces composed of polymer and phospholipid.

However, the regulation of blood-biomaterial surface interaction is difficult and much of the research based on the previously-mentioned concepts has had only partial success. The trend now is towards a combination of the previously-mentioned concepts and hybridisation of artificial materials with biological molecules. The following sections present the main approaches and techniques developed so far for creating biomaterials with improved blood compatibility, such as the creation of strong hydrophilic or super-hydrophobic surfaces, heparin- or albumin-coated surfaces, seeding of EC, coating with zwitterionic polymers, creation of micro- and nano-structured surfaces, and so on.

4.2 Strongly Hydrophilic and Strongly Hydrophobic Surfaces

This approach is based on the theoretical prediction of Ikada and co-workers [60], that the work of adhesion in water media approaches zero when the water contact angle (WCA) or the surface free energy approaches zero, e.g., when the surface is strongly hydrophilic or strong hydrophobic. It is well known that strongly hydrophilic uncharged surfaces reduce protein adsorption and thus platelet activation, as far as the platelet activation is mediated by denaturised fibrinogen, whereas strongly hydrophobic surfaces provide an inherently weak surface-cell interface and exploit shear stress caused by the blood flow.

4.2.1 Strongly Hydrophilic Surfaces

Creation of strong hydrophilic, low adhesive surfaces is relatively easy and the immobilisation of water-soluble biocompatible polymers on the surface is one of the possibilities. Such polymers strongly adsorb water. The presence of high water content on the surface is accepted as a potential advantage of the biomaterial because of its similarity to living matter and especially because it provides minimal interface tension in contact with blood, as well as reduced protein adsorption and cell adhesion [61]. To try and simulate the properties of the natural bloodstream some researchers have created highly flexible hydrophilic surfaces using water-soluble polymers with flexible chains, especially PEG. Such surfaces can be schematically represented as in Figure 4.1.
When proteins, platelets and other species approach such a surface, they in fact contact with water. Different approaches to polymer surface modification with PEG are used, such as covalent bonding to different polymer substrates; graft polymerisation of pendant PEG chains; introduction of PEG segments into the main polymer via block copolymerisation; direct adsorption of PEG-containing surface-active substances; layer-by-layer (LbL) deposition of PEG-containing polycations or polyanions. Most of these approaches are described in Chapter 2 and will not be repeated here. Low platelet adhesion is observed on acrylamide or other hydrogel coatings as well as on collagen coatings onto corona pre-activated polymer surfaces, reducing fibrosis around the biomaterial implants, as proved by *in vivo* animal experiments [62-65]. It is established experimentally that an increase in the surface hydrophilicity decreases the cell adhesion. However, low cell adhesion does not definitely prevent biological activation. Some researchers [65-67] found low platelet adhesion to strongly hydrophilic polar surfaces but high thrombin activation and coagulation. Morra and co-workers [68] review the role of polyethylene oxide (PEO) in the surface modification of polymeric materials for blood-contacting applications. The peculiar anti-bioadhesive properties displayed by PEO chains deeply affect thromboembolic phenomena, which are controlled by material-blood interfacial interactions. The physical basis of the anti-biofouling properties of PEO-covered surfaces are discussed in terms of the mobility of PEO chains in aqueous environments, steric stabilisation effects, the structural fit of PEO repeating units with the structure of water, and van der Waals interactions between PEO chains and the elements of blood. A number of
strategies are explored to produce thin strongly hydrophilic coatings. Glow discharge surface treatment with tetraglyme is one of them, producing surfaces that exhibit low fibrinogen adsorption [69, 70]. Surface-initiated free radical polymerisation of hydrophilic monomers promotes polymerisation as well as crosslinking in a limited region above the substrate. Thin hydrogel barriers prepared in this way inhibit thrombosis and intimal thickening [71, 72]. Adsorption of high molecular weight PEG-polylysine copolymers onto endovascular stents reduces re-stenosis after implantation [73], although long-term stability of the adsorbed copolymers may be a concern [74]. Copolymers of methyl methacrylate (MMA) and PEG are also quite effective in reducing the non-specific cell adhesion on a variety of surfaces [75-78]. A single or very thin crosslinked layer of ‘star’ (multi-arm) PEG reduces non-specific cell adhesion on the surface [79-81].

Covalent LbL methods in some cases yield coatings with resistance to protein adsorption and cell adhesion [82-84]. Covalent LbL methods of depositing dense PEG coatings are quite complicated and provide only a moderate reduction in cell adhesion [85]. Thin hydrogel coatings may be useful for reducing coagulation and thrombosis on blood-contacting devices with complex geometries, such as endovascular stents. However, the density of PEG chains in the coating must be high enough to achieve a substantial reduction in protein adsorption [86, 87]. Most single and even multiple PEG layers do not provide the required cell adhesion resistance for long-term effectiveness [85]. This suggests the use of relatively large aggregates of high-density PEG chains to create thin coatings, which could ensure adequate surface coverage of the biomaterial surface even with a limited number of attachment sites. Based on this idea, Scott and co-workers [88] report a dip coating strategy for covalent linkage of PEG to different substrates, producing PEG coatings ≤ 100 nm thick to address the long-term thrombosis of drug-eluting stents. The gelation of PEG octavinsulfone with amines in either bovine serum albumin (BSA) or PEG-octaamine is monitored by dynamic light scattering, which reveals the presence of the microgel before the macrogelation. Nuclear magnetic resonance (NMR) also reveals extremely high end-group conversions prior to macrogelation, consistent with the formation of highly crosslinked microgels and deviation from Flory–Stockmayer theory. The reacting solutions are diluted and incubated with nucleophile-functionalised surfaces before the macrogelation. Using optical waveguide light mode spectroscopy and quartz crystal microbalance with dissipation, a highly hydrated, protein-resistant layer with a thickness of approximately 75 nm was identified. Atomic force microscopy (AFM) in buffered water revealed the presence of coalesced spheres of various sizes but with diameters less than about 100 nm. Microgel coated glass or polyethylene terephthalate (PET) exhibits reduced protein adsorption and cell adhesion [89]. Cellular interactions with the surface are controlled by using different proteins to cap unreacted vinylsulfone groups within pH-responsive smart biomaterials of gelatine and poly(2-hydroxyethyl methacrylate-co-acrylic
Synthesised by redox polymerisation and characterised by Fourier-transform infrared spectroscopy (FTIR) and environmental scanning electron microscopy, environmentally responsive biomaterials containing polyelectrolyte segments prepared in this way are assessed for their water sorption potential under varying experimental conditions. A diffusion mechanism for the transport of water molecules arising from the solvent-polymer interaction is analysed to predict the behaviour of continuously relaxing macromolecular chains. The *in vitro* blood compatibility of these hydrophilic materials is evaluated by blood clot formation, platelet adhesion, percentage of haemolysis and protein-adsorption study of their surfaces [88]. Yao and co-workers [89] review the applications of bio-inspired special wettable surfaces, including their use as biomaterials. Trials to improve thromboresistance via creation of negatively charged hydrophilic surfaces are an example of bio-inspired approaches [90-94]. It is thought that the non-thrombogenicity is partially due to the surface negative charge, because most cells, including platelets, have negatively charged surfaces and so electrostatic repulsion from the negatively charged surface is significant [65, 95]. Because of the possible electrostatic interaction between cells and negatively charged surfaces, some researchers have created polymer surfaces that have been sulfonated to different extents and tried to correlate blood compatibility with the $\xi$-potential of the surface, but the expected correlation has not been found [95, 96].

### 4.2.2 Strongly Hydrophobic Surfaces

Mirzadeh, Korasani and co-workers [98-103] created strongly hydrophobic polymer surfaces by laser treatment and turned them into hydrophilic ones by grafting of hydroxyethylmethacrylate (HEMA) (after pre-activation by CO$_2$ pulse laser treatment). The data from their *in vitro* investigation demonstrated significantly reduced platelet adhesion and aggregation on the modified surfaces, both the strongly hydrophobic and the strongly hydrophilic, but the strongly hydrophobic surface appeared to be better with respect to blood compatibility [104]. Ion-beam radiation of siloxane rubber at a relatively high energy (50–150 keV) alters its surface chemical composition and wettability, which leads to lower thrombus formation on the ion-implanted haemodialysis catheters, as proved by *in vivo* experiments with animals [105-108].

### 4.3 Biomaterials with Micro- and Nano-domain Surfaces

Another, relatively old but still interesting, concept for blood compatibility is that of microdomain surfaces. Biomaterials with microdomain surfaces on which adsorbed proteins are able to self-organise according to the surface microheterogeneity are based
on this concept. It has been demonstrated that the low thrombogenicity of ABA-type block copolymers with hydrophilic-hydrophobic microdomain structure is due to significant reduction in the activation of the adhered platelets [109-114]. Typical of this group are the segmented polyether urethanes [115]. Some commercial segmented polyether urethanes are immobilised with a non-steroidal anti-inflammatory drug to improve their haemocompatibility [116]. p-Aminosalicylic acid (PAS) is a water-soluble non-steroidal anti-inflammatory drug with anti-aggregate platelet activity, which can be covalently immobilised on a SPU surface as follows: the SPU surface is modified by grafting of hexamethylenediisocyanate, and the free isocyanate remaining on the SPU surface is then coupled through a condensation reaction to the amine groups of PAS. The bonding of PAS from aqueous solution onto the SPU surface is studied by attenuated total reflectance – Fourier transform infrared spectroscopy (ATR-FTIR), ultraviolet (UV) and fluorescence spectroscopy. Plateau levels of coupled PAS are reached within 1.2 μg/cm² using PAS solution concentrations of 1 mg/ml. The surface wettability measured by WCA indicates that the immobilisation of PAS makes the surface more hydrophilic (θ_{water} = 43.1° ± 2.1°) when compared to the original SPU (θ_{water} = 70.3° ± 1.9°). The in vitro albumin (BSA) adsorption shows that the PAS-SPU surfaces adsorb more BSA (250 μg/mm²) than the unmodified SPU (112 μg/mm²). The PAS-immobilised polymer surfaces demonstrate better non-thrombogenic characteristics, assessed by measuring the thrombus formation and platelet adhesion. The low platelet adhesion, high adsorption of albumin relative to fibrinogen and low thrombus formation make them good potential candidates for biomedical applications. Generally, the SPU are block copolymers with good elasticity and thermoplasticity but they often need some modification to provide excellent biocompatibility. Ogawa and co-workers [117] blended 2-methacryloyloxyethyl phosphoryl choline (MPC) polymer and SPU to prepare an SPU/MPC polymer alloy with controlled domain structure. The effect of the molecular weight (M_w) of the MPC polymer on the microdomain structure and the mechanical properties of the polymer alloy were investigated. When MPC polymer with a higher M_w is blended with SPU, the SPU/MPC alloy undergoes a reduction in mechanical strength. On the other hand, differential scanning calorimetric (DSC) analysis revealed that the MPC polymer chains do not disrupt the crystallinity of the hard segments of SPU and the SPU/MPC alloy has the same physical properties as that of the original SPU when lower M_w MPC polymer is blended with the SPU. The blending of SPU with MPC polymer significantly improves the bio- and blood compatibility of the SPU evaluated by the surface adsorption of immunoglobulin.

Nanoscale modification of SPU with crosslinked MPC polymer can be performed to obtain biocompatible elastomers [117]. Various compositions of monomers are used to control the size and depth of the modified layer domains, including MPC, 2-ethylhexyl methacrylate (EHMA), and glycerol or 1,3-diglycerolate diacrylate. An SPU film is immersed in the monomer solution and visible light irradiation is
applied to initiate polymerisation of the SPU film, which is held by mica to condense MPC units at the surface. The surface of the obtained films is analysed by X-ray photoelectron spectroscopy (XPS) and WCA measurement. The surface density of MPC units changes with the monomer concentration, and is highest at an MPC:EHMA ratio of 7:3. In the modified SPU films, 6 to 25 nm MPC unit enriched domains are observed, the density of these domains gradually decreasing with the depth and their size, depending on the MPC composition in the monomer solution. The mechanical properties of the modified films, as evaluated by tensile strength under wet conditions, do not differ significantly from those of the unmodified SPU. With an increase in MPC unit enriched domains on the modified film surface, platelet adhesion and activation are significantly reduced when compared to the SPU film. This nanoscale surface modification is a useful technique for improving the haemocompatibility of elastic polymeric biomaterials. Poussard and co-workers [118] synthesise new SPU anionomers based on hydroxy telechelic polybutadiene via two environmentally friendly chemical routes. The effects of carboxylic group content and ion incorporation mode on the surface properties were investigated by water absorption analysis and static contact angle measurements using water, diiodomethane, formamide andethylene glycol (EG). Blood compatibility of the SPU was evaluated by in vitro adhesion assay using $^{111}$In radiolabelled platelet-rich plasma and $^{125}$I fibrinogen. The morphology of platelet adhesion was also observed by scanning electron microscopy (SEM). The carboxylic groups on the soft segments (S-α series), created by means of thioglycolic acid, increase the surface hydrophilicity, limit water uptake (5% for an ion content of 3.6 wt%), and reduce platelet adhesion and fibrinogen adsorption on the SPU’s surface. In contrast, the classical insertion of carboxylic groups onto the hard segment (H-α series), using dimethylolpropionate as chain extender, leads to high water uptake (18% for an ion content of 3.6 wt%) and promotes platelet and fibrinogen adhesion. SEM analyses of the non-ionic SPU exhibit surfaces with adhered platelets that undergo morphological modification. Similarly, the H-α ionic SPU exhibits adherent and activated platelets. On the contrary, no platelet morphology changes are observed on the S-α ionic surface. The insertion of carboxylic groups on the soft segments of SPU reduces their thrombogenicity.

Lina and co-workers [119] immobilised water-soluble chitosan (WSC)/dextran sulfate (DS) to improve blood compatibility of thermoplastic polyurethane (TPU) membranes. WSC/DS was immobilised on the surface of TPU membranes after ozone-induced graft polymerisation of polyacrylic acid (PAA). The surface is characterised by contact angle measurement and XPS. The adsorption of human plasma fibrinogen (HPF) follows the Langmuir adsorption isotherm. The surface density of the generated peroxides and the grafted PAA reaches a maximum value after ozone treatment for 20 minutes. The amount of immobilised WSC/DS increases with the increase of pH and the WSC molecular weight. The membrane-water interfacial free energy increases with PAA grafting and WSC/DS immobilisation,
indicating an increased wettability of the TPU membrane. The adsorption of fibrinogen on the TPU-WSC/DS membranes is effectively curtailed. Moreover, WSC/DS immobilisation effectively reduces the platelet adhesion and prolongs the blood coagulation time, thereby improving the blood compatibility of the TPU membrane. Thrombosis and intimal hyperplasia are the principal causes of small-diameter vascular graft failure. To improve the long-term potency of polyurethane (PU) vascular grafts, Taite and co-workers [120] incorporated both PEG and a diazenium diolate donor of nitric oxide (NO) into the backbone of the PU to improve the thromboresistance. Additionally, they incorporated a laminin-derived cell adhesive pentapeptide sequence: Tyr-Ile-Gly-Ser-Arg (YIGSR), to encourage endothelial cell adhesion and migration, while NO release encourages endothelial cell proliferation. NO production by PU films under physiological conditions demonstrates biphasic release, in which an initial burst of 70% of the incorporated NO is released within two days, followed by sustained release over two months. Endothelial cell proliferation in the presence of the NO-releasing material increases compared to the control PU, and platelet adhesion to PEG-containing PU decreases significantly with the addition of the NO donor. To improve the blood compatibility of a polyethylene (PE) film, Mao and co-workers [121] modified it by adding Pluronics F127, followed by crosslinking for stable entrapment in the PE matrix. The crosslinking is done by free radicals produced from the decomposition of dicumyl peroxide in the film through heating at 120 °C. The surface properties of the Pluronics F127 additive-containing PE films were investigated by FTIR, electron spectroscopy for chemical analysis and WCA measurements. The blood compatibility of these films was evaluated by platelet-rich plasma and blood cell adhesion tests. The blood compatibility of the Pluronics F127-containing film was better than that of the blank PE film. Blends of novel L-tyrosine based PU and polyphosphate with potential biomedical applications have been developed by Shah and co-workers [122] using an L-tyrosine based diphenolic monomer, desaminotyrosine-hexyl ester (DTH). Soft segments, polycaprolactone (PCL) diol and PEG, are used to synthesise two biodegradable L-tyrosine polyurethanes (LTU), PEG–C–DTH and PCL–C–DTH, respectively. These polymers have dramatically different physicochemical properties. By blending LTU with L-tyrosine polyphosphate (LTP), a family of materials with a wide range of thermal, morphological, surface, and derivative properties is produced. The hydrophilic nature of PEG is imparted to the PEG–based blends (PEG–C–DTH/LTP) as a significantly higher surface and bulk hydrophilicity compared to those of the PCL–based blends (PCL–C–DTH/LTP). The blends demonstrate a rapid initial hydrolytic degradation in phosphate buffered saline (PBS), followed by a significantly slower, prolonged degradation. Besteiro and co-workers [123] characterised PCL–based polyester urethane urea membranes prepared by extending a polypropylene oxide–based triisocyanate terminated PU pre-polymer with a PCL diol, and evaluated the in vitro haemocompatibility of the crosslinked bi-soft segment. The variation of the ratio of PU to PCL diol
in the membrane formulation yields alteration of the surface energy, and phase morphology both in the bulk and in the region near the surface, and, thus, affects the haemocompatibility. Nearly all membranes appear to be non-haemolytic for short-time contact with blood (15 minutes). The membranes prepared with 5% and 25% of PCL diol show almost no adherent platelets. These two membranes have higher hard segment aggregation in the region near the surface and mixing between the two soft segments in the bulk, but show contrasting surface energy characteristics. The surface energy and its polar and dispersive components do not correlate with any of the haemocompatibility aspects studied. In contrast, the phase morphology in the region near the surface is a major influencing factor on membrane haemocompatibility. Xu and co-workers [124] developed PU/dermatan-sulfate copolymers as haemocompatible, non-biofouling materials. While most approaches to the creation of non-biofouling materials use passive synthetic molecules, Xu and co-workers [124] described a way of preventing protein adsorption and adhesion of multiple cell types via incorporating a natural glycosaminoglycan molecule into a PU polymer chain.

4.4 The Immobilisation of Heparin and Other Bioactive Molecules

Various biologically active agents and molecules, e.g., heparin, prostaglandin and some enzymes, have been investigated as inhibitors of the coagulation process, and some devices with anticoagulant properties have already been created. It is known that mucopolysaccharides, heparin sulfate and chondroitin sulfate exist on the internal surfaces of blood vessels, making them non-thrombogenic [125]. On the basis of this observation, some researchers have tried to develop heparinised surfaces by looking for a correlation between the blood compatibility and the $\zeta$-potential of the surface but such a correlation has not been found [126-128].

4.4.1 Heparinised Surfaces

Surface heparinisation appears to be one of the most promising approaches to creating blood-compatible materials. The concept of heparinised surfaces is based on the acceptance that if heparin is immobilised onto a polymer surface while retaining its anti-thrombin activity, it will make the surface non-thrombogenic. Various ways of surface immobilising heparin are described in the literature [65, 129, 130]. Heparin naturally exists on the intravascular endothelium, together with other sulfated glucoaminoglycans [131]. Heparin has been in clinical use since the 1930s [132] as the most common anticoagulant preventing blood clotting during surgery and in the treatment of post-operative thrombosis and embolism. Pharmacologically, the
well-known anticoagulant activity of heparin is predominantly due to its ability to accelerate the reaction by which anti-thrombin III (ATIII) inactivates the thrombin, factor Xa and factor IXa [133, 134].

![Chemical structure of heparin](image)

**Figure 4.2** Chemical structure of heparin (a non-diffusing polyelectrolyte).
Adapted from page 34 of reference [55]

As is evident from **Figure 4.2**, heparin is a highly sulfated, anionic blood polysaccharide (5–25 kDa) that can bind to the blood protein anti-thrombin through ionic interactions, and the result is a several-fold increase in the rate at which anti-thrombin inactivates clotting factors such as thrombin. The enzyme thrombin plays a key role in the coagulation cascade by cleaving fibrinogen to produce fibrin monomers; the thrombin also increases platelet-platelet adhesion and stimulates platelet activation and degranulation. Thus, the inactivation of thrombin (by heparin-bound anti-thrombin) will inhibit blood coagulation. Surface tethered heparin is known to suppress platelet adhesion, and complement activation and protein adsorption [135]. Inactivation of the thrombin prevents transformation of the fibrinogen to crosslinked fibrin, i.e., prevents blood clotting. Therefore, it is accepted that surfaces with controlled release of heparin would be anti-thrombogenic. The easiest way to create such surfaces is by the physical adsorption of heparin. This approach is suitable for short-term release of heparin, up to several days, but the heparin adsorption is more stable if it forms a complex with the substrate surface [136]. Because of the strong anionic nature of its molecule, the heparin can form ionic complexes with cationic active substances. Heparin surface films can be formed through the adsorption of colloidal particles composed of an initial binary ionic complex, by mixing heparin and hexadecylamine hydrochloride in aqueous solution above the Krafft temperature, i.e., 48 °C [137]. The heparinisation of artificial surfaces is a successful strategy for preventing blood clotting and thrombus formation, as well as for improving the haemocompatibility of blood-contacting surfaces but generally, the binding of ATIII is most efficient when heparin is coupled by endpoint attachment [65, 138] (**Figure 4.3**).
Covalent bonding of nitrite-degraded heparin onto membrane oxygenators and tubing by end point attachment makes it possible to maintain a long-lasting, extracorporeal circulation without systematic heparinisation [138].

By a chemical process, polyamido-amine chains can be grafted onto the surface of PET (Dacron) devices. After such treatment, the devices are able to absorb significant amounts of heparin. Heparin recovery happens only by eluting at pH > 10 with sodium hydroxide solution [139]. The most significant challenge of the immobilisation is how to improve the heparin surface concentration while retaining its biological activity. Larm and co-workers [140] and Kim and co-workers [114] employed quasi-irreversible adsorption of branched high $M_w$ polyethylene imine (PEI) and heparin. The coated materials demonstrated a stable heparin attachment with retained biological activity. Heparin immobilisation via a spacer arm is preferable for keeping its biological activity. Effective immobilisation of heparin can be achieved either by grafting via spacer groups to a soluble polymer, followed by heparin polymer coating deposition on different biomaterial surfaces, or by surface bonding of heparin via spacer groups direct to the surface of non-soluble materials [141]. Ebert and co-workers [142] demonstrated that when heparin bonds to a substrate via an alkyl group, its activity increases with increasing spacer arm length. The nature of the spacer chains and the type of bonding control the amount of immobilised heparin and the speed of its release [143]. Spacer arm immobilised heparin acts conventionally by initially binding...
anti-thrombin, followed by formation of a ternary complex with the thrombin [144]. In vitro and in vivo tests of heparin-immobilised PU surfaces confirm the enhanced heparin activity when it is coupled via a spacer arm, in this case via flexible PEO spacer chains [114, 145]. Kim and co-workers [146] developed a similar method for surface immobilisation of heparin but thorough chemical reinforcing, e.g., a polyfunctional substance grafts firstly to the polymer surface by means of diisocyanate, followed by heparin immobilisation via a hydrophilic PEG spacer. This procedure leads to a four-fold increase in the amount of immobilised heparin. Ito and co-workers [143] investigated in vitro platelet adhesion and in vivo anti-thrombogenicity of polyether urethane urea, to which heparin is bonded covalently or ionically. When heparin is covalently bound to the polymers, platelet adhesion and platelet activation is increasingly suppressed with the increase in the bonded heparin amount. However, the adsorbed platelets activate to different degrees, depending on the method of heparinisation. Platelet adhesion and platelet activation upon adhesion appear to be regulated by the electrostatic repulsion between the platelet and the anionic surface of the covalently or ionically heparinised polymer, rather than by the physiological action of the bonded heparin. The effect of the heparinisation seems to be related to the molecular heterogeneity of the heparin. Heparinised PU that interacts very weakly with platelets in vitro have been tested for in vivo anti-thrombogenicity. The test was carried out by implanting a suture made of heparinised PU into canine veins. Ionically heparinised PU did not form a thrombus and maintained a smooth surface over a long period. On the other hand, covalently heparinised PU formed a small amount of thrombus and grew EC at the insertion point. Later experiments with polyvinyl alcohol-heparin hydrogels produced unsatisfactory results, which placed doubt on the possibility of in vivo applications for heparinised polymers [147].

Although graphitic carbon has been known and used as a biomaterial for a long time, the excellent biocompatibility of diamond-like carbon (DLC) films is only addressed in a few cases. Steffen and co-workers [148] anticipated the combination of bio-inert DLC films and surface-immobilised bioactive biomolecules with anti-thrombogenic properties, such as the polysaccharide heparin, as a straightforward concept to optimise haemocompatibility of a wide variety of materials (vascular grafts and so on), applying this strategy to polytetrafluoroethylene (PTFE), polydimethyl siloxane (PDMS) and polystyrene (PS). The DLC films are deposited on the polymer surface by an energetic acetylene plasma beam and subsequent exposure to ammonia plasma before covalent coupling of heparin to the surfaces functionalised in this way by an end point attachment. Heparin acts by binding to anti-thrombin and, thus, preventing clot formation.

Sandhu and Luthra [149] reported the development of a new haemocompatible material. This material is said to provide both anti-thrombogenic and non-thrombogenic properties, which provide an endothelial-like action that prevents
protein adsorption and inhibits thrombin at the same time. The new non-thrombogenic/
anti-thrombogenic polymer coating can provide triple endothelial-like action; the non-
thrombogenic and anti-thrombogenic polymer is commercialised by Medtronic Inc.,
under the name of Trillium and it is licensed for cardiopulmonary bypass products.

Michanetzis and co-workers [150] compared the improvement in haemocompatibility
of four polymeric biomaterials prepared by two different surface heparinisation
procedures introduced by Bamford and Al-Lamee [151] and Seifert and co-workers
[152], in order to compare their efficiency in improving the haemocompatibility of
four commercially available biomaterials: silicone rubber (SR), PE, polypropylene (PP)
and polyvinylchloride (PVC). The indirect method of Bamford and Al-Lamee [151]
produced a much better heparinisation yield (10.5% maximum) compared to the
direct method of Seifert and co-workers [152], with a yield of only 0.2% maximum.
Both methods provide a better response of the heparinised biomaterials compared
to the uncoated ones in terms of platelet retention and a significantly better response
in terms of activation of the coagulation system, suggesting that heparin molecules
remain biologically active in both cases. The results are particularly interesting for
PVC, where the maximum immobilisation yield is obtained by the indirect method,
also resulting in a pronounced improvement in haemocompatibility. SEM studies are
used to examine the adhered platelet morphology whereas AFM is used to determine
the surface morphology of heparinised and reference materials.

Kim and co-workers [153] designed sulfonated PEO (PEO–SO$_3$H) as a ‘negative cilia
model’ to investigate the synergistic effect of PEO and negatively charged –SO$_3$ groups.
PEO–SO$_3$H itself exhibits a heparin-like anticoagulant activity that is about 14%
of that of the free heparin. PU grafted with PEO–SO$_3$H (PU– PEO–SO$_3$H) increases
albumin adsorption significantly but suppresses other proteins, while PU–PEO
decreases the adsorption of all the proteins. The platelet adhesion is decreased on
PU–PEO but less so on PEO–SO$_3$H, demonstrating an additional effect of the –SO$_3$
groups. The enhanced blood compatibility of PU– PEO–SO$_3$H in the ex vivo rabbit
and in vivo canine implanting tests is confirmed. Furthermore, PEO–SO$_3$H exhibits
an improved biostability and suppresses calcification in addition to the enhanced
anti-thrombogenicity. The in vivo anti-thrombogenicity and biostability are improved
in the order of PU <PU–PEO <PU– PEO–SO$_3$H. The deposited calcium amounts
decrease in the order of PU >PU–PEO >PU– PEO–SO$_3$H in spite of the possible
attraction between negative –SO$_3$ groups and positive calcium ions. The bioprosthetic
tissue (BT) is grafted with H$_2$N– PEO–SO$_3$H via glutaraldehyde (GA) residues after
conventional GA fixation. BT– PEO–SO$_3$H also displays decreased calcification in
the in vivo animal models. The application of PEO–SO$_3$H is extended by designing
amphiphilic copolymers containing a PEO–SO$_3$H moiety and hydrophobic long alkyl
groups as anchors. The superior effect of PEO–SO$_3$H groups on thromboresistance
compared to PEO is also confirmed for copolymers coated or blended with other
polymers and the systems coupled by UV irradiation, photoreaction or gold-sulfur or silane coupling technology, and, therefore, it might be very useful for medical devices. Much earlier, a model of a heparinised surface (sulfated PE) had been developed and tested for blood compatibility by a Swedish research group [154].

Xu and co-workers [155] investigated heparin-coupled poly(PEG monomethacrylate)-Si(111) (PEG-MA) hybrids and their blood-compatible surfaces. Well defined (nearly monodispersed) poly(PEG-MA)–Si hybrids were prepared via surface initiated atom transfer radical polymerisation (ATRP) of the PEG-MA macromonomer on the hydrogen-terminated Si(111) surface (Si–H surface). Both the active chloride groups at the chain ends (from the ATRP process) and the chloride groups converted from some (~32%) of the –OH groups of the Si–C bonded PEG-MA polymer, or poly(PEG-MA) brushes can be used as leaving groups for the covalent coupling of heparin. For the heparinised poly(PEG-MA)–Si hybrid surfaces, protein adsorption and platelet adhesion are significantly suppressed. The well-defined and dense poly(PEG-MA) brushes, prepared from surface-initiated ATRP, allow the immobilisation of a relatively high amount of heparin (about 14 μg/cm²). The resulting silicon surface exhibits significantly improved anti-thrombogenicity with a plasma recalcification time (PRT) of about 150 minutes. The persistence of high bioactivity for the immobilised heparin on the hybrid surfaces can be attributed to the biocompatibility of the PEG-MA units, as well as their role as spacers in providing the immobilised heparin with a higher degree of conformational freedom in a more hydrophilic environment. Thus, the heparin-coupled poly(PEG-MA)–Si hybrids with anti-fouling and anti-thrombogenic surfaces are potentially useful in silicon-based implantable devices and tissue engineering. A membrane mimetic assembly incorporating surface-bound heparin can be fabricated as a system to improve the haemocompatibility of blood-contacting devices [156]. As a model system, heparin is chemically modified by end-point conjugation to biotin and immobilised onto membrane mimetic thin films via biotin-streptavidin interactions. Heparin surface density, determined by radiochemical titration, confirms that surface density is directly related to the molar concentration of the biotinylated lipid within the assembled membrane mimetic film. The capacity of surface bound heparin to promote ATIII-mediated thrombin inactivation is investigated in a parallel plate flow chamber under simulated venous and arterial wall shear rates of 50 and 500 s⁻¹, respectively. The rate of thrombin inactivation approaches a maximum at a heparin surface concentration greater than 4.4 pmol/cm² (61 ng/cm²). In the process, mass transport-limited regimes are identified for heparin-potentiated thrombin inactivation under both simulated venous and arterial conditions. New heparinisable modified polycarbonate urethane surfaces that diminish bacterial colonisation have been reported by Nardo and co-workers [157]. Ayres and co-workers [158] found that polymer brushes containing sulfated carbohydrate repeat units, which resemble surface-tethered heparin, result in significantly longer plasma recalcification clotting times than
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with non-sulfated polysaccharide polymer brush surfaces, used as a control. The sulfated brushes also reduce the production of complement factor products C3a, C4a and C5a, in comparison to the control surfaces. Nakayama and co-workers [159] prepare a heparin bioconjugate with a thermoresponsive cationic branched polymer. This is a novel, aqueous, anti-thrombogenic coating material comprising heparin bioconjugate that incorporates a thermoresponsive cationic polymer as a surfactant. The polymer is prepared by the sequential steps of initiator transfer agent terminator (iniferter) based living radical photopolymerisation of N-[3-dimethylamino-propyl]acrylamide (PDMAPAAm), followed by the polymerisation of N-isopropylacrylamide from tetra(N,N-diethyldithiocarbamylmethyl)benzene as a multifunctional iniferter. The polymer obtained has four branched chains, each consisting of a cationic PDMAPAAm block (M_n ~ 3000 g/mol) forming an inner domain for heparin binding and a thermoresponsive poly(N-isopropyl amide) block (M_n ~ 6000 g/mol) forming an outer domain for surface fixation; bioconjugation of the polymer with heparin occurs immediately upon simple mixing in an aqueous medium. Because the lower critical solution temperature (LCST) of the heparin bioconjugate is approximately 35 °C, it could be coated from an aqueous solution at room temperature. The excellent adsorptivity and high durability of the coating below 37 °C is demonstrated on several commonly used polymers by wettability measurement and surface chemical compositional analysis, and on collagen sheets and rat skin tissue by heparin staining. Blood coagulation is significantly prevented on the heparin bioconjugate coated surfaces.

Huang and co-workers [160] prepared a heparin-modified polysulfone membrane combining a specific recognition of low-density lipoproteins (LDL) and excellent blood compatibility, and, therefore, with great potential as an LDL absorber for applications in haemodialysis with simultaneous LDL removal. The modified membrane has three components: heparin, a negative charge and hydrophilicity. Non-leaching heparin molecules are covalently bonded onto the surface to provide similar beneficial effects to heparan sulfate in the natural endothelium. Sulfate and sulfonate groups, which carry a strong negative charge, are incorporated into the functional layer of the material to repel blood cells and proteins. Similarly, heparan sulfate in the vascular endothelium is a heavily sulfated molecule with a negative electrical charge, as heparin is in the novel material. The primary objective of Chen’s research [161] is to determine the optimal conditions for heparin immobilisation on collagen powder by varying pre-treatment methods, pH of the reaction environment and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to heparin weight ratio. This research is part of a series of investigations, with the ultimate purpose of developing an implantable small diameter (< 6 mm) vascular prosthesis that will remain functional while serving as a suitable interposition in the circulatory system.
4.4.2 Immobilisation of Other Bioactive Molecules

Mimicking biologically inert membrane surfaces is one of the most active areas of recent research. Other polysaccharide-based glycocalyx mimicking polymer coatings that reduce platelet adhesion and improve blood compatibility are also described in the literature [161-164]. Ferrer and co-workers [165] reported the creation of biomimetic polymer surfaces by photochemical attachment and patterning of dextran. The attachment of homogeneous and patterned dextran films works on PU and PS, with detailed analysis of surface morphology, swelling behaviour, and the protein resistance of these substrates. The photoactive dextran described and the attachment procedure is applicable to a wide variety of substrates, while accommodating surfaces with complex surface geometries. Dextran with azide content between 22 and 0.3 wt% is produced by esterification with \( p \)-azidobenzoic acid. Dextran (1.2 wt% azide) is photografted onto plasma-oxidised PU and PS with thicknesses of 5 ± 3 nm and 7 ± 3 nm, respectively. The dextran patterned onto oxidised PU is patchy, with a nominal height difference between dextranised and non-dextranised regions. The azidated dextran on oxidised PS exhibits a distinct step in height. In the presence of PBS, the dextranised regions became smooth and uniform, without affecting the height difference at the oxidised PU boundary. However, the dextranised regions on the oxidised PU swell by a factor of 3 relative to the dried thickness. These dissimilarities could be attributed to hydrogen bonding between the dextran and oxidised PU, which is confirmed by photo-immobilisation in the presence of lithium chloride. The resulting surface is the smoothest of all the azidated dextran samples (surface roughness - \( R_{\text{rms}} = 1 ± 0.3 \text{ nm} \)) and swells up to twice its dried thickness in PBS. The anti-fouling properties of dextran-functionalised surfaces are verified by selective adsorption of fluorescein isothiocyanate-labelled human albumin only on the non-dextranised regions of the patterned PU and PS substrates.

Covalent immobilisation of hirudin improves the haemocompatibility of polylactide-co-glycolide \( \text{in vitro} \). Seifert and co-workers [166] modify a biodegradable polymer, poly(DL-lactide-co-glycolide) RESOMER® RG756, by surface immobilisation of recombinant hirudin (r-Hir) with glutaraldehyde as coupling reagent to improve the blood-contacting properties of the polymer. The activity of the immobilised hirudin on the polymer surface is estimated by a chromogenic assay to about 2.5 r-Hir/cm\(^2\). The improvement in the haemocompatibility of the modified RG756 is evaluated in terms of platelet adhesion and activation, blood clotting times and clot formation rate. Fluorescence microscopy reveals that surface modification with r-Hir results in decreased platelet adhesion and activation. An enzyme-linked immunosorbent assay (ELISA) for P-selectin, a marker of platelet activation, was used to confirm this result. Clotting time experiments demonstrate significantly prolonged non-activated partial thromboplastin times, and a decreased clot formation rate of whole blood in contact with r-Hir modified RG756 compared with the plain polymer. A comparison
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of immobilised r-Hir with bound heparin yields equivalent improvement of blood-contacting properties of the investigated polymers. The immobilisation of r-Hir is a useful way of improving the blood-contacting properties of polylactide-co-glycolide and other polymers.

The immobilisation of fibrinolytic ferments, such as urokinase, also offers the possibility of creating anti-thrombogenic surfaces. Kimamoto and co-workers [167] immobilised the plasminogen activator, urokinase, to PU. The urokinase transforms the plasminogen in the active enzyme plasmin that digests the fibrin of the blood clots. Kim and co-workers [168] used amino alkylated surfaces, whereas Oshiro and Kosaki [169] used PEI to attach urokinase to polyamide. It appears that there is a critical length of the spacer chain for retention of urokinase bioactivity, which explains the need for mediated procedures. One of the reasons that platelets do not adhere to the surface of the conventional blood system is that potential inhibitors of platelet adhesion are situated on the surface of the blood vessels. Prostaglandins (PG), and especially PGI2, PGE1 and PGD2, are a group of such inhibitors. To utilise their anti-platelet activity researchers have tried to immobilise some of them to polymer surfaces [170, 171]. Bamford and co-workers [172] succeeded in attaching a covalently stable synthetic prostaglandin PGT2 analogue to different surfaces including polyester urethane. The design of lysine-containing anti-clotting coatings is based on the fact that surfaces incorporating a high density of lysine residues, in which the amino groups are free, are capable of selective adsorption of plasminogen from blood plasma, and virtually no other proteins [173]. In contrast, control surfaces that contain either no lysine, or lysine in which the amino group is not available, adsorb only very small amounts of plasminogen, and are unable to prevent clot formation. Nanocomposite fibrinolytic coatings can be obtained by tethering proteolytic enzymes to the surfaces of carbon nanotubes (CNT), which are then dispersed in polymethyl methacrylate (PMMA) [174]. Elias and co-workers [175] reported an increase in osteoblast adhesion and proliferation, as well as alkaline phosphatase activity and extracellular matrix (ECM) secretion, with decreasing carbon nanofibre diameters (in the range of 60–200 nm). Wang and co-workers [176] presented crosslinked chitosan (CS) microspheres with a smooth surface and evaluated blood coagulation. A crosslinking reaction occurred with the amino groups of the CS molecules. The swelling characteristics of the CS microspheres are influenced by the pH of the environment, being generally greater at low rather than higher pH values. The coagulation properties of the CS microspheres were evaluated by dynamic blood clotting, platelet adhesion and activation, erythrocyte adhesion, haemolysis, and protein absorption assays, and demonstrated reduced clotting time, induced platelet adhesion and activation. The shortening of the clotting time is related not only to platelet but also to erythrocyte aggregation.

Enzymes such as serine protease, subtilisin Carlsberg and trypsin can be loaded onto the CNT by physisorption. The extent of non-specific protein adsorption on these
biocatalytic films is 95% lower compared to the enzyme-free film. The incorporation of a fibrinolytic enzyme into the coating results in lowering of fibrinogen fouling by 92%. Clot-lysing coatings such as these could potentially prevent thrombosis in stents and other blood-contacting implants. Improved blood biocompatibility of a composite film of a CS/CNT complex was achieved by Takahashi and co-workers [177]. Single-walled CNT (SWCNT) are novel molecular-scale wires having excellent anti-adhesion properties with regard to platelets. On the other hand, CS is a partially de-acetylated derivative of chitin that has a critical role in cell attachment and growth. Therefore, ways in which CNT could improve the blood biocompatibility of CS film were investigated. Composite films with various concentrations of CS/CNT have been prepared and surface characterised (by Raman spectroscopy, XPS, AFM and contact angle measurements). The experimental results of cell attachment and platelet adhesion tests indicate that the novel composite film containing CS/CNT has two paradoxical characteristics, namely, good adherence of EC and minimum adherence and activation of platelets, making this film a promising anti-thrombogenic material for use in the biomedical field. PEGylation of PDMS surfaces confers resistance to non-specific protein adsorption. Moreover, the incorporation of free amino groups on the surface, by using PEG-lysine conjugates, renders the surface capable of dissolving fibrin clots because of adsorption of the fibrinolytic protein plasminogen from blood plasma [178]. Similar studies in the past had shown that PU surfaces coated with a lysine-derivatised acrylamide polymer dissolved fibrin clots by ready conversion of the adsorbed plasminogen to plasmin in the presence of tissue-plasminogen activator (TPA) [179].

Local NO release from polymeric surfaces can potently inhibit platelet adhesion and activation, making the surface resistant to clot formation [180, 181]. A low-leaching, NO-generating polyelectrolyte multi-layer thin film comprising sodium alginate and organoselenium-modified PEI can be prepared by LbL assembly [182]. The thin films are deposited on biomedical grade polymer substrates such as SR tubings and PU catheters, and produce NO even after prolonged contact with sheep whole blood. The multi-layers allow endogenous S-nitrothiols such as S-nitrosoglutathione (GSNO) and thiol-reducing agents such as glutathione to diffuse through the polymer matrix and reach the organoselenium sites, where the catalytic decomposition of GSNO to NO occurs. The LbL coatings show very low catalyst leaching. Sebra and co-workers [183] synthesised a NO donor polyester containing multiple S-nitrosothiol groups covalently attached to the polymer backbone through esterification of PEG with mercaptosuccinic acid, followed by the nitrosation of the –SH moieties. The poly(nitrosated polyester) (PNPE) obtained is blended with PMMA, yielding solid films capable of releasing NO. PNPE/PMMA coated surfaces inhibit platelet adhesion in contact with whole blood. A PNPE/PMMA blend can be used to coat blood-contacting surfaces, with the potential to inhibit thrombosis and restenosis after stenting.
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Liu and co-workers [184] performed surface modification of PET films with L-arginine (L-arg) to gain an improved anticoagulant surface. The surface chemistry changes of modified films are characterised by XPS and ATR-FTIR spectroscopy. The in vitro anticoagulant activities of the surface-modified PET films are evaluated by a blood clotting test, a haemolytic test, and the measurement of clotting time including PRT, activated partial thromboplastin time (APTT) and prothrombin time (PT). The blood coagulation index for L-arg modified PET films (PET-arg) is higher than that for PET at the same blood-sample contact time. The haemolysis ratio for PET-arg is lower than that for PET and within the accepted standard for biomaterials. The PRT and APTT for PET-arg are significantly prolonged, compared to those for the unmodified PET. All results suggest that the modification method described could be a possible candidate to create anti-thrombogenic PET surfaces for medical applications.

4.5 Albumin Coating

The empirical observation, that if a polymer surface, selectively adsorbing albumin, is fully covered with this protein, it demonstrates anti-thromobogenicity, inspired researchers to develop methods for the creation of albumin-coated surfaces [185]. Albumin-coated surfaces could be prepared by both covalent bonding and physical adsorption of albumin, to form albumin bonded and albumin affinity surfaces, respectively, the second one strongly adsorbing albumin from blood to make a passive coating (Figure 4.4).

Albumin consists of one single peptide chain forming three small globular units, the whole molecule constituting a rotational ellipsoid. The albumin is slightly negatively charged under physiological conditions and comparatively hydrophobic [186]. The hydrophobic groups are preferably localised in the interior. The albumin molecule easily expands and denaturises at local pH changes [187, 188]. Lee and Kim [189] demonstrated the importance of the interactions between the plasma proteins and the terminal groups of the platelet membrane glycoproteins. The albumin does not contain galactose and this is thought to be the reason for the absence of platelet affinity to this protein. It is still not fully understood why the albumin thrombogenicity is lower than that of other proteins, but it is known that the surface bound albumin does not leave ligands for platelets and can attach them only if it is denatured. The albumin acts as a bystander molecule for many surface contact activated biological reactions because it contains none of the peptide sequences known to interact with either adhesion receptors on the cell membranes or enzymes in the coagulation and complement cascades. An albumin coating may, therefore, increase the biocompatibility of the surface. As the adsorption is an equilibrium process followed by desorption, it is extremely important, from the point of view of effective protection, for the albumin to be attached strongly to the polymer surface. Some time ago, Brash and Davidson
[190] found that a material surface with a high selectivity for albumin adsorption can feasibly prevent the adhesion of platelets. The ‘combining’ state of albumin onto the material surface is a key for albumin to exert its function. However, a physically absorbed albumin layer has many shortcomings because the albumin does not ‘combine’ (attach) firmly to the substrate, which results in inter-protein exchange and gradually reduced anti-thrombogenicity. The results of a few long-running *in vivo* clinical experiments designed to evaluate this approach appear to be anecdotal.

**Figure 4.4** Surface bound albumin (a) and albumin affinity coating (b)

Direct surface alkylation or acylation with suitable polymers, such as PU, polyamides and cellulosics with 16 or 18 carbon chains, creates a more hydrophobic surface, efficiently adsorbing albumin [191, 192]. When the protein layer absorbed by the material surface consists mainly of fibrinogen or globin, and the conformation of the protein is changed, the coagulation factors and platelets are activated, which results in blood coagulation and thrombus formation. However, when the protein layer at the same material surface consists mainly of albumin, this layer can prevent blood
clotting. Munro and co-workers [191] reported a reversibly absorbed albumin layer on polymer surfaces with good anti-infection and anti-thrombogenicity. Platelet adhesion and activation are important factors in the formation of clots. Platelet activation not only triggers several coagulation factors, but the platelets are also an important ingredient of thrombus on the polymer surface. Thus, the behaviour of the platelets and their adhesion on the material surface can be considered to evaluate the anti-thrombogenicity of the materials produced. The surface immobilisation of albumin can be enhanced by surface pre-treatment using one of several well-known methods. For example, cold plasma discharge techniques can be used to improve albumin immobilisation onto various polymers [193, 194]. Henning and co-workers [195] prepared albumin-heparin conjugates and pre-adsorbed them on different materials to improve their blood compatibility. Glass, PVC, Biomer® and cellulose acetate are coated with albumin-heparin conjugate and its adsorption and desorption behaviour was studied using $^3$H and $^{51}$Cr radiolabelled conjugates. Pre-coated materials show a significant prolongation of the Lee-White clotting time compared to that of uncoated materials. The prolonged clotting time for pre-treated surfaces is due to the presence of surface-bound conjugate. Albumin-heparin conjugates with high affinity to ATIII give more prolonged clotting times (as low affinity conjugates) when used as coatings for glass. This indicates that the behaviour of heparin in the pre-adsorbed conjugates resembles that in the solution. Munro and Pelham [196] proposed an albumin binding method that allows formation of a renewable albumin buffer layer between the polymer and the blood. Noting that the albumin in the whole blood has a high affinity to circulating free fatty acids, they proposed a covalent binding of albumin to polymer surfaces via 16 or 18 carbon alkyl chains. These chains mimic the non-polar structure of the saturated fatty acids and, thus, develop a strong hydrophobic interaction with the albumin. Munro and co-workers [197] studied in vitro, the passivating tendencies of spontaneously adsorbed albumin and found that the adsorption from solution provides only sparse coverage. While a pre-adsorbed layer of albumin inhibits the subsequent adsorption of fibrinogen, a potentially thromboresistive effect, this albumin is easily desorbed in a fluid shear field. Methods for albumin surface immobilisation prior to implantation of different samples and devices have been developed but the thromboresistive effect is thought to be transitive owing the ultimate denaturation of the adsorbate.

Tsai and co-workers [198] developed a simple generalised coating process that could be tailored to industrial applications. The coating is a thin, transparent, biocompatible film based on SR but with increased albumin affinity compared to that of the SR. Two polymer forms have been developed: one with substituted hydroxyl groups (OH), and the other with 16 carbon acyl groups (C$_{16}$) in the siloxane side chains. Oxymercuration/demercuration or hydroboration reactions are used. SEM reveals that the film surfaces are smooth, uniform and featureless. ATR/FTIR spectra and advancing/receding WCA measurements confirm the presence of surface OH groups.
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and suggest the presence of surface acyl groups. Albumin adsorption and retention are markedly enhanced for surface OH and C\textsubscript{16} concentrations as low as 5% reaction yield. Kinetics, isotherm and competitive albumin/fibrinogen adsorption studies suggest that surface hydroxylation and perhaps C\textsubscript{16} acylation as well, markedly improve the albumin affinity, but not the fibrinogen affinity, of this material. The SR film can be durably coated on several materials, making it possible to favourably treat many blood-contacting devices, using a simple immersion/dipping process. Amiji and Park [199] reviewed methods for surface modification with water-soluble polymers, such as PEO, albumin and heparin. PEO is a synthetic, neutral, water-soluble polymer, while albumin and heparin are a natural globular protein and an anionic polysaccharide, respectively. When grafted onto the surface, all three macromolecules reduce the thrombogenicity of biomaterials. The reduced thrombogenicity is thought to be due to the unique hydrodynamic properties of the grafted macromolecules. In an aqueous medium, surface-bound water-soluble polymers are expected to be highly flexible and extended into the bulk solution. Biomaterials grafted with PEO, albumin or heparin are able to resist plasma protein adsorption and platelet adhesion predominantly by a steric repulsion mechanism. Kang and co-workers [200] immobilised albumin, collagen and gelatin on a plasma pre-treated and acrylic acid (AA) grafted PMMA surface. PMMA films were treated by oxygen plasma discharge followed by AA grafting. The carboxyl groups of the PMMA film surface previously activated with water-soluble carbodiimide were coupled with BSA, collagen and gelatin. The protein immobilisation onto the surface was confirmed by ATR-FTIR and XPS. The protein-immobilised PMMA films could be widely used as a biocompatible and haemocompatible material.

An albumin layer obtained by covalent grafting onto a material surface is a good way to improve the combining capacity of different substrates [201]. Hydrophilic side chains introduced on to the material surface not only reduce or resist protein adsorption [202] but also improve the blood compatibility of the materials because of the ability of the free fatty acid with 16 or 18 carbon alkyl groups to selectively absorb albumin [203]. McFarland and co-workers [204] study the in vitro activity of albumin-binding surfaces using immobilised monoclonal antibodies (Mab) to attract specific molecules to a solid surface from complex mixtures such as blood, plasma or serum, thereby directing the response to the modified substrate, a key goal in rational biomaterial design. The nature of the Mab dictates the nature of the response: anti-albumin antibodies are used to prevent cell and platelet adhesion in vitro, while anti-fibronectin Mab promote their attachment. Patterned surfaces can be formed, bearing Mab that generate adhesive and non-adhesive regions. Fibrinogen adsorption from plasma shows a Vroman peak of unmodified control polymer that is reduced by 64% in the presence of surface-bound anti-albumin Mab. The immobilisation of a control Mab reduces fibrinogen adsorption only slightly, implying an albumin-mediated effect. In static tests, the platelet adhesion from human platelet-rich plasma
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is significantly reduced by the immobilisation of anti-human serum albumin HSA Mab when compared to the untreated surface. When platelets and proteins (such as blood, plasma or serum) are introduced to the surface simultaneously, the generation of a defined protein film must be sufficiently rapid to shape the platelet or cell response. A method of reducing non-specific adsorption of proteins and enhancing selective absorption of albumin to improve the blood compatibility of PE films is reported by Ji and co-workers [205]. This method is based on albumin-selective adsorption by stearyl groups. PE film is firstly pre-treated with plasma so that polyacrylamide (PAAm) can be grafted by UV-induced polymerisation without a photoinitiator, and stearyl groups are then introduced via an alcoholysis reaction between polyacrylamide and octadecyl alcohol. Savay and co-workers [206] studied the potentiation of liposome-induced complement activation by surface-bound albumin. While highlighting a new modulatory mechanism of liposomal complement activation, their data raise the possibility of deposition of extravasated HSA at sites of tissue injury that may serve a hitherto unrecognised pro-inflammatory function.

Fu and co-workers [207] functionalised CNT with BSA in homogeneous aqueous solution. SWCNT and multiple-walled carbon nanotubes (MWCNT) were solubilised via esterification of the nanotube-bound carboxylic acids by oligomeric PEG compounds. The water-soluble samples were used as starting materials in reactions with BSA in ambient aqueous solutions. The reaction conditions were designed for thermodynamically favourable transformation from ester to amide linkages, yielding SWCNT-BSA and MWCNT-BSA conjugates. The use of soluble starting nanotube materials in an indirect functionalisation method represents a valuable approach to the biomodification of CNT. Roach and co-workers [208] presented data on protein adsorption (BSA and fibrinogen) onto model hydrophobic (–CH₃) and hydrophilic (–OH) surfaces, investigated using a quartz crystal microbalance (QCM) and grazing angle infrared spectroscopy (GA-IR). The data suggest that albumin undergoes adsorption via a single step whereas fibrinogen adsorption is a more complex, multi-stage process. Albumin has a stronger affinity toward the –CH₃ when compared to the –OH terminated surface. In contrast, fibrinogen adheres more rapidly to both surfaces, having a slightly higher affinity toward the hydrophobic surface. Conformational assessment of the adsorbed proteins by GA-IR shows that after initial one hour incubation, few further time-dependent changes are observed. Both proteins exhibited a less organised secondary structure upon adsorption onto a hydrophobic surface than onto a hydrophilic surface, with the greatest effect observed for albumin. This study demonstrates the ability of simple tailor-made monochemical surfaces to influence binding rates and conformation of bound proteins through protein-surface interactions. Current interest in biocompatible materials has focused on surface modifications to induce rapid healing, both of implants and for wound care products. This effect may also be significant at the next stage of implant integration, as cell adhesion occurs through the surface protein layer [209].
Tan and co-workers [210] prepare BSA surface imprinted sub-μm particles with magnetic susceptibility through core-shell mini-emulsion polymerisation. Molecular imprinting is a state-of-the-art technique for preparing mimics of natural, biological receptors. Nevertheless, the imprinting of macromolecules such as proteins, remains a challenge, due to their bulkiness and sensitivity to denaturation. In this work, a surface imprinting strategy based on covalently immobilised template molecules was adopted for protein imprinting. BSA surface imprinted sub-μm particles (500–600 nm) with magnetic susceptibility were prepared through a two-stage core-shell mini-emulsion polymerisation system using MMA and ethylene glycol dimethacrylate as functional and crosslinking monomers, respectively. The particles possess a novel red blood cell-like structure and exhibit a very favourable recognition property toward the template BSA molecules in an aqueous medium. In addition, the importance of template immobilisation for successful protein imprinting had also been illustrated to demonstrate the potential of this approach as a general methodology for protein imprinting. Fang and co-workers [211] modify the surface of polyethersulfone (PES) membranes by grafting BSA. They synthesised poly(acrylonitrile-co-vinyl pyrrolidone-co-AA) (P(AN-VP-AA)) via free radical copolymerisation, and used it to blend with PES to prepare a PES membrane followed by grafting of BSA onto the surface of the membrane. Compared to binary copolymers, such as P(VP-AA) and P(AN-AA), the amount of blending of the terpolymer and the amount of BSA grafting increase. The WCA, protein adsorption and platelet adhesion obviously decrease after grafting BSA onto the membrane surface. Furthermore, the anti-thrombogenicity of the modified membranes, such as the PT and the APTT increase, and the cytocompatibility also increases. The adsorption of BSA onto the surfaces of PMMA and of MMA copolymer with 2,3-epoxypropyl methacrylate was also investigated [212]. The polymeric matrices are obtained through radical emulsion polymerisation with and without the presence of a continuous external magnetic field (MF) of 1500 Gs intensity. Two types of surfactant agents are used to synthesise the polymers: a classic one, sodium lauryl sulfate (SLS), and a second one, β-cyclodextrin (CD). Protein adsorption is conducted both in the presence and in the absence of a MF, by varying the coupling conditions, the temperature, pH and albumin/polymer ratio. The study underlines the assistance of a MF during the adsorption process, materialised into growth of the BSA adsorbed quantity. Thus, the presence of a MF during adsorption ensures that the quantity of BSA adsorbed onto the surface of polymers prepared in the MF doubles. The adsorption process is also related to the surfactant substances used for the synthesis of polymeric matrices, which affect the surface tension. The higher content of the adsorbed BSA corresponds to the polymers with CD instead of SLS. This fact is attributed to the catalytic activity of the MF, which determines the molecule’s distortions, the growth of distance interactions and the modifications of the angles between bonds, with a beneficial effect upon adsorption.
4.6 Endothelial Cells Attachment

Lining natural blood vessels have fibrinolytic activity and are, thus, able to hydrolyse fibrin. The natural bloodstream system has an internal surface coated with EC, and the blood does not coagulate. This suggested the idea of creating hybrid polymeric materials with quasi-intima coated surfaces formed by EC [96, 213]. Several research groups tried to immobilise natural EC (taken from the surface of blood vessels) onto different polymer substrates. In these experiments, the EC are differentiated onto a fibrin network formed by coagulation of blood. The non-thrombogenicity in this case is due not to the polymer surface itself but to the surface of the EC.

Unfortunately, the small diameter (< 6 mm) of the artificial cardiovascular prostheses occluded due to thrombi formation before the quasi-intima could be created [214]. One approach to solving this problem of endothelialisation [215] is to seed autologous EC onto the luminal surface of the vascular grafts to allow the formation of a monolayer of EC before implantation. This approach is able to increase patency of the vascular grafts [216]. An OH-functionalised polycarbonate urethane (PCU) is used to develop a new anti-thrombogenic surface. Biomolecules such as glycine or the fibronectin fragment gly-arg-gly-asp-ser are covalently bound to the PCU surface by succinyl dichloride coupling. The modification steps are controlled by infrared spectroscopy and amino acid analysis. Successfully modified films are tested under stationary cell culture conditions [217]. Kawamoto and co-workers [218] studied the adhesion strength, anti-thrombogenicity and cultivation of EC in tubes. When the surface is of SPU, EC are not able to proliferate, but if it is modified by plasma treatment, the adhesion and proliferation of bovine aortic EC (BAEC) is drastically improved. The cells are capable of proliferating on the inner surface of a plasma-treated, SPU-coated tube. When a steady flow shear stress of 9 Pa is applied to the cells proliferated on the modified SPU surface for 90 minutes, most cells do not detach from the surface. From an in vitro evaluation test of anti-thrombogenicity, the cell surface can be considered to provide an inert surface against thrombus formation and blood coagulation. The improvements in BAEC proliferation and adhesion after plasma treatment are due to the change in surface wettability. The plasma treatment could be useful for the development of small diameter hybrid vascular grafts.

Nanoscaled surface texture has a significant influence on cell behaviour. Nanoscaled random surface roughness enhances cell adhesion and functions [219]. The basement membrane is mainly composed of type IV collagen and laminin fibres embedded in heparan sulfate proteoglycan hydrogels. The protein fibres in the basement membrane have nanoscaled diameters, ranging from several to several tens of nanometres [220]. The cells attach and organise well around fibres with a diameter smaller than the cell size [221]. Although PTFE and PET (Dacron MT) are used successfully in treating the pathology of large diameter arteries (> 6 mm, inner diameter), no materials are
Surface engineering of polymeric biomaterials is successful in replacing small diameter blood vessels (< 6 mm). The main reason for the long-term failure of the small diameter vascular graft is the incomplete cover of EC on the vascular graft surfaces and the subsequent myointimal hyperplasia [222, 223]. Kaibara and co-workers [224] attempted to construct a small diameter anti-thrombogenic hybrid vascular graft. To prepare a porous SPU tube, a solution of SPU containing different concentrations of sodium chloride (NaCl) was coated on a glass rod and the coated SPU is immediately immersed in water. The BAEC are not normally capable of adhering and proliferating on the surface of the porous SPU but after modification by plasma treatment, the proliferation of EC is drastically improved. The cells proliferate confluently on the porous SPU surface prepared at low concentrations of NaCl below 10 g per 100 ml, but poorly on the porous surface prepared at high concentrations of NaCl. The construction of a hybrid vascular graft consisting of a porous SPU tube (2 mm in inner diameter, 5 cm in length) and EC was attempted. The cells cultured on the inner surface of the tube proliferate to cover it completely. From an in vitro anti-thrombogenic evaluation test, which involves the use of human blood, the present hybrid graft provides an inert surface against thrombus formation and blood coagulation. Negligible changes in the shape of human leucocytes in contact with the surface of BAEC occur, suggesting that the BAEC used are immunologically less active against human blood. Birchall and co-workers [225] investigated the adherence and retention under in vitro flow conditions of endothelium grown on the luminal surface of 4 mm internal diameter biomatrix vascular conduits. The biomatrix vascular conduits are produced in living animals and consist of a naturally formed ECM wall incorporating a polyester mesh. They proposed that the microarchitecture of the luminal surface may be conducive to EC seeding and to the formation of a firmly adherent endothelium without prior treatment of the surface with cell adhesives. EC are isolated from segments of human saphenous vein and cultured to cover the surface entirely. Cultured cells are characterised by morphology and immunocytochemistry with anti-CD31, von Willebrand factor, smooth muscle actin cytokeratin, and the lectin Ulex europaeus agglutinin. After culture, EC are seeded (1 x 10^6 cell/ml) by rotation onto the luminal surface of 20 cm long biomatrix vascular conduits (n = 3). Quantification of the extent of luminal surface endothelialisation, pre-flow and post-flow, and cell densities at confluence is performed with digital imaging light microscopy and image analysis software. Confluent EC monolayers are established on the luminal surface of biomatrix vascular conduits within 48 hours. The endothelium formed is firmly adhered and is retained under a blood flow within the physiological range.

Surface engineering of electrospun PET nanofibres towards development of a new material for blood vessel engineering was reported by Ma and co-workers [226]. Non-woven PET nanofibre mats (NFM) were prepared by electrospinning technology and surface modified to mimic the fibrous proteins in native ECM so as to construct a biocompatible surface for EC. The electrospun PET NFM is first treated in...
formaldehyde to yield hydroxyl groups on the surface, followed by the grafting polymerisation of methacrylic acid initiated by Ce(IV). Finally, the PMAA-grafted PET NFM is grafted with gelatine using water-soluble carbodiimide as a coupling agent. Plane PET film is also surface modified and characterised for basic understanding of the surface modification process. The grafting of PMAA and gelatine on the PET surface is confirmed by XPS spectroscopy and quantitatively analysed by colorimetric methods. Epithelial cells are cultured on the original and gelatine-modified PET NFM and the cell morphology, proliferation and viability are studied. Three characteristic surface markers expressed by EC are studied using immunofluorescent microscopy. The gelatine grafting method can obviously improve the spreading and proliferation of the EC on the PET NFM, and moreover, can preserve the EC’s phenotype. Sarkar and co-workers [227] discussed the possibilities for addressing thrombogenicity in vascular graft construction. The self-endothelialising synthetic graft is an attractive proposition as a confluent endothelial layer incorporates many of the anti-thrombogenic properties of arteries. Surface modification to promote this behaviour shows good results in animal models. The difficulties experienced in achieving spontaneous endothelialisation in humans led to the investigation of pre-implantation in vitro EC seeding. The aim of these approaches ultimately is to produce novel synthetic grafts that are anti-thrombogenic and, thus, suitable for coronary and distal infra-inguinal bypass. A novel strategy to graft arg-gly-asp (RGD) peptide on biomaterial surfaces for endothelialisation of small diameter vascular grafts and tissue engineering of blood vessels was proposed by Li and co-workers [228]. To improve the performance of small diameter vascular grafts, endothelialisation of biomaterial surfaces and tissue engineering are more promising strategies for fabricating small diameter vascular grafts. In this study, a gly-arg-gly-asp-ser-pro (GRGDSP) peptide was grafted on the surfaces of PCU, with photoactive 4-benzoylbenzoic acid (BBA) by UV irradiation. The photoactive peptides (BBA-GRGDSP) were synthesised in a classical way and the grafted surfaces characterised by WCA measurement and XPS. The results suggest that the peptides are successfully grafted on to the PCU surfaces. The effect of these modified surfaces on EC adhesion and proliferation is examined over 72 hours. PCU surfaces coupled with the synthetic photoactive RGD peptides, as characterised with phase contrast microscope and the metabolic activity (methylthiazolteletrazolium) assay enhanced EC proliferation and spreading with increasing concentration of RGD peptides grafted on their surfaces. Increased retention of EC is also observed on the polymer surfaces under flow shear stress conditions. The results demonstrate that GRGDSP peptides grafted on the surfaces of polymers with photoactive BBA could be an efficient way of fabricating small diameter blood vessels. To improve the attachment, growth and adhesion of EC and thus to accelerate the re-endothelialisation of stents, Yina and co-workers [229] immobilised a synthesised mussel adhesive polypeptide mimic, containing dihydroxyphenylalanine and L-lysine (MAPDL) onto 316L stainless steel (316LSS) with a PEG molecule as spacer arm using a cold plasma-induced grafting technique. To immobilise MAPDL
effectively, ethylene vinyl acetate (EVA) should first be coated onto the 316LSS. Different molecular weight PEG and different grafting times were tested to obtain the optimal cell bioactivity. XPS and WCA measurements indicated the successful immobilisation of MAPDL. In vitro cell culture results show that compared with the control of 316LSS, the attachment, adhesion and growth of cells on the MAPDL-coated EVAc surface, in particular with PEG as a spacer arm, are significantly enhanced, and a complete EC layer is formed after a two day culture. A platelet adhesion experiment revealed that the platelet adhesion is also reduced on the MAPDL-coated EVAc surface. The in vitro inflammatory assessment showed that the MAPDL coating inhibits TNF-α and IL-1β release from monocyte cells, which is indicative of good anti-inflammatory properties. The MAPDL coatings appear to be a promising strategy for rapid re-endothelialisation of intravascular stent devices. Effective surface modification with biocompatible molecules is known to be effective in reducing the life-threatening risks related to artificial cardiovascular implants. In recent strategies in regenerative medicine, the enhancement and support of natural repair systems at the site of injury by designed biocompatible molecules succeeds in rapid and effective injury repair. Therefore, such a strategy could also be effective for rapid endothelialisation of cardiovascular implants to lower the risk of thrombosis and stenosis. To achieve this enhancement of the natural repair system, a biomimetic molecule that mimics proper cellular organisation at the implant location is required. Although many reported peptides have cell-attracting properties on material surfaces, there have been few peptides that could control cell-specific adhesion. For advanced cardiovascular implants, peptides that can mimic the natural mechanism that controls cell-specific organisation have been eagerly awaited. To obtain such peptides, Kanie and co-workers [230] hypothesised that there was a cellular bias toward certain varieties of amino acids and examined the cell preference (in terms of adhesion, proliferation and protein attraction) of the many varieties of peptide arrays. To investigate the role of specific peptides in controlling the organisation of various cardiovascular-related cells, they compare EC, smooth muscle cells and fibroblasts. A clear, cell-specific preference was found for amino acids (longer than 5-mer) using three types of cells, and the combined effect of the physicochemical properties of the residues was analysed to interpret the mechanism. Yang and co-workers [231] reported bioactive plasma polymerised bipolar films for enhanced EC mobility. XPS revealed that polar entities exist at the interface between PAAm and PAA nanolayers. They induce strong dipolar orientation polarisability and cause the redistribution of charges, which results in a remarkable increase in polar surface energy and hydrophilicity of the multi-stack bipolar films. In particular bipolar films with amine groups on their outermost surface show strongly enhanced cellular mobility. The attachment, adhesion, proliferation, migration and coverage of EC are significantly enhanced on such films. They are therefore promising as vascular implant materials, and could have applications as coating materials for tissue engineering. Among the strategies to improve a material’s haemocompatibility, pre-coating with the tripeptide RGD is
used to favour endothelialisation, thus, lowering thrombogenicity. Andrade and co-workers [232] were the first to study the blood compatibility of native and RGD-modified bacterial cellulose (BC). The PRT and whole blood clotting results demonstrate the haemocompatibility of BC. A significant amount of plasma protein adsorbs to BC fibres, however, according to analysis by intrinsic tryptophan fluorescence techniques when albumin, globulin and fibrinogen from pure protein solutions adsorb to BC they do not undergo detectable conformational modifications. Human microvascular EC cultured on RGD-modified BC readily form a confluent cell layer, inhibiting the adhesion of platelets. Therefore both native and RGD-modified BC may be classified as haemocompatible materials.

### 4.7 Natural Biomembrane Mimetic Surfaces

The concept of creating surfaces that mimic natural biomembranes is relatively new. The biomembrane is a hybrid material, consisting of phospholipids and proteins whose molecules are not covalently bonded. Therefore, the biomembrane surface is heterogeneous and dynamic [233]. This fact was remarked on by some researchers [234] who tried to use it to create an ideal anti-thrombogenic surface, i.e., the concept of natural biomimetic surface creation is based on the phospholipid’s own properties. To regulate the adsorption of phospholipids and to create a surface that mimics a biomembrane, polymers with suitable properties are needed. Chapman and Hoyward [235] developed new biomaterials based on the mimicry of a simple component, presenting on the extracellular surface of the lipid double layer, which forms the matrix of the plasma membrane of the blood cells, namely the PC group of phosphatidylcholine and sphingomyelin. Covalent bonding of PC functional groups to a number of commercially available polymers to enhance their blood compatibility was reported recently by Chapman and co-workers [236-238]. They prepared reactive compounds that allow attachment of PC units to the surface of polymers containing carboxyl or hydroxyl groups. The modified polymers retain their mechanical properties and exhibit characteristics of the membrane surfaces, but this method is based on weak interactions of PC with biomaterial surfaces and proteins and cells in the blood. Therefore, it appears that the phospholipid-coated surfaces are not stable enough and have very poor mechanical properties, limiting their applications.

There are other concepts for creating blood-compatible polymeric materials that are based on the characteristics of the natural phospholipids presenting in the blood. Nakabayashi and co-workers [240] accepted that if the polymer surface has a structure similar to the phospholipids, a large amount of the plasma natural phospholipids can be adsorbed on the surface due to their propensity to self-organisation. Focusing on this idea, they synthesised a phospholipid polar group containing a methacrylate
monomer, namely MPC. However, the effects of the MPC chains on lipid adsorption and cell adhesion remain unclear because of the low synthetic yield and/or low purity of the MPC and its copolymers, which are insufficient to evaluate the blood compatibility. Recently, the synthesis of MPC and its copolymers was improved [240, 241] and it is found that the amount of adsorbed phospholipids on MPC obtained in this way increases. In addition, DSC and XPS studies confirm that the surface-adsorbed phospholipids are self-organised in a structure similar to that of the biological bilayer membrane [242, 243]. The MPC copolymers are characterised by low thromobogenicity, insignificant protein adsorption and good permeability, and they are expected to find applications as haemodialysis membranes, biosensors and drug delivery systems.

Yianni [244] reports attachment and coating of PC polymers to PVC. PVC is one of the most commonly used polymers for disposable medical devices working in direct contact with blood. PVC is coated with MPC-laurylmethacrylate copolymer. This PC copolymer is dissolved in a suitable solvent such as ethanol, passed through a filter and PVC strips are then dipped into this solution. The coated PVC strips are air dried before the haemocompatibility testing. The platelet adhesion and activation are greatly reduced on the PC copolymer coated PVC. The coated surface is stable and resists degradation during sterilisation. All results indicate that the haemocompatibility of PVC could be significantly enhanced by coating with phosphorylcholine containing polymers. Liu and co-workers [245] describe a surface modification of PE membranes using PC derivatives to enhance platelet compatibility. AA is graft copolymerised onto the surface of PE by UV irradiation. Prior to this process, PE film is immersed in an aqueous solution containing a photoinitiator, sensitisser and various organic solvents. PC with various spacer lengths are introduced onto the PE surface by a series of chemical reactions. EG, butanediol, polypropylene glycol and polytetramethyl glycol (PTMG) are used as spacers. The platelet compatibility of PC-modified PE film is evaluated by a platelet adhesion test indicating that the platelet compatibility of the PE film is affected by the existence of various functional groups on the film surface. The amount of the adhered platelets decreases in the order: PE-POC (phosphoryl oxychloride (POC) treated PE film) > PE-PTMG > PE-AA > PE-PC. The length of the lipophilic spacer between the PC groups and the PE surface affects the stability of the film surface. It is believed that the self-assembly of polymerisable lipids in association with transmembrane proteins [246], as well as peptide or carbohydrate lipophilic conjugates establish a versatile scheme for generating chemically heterogeneous films with tailored biological functionality [247, 248]. Feng and co-workers [246] report the preparation of a membrane mimetic thin film composed of mixed polymerisable lipids containing both phosphatidylcholine and biotin head groups. This allows the possibility of fabricating a further series of model membrane mimetic films derivatised at varying surface densities of biotinylated heparin terminated chains.
On the luminal surface of the common synthetic vascular prostheses, blood coagulation can occur and a thrombus membrane is formed when blood flow passes through it. The thrombus membrane should be organised according to the wound healing process and it becomes a pseudointima, which could serve as a blood conduit. However, the small diameter vascular prosthesis may be quickly occluded by the initial thrombus. Therefore, no clinically applicable small diameter prostheses have been developed to date. MPC polymers resemble the structure of an outer cell membrane similar to the fluid mosaic model and demonstrate excellent anti-thrombogenicity. Therefore, Yoneyama and co-workers [249] try to develop a clinically applicable small diameter prosthesis based on the new concept of the MPC polymer, preparing vascular prostheses (2 mm internal diameter) from a polymer blend composed of SPU and MPC polymers. The prostheses were placed in rabbit carotid arteries. The luminal surface retrieved at eight weeks after implantation appears to be clear without thrombus and pseudointima. This shows that the MPC containing polymer mix studied can be applied for fabrication of small diameter prostheses.

SR has been used for a long time as a biomaterial, because of good mechanical and optical properties, but its chemical nature, hydrophobicity, and poor anti-thrombogenicity limit its use in many demanding biomedical applications. In order to provide modified silicone with enhanced haemocompatibility, varied surface modification techniques are used. Xu and co-workers [250] perform an ozone-induced grafting of phosphorylcholine polymer (2-methacryloyloxyethyl phosphorylcholine) onto silicone film to improve its haemocompatibility. The surface graft polymerisation is confirmed by XPS and ATR-FTIR. Contact angle measurements demonstrate a significant increase in the hydrophilicity of the MPC grafted silicone film. The blood compatibility of the grafted film is evaluated by a platelet rich plasma (PRP) adhesion study and SEM, using a bare silicone film as a reference. No platelet adhesion is observed on the grafted film incubated with PRP at 37 °C for 60 and 180 minutes. The ozonisation is an effective pre-treatment method to graft MPC onto silicone surfaces with many advantages and, therefore, it promises to be very useful for practical application when creating blood contacting medical devices. SB monomers are also grafted onto silicone surfaces to improve their haemocompatibility [251]. Ozonisation is used to introduce active peroxide groups onto the silicone film surface and, subsequently, graft polymerisation of \(N,N'-\text{dimethyl-N-methacryloyloxyethyl-N-(3-sulfopropyl)}\) ammonium (DMMSA) zwitterionic SB structure, onto the ozone activated silicone surface is conducted. Surface analysis is accomplished by means of ATR-FTIR, XPS, SEM and contact angle measurements. ATR-FTIR and XPS confirm the successful graft polymerisation. The grafted films possess relatively hydrophilic surfaces as indicated by contact angle measurement. The blood compatibility of the grafted films is evaluated by platelet adhesion in PRP and protein adsorption in bovine fibrinogen, using bare silicone film as a reference. No platelet adhesion is observed for the grafted films.
incubated in PRP for 120 minutes. The protein adsorption is reduced on the grafted films after incubation in bovine fibrinogen for 120 minutes. The results confirm the improved blood compatibility obtained by grafting the previously mentioned new zwitterionic SB structure onto the silicone film. Yuan and co-workers [252] developed a PU vascular catheter, surface grafted with zwitterionic SB monomer after pre-activation by ozone. This surface modification is aimed at improving the haemocompatibility and anti-thrombogenicity of the PU by grafting the zwitterionic SB structure monomer, DMMSA. The DMMSA-grafted PU vascular catheter is characterised by ATR-FTIR and XPS. The blood compatibility was evaluated by a platelet adhesion study using PRP and SEM to observe the morphology of the platelets. A PU vascular catheter is used as a reference. It is significant that this new zwitterionic SB grafted PU vascular catheter demonstrates an improved anti-thrombogenicity and it is effective if the inner diameter of the vascular catheter is only 3 mm.

Kobayashi and co-workers [253] modified the blood-contacting surface of the SPU diaphragm used in an electromechanical pulsate ventricular assist device to improve the biocompatibility. They introduced MPC units onto its surface and form an interpenetrating polymer network (IPN) structure, which contains independently crosslinked MPC polymer and SPU. The SPU diaphragm, modified with the IPN structure, is then assembled into a target test pump and undergoes continuous pump operation at 37 °C for two weeks in a simulated systemic circulation, using a mock circulatory loop. The IPN-modified diaphragm prevents protein adsorption as well as cell adhesion when compared to the unmodified SPU surface. The result proves that the IPN structure firmly secures the MPC units to the SPU surface even under a high mechanical stress and in a high shear environment. The anti-thrombogenic power of MPC units remains unchanged after two weeks of continuous exposure to a high-shear environment; the SPU, modified with crosslinks by MPC IPN is a powerful anti-thrombogenic surface for blood pumps used for chronic circulatory support of cardiac patients. Ito and co-workers [254] developed a new type of copolymer coating composed of L-histidine, a zwitterion, and n-butyl methacrylate (BMA), as a hydrophobic moiety. Coated with this copolymer, PS surfaces are characterised with a significantly lower non-specific protein adsorption and cell adhesion when compared to the BSA passivated surfaces. Chung and co-workers [255] screened SAM of different phospholipid molecules on gold, for fibrinogen adsorption and platelet adhesion. Their comparative study of the bromoethylphosphorate, phosphorylcholine, phosphorylethanol amine, and hydroxyl terminated SAM shows that the phosphorylcholine terminated SAM show the best anti-fouling properties. CB-based SAM and polymers show a very low fibrinogen adsorption combined with a very low platelet adhesion. Moreover, the PCBMA polymer also exhibits anticoagulant activity and increased blood clotting time, which made it a promising candidate for coating of blood contacting devices and implants [256, 257].
The surface design to reduce non-specific biofouling is one of the most important steps in the fabrication of medical devices. Iwasaki and co-workers [258] present a newly synthesised carbohydrate immobilised phosphorylcholine polymer for surface modification of medical devices to control the interface with living cells. A random copolymer composed of methacryloyloxyethyl phosphorylcholine (MPC), BMA and 2-lactobionamidoethyl methacrylate (LAMA) is synthesised by conventional radical polymerisation. The monomer feeding ratio in the copolymer is adjusted to 24/75/1 MPC/BMA/LAMA (PMBL). The copolymer, PMBL, containing 1.0 mol% LAMA could be coated by solvent evaporation from an ethanol solution. Cells of the human hepatocellular liver carcinoma cell line (HepG2) having a sialoglycoprotein receptors (ASGPR) are seeded on PMBL 1.0 or poly(BMA) (PBMA) coated PET plates. On PBMA, many adherent cells are observed and are well spread with monolayer adhesion. HepG2 adhesion is observed on PMBL 1.0 because of the cells ASGPR. Furthermore, some of the cells adhering to PMBL 1.0 have a spheroid formation and similarly shaped spheroids are scattered on the surface. According to confocal laser microscopic observation, albumin production preferentially occurs in the centre of the spheroid after 96 hours of cultivation. The albumin production of the adhered to PBMA cells is sparse. The amount of albumin production per unit cell that adhere to PMBL 1.0, determined by ELISA is significantly higher than those which adhere to PBMA. Long-term cultivation of HepG2 is also performed using hollow fibre mini modules coated with PMBL 1.0. The concentration of albumin produced from HepG2 increases continuously for one month. Spheroid formation of HepG2 cells is also seen on the hollow fibre membrane. Evidently, PMBL 1.0 can provide a suitable surface for the cultivation of hepatocytes and has great potential for producing reliable bioartificial liver devices.

Preparation, characterisation and blood compatibility of polylactide-based phospholipid polymers were presented by Chen and co-workers [259]. L-a-glycerophosphorylcholine (GPCh) is obtained by hydrolysis of lecithin extracted from eggs. FTIR and 1H-NMR analyses indicate a successful preparation of GPCh. A polylactide-based phospholipid polymer (PLLA-PC) is synthesised by ring-opening polymerisation of L-lactide in the presence of GPCh to improve the cell-material interfacial reaction of PLLA for tissue engineering applications. The yield of the reaction strongly depends on the reaction time. Values above 80% are obtained which are much higher than those reported in literature. Copolymers with the largest molecular weights are obtained after 48 hours at 122 °C. Surface rearrangement is detected due to a dynamic molecular motion, according to XPS data. An increase in the hydrophilicity and a decrease in the fibrinogen adsorption and platelet adhesion are observed due to the presence of hydrophilic PC moieties in the copolymer. A current review (2010) of Xu and co-workers [260] summarised recent achievements and progress in the development of various functional MPC polymer bio-interfaces for lab-on-a-chip devices and applications. As phospholipid polymers, the MPC polymers
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can form cell membrane-like surfaces by using suitable surface chemistry and physics and thereby to provide bio-interfaces capable of suppressing protein adsorption and many subsequent biological responses. In order to enable application to microfluidic devices, a number of MPC polymers with diverse functions are especially designed and synthesised by incorporating functional units such as charged and active ester units for generating a desirable microfluidic flow and to conjugate biomolecules, respectively. Furthermore, these polymers are incorporated with silane or a hydrophobic moiety to construct stable interfaces on various substrate materials such as glass, quartz, PMMA and PDMS, via a silane coupling reaction or hydrophobic interactions. The basic interfacial properties of these interfaces are characterised from multiple aspects of chemistry, physics and biology, and the suppression of non-specific bioadsorption and control of microfluidic flow are successfully achieved using these bio-interfaces on a chips. Many chip-based biomedical applications such as immunoassays and DNA separation are accomplished by integrating these bio-interfaces on a chip. All results indicate that the functional phospholipid polymer interfaces are promising and useful for application to lab-on-a-chip devices in biomedicine. Later review by Zong and Gong [261] presents the fabrication and biocompatibility of cell outer membrane mimetic surfaces. The surface design used for improving biocompatibility is one of the most important issues for the fabrication of medical devices. To mimic the ideal surface structure of the cell outer membrane, a large number of polymers bearing PC groups are used to modify the surfaces of biomaterials and medical devices. The bio- and blood compatibility of the modified materials whose surface is required to interact with a living organism is obviously improved by introducing PC groups. The fabrication strategies of cell outer membrane mimetic surfaces and their resulting bio- and blood compatibility are summarised.

4.8 Polyelectrolyte Multi-layers

There is an increasing interest in the preparation of anti-thrombogenic thin films using the LbL assembly technique based mainly on the electrostatic interaction between opposite-charged surfaces [262-273] or in some cases on covalent bonding [274-276]. A LbL assembly has been used for in vivo repair of damaged blood vessels by multi-layer anionic hyaluronic acid (HA)-cationic CS coating formation on the arterial walls [277]. CS, with its excellent bioadhesive properties on negatively charged surfaces (such as those presented by the damaged arterial lumen), is deposited as the first layer to ensure a strong adhesion of the coating. The growth of blood clot on damaged arterial surfaces is significantly inhibited by the (CS/HA)\textsuperscript{n} multi-layers. The incorporation of L-arginine, which is known to inhibit monocyte and platelet adhesion, into the multi-layer results in a 91% reduction in platelet adhesion when compared to the unprotected damaged arteries.
Polyelectrolyte multi-layer coatings of CS and DS with DS as the outermost layer on polytetramethylene adipate-co-terephthalate membranes resist the platelet adhesion and HPF adsorption [278]. Yu and co-workers [279] developed antithrombogenic polyelectrolyte multi-layer on TPU via a LbL self-assembly technique to improve the polymer hydrophilicity and haemocompatibility. Polysaccharide polyelectrolyte multi-layers including CS as a positively charged agent and DS as a negatively charged anti-adhesive agent are prepared on an aminolysed TPU film employing a LbL self-assembly approach. XPS, field emission scanning electron microscopy and AFM data verify the progressive build-up of the polyelectrolyte multi-layers. The WCA and the $\zeta$-potential reaches the steady value after coating of four bilayers, thus, proving that the full coverage with polyelectrolyte multi-layers is achieved. The growth inhibition index of L929 fibroblast proliferation suggests that these TPU films are non-cytotoxic. Such an easy, valid, shape independent and non-cytotoxic processing has a potential for modification of TPU substrates with application in haemodialysis and cardiovascular devices. ECM-like biomimetic surface modification of cardiovascular implants is a promising method for haemocompatibility improvement. Li and co-workers [280] prepare collagen (Col)-sulfated CS (SCS) multi-layer coatings on pure titanium using a LbL self-assembly technique. The Col-SCS multi-layer growth is carried out by first deposition of a single layer of positively charged poly-L-lysine (PLL) on a sodium hydroxide treated titanium substrate (negatively charged surface), followed by alternate deposition of negatively charged SCS and positively charged Col, and terminated by an outermost layer of SCS. Platelet adhesion in vitro, partial APTT and PT assays are used to evaluate the haemocompatibility of the Col-SCS multi-layer-coated titanium. The multi-layer processed surfaces display reduced platelet adhesion and activation, and prolonged clotting time of APTT and PT when compared to the untreated titanium. Therefore, the approach described here may provide a basis for the preparation of modified titanium surfaces for application in cardiovascular implants. Zhang and co-workers [281] prepare five SAM and three polymeric brushes with very low fibrinogen adsorption. The five SAM are oligoethylene glycol (OEG), PC, oligo( phosphoryl-choline), and two mixed positively and negatively charged SAM of $\text{SO}_3^-/N^+\text{(CH}_3)_3$ and $\text{COO}^-/N^+\text{(CH}_3)_3$. Three polymer brushes are prepared on gold surfaces via SI-ATRP using three monomers, sulfobetaine methacrylate (SBMA), carboxybetaine methacrylate (CBMA), and oligo(ethylene glycol) methyl ether methacrylate (OEG-MA). Surface plasmon resonance measurements show that although all of these surfaces are ‘non-fouling’ to fibrinogen adsorption from buffer solution, their protein adsorption from undiluted human blood plasma varies widely. The polymer brushes exhibit much lower protein adsorption from plasma than any of the five SAM tested. However, platelet adhesion measurements on plasma-pre-adsorbed surfaces show that all of these surfaces have very low platelet adhesion. Clotting time measurements using recalcified platelet poor plasma (PPP) incubation with
the eight types of surfaces show that they do not shorten clotting times. Linear polymers of polySBMA and polyCBMA with similar molecular weights have also been synthesised and characterised. In the presence of polyCBMA linear polymers, the clotting time of PPP is prolonged and increased with the concentration of the polymer, while no anticoagulant activity is observed for the polySBMA or PEG polymers. The unique anticoagulant activity of polyCBMA, as well as its high plasma protein adsorption resistance, makes polyCBMA a candidate for blood-contacting applications.

4.9 Micro- and Nanostructured Blood Contacting Surfaces

Biomaterials are revolutionising many aspects of preventive and therapeutic healthcare. One of the prime requirements to these materials is bio- and haemocompatibility, that is, the bio-inertness or ability of the material to perform with an appropriate response in a specific application [282]. The search for non-fouling materials to increase bio- and haemocompatibility of implantable and other biomedical devices has never stopped. However, only a limited number of such materials are still available, which efficiently restrict non-specific protein adsorption and cell adhesion. Surface roughness, surface chemistry, surface charge distribution, and interfacial free energy are typical indices of surface properties of any material [283] and, as it is well known, all of them influence the protein adsorption and cell-material surface interaction. Combining of physical and chemical surface features with suitable surface textures is the current approach to solve the problems connected to blood compatibility and especially to biomaterials for long-term blood contacting devices. The design of high performance biomaterials by architectural manipulation with regard to surface texture for improvement of the biomaterials’ blood compatibility is discussed in some reviews [282, 284].

For many applications, it is essential to be able to control the interface between device and biological environment by micro- and/or nano-scale control of the surface chemical composition and topography. Molecular thickness coatings made by biologically active macromolecules provide predictable interfacial control over interactions with biological media. Covalent immobilisation of polysaccharides, proteins and synthetic oligopeptides can be achieved, in addition to other ways, via nanometre thick, interfacial bonding layers deposited on the surface by gas plasma treatment and multi-step coating schemes [285]. Up to now, a number of non-fouling, bio- and haemocompatibility increasing coatings for biomaterials have been explored that are divided generally into two major categories: (i) based on PEG, and other hydrophilic or super hydrophobic synthetic polymers, discussed in Chapter 2 and Chapter 3 and (ii) biomimetic materials which include PC, SB zwitterionic polymers, oligo/polysaccharides (dextran, heparin), BSA coatings, other proteins as well as seeded EC.
This section focuses on the influence of the surface roughness and topography on the interactions with cells and on surface engineered micro- and nano-structures aimed at prevention of non-specific protein adsorption and cell adhesion, and thus, at the improvement of the haemocompatibility. The interaction of the adherent cell with its surroundings can ultimately determine cell fate. It is known that cells require a minimal contact area with the substrate to survive [286], and that the nature of this contact area can control the formation of connections with the outside environment [287]. As already mentioned, on an artificial substrate, such as the biomaterial is, this proceeds via four steps [288]: initial cell attachment; cell spreading; organisation of actin cytoskeleton; and formation of specific focal contacts. The cell adhesion and activity on the biomaterial surface depends strongly on many other surface physicochemical properties such as hydrophilicity, chemical composition and charge, steric hindrance, existence of a ‘conditioning layer’, and so on, on the surface roughness and topography [289]. The surface roughness influences the spreading of liquid cements secreted by the cells to increase adhesion on engineered topography. Even the smoothest substrate has some molecular roughness on the surface. Depending on the viscosity of the liquid, the adhesive might not fill all the small crevices. The unfilled crevices can be on a molecular or micron scale level depending on the size of the biological organism [290]. The trends observed from many studies are that as the roughness increases, the advancing angle also increases and the receding angle decreases. This means that when static conditions are examined, as the roughness increases, the contact angle increases and, thus, the calculated critical surface tension increases. However, this statement does not consider the size, the shape and the exact location that the droplet edge falls compared to the rough features but this roughness influences the spreading of liquid adhesives. A number of reports on cellular responses to topographical cues at both the nanometre and micrometre scale have appeared in the past few decades. However, it is argued by a number of authors that these structural features are of less significance in the initial stages of the attachment process than the intrinsic thermodynamic factors involved [291, 292] and a number of detailed studies support this assertion [293]. The change of surface wettability due to surface roughness, i.e., topography, is likely to be a contributing factor to these responses. Studying the interfacial properties and protein resistance of nanoscale polysaccharide coatings, Griesler and co-workers [285] prove the influence of nanoscale topography on the hydrophobicity (the WCA and its hysteresis), confirmed later for fluorine-based polymer thin films [294]. Studying the influence of natural surface microtopographies on biofouling, Bohringer [295] and Bers and Wahl [296] reported promising anti-fouling properties of microstructured surfaces. Carman and co-workers [297] demonstrated experimentally, the importance of wettability models in predicting cellular contact guidance for engineered topographies and patent surface topography for non-toxic bioadhesion control [298], but did not fully explain the process. Bioadhesion is a complex and very specific process. The material modulus and surface elasticity of the cell membrane are other factors to consider, in addition
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to the variety of adhesive proteins, glycoproteins, and polysaccharides that organisms secrete. The wettability models are limited by the assumption that the liquid droplet is much larger than the topographical features. This allows line tension effects to be neglected. Measurements with smaller drop sizes are believed to enable the inclusion of line tension effects.

The effect of surface topography on cellular response is of fundamental importance, especially where living systems encounter biomedical device surfaces. To understand these biological processes, there is a widespread interest in tailored surface-active materials produced by surface chemistry coupled with advanced patterning processes [299]. In addition to the traditional approaches to improve the blood compatibility, based on the minimisation of the blood-biomaterial interactions, the chemical immobilisation of drugs or biomolecules on biomaterial surfaces or the seeding of vascular endothelial cells, still do not meet all requirements of the practical applications.

Chen and co-workers [300] proposed a bio-inspired strategy to mimic the multi-scale micro/nanostructures on the inner surface of natural blood vessels, and review recent progress in the design and fabrication of micro-/nano-scale topography. During millions years of evolution, various structures and functions have been developed by the nature from which researchers can learn very much to develop new technologies not only mimicking the structure of some plants and animals, but also to understand the secrets of their functional mechanisms [301, 302]. Investigations on the superhydrophobic lotus leaf [303], the superoleophobic fish scale [304], the dew collecting spider’s web [305], the water-repellent water strider’s leg [306], and so on show that the micro- and nano-structures play a very important role in the properties and interactions at the bio-interfaces. In many cases, ultrastructures, especially micro/nanostructures determine ‘how it works’ in practical applications. Biomimetic approaches (non-fouling lotus leaf or dolphin skin mimic) have already been investigated for use in the development of practically applicable marine biofouling preventing surfaces (AMBITO FP6 research project) as an biocide-free alternative for non-toxic biofouling control.

There is no doubt that the natural blood vessel is the best blood compatible ‘material’. It is known that within the blood vessel, a layer of EC is present on the self-assembling basal membrane consisting of collagen, proteoglycans, and glycoproteins such as fibronectin and laminin [47]. Therefore, guided by a bio-inspired strategy, some researchers [307] try to reduce platelet adhesion simply by building suitable nanostructures on the material surface. They directly coat carbon nanotube arrays with fluorinated polycarbonate urethane (FPCU) that lead to the formation of a nanostructured biomaterial surface of low surface free energy. Compared to a flat FPCU surface, the nanostructured surface is much more hydrophobic and indeed, it
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could be considered superhydrophobic. In platelet adhesion experiments, almost no platelets adhere to this surface. Further immunofluorescence experiments reveal much less platelet activation on the nanostructured materials compared to the corresponding smooth ones. The excellent resistance to platelets that suggests outstanding blood compatibility of such materials make them useful in various medical applications. These observations are opposite to the accepted knowledge that smooth surfaces are preferable for good blood compatibility but maybe for other biomaterials a smooth surface may not be necessary [308]. Siedlecki and co-workers [309] developed polyetherurethane urea (PUU) surfaces with square pillars with width and separation of 700 nm or 400 nm, which reduce the platelet adhesion from plasma at low shear stress. In addition, no significant increase in the activation of non-adherent platelets in the bulk fluid is observed. This indicates that although the pillars lead to an increase of the total surface area, the contact opportunity between platelets and the actual surface is reduced and restricted to the top of the pillars. Minelli and co-workers [310] fabricate structured polymer films with typical topographic features ranging in size from nm to μm using the demixing behaviour of an immiscible polymer blend. Their findings indicate that increasing the feature size of the surface structures from 27 nm to 1240 nm encouraged by the Willebrand factor adsorption, leads to platelet adhesion and consequent thrombus formation. Koh and co-workers [311, 312] prepared a nanostructured polylactic-co-glycolic acid (PLGA)-MWCNT composite with significantly reduced platelet adhesion and activation on its surface. Due to its excellent anti-adhesion and low platelet activation properties, such composite has a potential efficiency in suppressing blood coagulation. In a following study, Koh and co-workers [313] fabricated nanostructures on PLGA films in the submicron to nanometre range with high aspect ratio surface features. In this way, they prove that platelet adhesion and activation can be greatly reduced by surface topographic features at the sub-μm scale. In contrast, dimensions at the μm scale do not reduce the platelet response compared to the controls, suggesting that the effective features are in the range of the size of platelets or below.

Zhou and co-workers [314] observed surfaces of rabbit heart valves by SEM and find that a dual scale structure, consisting of cobblestone-like structures (of 8 μm underside diameter and 3 μm in height), and cilia (of about 150 nm in diameter), improves the blood compatibility of the valves. They fabricated similar hierarchical microstructures on the surface of PDMS using femtosecond laser and soft lithography methods. A comparative study demonstrates that the textured surface has improved wettability and anticoagulation properties when compared to the analogous smooth material. Chen and co-workers [315] successfully constructed nanoscale topographic features on a thermally responsive poly(N-isopropylacrylamide) (PNIPAAm) surface by grafting the polymer from silicon nanowire arrays (SiNWA) via surface initiated ATRP. The surface prepared in this way shows largely reduced platelet adhesion in vitro both below and above the LCST of PNIPAAm (~32 °C), while a smooth PNIPAAm surface
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exhibits resistance to platelet adhesion only at temperatures below the LCST. Further investigations demonstrated that the nanoscale topography gives rise to relatively high water content on the SiNWA-PNIPAAm surface which plays a key role in reducing the platelet adhesion. These results suggest additional applications of PNIPAAm in biomaterials and biomedicine at body temperature (37 °C). The combination of stimuli responsiveness and multi-scale topography may lead to improved blood compatibility: it mimics the natural blood vessel in the sense that the vessel changes dynamically in response to multiple factors such as signalling molecules, blood pressure and shear forces. Mao and co-workers [316] recently developed a method for in vivo imaging of blood vessel tissue directly in living mammalians using AFM. To mimic the inner surface of blood vessels, multi-scale structures are developed on the surface of PDMS consisting of interlaced sub-μm ridges and nanoprotuberances by simply combining self-assembly and physical treatment [317]. Platelet adhesion measurements show that such a multi-scale structured PDMS surface reduces the adhesion of adenosine diphosphate activated platelets under flow conditions. Further investigations demonstrate that this multi-scale topography, which matches that of the μm-sized platelets, provides low adhesion interactions. The multi-scale structures on this surface may also remodel the boundary conditions at the liquid interface, leading to a low collision frequency of platelets. This surface has considerable potential as a non-thrombogenic material in medical and surgical applications. Vrlič [318] developed a new anti-bioadhesive surface that is able to control the adhesion of specific proteins, responsible for the development of neurodegenerative diseases such as Creutzfeldt-Jakob, Alzheimers, Parkinson’s and Lewis disease. The approach is focused on problems prior to the detection step, which have never been considered before, particularly on the improvement of Eppendorf tubes that are used for the storage of body fluids such as cerebrospinal fluid and blood. These tubes made of PP induce a depletion of biological material, in some cases over 70%, resulting in a low protein concentration for the further immunoenzymic detection. With the purpose of reducing the specific proteins’ adhesion on the support surfaces, two courses of treatments are anticipated. The first one consists of a surface modification by a highly reactive fluorine plasma treatment and the second one incorporates development of new hydrophilic surfaces by coupling two techniques, plasma activation and subsequent grafting of polymer molecules. Finally, with the latter approach, an original method of surface modification is attained by using complex solutions of polymers and surfactants that permits controlled configuration of the nanostructured surfaces. An increase of the surface roughness usually increases the thrombogenicity thought to be due to the larger contact area with the platelets [47, 320, 321]. According to Chen and co-workers [321], the term ‘roughness’ is not precise enough to describe the surface topography because it does not reflect whether the roughness dimensions are at the macroscale, microscale or nanoscale. The surface roughness could be divided into three groups: > 2 μm (dimension of the platelets), < 2 μm and > 50 nm (dimension of the proteins), and < 50 nm (below the dimension of the proteins). In the first range only (> 2 μm, the
dimension of the platelets) an increase in the surface roughness will result in a larger contact area for platelet surface adhesion, leading to a more thrombogenic surface. In the second range (< 2 μm and > 50 nm, dimension of the proteins), particular surface topographies, such as pillars and grooves, may reduce the contact area for platelets, that will be able to adhere on the top of the topographic features only and, thus, the platelet adhesion and thrombus formation may be reduced. In the third range (< 50 nm), the surface structures are even smaller than the platelets pseudopods and the surface can be considered to be smooth for platelets.

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