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Polymer Thin Films for Biomedical Applications

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1.1
Introduction

Modern medicine uses a variety of synthetic materials and devices to treat medical conditions and diseases. Biomedical devices such as coronary stents, vascular grafts, heart valves, blood bags, blood oxygenators, renal dialyzers, catheters, hip prostheses, knee prostheses, intraocular lenses, contact lenses, cochlear implants, and dental implants have definitely played an important role in transforming lives and improving the quality of living. Advances in protein-based drugs, gene therapy, targeted drug delivery, and tissue engineering have the potential to revolutionize contemporary medicine. Artificial skins to treat burn victims, artificial pancreas for people with diabetes, and cardiac patches to regenerate cardiac muscle damaged by a heart attack, no longer seem far-fetched, because of developments in tissue engineering. Thus, a wide range of synthetic materials are used to evaluate, treat, augment or replace any tissue, organ or function of the body. "Biomaterial" is a term used to categorize such materials and devices that directly "interact" with human tissues and organs [1]. The interactions may involve, for example, platelet aggregation and blood coagulation in the case of blood-contacting devices, immune response and foreign body reactions around biomaterials or devices implanted in the body, or more desirably, structural and functional connection between the implant and the host tissue (this is termed osseointegration in the case of dental and orthopedic implants).

Biomaterials interact with biological systems through their surfaces. It is, therefore, vitally important to control the surface properties of a biomaterial so that it integrates well with host tissues – that is, to make the material "biocompatible" [2]. Organic thin films and coatings, particularly those of polymers, are very attractive as biomaterial coatings because they offer great versatility in the chemical groups that can be incorporated at the surface (to control tissue–biomaterial interactions); the coatings also have mechanical properties that are similar to soft biological tissues. The relative ease of processing is another reason for the extensive interest in organic thin films. Biomaterial surfaces can be coated with polymers using

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simple techniques such as dip-coating, spray-coating, spin-coating, or solvent casting. Coating techniques involving the chemical grafting of molecules onto the biomaterial surface are also available. Nanothin coatings based on self-assembled monolayers (SAMs), surface-tethered polymers (polymer brushes), or multilayer coatings based on layer-by-layer assembly offer precise control on the location and orientation of chemical groups and biomolecules on the surface of the coating.

In this chapter we discuss polymer thin films and coatings that have potential biomedical applications. Three main areas are covered: (i) biocompatible coatings for implants (e.g., protein-repellant coatings, antithrombogenic coatings that can prevent blood coagulation around implants, and antibacterial coatings); (ii) polymer thin films for tissue engineering; and (iii) polymer thin films for drug delivery and gene therapy. Emphasis is placed on the material and processing aspects of the coating, the physico-chemical properties of the coating, and its protein-adsorption and cell-adhesion characteristics.

1.2 Biocompatible Coatings

1.2.1 Protein-Repellant Coatings

Protein adsorption on biomaterial surfaces plays a critical role in determining cellular events at the tissue–implant interface. The adsorption of plasma proteins onto the surface of a blood-contacting implant can trigger a cascade of chemical reactions, leading to the formation of a blood clot surrounding the implant. The blood protein factor XII is known to be activated by negatively charged surfaces such as glass, and some polymers such as poly(vinyl chloride) [3] may initiate the intrinsic pathway of blood coagulation. Protein adsorption can also activate the host foreign-body response, by switching on the complement system. The adhesion of neutrophils and macrophages, which are associated with the host foreign-body response, can subject the biomaterial surface to attack by destructive enzymes, superoxide anions, and hydrogen peroxide [4]. Finally, protein adsorption can promote bacterial colonization on the implant surface, that in time will require the reoperation of infected implants. There is, therefore, a great interest in developing biomedical coatings that can resist protein adsorption.

1.2.1.1 PEGylated Thin Films

The ability of hydrophilic surfaces, especially coatings based on poly(ethylene glycol) (PEG), to resist protein adsorption has been demonstrated in several studies [5–14]. PEG surfaces seem to be the benchmark in protein-adsorption studies, because of their exceptional resistance to protein adsorption. Silane-based PEG SAMs were found to maintain their integrity after sterilization in an autoclave using 20 psi steam at 120 °C for 1 h. Strategies to bind PEG molecules to the surfaces of metallic implants, such as the use of PEG conjugates with the adhesive

amino acid L-2,4-dihydroxyphenylalanine (DOPA) [15–17], or with the cyanobacterial iron chelator anachelin [18], have been reported.

Amphiphilic block copolymer coatings containing PEGylated and fluoroalkyl moieties have shown to have extremely low forces of interaction with protein molecules (C.J. Wienman *et al.*, unpublished results). Novel architectures, such as multi-armed molecules, have been used to prepare monolayer coatings on surfaces, which decreased the protein adsorption [19]. Hydrogel thin films were prepared on amine-functionalized surfaces by spin-coating of a six-armed, star-shaped poly(ethylene glycol-*ran*-propylene glycol) polymer, with crosslinkable peripheral isocyanate groups [20]. Crosslinking of the star-shaped molecules occurred by reaction between the isocyanate groups in an aqueous environment to form urea linkages. The coatings prevented adsorption of avidin (over a pH range of 5 to 9.5), and were stable over several months when stored under ambient conditions. A multicomponent coating formulation to obtain robust, readily functionalized PEG-based thin films has been reported by Lochhead and coworkers for imparting resistance to protein adsorption, fibroblast cell adhesion, and bacterial adhesion [21]. The formulation consisted of: (i) the active component, NHS–PEG-aminosilane (NHS = *N*-hydroxysuccinimide); (ii) the matrix-forming component, polyoxyethylene sorbitan tetraoleate; and (iii) the molecular crosslinker, 6-azidosulfonylhexyltriethoxy silane. Solutions of these three components in dimethylsulfoxide (DMSO) were spin-coated onto glass, silicon, or tissue culture grade polystyrene (TCPS) substrates and thermally cured at 0.1 mmHg pressure for 75 min. The coatings showed significant inhibition of fibrinogen and lysozyme adsorption, microbial adhesion, and fibroblast cell adhesion. The *in situ* crosslinked PEG-based coatings could be functionalized with an RGD (Arg-Gly-Asp) peptide to promote cell adhesion for tissue engineering applications. PEGylated surfaces were also made biofunctional using latent aldehyde groups that were used to covalently tether proteins and bioligands [22]. Similarly, PEGylated polymer brushes were functionalized with cell-adhesive proteins to promote the osseointegration of bone and dental implants [23].

Coatings prepared using the polysaccharide, chitosan (CS) that was grafted with PEG side chains, showed good resistance to the adsorption of fibrinogen and bovine serum albumin (BSA) [24]. In the chitosan polymer, 72% of the sugar units were deacetylated, and 56% of all sugar units carried a $-(CH_2CH_2O)_{44}CH_3$ side chain. SAMs of 16-mercaptodecanoic acid on gold were used as substrates for the PEGylated chitosan coatings. The coating adhered to the substrate via electrostatic as well as covalent coupling between the primary amine groups in chitosan and carboxylic acids in the SAM.

1.2.1.2 **Non-PEGylated Hydrophilic Thin Films**

A variety of hydrophilic thin films have been investigated as alternatives to PEGylated coatings for biomedical applications [25, 26]. There is significant interest in developing biomedical coatings that have better thermal and oxidative stability than PEG, but with protein-repellency comparable to that of PEG. A recent review highlighted some developments in the design and synthesis of

protein-resistant polymer coatings, and provided a discussion of the mechanism of antifouling activity [27]. Pulsed plasma-deposited poly(*N*-acryloylsarcosine methyl ester) coatings were shown to be resistant towards the adsorption of fibrinogen and lysozyme [28], while hydrophilic tertiary amine oxide surfaces were found to compare favorably with PEGylated coatings in preventing nonspecific protein adsorption [29]. Hydrogel thin films of poly(*N*,*N*′-methylene bisacrylamide) showed less than 0.5 ng cm^{-2} adsorption of BSA. The highly hydrophilic polyacrylamide was chemically tethered to the hydrophobic substrate of an alkyl thiol SAM using a photoinitiator, benzophenone, which induced crosslinking with the substrate via hydrogen abstraction [30]. Zwitterionic polymers have found renewed interest as protein-resistant coatings. These polymers attempt to mimic the excellent resistance to protein adsorption and hemocompatibility of the zwitterionic phosphorylcholine group that is a major component of cell membranes [31]. Thin film coatings of zwitterionic SAMs and polymer brushes, of phosphobetaines, carboxybetaines and sulfobetaines [32], have shown excellent resistance to protein adsorption [33–39]. In addition to their protein repellency, the carboxylic acid groups of carboxybetaine thin films have been used to covalently immobilize cell-adhesive peptides for tissue engineering applications [37]. Interpenetrating polymer networks of polyurethanes and zwitterionic polymers combined the desirable mechanical properties of polyurethane with the antifouling properties of the zwitterionic polymer [40]. In a novel approach, zwitterionic amino acids were used to prepare thin film coatings on gold-coated glass slides [41]. SAMs of *N*-3-mercaptopropylamino acids, prepared from the 19 natural amino acids, were investigated for their ability to resist the nonspecific adsorption of proteins. When the SAMs were exposed to a solution of BSA (76 mg ml^{-1}) in phosphate-buffered saline (PBS) for 20 min, the concentration of nonspecifically bound proteins ranged from approximately 400 ng cm^{-2}, with polar and ionic amino acids, to approximately 800 ng cm^{-2}, with amino acids of increased hydrophobicity. The nonspecific adsorption of BSA increased in the following order: Asp < Asn < Ser < Met < Glu < Gln < Thr < Gly < His < Cys < Arg < Phe < Trp < Val < Pro < Ile < Leu < Ala < Tyr. Thin-film coatings prepared using poly(*N*-substituted glycine) (polypeptoids) exhibited significant reductions in the adsorption of lysozyme, fibrinogen, and serum proteins [42]. Poly(2-methyl-2-oxazoline), a peptide-like polymer, has been used to prepare surfaces with a protein adsorption below a level of 2 ng cm^{-2} [43]. The polyoxazoline surfaces had protein repellency comparable to that of the best PEG-based coatings.

1.2.1.3 Thin Films of Hyperbranched Polymers

Hyperbranched polymers with hydrophilic groups have attracted interest because of their similarity to the antifouling glycocalyx (extracellular matrix; ECM) of cells [44–47]. Dendritic polyglycerols (cf. Figure 1.1) combine the characteristics of highly protein-resistant polymers, namely a highly flexible aliphatic polyether segments, hydrophilic surface groups, and a highly branched architecture [49]. Fibrinogen adsorption on these surfaces was comparable to that on a PEGylated SAM of $HS(CH_2)_{11}(OCH_2CH_2)_3OH$, and better than the dextran-coated surfaces which

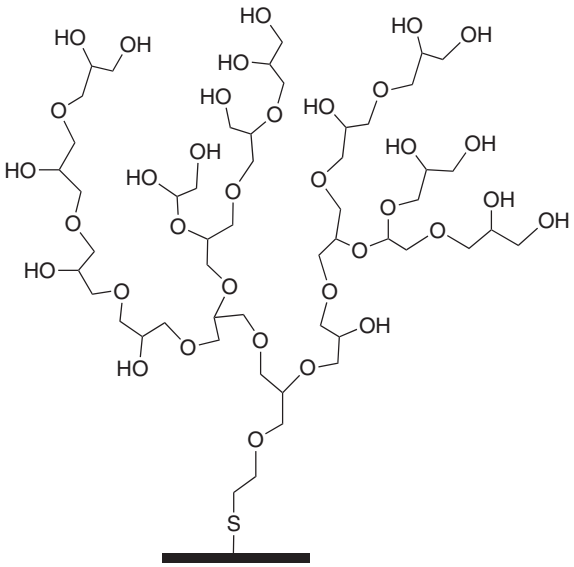

Figure 1.1 A dendritic polyglycerol monolayer. Adapted with permission from Ref. [48]; © 2008, American Chemical Society.

have been used for decades as low-protein-binding substrates [50]. Self-assembled monothiol-terminated hyperbranched polyglycerols on gold surfaces have shown a better resistance to the adsorption of BSA and immunoglobulin adsorption than the SAMs of linear PEG thiols [48]. Dendritic or hyperbranched hydrophilic polymers will result in a highly hydrated surface, which is an important characteristic of most antibiofouling surfaces; however, similar hyperbranched polyglycerols have shown a lower resistance to bacterial adhesion than linear PEG molecules [51]. The combined effects of steric repulsion, which is expected to be higher for linear architectures because of higher polymer flexibility, and the packing density of hydrophilic groups, which will be higher for dendritic architectures; this plays a critical role in determining the protein and cell repellency of the dendritic coatings. In contrast, Jiang and coworkers have argued that conformational flexibility is not required for protein resistance, and that only hydration played a dominant role in surface resistance to nonspecific protein adsorption [52]. Thus, the role of steric repulsion and polymer conformation on resistance to protein adsorption requires further investigation.

1.2.1.4 **Multilayer Thin Films**

Polyelectrolyte multilayer (PEM) thin films have been widely explored as functional coatings in biomedical engineering, particularly in tissue engineering and gene/drug delivery. These coatings are, however, less protein-resistant than uncharged hydrophilic coatings such as PEG, or zwitterionic coatings such as those containing phosphorylcholine groups [53]. The interactions of proteins with PEMs have been characterized using human serum albumin and poly(sodium

4-styrenesulfonate) (PSS)/poly(allyl amine hydrochloride) (PAH) [$(PSS/PAH)_n$] multilayer thin films [54]. The protein adsorbed onto the multilayer coatings, regardless of whether PAH or PSS was the terminal layer. On PSS-ending multilayers, the human serum albumin adsorption was limited to a dense monolayer, whereas on PAH-ending multilayers protein films with thicknesses exceeding several-fold the native protein dimension could be formed. Protein interaction with PEMs was found to be electrostatic in origin. PEM coatings with balanced charges have shown resistance to protein adsorption. By controlling the amounts of the cationic poly(2-aminoethyl methacrylate hydrochloride) and anionic poly(2-carboxyethyl acrylate), the net charge (as estimated by direct force measurements) of the PEM thin film could be minimized; this minimum in net charge corresponded to a minimum in protein adsorption on the polyelectrolyte blends [55].

In order to make $(PAH/PSS)_n$ multilayer films resistant to protein adsorption, PEM coatings terminated with the anionic PSS polymer were additionally coated with a terminal layer of poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) [56]. The PEG grafts imparted protein resistance to the polyelectrolyte coatings. Protein adsorption from full serum on the PEGylated surfaces of $(PAH/PSS)_n$(PLL-*g*-PEG) multilayers ($n = 1–4$) was three orders of magnitude lower in comparison to $(PAH/PSS)_n$ surfaces that did not contain the PEG grafts. The layer-by-layer (LBL) assembly of multiarm PEG with reactive vinylsulfone end groups and dithiothreitol (DTT) was found to be resistant to protein adsorption and cell adhesion [57]. The vinylsulfone end groups of PEG reacted with thiol groups on DTT through a Michael-type reaction, producing a thin, crosslinked PEG coating. The resistance to cell adhesion then increased with an increase in the number of layers in the coating. For the same number of layers, multilayers prepared with eight-arm PEG molecules were more resistant to cell adhesion than multilayers containing four-arm PEG. An RGD-containing peptide, acetyl-GCGYGRGDSPG-NH_2, could be covalently immobilized on the surface, through reaction between the vinylsulfone end groups of PEG and the cysteine residue in the peptide, to enhance cell attachment by binding cell-surface receptors of the integrin family.

Polyelectrolytes bearing zwitterionic groups have been used in PEM coatings to impart resistance to protein adsorption [58, 59]. The polyelectrolytes shown in Figure 1.2 were obtained by modification of poly(L-glutamic acid) (PGA), poly(acrylic acid) (PAA), and poly(L-lysine) (PLL) [58]. An earlier study had also reported that fibronectin was adsorbed onto poly(allylamine hydrochloride)-terminated and Nafion™-terminated polyelectrolyte multilayer coatings, but fibronectin adsorption was low on multilayers terminated with a poly(acrylic acid-*co*-3-[2-(acrylamido)-ethyl dimethylammonio]propane sulfonate) copolymer that contained the zwitterionic sulfobetaine monomer [60].

1.2.2 Antithrombogenic Coatings

1.2.2.1 Surface Chemistry and Blood Compatibility

The blood compatibility of coatings is strongly influenced by chemical groups present at its surface. Sperling *et al.* found that leukocytes did not adsorb onto a

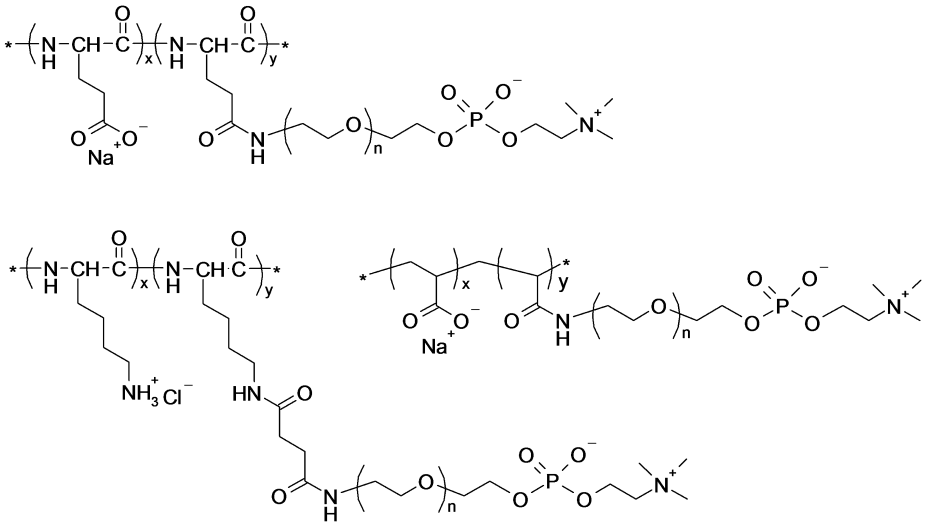

Figure 1.2 Polyelectrolytes bearing zwitterionic moieties. Adapted with permission from Ref. [58]; © 2009 Wiley-VCH Verlag.

–CH_3-terminated alkanethiol SAM, but their adhesion was greatly enhanced on surfaces with –OH groups [61]. The opposite was detected for the adhesion of platelets. A strong correlation between the activation of the complement system and the adhesion of leukocytes with the content of –OH groups was observed. Complement activation was also scaled with the amount of –COOH groups at the surface. Rodrigues *et al*. showed that fibrinogen adsorption decreased linearly with an increase of –OH groups on a SAM surface [62]. Platelet adhesion and activation were also seen to decrease with an increase of surface hydrophilicity. The adsorption of plasma albumin onto the surfaces passivated the surfaces and lowered platelet adhesion.

1.2.2.2 **Membrane-Mimetic Thin Films**

Chung *et al*. screened SAMs of different phospholipids molecules, on gold, for fibrinogen adsorption and platelet adhesion [63]. It was found that, of the bromoethylphosphorate-, phosphorylcholine-, phosphorylethanolamine-, and hydroxyl-terminated SAMs, the phosphorylcholine-terminated SAMs showed the best antifouling properties. Carboxybetaine-based SAMs and polymers showed not only a very low fibrinogen adsorption but also a very low platelet adhesion. Moreover, the poly(carboxybetaine methacrylate) polymer also exhibited anticoagulant activity and increased the clotting time of blood, which made it a promising candidate for coating blood-contacting devices and implants [64, 65]. Ito and coworkers have developed a new type of copolymer coating composed of L-histidine, a zwitterion, and *n*-butyl methacrylate, a hydrophobic moiety [66]. Polystyrene surfaces coated with the copolymer were found to have a significantly low nonspecific adsorption of proteins and adhesion of cells in comparison with BSA-passivated surfaces.

1.2.2.3 Heparin-Mimetic Thin Films

Heparin is a highly-sulfated, anionic, polysaccharide (5–25 kDa) (cf. Figure 1.12) that can bind to the blood protein antithrombin through ionic interactions, and result in a several-fold acceleration of the rate at which antithrombin inactivates clotting factors such as thrombin. The enzyme thrombin plays a key role in the coagulation cascade by cleaving fibrinogen to produce fibrin monomers; thrombin also increases platelet–platelet adhesion and stimulates platelet activation and degranulation. Hence, the inactivation of thrombin (by heparin-bound antithrombin) will inhibit blood coagulation. Surface-tethered heparin is also known to suppress platelet adhesion, complement activation and protein adsorption [67]. Ayres *et al*. found that polymer brushes containing sulfated carbohydrate repeat units, which resembled surface-tethered heparin, resulted in significantly longer plasma recalcification clotting times than with nonsulfated polysugar polymer brush surfaces used as a control [67]. The sulfated brushes also reduced the production of complement factor products C3a, C4a, and C5a, in comparison to the control surfaces. Other polysaccharide-based glycocalyx-mimicking polymer coatings that reduce platelet adhesion and improve blood compatibility have also been described [68, 69].

1.2.2.4 Clot-Lyzing Thin Films

The PEGylation of poly(dimethyl siloxane) (PDMS) surfaces conferred resistance to nonspecific protein adsorption. Moreover, the incorporation of free ε-amino groups on the surface, by using PEG–lysine conjugates, rendered the surface capable of dissolving fibrin clots because of adsorption of the fibrinolytic protein, plasminogen, from blood plasma [70]. Similar studies in the past had shown that polyurethane surfaces coated with a lysine-derivatized acrylamide polymer dissolved fibrin clots by a ready conversion of the adsorbed plasminogen to plasmin in the presence of tissue-plasminogen activator (TPA) [71]. The design of these lysine-based anticlotting coatings is based on the fact that surfaces incorporating a high density of lysine residues, in which the ε-amino groups are free, are capable of selective adsorption of plasminogen from blood plasma (up to a level of $1.2\,\mu g\,cm^{-2}$, corresponding to a compact monolayer of plasminogen), and virtually no other proteins [72]. In contrast, control surfaces that contained either no lysine, or lysine in which the ε-amino group was not available, adsorbed only very small amounts of plasminogen, and were unable to prevent clot formation.

Nanocomposite fibrinolytic coatings were obtained by tethering proteolytic enzymes to the surfaces of carbon nanotubes (CNTs), which were then dispersed in poly(methyl methacrylate) (PMMA) [73]. Enzymes, such as serine protease subtilisin Carlsberg (SC) and trypsin, were loaded onto the CNTs by physisorption. The extent of nonspecific protein adsorption on these biocatalytic films was 95% lower compared to the enzyme-free film. The incorporation of a fibrinolytic enzyme into the coating resulted in a lowering of fibrinogen fouling by 92%. Clot-lyzing coatings such as these could potentially prevent thrombosis in stents and other blood-contacting implants [74].

Local nitric oxide (NO) release from polymeric surfaces can potently inhibit platelet adhesion and activation, making the surface resistant to clot formation [75, 76]. A low-leaching, NO-generating PEM thin film comprising sodium alginate (ALG) and organoselenium-modified polyethyleneimine was prepared by LBL assembly [77]. The thin films were deposited on biomedical-grade polymer substrates (such as silicone rubber tubings and polyurethane catheters), and produced NO even after prolonged contact with sheep whole blood. The multilayers allowed endogenous *S*-nitrothiols such as *S*-nitrosoglutathione (GSNO) and thiol-reducing agents such as glutathione (GSH), to diffuse through the polymer matrix and reach the organoselenium sites, where the catalytic decomposition of GSNO to NO occurred. The LBL coatings showed a very low catalyst leaching.

1.2.2.5 **Polyelectrolyte Multilayer Thin Films**

There is an increasing interest in preparing antithrombogenic polymer thin films using the LBL assembly technique [78–90]. When PEM coatings of chitosan and dextran sulfate (DS) were prepared on poly(tetramethylene adipate-*co*-terephthalate) membranes [91], it was found that coatings with dextran sulfate as the outermost layer could resist platelet adhesion and human plasma fibrinogen adsorption. A LBL assembly approach has also been used for the *in vivo* repair of damaged blood vessels by forming a multilayer coating of anionic hyaluronic acid (HA) and cationic chitosan on the arterial walls [92]. Chitosan, with its excellent bioadhesive properties on negatively charged surfaces (such as those presented by the damaged arterial lumen), was deposited as the first layer to ensure a strong adhesion of the coating. The growth of blood clot on damaged arterial surfaces was significantly inhibited by the $(CS/HA)_n$ multilayers (87% reduction in platelet adhesion). The incorporation of L-arginine (which is known to inhibit monocyte and platelet adhesion) into the multilayer resulted in a 91% reduction in platelet adhesion compared to the unprotected damaged arteries.

1.2.2.6 **Polyurethane Coatings**

Novel polyurethane coatings that incorporated hyaluronic acid as a chain extender during polyurethane synthesis have been reported [93]. The surface hydrophilicity was increased with an increase in hyaluronic acid content. Ultimately, a 20-fold decrease in platelet adhesion was identified due to the inclusion of hyaluronic acid, when compared to polyurethane that did not contain the polysaccharide. The polyurethane–hyaluronic acid coatings were cytocompatible and supported endothelial cell adhesion and viability. The surfaces of the poly(ester urethane) guiding catheters were dip-coated with an amphiphilic conjugate of stearyl poly(ethylene oxide) (SPEO) with 4,4′-methylene diphenyl diisocyanate [94]. In order to improve adhesion of the SPEO–diisocyanate surface-modifying additive to the poly(ester urethane) substrate, a "film-building additive" was used in addition to the SPEO–diisocyanate conjugate, while preparing the coating formulation. The film-building additive was a poly(ether urethane), Pellethane® 2363-80AE (Dow Chemical Co.), a polytetramethylene glycol-based polyurethane elastomer.

The resultant coated surfaces resisted blood clotting much more effectively than did the uncoated polyurethane.

1.2.2.7 Vapor-Deposited Thin Films

Chemical vapor deposition (CVD) represents another attractive technique for preparing polymer thin film coatings for biomedical applications. Polymer coatings of various [2.2] paracyclophane (PCP) derivatives were codeposited in controlled ratios by CVD, and the multifunctional coatings evaluated for their biocompatibility and antithrombotic properties [95]. The functionalized PCPs were polymerized into poly(*p*-xylylenes) during the deposition process.

1.2.3 Antimicrobial Coatings

Of the three million cases of central venous catheter insertions per year in the USA, 30% result in infection-related mortality. This occurs because pathogens (e.g., bacteria) are inadvertently transferred from the skin or air into the wound site during the surgical insertion of implants. Bacterial infection also represents a serious complication in the case of orthopedic implants. When dealing with an infected fibrous capsule (through which antibiotics cannot easily penetrate), or with infections resulting from antibiotic-resistant bacterial strains, surgical removal of the implanted biomaterial very often becomes necessary. It follows that biomedical coatings that could resist bacterial adhesion and colonization would help to prevent implant-associated bacterial infections.

1.2.3.1 Cationic Polymers

Many bactericidal coatings are based on the membrane-disrupting activity of quaternary ammonium, phosphonium, or pyridinium groups [96–98]. Klibanov and coworkers found that surface-tethered poly(4-vinyl-*N*-hexyl pyridinium bromide) chains were highly effective against Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermis*, as well as the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* [99]. The antibacterial activity was found to depend on the molecular weight of the tethered polymer chains; thus, it was proposed that only sufficiently long and flexible chains would be able to penetrate the bacterial cell walls and disrupt the cell membrane. In contrast, Isquith *et al.* reported that even monolayers of 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride exhibited antibacterial activity while chemically bonded to a variety of surfaces [100]. The antibacterial activity of these coatings was due to the presence of the surface-bonded molecules, and not to the slow release of membrane-disrupting molecules from the surface. By using spray-coated coatings of quaternized polystyrene-*block*-poly(4-vinylpyridine) block copolymers, the molecular weight was found not to be a limiting factor of antibacterial activity [101], as even a polymer with a relatively low-molecular-weight pyridinium block showed a high bactericidal activity. Similar results were obtained in recent studies involving poly(butylmethacrylate-*co*-Boc-aminoethyl methacrylate) polymer brushes of con-

trolled layer thicknesses and grafting density [102]. Here, the bactericidal efficiency of these surfaces was shown to be independent of the polymer layer thickness, within the range of surfaces studied.

The surface concentration of pyridinium groups is critical in determining bactericidal efficiency [101, 103]. Antibacterial activity was higher when the surface concentration of the quaternary nitrogen [characterized using X-ray photoelectron spectroscopy (XPS) and near edge X-ray absorption fine structure (NEXAFS)] was higher [101]. The bactericidal effect was also higher when a semifluorinated alkyl bromide, $F(CF_2)_8(CH_2)_6Br$, was used for quaternization along with *n*-hexyl bromide. The fluorinated side chains increased the surface concentration of the high-surface energy pyridinium rings, and this resulted in an enhanced antibacterial activity. Hydrophobic interactions of the highly nonpolar fluoroalkyl groups with the bacterial cell membrane may also have contributed to the antibacterial effect. The extensive literature on antibacterial polymers and coatings based on cationic polymers is discussed elsewhere [101]. Polymeric surface modifiers (PSMs) with soft blocks comprising semifluorinated ($-CH_2OCH_2CF_3$) and 5,5-dimethylhydantoin or alkyl ammonium side groups were found to have good biocidal properties [104–106]. The near-surface amide groups of the hydantoin side groups were converted to antibacterial chloramide groups using hypochlorite. The surfaces of conventional polyurethane (isophorone diisocyante/1,4-butanediol-derived hard block and poly(tetramethylene oxide) soft block) blended with PSM were resistant to both, Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* and *E. coli*. Indeed, only 1.6 wt% of the PSM was found to be sufficient to completely kill *P. aeruginosa* within a 15 min period. Polyurethane coatings have also been rendered antibacterial by a UV-induced surface-initiated polymerization of 4-vinylpyridine from the polyurethane surface [107].

Hydrophobic fluoroalkyl groups enhanced the bactericidal activity of pyridinium and ammonium polymer coatings [101, 104]. Interestingly, the incorporation of a hydrophilic comonomer such as poly(ethylene glycol)-methyl ether methacrylate or hydroxyethyl methacrylate was also found to increase the antibacterial activity of 4-vinyl-*N*-hexyl pyridinium coatings [108].

1.2.3.2 **Nanocomposite Polymer Thin Films Incorporating Inorganic Biocides**

The antibacterial activity of silver has been used to prepare bactericidal thin films for biomedical surfaces. Hybrids of silver particles with highly branched amphiphilically modified polyethyleneimines (PEIs) were found to be bactericidal [109]. Liposomes loaded with silver ions were embedded in poly(L-lysine)/hyaluronic acid multilayer thin films. The controlled release of encapsulated $AgNO_3$ from the coating resulted in a 4-log reduction in the number of viable *E. coli* cells in contact with the coating [110]. Rubner and coworkers prepared antibacterial coatings based on hydrogen-bonded multilayers containing *in situ*-synthesized silver nanoparticles, and found these coatings to be efficient against both Gram-positive and Gram-negative bacteria [111]. The same authors also reported a dual-function antibacterial coating with both quaternary ammonium salts and silver [112]. The coatings were prepared by a LBL assembly of poly(allylamine hydrochloride) and

poly(acrylic acid); the polymer layers were then coated with silica nanoparticles that were later functionalized with quaternary ammonium silane. The silver nanoparticles were created *in situ* in the polymer layer.

Antibacterial surfaces with a wide range of surface wettability (water contact angles of 30–140°) have been prepared using hydrolytically stable *N*-alkylmethoxysilane pyridinium polymers [113]. Antibacterial agents such as silver bromide nanoparticles and triiodide ions were also incorporated into these coatings. An electrochemical deposition technique was used to prepare thin films of silver/polymer nanocomposites on stainless steel surfaces; here, the polymer matrix consisted of an inert poly(ethyl acrylate), a macroinitiator of controlled radical polymerization poly(2-phenyl-2-(2,2,6,6-tetramethylpiperidin-1-yloxy)ethyl acrylate), or poly(8-quinolinylacrylate) (P8QA) that has broad antibacterial activity and complexation ability toward metal ions [114]. The silver-containing, electro-grafted, acrylic polymer films showed significant bactericidal activity against *S. aureus*, with the P8QA-based coatings showing the highest antibacterial activity. Antibacterial coatings were also prepared on stainless steel substrates by first depositing an adhesion-promoter layer of hexamethyldisiloxane using a low-pressure plasma technique, followed by the plasma-deposition of ethylene diamine polymer [115]. The PEI coatings were quaternized with alkyl halides to impart antibacterial activity.

The two naturally occurring polymers, alginate and gelatin, have each been used to prepare antibacterial, biodegradable polymer coatings [116]. These coatings were loaded with silver nanoparticles to impart antibacterial activity. Moreover, the pH-responsive swelling/shrinking behavior, resulting from the alginate carboxylic acid groups, could potentially be used for the controlled storage and release of biomolecules. Surface immobilization with bioactive proteins was also shown to be possible. The catechol-functionalized polyelectrolytes were found to form stable LBL assemblies on a variety of substrates, such as poly(tetrafluoroethylene) (PTFE), polyethylene, poly(ethylene terephthalate), and polycarbonate [117]. The catechol groups were used to bind the polyelectrolytes to the substrate, and also for the *in situ* deposition of silver nanoparticles, imparting an antibacterial activity to the multilayer film.

PDMS substrates have been coated with titanium dioxide thin films by liquid-phase deposition, from water, under near-ambient conditions [118]. Such coatings reduced the adhesion of both Gram-positive and Gram-negative bacteria. Moreover, the bacterial adhesion was further reduced if the TiO_2 over-layer was irradiated with UV light before introduction of the bacteria. Antibacterial coatings have also been prepared using chitosan/heparin multilayer thin films that consisted of embedded TiO_2 or silver nanoparticles [119].

1.2.3.3 Antibiotic-Conjugated Polymer Thin Films

Atom transfer radical polymerization (ATRP) was used to grow poly(2-hydroxyethyl methacrylate) polymer brushes on titanium surfaces. For this, the pendent hydroxyl groups were converted into carboxyl or amine groups, which were used to covalently immobilize antibiotics such as gentamicin and penicillin. These coatings showed a significant decrease in the viability of *S. aureus* [120]. Surfaces

of PTFE grafted with penicillin also showed antibacterial activity [121]. Poly(dimethylaminomethyl styrene), which is cationic by virtue of protonation, was coated onto substrates using an initiated chemical vapor deposition (iCVD) method, and found to be very effective against Gram-negative *E. coli* and Gram-positive *B. subtilis* [122]. Other biocompatible coatings and antimicrobial coatings have also been prepared using iCVD (for a review, see Ref. [123]).

1.2.3.4 **Biomimetic Antibacterial Coatings**

Facially amphiphilic polymers that mimic the physico-chemical properties of natural host defense peptides have been found to have excellent antibacterial activity coupled with selectivity, which in turn makes them promising candidates for imparting antibacterial activity to biomedical surfaces [124]. Etienne *et al.* prepared antifungal coatings by embedding the antifungal peptide chromofungin into PEM thin films [125]. Despite being embedded in the film, the chromofungin was able interact with the membrane of the fungi and demonstrate antimicrobial activity. The antifungal coatings did not exhibit any cytotoxicity towards eukaryotic cells (e.g., human gingival fibroblast cells). The antibacterial peptide, defensin, was also embedded in PEM films to impart bactericidal activity to the coatings [126]. In this way, copolymer brushes of 2-(2-methoxyethoxy)ethyl methacrylate and hydroxyl-terminated oligo(ethylene glycol) methacrylate were grafted with the natural antibacterial peptide, magainin I, and found to be effective against different strains of Gram-positive bacteria [127].

1.2.3.5 **Thin Films Resistant to the Adhesion of Viable Bacteria**

Nonbiocidal coatings have also been investigated for their ability to resist bacterial colonization. Coatings of "surfactant polymers" with a structure consisting of a poly(vinyl amine) backbone and hydrophilic PEG and hydrophobic *n*-hexyl grafts, were successful in suppressing bacterial adhesion on biomaterial surfaces [128]. The PEG packing density and hydration thickness were found to be critical in determining the ability of the coating to shield the surface against bacterial interactions. PEMs prepared using poly(L-lysine) and poly(L-glutamic acid)-*graft*-poly(ethylene glycol) were similarly found to drastically reduce both protein adsorption and bacterial adhesion [129].

Poly(L-lysine)-*graft*-poly(ethylene glycol) polymers, functionalized with bioligands such as RGD, were adsorbed from aqueous solutions onto negatively charged metal oxide surfaces, reducing protein adsorption as well as the adhesion of *S. aureus*, *S. epidermidis*, *S. mutans*, and *P. aeruginosa* to titanium surfaces [130]. The RGD-functionalized thin films selectively allowed cells such as fibroblasts to attach, which makes them useful as coatings for biomedical implants that can mediate the adhesion of host cells to the implant surface, but do not allow bacterial colonization. Zwitterionic poly(sulfobetaine methacrylate) polymer brushes resulted in a more than 90% reduction in the adhesion of viable bacterial cells relative to glass controls [131].

SAMs presenting methyl, L-gulonamide (a sugar alcohol tethered with an amide bond), and triethylene glycol were tested for resistance to *E. coli* biofilm formation

[132]. The triethylene glycol-terminated SAM was the most resistant. PEM coatings on glass, prepared using chitosan and hyaluronic acid, lowered the attachment density of *E. coli* by approximately 80% if the films were sufficiently thick (~300 nm) and hydrated (with low rigidity) [133]. The attachment density did not depend on the terminal layer, and was similar on both $(CS/HA)_{10}$ and $(CS/HA)_{10}CS$ films. However, if the LBL assembly was performed at a lower ionic strength (0.01 *M* NaCl instead of 0.15 *M* NaCl), then thinner coatings were obtained (~120 nm for a $(CS/HA)_{20}$ multilayer film), the surfaces were more rigid, and the bacterial adhesion was greater.

Lichter *et al*. prepared PEM thin films comprised of poly(allylamine hydrochloride) and poly(acrylic acid), with dry a thickness of about 50 nm, and found that the adhesion of viable *S. epidermis*, a Gram-positive bacterium, correlated positively with the stiffness of the polymeric substrates [134]. The elastic moduli of the hydrated films was controlled using the pH-modulation of the extent of ionic crosslinking in the thin films, and ranged over two orders of magnitude (from 0.8 to 80.4 MPa). The colony density was lowest on the most compliant films, with $E \sim 0.8$ MPa; similar trends were observed for Gram-negative *E. coli*. Thus, the mechanical stiffness of biomedical coatings could play an important role in regulating the adhesion and subsequent colonization of viable bacteria.

UV-induced grafting/polymerization of poly(*N*-vinyl-2-pyrrolidone) onto poly(ethylene terephthalate) (PET) surfaces, in an aqueous medium, prevented colonization of the surface by *S. aureus* [135]. The covalent immobilization of silk sericin on poly(methacrylic acid)-functionalized titanium surfaces was found to significantly reduce the adhesion of *S. aureus* and *S. epidermis*, while promoting the adhesion, proliferation, and alkaline phosphatase activity of osteoblasts [136]. The hydrophilic poly(methacrylic acid) lowered bacterial adhesion, while the silk sericin protein enhanced osteoblast attachment and proliferation. Such thin film coatings on titanium surfaces could potentially be used to prevent the bacterial infection of bone implants, while promoting osseointegration.

Puskas *et al*. reviewed the biomedical applications of polyisobutylene-based biomaterials [137]. The arborescent (randomly branched, tree-like) polyisobutylene-polystyrene block copolymers (PIB-PS) surfaces showed a greatly reduced attachment of a common uropathogenic species, *E. coli* 67, compared to medical-grade silicone rubber (SIL-K™). The relatively hydrophobic PIB-PS surfaces resulted in a strong binding of the adsorbed proteins; hence, when the surfaces of both PIB-PS and SIL-K™ were coated with a 29 kDa neutrophil protein, p29, further significant reductions in uropathogen attachment were observed (approximately 90% on PIB-PS and 60% on SIL-K™).

1.3 Coatings for Tissue Engineering Substrates

Polymers such as polystyrene (e.g., TCPS substrates) and PDMS [138] have frequently been used as substrates for cell culture and tissue engineering applications. A variety of thin-film coating strategies have been developed to

impart biocompatibility and biofunctionality to these substrates. The different techniques available for the immobilization of bioactive molecules onto surfaces have been reviewed by Goddard *et al*. [139]. Here, we will highlight polymeric thin films that have been used to control protein adsorption and cell adhesion onto tissue engineering substrates.

1.3.1 PEGylated Thin Films

Poly(ethylene glycol) is used extensively in biomedical surface modification. In a review, Krsko and Libera have discussed strategies for controlling the interaction of cells and proteins with PEG-based coatings [140]. The different methods for attaching PEG molecules to a surface include:

- The use of SAMs of short oligomers.
- The adsorption of triblock copolymers such as poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), known as Pluronic™.
- Surface-grafted PEGylated brushes.
- Thin-film hydrogels obtained by chemical crosslinking techniques that include irradiation with ionizing radiation, such as high-energy electron-beam or gamma rays.

Mrksich and coworkers have reported a strategy for the controlled, irreversible immobilization of adhesion proteins on biologically inert surfaces of PEG-terminated SAMs [141].

Low-friction surfaces for biomedical applications were obtained by functionalizing PDMS surfaces with PEG–DOPA–lysine conjugates [142]. The DOPA (L-3,4-dihydroxyl-L-phenylalanine) and lysine peptide mimics of mussel adhesive proteins resulted in a strong attachment of the PEGylated thin films to PDMS. The resultant surfaces had an extremely low friction coefficient (~0.03) compared to bare PDMS (~0.98), although a lowering of the friction coefficient occurred only when DOPA was bound to lysine. Modification with PEG–DOPA did not have any effect on the friction coefficient. Rather, lysine played a critical role in lowering the friction coefficient.

1.3.2 Zwitterionic Thin Films

Poly(carboxybetaine methacrylate) homopolymer brushes (of 10–15 nm thickness) were prepared using surface-initiated ATRP [143]. The zwitterionic nature of the thin-film resulted in a well-hydrated surface, and prevented any nonspecific adsorption of fibrinogen, lysozyme and human chorionic gonadotropin proteins. The carboxylic acid side groups were also used to selectively immobilize fibronectin, after which the surfaces showed a good adhesion and spreading of aortic endothelial cells. These dual-function polymer brushes could potentially

be used as coatings for tissue engineering substrates and biosensors. The effects of incorporating cationic charges into zwitterionic polymers (based on phosphorylcholine; PC) on biocompatibility, specific cell–surface interactions and cytotoxicity have also been studied [144]. The cationic comonomer, choline methacrylate (CMA), was used to introduce cationic groups into the zwitterionic polymer. While phosphorylcholine $\left[Me_3^+N–CH_2–CH_2–O–P(=O)(O^-)OH\right]$ is a zwitterion, with no net charge, choline $\left[Me_3^+N–CH_2–CH_2–OH\right]$ is cationically charged. In general, although protein- and cell-repellant surfaces are necessary for biocompatibility, the ability to promote cell adhesion and growth is important in the surface modification of biomaterials such as vascular grafts. The cationic surfaces promoted both cell adhesion and growth; indeed, the presence of a cationic charge in the PC-based coatings led to a significant increase in the adsorption of different proteins, as well as in the adhesion of fibroblasts, epithelial cells, granulocytes, and mononuclear cells. The surfaces remained noncytotoxic up to 30 mol% of the cationic moiety. The coating stability in PBS, however, was greatly reduced when the CMA content exceeded 15 mol%, evidently because of an interaction of the ions in PBS with the cationic groups in the coating.

1.3.3
Thin Films of Hyperbranched Polymers

Fluoropolymer surfaces have been functionalized with thin films of a hyperbranched glycopolymer for biocompatibility. The glycopolymer was prepared by the atom transfer radical copolymerization of 2-(2-bromopropionyloxy)ethyl acrylate inimer (initiator + monomer) and a sugar-carrying acrylate, 3-*O*-acryloyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranoside, and was grafted onto the fluorocarbon substrate using low-pressure argon plasma [145]. The hyperbranched glycopolymer thin films were found to promote fibronectin adsorption and human umbilical vein endothelial cell (HUVEC) adhesion, which would make them useful as coatings for tissue engineering substrates. Spin-coated thin films of sorbitol-containing polyesters, which were synthesized via a one-pot, lipase-catalyzed condensation polymerization, were found to elicit fibroblast 3T3 cell behavior similar to that of a biocompatible poly(ε-caprolactone) control [146]. The polymer could be functionalized with biological molecules such as oligopeptides or oligosaccharides by attachment to the hydroxyl groups of sorbitol. Moreover, the lipase-catalyzed polymerization avoided the use of potentially toxic catalysts.

The adhesion and proliferation of human corneal epithelial cells (HCECs) on thin film coatings of hydroxyl-terminated aliphatic polyester dendrons were compared to the interaction of these cells with hydroxyl-terminated PEG SAMs on gold [147]. Whilst little or no HCEC adhesion was observed on the PEG SAMs, the HCEC proliferation was increased exponentially on the dendronized surfaces. The attachment density increased in line with an increase of the generation number of the dendrimer. When the peripheral hydroxyl groups of the dendronized surfaces were further reacted with methoxy-terminated PEG chains, HCEC adhesion

was significantly reduced. Two facts became evident from this study. First, the surface hydrophilicity alone does not confer cell repellency to a surface. The hydroxyl-terminated PEG SAMs and hydroxyl-terminated dendrimers were both hydrophilic and hydrated, but the latter was cell-adhesive and the former cell-repellant. Second, the dendronized surfaces induced the adsorption of adhesive proteins (e.g., fibronectin) that are secreted by the cells, and thus promoted cell adhesion. When the peripheral hydroxyl groups of the dendrimers were PEGylated, however, the cell density was even lower than that of the PEG SAMs. Consequently, the high density of PEG groups at the surface caused the surface to become repulsive to both proteins and cells.

1.3.4 Polyurethane Coatings

By using combinations of five different polyols, six different isocyanates, and nine different chain extenders, a library of 120 polyurethanes has been synthesized and evaluated for cell adhesion, using microarray-based assays [148–150]. The polyurethanes which showed the highest number of adhered primary renal tubular epithelial cells contained 4,4′-methylenebis(phenylisocyante) (MDI) as the diisocyanate, and poly(tetramethylene glycol) (PTMG) as the diol [148]. Clearly, such polymer chemistry will influence factors such as surface wettability, surface roughness, and coating modulus, all of which will in turn affect cell adhesion. Similarly, those polyurethanes that successfully bound immature bone marrow dendritic cells also contained MDI as the diisocyanate, PTMG (250 or 1000 Da) as the diol, and either propyleneglycol, 1,4-butanediol, or no chain extender [149]. The immobilization of dendritic cells, which play a key role in the initiation of immune response by acting as antigen-presenting cells, is important in the development of vaccines, particularly those against tumors. It is important to identify efficient substrates for the immobilization of these cells in their immature state, as maturation can affect their ability to capture antigens by phagocytosis. The polyurethane coatings were also screened for applications such as human skeletal progenitor cell isolation and surface modification of tissue engineering scaffolds aimed at enhancing skeletal cell growth and differentiation. Among 120 polyurethanes analyzed in the present study for their ability to bind to skeletal progenitor cells from human bone marrow, only four exhibited high binding affinities for STRO-1+ cells from human bone marrow. Each of these high-affinity polyurethanes was hydrophilic ($\theta_w \sim 30°$), contained PEG (2000 or 900 Da) as the polyol, contained either MDI or 1,4-phenylene diisocyanate (PDI) as the diisocyanate, and either 1,4-butanediol or no chain extender. Alperin *et al.* used solvent-cast thin films of a biodegradable polyurethane to prepare cardiac grafts for heart tissue regeneration [151]. In myocardial infarction, a macroscopic area of the heart muscle tissue is damaged due to an inadequate supply of blood. As cardiomyocytes are terminally differentiated cells, they are unable to regenerate heart tissue after infarction. Hence, embryonic stem cell-derived cardiomyocytes were seeded onto polyurethane films, and coated with ECM proteins such as laminin or collagen type

IV to promote cell adhesion; the result was an elastomeric film that could respond to contractile forces produced by the cultured cardiomyocytes.

1.3.5 Polysaccharide-Based Thin Films

The presence of specific biomolecules such as chitosan or *O*-carboxymethylchitosan (OCMCS) at the surfaces of tissue-engineering substrates was found to be as important as surface-wettability or charge in influencing cell–surface interactions [152]. The surface-bound free radicals, which were generated by the treatment of a PET surface with argon plasma, were used to grow poly(acrylic acid) (PAA) brushes from the PET surface. Both, CS and OCMCS were covalently immobilized on the PAA brush using a carbodiimide-mediated reaction of the acid groups on the surface with amine groups of CS or OCMCS. The distinctly different morphologies of smooth muscle cells on the CS and OCMCS surfaces were attributed to specific interactions of OCMCS with the cell membrane. The OCMCS-modified surfaces were also found to be protein-repellant, with excellent antithrombogenic properties.

By using an *in vitro* cell culture of human mesenchymal stem cells and *in vivo* subcutaneous implantation into mice, it was found that covalently immobilized collagen coatings clearly improved the cytocompatibility of stainless steel implants [153]. The stainless steel surfaces were first coated with a 200 nm-thick tantalum coating (using magnetron sputtering from a tantalum target in argon atmosphere), followed by a 70 nm-thick tantalum oxide coating (by introducing oxygen during the sputtering process), to improve the corrosion resistance of stainless steel. These tantalum oxide-modified stainless steel surfaces were functionalized with aminopropyl triethoxy silane, and further activated by immersing the surfaces in *N*,*N*′-disulfosuccinimidyl suberate. Collagen molecules were covalently tethered to these activated surfaces, and chemically crosslinked using a carbodiimide chemistry. Such chemical crosslinking greatly improved the resistance against biodegradation and mechanical stability of the bioactive coating. The collagenous layer would also enhance cell adhesion and integration of the biomaterial with the surrounding tissue.

1.3.6 Polyelectrolyte Multilayer Thin Films

Currently, there is an active interest in using multilayer polymer thin films for tissue engineering, which is evident from the number of reports in this area; comprehensive reviews on this topic are available, produced by the groups of Schlenoff and Kotov [154, 155]. Hubbell and coworkers were among the first to investigate the interaction of cells with LBL-assembled PEM thin films [156]. By using human fibroblast cells, which are known to spread aggressively on most surfaces when cultured in serum-containing medium (the medium would thus

contain proteins such as fibronectin to promote cell attachment), Hubbell's group showed that poly(L-lysine)/alginate multilayers could prevent the fibroblasts from spreading. The LBL assemblies were shown to be stable in cell culture medium for over 24 h and to remain attached to the substrate under fluid flow, even at a wall shear rate of $1000\,s^{-1}$. Additional tissue engineering applications of PEM assemblies were proposed by Ogier and coworkers, who investigated the cell adherence, viability, phenotype expression and inflammatory response [via tumor necrosis factor-α (TNF-α) and interleukin (IL)-8 secretion] of human osteoblast-like SaOS-2 cells and human periodontal ligament cells, on multilayer thin films [157–159]. Except for PEI, which was cytotoxic, poly(sodium 4-styrenesulfonate), poly(allylamine hydrochloride), poly(L-glutamic acid) and poly(L-lysine) were all shown to be biocompatible, which suggested that multilayer coatings prepared using these polyelectrolytes would be suitable for implant coatings. Subsequently, Chluba *et al.* immobilized a peptide hormone, α-melanocortin, on $(PGA/PLL)_n$ multilayer assembly by covalently binding the hormone to PLL forming the outer layer [160]. Chluba's group showed the immobilized hormone to be as biologically active as the free hormone. It follows that the covalent immobilization of growth factors on biomaterial surfaces may have important tissue engineering applications [161–165].

Boura *et al.* investigated the possibility of using PEM thin films as coatings to improve the biocompatibility of small-diameter vascular grafts. Specifically, the group determined the ability of these coatings to support and maintain a confluent layer of healthy HUVECs [166]. In these studies, the $(PSS/PAH)_n$ and poly(L-glutamic acid)/poly(D-lysine) $(PGA/PDL)_n$ multilayers were shown to be noncytotoxic, nor to alter the phenotype of the endothelial cells. The PEMs also showed a higher initial cell attachment compared to polyelectrolyte monolayers. Cell growth on these multilayer thin films was similar to that on TCPS. The $(PSS/PAH)_n$ multilayers showed excellent biocompatibility and a greater growth and adhesion of HUVECs than did the $(PGA/PDL)_n$ multilayers.

Mendelsohn *et al.* have reported the influence of processing conditions on the cell-adhesive properties of $(PAH/PAA)_n$ PEM thin films [167]. The degree of ionization of the polyelectrolytes (i.e., the relative number of NH_3^+ versus NH_2 groups for PAH, $pK_a \sim 9$, and the number of COO^- versus COOH groups for PAA, $pK_a \sim 5$), as well as the crosslink density (i.e., the number of ionic bonds, $COO^- \cdots NH_3^+$) was tuned using the deposition pH conditions. When PAH and PAA were both deposited from solution at pH 6.5 (denoted as 6.5/6.5 PAH/PAA), both polymers were fully charged molecules and formed thin, flat layers because of the high ionic crosslink density (cf. Figure 1.3). The film swelled by only ~115% of its original dry height in PBS. In the 7.5/3.5 PAH/PAA multilayers, both PAH and PAA were partially ionized and adsorbed in loop-rich conformations, forming thick layers with a high degree of internal charge pairing. The multilayers did not possess well-blended surfaces, and the chemical groups of the last-deposited polymer dominated the surface. In the 2.0/2.0 PAH/PAA multilayers, both the interior and the surface of the film were enriched by PAA chains, irrespective of the outermost

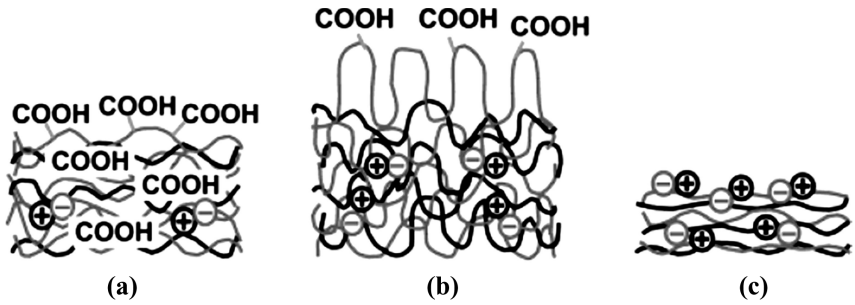

Figure 1.3 Schematics of the (a) 2.0/2.0, (b) 7.5/3.5, and (c) 6.5/6.5 PAH/PAA [poly(allyl amine hydrochloride)/poly(acrylic acid)] multilayer assemblies, shown with PAA as the outermost layer. Reproduced with permission from Ref. [167]; © 2003, American Chemical Society.

layer. The extent of ionic crosslinking was low because most of the PAA groups existed in their uncharged, protonated COOH state. The film swelled by almost 400% of its original thickness.

The PEM thin films were investigated for their *in vitro* interactions with a highly adhesive murine fibroblast cell line. Prior to seeding with the fibroblast cells, which were suspended in normal serum-containing media, the coatings were sterilized with 70% (v/v) ethanol (this sterilization did not affect the mechanical integrity of the films). The 2.0/2.0 PAH/PAA films completely resisted attachment of the NR6WT fibroblast cells, and were not cytotoxic; however, it was found that at least 15 layers were required to create a surface that resisted cell attachment. In contrast, cell attachment was observed on the TCPS control and the 6.5/6.5 and 7.5/3.5 PAH/PAA films. Interestingly, the 6.5/6.5 and 7.5/3.5 PAH/PAA multilayers were always cell-adhesive, irrespective of whether PAH or PAA was the last layer adsorbed; similarly, the 2.0/2.0 multilayers were always cell-resistant, irrespective of the last polyion deposited. Both, the cytophilic 7.5/3.5 and the cytophobic 2.0/2.0 PAH/PAA films, readily adsorbed model proteins from a solution in PBS (Figure 1.4). However, in comparison to an uncoated gold surface, the PEMs showed a lower adsorption of the predominantly anionic protein fibrinogen. All of the PEM coatings adsorbed the highly cationic lysozyme, regardless of their net surface charge. Notably, the cell-resistant 2.0/2.0 PAH/PAA coatings adsorbed more of each protein than did the 7.5/3.5 system. A similar study, on the influence of the type of the outermost layer, the presence of proteins, and the number of layers in the film on cell interactions, was reported earlier by Richert *et al.* [168].

Schneider *et al.* have proposed that $(PLL/PGA)_n$ PEMs grafted with sugar molecules (e.g., mannose) could be used not only as nonviral vectors but also as cell-adhesive substrates in tissue engineering [169]. PGA was selected as the terminal layer in this study because it showed a higher cell viability compared to PLL. Specific interactions were identified of the primary chondrocytes with the glycated thin films, because of which the cells adhered well to these films. On the other

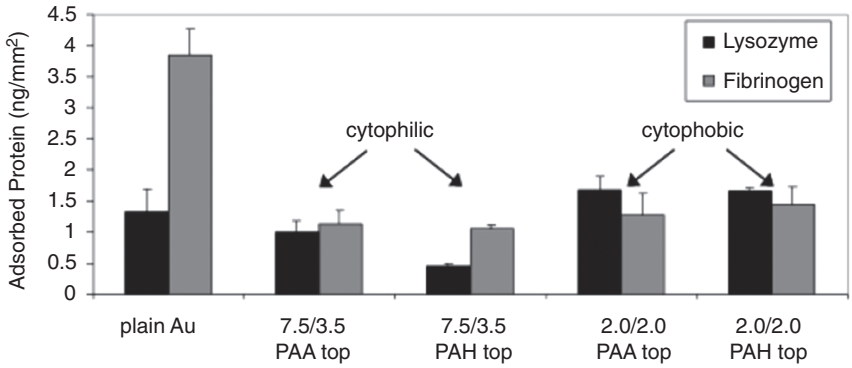

Figure 1.4 Surface plasmon resonance (SPR)-derived adsorption data for lysozyme and fibrinogen on an uncoated gold surface and on gold coated with 10 to 11 layers of the cytophilic 7.5/3.5 or 14 to 15 layers of the cytophobic 2.0/2.0 PAH/PAA multilayer system. Reproduced with permission from Ref. [167]; © 2003, American Chemical Society.

hand, chondrosarcoma cells did not grow well on the mannose-grafted film. Moreover, while cell adhesion was strongly influenced by the mannose, the effect of lactose was much less obvious. The specific interaction of primary chondrocytes with the surface was attributed to the large number of mannose receptor transmembrane proteins present at the cell surfaces. Such preferential adhesion of primary cells to a biomaterial surface, when compared to that of tumor cells, would be an important factor for improving the biocompatibility of implanted prostheses following surgical ablation.

Wittmer *et al.* studied protein adsorption and HUVEC attachment on fibronectin-terminated PEM thin films consisting of PLL and dextran sulfate [170]. It was observed that fibronectin, which enhanced cell adhesion, was adsorbed in an irreversible manner and to a greater extent on the positively charged and less hydrated PLL-terminated films, than on the DS-terminated films. The adsorbed fibronectin subsequently promoted cell spreading. Moreover, positively charged PLL-terminated films showed a greater degree of cell-spreading than with negatively charged DS-terminated films. Fibronectin adsorption on the LBL assembly resulted in a lower film hydration, a higher surface charge, and also enhanced cell spreading on the thin film.

Menu and coworkers used PEM thin films of PSS and PAH to coat the luminal side of cryopreserved human umbilical arteries, in order to promote re-endothelialization, so that the coated arteries could be used as vascular grafts [171]. The internal walls of the de-endothelialized arteries were coated with a $(PAH/PSS)_3PAH$ film by sequential injection of the PAH and PSS solutions, with a 15 min incubation period followed by a 15 min rinse period after each injection. The biomechanical properties of the LBL-coated umbilical arteries were similar to those of fresh arteries. Notably, the PEM coating greatly promoted

endothelialization of the coated surface via differentiation of endothelial progenitor cells into mature endothelial cells. A confluent endothelial cell monolayer was formed within two weeks, while previous fibronectin-coated surfaces required about two months to achieve confluence [172].

Wittmer *et al.* determined the factors affecting the attachment and function of hepatic cells on multilayer nanofilms formed by LBL assembly [173]. The group also studied the role of chemical crosslinking after LBL assembly. Both, biopolymers [e.g., chitosan and alginate (ALG)] and synthetic polymers (e.g., PAH and PSS) were used to prepare the multilayer films. The types of polymer which comprised the multilayer film played an important role in the bioresponse. Although, none of the pure polysaccharide films promoted attachment and growth of the human hepatocellular carcinoma (HepG2) cells, one polysaccharide–polypeptide multilayer, which was composed of PLL and ALG, promoted a strong attachment. Whilst the overall film charge was found to be unimportant in influencing cell behavior, the film terminal layer had a quite strong influence on hepatic cell attachment and growth. Multilayers which terminated with the anionic PSS, in $(PAH/PSS)_n$ thin films, promoted HepG2 attachment and growth. Likewise, a cationic terminal layer, in a PLL/ALG assembly, also resulted in confluent culture of the HepG2 cells. Film rigidity, which was engineered by chemical crosslinking of the layers, was found also to affect cell response; indeed, HepG2 attachment and growth was significantly enhanced by chemical crosslinking (and therefore film rigidity). The $(PAH/PSS)_n$ films, crosslinked $(PLL/ALG)_n$ films, and crosslinked PLL/PGA films with a terminal PLL layer, were each identified as the most promising candidates for *in vivo* human liver tissue engineering applications.

Ren *et al.* observed that the initial adhesion and proliferation of skeletal muscle cells (C2C12 cells), on 1 μm-thick PEM films, and their differentiation into myotubes, depended on the stiffness of the film [174]. The surface elastic moduli, E, were measured using atomic force microscopy (AFM) nanoidentation experiments, and were varied by varying the crosslink density of the films. Stiff films ($E > 320$ kPa) of crosslinked poly(L-lysine)/hyaluronic acid (PLL/HA) multilayers promoted the formation of focal adhesions and enhanced proliferation, whereas soft films were not favorable for cell anchoring, spreading, or proliferation. The crosslinked $(PLL/HA)_n$ films did not require specific protein or ligand precoating to promote cell adhesion. In fact, the crosslinked films were very hydrophilic (water contact angle, $\theta_w < 10°$), and showed a low adsorption (~100 ng cm^{-2}) of proteins from fetal bovine serum (FBS). Interestingly, the un-crosslinked PEM films were moderately hydrophobic and showed a high adsorption of FBS proteins (~2000 ng cm^{-2}).

Sallolum *et al.* studied the effect of surface charge, film thickness, hydrophobicity, and the presence of zwitterionic groups, on the adhesion and spreading of vascular smooth muscle cells on different PEM coatings [175]. Polyelectrolytes such as PAA, poly(methacrylic acid)-*block*-poly(ethylene oxide) (PMA-*b*-PEO), PSS, a perfluorosulfonate ionomer (Nafion™), PAH, poly(diallyldimethylammonium chloride) (PDADMA), poly(2-vinylpyridine)-*block*-poly(ethylene oxide) that was 86% quaternized with methyl iodide (PM2VP-*b*-PEO), and poly(4-vinylpyridine)

that was 45% quaternized with 1*H*,1*H*,2*H*,2*H*-perfluorooctyl iodide (PFPVP), were used. The fluorinated polyelectrolytes were found to promote cell adhesion. In general, hydrophobic polyelectrolyte film surfaces, regardless of their formal charge, were more cytophilic than hydrophilic surfaces. Moreover, the number of multilayers had no effect on cell adhesion and growth. Thin films prepared from a copolymer of acrylic acid and (3-[2-(acrylamido)-ethyldimethyl ammonio]propane sulfonate) (AEDAPS) were used to study the effect of zwitterionic groups. Cell adhesion decreased with an increase in the fraction of AEDAPS in the copolymer. Interestingly, the negatively charged surfaces of (PM2VP-*b*-PEO/PMA-*b*-PEO)$_2$ showed a greater cell spreading ability than the positively charged surfaces of (PM2VP-*b*-PEO/PMA-*b*-PEO)$_2$(PM2VP-*b*-PEO). The images of rat aortic smooth muscle A7r5 cells cultured on the diblock surfaces reported by these authors, however, indicated that more cells had settled on the positively charge surface, as expected. A micropatterning of the cells could be achieved by stamping Nafion™ on the PAA–PAEDAPS copolymer.

Wu *et al*. found that PEMs prepared using HA and poly(allylamine hydrochloride) or collagen (COL), supported neural cell adhesion, neurite elongation, and neural network formation [176]. These films, when deposited onto amino-functionalized glass slides, were found to be cytocompatible with hippocampal and cortical neurons. The hippocampal neurons preferred the (HA/PAH)$_n$ films, while the cortical neurons preferred the (HA/COL)$_n$ films. Neurite outgrowth could not be simply correlated to the terminal layer, and was also found to depend on the number of bilayers. Nadiri *et al*. have reported that bone morphogenetic proteins (BMP) and the BMP antagonist ("Noggin"), which were embedded in poly(L-glutamic acid)/poly(L-lysine) PEM thin films, could be used to induce or inhibit cell death. Such a control on cell apoptosis could find applications in tissue repair, and in the specific "shaping" of artificial organs [177]. PEM thin films have also been prepared using proteins. Haynie *et al*. have reviewed the biomedical applications of polypeptide multilayer films [178].

Moby *et al*. used the LBL technique to coat the luminal surfaces of expanded PTFE tubes with PEM thin films composed of PEI, PSS, and PAH [179]. The PEI(PSS/PAH)$_3$ coatings promoted endothelial cell adhesion and resulted in a healthy confluent cell monolayer formation. The cell viability on the multilayer thin films was greatly improved compared to the nonmodified PTFE surface. The presence of a confluent endothelial layer is necessary for the successful replacement of diseased vessels by synthetic vascular grafts.

He *et al*. have used LBL assemblies of polycations such as PEI or CS, with polyanions such as gelatin or laminin (LN) to coat silicon microelectrode arrays of neural implants [180]. Neural implants are used for the *in vivo* recording of neural activity, or for stimulating neurons with electrical impulses from an external source. The insertion of rigid metal electrodes into soft neural tissue triggers the formation of a scar around the metal electrode, which electrically insulates the electrode from the neurons. Coatings that can prevent scar formation and promote the adhesion of neurons can avoid electrical isolation of the electrode due to scar tissue. *In vitro* experiments showed that the (PEI/LN)$_n$ coatings promoted the

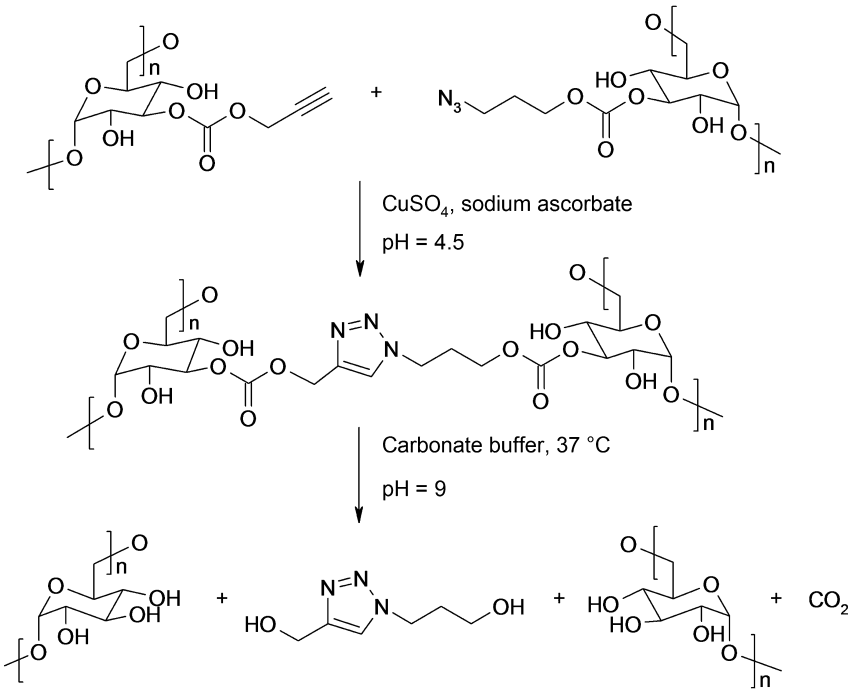

Figure 1.5 1,3-Dipolar click cycloaddition reaction between dextran-propargyl carbonate and dextran-azidopropyl carbonate to from triazole ring linkages between the layers. Hydrolysis of the carbonate esters that linked the triazole ring and dextrose resulted in a disintegration of the multilayered film. Adapted with permission from Ref. [183]; © 2008, Wiley-VCH Verlag.

adhesion and differentiation of chick cortical neurons, without increasing the impedance of the electrodes. Single-walled CNT PEMs have also been proposed as biocompatible platforms for neuroprosthetic implants [181].

Lee *et al.* [182] prepared tissue-engineering scaffolds using inverted colloidal crystals. For this, the internal surfaces of the scaffolds were coated with clay/poly(diallyl dimethylammonium chloride) LBL multilayers to enhance cell adhesion. Cocultures of adherent and nonadherent cells were obtained using these scaffolds, which were fabricated with the goal of an *in vitro* replication of the differentiation microenvironments, or niches, of hematopoietic stem cells.

De Geest *et al.* have prepared polyelectrolyte-free, polymeric multilayer films containing alkyne- and azide-functionalized dextrans using the LBL assembly technique [183]. The interlayer crosslinking was achieved via the triazole linkages formed by the Huisgen 1,3-dipolar cycloaddition reaction between the alkyne and azide groups (Figure 1.5). The coatings were biodegradable as a result of the hydrolysis of carbonate ester links present in the polymers. Such biodegradable

multilayers, which do not use potentially cytotoxic polyelectrolytes, have shown promise in tissue engineering and drug delivery applications.

1.3.7 Temperature-Responsive Polymer Coatings

Thin films and coatings of temperature-responsive polymers, such as poly(*N*-isopropylacrylamide) (PNIPAAm), have been widely investigated in the area of cell sheet engineering [184]. This process, which involves tissue reconstruction from cell sheets rather than from single cells, was developed to overcome the limitations of tissue reconstruction using biodegradable scaffolds or by the injection of cell suspensions (Figure 1.6) [185–187]. In this approach, temperature-responsive polymers are covalently grafted onto tissue culture dishes, which allows various types of cell to adhere and proliferate at a temperature above the lower critical solution temperature (LCST) of the grafted polymer (a state wherein the surface is hydrophobic) [188]. The cells detach spontaneously when the temperature is lowered below the LCST, because of spontaneous hydration of the grafted polymer chains [189]. Cell detachment from the thermally responsive surfaces was a result of active cellular metabolic processes triggered by surface-wettability changes [190, 191]. A covalently grafted layer of PNIPAAm of about 20 nm thickness allows thermally responsive cell adhesion and detachment [192]. The confluent cells can be harvested noninvasively as single, contiguous cell sheets with intact cell–cell junctions

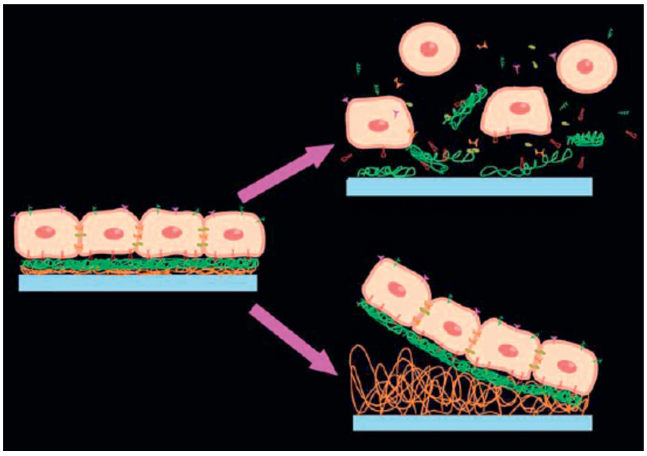

Figure 1.6 Cell sheet harvesting. Trypsin degrades the deposited extracellular matrix (ECM; green), as well as the membrane proteins, so that confluent, monolayer cells are harvested as single cells (upper right). The temperature-responsive polymer (orange), covalently immobilized on the dish surface, hydrates when the temperature is reduced; this decreases the interaction with the deposited ECM. All the cells connected via cell–cell junction proteins are harvested as a single, contiguous cell sheet, without the need for proteolytic enzymes (lower right). Reproduced with permission from Ref. [185]; © 2004, Elsevier.

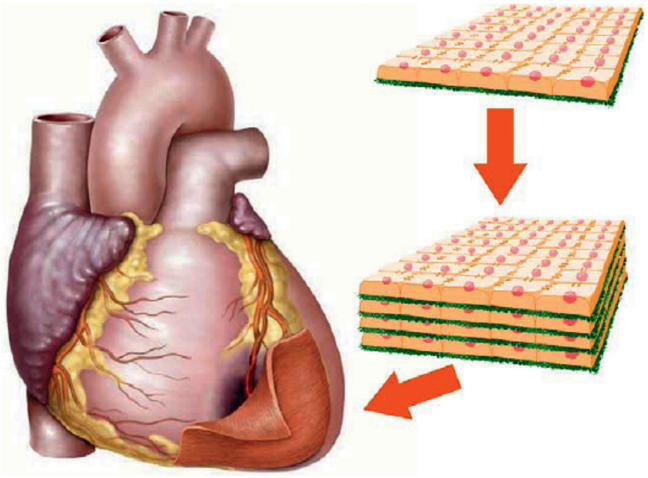

Figure 1.7 Cardia patch cell sheet engineering. Cardia myocyte sheets are harvested from temperature-responsive culture dishes. Four cell sheets are then stratified and transplanted to ischemic hearts as cardia patches. Reproduced with permission from Ref. [185]; © 2004, Elsevier.

and a deposited ECM. The low-temperature liftoff from PNIPAAm surfaces is less damaging to the ECM proteins than enzymatic digestion and mechanical dissociation methods of cell harvesting [193]. A cell sheet composed of different types of cell (patterned cocultured cell sheets) can be obtained by patterning the substrate with thermoresponsive polymers with different LCSTs [194]. Double-layered cell sheets can also be engineered in this way [195]. Corneal epithelial cell sheets and retina pigment epithelial cell sheets for ocular surface regeneration [196–199], periodontal ligament cell sheets for regenerating the connective tissue that attaches a tooth to the alveolar bone, urothelial cell sheets for bladder augmentation, and cardiomyocyte sheets for engineering electrically communicative, pulsatile, three-dimensional (3-D) cardiac constructs (Figure 1.7) [200–202], have each been obtained using cell sheet engineering.

Patterned surfaces have been used to produce heterotypic cell cocultures by covalently grafting PNIPAAm onto the tissue culture surfaces [203, 204]. PNIPAAm brushes were grown from TCPS surfaces by polymerization of the *N*-isopropylacrylamide (NIPAAm) monomer upon exposure to an electron beam through a patterned metal mask. The patterned surfaces were used for the culture of hepatocytes. When the temperature was reduced below 37 °C, hepatocytes became selectively detached from the PNIPAAm regions of the patterned substrate and were replaced by endothelial cells that were seeded and cocultured (with the remaining hepatocytes) at 37 °C.

Okajima and coworkers reported the details of a thin film coating that could regulate cell adhesion by controlling the potassium ion concentration in the cell

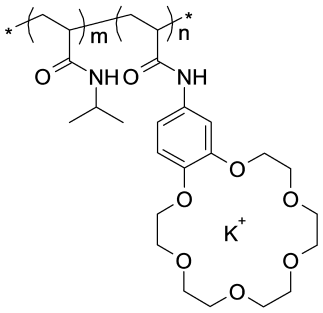

Figure 1.8 Structure of the NIPAAm-*co*-BCAAm polymer. Upon capturing a potassium ion, the copolymer of the thermoresponsive polymer NIPAAm and the crown ether polymer BCAAm swells and releases adhered cells. Adapted with permission from Ref. [206]; © 2005, American Chemical Society.

culture medium [205]. For this, polyethylene films were grafted with a copolymer of NIPAAm and benzo-18-crown-6-acrylamide (BCAAm) (see Figure 1.8), using radical copolymerization of the monomers on argon plasma-treated polyethylene substrates. In a cell culture medium, a complex formation of the pendant crown ether of the grafted polymer with potassium ions in the medium caused an increase in the LCST of the copolymer, and a switch to a more wettable hydrophilic state at 37 °C. This allowed the cell sheets to be detached from the substrate, without using proteolytic enzymes or changing the cell culture temperature. In this way, Okajima and colleagues were able to develop a substrate that could sense and selectively release dead cells in the culture [206]. Living cells concentrate potassium ions internally, and release them when they die; the polymer thin film coating was able to detect, locally, any potassium ions released from the dead cells, which were then selectively removed.

Cell-adhesive RGDS (Arg-Gly-Asp-Ser) peptides were immobilized on a temperature-responsive poly(*N*-isopropylacrylamide-*co*-2-carboxyisopropylacrylamide) copolymer grafted onto TCPS dishes [207, 208]. These surfaces facilitated both the adhesion and spreading of HUVECs and bovine aortic endothelial cells at 37 °C. The spread cells were seen to detach spontaneously from the surfaces when the temperature was lowered below the LCST of the polymer. In this way, the binding of cell integrin receptors located on cell membranes to immobilized RGDS located on cell culture substrates could be reversed simply by using a mild temperature stimulus, without enzymatic or chemical treatments. As these surfaces can be used to culture cells under serum-free conditions, they would be suited to applications where the use of animal-derived materials (e.g., serum) may not be desirable for reasons of cost and/or safety.

1.3.8 Electroactive Thin Films

Yeo and Mrksich prepared cell culture substrates that could be triggered to release tethered ligands by the oxidation or reduction of electroactive linkage groups [209]. The surface immobilization of ligands was carried out on two types of monolayer. The first type consisted of a maleimide group tethered to an electroactive quinone

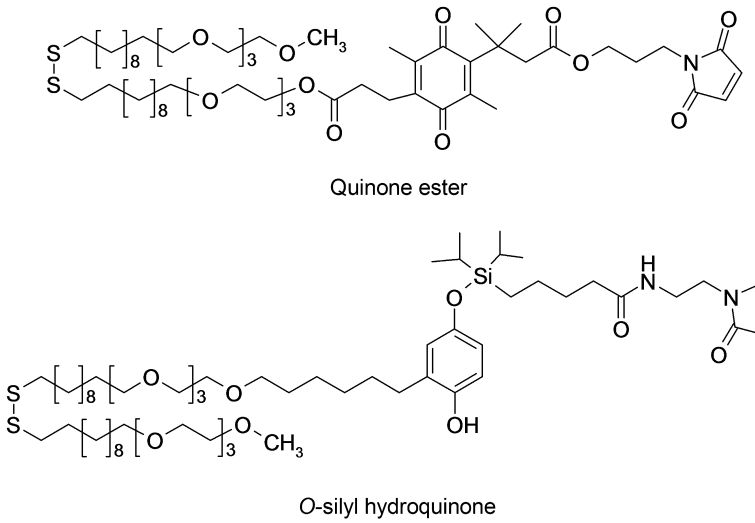

Figure 1.9 Redox molecules for preparing self-assembled monolayer (SAM) coatings for cell sheet engineering. Adapted with permission from Ref. [209]; © 2006, American Chemical Society.

ester, while the second type consisted of a maleimide group tethered to an electroactive *O*-silyl hydroquinone moiety (Figure 1.9). An RGD-containing peptide (CGRGDS) was immobilized on the monolayer surfaces by reaction of the maleimide groups with the terminal cysteine residue of the peptide. In the former type of monolayer, the electrochemical reduction of the quinone to hydroquinone was followed by a cyclization reaction, to give a lactone and release the RGD ligand. In the latter type of monolayer, the electrochemical oxidation of *O*-silyl hydroquinone to benzoquinone resulted in a hydrolysis of the silyl ether and the selective release of RGD ligands. Swiss 3T3 fibroblast cells that had adhered to the RGD-presenting monolayers could be electrically triggered to release from the surface by applying an electrical potential to the monolayer. The same group also demonstrated that such electrochemical strategies could be used to release cells from surfaces in a selective and noninvasive manner, and may also be useful in directing stem cell differentiation, maturation, and function. The applications of other stimulus-responsive surfaces in areas such as biofouling, cell culture, tissue engineering and regenerative medicine have been reviewed recently by Mendes [210].

1.3.9
Other Functional Polymer Coatings

Fluoroalkyl groups, with a relatively low surface energy, can be used to produce a surface enrichment of bioactive molecules in a coating [211, 212]. Santerre and

coworkers have used bioactive fluorinated surface modifiers to deliver vitamin E antioxidants [213] and cell-adhesive RGD peptides [214] to the surfaces of polycarbonate polyurethanes. Poly(trivinyltrimethylcyclotrisiloxane) thin films synthesized by iCVD were seen to show promise as electrical insulating coatings for neural implants (and as an alternative to the currently used Parylene-C coatings) on the basis of their high resistivity, hydrolytic stability, pin-hole-free smooth surface morphology, and biocompatibility [215].

Karp *et al.* found that spin-coated thin films of poly(DL-lactide-*co*-glycolide) (PLGA) of <100 nm thickness supported the formation of a bone matrix when seeded with rat bone marrow cells in an α-minimal essential medium. A confluent 0.5 μm-thick cement line, comprising a collagen-free layer of calcium hydroxyapatite, was formed on the PLGA surface despite the fact that the acidic products formed by the degradation of PLGA would be expected to dissolve calcium hydroxyapatite. The cement line served as a scaffold for the assembly of mineralized collagen, and would (potentially) connect new bone to the old bone surface in bone implants [216].

Biologically active dopants, such as growth factors, have been used in conductive polymer coatings. When nerve growth factor (NGF) was incorporated into electrochemically deposited polypyrrole and poly(3,4-ethylene dioxythiophene) thin films, the PC-12 cells adhered to the NGF-modified substrates and extended neurites [217]. Subsequently, NGF was shown to increase the conductivity and lower the impedance of the conducting polymer films, which can be used as coatings for electrodes that interface with neurons.

Li *et al.* prepared carboxylic acid gradients on the surfaces of PET films, and found that neurite outgrowths could be guided by the chemical gradient on the surface [218]. The exposure of PET surfaces to UV light resulted in the formation of surface peroxides that could be used to polymerize surface-located acrylic acid. The gradients were created by subjecting different areas of the substrate to different durations of UV exposure; neurite growth was shown to occur preferentially along the direction of decreasing –COOH density. Jhaveri *et al.* prepared 300 μm-thick hydrogel coatings on the surfaces of neural implants via the photopolymerization of lysine-conjugated 2-hydroxyethyl methacrylate and an ethylene glycol dimethacrylate crosslinker [219]. The coating was used to encapsulate and supply nerve growth factor (NGF) to the dorsal root ganglion (DRG) neurons in cell-culture experiments. By comparison with bath-applied NGF, a controlled release of NGF from the hydrogel coatings resulted in significantly longer neuronal processes.

Conformal coatings of hydrogel microparticles have been used to attenuate not only biofouling, leukocyte adhesion and activation, but also adverse host responses in biomedical and biotechnological applications [220]. Bridges *et al.* prepared thin films of poly(*N*-isopropyl acrylamide) hydrogel microparticles crosslinked with ethylene glycol diacrylate by using a spin-coating process. These particles were then grafted covalently, by exposure to UV light, onto aminobenzophenone-tethered poly(ethylene terephthalate) surfaces in order to obtain biocompatible coatings [221].

1.3.10
Multilayer Thin Films for Cell Encapsulation

Krol *et al*. created conformal coatings on individual human pancreatic islets using a polyelectrolyte LBL assembly [222]. In the islet transplantation approach for the treatment of diabetes, the islets must be encapsulated in semipermeable microcapsules so as to protect the donor cells from the host immune system, while allowing the transport of glucose, insulin, and other nutrients. Polyelectrolyte multilayer coatings seem to show promise in achieving these goals. Alternatively, the islet of Langerhans cells were deposited with multilayers of cationic PAH or poly(diallyldimethyl ammonium chloride), and anionic PSS. The surface charge of the islets was used as a binding site for the polyions, while the functionality of the encapsulated islets and permeability of the capsules were characterized by determining insulin release at different glucose levels. The coated islets accounted for about 40% of the insulin release by uncoated islets, under stimulation with a low concentration (3.3 *M*) of glucose. Both, the polymer nature and the molecular weight played important roles in the release behavior of the coated islets. Wilson *et al*. achieved pancreatic islet microencapsulation by an LBL assembly of PLL with biotinylated PEG side chains (PPB), and streptavidin (SA) (Figure 1.10) [223]. By

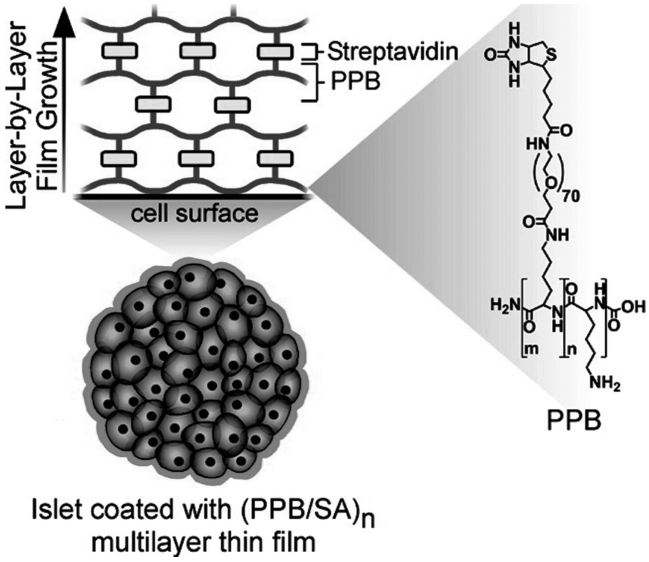

Figure 1.10 Nanothin conformal islet coatings via layer-by-layer (LBL) deposition of poly(L-lysine) with biotinylated PEG grafts (PPB), and streptavidin (SA). The PPB interacts electrostatically with negatively charged cell surfaces, facilitating the binding of SA. Unoccupied biotin binding sites of immobilized SA allow a second layer of PPB to be added, thereby enabling the incorporation of a second SA layer. This process may be repeated to generate thin films assembled via alternating deposition of PPB and SA. Reproduced with permission from Ref. [223]; © 2008, American Chemical Society.

controlling the extent of grafting with biotin–PEG, it was possible to avoid polycation-mediated cell death. The islets could be coated with $(PPB/SA)_8$ multilayer films without any loss of viability or function, and the coated islets also performed comparably to untreated controls *in vivo*. Pancreatic islets have also been encapsulated in photopolymerized PEG diacrylate hydrogel coatings using a novel apparatus [224].

1.3.11 Patterned Thin Films

Cell–biomaterial interactions in tissue engineering are influenced not only by topographical features comparable to cell size (1–100 μm) [225], but also by the nanoscale details of the biomaterial surface [226–228]. Surfaces can provide physical and chemical guidance to growth and alignment of cells such as neurons, Schwann cells, epithelial cells, and bone-derived cells [229]. The "contact guidance" or mechanical cues provided by grooves on a polymer surface – and also the chemical cues offered by micropatterns of molecules that promote or prevent cell adhesion – have attracted great interest in biomedical engineering.

Cheng *et al.* used a microheater array to pattern single or multiple types of cell onto plasma-polymerized NIPAAm thermoresponsive coatings [230]. Site-specific cell adhesion was achieved by temperature-controlled polymer conformation and surface wettability. By using a nanoembossing technique, Mills *et al.* prepared patterned poly(lactic acid) (PLA) structures, with dimensions much smaller than the size of an individual cell [231, 232]. Free-standing films of topographically patterned PLA were obtained by embossing a PLA film with microstructured or nanostructured silicon master patterns; feature dimensions ranging from tens of micrometers down to hundreds of nanometers, covering areas up to 1 cm^2, could be produced using this method (Figure 1.11). Such surfaces could, in principle, be used to examine the effects of local interactions of surface topography with cell surfaces. The optical clarity of the embossed films depended mainly on the surface roughness, which could be significantly decreased simply by sandwiching the film against an unstructured silicon master. Optically transparent patterned films are useful in studies employing optical microscopy. For example, Fernandez *et al.* produced optically transparent nanostructured chitosan thin sheets using soft lithography [233]. The technique here consisted of forming a film of chitosan on a topographically patterned silicon master, and obtaining a free-standing film by peeling off the coating from the silicon mold, after having evaporated off the solvent. This step was facilitated by a surface modification of the mold with a nonadhesive silane. One problem with this approach was the poor mechanical stability of the topographically patterned polymer film, when the polymer was not elastomeric in nature.

Feinberg *et al.* have fabricated microtopographies in PDMS elastomers by using micromachined silicon wafers having patterned geometries [227]. In order to form these microtopographies, the PDMS films were cast and cured on the patterned silicon substrates. The adhesion and growth of porcine vascular endothelial cells

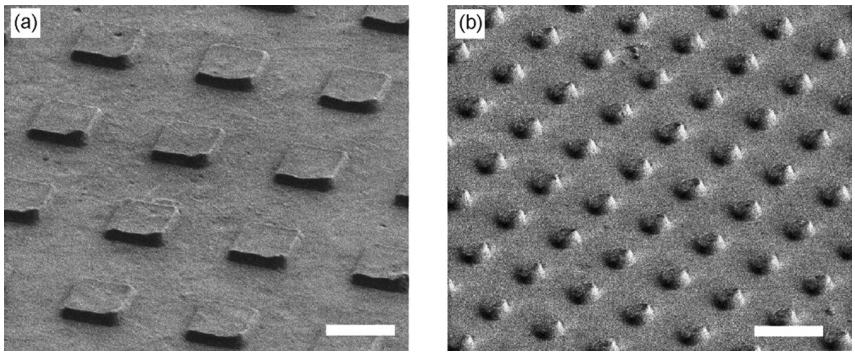

Figure 1.11 Scanning electron microscopy images of: (a) 25 μm^2, 500 nm-tall square posts with a 10 μm period; and (b) 25 μm-diameter, 500 nm-tall round posts with a 5 μm period nanoimprinted into a freestanding sheet of poly(lactic acid). Reproduced with permission from Ref. [231]; © 2005, Wiley Periodicals, Inc.

(ECs) on the topographically patterned surfaces were affected by the interaction of factors such as surface chemistry (i.e., whether or not the PDMS surface was treated with a radiofrequency glow discharge argon plasma to make the surface hydrophilic, and whether or not the surfaces were coated with the protein fibronectin that promotes cell adhesion), the elastic modulus of the coating, and the topography (i.e., the height and spacing of the microridges at the surface).

One interesting application of micropatterned polymer films in tissue engineering is the use of a biodegradable thin and thick film scaffolds prepared from a blend of poly(L-lactic acid-*co*-glycolic acid) and poly(hydroxybutyrate-*co*-hydroxyvaleric acid) [229]. The films were prepared by casting a solution of the polymer blend onto a micropatterned silicon template that incorporated 21 μm- and 42 μm-wide grooves on the surfaces (with 20 μm ridge width and depth). The polymer film was detached from the template by immersing the coated template in distilled water, after which the topographically patterned film was seeded with photoreceptor cells. The effects of not only physical constraint (i.e., the grooves) but also the surface chemistry (chemical cues from laminin, a noncollagenous adhesive glycoprotein for neuronal cells) on cell adhesion, survival, and alignment of the photoreceptor cells were investigated. The rod and cone photoreceptor cells showed a clear preference for grooves on the surface rather than ridges, which highlighted the possibility of reconstituting rod–cone mosaics through the use of patterned scaffold surfaces. Such micropatterned thick/thin film scaffolds have the potential to deliver photoreceptor cells to the subretinal space of patients with blinding retinal diseases.

Shi and coworkers prepared biodegradable, topographically patterned thin films with unidirectional grooves that were, for example, 150 nm high and 1 μm wide, using a holographic diffraction grating as templates. For the nonphotolithographic approach, grooves with a sinusoidal cross-section were created by casting a

solution of PLA onto the template, followed by peeling off the dried film from the template [234, 235]. The neurites of cells from chick sympathetic ganglia were found to align parallel to the grooves, and to be longer on patterned films than on unpatterned controls. Such well-controlled contact guidance of neurites has practical applications in nerve regeneration and reconnection, for the treatment of nerve injury. Libera and coworkers have described the fabrication and use of sub-micron-sized cell-repulsive PEG hydrogels patterned on an otherwise cell-adhesive substrate, to enable (in selective fashion) the growth of neurons and neuronal processes, but to repel astrocytes [236]. The approach of Libera *et al*. was based on differences in the shapes and sizes of the two cells. The *axons* are high aspect-ratio neuronal processes with diameters on the order of 1 μm and lengths exceeding centimeters, whereas the star-shaped *astrocytic glial cells* are substantially larger than 1 μm in size. When the hydrogel patterns were sufficiently closely spaced, the neurites could grow on the adhesive surface between the hydrogels, whilst the astrocytes were unable to adhere. One potential application of this concept might be to engineer an implantable nerve-guidance device that would selectively enable regrowing axons to bridge a spinal cord injury, without interference from the glial scar.

1.4 Polymer Thin Films for Drug Delivery

Leugen *et al*. have discussed several examples where polyelectrolyte LBL assemblies that are functionalized by embedded proteins, peptides or drugs, could control cell activation or act as local drug delivery systems [237]. Among the early reports on the pH-dependent deconstruction of PEM thin films for controlled release application are those of Hammond and coworkers [238, 239]. Here, hydrolytically degradable LBL thin films were prepared using a degradable poly(β-amino ester) as the cationic polymer, and a series of model therapeutic polysaccharides (e.g., heparin, low-molecular-weight heparin, chondroitin sulfate) that contain a large number of anionic sulfate groups. These degradable multilayer films were capable of both parallel and serial release of multiagents [239]. “Barrier” layers consisting of covalently crosslinked PEMs were used to block the interlayer diffusion of the model drugs. Dextran sulfate (a diffusing polyelectrolyte) and heparin (a nondiffusing polyelectrolyte) were used as model macromolecular drugs (Figure 1.12). This classification, as “diffusing” and “nondiffusing”, was based on the interlayer diffusion characteristics of the polyelectrolytes in the PEM assemblies. Diffusing polysaccharides (e.g., many polypeptides and polysaccharides) rapidly diffuse throughout LBL architectures during assembly, and this results in poorly organized, blended structures. In contrast, nondiffusing polyelectrolytes (e.g., most synthetic, strong polyelectrolytes) do not diffuse across layers [239]. It was found that a covalently crosslinked barrier layer composed of nondiffusing polyelectrolytes would prevent the interlayer diffusion of model drugs, and could be used to tailor, in precise fashion, the sequential release of these drugs.

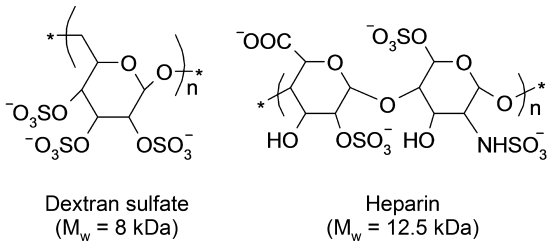

Figure 1.12 Chemical structures of dextran sulfate (a diffusing polyelectrolyte) and heparin (a nondiffusing polyelectrolyte) [239].

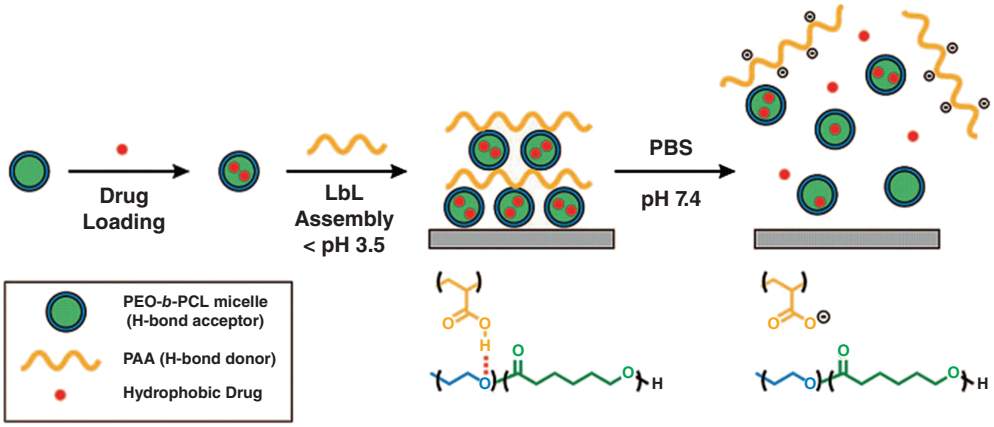

Figure 1.13 Schematic representation of the hydrogen-bonding LBL assembly of block copolymer micelles for hydrophobic drug delivery vehicles from surfaces. Reproduced with permission from Ref. [241]; © 2008, American Chemical Society.

Protein delivery from hydrolytically degradable and biocompatible LBL films has also been investigated by the Hammond research group [240]. The embedded protein molecules were found to retain 100% functionality after release from the multilayer thin films, showing that the processing conditions in LBL assembly were sufficiently gentle to avoid protein denaturation. Here, micelle-containing LBL films were prepared by the integration of biodegradable block copolymer micelles as nanosized carriers for hydrophobic drugs within LBL films, using hydrogen-bonding interactions as the driving force for assembly [241, 242] (Figure 1.13). For this, PAA was the H-bond donor, while biodegradable poly(ethylene oxide)-*block*-poly(ε-caprolactone) (PEO-*b*-PCL) was the H-bond acceptor. In this way, free-standing 3.1 μm-thick micelle LBL films of $(PEO\text{-}b\text{-}PCL/PAA)_{60}$ were isolated. This approach is useful for the surface delivery of hydrophobic and neutral drugs, which are difficult to encapsulate directly in PEMs. As an example, when the hydrophobic antibacterial drug, triclosan, was loaded into the micelles,

the drug-loaded LBL film was found to release significant amounts of triclosan to inhibit the growth of *S. aureus*. Notably, the thermal crosslinking of PAA retarded drug release to the surrounding medium, enabling a sustained release which persisted for several days.

Addison *et al.* have demonstrated that stimulus-responsive block copolymer micelles can be used as triggerable delivery systems when incorporated within multilayer films deposited on polystyrene latex particles [243]. This approach of using core–shell architectures for the encapsulation and release of actives was pioneered by Caruso and others [244]. Cationic, pH-responsive micelles of poly[2-(dimethylamino)ethyl methacrylate-*block*-poly(2-(diethylamino)ethyl methacrylate)] (PDMA-*b*-PDEA) micelles and anionic poly(sodium 4-styrene sulfonate) polymer were deposited on the surface of anionic polystyrene latex particles using the LBL technique. The block copolymer micelles can be loaded with bioactive molecules such as a hydrophobic small-molecule drug. The block copolymer micelles were found to retain their micelle structure at pH 9.3, with very little release of the hydrophobic actives; however, at pH 4 the micelles underwent a transition to a polymer brush-like structure, resulting in a rapid release of the active agents.

Erel-Unal and Sukhishvili have reported the construction of hydrogen-bonded hybrid polymer multilayers comprising of poly(*N*-vinylcaprolactam) (PVCL)/poly(L-aspartic acid) (PLAA) bilayers with a critical disintegration pH of ~3.3, and poly(*N*-vinylcaprolactam) (PVCL)/tannic acid (TA) bilayers with a critical disintegration pH of 9.5 [245]. These authors have proposed that such biodegradable thin films could be used for the pH-responsive release of active molecules for future biomedical applications. PEMs have also been used for microencapsulation in drug delivery. For example, de Geest *et al.* coated dextran-based hydrogels with $(PSS/PAH)_n$ PEM thin films, using the LBL technique, to obtain pH-responsive self-rupturing microcapsules for both protein and drug delivery [246].

Schneider *et al.* investigated the release of model drugs – sodium diclofenac (an anti-inflammatory drug) and paclitaxel (an anticancer drug) – from covalently crosslinked CS/HA and $(PLL/HA)_n$ PEM thin films. When both crosslinked and uncrosslinked PEMs were compared, the crosslinked films were found to have the desired combination of properties – namely, mechanical resistance, biodegradability, and bioactivity. Paclitaxel for example, was found to remain active when loaded in crosslinked $(PLL/HA)_n$ films, and this led to a dramatic decrease in human colonic adenocarcinoma cell viability over a three-day period.

Chen *et al.* prepared a drug-eluting coronary stent to treat coronary arterial stenosis by coating the stent with LBL assemblies of collagen and sirolimus, an immunosuppressant drug. The collagen layers were chemically crosslinked using genipin, a naturally occurring crosslinking agent, so as to control the sirolimus release rate [247]. During use, a balloon expansion of the coated stent could be achieved without causing the coatings to crack or peel away from the stent wire.

Jewell *et al.* have reported that, by conjugating cationic protein transduction domains to therapeutic proteins, the extent to which the proteins were internalized by the cells could be increased [248]. Most likely, LBL assemblies of therapeutic

functionalized proteins with PSS would allow both spatial and temporal control over the delivery of proteins to the cells and tissues.

Currently, CVD represents a convenient, single-step synthesis of high-quality polymer thin films on a variety of substrates, including microparticles and nanoparticles [249, 250]. For example, Lau *et al*. used iCVD to synthesize methacrylic acid copolymer thin film coatings for encapsulating drug microcrystals (<100 μm) for controlled release in the gastrointestinal tract. The thin film coatings showed an abrupt transition in swelling (from 5% to 30%) when the pH rose from 5 to 6.5 that would allow the drug to be protected while in the acidic environment of the stomach, but released on entering the more alkaline small intestine.

Thermoplastic elastomers, such as polystyrene-*block*-polyisobutylene-*block*-polystyrene, are easy to process and relatively stable in biological environments. This renders them attractive as a materials for the construction of, or the coating of, biomedical devices and implants. Recently, Ranade *et al*. have discussed the possible use of a polystyrene-*block*-polyisobutylene-*block*-polystyrene copolymer as a matrix for paclitaxel delivery from Boston Scientific's TAXUS™ coronary stent [251].

1.5 Polymer Thin Films for Gene Delivery

Polymer thin films that support cell adhesion and incorporate plasmid DNA for sustained release are of great interest in gene therapy and tissue engineering [252–256]. Among the several approaches available for gene delivery, DNA–polycation multilayer thin films have shown much promise as nonviral vectors for localized and sustained transfection, both *in vitro* and *in vivo*. The incorporation of DNA into multilayered films was first reported in 1993 by Lvov, Decher and Sukhorukov [257]. Lynn and coworkers have demonstrated this approach using a LBL assembly of a poly(β-amino ester) and a plasmid DNA encoding for enhanced green fluorescent protein (EGFP) [252–255]. The release of DNA occurred by hydrolytic degradation of the poly(β-amino ester) matrix. COS-7 line fibroblast cells began to express the protein after contacting these 100 nm-thick multilayer films. The experimentally observed increase in EGFP expression was consistent with the decrease in average film thickness, because of release of the embedded plasmid DNA, and correlated well with the DNA release profile. A sustained release over a period of about 31 h could be achieved in this way, although the majority of the plasmid was released from the film during the first 16 h of incubation. Approximately 19 ± 8% of the total number of cells expressed the protein after 48 h of contact with coated slides. The rate of DNA release could be controlled by tailoring the hydrophobicity of the poly(β-amino ester) (Figure 1.14a) [255]. In a similar approach, Lu *et al*. have discussed controlled release of DNA using LBL-assembled multilayer thin films composed of a cationic polymer poly(2-aminoethyl propylene phosphate) (PPE-EA) [258] and plasmid DNA [259]. The biodegradable

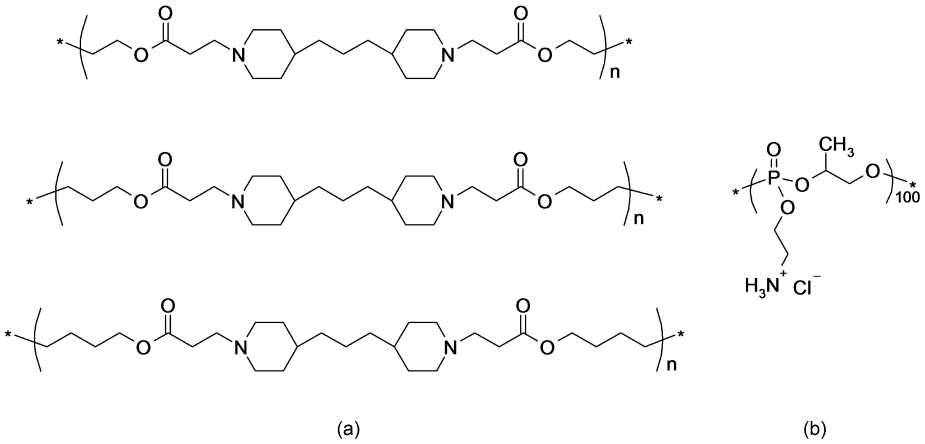

Figure 1.14 Biodegradable polymers for preparing DNA–polycation multilayer thin films. (a) Poly(β-amino ester)s with different hydrophobicities [255]; (b) Poly(2-aminoethyl propylene phosphate) [258, 259].

polyphosphoester (see Figure 1.14b) degraded upon incubation in PBS, and provided a local and sustained delivery of bioactive plasmid DNA for up to two months. The multilayer thin films were found to be cytocompatible with osteoblast cells, and a sustained expression of GFP by the cells was detected for up to 20 days. About 47% of the cells were transfected after 10 days of contact with multilayer films with PPE-EA as the terminal layer. The polyphosphoester has a greater hydrolytic stability than poly(β-amino ester)s, and may be more suitable for long-term plasmid delivery in gene-induced tissue-engineering applications.

In a different approach towards preparing degradable PEM films, Lynn and coworkers have reported a new class of ester-functionalized "charge-shifting" polyamines [260]. Here, PAH was treated with an excess of methyl acrylate to obtain a cationic polymer with "charge-shifting" ester side chains (Figure 1.15). DNA-containing polyelectrolyte multilayers were prepared using this polymer. A gradual hydrolysis of the ester-functionalized side chains introduced carboxylate groups and reduced the net charge of the polymer, and resulted in film erosion and release of the entrapped DNA. The rate of release could be controlled by varying the degree of substitution of the amine groups with the methyl acrylate side chains.

Charge-shifting polymers were used to fabricate PEM films that were stable at neutral pH, but eroded over a period of several days at pH ~5 [261]. The addition of citraconic anhydride to poly(allyl amine) resulted in an anionic carboxylate-functionalized polymer that readily converted to the cationic poly(allyl amine) in an acidic environment. It was proposed that this approach could lead to a significant expansion of the range of different cationic agents (e.g., cationic proteins,

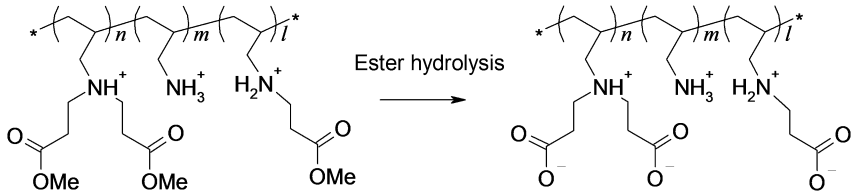

Figure 1.15 "Charge-shifting" polyamines used for preparing multilayer thin films incorporating DNA, for controlled release. Adapted with permission from Ref. [260]; © 2008, Wiley-VCH Verlag.

peptides, polymers, nanoparticles) that can be released or delivered from surfaces using PEMs.

Zhang *et al.* have investigated the transfection ability and intracellular DNA pathway of gene-delivery systems based on $(PLL/HA)_n$ thin films [262]. For this, plasmid DNA was complexed with PLL, β-cyclodextrin (CD) or β-cyclodextrin-grafted PLL (PLL-CD), in solution. Subsequently, $(PLL/HA)_5$ coatings were incubated with the plasmid DNA complexes for 90 min, followed by the assembly of another multilayer film of $(PLL/HA)_5$. When the films had been dried and sterilized by exposure to UV light, the transfection efficiency was found to be higher when the DNA complexes were delivered from the multilayer system than from solution. This higher efficiency was achieved because of an efficient internalization in the cytoplasm (through a nonendocytic pathway) and, subsequently, in the nuclei of the transfected cells. In contrast, internalization in solution was via endocytosis, when the complexes were trapped in endosomes and lysosomes. Degradation in the lysosomes resulted in a lower transfection efficiency.

Wang *et al.* demonstrated the release of DNA from an LBL-assembled thin film of DNA with an inorganic compound that had a relatively low binding affinity for DNA [263]. Zr^{4+}/DNA multilayer films were prepared through the LBL assembly of zirconyl chloride octahydrate ($ZrOCl_2 \cdot 8H_2O$) and sodium salt of fish sperm DNA. The addition of a chelator (e.g., sodium citrate) cleaved the electrostatic/coordinate covalent linkages between Zr^{4+} and the phosphate groups of the DNA, and this resulted in disintegration of the film and a release of DNA. The ability of the chelators to disassemble the multilayer film varied, in general, as sodium citrate > sodium tartarate > sodium fluoride > sodium acetate. About 97% of the $(Zr^{4+}/DNA)_{11}$ film was released within 6 h in a sodium citrate solution (5 m*M*), compared to 86% in sodium tartarate and 70% in sodium fluoride, for the same duration. Only about 17% of the film was released from the surface in 15 h when sodium acetate was used.

Sakai *et al.* coated electrospun fibrous mats of PLA with LBL thin films of PEI/plasmid-DNA multilayers. Because of the large surface-to-volume ratio, and flexibility, these mats could function as improved substrates for gene therapy and tissue engineering [264].

1.6 Conclusions

Among all the coatings and thin films discussed so far, those coatings based on PEG and zwitterionic polymers are exceptional in their ability to resist protein adsorption. The strong interaction of these coatings with water molecules is evidently the reason for their antibiofouling properties. These hydrophilic coatings are also successful in imparting blood compatibility and preventing blood coagulation, as well as rendering the biomaterial inert to the body's immune responses. Strategies to promote cell adhesion and proliferation on these otherwise nonadhesive coatings are available so that the coated implant can integrate with the host tissue. PEGylated and zwitterionic polymer coatings, when functionalized with reactive groups such as carboxylic acid, amino or aldehyde, have been used to tether biomolecules such as growth factors to facilitate tissue integration. Novel polymeric materials, including polymers with branched architectures, are currently being investigated and compared with PEGylated coatings for their protein resistance and biocompatibility. The influence of polymer chemistry and polymer conformation on biological interactions is an ongoing area of research. Among the different coating techniques available for preparing polymer thin films, the LBL assembly technique, employing polyelectrolytes, is currently a highly active area of research, the main reasons for this being the processing simplicity and ability to form conformal coatings. The CVD approach shares some of these traits. Stimulus-responsive coatings — and especially thermally responsive coatings based on poly(*N*-isopropylacrylamide) — have shown much promise for harvesting cells in the form of sheets for novel tissue-engineering applications. The use of polymer thin films as nonviral vectors for localized gene delivery from surfaces represents another highly interesting area of biomedical research with great potential.

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