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Antifouling surfaces in medical application

Summary — The uncontrolled adhesion of biological compounds on the surface of implant materials is a harmful phenomenon that causes the function of medical devices to deteriorate. The design of surfaces that resist nonspecific protein, cell or bacteria adsorption (so-called antifouling surfaces) is of special interest as it is critical for the development of medical devices that have contact with physiological fluids. Significant efforts have been made in coating surfaces with bioinert *macromolecules*. Both self-assembled monolayers (SAM) and polymer brushes have attracted considerable attention due to their facile synthesis, their diverse physicochemical properties (composition, molar mass or topology) and their tunable surface chemistry (film thickness, grafting density, conformation and flexibility). In this article, a general description of surfaces with nonfouling properties is provided. Two basic classes of nonfouling polymers (hydrophilic and zwitterionic) are discussed with a series of practical examples.
Keywords: antifouling surfaces, protein adsorption, self-assembled monolayers, poly(ethylene glycol), zwitterionic polymers.

POWIERZCHNIE PRZECIWDZIAŁAJĄCE OSADZANIU SIĘ ZWIĄZKÓW BIOLOGICZNIE AKTYWNYCH W ZASTOSOWANIACH MEDYCZNYCH
Streszczenie — Artykuł stanowi przegląd literaturowy dotyczący powierzchni przeciwdziałających osadzaniu się związków biologicznie aktywnych. Niekontrolowana adhezja związków biologicznie aktywnych na powierzchni implantów to niekorzystne zjawisko pogarszające prawidłowe funkcjonowanie wszczepialnych urządzeń medycznych. Kluczowa więc jest możliwość utworzenia powierzchni odpornych na adhezję białek, komórek bądź bakterii (tzw. powierzchni przeciwdziałających osadzaniu się związków biologicznie aktywnych). W ostatnich latach dużym zainteresowaniem cieszą się powierzchnie zdolne do przeciwdziałania adsorpcji, pokryte powłoką wytworzoną na bazie biokompatybilnych i nietoksycznych polimerów. Najwięcej uwagi poświęcono samoorganizującym się warstwom (SAM) i powierzchniom ze szczepionymi łańcuchami polimerowymi. Zainteresowanie to wynika ze stosunkowo łatwej syntezy powierzchni, różnorodności fizykochemicznych właściwości stosowanych polimerów (skład, masa molowa i topologia) oraz samej powierzchni polimeru (grubość warstwy, gęstość szczepienia, konformacja łańcuchów). Omówiono dwie podstawowe klasy polimerów przeciwdziałających adhezji białek — hydrofilowe polimery oraz polimery zawierające jony obojnacze.
Słowa kluczowe: powierzchnie przeciwdziałające osadzaniu się związków biologicznie aktywnych, adsorpcja białek, samoorganizujące się warstwy, poli(glikol etylenowy), polimery zawierające jony obojnacze.

INTRODUCTION

For applications in biosensing, diagnostics, and medical devices, the precise control of interactions between the material surface and the biological milieu is an important but challenging goal. Preventing the nonspecific interaction of cells, proteins, and microorganisms with the material surface is critical because these interactions are highly problematic in terms of device efficacy and safety.

Generally, medical implants, regardless of their composition, will become coated with a layer of proteins within a few seconds of contact with physiological fluids and tissues. This phenomenon is known as the biofouling process [1]. In many cases, biofouling is harmful and causes the function of medical devices to deteriorate. The host defense mechanism is activated; this results in the occlusion of cardiovascular implants by thrombus, block up the pores of the membranes or sensor because of protein accumulation or prevents the release of drug from a delivery device.

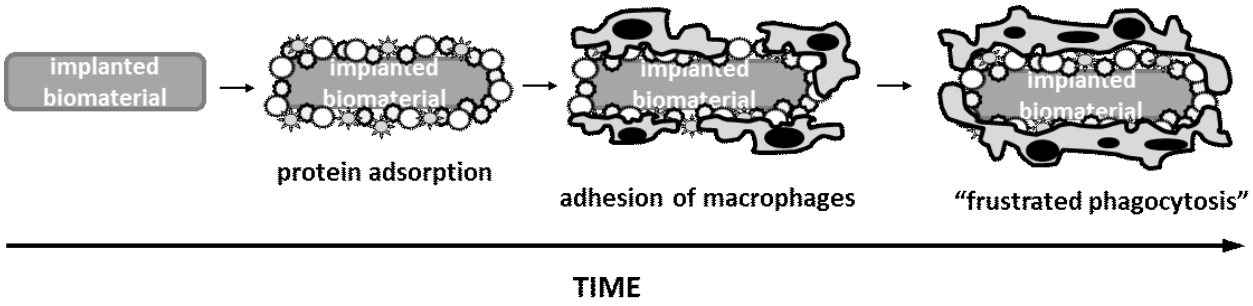

Fig. 1. The foreign body reaction to an implanted biomaterial

The reviews of Ratner and Williams provide a good explanation how the body reacts to the implantation of foreign materials [2, 3]. Nonspecific protein adsorption is the first interaction event, which occurs within seconds at the interface between a foreign material and human fluids and constitutes the initial step of the inflammatory cascade. After approximately one day, the adhesion of macrophages, that secrete inflammation mediators and tissue destruction, occurs. Because the size of most implants is orders of magnitude larger than that of the cells themselves, the cells begin to join together to form giant cells. Within the next few weeks, the giant cells release chemical signals that attract fibroblasts to the implant region in a process called „frustrated phagocytosis". A collagen layer is formed that separates the implant from the rest of the body. This process is represented schematically in Figure 1.

This situation leads to an inflammatory response, and the host finally begins to treat the implanted device as a foreign body [4]. Many devices eventually fail because of their inability to effectively communicate with the surrounding tissues [5].

Therefore, materials with antifouling properties have been the subject of much interest and extensive research within the last few years [5—11]. The antifouling (nonfouling) capability of devices ensures their resistivity to the nonspecific adsorption of proteins, cells or other biological species and makes the device significantly less detectable by immune system cells. This antifouling behavior is usually achieved by coating the surface with bioinert *macromolecules*. Generally, two major classes of polymers have been investigated for the minimization of nonspecific adsorption: hydrophilic and zwitterionic. Within these groups, poly(ethylene glycol)-based surfaces have been extensively studied as nonfouling materials (a detailed description is provided further below). A number of different approaches have been developed to couple nonfouling materials to a solid substrate, including the following: physical adsorption, chemisorption or grafting-from and grafting-to the surface. Proper control over the structural parameters of the surface, such as polymer density, thickness and chain conformation produces materials with effective protein adsorption resistance.

The purpose of this review is to provide an overview of surfaces with nonfouling properties, followed by a discussion on several representative polymers that have been used to inhibit protein adsorption. Although the mechanism leading to protein adsorption is still not fully understood, general structure-property relationships will be discussed.

FACTORS CONTROLLING PROTEIN ADSORPTION AT THE SURFACE

Protein adsorption is a very complex process driven by different protein-surface forces, including van der Waals, hydrophobic and electrostatic forces. The parameters that influence protein-surface interactions include the properties of the protein and the solution environment on the one side and the surface properties on the other side [12, 13].

Proteins are complex biopolymers containing different amino acids in the backbone and different compounds, such as oligosaccharides, lipids or phosphates, attached to the amino acids as side chains. This diversity of proteins and their structure (four distinct levels of protein structure) makes the understanding of their adsorption behavior difficult. Moreover, the properties of proteins, such as the charge, size, structural stability (so-called hard and soft proteins) and steric conformation, may also affect their surface adsorption properties. The stability of a protein's structure is of particular importance. As proteins contain a specific distribution of hydrophilic, hydrophobic, and positively or negatively charged side chains, they can adapt certain orientations when approaching a solid (hydrophobic, hydrophilic or charged) surface [9, 12]. As a result, different protein regions exhibit different affinities to various surfaces, which determines their adsorption characteristics. Proteins with a high internal stability, the so-called hard proteins [*e.g.* α-chymotrypsin, ribonuclease, lysozyme and β-lactoglobulin (β-Lg)], usually exhibit low levels of adsorption on hydrophilic surfaces (unless there is electrostatic attraction). However, these proteins readily adsorb on hydrophobic surfaces that lead to a change in the conformation of the protein. In contrast, proteins with a low internal stability, the so-called soft proteins [*e.g.* bovine or human serum albumins, immunoglobulin (IgG), α-lactoalbumin, β-casein or hemoglobin], generally tend to adsorb on all surfaces regardless of electrostatic interactions [9].

External parameters, such as temperature, pH and ionic strength, also influence protein adsorption [12, 13].

The driving force of protein adsorption is an entropy gain arising from the release of surface-adsorbed water molecules and the structural rearrangement inside the protein. Thus, the amount of surface-adsorbed proteins increases at elevated temperatures [13].

The pH determines the electrostatic state of the proteins. When the pH equals the isoelectric point (pI) of a protein, the overall charge of the molecule is neutral. The proteins are positively charged at low pH values (pH < pI) and negatively charged at high pH values (pH > pI). The adsorption rate is high when the protein and the substrate bear opposite charges due to the electrostatic attractions, which may accelerate migration towards the surface [12].

The influence of surface properties on protein adsorption will be discussed in detail further below.

FUNDAMENTALS OF NONFOULING SURFACE COATING

A common approach to avoid problems arising from protein adsorption is coating a device used in biomedical applications with a layer of a material that prevents nonspecific interactions. This approach involves the formation of a self-assembled monolayer (SAM) on the surface or the coverage of the surface with a polymer layer (Figure 2).

There are two major classes of polymer materials that exhibit antifouling properties: hydrophilic polymers and polyzwitterions. The main polymers applied are summarized in Table 1.

Most hydrophilic nonfouling materials include poly(ethylene glycol) (PEG), oligo(ethylene glycol) (OEG), poly[oligo(ethylene glycol)] methacrylates (POEGMA), polyacrylamides, polyglycidols and polysaccharides. Among these polymers, the best-known and most-studied over decades surfaces include those covered by poly(ethylene glycol) or oligo(ethylene glycol) [14—16, 28—32]. This interest results from the fact that PEG is non-toxic, non-immunogenetic, non-antigenetic and biocompatible [33].

The zwitterionic antifouling polymers can be divided into polybetaines and polyampholytes. Polybetaines are derived from 2-methacryloyloxyethyl phosphorylcholine (MPC), sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA) and contain a positive and negative charge on the same monomer unit (Fig. 3A). Polyampholytes are formed by a pair of separate monomers with two oppositely charged moieties. The overall neutral charge (which is necessary for protein repulsion) can be achieved by mixing the positively and negatively charged monomers in a 1:1 ratio prior to co-polymerization (Fig. 3B).

T a b l e 1. Overview of hydrophilic and zwitterionic antifouling polymers

Polymer	Ref.
Hydrophilic	
SAM-OEG	[14], [15]
PEG	[16]
POEGMA	[17]
Polyglycidol-based materials	[18], [19]
PHEMA [poly(2-hydroxyethyl methacrylate)]	[20]
PAAm (polyacrylamides)	[21]
Dextran	[22]
Polysaccharide	[23]
Mannitol	[24]
Zwitterionic	
polyMPC	[25]
polySBMA	[26]
polyCBMA	[27]

Approaches for the design of inert surfaces

Numerous methods have been used to cover the surface with antifouling materials, including the covalent attachment or adsorption (chemi- or physisorption). The covalent attachment is usually achieved during the preparation of the polymer layer *via* the grafting-to or grafting-from techniques [17, 34]. The first technique consists of the immobilization of the pre-synthesized polymer chains with reactive end-groups on a surface containing chemical anchoring groups (Fig. 4A). This method can be performed relatively easily, but only a limited amount of polymer can be tethered to the substrate. The second technique utilizes the polymerization of monomers ini-

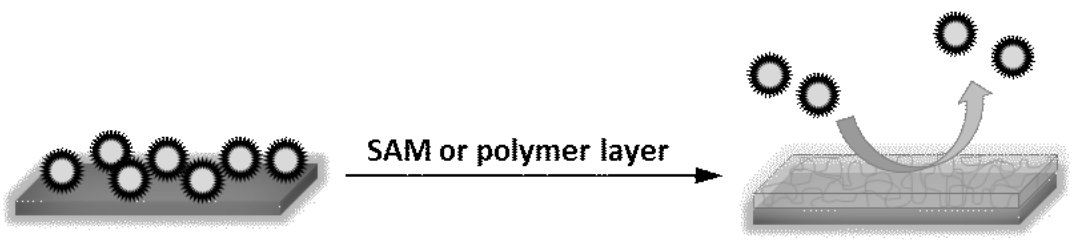

Fig. 2. Schematic illustration of surface structures that resist protein adsorption

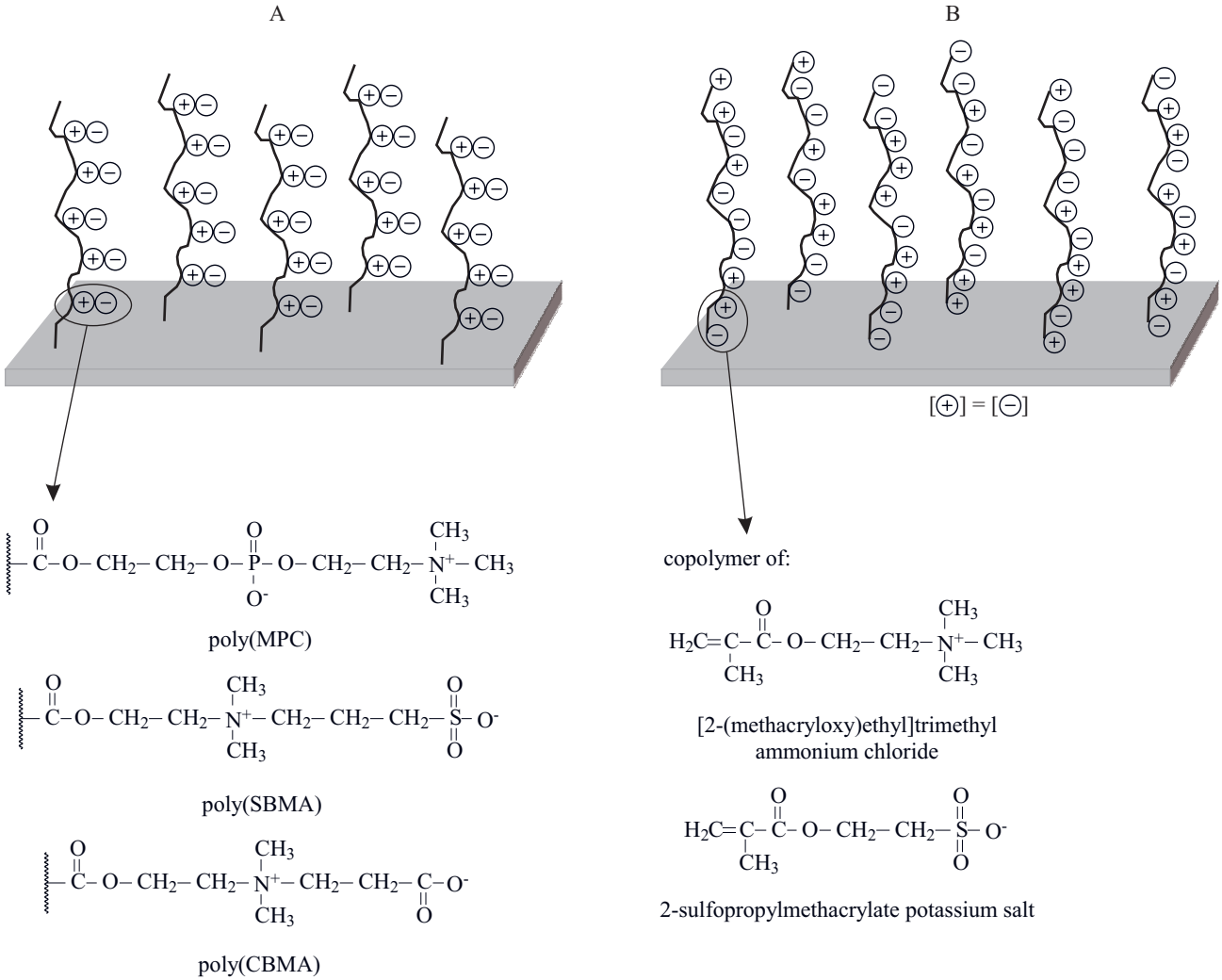

Fig. 3. Polybetaines (A) and polyampholytes (B) used as antifouling layers

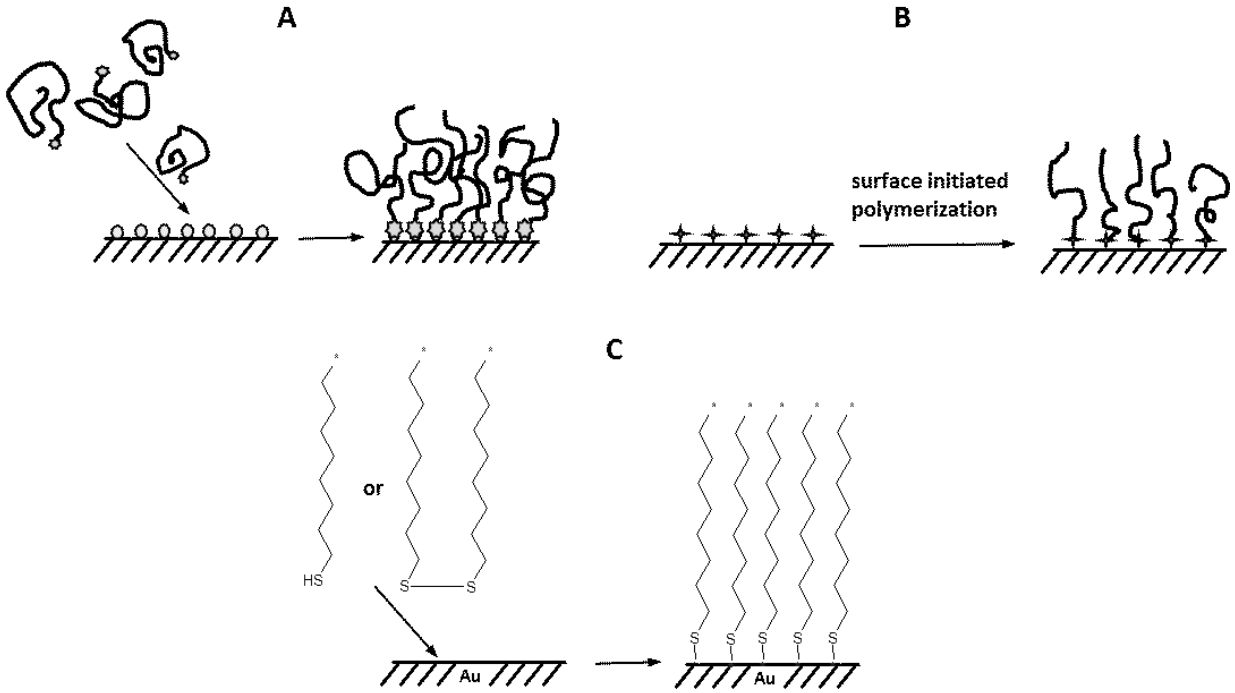

Fig. 4. Grafting-to (A), grafting-from (B) and chemisorption (C) strategies for modifying surfaces with antifouling layers

tiated by an initiator that is attached to the surface [so-called surface-initiated polymerization (SIP)], applying living/controlled polymerization processes, where ATRP (Atom Transfer Radical Polymerization) is the most commonly used (Fig. 4B) [34, 35]. Well-defined polymer brushes with a high grafting density can be obtained using this method.

In contrast, chemisorption involves the formation of strong chemical bonds between the thiol-terminated polymer chains or low molar mass compounds and Au-surfaces. This technique is generally used to obtain antifouling SAM surfaces (Fig. 4C) [36]. The covering of the surface with antifouling material by physisorption relies on relatively weak electrostatic and hydrophobic interactions that force the polymers to adhere to the surface. The advantage of the adsorptive methods is that they are usually conducted under relatively mild conditions. The physisorption however is reversible, and proteins may replace the physisorbed polymer over time.

What makes the surface antifouling?

Even though proteins tend to adsorb on hydrophobic surfaces through hydrophobic interactions like van der Waals forces, other factors, such as electrostatic forces, hydrogen bonding and pH, strongly influence the adsorption process. Proteins may also adsorb on the hydrophilic surfaces due to the charge interactions [37].

There are few physical and chemical theories describing the general rules that must be fulfilled for a surface to become resistant to proteins [38—41].

It is generally accepted that the antifouling properties of both hydrophilic and zwitterionic materials are correlated with the formation of a hydration layer on the surface [40, 42]. The water molecules bound with polymer layer form a physical and energetic barrier that hinders protein adsorption. The „surface-bound" water molecules are formed by hydrogen bonding for hydrophilic materials or by ionic solvation for zwitterionic materials. The strength of the surface hydration is determined by the physicochemical properties of material on the surface (molar mass and surface chemistry) and the surface packing (film thickness, grafting density, conformation and flexibility, especially for long-chain polymers).

The fouling resistance is also often explained using the steric repulsion model (model created for the PEG brushes) [38, 39]. When a protein approaches the surface, water molecules associated with the polymer chains are released, and the chains become compressed. The increase in enthalpy due to chain dehydration and the decrease in entropy due to chain compression are both unfavorable and provide a thermodynamic basis for the antifouling properties of the coating [15, 38]. Moreover, this model highlights the importance of the polymer chain length and the surface density of grafted chains regarding the resistance to protein adsorption [39, 41]. Longer polymer chains and higher grafting densities lead to better antifouling surfaces.

Based on the aforementioned theories, it can be stated that nonfouling polymer surfaces can be achieved when surface hydration and steric repulsion forces work together. If chain flexibility plays a small role (*e.g.*, for self-assembled monolayers), chain hydration is the driving force that resists protein adsorption (Figure 5A). However, in the case of flexible chains (*e.g.*, for polymer brushes), both the chain flexibility and hydration are important (Fig. 5B and 5C).

The first general rule for elucidating structure-activity relationships between different chemical structures of small molecules on the surface and their potential to suppress protein adsorption was postulated by Whiteside and co-workers [43, 44]. They prepared a number of SAM surfaces composed of alkane thiols (C=15) containing different functional end-groups and used them to investigate protein adsorption. Based on the obtained results, they postulated that nonfouling surfaces should be hydrophilic, electrically neutral and possess hydrogen bond acceptors but not hydrogen bond donors. These suggested properties were compatible with the hypothesis that the interaction of the functional groups with water is important for creating surfaces inert with regard to proteins. However, the structural characteristics of nonfouling materials described by Whiteside are not adequate for all polymers. It has been shown that SAMs with

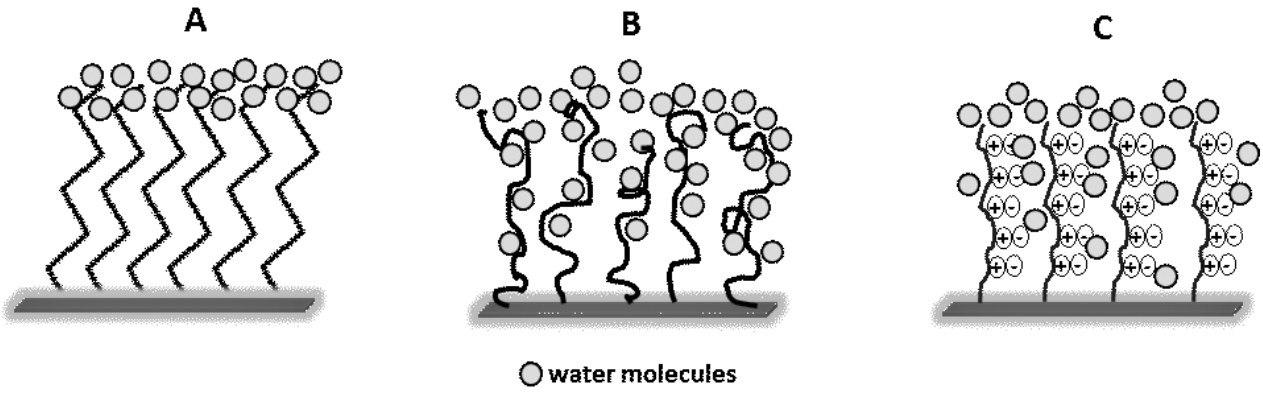

Fig. 5. Schematic image of the chain hydration and flexibility of: A) SAMs, B) hydrophilic polymers and C) zwitterionic polymers

hydroxyl-terminated OEG [28] or mannitol [24] show high resistance to proteins, even though they contain hydrogen bond donors.

The concepts discussed above regarding inert surfaces focus on the characteristics of various types of polymer chains tethered to the surface and their interactions with water. However, the interaction between polymer chains and the protein is often neglected. Thus, a complete explanation of protein resistance mechanisms is not provided.

Latour [45] provided a thermodynamic analysis of protein adsorption to surfaces. The specific molecular-level interactions that contribute to enthalpy, entropy and free energy changes during the protein adsorption process were addressed. The interactions between the proteins, the surface-tethered chains and water were considered. It was suggested that two independent factors provide thermodynamically favorable conditions for protein adsorption resistance. The first factor requires well-hydrated, long and flexible surface-tethered chains with a sufficiently low surface density that allows for chain mobility while ensuring complete surface coverage. This factor supports a sufficiently high entropy compared with that resulted from the formation of stable bonds between the protein and the surface-tethered chains. The second factor requires polymers that have hydrogen bonding groups that are readily accessible to water molecules but not to the hydrogen bond-forming groups of the protein. The combination of these two factors results in a situation in which the thermodynamic state of the system when proteins are adsorbed has higher free energy than the state when the proteins are not adsorbed, thus providing protein adsorption resistance.

ANTIFOULING POLYMER-COATED SURFACES

This chapter discusses the characteristics of surfaces covered with hydrophilic and zwitterionic polymers. The influence of chain length, grafting densities and topology on the surface resistivity to protein adsorption is described in detail.

PEG-containing surfaces

Conventional methods for immobilizing PEG coatings on substrates include direct attachment of self-assembled PEG monolayers, graft polymerization of PEG macromonomers (leading to polymer brushes on surface) and adsorption of PEG (co)polymers at multiple sites on the surface (leading to branched molecules on the substrate).

SAM of alkanethiols on gold surfaces with short oligomers of the ethylene glycol units [$HS(CH_2)_{11}$-$(OCH_2CH_2)_nOH$, where $n = 2—17$] were the first developed SAMs that resisted the adsorption of several model proteins [14, 46]. It was shown that the efficiency of protein resistance increased as the number of OEG units increased [14]. The antifouling behavior of the short-chain SAM-OEG was explained by the surface hydration (see Fig. 5A) as the water molecules bound to the top of the SAMs are the only source of a large repulsive force repelling the protein adsorption. The conformational flexibility of the OEG chains was not responsible for the reduced protein adsorption because the densely packed, short chains have less freedom to undergo conformational changes upon protein adsorption [47]. However, it has been shown that depending on the preparation conditions, the resultant SAM-OEG can also form incomplete monolayers (neither highly ordered nor densely packed) with different OEG chain conformations [48, 49], which changes the nonfouling behavior of the surface. However, although a higher packing density of OEG was observed on silver (planar all-trans conformation) than on gold (helical conformation) surfaces, better protein resistance was observed on the latter [28, 50]. This effect was ascribed to the improved penetration of water molecules into the monolayer on the gold surface [48, 51]. As a result, it was postulated that for SAM-OEG, the combination of several factors, such as the philicity of the end-groups, the OEG chain length, the conformation-dependent degree of solvation and, consequently, the stability of the interfacial water layer, influence the formation of a polymer film that is completely protein resistant [28, 50]. If the ability of a SAM to coordinate water both in its interior and on its surface is reduced when one of these factors is unfavorable or absent, the overall protein resistance decreases.

For the surfaces covered with longer PEG chains, not only the surface hydration and chain flexibility but also the surface packing (grafting density) and polymer topology determine the nonfouling properties of the surface [16, 29, 30, 32, 52, 53].

It was observed that the grafting density is more important than the molar mass of PEG in preventing protein adsorption [16, 29, 54]. Sofia *et al.* [29] reported that no specific PEG molar mass (studied surfaces with PEG 3400 to 20 000 g/mol) is necessary for the prevention of protein adsorption. Instead, for a given molar mass, there is a specific grafting density above which the surfaces become impenetrable to the proteins [29]. This is connected with the overlapping of polymer chains at a certain grafting density, which results in a completely covered surface. Moreover, Unsworth *et al.* [54] observed an optimum grafting density for protein resistance over which the adsorption increased again. They prepared gold-coated silicon surfaces covered with PEGs with M_n of 750 g/mol, 2000 g/mol and 5000 g/mol. A grafting density of ~ 0.5 chain/nm^2 for PEG 750 and 2000 g/mol occurred to be the most effective density for protein resistance. At higher grafting density values, the resistance decreased. For surfaces covered with PEG 5000 g/mol, the maximum efficiency in protein resistance was observed for ~0.28 chain/nm^2. This behavior might be possibly ex-

plained by the decrease in the polymer chain hydration and mobility as the grafting density increases, which results in a loss in nonfouling properties.

As mentioned earlier, the dense coverage of a surface is the important parameter in determining the ability of a polymer layer to prevent protein adsorption. Thus, the branched polymer architecture (star-shaped or dendronized molecules on the surface) should be superior for the prevention of unspecific protein adsorption [29, 52, 53, 55, 56]. Star-shaped hydroxyl-terminated PEG molecules (70 and 20 arms of $M_{PEG,arm}$ = 5200 g/mol and 10 000 g/mol, respectively) tethered to silicon surfaces appeared to sufficiently resist the adsorption of larger proteins (albumin and fibronectin) [57]. However, star molecules were only attached to the surface and did no undergo intermolecular crosslinking, leaving open spaces between the molecules. This inefficiently packed layer of star-PEG resulted in the adsorption of small proteins (cytochrome-c). To overcome this problem, Moeller *et al.* [53, 56] prepared surfaces with a layer made from isocyanate-terminated, star-shaped poly(ethylene glycol-ran-propylene glycol) prepolymers (six arms, M_n = 3000, 12 000 and 18 000 g/mol). Due to the highly reactive end-groups, these molecules formed a densely packed polymer network *via* an intermolecular crosslinking reaction on the substrate, leading to a high polymer surface coverage. No protein (lysozyme and insulin — small-sized proteins) adsorption was detected on the coatings prepared using these star-PEG polymers. The adsorption of fibrinogen and lysozyme was also studied on the PEG-functionalized dendronized surfaces [52, 55]. Poly(ethylene glycol) monothiol was chemisorbed onto gold-silicon wafers, followed by the modification of the OH-end groups with aliphatic polyester dendrons (generation 1—4). The peripheral hydroxyl groups of the dendronized surfaces were subsequently modified with PEG monomethyl ether chains with various molar masses (350 up to 5000 g/mol). It was shown that protein adsorption gradually decreased with increasing molar mass and reached an optimum level for structure with PEG 2000 g/mol. Additionally, for a certain molar mass, a decrease in protein adsorption (regardless of protein size) was observed as dendron generation increased.

In recent years, a great variety of surfaces covered with poly[oligo(ethylene glycol)] methacrylates (POEGMA) have been prepared as an alternative approach to produce surfaces with protein resistance characteristics [27, 58—68]. Synthetic methods for obtaining such surfaces are detailed in a review by Klok *et al.* [17]. These surfaces, mostly obtained by surface-initiated controlled radical polymerization, can provide ultrathin polymer coating. Because the brush thickness and grafting density can be easily adjusted, this method is advantageous for elucidating nonfouling properties. Table 2 provides an overview of some POEGMA surfaces that have been shown to effectively resist protein adsorption.

Surfaces covered with other hydrophilic polymers

Recently, efforts have focused on the development of alternative bioinert, antifouling polymers as a substitute for PEG-coated surfaces. Alternatives include surfaces covered with polyoxazolines [74—78], polyglycidol dendrons [18, 19, 79] and polysaccharides [23].

The close structural similarity of polyglycidol (PG) to PEG renders the polymer predetermined for biological applications. This polymer is biocompatible and non-cytotoxic and possesses free hydroxyl groups that are available for further functionalization [80]. Most available data concerning the antifouling properties of PG surfaces are based on dendritic or hyperbranched polymers [18, 19, 79, 81—84]. The antifouling properties of the dendritic or hyperbranched polyglycidols are ascribed to the presence of highly flexible aliphatic polyether, hydrophilic surface groups and to a highly branched architecture. Haag *et al.* [81] prepared the disulfide-functionalized hyperbranched PGs that were self-assembled on a gold surface. The resulting materials prevented the adsorption of fibrinogen at the same level as PEG SAMs. It was revealed that the more globular the structures, the more inert surface toward protein adsorption, while the molar mass changes did not appear to have a significant effect. A different approach to hyperbranched PGs surfaces involved the polymerization of glycidol (M_n = 1600 g/mol and 4300 g/mol) with a disulfide initiator, followed by the reduction of the disulfide group and the subsequent self-assembly of the branched PGs [18]. The branched polymers produced more uniform and smooth surface compared with linear PEG. Although the low molar mass PG produced a higher grafting density, the surface coated with the high molar mass PG was more resistant to protein adsorption. Additionally, the degree of protein adsorption decreased as the grafting density of chains on the surface increased. The capability of polyglycidol dendrons to resist nonspecific protein adsorption was also demonstrated in [82, 83]. Two different approaches were used to modify gold surfaces. In the case of the „anhydride method," the monoamino glycidols derivatives with different structure (dendritic and linear), size and functionality were coupled to the gold surfaces coated with mercaptoundecanoic acid. In contrast, in the case of chemisorption, the alkanethiolate-based polyglycidol dendronds with terminal hydroxyl or methoxy functionalities were directly chemisorbed on the gold surface. For the first method, the studies revealed that the capability of PG dendrons to resist protein adsorption depends on the coating density reaching minimum adsorption for [G2] generation [82]. As the grafting of higher generations was worse, the lower protein resistance was observed. Surfaces obtained *via* the latter method formed better and more defined monolayers. It was presented [83] that the surfaces with [G1] generation showed already a dramatic decrease of fibrinogen adsorption, indicating that oligoglycidols of low molar

T a b l e 2. Examples of antifouling surfaces composed of POEGMA

Polymer on the surface	Substrate	Polymer layer description	Protein adhesion description	Remarks	Ref.
Poly(ethylene glycol) methyl ether methacrylate PEGMEMA (4 ethylene glycol units)	Stainless steel	• UV-induced graft polymerization of monomer on plasma pre-treated steel • Concentration of grafted PEGMEMA increased with monomer concentration during grafting	• Bovine serum albumin and γ-globulin (2 mg/cm^3) • Adhesion at 25 °C over 24 h	Protein adhesion resistance increased with PEGMEMA graft concentration of	[69]
Poly(ethylene glycol) methacrylate PEGMA (10 ethylene glycol units)	Glass or silicon	• SI-ATRP • Layer thickness of 10—90 nm • Brush density controlled by the use of ATRP "active" and "inactive" initiator mixture	• Fibrinogen (1 μM) • Adhesion at 25 °C over 24 h	Protein adhesion resistance increased with "active"/"inactive" initiator ratio (most efficient ratio >0.6/0.4) Protein adsorption increased with incubation time	[60]
Poly(ethylene glycol) methyl ether acrylate PEGMEA (8 ethylene glycol units)	PHEMA-*co*-PMMA hydrogel	• SI-ATRP • Length of PEGMEA grafts $2\,050 < M_n < 33\,500$ g/mol	• Green fluorescent protein (0.15 mg/cm^3), β-lactamase (0.3 mg/cm^3), lens epithelial cells • Adhesion at 37 °C over 150 min or 3 days	Protein adsorption investigated for PEGMEA of 33 500 g/mol; protein adsorption reduced by approximately 70 % in the presence of PEGMEA brushes	[59]
Poly(ethylene glycol) methyl ether methacrylate PEGMEMA (9 ethylene glycol units)	Gold	• SI-ATRP • Layer thickness of 4—50 nm • Brush density controlled by the use of ATRP "active" and "inactive" initiator mixture	• Fibronectin and fibrinogen (1 mg/cm^3), foetal bovine serum, proteins from plasma, blood platelet	Amount of adsorbed fibronectin and fibrinogen at or below the detection limit of SPR (<1 ng/cm^2). Only 9.2 ng of plasma proteins per cm^2 of surface were adsorbed Low adsorption of platelet detected ($9.57 \cdot 10^3$/cm^2)	[27, 70]
Poly(ethylene glycol) methyl ether methacrylate PEGMEMA (1, 2 and 3 ethylene glycol units)	Silica	• SI-ATRP • Brush density controlled by the use of ATRP "active" and "inactive" initiator mixture	• Bovine serum albumin (0.4 mg/cm^3) • Adhesion at 37 °C over 2 h	The amount of protein decreased with increasing grafting density to an optimal value of 0.56 chain/nm^2 and then increased again The BSA amount was also reduced with decreasing in ethylene glycol length	[71]
Poly(ethylene glycol) methyl ether methacrylate (8 ethylene glycol units)	Gold	• SI-ATRP • Brush density controlled by the use of ATRP "active" and "inactive" initiator mixture	• GRGDS (1.5 mg/cm^3), adhesion at 37 °C over 100 s • Mouse fibroblast cell MC3T3, adhesion at 37 °C over 8 h	The GRGDS and cell adsorption reduced for surface with higher grafting densities	[72]
Poly(ethylene glycol) methyl ether methacrylate (4 and 9 ethylene glycol units) Poly[hydroxy(ethylene glycol)] methacrylate (8 and 10 ethylene glycol units)	Silicon or Gold	• SI-ATRP • Polymer layer thickness (up to 80 nm) and density controlled by the polymerization conditions	• Bovine serum albumin, fibrinogen, streptavidin (1 mg/cm^3) • Adhesion at 30 min	No protein adsorption detected (within experimental error)	[73]

mass can be protein resistant. The elimination of the hydrogen-donor group by methylation did not influence the nonfouling properties of the monolayer. Recently, novel polymer brushes were obtained by the surface-initiated ATRP of macromonomers with dendritic or linear oligoglycidols [79]. All brushes exhibited good resistance to single fibrinogen adsorption. The brushes were also verified towards human serum and blood plasma. In this case, a dependence of protein adsorption on the brush architecture was observed. The best performance was achieved for brushes with dendritic side chains because they offer a route for increasing the local polymer density. The brushes with linear, hydroxy-terminated oligoglicydols side chains were more efficient in resisting protein absorption than the methoxy-terminated side chains, illustrating the importance of forming a tightly bound hydration layer for antifouling properties. Macromonomers of linear polyglycidols were also polymerized from the surface *via* ATRP [85] and photopolymerization [86] and applied as antifouling devices. The studies of albumins adsorption to glassy carbon, gold and stainless steel covered with polyglycidol grafts revealed a good proteins resistance of investigated surfaces.

Poly(2-oxazoline)s (POx) have just begun to attract interest as biomaterials with a potential application in medicine. Studies have shown that hydrophilic POx [especially the poly(methyl-2-oxazoline) — PMOx and poly(2-ethyl-2-oxazoline) — PEOx] are non-cytotoxic and exhibit low acute toxicity. Moreover, they do not accumulate in a specific organ but are rapidly cleared from the blood in its free form [87—90]. Additionally, they are also peptidomimetics, meaning that their structure is isomeric with that of polypeptides. It has been shown that polymer brushes of PMOx and PEOx on substrates can efficiently reduce protein adsorption. Textor *et al.* [74—76] designed comb copolymers consisting of a polycationic poly(L-lysine) backbone and PMOx side chains that were self-assembled on negatively charged Nb_2O_5 surfaces. The PMOx-based surfaces with an optimal side-chain grafting density (regulated by the molar mass of the PMOx chains) were shown to prevent the adsorption of proteins from human serum to a level of < 2 ng/cm^2. This result is similar to the protein resistance properties of the most effective PEG-based coatings [75, 76]. Additionally, films prepared from poly(L-lysine)-PMOx have shown considerably higher stability in all model environmental tests when compared with poly(L-lysine)-PEG [76]. In early studies, Lahmann and Ruhe reported the first results concerning PEOx brushes on gold surfaces with protein resistance properties [91]. PEOx with different molar masses (*DP* = 5—150) and end-groups were chemisorbed onto gold surfaces (layer thickness 3—5 nm). The amount of adsorbed fibrinogen decreased with increasing polymer chain length. Recently, Yan *et al.* [78] employed photoimmobilization chemistry to produce PEOx films (M_n = 5 000—500 000 g/mol) on solid surfaces and then used these films to monitor bovine serum albumin adsorption. The low protein adsorption observed for surfaces covered with the polymer of the highest molar mass is explained by the high concentration of polymer on the surface. Jordan *et al.* [77] performed detailed studies on POx bottle-brush brushes (BBB) and the influence of side chain composition [PMOx, PEOx or poly(*n*-propyl-2-oxazoline)], length (*DP* = 6—18 for PMOx) and side chain end-group (groups with different philicity) on the effective control of fibronectin adsorption. BBBs with hydrophilic (PMOx and PEOx) side chains with a sufficient length (*DP* up to 10) afforded very low (< 6 ng/cm^2) protein adsorption. Side chain end-group functionalization was found to have a slight but not significant effect on the protein adsorption properties.

Surfaces covered with polyzwitterions

Although a large group of hydrophilic polymers on the surfaces have been widely used to create antifouling surfaces, another class of protein-resistant materials based on zwitterionic polymers have also been developed [7, 17, 92]. An example of the structure of polyzwitterions is presented in Fig. 3. A critical factor determining the nonfouling properties of polyzwitterionic materials is the control of both the uniformity of the charge distribution and the charge neutrality (of two opposite charge moieties on the surface). This allow to maximize the surface hydration and reduce the charge interactions with the protein molecule. The surface hydration, in the case of polyzwitterions, is formed by the ionic solvation of both positive and negative charge units by the water molecules, in addition to hydrogen bonding [93, 94].

Of the zwitterionic polymers, phosphorylcholine (MPC)-based polymer surfaces mostly obtained *via* surface-initiated ATRP [25, 95—98] have been shown to effectively reduce protein adsorption and cell adhesion. A series of MPC-grafted silicon surfaces with different graft chain lengths but similar graft densities were prepared [97]. At a graft density of 0.39 chains/nm^2, the adsorption of fibrinogen and lysozyme decreased with increasing chain length. Another study of MPC-grafted silicon surfaces revealed that increasing the thickness of the coating layer and the graft density leads to an improvement in protein resistance, reaching a level of <10 ng/cm^2 at a graft density of >0.29 chains/nm^2 and a chain length of >100 unit [96]. Brash *et al.* [25] compared the antifouling properties of PEGMEMA (M_n = 300 g/mol, PEG side chains of length n = 4,5) and poly(MPC) M_n = 295 g/mol) grafted on silicon wafers. Protein adsorption to both grafted surfaces was found to decrease with increasing graft density and chain length. Further, for a given chain length and graft density, the adsorption of lysozyme and fibrinogen on the poly(MPC) and PEGMEMA surfaces was essentially the same. Based on these results, it was suggested that „the water barrier layer" associated with polymers affords protein-resistant surfaces.

Polymer brushes obtained by surface-initiated ATPR of sulfabetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA) have also been used as nonfouling surfaces [26, 27, 99, 100]. Surfaces grafted with these polymers efficiently reduced the adsorption of single proteins, plasma proteins and proteins from human serum to levels comparable with those of PEG-like films. Moreover, CBMA-based surfaces exhibited improved protein resistance compared with that of PEGMEMA and SBMA-surfaces, indicating that zwitterionic polymers are a viable alternative to PEG-based materials [27, 99]. The improvement in CBMA over SBMA polymer surfaces may be due to the shorter distance between the charge groups on the monomer, which results in a stronger hydration layer on the surface [27, 99].

CONCLUSIONS

Understanding the interactions between proteins and biomaterial surfaces is critical for designing biocompatible medical devices. Polymers play a significant role in the creation of antifouling materials. In general, approaches to reduce protein adsorption at the surface include surface modification with hydrophilic polymers or polymers containing zwitterionic species. The nonfouling properties of such surfaces are mainly attributed to the surface hydration that depends on the surface chemistry, such as the type of end-groups (in case of SAMs), the chain length and the degree of surface packing (film thickness, grafting density, conformation and flexibility, especially for long-chain polymers).

Although great progress has been made in controlling the protein adsorption on biomaterial surfaces, many challenges still remain for biomedical applications. For example, the lifetime of the prepared device requires further investigation. Some nonfouling surfaces discussed in this article exhibit stability for up to few weeks under protein adsorption conditions. However, questions like what is the optimal lifetime of the nonfouling coating, and related to that, what attachment methodology should be used to match this time scale or what is the mechanism of layer removal from the surface still remains. Another challenge involves the use of antifouling surfaces with attached bioactive molecules, which can promote or support desired reactions with the biological environment. It has been shown that it is possible to immobilize various biomolecules onto surfaces [100]; however, the optimization of the biomolecule density and the maintenance of the resulting structure and activity are still to be resolved.

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