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Feature Article

Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials

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ABSTRACT

Surface resistance to nonspecific protein adsorption, cell/bacterial adhesion, and biofilm formation is critical for the development and performance of biomedical and analytical devices. Significant needs and efforts have been made in the development of biocompatible and bioactive materials for antifouling surfaces, but much of the work retains an empirical flavor due to the complexity of experiments and the lack of robust theoretical models. In this review, two major classes of nonfouling materials (i.e. hydrophilic and zwitterionic materials) and associated basic nonfouling mechanisms and practical examples are discussed. Highly hydrated chemical groups with optimized physical properties of the surface, along with appropriate surface coating methods, are the keys to developing effective and stable nonfouling materials for long-term biomedical applications. The zwitterionic polymers are promising nonfouling biomaterials due to the simplicity of synthesis, ease of applicability, abundance of raw materials, and availability of functional groups.

1. Introduction

There is a significant need for the development of novel antifouling materials to resist nonspecific protein adsorption and cell adhesion for medical implants, drug delivery carriers, biosensors, and marine coatings [1]. It is generally believed that reducing biofouling could significantly attenuate subsequent adverse inflammatory responses including leukocyte activation, tissue fibrosis, thrombosis coagulation, and infection. On the other hand, even 10 ng/cm^2 fibrinogen adsorption can induce a full-scale blood platelet adhesion, resulting in implantable device failure and adverse outcomes to the patients. Poly (ethylene glycol) (PEG)-based materials [2] have been well demonstrated for their super-low-fouling ability to resist nonspecific protein adsorption and cell adhesion. But, the PEG-based materials are readily subject to oxidation in most biochemically relevant solutions. Significant effort has been invested in the search for alternative antifouling materials with stability higher than PEG and this has resulted in substantial progress in developing improved materials.

There are two major classes of antifouling materials, namely polyhydrophilic and polyzwitterionic materials as summarized in Table 1. A number of low-fouling or nonfouling polyhydrophilic materials including PEG, polysaccharides, and polyamides have been found to mostly share some common structural and chemical properties: hydrophilic nature, electrically neutrality, and hydrogen-bond acceptors/donor [3,4]. For the polyzwitterionic materials, they can be further classified into polybetaines carrying a positive and a negative charge on the same monomer unit such as 2-methacryloyloxylethyl phosphorylcholine (MPC), sulfobetaine methacrylate (SBMA), and carboxybetaine methacrylate (CBMA) and polyampholytes carrying 1:1 positive and negative charge on two different monomer units such as mixed charge complex $-N^+(CH_3)_3$ and $-SO_3^-/-COO^-$ and natural amino acids (Glu−, Asp−, Lys+, and Arg+). Since all proteins have positively and negatively charged residues randomly distributed on its surface, they can be easily adhered to either positively or negatively charged surfaces [5]. It is suggested that a nanoscale homogenous mixture of balanced charge groups from polyzwitterionic materials is the key to controlling nonfouling properties. Apart from functional materials, surface packing also plays an important role in achieving nonfouling properties of a surface. Surface coating and modification methods include physical adsorption via hydrophobic interactions or spin-coating [6–8], chemical linkage, self-assembled monolayers (SAMs) [9,10], “grafted-from-surface” via surface-initiated atom transfer radical polymerization (ATRP) [11,12], “graft-to-surface” via dipping, cross-linking, or reaction of the

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Table 1
Overview of hydrophilic and zwitterionic antifouling materials.

Materials	Protein adsorption	Cell adhesion
Hydrophilic materials		
PEG-based materials		
PS-g-PEGMA and PMMA-g-PEGMA [92]	Yes	No
PEG-poly(phosphonate) terpolymer [93]	Yes	No
PLL-g-PEG [94,95]	Yes	Yes
PEGMA [96,97]	Yes	No
$PPEG_xLys$ [98]	Yes	No
POEGMA [99–102]	Yes	Yes
PEO-PU-PEO [61,103–105]	Yes	No
PEO-PPO-PEO [106]	Yes	No
PEO [33]	Yes	No
PEG [8,68,107–109]	Yes	Yes
Py-g-PEG [63]	Yes	No
mPEG-DOPA [60,110]	Yes	No
mPEG-MAPD [110]	Yes	No
OEG-SAM [10,87,102]	Yes	Yes
PMOXA [77,111]	Yes	No
Dendron		
Glycerol dendron [75]	Yes	No
HPG [76]	Yes	No
Tetraglyme [112–115]	Yes	Yes
Dextran [57,116]	Yes	No
Polysaccharide [117]	Yes	No
Poly (HEMA) [118,119]	No	Yes
PVA [120]	Yes	No
Polyamines functionalized with acetyl chloride [121]	Yes	Yes
Mannitol-SAM [73]	Yes	Yes
Peptide-based SAM [74]	Yes	No
Polybetaine		
Poly(CBAA) [43,62,85,102,122,123]	Yes	Yes
Poly(SBMA) [100,102,124,125]	Yes	Yes
Poly(CBMA) [83,126]	Yes	Yes
Poly(MPC) [119,127,128]	Yes	Yes
PC-SAM [87,102]	Yes	Yes
OPC-SAM [87,102,129]	Yes	Yes
Polyampholyte		
SA/TMA-SAM [19,87,102]	Yes	Yes
CA/TMA-SAM [19,87,102]	Yes	Yes
PM/TMA-SAM [19,87,102]	Yes	No
Peptide surfaces derived from natural amino acids [90]	Yes	No
Poly(TM-SA) [12]	Yes	No
Poly(METMA-MES) [14]	Yes	No
PDDA/PSS [130]	Yes	No

specific groups of polymers with the substrate [13,14], and plasmas treatments [15].

It is hypothesized that the nonfouling ability of both polyhydrophilic and polyzwitterionic materials are tightly correlated with a hydration layer near the surface [16–18], because a tightly bound water layer forms a physical and energetic barrier to prevent protein adsorption on the surface. Water molecules residing on and/or penetrating into nonfouling materials can be classified into two types of "surface-bound" waters formed by hydrogen bonding for hydrophilic materials and by even more strongly ionic solvation for zwitterionic materials. Expulsion of water molecules from both surface and protein is the first and obligatory step to facilitate protein adsorption by reducing free energy barrier arising from dehydration entropic effects [19]. This is an intrinsic cause for nonfouling materials. The strength of surface hydration is primarily determined by physicochemical property of materials (i.e. molecular weight and surface chemistry) and their surface packing (i.e. film thickness, packing density, and chain conformation). Aside from surface hydration, chain flexibility also plays an important role in protein resistance especially for long-chain polymers. When protein approaches to the surface, the compression of the polymer chains causes steric repulsion to resist protein adsorption due to unfavorable decrease in entropy [20,21]. Although most of water-soluble polymers can reduce protein adsorption to some extent, the best nonfouling ability of polymers can only be achieved when surface hydration and steric repulsion work together. It is speculated that if chain flexibility plays a little role, chain hydration is an only source to resist protein adsorption (Fig. 1c), while if chain flexibility plays a certain role, both chain flexibility and chain hydration are important (Fig. 1a and b).

Several nonfouling material reviews were published recently [22,23]. These articles present a variety of antifouling materials synthesized by different methods. However, we believe that a more detailed and comprehensive review that combines design principles and biological applications would be useful for the biomaterial community. This review includes three major parts. The first part discusses the importance of surface hydration in protein resistance using both experimental and computational evidences. The second part focuses on the hydrophilic materials while the third part on the zwitterionic materials, both following the concept of enhancing surface hydration. Examples of nonfouling materials and associated surface-grafting methods in each part are given to illustrate basic principles and practical applications. Finally, recent advances and future perspective are discussed.

2. Surface hydration: hydrogen bonding or ionic solvation

2.1. Experimental evidence of hydration layer

Numerous studies have been carried out to characterize the dynamics and structure of interfacial waters and their correlation with nonfouling properties. Tanaka et al. classified interfacial waters in poly(2-methoxyethyl acrylate) (PMEA) into three categories: nonfreezing water (non-crystallize at −100 °C), freezing bound water (crystallize below 0 °C), and free water (crystallize at 0 °C), they found that freezing bound water at about −40 °C displayed an excellent blood compatibility due to strong interactions with polymer via hydrogen bondings [24–26]. Similarly, Morisaku et al. measured and compared the contents of freezable (non-crystallizable at −70 °C) and non-freezable (crystallizable at about 0 °C) waters in poly(MPC) and poly(methoxy oligo(ethylene glycol)-monomethacrylate) [(Me(EG)nMA)] hydrogels [27]. They found that the poly(MPC) hydrogels had larger non-freezable water than the poly(Me(EG)nMA) hydrogels although the equilibrium water contents were similar and they thus proposed that the amount of non-freezable water around polymer chains may influence the degree of protein adsorption resistance. Along the similar line, more information about structure transition of nonfouling materials before and after exposed to water was investigated. Fick et al. showed the swelling behavior of self-assembled monolayers of alkanethiol-terminated poly(ethylene glycol) by neutron reflectometry [28]. Zolk et al. used IR–vis SFG to demonstrate the conformational change of OEG-SAMs on gold from an ordered crystallize state to a disordered amorphous state when the OEG-SAMs were switched from dry air condition to aqueous solution [29]. Wang et al. applied vibrational sum-frequency generation (VSFG) to confirm that interfacial water molecules were able to not only retain in SAM film even after the sample was removed from the fluid, but also recover the disturbed SAM structure after re-immersing the sample into waters [30]. All theses results indicate that water molecules can be associated with the hydrophilic film due to their great capability of hydration.

From a more universal point of view, researchers who pay more attention on the strong interactions between water molecules and polymers provided further evidences in such a correlation between strong hydration of polymers and their resistance to protein adsorption. When hydrophilic polymers contact with bulk water,

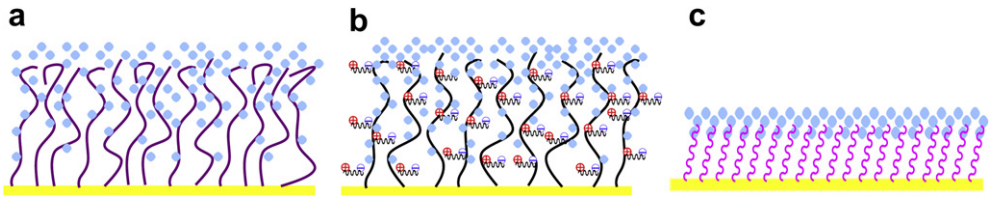

Fig. 1. Illustration of chain hydration and chain flexibility of (a) hydrophilic polymers, (b) zwitterionic polymers, and (c) SAMs, which attribute to surface resistance to nonspefic protein adsorption in different ways.

water molecules penetrate into the polymer film to form a hydrogen-bond network in the polymers. The highly hydrated polymer films exhibit nonfouling property, thus any decrease in the surface hydration might lead to the decrease of resistance to nonspecific protein adsorption. Since hydrogen bonds are relatively easily to break and reform, hydrophilic materials such as polyamide, mannitol, and PEG often experience the transition from nonfouling to fouling upon the change in surface hydration [31] caused by increase of packing density [10,32], increase of hydrophobicity when copolymerized with hydrophobic monomers [17], and raise of temperature [33,34].

On the other hand, zwitterionic materials containing both positive and negative charged units can bind water molecules even more strongly and stably via electrostatically induced hydration, as compared to those hydrophilic materials to achieve surface hydration via hydrogen bonding. Thus, materials with more strongly ionic solvation fall into the scope of searching nonfouling materials. pMPC, a big group of bio-mimetic polymer, has been extensively investigated with a long history in its biocompatibility for numerous applications [35–38]. Kitano et al. found that more bound water around the surface of zwitterionic materials than other hydrophilic or hydrophobic materials [39], which revealed the differences in the nature and strength of hydration between ionic solvation and hydrogen bonding. Holmlin et al. [40] demonstrated that the resistance to nonspecific protein adsorption of a mixed SAMs formed by a 1:1 combination of a thiol terminated in a trimethylammonium group and a thiol terminated in a sulfonate group is comparable to the best known OEG-SAM system. This result indicates that the flexibility in short-chain OEG-SAM systems is not required, only strong hydration of SAMs became a necessary prerequisite for protein resistance. On the other hand, the hydration of zwitterionic groups could be greatly compromised to cause the increase of nonspecific protein adsorption at certain experimental conditions such as very low ionic solution, very large molecular weight, extreme high or low packing density, or low temperature [10,41–43].

2.2. Molecular simulations of nonfouling materials

In parallel to extensive experimental efforts, molecular simulations provide atomic-level information to understand the nonfouling mechanism. De Gennes and co-workers [20] reported the first theoretical studies of the resistance of poly(ethylene glycol) (PEG) polymers to protein adsorption. They concluded that steric repulsion resulting from the compression of PEO chains as protein approaches the surface was mainly responsible for prevention of protein adsorption. It was predicted that the longer chain lengths and higher surface densities lead to better protein resistance. Szleifer et al. [44,45] improved the De Gennes's model using the single chain mean field (SCMF) theory. They found that polymers grafted to a hydrophobic surface can reduce protein adsorption simply because the polymers blocked protein adsorption sites. In the Szleifer model, surface density was a key factor to control protein adsorption, but chain length had a little effect on protein adsorption. Thus, the Szleifer model is able to interpret experimental results for both long-chain PEG and short-chain oligo (ethylene glycol) (OEG) polymers. Both De Gennes and Szleifer models highlight the importance of surface density of grafted polymers to resist protein adsorption, but their models do not provide atomic-level information about nonfouling mechanism. In their work, the protein was modeled as a structureless, spherical particle, while water was treated as a continuous medium in the De Gennes model or as a homogeneous spherical noninteracting molecule in the Szleifer model. While simplified models allow simulations to be performed in the time scale of seconds, the detailed conformational change of a protein and polymer chains was ignored. Later, the Grunze group [17,46,47] performed grand canonical Monte Carlo (GCMC) simulations to study the effect of conformation of OEG-terminated alkanethiol SAMs on gold and silver substrates on the interaction of waters with OEG-SAM. They attributed OEG-SAMs with helical conformation on Au (h-SAM) to reduce protein adsorption, while those SAMs with all trans conformation on Ag (t-SAM) enhance protein adsorption. Their results showed that more water molecules penetrated into h-SAM than t-SAM to form hydrogen bonds with OEG chains, leading to the prevention of protein adsorption on the surfaces.

Since molecular details of a surface are of great importance to protein adsorption, a series of explicit-solvent molecular dynamics (MD) simulations [16,48,49] were performed to examine the effect of OEG surface density on the lysozyme adsorption by varying the composition of $\chi_{OEG} = 0.2$, 0.5, 0.8, and 1.0 in a mixed SAMs of $S(CH_2)_4(OCH_2CH_2)_4OH$ (OEG-SAM) and $S(CH_2)_4OH$ (OH-SAM). Simulation results show that more water molecules are tightly bound at the protein/SAMs interface via hydrogen bonding at medium OEG surface densities $\chi_{OEG} = 0.5$ and 0.8 than those at too high ($\chi_{OEG} = 1.0$) or too low ($\chi_{OEG} = 0.2$) OEG surface densities. Recent surface plasmon resonance (SPR) studies of protein adsorption on the OEG-SAMs [10] also show that OEG-SAMs resist protein adsorption within a certain range of the surface OEG densities (0.6–0.8), yet adsorb proteins when the OEG surface density is too high or too low (Fig. 2a). By the comparison of experimental and simulation results, it appears that there is a correlation between the nonfouling properties of the OEG-SAM surface and the hydration of OEG chains (or appropriate OEG surface density). To directly correlate hydration force with nonfouling ability of a surface, restrained MD simulations [16,50] are performed to calculate the interaction forces exerted onto a protein from an OEG-SAMs and interfacial solvent as the protein approaches to the surface from a large separation down to intimate contact. Force vs distance profiles (Fig. 2b) show that relative strength of repulsive force acting on the protein is in the decreasing order of OEG-SAMs > OH-SAMs > CH_3-SAMs. Moreover, the total repulsive force is decomposed into two contributions: one from the SAM surface and the other from

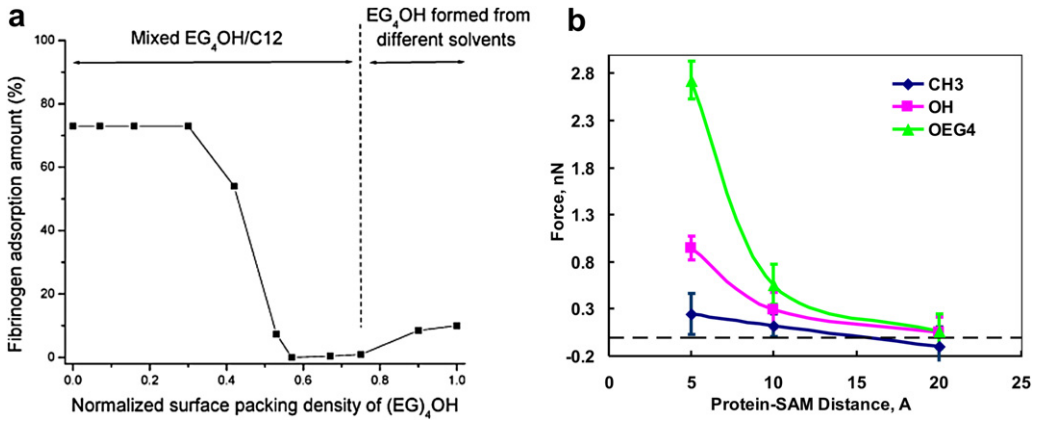

Fig. 2. (a) The amount of adsorbed fibrinogen as a function of the surface packing density of (ethylene glycol)$_4$ OH-SAM [10]. Copyright 2005 American Chemical Society. (b) Interaction force acting on the Lysozyme from three SAM surfaces [16]. Copyright 2005 Biophysical Journal.

interfacial water. Results show that the repulsive force mainly comes from interfacial waters (over 95% contribution to total force), not from the SAM surface. On the other hand, the dynamic, structural, and binding properties of interfacial waters are indeed affected by the physicochemical properties of surfaces, indicating that the interplay interactions between waters and surfaces are essential for nonfouling ability.

Along the same line, although simulations demonstrate the hydration force originated from interfacial waters at the hydrophilic OEG-SAMs is dominant driving force to resist protein adsorption, "surface hydration hypothesis" derived from hydrophilic surfaces is needed to be further examined for zwitterionic surfaces to establish the generality of nonfouling mechanism. Additional MD simulations are performed to study the interactions of zwitterionic phosphorylcholine-terminated SAM [$S(CH_2)_{11}PO_4(CH_2)_2N(CH_3)_3$, PC-SAM] [49,51] with a model protein of lysozyme. The PC-SAMs display a much stronger repulsive force to protein raised from interfacial waters than the OEG-SAMs. Although both PC-SAM and OEG-SAM have been demonstrated as nonfouling surfaces by experiments [10,52], comparison of interfacial waters near the PC-SAM and OEG-SAM surfaces from simulations reveals different structural and dynamic properties. First, interfacial waters near zwitterionic PC-SAM surface have longer residence time (stay longer) and smaller self-diffusion coefficient (move slower) than those near the hydrophilic OEG-SAM surface, indicating that PC-SAM surface binds water molecules more strongly than the OEG-SAM surface. Secondly, interfacial waters at the PC-SAMs have much larger reorientational dynamics than those at the OEG-SAMs, indicating that the PC-SAM binds waters via ionic solvation while the OEG-SAM via hydrogen bonding. Furthermore, based on the fact that surface hydration is well correlated with nonfouling properties derived from PEG and PC systems, a simple computational method is developed to quickly evaluate the intrinsic hydration capacity of model surface coating functional groups by computing hydration volume and number of hydrating solvent molecules [53]. Fig. 3 shows a range of hydration capacity and nonfouling performance of three class materials of ethylene glycols, sugar alcohols, and glycine analogues. With known as nonfouling materials from experiments, OEG with four ethylene glycol repeat units, MAN (mannitol), SORB (sorbitol), and TMG (quartenary amine trimethylglycine) have scaled number of associated water molecules greater than 0.15, while GLY (primary amine glycine), 1 MG (secondary amine sarcosine), and DMG (tertiary amine dimethylglycine), all known to allow nonspecific protein adsorption, are less than 0.15. Consistent experimental measurements and simulation calculations suggest a critical threshold value of hydration capacity (~0.15) for initially screening nonfouling materials.

3. Hydrogen bonding-rich materials

Many hydrophilic materials have been demonstrated their nonfouling ability to reduce not only nonspecific protein adsorption, but also cell and bacteria adhesion. Hydrophilic PEG-based polymers [2,54–56] and polymers incorporating oligosaccharide moieties [57,58] are inherently anti-biofouling in nature. Hydration of materials via hydrogen bonding is often used to interpret the molecular basis for nonfouling behavior by experimental and computational works.

3.1. Short-chain hydrophilic SAMs

Molecular details of the surface are of great importance to protein adsorption. SAMs are well suitable platforms to study protein adsorption at the molecular-level since nanoscale surface properties can be precisely controlled via varying the abundance, type, and spatial (both normal and lateral) distribution of tail groups of the SAMs. Over the last 10–15 years, among many studies to search for nonfouling materials beyond PEG, Whitesides and his co-workers have used SPR and SAMs as a platform to systematically test and indentify a large number of protein-resistant SAMs with

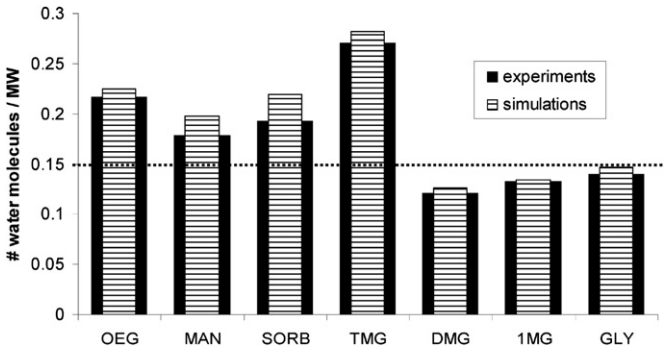

Fig. 3. Hydration capacity (i.e. number of hydrated waters) for a series of nonfouling materials evaluated by experiments and simulations, where OEG represents Oligo (ethylene glycol) with four ethylene glycol repeat units, MAN (mannitol), SORB (sorbitol), GLY (primary amine glycine), 1MG (secondary amine sarcosine), DMG (tertiary amine dimethylglycine), and TMG (quartenary amine trimethylglycine). Dash line indicates a threshold intrinsic hydration capacity for nonfouling surfaces. Copyright 2008 American Chemical Society.

different functional groups [3,4]. Four common features for those surfaces resisting protein adsorption are summarized – (i) hydrophilic, (ii) electrically neutral, (iii) hydrogen bond acceptors, and (iv) not hydrogen bond donors. However, this generalization does not explain all nonfouling surfaces especially carbohydrates terminated SAMs. For example, a SAM terminated with mannitol groups only containing H-bond donor were found to prevent the adsorption of several proteins and the attachment of cell and was indistinguishable from a SAM presenting oligo (ethylene glycol) ($-(EG)_nOH$ and $-(EG)_nOCH_3$, $n = 3-6$) groups [50]. This result further indicates that the formation of tightly bound water via hydrogen bonds between interfacial waters and surfaces does not necessarily require "H-bond acceptors" materials, rather than "H-bond forming" materials.

Both nanoscale surface structural and chemical properties of SAMs affect protein adsorption, including adsorption kinetics, adsorption isotherm, and protein morphology on surfaces. As can been seen in Fig. 4a, COOH-terminated SAMs formed at higher temperature of 50 °C have more compact structures than those formed at room temperature of 22 °C. The difference in nanoscale structure was revealed by low-current STM but was not detectable from X-ray photoelectron spectroscopy (XPS) and contact angle measurements. Carboxylic-terminated SAMs formed at higher temperatures generally promote protein adsorption because of their compact structures and fewer adsorbed water molecules. However, the adsorbed amount of protein on hydrophobic methyl-terminated SAMs prepared at different solution temperatures (55 °C and 22 °C) is similar because of similar adsorbed water molecules despite their nanoscale structural difference, highlighting the importance of surface chemistry. Similarly, surface density and structure can also be controlled by using different solvents to assemble SAM chains. XPS reveals that OEG-SAMs assembled from a 95% ethanol and 5% water solution show higher packing density on gold than those from pure ethanol solution [10]. SPR results show that more fibrinogens adsorb on the densely packed OEG-SAMs prepared by mixed ethanol and water solution than those on relatively loosely packed OEG-SAMs prepared by pure ethanol solution (Fig. 4b). The normalized coverage of fibrinogen and lysozyme on OEG-SAMs assembled from the 95% ethanol solution is about 8.5% and 6%, respectively, assumed a full monolayer coverage of protein on C_{16}SH-SAM surface and use this coverage to normalize the amount of protein adsorbed on the OEG-SAMs. Although the adsorbed amount of protein is small ($\sim$18 ng/cm^2 or $\sim$8.5% surface coverage) on the OEG-SAMs prepared from the 95% ethanol solution, previous studies show that as little as 10 ng/cm^2 adsorbed fibrinogen could induce monocyte adhesion *in vitro* [59]. Early studies [10,17,32] also show that too high or low OEG surface densities will lead to protein adsorption. Appropriate OEG surface densities are thus required for its nonfouling behavior (Fig. 2).

Moreover, mixed SAMs prepared from a 1:1 mixture of positively charged 11-mercapto-N,N,N-trimethylammonium chloride (TMA) and one of the following negatively charged compounds: SA, 12-mercaptododecanoic acid (CA), 11-mercaptoundecylphosphonic acid (PA), or methyl 11-mercaptoundecylphosphonate (PM) are further studied to examine their protein-resistant properties [19,40]. SPR results in Fig. 5 show that all these mixed SAMs (TMA/SA, TMA/CA, and TMA/PM) had been shown to have $<$0.3 ng/cm^{-2} protein adsorption from single fibrinogen, lysozyme, and BSA solution. Although SAM is a model system to study the interactions between protein and surface due to ease of preparation and modification, non-covalent bonds between thoil and gold may not be stable enough in complex media and any surface defect in SAM structures can greatly and quickly reduce nonfouling property.

3.2. Long-chain hydrophilic polymers

Surface hydration is often used to interpret the nonfouling behavior of the short-chain SAMs because the tightly bound water molecules on the topmost part of SAMs are only source of large repulsive forces to repel protein adsorption, whereas chain flexibility plays little role in protein resistance due to tightly packed density and short rigid chains. But, for the long-chain polymer, both surface hydration (i.e. water barrier) and chain flexibility (i.e. steric repulsion) contribute to surface resistance to nonspecific protein adsorption. Unlike surface hydration, the steric repulsion results from the compression of long polymer chains to yield repulsive forces to resist protein adsorption. It is generally accepted that the nonfouling properties of a surface are determined by both functional groups and their surface packing. There are two ways to fabricate polymer brushes: physisorption and covalent attachment. Covalent attachment can be accomplished by either "graft-to" or "graft-from" approaches. The "graft-from" approach is a more promising method in the synthesis of polymer brushes with a high grafting density and well-controlled film thickness, while "graft-to" method is more convenient for practical applications [14].

Numerous methods have been developed to immobilize PEG-based materials (i.e. the most commonly used nonfouling materials today) on surfaces including physisorption [6,13,60], chemisorption [61,62], covalent grafting of PEG onto surfaces [63,64], and plasma polymerization of OEG precursors [12,15]. The nonfouling properties of PEG are primarily due to their high chain mobility, large exclusion volume, and steric hindrance effect of highly hydrated layer. Luesse and Arnold applied proton and deuterium NMR relaxation time measurements to determine the number of water molecules per ethylene glycol repeat unit ($-CH_2CH_2-O-$) in PEG. They observed that the maximum water content was one water molecule per ethylene glycol unit [65]. The enthalpy of hydration per mole of oxyethylene group is $\sim$7 kJ measured by both microtitration calorimetry [66] and NMR [67]. Although PEG displays excellent protein resistance ability *in vitro*, *in vivo* studies of cell/bacteria adhesion on PEG surfaces showed that bacteria can attach to PEG-based surfaces at the low surface density [68] or to the nanopatterned PEG-based surfaces [69], highlighting the importance of surface density and topology for nonfouling properties. The cell/bacteria adhesion on the PEGylated surfaces might be caused by following possible reasons: (i) The PEG-based materials are readily subject to oxidation in most biochemically relevant solutions [59,70–72]. The short-term stability of PEG is one of its major limits responsible for decreased performance in preventing nonspecific protein adsorption and (ii) Defects on the PEG-based surfaces always induce nonspecific protein adsorption and cell attachment. For example, the "graft-to-surface" methods via physical adsorption, dipping, and cross-linking usually result in more defects and less coverage on the surface than the "graft-from-surface" method such as SI-ATRP, thus leading to more protein adsorption and cell adhesion. It should be noted that the nonfouling mechanism of PEG-based surfaces to cell/bacterial adhesion is still not conclusive due to the complex response and interactions among PEG, cell/bacterial, and associated environmental stimuli, especially in complex medium such as *in vivo* or conditions with full nutrition for biofilm formation.

Recently, substantial efforts have been made toward the development of alternative bioinert polymers as a substitute for PEG, including polyoxazolines [73,74], polyglycerol (PG) dendrons [75,76], polysaccharides [57], and zwitterionic polymers. Concepts learned from Nature provide a basis for designing new interfacing materials. The blood contacting face of the vascular endothelial cells is nonfouling and non-activating, suggesting biomimetic design ideas. Poly-2-methyl-2-oxazoline (PMOXA), a peptide-like polymer [77], exhibited a significant reduction in full human serum

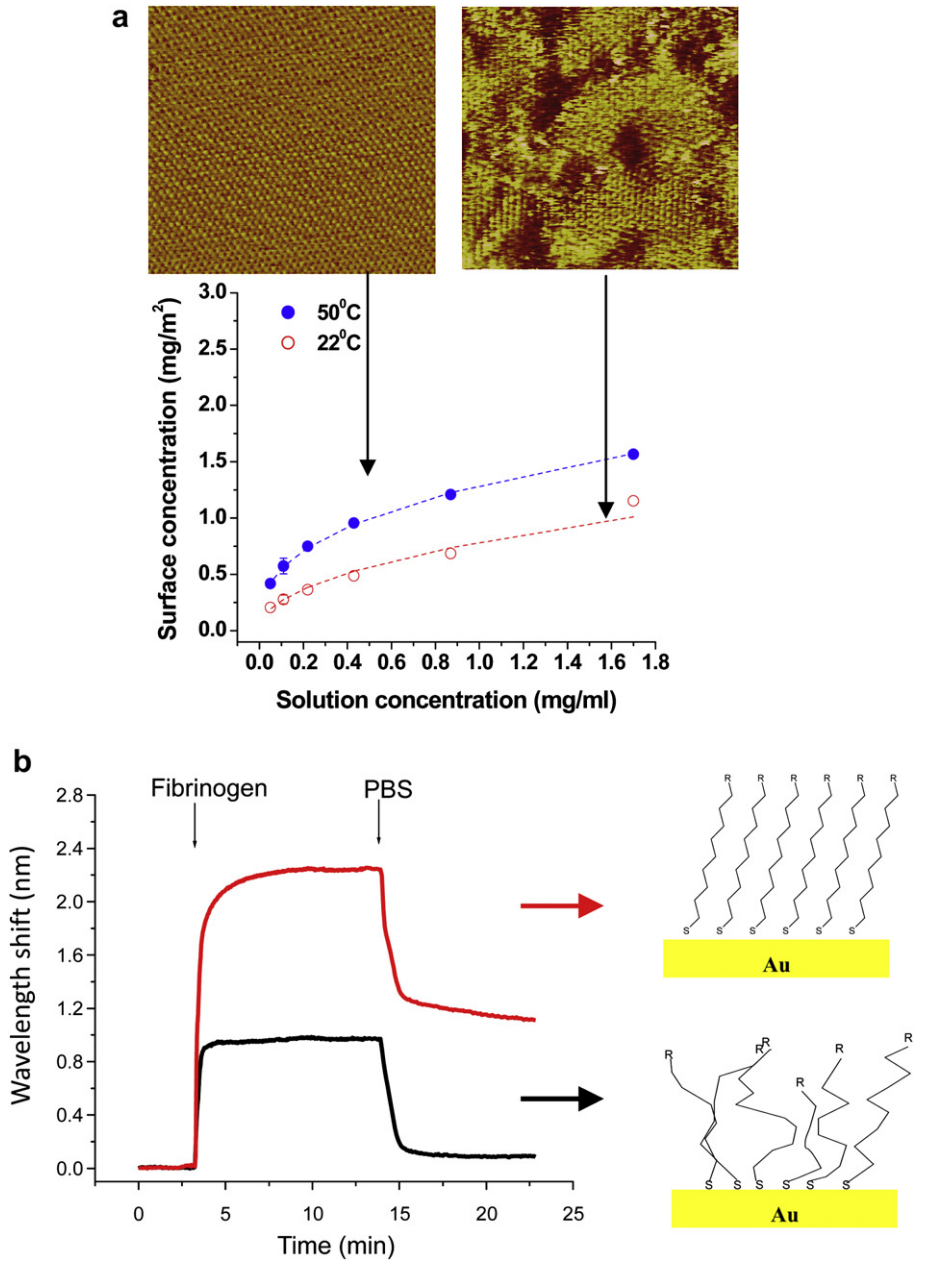

Fig. 4. The effect of surface packing density of SAM chains induced by temperature and solvent on the protein adsorption. (a) Protein adsorption is different on COOH-SAMs prepared at higher or room temperature from SPR experiments. STM images (20 nm × 20 nm) show more uniform SAMs form at higher temperature. (b) Fibrinogen (Fn) has 8.5% monolayer coverage on highly packed OEG-SAMs prepared from mixed 95% ethanol and 5% water solution while loosely packed OEG-SAMs prepared from pure ethanol resist Fn adsorption.

protein adsorption quantitatively to the same level as for PLL-g-PEG (<2 ng/cm^2 as determined by optical waveguide lightmode spectroscopy). Dendritic polyethers based on glycerol dendrimers and linear/hyperbranched polyglycidols [78] are highly biocompatible with well cytotoxic toleration by mice even when injected in high does. Mimicking biological cell membranes containing highly hydrated glycocalyx provides another approach for conferring antifouling properties to surfaces [57] because carbohydrates – the principal component of the glycocalyx – are thought to be mainly responsible for its ability to prevent undesirable nonspecific adsorption of protein. A series of oligosaccharide surfactant polymers consisting of a flexible polyvinylamine (PVAm) backbone with hydrophilic dextran or oligomaltose and hydrophobic hexanoly side chains [58,79] were synthesized and coated on the hydrophobic surface such as graphite, medical-grade polycarbonate, and OTS via a simple dipping method. On hydrophobic surface, the hydrophobic hexanoly chains assemble through hydrophobic interactions and are constrained to lie parallel to the substrate, while solvated hydrophilic dextran side chains protruding into the aqueous phase to shield a biomaterial from adhesive bacterial interactions. The application of thin oligosaccharide surfactant polymers has been shown to reduce non-adhesive interactions with fibrinogen and reduce infection and thrombus formation via non-adhesive interactions with proteins, platelets, cells and bacteria.

4. Zwitterionic materials

Zwitterionic materials are considered as biocompatible because they are bio-mimetic ones. Two types of nonfouling

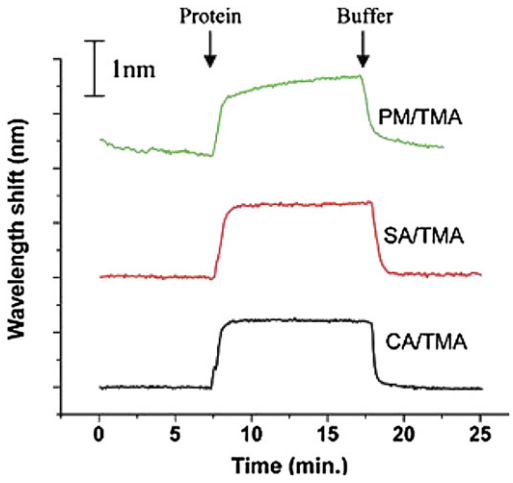

Fig. 5. Fibrinogen adsorption on three 1:1 mixed SAMs of positively and negatively charged components of SA/TMA, CA/TMA, and PM/TMA. All these mixed charged SAMs have demonstrated their highly resistant to Fn adsorption. Copyright 2006 American Chemical Society.

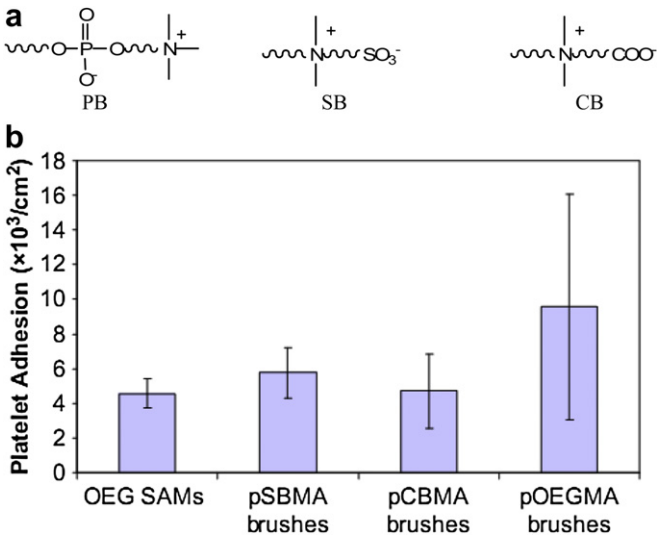

Fig. 7. (a) Chemical structures of phosphonate-betaines (PB), sulfonate-betaines (SB), and carboxylate-betaines (CB). (b) Platelet adhesion on OEG-SAM and different polymer brush surfaces: polySBMA, polyCBMA, and polyOEGMA. Copyright 2008 Elsevier Ltd.

polyzwitterionic materials are polybetaines with positively and negatively charged moieties on the same monomer unit and polyampholytes with positively and negatively charged moieties on different monomer units. A critical factor determining nonfouling property of polyzwitterionic materials is to control both uniformity of charge distribution and charge neutrality of two opposite charge moieties on the surface (Fig. 6), which is the guide line to design new nonfouling polyzwitterionic materials. In such a way nonfouling polyzwitterionic materials could maximize the surface hydration and reduce charge interaction with protein molecules. In this section, we will focus on the most recent advance in the design of various polybetaines and polyampholytes following this design guide line on surfaces for biological applications.

4.1. Polybetaines

Polybetaines can be further classified into three major groups based on the negatively charged groups: sulfonate-betaines (SB), carboxylate-betaines (CB), and phosphonate-betaines (PB) (Fig. 7). The first synthetic polybetaine is polycarboxybetaine through the hydrolysis of poly(4-vinylpyridine) reported by Ladenheim and Morawetz in 1957 [80]. A number of polybetaines such as phosphorylcholine (PC)-based copolymers [35,81,82], polySB, and polyCB materials [41,62,64,83–85] have been synthesized and characterized for demonstrating their biocompatibility and

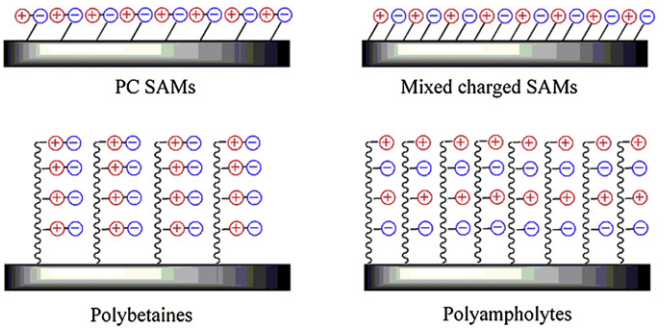

Fig. 6. Illustration of the guide line to design new nonfouling polyzwitterionic materials. All these surfaces have both features in uniformity of charge distribution and charge neutrality of two opposite charge moieties on the surface, which show excellent resistance to proteins.

hemocompatibility to reduce nonspecific protein adsorption and consequent platelet adsorption in various biological applications.

To provide insights into the molecular-level nonfouling mechanism of zwitterionic materials, key factors leading to the strong resistance of zwitterionic phosphorylcholine (PC)-terminated SAMs on gold were examined by both experimental and molecular simulation methods [49,51,52]. Results indicated that three key properties, including the charge balance, the minimized dipole and the close packing density, lead to fibrinogen adsorption of $<$0.3 ng/cm^2 and BSA adsorption of $<$0.1 ng/cm^2 when 1:1 N/P ratio is presented to maintain the charge neutrality of PC-SAMs where N atom represents a positive charge and P atom represents a negative charge in PC [52]. The thickness of PC-SAMs is lower than the configuration of PC group pointing to solution, indicating that PC groups are in an antiparallel orientation through charge and dipole interaction. An additional MD simulation revealed that the PC-SAMs could form 2-D crystalline structure with the same packing configuration as PC head groups of membrane lipids, in agreement with the observation in experiments [49,51,52].

Upon these PC results, the nonfouling property of polySB and polyCB were explored since those groups possess the similarity in charge distribution and neutrality as PC [11,84]. The nonfouling properties of SB and CB have been demonstrated by several well-controlled methods to form polymer thin film via surface-initiated atom transfer radial polymerization (SI-ATRP) [86], self-assembly [84], free radical polymerization [87], and 3,4-dihydroxyphenylalanine (DOPA) linkage [14]. Ladd et al. showed the $<$5 ng/cm^2 adsorbed proteins from 100% blood plasma on gold surface coated with polyCB by surface-initiated-ATRP [64], Yang et al. showed the $<$0.3 ng/cm^2 adsorbed proteins from 100% blood plasma when the polyCB film thickness is optimized at $\sim$20 nm [43]. Yang et al. also demonstrated that the nano gold particles keep the unchanged diameters in undiluted human blood serum by dynamic light scattering method [88]. With these three experiments, it is believed that the ultralow-fouling of the proteins on a material surface leads to 'invisible' nanoparticles in blood, which can avoid blood clot process and possibly escape from immune response. These results also indicated that multiple proteins in a complex blood plasma require even more stringent surface properties in nanoscale and macroscale to prevent protein

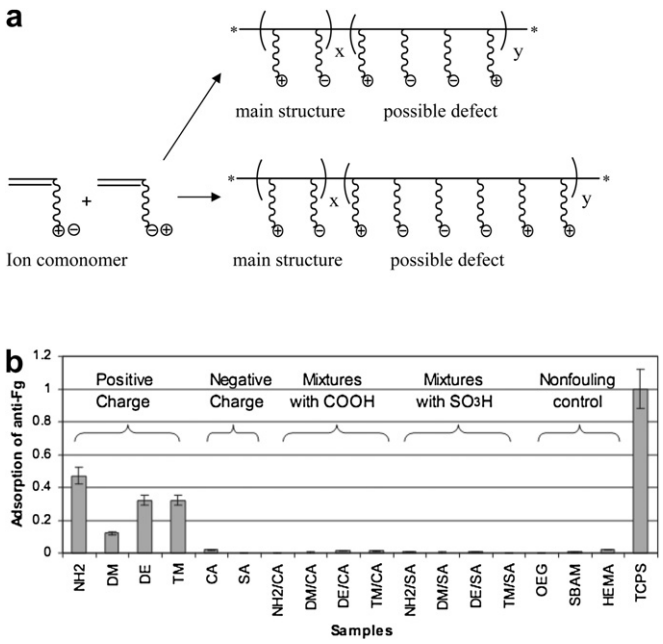

Fig. 8. (a) Illustration of the polyampholytes surface with perfect main structure and possible surface defects. (b) Anti-fibrinogen adsorption on various hydrogels. Copyright 2008 Wiley-VCH, Weinheim.

adsorption. The optimization of surface roughness, pattern, and thickness is critical for avoiding uncertain surface defects and thus minimizing protein adsorption [41].

Up to cell level, the resistance to microbial adhesion on material surfaces is also a property being examined since the adhesion of platelet or bacteria could lead to infection when medical devices are inserted into or implanted in patients. Fig. 7b shows platelet adhesion level of $5.76 \times 10^3/cm^2$, $4.71 \times 10^3/cm^2$, and $9.57 \times 10^3/cm^2$ on three polymer brushes of polySBMA, polyCBMA, and polyOEGMA, respectively, in comparison to $4.57 \times 10^3/cm^2$ platelet adhesion on the OEG-SAM. In blood, the presence of adherent platelets usually shortens the plasma clotting time because platelets catalyze undesirable thrombin formation. Blood clotting tests have also shown that all these polymer brushes did not alter plasmas clotting time, especially for polyCBMA brush that exhibits significant anticoagulant activity. PolyCB was further subject to the study of long-term biofilm formation. The surface coated with 30 nm polyCB can greatly suppress the adhesion of *Pseudomonas aeruginosa* over ten days, having less than 7% of *P. aeruginosa* coverage on the untreated glass surface [89]. Control biofilm experiments of OEG-SAM that has no protein adsorption and low platelet adhesion fail to resist long-term bacterial formation. Based on the experiments illustrated above, it is not necessarily true that the surface to resist protein adsorption is able to prevent platelet adhesion and biofilm formation, because the latter involves more harsh and complex environmental conditions such as bacteria strength and growth medium. Meanwhile polyCBMA is a promising polymer for blood-contacting applications, as well as for environmentally friendly coatings for anti marine fouling, due to its excellent resistance to protein, platelet, and bacteria adhesion [41,64].

4.2. Polyampholytes

Besides three classic zwitterionic polybetaines such as MPC, SB and CB, polyampholytes is another big group of materials with the closest structure to polybetaine polymers. Polyampholyte polymers are formed by a pair of separate mononers with two opposite charge moieties respectively. The uniformity of charge distribution and charge neutrality of polymers is mostly achieved by 1:1 homogeneous reaction mixture of the two oppositely charged monomers before co-polymerization. Although there is a possibility to form minor defects with two or more same charge connected together in ion-pair comonomer polymer (Fig. 8a), recent works of ion-pair comonomers polymer [12,14] demonstrates that such minor defects are negligible for protein adsorption. It is believed that the statistically distribution of charge groups are rather homogenous in nanometer scale. Possible rearrangement of charge groups through soft polymer backbone might also reduce the defects in charge distribution. Thus, it is possible to design a wide range of new nonfouling polyampholytes through mixed charge monomers.

To demonstrate the importance of uniform charge distribution on the surface in the protein adsorption, different polyampholytes with net and neutral surface charges were examined for their resistance to protein (Fig. 8b). Similar to well-known nonfouling surfaces of OEG and SBMA, all mixed charge polyampholytes with homogenous charge balance including 2-carboxyethyl acrylate (CA)-based mixtures with NH_2 (NH_2/CA), (Dimethylamino)ethyl methacrylate (DM/CA), 2-(Diethylamino)ethyl methacrylate (DE/CA), 2-(Methacryloyloxy)ethyl trimethylammonium chloride (TM/CA) and 3-Sulfopropyl methacrylate potassium salt (SA)-based mixtures of NH_2/SA, DM/SA, DE/SA, TM/SA exhibit extremely low fibrinogen adsorption at a wide range of pH and ionic strength. As expected, deviation from charge neutrality can induce electrostatic interactions between proteins and surface, leading to protein adsorption. This fact indicates that charge balanced polyampholytes are equivalent to polybetaines with very stable and robust structures at nonphysiological conditions.

SAMs with mixed positively and negatively charge groups are another format of mixed charge polymers. Even a 1:1 mixture of cationic and anionic SAM displays excellent resistance to nonspecific protein adsorption, Chen et al. closely investigated these zwitterionic SAMs. They show better resistance to protein adsorption than the statistical copolymers [19,40] since the mobility of thiols on gold surface improved uniformity of charge distribution. The molecular simulation shows a 2-D crystalline structure formed in the mixed SAMs of 1:1 mixture of TMA/SA on Au(111). The lattice of mixed TMA/SA SAMs revealed by atomic force microscopy is about (5.2 ± 0.2 Å $\times$ 5.2 ± 0.2 Å) 60°, which is similar to the same packing configuration as PC head groups of membrane lipids. This indicated that the packing structure of mixed SAMs is determined by strong charge–charge interactions of the terminal groups rather than S–Au and chain–chain interactions. Simulations also indicate that a balanced charge and minimized dipole are essential for enhancing hydration capacity at the topmost layer via electrostatic interactions [52].

Besides the statistical copolymers from the two charged components, natural peptides with alternating negatively charged Glu or Asp and positively charged Lys or Arg are another candidates as biodegradable nonfouling materials since their final metabolized products are natural amino acids. Based on the design principle learned from mixed charged polyampholytes polymers and SAMs, results show that peptides with alternating Glu/Lys or Asp/Lys via self-assembly are highly resistant to the nonspecific adsorption of fibrinogen, lysozyme, and albumin (<0.3 ng/cm^2) [90], comparable to PEG-based materials. This work demonstrates for the first time the development of ultralow-fouling materials from natural peptides, providing a convenient way to synthesize super stealth natural peptides by genetic engineering methods. Furthermore, these novel peptides mimic natural proteins and are biodegradable and nontoxicity through metabolism of peptides and may help understand how various peptide sequences play their roles in

protein–protein interactions and protein stability. Overall, strong hydration ability through ionic solvation of polyampholyte plays a key role in their nonfouling properties. A nanometer-scale homogenous mixture of balanced charge groups provide an excellent condition for charge groups to be fully ionized through the electrical field effects of the opposite ions, which override the weakening effect of weak acid or base in crowded conditions, which leads to the best condition for surface hydration. This homogeneity of opposite charge groups could avoid protein adsorption induced local charge interaction.

5. Conclusions and perspectives

The development of nonfouling materials and associated surface coating methods is critically important for many biomedical applications. Zwitterionic materials, especially for polyampholyte polymers, are very well positioned to play a role in the development of next-generation antifouling and antimicrobial materials. The advantages of nonfouling zwitterionic materials include the simplicity of synthesis, ease of applicability, abundance of raw materials, and availability of functional groups. With the understanding of the nonfouling mechanism and the design principle that the nonfouling properties are mainly attributed to surface hydration depending on the surface chemistry and physical packing of materials, future research direction could focus on the development of nonfouling materials with the general property and special function to meet challenge of the long-term stable performance in more complex condition *in vivo* [91]. Specially, (1) zwitterionic functional groups that possess the maximum polarity are used to enhance surface hydration via electrostatic interactions; (2) functional groups, such as carboxyl or amino groups, will be a part of nonfouling materials. For example, dual functional polyCB can achieve both protein resistance and ligand immobilizability [83,85]; (3) apart from surface chemistry, novel synthesizing and surface coating approaches need to be developed to optimize the physical properties of the surface. The basic information is critical to our understanding of the factors involved in nonfouling polymers and this information is the basis of efforts to design new and more effective zwitterionic materials.

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References

[1] Ratner BD, Bryant SJ. Ann Rev Biomed Eng 2004;6(1):41–75.
[2] Harris JM. Poly(ethylene glycol) chemistry - biotechnical and biomedical applications. New York and London: Plenum Press; 1992.
[3] Chapman RG, Ostuni E, Takayama S, Holmlin RE, Yan L, Whitesides GM. J Am Chem Soc 2000;122(34):8303–4.
[4] Ostuni E, Chapman RG, Holmlin RE, Takayama S, Whitesides GM. Langmuir 2001;17(18):5605–20.
[5] Chen Y, Thayumanavan S. Langmuir 2009;25(24):13795–9.
[6] Chang Y, Chu WL, Chen WY, Zheng J, Liu L, Ruaan RC, et al. J Biomed Mater Res A 2010;93A(1):400–8.
[7] Ma C, Hou Y, Liu S, Zhang G. Langmuir 2009;25(16):9467–72.
[8] Harbers GM, Emoto K, Greef C, Metzger SW, Woodward HN, Mascali JJ, et al. Chem Mater 2007;19(18):4405–14.
[9] Prime KL, Whitesides GM. Science 1991;252(5009):1164–7.
[10] Li L, Chen S, Zheng J, Ratner BD, Jiang S. J Phys Chem B 2005;109(7):2934–41.
[11] Zhang Z, Chen S, Chang Y, Jiang S. J Phys Chem B 2006;110(22):10799–804.
[12] Bernards MT, Cheng G, Zhang Z, Chen S, Jiang S. Macromolecules 2008;41 (12):4216–9.
[13] Gao C, Li G, Xue H, Yang W, Zhang F, Jiang S. Biomaterials 2010;31 (7):1486–92.
[14] Li GZ, Xue H, Gao CL, Zhang FB, Jiang SY. Macromolecules 2010;43(1):14–6.
[15] Johnston EE, Bryers JD, Ratner BD. Langmuir 2004;21(3):870–81.
[16] Zheng J, Li L, Tsao HK, Sheng YJ, Chen S, Jiang S. Biophys J 2005;89 (1):158–66.
[17] Herrwerth S, Eck W, Reinhardt S, Grunze M. J Am Chem Soc 2003;125 (31):9359–66.
[18] Chen S, Li L, Boozer CL, Jiang S. Langmuir 2000;16(24):9287–93.
[19] Chen S, Yu F, Yu Q, He Y, Jiang S. Langmuir 2006;22(19):8186–91.
[20] Jeon SI, Lee JH, Andrade JD, De Gennes PG. J Colloid Interface Sci 1991;142 (1):149–58.
[21] Jeon SI, Andrade JD. J Colloid Interface Sci 1991;142(1):159–66.
[22] Gong P, Grainger DW. Methods Mol Biol 2007;381:59–92.
[23] Jiang S, Cao Z. Adv Mater 2010;22(9):920–32.
[24] Tanaka M, Sackmann E. Nature 2005;437(7059):656–63.
[25] Tanaka M, Motomura T, Ishii N, Shimura K, Onishi M, Mochizuki A, et al. Polym Int 2000;49(12):1709–13.
[26] Tanaka M, Mochizuki A, Ishii N, Motomura T, Hatakeyama T. Biomacromolecules 2001;3(1):36–41.
[27] Morisaku T, Watanabe J, Konno T, Takai M, Ishihara K. Polymer 2008;49 (21):4652–7.
[28] Fick J, Steitz R, Leiner V, Tokumitsu S, Himmelhaus M, Grunze M. Langmuir 2004;20(10):3848–53.
[29] Zolk M, Eisert F, Pipper J, Herrwerth S, Eck W, Buck M, et al. Langmuir 2000;16(14):5849–52.
[30] Wang RY, Himmelhaus M, Fick J, Herrwerth S, Eck W, Grunze M. J Chem Phys 2005;122(16):164702.
[31] Gil ES, Hudson SM. Prog Polym Sci 2004;29(12):1173–222.
[32] Vanderah DJ, La H, Naff J, Silin V, Rubinson KA. J Am Chem Soc 2004;126 (42):13639–41.
[33] Leckband D, Sheth S, Halperin A. J Biomater Sci Polym Ed 1999;10:1125–47.
[34] Lutz JF, Akdemir Ö, Hoth A. J Am Chem Soc 2006;128(40):13046–7.
[35] Lewis AL. Colloids Surf B Biointerfaces 2000;18(3–4):261–75.
[36] Ishihara K, Nomura H, Mihara T, Kurita K, Iwasaki Y, Nakabayashi NJ. Biomed Mater Res Part A 1998;39(2):323–30.
[37] Iwasaki Y, Ijuin M, Mikami A, Nakabayashi N, Ishihara K. J Biomed Mater Res 1999;46(3):360–7.
[38] Xu Y, Takai M, Ishihara K. Biomacromolecules 2008;10(2):267–74.
[39] Kitano H, Mori T, Takeuchi Y, Tada S, Gemmei-Ide M, Yokoyama Y, et al. Macromol Biosci 2005;5(4):314–21.
[40] Holmlin RE, Chen X, Chapman RG, Takayama S, Whitesides GM. Langmuir 2001;17(9):2841–50.
[41] Yang W, Chen S, Cheng G, Vaisocherovaćć H, Xue H, Li W, et al. Langmuir 2008;24(17):9211–4.
[42] Azzaroni O, Brown AA, Huck WTS. Angew Chem Int Ed 2006;45 (11):1770–4.
[43] Yang W, Xue H, Li W, Zhang J, Jiang S. Langmuir 2009;25(19):11911–6.
[44] Szleifer I. Biophys J 1997;72(2):595–612.
[45] Fang F, Szleifer I. PNAS 2006;103(15):5769–74.
[46] Wang RLC, Kreuzer HJ, Grunze M. J Phys Chem B 1997;101(47):9767–73.
[47] Pertsin AJ, Grunze M. Langmuir 2000;16(23):8829–41.
[48] Zheng J, Li L, Chen S, Jiang S. Langmuir 2004;20(20):8931–8.
[49] Zheng J, He Y, Chen S, Li L, Bernards MT, Jiang S. J Chem Phys 2006;125 (17):174714–7.
[50] He Y, Chang Y, Hower JC, Zheng J, Chen S, Jiang S. Phys Chem Chem Phys 2008;10(36):5539–44.
[51] He Y, Hower J, Chen S, Bernards MT, Chang Y, Jiang S. Langmuir 2008;24 (18):10358–64.
[52] Chen S, Zheng J, Li L, Jiang S. J Am Chem Soc 2005;127(41):14473–8.
[53] Hower JC, Bernards MT, Chen S, Tsao HK, Sheng YJ, Jiang S. J Phys Chem B 2008;113(1):197–201.
[54] Bailey Jr FE, Koleske JY. Poly(ethylene oxide). New York: Academic Press; 1984.
[55] Pale-Grosdemange C, Simon ES, Prime KL, Whitesides GM. J Am Chem Soc 1991;113(1):12–20.
[56] Prime KL, Whitesides GM. J Am Chem Soc 1993;115(23):10714–21.
[57] Holland NB, Qiu YX, Ruegsegger M, Marchant RE. Nature 1998;392 (6678):799–801.
[58] Ruegsegger MA, Marchant RE. J Biomed Mater Res 2001;56(2):159–67.
[59] Shen M, Pan YV, Wagner MS, Hauch KD, Castner DG, Ratner BD, et al. J Biomater Sci Polym Ed 2001;12:961.
[60] Dalsin JL, Lin LJ, Tosatti S, Voros J, Textor M, Messersmith PB. Langmuir 2005;21(2):640–6.
[61] Tan J, Brash JL. J Biomed Mater Res A 2008;85A(4):862–72.
[62] Zhang Z, Vaisocherova H, Cheng G, Yang W, Xue H, Jiang SY. Biomacromolecules 2008;9(10):2686–92.
[63] Otsuka H, Satomi T, Ueno K, Mitamura Y, Tateishi T. Synthesis of polypyridine-graft-PEG copolymer for long-term stability of nonfouling character. In: Somiya S, Doyama M, editors. Transactions of the materials research Society of Japan, vol. 31. Amsterdam: Elsevier Science Bv; 2006. p. 627–31. No. 3.
[64] Ladd J, Zhang Z, Chen S, Hower JC, Jiang S. Biomacromolecules 2008;9 (5):1357–61.
[65] Lusse S, Arnold K. Macromolecules 1996;29(12):4251–7.

[66] Olofsson G. J Phys Chem 1985;89(8):1473–7.
[67] Klose G, Eisenblaetter S, Galle J, Islamov A, Dietrich U. Langmuir 1995;11 (8):2889–92.
[68] Kingshott P, Wei J, Bagge-Ravn D, Gadegaard N, Gram L. Langmuir 2003;19 (17):6912–21.
[69] Schulte VA, Dićez M, Moller M, Lensen MC. Biomacromolecules 2009;10 (10):2795–801.
[70] Li L, Chen S, Jiang S. J Biomater Sci Polym Ed 2007;18:1415–27.
[71] Ostuni E, Chapman RG, Liang MN, Meluleni G, Pier G, Ingber DE, et al. Langmuir 2001;17(20):6336–43.
[72] Herold DA, Keil K, Bruns DE. Biochem Pharmacol 1989;38(1):73–6.
[73] Luk YY, Kato M, Mrksich M. Langmuir 2000;16(24):9604–8.
[74] Chelmowski R, Koster SD, Kerstan A, Prekelt A, Grunwald C, Winkler T, et al. J Am Chem Soc 2008;130(45):14952–3.
[75] Wyszogrodzka M, Haag R. Biomacromolecules 2009;10(5):1043–54.
[76] Yeh PYJ, Kainthan RK, Zou YQ, Chiao M, Kizhakkedathu JN. Langmuir 2008;24 (9):4907–16.
[77] Konradi R, Pidhatika B, Muhlebach A, Textort M. Langmuir 2008;24 (3):613–6.
[78] Kainthan RK, Janzen J, Levin E, Devine DV, Brooks DE. Biomacromolecules 2006;7(3):703–9.
[79] Vacheethasanee K, Marchant RE. J Biomed Mater Res A 2000;50(3):302–12.
[80] Ladenheim H, Morawetz H. J Polym Sci 1957;26(113):251–4.
[81] Ueda T, Oshida H, Kurita K, Ishihara K, Nakabayashi N. Polym J 1992;24 (11):1259–69.
[82] Nakaya T, Li Y-J. Prog Polym Sci 1999;24(1):143–81.
[83] Zhang Z, Chen S, Jiang S. Biomacromolecules 2006;7(12):3311–5.
[84] Chang Y, Chen S, Zhang Z, Jiang S. Langmuir 2006;22(5):2222–6.
[85] Vaisocherovaćć H, Yang W, Zhang Z, Cao Z, Cheng G, Piliarik M, et al. Anal Chem 2008;80(20):7894–901.
[86] Ma H, Hyun J, Stiller P, Chilkoti A. Adv Mater 2004;16(4):338–41.
[87] Chen SF, Jiang SY. Adv Mater 2008;20(2):335.
[88] Yang W, Zhang L, Wang S, White AD, Jiang S. Biomaterials 2009;30(29):5617–21.
[89] Cheng G, Li G, Xue H, Chen S, Bryers JD, Jiang S. Biomaterials 2009;30 (28):5234–40.
[90] Chen SF, Cao ZQ, Jiang SY. Biomaterials 2009;30(29):5892–6.
[91] Laughlin RG. Langmuir 1991;7(5):842–7.
[92] D'Sa RA, Meenan BJ. Langmuir 2009;26(3):1894–903.
[93] Zoulalian V, Zurcher S, Tosatti S, Textor M, Monge S, Robin JJ. Langmuir 2010;26(1):74–82.
[94] Schuler M, Hamilton DW, Kunzler TP, Sprecher CM, de Wild M, Brunette DM, et al. J Biomed Mater Res B Appl Biomater 2009;91B(2):517–27.
[95] Maddikeri RR, Tosatti S, Schuler M, Chessari S, Textor M, Richards RG, et al. J Biomed Mater Res A 2008;84A(2):425–35.
[96] Tugulu S, Klok HA. Macromol Symp 2009;279:103–9.
[97] Tugulu S, Klok HA. Biomacromolecules 2008;9(3):906–12.
[98] Wang J, Gibson MI, Barbey R, Xiao SJ, Klok HA. Macromol Rapid Commun 2009;30(9–10):845–50.
[99] Hucknall A, Simnick AJ, Hill RT, Chilkoti A, Garcia A, Johannes MS, et al. Biointerphases 2009;4(2):FA50–7.
[100] Cheng G, Zhang Z, Chen SF, Bryers JD, Jiang SY. Biomaterials 2007;28 (29):4192–9.
[101] Hucknall A, Kim DH, Rangarajan S, Hill RT, Reichert WM, Chilkoti A. Adv Mater 2009;21(19):1968–71.
[102] Zhang Z, Zhang M, Chen SF, Horbetta TA, Ratner BD, Jiang SY. Biomaterials 2008;29(32):4285–91.
[103] Tan J, Brash JL. J Biomed Mater Res Part A 2009;90A(1):196–204.
[104] Tan J, McClung WG, Brash JL. J Biomed Mater Res Part A 2008;85A(4):873–80.
[105] Tan J, Brash JL. J Appl Polym Sci 2008;108(3):1617–28.
[106] Sheu MS, Hoffman AS, Feijen J. J Adhes Sci Technol 1992;6(9):995–1009.
[107] Yoshimoto K, Hirase T, Nemoto S, Hatta T, Nagasaki Y. Langmuir 2008;24 (17):9623–9.
[108] Hyun J, Jang H, Kim K, Na K, Tak T. J Memb Sci 2006;282(1–2):52–9.
[109] Sharma S, Johnson RW, Desai TA. Langmuir 2004;20(2):348–56.
[110] Dalsin JL, Hu BH, Lee BP, Messersmith PB. J Am Chem Soc 2003;125(14):4253–8.
[111] Pidhatika B, Moller J, Vogel V, Konradi R. Chimia 2008;62(4):264–9.
[112] Zhang M, Horbett TA. J Biomed Mater Res Part A 2009;89A(3):791–803.
[113] Cao L, Chang M, Lee CY, Castnet DG, Sukavaneshvar S, Ratner BD, et al. J Biomed Mater Res Part A 2007;81A(4):827–37.
[114] Shen MC, Wagner MS, Castner DG, Ratner BD, Horbett TA. Langmuir 2003;19 (5):1692–9.
[115] Shen M, Martinson L, Wagner MS, Castner DG, Ratner BD, Horbett TA. J Biomater Sci Polym Ed 2002;13(4):367–90.
[116] Martwiset S, Koh AE, Chen W. Langmuir 2006;22(19):8192–6.
[117] McArthur SL, McLean KM, Kingshott P, St John HAW, Chatelier RC, Griesser HJ. Colloids Surf B Biointerfaces 2000;17(1):37–48.
[118] Mrabet B, Nguyen MN, Majbri A, Mahouche S, Turmine M, Bakhrouf A, et al. Surf Sci 2009;603(16):2422–9.
[119] Ishihara K, Ishikawa E, Iwasaki Y, Nakabayashi N. J. Biomater. Sci. Polym. Ed. 1999;10(10):1047–61.
[120] Amanda A, Mallapragada SK. Biotechnol Prog 2001;17(5):917–23.
[121] Chapman RG, Ostuni E, Liang MN, Meluleni G, Kim E, Yan L, et al. Langmuir 2001;17(4):1225–33.
[122] Vaisocherova H, Zhang Z, Yang W, Cao ZQ, Cheng G, Taylor AD, et al. Biosens Bioelectron 2009;24(7):1924–30.
[123] Zhang Z, Cheng G, Carr LR, Vaisocherova H, Chen SF, Jiang SY. Biomaterials 2008;29(36):4719–25.
[124] Li GZ, Cheng G, Xue H, Chen SF, Zhang FB, Jiang SY. Biomaterials 2008;29 (35):4592–7.
[125] Li GZ, Xue H, Cheng G, Chen SF, Zhang FB, Jiang SY. J Phys Chem B 2008;112 (48):15269–74.
[126] Cheng G, Xite H, Zhang Z, Chen SF, Jiang SY. Angew Chem Int Ed 2008;47 (46):8831–4.
[127] Ishihara K, Hasegawa T, Watanabe J, Iwasaki Y. Artif Organs 2002;26 (12):1014–9.
[128] Hasegawa T, Iwasaki Y, Ishihara K. J Biomed Mater Res 2002;63 (3):333–41.
[129] Chen SF, Liu LY, Jiang SY. Langmuir 2006;22(6):2418–21.
[130] Yi SJ, Yuk JS, Jung SH, Zhavnerko GK, Kim YM, Ha KS. Mol Cell 2003;15 (3):333–40.

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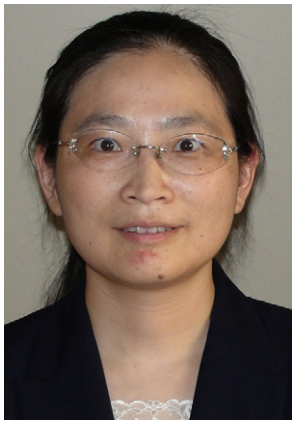

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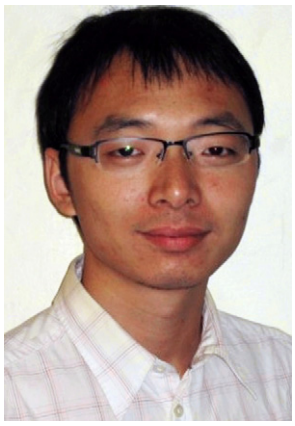

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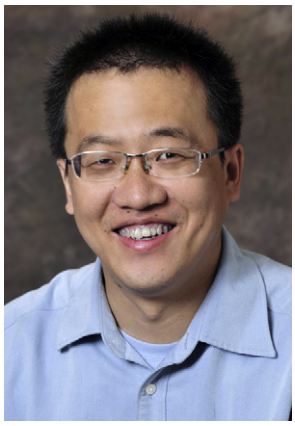

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