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Historical perspective

Heterogeneous surfaces to repel proteins

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ABSTRACT

The nonspecific adsorption of proteins is usually undesirable on solid surfaces as it induces adverse responses, such as platelet adhesion on medical devices, negative signals of biosensors and contamination blockage of filtration membranes. Thus, an important scheme in material science is to design and fabricate protein-repulsive surfaces. Early approaches in this field focused on homogeneous surfaces comprised of single type functionality. Yet, recent researches have demonstrated that surfaces with heterogeneities (chemistry and topography) show promising performance against protein adsorption. In this review, we will summarize the recent achievements and discuss the new perspectives in the research of developing and characterizing heterogeneous surfaces to repel proteins. The protein repulsion mechanisms of different heterogeneous surfaces will also be discussed in details, followed by the perspective and challenge of this emerging field.

Contents

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1. Introduction

A great challenge in material science is to prevent the nonspecific protein adsorption on solid surfaces in the presence of blood plasma as this induces undesirable responses such as inflammations on implants, negative signals of biosensors and contamination blockage of filtration membranes [1–7]. Protein adsorption is also a major problem for *in vivo* applications of nanoparticles (NPs) since the adsorbed protein corona can adversely influence their abilities to target biomolecules and tissues, the bioactivity of antibodies immobilized on the NPs, and the removal pathway of NPs by the immune systems [8–10]. A number of hydrophilic surfaces, such as Poly(ethylene glycol) (PEG) [11–14], heparin [15–17], poly(vinyl alcohol) [18], and polysaccharides [19–21], among others, can reduce nonspecific protein adsorption. We refer to these systems as "homogeneous" surfaces with single type functionality. However, these homogeneous surfaces usually lose their protein resistance properties in complex biological media (*e.g.*, blood stream) because of their fragilities by oxidation in the presence of dehydrogenases, dioxygen and transition metal ions[22–25]. Even in single component solution, the adsorptions of cell adhesion proteins on these homogeneous surfaces are not low enough (<5 ng/cm^2) so as to induce leukocyte adhesions [26,27].

Recently, there has been considerable attention on creating surfaces with "heterogeneous" properties (chemistry and topography) to repel proteins. Compared to homogeneous surfaces, there are three main advantages of heterogeneous surfaces in repelling proteins: (1) Heterogeneous surfaces can generate an enhanced degree of protein resistance performance. For example, hyperbranched fluoropolymer-poly(dimethylsiloxane)-PEG (HBFP-PDMS-PEG) amphiphilic networks developed by Wooley's group displayed a 60% greater resistance to protein adsorption in comparison to a commercially available antibiofouling PDMS coating [28]. (2) Heterogeneous surfaces can maintain their protein resistances for long-term stability under physiological conditions. Surfaces with a 1:1 molar ratio of positive and negative charge functionalities, *e.g.*, the quaternary ammonium and carboxyl groups, were reported to continue to resist proteins and cells even after 10 days, while in some cases PEG lost its repellency after 2 h in the bacteria solution [29]. (3) Heterogeneous surfaces offer multifunctional and/or morphological variations for easy synthesis and incorporation of functionalities at the interface to interact with biomolecules and cells in living systems. For example, NPs bearing amphiphilic heterogeneous surfaces have been shown to efficiently achieve their specific bio-targeting by preventing the nonspecific protein adsorption [30]. Therefore, heterogeneous surfaces have gained a wide range of medical and marine applications as an alternative to homogeneous coatings.

The idea of exploring heterogeneous surfaces to repel proteins is actually inspired by nature's approaches. For example, the cell surface consisting of various amphiphilic molecules is highly resistant to proteins in the blood stream. The heterogeneous surface of the cell membrane also allows for the multi-level interactions between molecular recognition and biological functions. One of the heterogeneous functionalities on cell surface is zwitterionic phospholipids with both positive and negative charges. Experiments and simulations have both demonstrated the stronger hydration on zwitterions induced by electrostatic interactions compared with the hydration of homogeneous PEG surface *via* hydrogen bonding, which contributes to the ultralow fouling properties of cell surface [31–38]. Besides the compositional heterogeneity, the topographic variations of a lotus leaf display superhydrophobic and are highly stealth-like to bio-contaminations. This type of heterogeneous surface has been both theoretically and experimentally demonstrated to possess good blood compatibility [39,40].

The types of surface heterogeneities created in the laboratory to repel proteins can be either compositional or topographical variations. For example, the compositional heterogeneity can be synthetically adjusted by positive and negative charges or hydrophobic and hydrophilic characters. The morphologies of heterogeneity can be technically tuned from the molecular dimensions, microscopic size to macroscopic level. Both the composition and dimension of surface heterogeneity can energetically discourage the interaction between proteins and surfaces, which in turn will limit protein adsorption. However, to date, there is a lack of a specific review on heterogeneous protein-repulsive surfaces. Despite this, some reviews mention that the heterogeneous surfaces are able to repel zoospores for fouling-resistant marine applications. In fact, most of these surfaces are not efficiently repulsive to proteins because the interaction of zoospores with surfaces is significantly different from that of amphiphilic protein molecules [41–43]. In order to deepen the understanding of the role of surface heterogeneity in repelling proteins and take the advantage of heterogeneity concepts in designing/controlling protein–surface interactions for various bio-interface based technologies, this review is intended to highlight the promise of heterogeneous surfaces for protein-resistance applications and discuss the theoretical explanations of the repulsion mechanism behind different types of surface heterogeneities.

2. Strategies for designing heterogeneous surfaces to repel proteins

In general, representative heterogeneous surfaces for repelling proteins can be summarized into three categories: zwitterionic surfaces with both positive and negative charges on different atoms, amphiphilic coatings possessing both hydrophilic and hydrophobic phases, and topographic surfaces with different feature geometries. With different patches of hydrophobic/hydrophilic, positively/negatively charged, and polar/nonpolar characters, a protein molecule tends to interact with surfaces mainly through electrostatic interactions and van der Waals forces. By eliminating these direct protein–surface interactions, the heterogeneous surface with chemical variations can thermodynamically hinder protein adsorption. On the other hand, the length of heterogeneous patches on the surface is also believed to determine how a protein interacts with a surface. While a typical protein has a surface area of 10-1000 nm^2, the initial contact area between a protein molecule and a surface has been demonstrated to be only 1 to 2 nm^2 which is independent of the size of the protein [44]. After the initial interaction stage, the protein molecule will increase its contact area by reorganizing, unfolding and spreading on the surface, which is known as the growing disk model for the dynamic of macromolecule adsorption [45–48]. With a molecular-scale similar to the dynamic path sampled by a protein, the surface heterogeneities can kinetically discourage the initial adsorption stage and inhibit protein adsorption. Therefore, the heterogeneous surfaces can lower the entropic and enthalpic driving forces for the adsorption of protein molecules. We will discuss each surface heterogeneity as follows.

2.1. Zwitterionic heterogeneity

Depending on the amino acid sequences in a given region, the outer hydrophilic surface of a protein molecule is heterogeneously charged at neutral pH. For a surface with homogeneous charge, certain regions of the protein will react favorably with the surface *via* opposite charges attraction (see Fig. 1a and b for negatively and positively charged surface, respectively) while others will not. Such direct electrostatic interaction between protein and homogeneously charged surface results in the formation of ion pairs and the release of conterions [49]. Any factor, such as high salt concentrations, to suppress the counterion evaporation effect, can make the homogeneously charged surface repulsive to proteins [50].

Unlike the homogeneously charged surfaces, the zwitterionic heterogeneous surfaces present an equal number of both positively and negatively charged functionalities. This typical surface (Fig. 1c) can be generated by using a 1:1 molar mixture of positively and negatively charged molecules or zwitterionic molecules that combine positively charged moiety and a negatively charged moiety in the same molecule.