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Biomaterials 26 (2005) 5972–5982
Biomaterials
www.elsevier.com/locate/biomaterials

Protein binding properties of surface-modified porous polyethylene membranes

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Received 14 December 2004; accepted 23 March 2005
Available online 10 May 2005

Abstract

In this study, we quantified the adsorption of immunoglobulin G (IgG) protein onto several polyelectrolyte-modified sintered porous polyethylene (PPE) membranes. The polymer surfaces had both cationic and anionic charges obtained via the adsorption of polyethylenimine (PEI) and polyacrylic acid (PAA), respectively, onto plasma-activated PPE. The amount of IgG adsorption was determined by measuring the gamma radiation emitted by [^{125}I]-IgG radio labeled protein. By studying the impact of pH and ionic strength on IgG adsorption, we attempted to characterize the role and nature of the electrostatic interactions involved in the adsorption process to better understand how these interactions were influenced by the charge and structure of immobilized polyelectrolyte complexes at modified membrane surfaces. We were able to show that surface modification of PPE membranes with adsorbed PEI monolayers and PEI–PAA bilayers can greatly improve the IgG binding ability of the membrane under optimized conditions. We also showed that the observed improvement in the IgG binding is derived from electrostatic interactions between IgG and the polyelectrolyte surface. In addition, we found that the greatest IgG adsorption occurred when the IgG and the surface possessed predominantly opposite charges, rather than when the surface possessed the greatest electrostatic charge. Finally, we have found that the molecular weight of the terminating polyelectrolyte has a noticeable effect upon the electrostatic interactions between IgG and the PEI–PAA bilayer-modified PPE surfaces.

Keywords: Protein adsorption; Specific interactions; Polyelectrolytes; Immunoglobulin

1. Introduction

Materials capable of binding large amounts of protein and other biomolecules are desirable media for applications such as protein separation and purification, peptide synthesis, and lateral flow diagnostic assays [1–8]. Ideal materials for these applications should have stable and controllable surface properties, combine a high surface affinity for protein with a large surface area, allow wicking of aqueous fluids, and be inexpensive and simple to handle [5–11]. For instance, a membrane with high density of positively charged groups would be desirable for adsorption of nucleic acid, which are negatively charged. Common materials utilized in these applications fall short of the ideal in several areas. Inorganic materials, such as metals and ceramics, have been employed as materials for solid-phase applications as the electronic nature of these materials engender surfaces that can be made readily charged and, thus, may be easily and effectively modified. Despite such advantages, such materials are often costly as a result of natural scarcity and/or expensive manufacturing processes required for their fabrication.

Synthetic porous polymers are a preferred alternative, when applicable, due to their low cost, simple processing, and high surface area. Unfortunately, few commercially available polymers possess surfaces containing

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0142-9612/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2005.03.025

desirable chemical functionalities in worthwhile concentrations. Those polymers having the necessary chemical properties, such as nitrocellulose, chitosan, and polyelectrolytes, often lack the mechanical and physical properties needed in solid-phase applications.

Sintered porous polyethylene (PPE), on the other hand, has many advantages over current materials used in many pharmaceutical and biomedical applications. Sintered PPE can be cheaply manufactured with a higher degree of control over such properties as pore size, pore size distribution, pore volume, surface area, and material density than is found in currently used porous materials, specifically nitrocellulose, as shown in Fig. 1. In addition, PPE can be easily manufactured into a wide range of complex geometries with a low degree of geometric variability. However, despite the flexibility to control the geometric and physical properties of the sintered polymer material, the intrinsic chemical properties of polyethylene fail to provide any reactive functionality or surface hydrophilicity that is capable of binding proteins [12–15]. Unfortunately, unmodified polyethylene, given its hydrophobic chemical make-up, does not have favorable surface properties, and hence, exhibits poor protein binding capabilities.

There are several approaches to the modification of PPE membranes that would endow them with controllable wicking and protein binding properties, thermal and environmental stability, long shelf life, and minimal impact upon their original physical and mechanical properties. The chemical modification of the sintered polyethylene is accomplished through the use of reactive oxygen plasma to form negatively charged functional groups at the polyethylene surface, the adsorption of a positively charged polyelectrolyte onto the polyethylene surface, and finally, the formation of a polyelectrolyte bilayer by the additional adsorption of a negatively charged polyelectrolyte [16–28]. The formation of highly charged centers within the polymer chains of polyelectrolytes in solution leads to strong interactions between polyelectrolytes and solids with oppositely charged surfaces [17–19,23]. Many applications involving polyelectrolytes such as water treatment and paper processing are centered around this strong electrostatic interaction and consequential adsorption of the polymer onto charged surfaces [20–28]. Because the surface adsorption of polyelectrolytes is electrostatic in nature and thus reversible, the surface affinity and stability of the polyelectrolyte is highly dependent upon many parameters that include system solvent, solvent pH, surface charge, surface charge density, polyelectrolyte charge, and polyelectrolyte charge density. Changing ionic strength of such a system may cause a dramatic change in the local charge of an adsorbed polyelectrolyte, which can, in turn, result in local pH values that are two units different from the expected bulk value [29]. Hence, the polyelectrolyte monolayer and polyelectrolyte bilayer complexes adsorbed on the plasma-activated surface afford the necessary flexibility for the optimization of the surface properties. The protein binding characteristics of the chemically modified sintered PPE membranes may be therefore controlled as a function of all the above-mentioned surface modification variables.

The mechanisms governing the interactions of proteins with surfaces is quite complex and, despite substantial research, is still poorly understood [30–35]. It is generally accepted, however, that the interactions of a protein with a surface consists of two distinct processes involving non-specific and specific binding. Non-specific binding is based upon hydrophobic interactions between the protein and the surface and is related to the hydrophobicity of the surface. During the non-specific adsorption of proteins, there is no correlation between protein adsorption and protein orientation on the surface. As a result, non-specifically bound proteins are randomly oriented on the surface and only those proteins with proper alignment retain their activity. On the other hand, specific adsorption implies

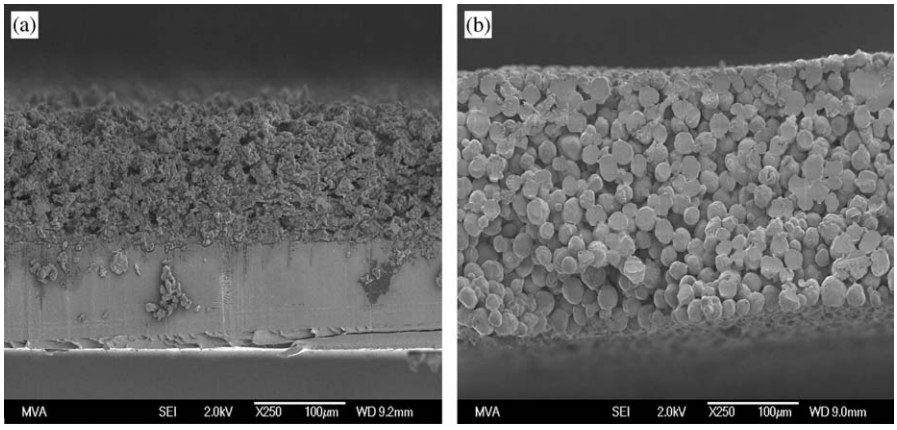

Fig. 1. SEM micrographs of the structure of a longitudinal cross-section of a porous nitrocellulose membrane (a), and of a porous sintered high-density polyethylene membrane (b).