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Protein adsorption on polyanion/polycation layer-by-layer assembled polyelectrolyte films

Jen Ming Yang [a,*], Rong-Ze Tsai [a], Chih-Chin Hsu [b,c]

[a] Department of Chemical and Materials Engineering, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan
[b] Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[c] College of Medicine, Chang Gung University, Taoyuan, Taiwan

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ABSTRACT

As layer-by-layer self-assembly deposition (LbL) is a versatile technique for surface modification, protein adsorption on the LbL modified glass is evaluated in this study. At the beginning, glass slides was silanized by 3-aminopropyltriethoxysilane (APTES). Sodium alginate (Alg), poly(γ-glutamic acid) (PGA) and poly(aspartic acid) (PAsp) were selected as polyanion electrolytes and chitosan (CS) was used as the polycation electrolyte. Both polyanion and polycation electrolytes alternately deposited on the silanized glass slide surface by the LbL technique to get three different polyanion/chitosan series of LbL films ([Alg/CS], [PGA/CS], and [PAsp/CS]). Three kinds of kinetic model including pseudo-first-order, second-order kinetic and intraparticle diffusion model were used to evaluate the adsorption of albumin on the three different polyanion/chitosan series of LbL films. It is found that the adsorption of albumin on the polyanion/chitosan series of LbL films can be described well with the pseudo-second-order kinetic mechanism. To make sure if the pseudo-second-order kinetic mechanism of protein adsorbed on the other polyanion/polycation LbL films is also suitable, poly(allylamine hydrochloride) (PAH) and poly(L-lysine) (PLL) are used as two other polycations. The [polyanion/PAH] and [polyanion/PLL] series of LbL films were prepared with the same LbL technique for albumin, fibrinogen, and fibronectin adsorption. From the results, it is found that albumin, fibrinogen, and fibronectin adsorption on the various polyanion/polycation LbL films can be described well with the pseudo-second-order kinetic mechanism. The protein adsorbed at equilibrium and rate constant of protein adsorbed on the various LbL films can be determined.

1. Introduction

A working hypothesis that has emerged as a fundamental biomaterials surface science tenet is that protein adsorption is the first step in the acute biological response to artificial materials. If the number and kind of proteins adsorbed to a surface is not clearly known, then evidence-based biochemical mechanisms of the biological response to materials cannot be responsibly proposed. If mechanisms of the biological response to materials remain obscure, then structure-property relationships cannot be formulated, leaving biomaterials development dependent on design-directed or trial-and error approaches [1–3]. The entirety of biomaterials surface science seems critically dependent on a thorough understanding of protein adsorption [4]. Consequently; surface chemistry has been one of the most crucial subjects in the studies of biomedical materials. Surface modification is an effective means to alter biological interactions to a material and offers a number of advantages. It include the improvement of blood compatibility, reduction of tissue adhesion, the improvement of lubricity, the increase of the wettability on a surface, addition of biologically active substances to the surface layer, and altering protein adsorption characteristics.

Polyelectrolytes are used in a variety of biomedical systems such as dental adhesives and for pulp regeneration, controlled-release devices, and drug delivery [5–9]. The layer-by-layer (LbL) method initially introduced by Moehwald, Decher, and Lvov consists in alternately depositing polyelectrolytes that self-assemble and self-organize on the material's surface, leading to the formation of polyelectrolyte multilayer (PEM) [10–14]. Polyelectrolyte multilayer (PEM) coating generated via layer-by-layer self-assembly of oppositely charged polymers show great potential to functionalize almost any kind of substrate such as rubbers, plastics, metals, or

* Corresponding author.
E-mail address: jmyang@mail.cgu.edu.tw (J.M. Yang).

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glasses with the advantages of simplicity, versatility and easy fabrication [15–52], so the studies about the modification of materials with the LbL technique in wide range have been reported [15–52].

Although the wide range of the studies about the studies of charged polyelectrolytes have been reported [15–52], the evaluation of the kinetic model of protein adsorption on the LbL films has not been reported. In this study, the surface of glass slides was modified by 3-Aminopropyltriethoxysilane (APTES) to bear the positively charged terminal amino groups. Chitosan (CS), poly(allylamine hydrochloride) (PAH) and Poly(L-lysine) (PLL) were used as the polycation electrolyte and sodium alginate, poly(γ-glutamic acid) (PGA) and poly(aspartic acid) (PAsp) were selected as polyanion electrolytes to deposit on the APTES-modified glass slide surface by the LbL self-assembly deposition technique to get nine polyanion/polycation series of LbL multilayer films including [Alg/CS], [PGA/CS], [PAsp/CS], [Alg/PAH], [PGA/PAH], [PAsp/PAH], [Alg/PLL], [PGA/PLL], and [PAsp/PLL]. Protein adsorption with albumin, fibrinogen and fibronectin as model proteins on the nine polyanion/polycation series of LbL multilayer films system was conducted. In order to investigate the mechanism of protein adsorption, three kinds of kinetic model [53–58] including pseudo-first-order, second-order kinetic and intraparticle diffusion model were used to evaluate the adsorption of protein on LbL film.

2. Materials and methods

2.1. Materials

Hydrogen peroxide (35%), acetone (99.9%), acetic acid (99.8%), and sulfonic acid (98%) were purchased from Acros Organics (New Jersey, USA). 3-aminopropyltriethoxysilane (99%) was obtained from Sigma-Aldrich, USA for the modification of glass slide. Chitosan, poly(allylamine hydrochloride) (PAH), Poly(L-lysine) (PLL), sodium alginate, poly(γ-glutamic acid), and poly(aspartic acid) used as polycation and polyanions (scheme S1). Poly(allylamine hydrochloride) (PAH), Poly(L-lysine) (PLL), sodium alginate, and poly(aspartic acid) were purchased from Sigma-Aldrich, USA. They were all reagent grade and used without further purification. Chitosan was purchased from Chitin, Chitosan Inc., Taiwan. Poly(γ-glutamic acid) was provided by Vedan Enterprise Corporation, Taiwan. The deactylation for chitosan is about 97%. The molecular weight for chitosan, poly(allylamine hydrochloride), poly(γ-glutamic acid), and poly(aspartic acid) were about 360.8 kDa, 56 kDa, 400 kDa, and 5–15 kDa, respectively. Albumin, fibrinogen and fibronectin were all reagent grade and obtained from Sigma-Aldrich, USA for the evaluation of protein adsorption.

2.2. Silanization of glass substrate by 3-aminopropyltriethoxysilane (APTES) and fabrication of polyanion/polycation series of LbL multilayer films

Glass slides cut into squares 1.3 cm × 1.3 cm were cleaned by immersion in hot piranha solution (H_2O_2:H_2SO_4 = 3:7, v/v) for 4 h, and then washed by pure water. The dried glass slides were immersed in 10% (v/v) solution of APTES in freshly distilled toluene at room temperature for 12 h. Finally, the silanized glass slides were removed from the solution, rinsed with toluene and dried in oven. The process of the fabrication of [polyanion/polycation] LbL multilayer films was performed following the previous study [52]. The preparation of the polyanion/polycation series of LbL multilayer films are briefly shown in Fig. 1. The surface atomic percentage was obtained with the characterization of X-ray photoelectron spectroscopy (XPS). The roughness on polyanion/polycation series of LbL multilayer films was obtained with atomic force microscopy (AFM) images by a MFP-3D™ microscope (Asylum Research; CA, USA). The hydrophilicity on the polyanion/polycation series of LbL multilayer films were measured with static air-water contact angles by a sessile drop method using a contact angle meter (Face CA-D, Kyowa Interface Science Co. LTD.; Japan) at ambient humidity and temperature. For each polyanion/polycation LbL multilayer film, 4 drops were examined to obtain the average contact angle values. The surface density of amino group on polyanion/polycation series of LbL multilayer films was evaluated from uptake of an acid dye as described elsewhere [52].

2.3. Kinetics study of protein adsorption on LbL films

The process of the protein adsorption was performed following the previous study [53]. At first, CPBS buffer solution including 0.01 M sodium citrate, 0.01 M sodium phosphate, and 0.12 M sodium chloride mixed solution with pH 7.4 was prepared. The films were immersed into the buffer solution for about 12 h. Then the films were filled with 2 ml of protein solution at 30 °C for 1 h. The concentration of protein solutions for Albumin, Fibrinogen, and Fibronectin is 0.1 g, 0.01 g, and 0.002 g in 100 ml CPBS buffer solution, respectively. After adsorption, the films were rinsed with deionized water. The adsorbed protein was desorbed with 1% Triton X-100 and 1% sodium dodecyl sulfate in 0.01 N NaOH at 30 °C with shaking. The desorbed protein with 1.5 ml boric acid buffer solution was mixed with fluorescamine in acetone with vigorous stirring. The quantity of protein was then determined with a fluorescence spectrophotometer. Three kinds of kinetic model including pseudo-first-order, second-order kinetic and intraparticle diffusion model [54–58] were used to evaluate the adsorption data of protein on LbL film.

If the diffusion of the protein to the multilayer film surface is the rate determining step of the adsorption process and could be adequately described by the pseudo-first-order equation [54,56]:

$$dq_t/dt = k_1(q_e - q_t) \quad (1)$$

where $\boldsymbol{q_t}$ and $\boldsymbol{q_e}$ are the amount of protein adsorbed at time $\boldsymbol{t}$ and at equilibrium (μg/cm^2), respectively, and $\boldsymbol{k_1}$ is the rate constant of the pseudo-first-order adsorption process (1/min). The integrated rate law, after applying the initial conditions of $\boldsymbol{q_t} = 0$ at $\boldsymbol{t} = 0$, is

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \quad (2)$$

Plots of $\boldsymbol{\ln(q_e - q_t)}$ versus $\boldsymbol{t}$ will give a straight line for pseudo-first-order kinetics, which allows computation of the adsorption rate constant, $\boldsymbol{k_1}$.

If the rate of adsorption of protein on the polyelectrolyte films was assumed as a second-order mechanism, the following equation could be used to test the experimental data, expressed as [55,57]:

$$dq_t/d_t = k_2(q_e - q_t)^2 \quad (3)$$

Integrating this equation for the boundary conditions, gives:

$$1/(q_e - q_t) = 1/q_e + k_2 t \quad (4)$$

where $\boldsymbol{q_e}$ is the amount of protein adsorbed at equilibrium (μg/cm^2) and $\boldsymbol{k_2}$ is the rate constant of adsorption (cm^2/μg-min). The above equation can be rearranged to obtain a linear form

$$t/q_t = 1/(k_2 q_e{}^2) + t/q_e = 1/H + t/q_e \quad (5)$$

where H equals to the value of $\boldsymbol{(k_2 q_e{}^2)}$ and is the initial adsorption rate (μg/cm^2-min) of protein on LbL films. Therefore a plot of $\boldsymbol{t/q_t}$ versus time will give a straight line, and from the gradient and intercept, $\boldsymbol{q_e}$, $\boldsymbol{k_2}$ and $\boldsymbol{H}$ can be obtained.

If pore diffusion of protein is generally the rate determining step in the adsorption process, the intraparticle diffusion model could be applied. It can be written as follows [54,58]:

$$q_t = k_i t^{1/2} + C \quad (6)$$