Journal of Colloid and Interface Science 485 (2017) 288–295

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

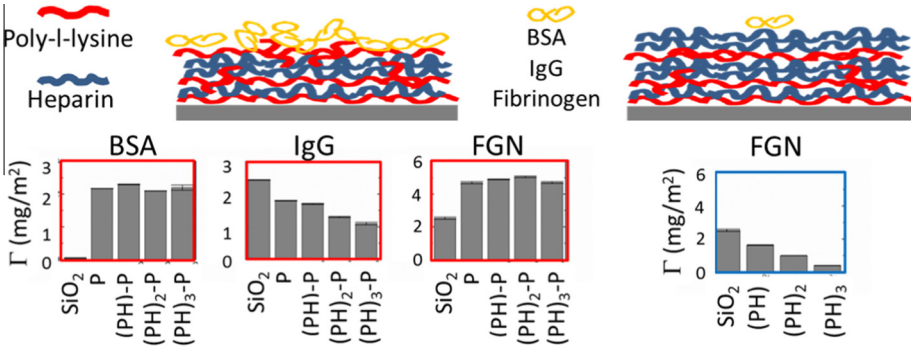
Regular Article

Poly-L-lysine/heparin multilayer coatings prevent blood protein adsorption

Alejandro Barrantes [a,b,*], Jonas Wengenroth [a], Thomas Arnebrant [b], Håvard J. Haugen [a]

[a] Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, P.O. Box 1109 Blindern, 0317 Oslo, Norway
[b] Biomedical Sciences, Health and Society, Malmö University, 20560 Malmö, Sweden

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 June 2016
Revised 19 September 2016
Accepted 21 September 2016
Available online 22 September 2016

Keywords:
Layer by layer
Protein adsorption
Ellipsometry
QCM-D
Heparin coating

ABSTRACT

The adsorption of blood proteins, serum albumin (BSA), immunoglobulin G (IgG) and fibrinogen (FGN), onto model SiO_2 planar surfaces coated with poly-L-lysine/heparin multilayers (PLL/HEP) has been investigated by means of ellipsometry and quartz crystal microbalance with dissipation. Aiming at the development of low fouling coatings, this study has been focused on the effects that the number of layers and the type of polyelectrolyte present on the topmost layer have on the adsorption of these proteins. The three proteins interact with PLL-ended coatings whereas HEP-ended coatings prevent the adsorption of both BSA and IgG and induce a decrease in the adsorbed amount of FGN, down to 0.4 mg/m^2 for three bilayers, as the number of PLL/HEP bilayers increases. These results suggest that heparin-ended multilayers prevent protein adsorption, which is an indicative of good blood compatibility. As a consequence we propose that PLL/HEP coatings could be used for the development of vascular medical devices.

1. Introduction

Protein adsorption has become a major problem for the successful performance of biomaterials. As soon as a foreign material gets in contact with blood the proteins present in the fluid will adsorb at the interface either in a reversible or in an irreversible manner depending on the nature of both the material and the protein [1]. Because some of these proteins, like immunoglobulin G (IgG) and fibrinogen (FGN), are involved in the activation of the complement system or in the clotting cascade [2,3], fouling may lead to the degradation of the material [4] or clot formation onto it [3].

Many efforts have been made control protein adsorption onto surfaces, especially by coating the surfaces by means of self-assembled monolayers (SAMs) [5–7] and, in recent years,

* Corresponding author at: Department of Biomaterials, Institute of Clinical Dentistry, University of Oslo, P.O. Box 1109 Blindern, 0317 Oslo, Norway.
E-mail addresses: a.b.bautista@odont.uio.no, barrantes_ale@yahoo.es (A. Barrantes), jonas.wengenroth@odont.uio.no (J. Wengenroth), thomas.arnebrant@mah.se (T. Arnebrant), h.j.haugen@odont.uio.no (H.J. Haugen).

http://dx.doi.org/10.1016/j.jcis.2016.09.046
0021-9797/

polyelectrolyte multilayers (PEMs) [8,9]. The purpose of most of these strategies is to change the physicochemical properties of the surface rendering either a low fouling interface or a selective one that only binds specific biomolecules. PEMs fabricated by the layer by layer method have become one of the most popular ways to modify planar surfaces [8–15] and colloidal particles [16,17] providing them with tailor made properties such as controlled thickness, roughness, wettability and rheology. This method consists in alternatively depositing either natural or synthetic polymers onto a surface by exposing it to solutions that contain the polymer of interest. The interaction forces that drive the build-up of the multilayer are mainly electrostatic [10,11,13,15,18] however, coatings assembled through hydrophobic forces [12] and hydrogen bonds have also been developed [19]. The popularity of PEMs does not come only from its simplicity but also from the wide range of applications they have. Apart from antifouling coatings [8,9], they have been extensively used in drug delivery [17] and biosensing devices [14,20].

We have previously studied the effects that pH and the underlying substrate have on the build-up of poly-L-lysine/heparin (PLL/HEP) multilayers [11]. Preventing protein fouling onto the surface of vascular medical devices is essential for their proper operation. Therefore, in the present study, as a step forward towards their application as hemocompatible coatings for biomedical devices and with the aim of developing low fouling coatings, we have investigated what are the effects on protein adsorption of both the polymer present on the outermost layer of the coating as well as the number of layers. For this purpose we have chosen three relevant blood proteins such as serum albumin, which is the most abundant protein present in blood, and immunoglobulin G and fibrinogen known for their central role in the activation of complement system and the clotting cascade [2].

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich. Poly-L-lysine hydrobromide (MW 30,000–70,000 g/mol), heparin sodium salt from intestinal mucosa, bovine serum albumin, human IgG and human fibrinogen were also purchased from Sigma-Aldrich and used without further purification. All solutions were prepared with Mili-Q water (18.2 MΩ cm, Millipore, Billerica, MA, US).

2.2. Surfaces

Ellipsometry experiments were performed on silicon wafers with a silica layer of approximately 300 Å (Semiconductor Wafer Inc. Hsinchu, Taiwan). Hydrophilic silica substrates were cleaned following the procedure developed at RCA laboratories [21]. The silica substrates were boiled for 5 min in an alkaline solution; rinsed extensively with water; boiled again for 5 min in an acidic solution; and finally rinsed with water and ethanol. The components of the alkaline solution were: NH_4OH (25%), H_2O_2 (30%), and water with a volume proportion of 1:1:5 respectively. The components of the acidic solution were: HCl (37%), H_2O_2 (30%), and water with a volume proportion of 1:1:5 respectively. At the end of the cleaning procedure the surfaces were stored in ethanol. Prior to use, the surfaces were plasma cleaned for 5 min in low pressure residual air using a glow discharge unit (PDC-32 G, Harrick Scientific Corp., USA).

QCM-D measurements were performed on AT-cut 5 MHz quartz crystals (Q-Sense E4, Biolin Scientific AB, Sweden) and had silica as outermost layers. The substrates were cleaned according to the instructions from the manufacturer: (1) 10 min plasma treatment; (2) 30 min immersion into 2% SDS solution (3) extensive rinsing with water; (4) 10 min plasma treatment. Both cleaning procedures yielded hydrophilic surfaces with water contact angles less than 10° as measured with a drop shape analyzer, (DSA100, Krüss GmbH, Hamburg, Germany).

2.3. Multilayer build-up and protein adsorption studies

All measurements were done in Dulbecco's phosphate buffered saline (ionic strength, I = 0.148 M), pH 7.4 (DPBS) at 25 °C and concentrations of 0.02 mg/ml and 0.2 mg/ml for the polyelectrolytes and protein adsorption experiments respectively. The adsorption of PLL and HEP was monitored in solution for 5 min with a 5 min long rinsing step with polyelectrolyte-free buffer solution between each polyelectrolyte addition. After the last polyelectrolyte layer was formed and rinsed the protein solution was supplied and its interaction with the coated surface was monitored for 30 min. In order to elucidate whether protein adsorption was reversible or irreversible, a final 30 min rinsing step with DPBS was done.

The instruments employed were a Rudolph thin film ellipsometer (type 43603-200E, Rudolph Research, USA) automated according to the concept of Landgren and Jonsson [22] with an experimental setup based on null ellipsometry according to the principles of Cuypers [23]. A xenon arc lamp was used as the light source, and light was detected at 442.9 nm using an interference filter with UV and infrared blocking (Melles Griot, The Netherlands). The trapezoid cuvette made of optical glass (Hellma, Germany) was equipped with a magnetic stirrer (325 rpm). The adsorbed amount, Γ, was calculated by using de Feijter's equation [24] (Eq. (1)), where n_f is the refractive index and d the thickness of the mixed polyelectrolyte multilayer. Although the dn/dc value for heparin is 0.13 ml/g [25], the value for PLL, dn/dc = 0.15 ml/g [26], has been used for the whole multilayer. This procedure will introduce an underestimation for the adsorbed amount of heparin [27].

$$\Gamma = d\frac{n_f - n_{Buffer}}{dn/dc} \quad (1)$$

Proteins adsorbed amounts were estimated in the same way as the ones obtained for the PEMs considering the PEM-protein complex as a single layer, and by using a refractive index increment value of 0.18 ml/g [24]. The adsorbed amount of the protein layers was then obtained by applying the following expression: $\Gamma_{PEM+Prot}(0.18)-\Gamma_{PEM}(0.18)$ (the same procedure was applied to the thickness, d_{Ellips}) under the assumption that protein adsorption did not induce any changes in the optical properties of the multilayers.

The QCM-D measurements were performed by using a (Q-Sense E4 system, Biolin Scientific AB, Sweden). Both polyelectrolyte and protein solutions were supplied by means of a peristaltic pump at a flow rate of 0.1 ml/min. A detailed description of the technique and its basic principles can be found elsewhere [28]. Briefly, an alternating-current voltage is applied through a gold-coated quartz chip to stimulate the shear mode oscillation of the quartz crystal. When a certain amount of mass is adsorbed onto the sensor chip, a proportional decrease in the resonance frequency, Δf, will be detected as stated in Eq. (2), known as Sauerbrey's equation Sauerbrey [29]:

$$\Delta f = -\frac{n\Delta m_s}{C} \quad (2)$$

where n is the overtone number (n = 1, 3, 5, ...), C is the mass-sensitivity constant (C = 0.177 mg/m^2), and the subscript 's' stands for Sauerbrey. From this relation, a rough estimate of the mass can be made when the film deposited onto the chip can be considered rigid. Therefore, Sauerbrey's equation was used to determine the PEMs as well as the BSA and IgG adsorbed amounts. However,