Impact of thermal annealing on wettability and antifouling characteristics of alginate poly-L-lysine polyelectrolyte multilayer films

Eleftheria Diamanti[a], Nicolas Muzzio[b], Danijela Gregurec[a], Joseba Irigoyen[a], Miguel Pasquale[b], Omar Azzaroni[b], Martin Brinkmann[c], Sergio Enrique Moya[a,*]

[a] Soft Matter Nanotechnology Group, CIC biomaGUNE, Paseo Miramón 182C, 20009 San Sebastián, Guipúzcoa, Spain
[b] Instituto de Investigaciones Fisicoquimicas Teóricas y Aplicadas (INIFTA), (UNLP, CONICET), Sucursal 4, Casilla de Correo 16, 1900 La Plata, Argentina
[c] Experimental Physics, Saarland University, 66123 Sarbruecken, Germany

ARTICLE INFO

ABSTRACT

Polyelectrolyte multilayers (PEMs) of poly-L-lysine (PLL) and alginic acid sodium salt (Alg) are fabricated applying the layer by layer technique and annealed at a constant temperature; 37, 50 and 80 °C, for 72 h. Atomic force microscopy reveals changes in the topography of the PEM, which is changing from a fibrillar to a smooth surface. Advancing contact angle in water varies from 36° before annealing to 93°, 77° and 95° after annealing at 37, 50 and 80 °C, respectively. Surface energy changes after annealing were calculated from contact angle measurements performed with organic solvents. Quartz crystal microbalance with dissipation, contact angle and fluorescence spectroscopy measurements show a significant decrease in the adsorption of the bovine serum albumin protein to the PEMs after annealing. Changes in the physical properties of the PEMs are interpreted as a result of the reorganization of the polyelectrolytes in the PEMs from a layered structure into complexes where the interaction of polycations and polyanions is enhanced. This work proposes a simple method to endow bio-PEMs with antifouling characteristics and tune their wettability.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Polyelectrolyte multilayers (PEMs) fabricated by means of the Layer by Layer (LbL) technique have found multiple applications in the last years for surface engineering and device fabrication [1–3]. PEMs have potential applications in diverse areas such as optoelectronics, nanofiltration and tissue engineering [4–8]. Recently, PEMs have attracted attention as antifouling coatings, as they can be easily assembled on almost any charged surface and the stepwise assembly of polyelectrolytes allows for a precise control of composition of the multilayers in the vertical plane combining different polyelectrolytes and nanomaterials, with synergic antifouling effects. In this regards several interesting strategies have been followed i.e. the use of perfluorinated PEG co-polymers as layer constituents, the control of the surface charge density or the use of a sacrificial top layer that can be removed together with the foulant and later reassembled [9–12].

PEMs are assembled by the alternating deposition of polycations and polyanions on top of charged surfaces, based on attractive electrostatic interactions and entropy considerations, since counter ions are released during the PEM assembly [13–15]. The LbL process can be considered as a special case of polycation/polyanion complex formation. The layered structure of the film is a consequence of the step-wise assembly of polycations and polyanions. However, the layers are not fully stratified, as there is a certain degree of interdigitation among them. One layer in PEMs can be sensed up to 4 layers below [16]. Interdigitation is partially a consequence of the presence of free space within the film, which is filled by the following depositing layers [17].

PEMs are very stable; they are not easily removed, unless at least one of the polyelectrolytes is weak and loses charges by changing the pH, or there is a specific ion or surfactant interacting with the monomers that can weaken the electrostatic attraction between oppositely charged polyelectrolytes [18,19]. Only at very high ionic strength the films can be partially erased [20]. Despite PEM stability, the layered arrangement of the polyelectrolyte is not the energetically optimal. From an intuitive point of view the best arrangement of the oppositely charged polyelectrolytes would not

be as separated layers but as complexes, where there is a maximal compensation of the negative and positive charges. In a complex the oppositely charged polyelectrolytes will be closer than in the LbL assembly. However, a reorganization of the PEMs into complexes, with the disappearance of the characteristic stratified structure could compromise the stability of the PEM itself.

Thermal annealing, exposing the PEMs to heat for a defined period of time, gives the polyelectrolyte molecules the energy to rearrange in the films and find more convenient conformations. It has been shown that annealing to capsules of PDADMAC/PSS leads to the rearrangement of the polymers with a consequent loss of internal volume and increase of the layer thickness. This phenomenon occurs only when PDADMAC is the last layer [21–24]. Glinel et al. show the impact of annealing on the responsive behavior of polyelectrolyte films in relation with changes on the architecture of the multilayers [25]. Besides these works there are no other reported examples related to the thermal annealing of PEMs. In this work we study the effect of thermal annealing on PEMs constructed upon two biopolymers that have been extensively used for biomedical applications as they provide enhanced surface biocompatibility; these are the positively charged poly-L-lysine (PLL) and the negatively charged alginic acid sodium salt (Alg) [26–30]. We will demonstrate that by applying annealing on these films their wetting properties will dramatically change with variations in the contact angle in water of 60°, from highly hydrophilic to hydrophobic. This variation in wetting behavior is a consequence of the rearrangement of the polymer chains, which could be visualized by Atomic Force Microscopy (AFM) [31,32]. Thermal annealing of PLL/Alg multilyers has also a clear effect on the interaction of the PEMs with proteins, decreasing protein deposition while increasing the antifouling character of the PEMs. The use of annealing at different temperatures to decrease protein deposition provides a simple procedure to tune the antifouling characteristics of surfaces without altering the chemistry of the multilayers and employing commercial and biocompatible polymers, which are amenable with biomedical applications, where limited protein deposition on surfaces may be required.

2. Material and methods

Poly-L-lysine solution 0.1% (w/v) in H_2O (PLL, Mw 150–300 kDa), bovine serum albumin hydrolyzed powder, pH 7 ≥98% (BSA, Mw ~66 kDa), was obtained from Sigma-Aldrich. Alginic acid sodium salt (Alg, Mw 10–600 kDa) was acquired from Acros Organics. Fluorescent label ATTO 488 NHS-ester (1 mg) was purchased from ATTO – TEC – Fluorescent Labels and Dyes. Disposable PD-10 desalting columns filled with Sephadex® G-25 gel filtration medium were purchased from GE Healthcare Life Sciences. SiO_2 particles with a diameter of 3 μm were purchased from Attendbio.

Sodium chloride (NaCl), phosphate buffer saline (PBS), sodium bicarbonate ($NaHCO_3$), sodium hydroxide (NaOH), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (Hepes) dimethyl sulfoxide (DMSO) solvent and ethylene glycol (EG) was purchased from Sigma-Aldrich.

2.1. Build up of polyelectrolyte multilayer films

Each polyelectrolyte was dissolved in 10 mM Hepes/150 mM NaCl buffer (pH 7.4) at a final concentration 1 mg ml^{-1}. Following the LbL technique 15 layers of PLL/Alg with PLL as the outermost layer; (PLL/Alg)$_{7.5}$, were coated on top of negatively charged surfaces. The surfaces employed for the assembly varied according to the requirements of each experimental configuration. Samples were incubated for 10 min in each polyelectrolyte solution. Each

step of assembly was followed by washing with Hepes/NaCl buffer for the removal of the surplus polyelectrolyte.

2.2. Annealing process

The annealing of the samples was conducted during 3 days in a Memmert UNE 200–300 oven with a range of temperature 5–250 °C under atmosphere conditions for three different temperatures; 37 °C, 50 °C and 80 °C. For annealing all samples was first dried in air.

2.3. Atomic force microscopy

Structural details of the PLL/Alg films were investigated using a Nanowizard II AFM (JPK, Berlin, Germany). Images were acquired on dry samples, scanning in air. Prior to imaging polyelectrolyte films were washed with nanopure water and left to dry at room temperature. TESP-V2 cantilever (Brucker, AFM Probes) with a nominal spring constant of 40 N/m was used for imaging in intermittent mode. The resonant frequency was in the range of 280–320 kHz. The root mean square (RMS) roughness is calculated as the average of RMS line profiles across vertical and horizontal direction of the AFM images using Gwyddion software.

2.4. Contact angle measurements

The wetting properties of the PEM film were characterized before and after the annealing in a DSA 100 contact angle measuring system with a DSA 100 control from the Kruss Company at room temperature (20 °C) and ambient atmospheric conditions. During the measurement, the liquid drops remain attached to the steel tip with outer diameter, $d = -0.5$ mm. Still images of a 3 μL drop with 500 μL min^{-1} velocity were captured after the liquid volume was slowly inflated or deflated until the contact line moved gradually outward or inward, respectively. Drop profiles were recorded and fitted with the included software package (DSA 3). Apparent contact angles of the drops for 5 repetitions of each sample were obtained with a stdev = 1.9.

Contact angle measurements were performed using different test liquids to characterize the surface energy of PEMs before and after annealing. Water, ethylene glycol (EG), and dimethylsulfoxide (DMSO) were chosen as test liquids to determine the dispersion component γ_s^D and the polar component γ_s^p, as well as the total interfacial free energy of the bare substrate, γ_s for each test liquid, we measure the static advancing contact angle, θ_a, and the static receding contact angle, θ_a.

2.5. Polyelectrolyte assembly on colloids

For ζ-potential measurements PEMs were assembled on top of SiO_2 particles (3 μm). To do so, SiO_2 particles were first suspended in 10 mM Hepes/150 mM NaCl (pH 7.4) buffer at 1 mg ml^{-1}. Subsequently the particles were incubated at the polyelectrolyte solution (1 mg ml^{-1}) for 15 min. The procedure was repeated for every layer deposition for the construction of 15 layers. In between polyelectrolyte depositions three washing steps were performed via centrifugation.

2.6. ζ-potential measurements

Changes on the surface charge of the PEM coated colloids were recorded using a Zetasizer (Malvern, of the UK). ζ-potential measurements were conducted in a disposable folded capillary cell at 25 °C and they were performed at a cell drive voltage of 30 V, using a monomodal analysis model. Five repetitions were conducted for