

Tuning Fibrinogen Adsorption on Layer-by-Layer Built PAA/PAH Multilayer Films: Effect of Deposition Conditions and Hydrophilicity

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Abstract

This work investigates the relationship between adsorption of fibrinogen (FNG) on solid surfaces modified by polyelectrolyte multilayer films (PEM) and their surface properties. A pair of weak polyelectrolytes, polyacrylic acid and poly (allylamine hydrochloride), was used to prepare four different PEM films by assembly under different deposition conditions. The thickness of the films was examined by ellipsometry and surface wettability was analyzed by the contact angle method. The Bradford assay and ELISA method were used to quantify FNG adsorption and conformation onto different PEMs. The study contributes to understanding the impact of hydrophilicity and film structure on protein adsorption, and can be used to design biocompatible biomedical devices.

Keywords: protein adsorption, surface modification, polyelectrolyte multilayers, biofunctionalization

1. Introduction

Polyelectrolyte (PE) multilayers (PEM) are now a well-established concept for modifying the properties of solid substrates as biomaterials by creating nano-thick films to improve their biocompatibility [1-2]. PEMs provide precise and controlled modification of surface properties without affecting the micro topology of the materials or their bulk properties. The selection of polyelectrolyte pairs and process parameters during PEM fabrication dictates their physicochemical properties and enables the fine-tuning of protein adsorption onto PEM-coated biomaterials, which is critical in biomedicine field, particularly for the design of blood-contacting devices [3]. Fibrinogen (FNG) adsorption pattern onto biomaterial surfaces is crucial for their blood compatibility [4]. Several studies suggested that platelet adhesion and activation onto biomaterial surfaces, which are prerequisites for thrombus formation, might be affected particularly by the conformation of FNG upon adsorption [5-7]. The adsorption profile of FNG depends strongly on the physicochemical properties of the surface such as surface wettability, surface charge density, and others [8-9]. In this study we used PEM to create biocompatible coatings on biomaterials with diverse well-defined surface properties. The results indicate a correlation between the nature of the outermost polyelectrolyte layer, surface wettability and FNG adsorption. The obtained data can guide the rational design of biocompatible surfaces with controlled protein adsorption.

2. Materials and methods

2.1. PEM build-up

Polyelectrolyte multilayer films were assembled using a layer-by-layer dip coating technique described in more details in [10], applying polyethyleneimine (PEI, Sigma Aldrich, branched, Mw ~750 kDa) as a precursor layer and continuing with polyacrylic acid (PAA, Sigma Aldrich, Mw ~100 kDa) and poly (allylamine hydrochloride) (PAH, Thermo Scientific, Mw 120-200 kDa). The deposition was performed from water solutions with PE concentration of 2 mg/ml in presence of 0.5 M NaCl. Four types of PEM films were built and further studied. Two were created using PAA and PAH solutions at pH 7.0: PAA 7.0 CL, which had PAA as the outermost layer, and PAH 7.0 CL, which had PAH as the outermost layer. The other two films were made from PAA and PAH solutions at pH 3.5, with PAA (designated PAA 3.5 CL) and PAH (designated PAH 3.5 CL) as the outermost layers, respectively. All samples were thermally cross-linked (CL) in an oven for 2 hours at 180°C. Silicon (100) wafers (10 × 10 mm, CrysTec GmbH, Berlin, Germany) were used as substrates for the ellipsometry and ELISA experiments and glass coverslips (Carl

Roth GmbH, Karlsruhe, Germany) for the wettability studies and Bradford assay. PEM films were prepared and analyzed at triplicate.

2.2. Thickness

The thickness and refractive indices of PEM films were determined using a Sentech SE800 spectroscopic ellipsometer. Data was evaluated using a four-layer model considering the bulk Si, SiO₂ layer, PEM, and surrounding air.

2.3. Contact angle and surface free energy

Water contact angle (CA) of PEM was measured by sessile drop method using a contact angle goniometer (Dataphysics GmbH, Filderstadt, Germany). Young-Laplace equation was applied to analyze droplet shape. The surface free energy (SFE) of PEMs was analyzed using the Owens, Wendt, Rabel, and Kaelble approach. The surface free tension of deionized water and glycerol were calculated with data from Ström et al. [11] and the surface free tension of n-hexadecane was estimated according to Jasper et al. [12].

3. Protein adsorption

3.1. Bradford assay

A human FNG (Sigma Aldrich-F4883, 100 µg/ml) dissolved in phosphate buffered saline (PBS) with pH 7.4 was incubated with PEM-coated substrates for 1 hour at 37°C. The amount of adsorbed FNG was quantified using the Bradford assay [13] by subtracting the concentration of FNG remained in the solution after incubation with PEM coated samples from the initial FNG concentration. UV absorbance measurements were taken using a Lambda Bio+ spectrophotometer (PerkinElmer, Waltham, MA, USA) at 460 nm and 640 nm. A calibration line was used to calculate the amount of adsorbed FNG.

3.2. Indirect ELISA

PEM were preadsorbed with 100% human citrate plasma or FNG (10 µg/mL) solution for 1 hour at 37°C. The amount and conformational state of adsorbed FNG was studied using a modified enzyme immunoassay as previously described [14]. Briefly, monoclonal mouse anti-FNG antibody (Sigma Aldrich, Clone 85D4, 1: 8000) which identifies a conformational sensitive epitope of the α chain (302– 303) called D-domain, was used to measure the accessibility of this domain for platelet binding. The optical density (OD) was read at 450 nm using a plate reader Infinite F200 PRO (Tecan Trading GmbH, Männedorf, Switzerland).

3.3. Statistical analysis

Statistical analysis was performed using one-way ANOVA with Tukey's post-hoc test to determine significant differences between the various surfaces.

4. Results and discussion

4.1. Physicochemical properties of the PEM films

Thickness of dry PEM was measured (Figure 1 A) and showed substantial variations among PEM assembled under different pH conditions, consistent with the findings of Shiratori and Rubner [15]. In acidic pH media (pH 3.5), thicker films (50-55 nm) with higher refractive indices ($n \approx 1.6$), therefore higher density were formed, while in neutral pH conditions (pH 7.0), the films tended to be thinner (10-12 nm) and less dense, having lower refractive indices ($n \approx 1.4-1.5$). The increase in thickness at lower pH levels can be attributed to reduced ionization and a prevalence of more coiled conformation of the polyelectrolyte chain, especially in the case of PAA [16]. Additionally, film thickness was found to be independent on the type of the outermost layers when both polyelectrolytes are nearly fully charged [15].

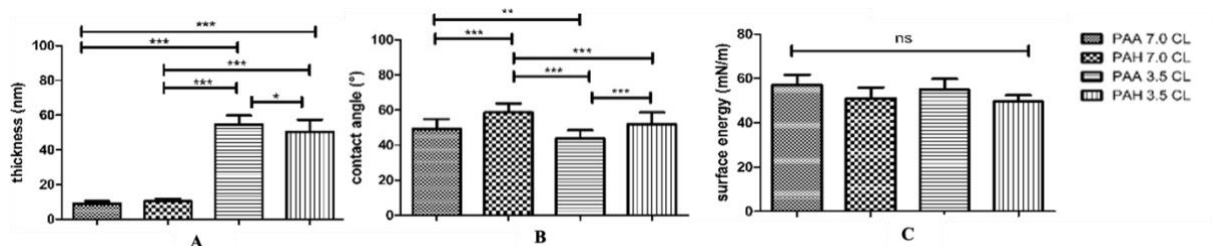


Figure 1 PEM thickness (A), Contact angle (B) and Surface free Energy (C). ns - non-significant ($P > 0.05$), * - $P \leq 0.05$, ** - $P \leq 0.01$, *** - $P \leq 0.001$.

All four PAA/PAH films exhibited moderate hydrophilicity with contact angles between 44° and 59° (Figure 1 B). The thermal cross-linking of PAA/PAH multilayers likely contributed to this similar hydrophilicity by minimizing the effect of pH on surface properties [17]. The wettability of PAA/PAH multilayers was influenced by the chemical composition of the outermost layer and to a lesser degree, by the assembly pH. PAA-terminated films were generally more hydrophilic than PAH-terminated ones, in line with previous studies [17]. No statistically significant differences in surface free energy were observed among the films (Figure 1 C), possibly due to similar levels of dehydration following thermal cross-linking. The assembly pH affects the ionization state, layer thickness and wettability, while the outermost layer determines the overall hydrophilicity.

4.2. Protein adsorption

The colorimetric Bradford assay was applied to measure the total amount of FNG adsorbed to PEM surfaces from a single protein solution (Figure 2 A). By this method, maximum adsorption levels were found on the surface of PAA 3.5 CL.

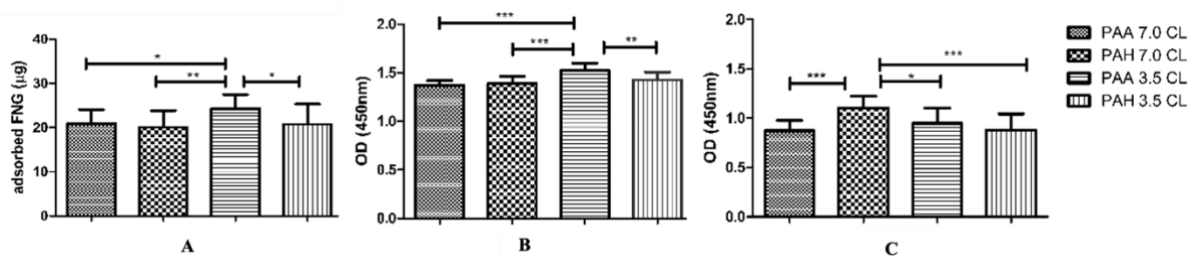


Figure 2 Adsorbed FNG from single solution, assessed by Bradford assay (A), from single solution assessed by ELISA (B) and from human citrate plasma assessed by ELISA (C). ns-non-significant ($P > 0.05$), * - $P \leq 0.05$, ** - $P \leq 0.01$, and *** - $P \leq 0.001$.

Using the ELISA method (Figure 2 B and C) we assessed the amount and conformational state of the adsorbed FNG by using a specific antibody against the conformationally sensitive D-domain in the FNG molecule, which is responsible for platelet adhesion [7]. The highest amount of FNG adsorption from single FNG solution as assessed by ELISA was also found on PAA 3.5 CL - the most hydrophilic surface (Figure 2 B). However, when adsorbed from human plasma, the highest level of FNG was found on PAH 7.0 CL - the least hydrophilic surface (Figure 2 C). The differences in FNG adsorption from single protein solution or plasma are most likely due to the competitive protein adsorption (Vroman effect) from complex protein fluids such as plasma [18]. The protein adsorption mainly correlates with the surface wettability of PEM and the functional groups of the outermost layer, which are the primary parameters contributing to the hydrophilicity. Small changes in contact angle impact the rate of protein adsorption, as also indicated by Vogler [19]. In this way, the outer layer of the PEM can determine the surface wettability and the conformational state of FNG adsorbed from complex protein fluids such as human plasma.

Conclusion

This study highlights the ability of PEM to modulate FNG adsorption on biomaterial surfaces. Surface wettability, primarily influenced by the outermost layer, significantly influences FNG adsorption. The pH of the polyelectrolyte solutions strongly affects the thickness of the films but does not lead to different FNG adsorption. This is most likely due to thermal cross-linking, which minimizes the effect of pH on the surface properties of PEMs.

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