

Hofmeister anions effect on the thickness and morphology of polyelectrolyte multilayers for biofunctionalization of cardiovascular stents

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The effect of Hofmeister anions on the surface properties of polyelectrolyte multilayers built from hyaluronan and chitosan by layer-by-layer deposition is studied by ellipsometry and atomic force microscopy. The thickness, roughness and morphology of the resulting coatings were found to depend on the type of the anion. Relationship between the surface properties and the biological response of the polyelectrolyte multilayers is established by assessing the degree of protein (albumin) adsorption.

Key words: polyelectrolyte multilayers, ellipsometry, AFM, Hofmeister anions

INTRODUCTION

Coronary artery disease (CAD) is globally one of the major causes of morbidity and mortality, affecting over 17 million people per year (4 million in Europe) (data from the World Health Organization). In addition to medical treatment, coronary stent implantation is for many patients the method of choice for the management of coronary atherosclerosis. However bare metal coronary stents can fail to maintain vessel patency due to either restenosis or stent thrombosis. Metal stents coated with an outer layer of polymer (bioabsorbable or non-bioabsorbable) can be drug-loaded, thus providing more controlled and sustained drug delivery, that might allow optimal drug-tissue interactions [1].

In the recent years the use of polyelectrolyte multilayers (PEMs) fabricated by layer-by-layer (LbL) self-assembly of polycations and polyanions has emerged as a powerful and versatile strategy to engineer surface films for biofunctionalization and drug delivery [2-4]. A wide variety of polyelectrolytes (PE) ranging from designed synthetic polymers to naturally derived biopolymers may be employed for film build-up. The resulting biomimetic PEM films are of special interest for coating dental and orthopedic implants, particularly when they contain components of the native extracellular matrix of bone.

The physicochemical characteristics of the PEM films, such as thickness, stiffness, chemistry, stability, permeability, composition, biofunctionality and dynamics, can be tunable to a large extent and they can act as a key to modulate the interaction with biomolecules and cells [5, 6]. Scanning- or atomic force microscopy have been widely used for studying the topography and the mechanical properties of PEMs [7-11].

This study addresses the optimization of biocompatibility of PEM films built from the biodegradable linear polysaccharides hyaluronan (HA) and chitosan (Chi), possessing an enhanced thrombo-resistance [12], by addition of various Hofmeister anions and monitoring of the thickness and morphology of the resulting coatings. The exchange of counterions between the dipping solutions and the multilayers represents a promising way to tune PEMs' hydrophobicity [13, 14]. We focus on the effect of monovalent chaotropic (ClO_4^-), kosmotropic (F^- and CH_3COO^-), and neutral (Cl^-) anions on the biological response of the PEMs, evaluating the albumin adsorption. Thereby we establish proper conditions for optimal biocompatibility of the studied matrices for blood-exposed cardiac stents.

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EXPERIMENTAL

Materials. Polyethylenimine (PEI) (MW \approx 750 kDa, 50 wt.% solution), chitosan (Chi) (MW \approx 50–190 kDa, 75–85% deacetylated), both from Sigma Aldrich (Steinheim, Germany), and hyaluronic acid (HA) (MW \approx 360 kDa) from Lifecore Biomedical, LLC (Chaska, USA) were used as received. HA and Chi were dissolved in 250 mM solutions of NaCl, NaF, NaClO₄ or CH₃COONa (all from Sigma-Aldrich) to a concentration of 1 mg/ml, pH 5.5. PEI was dissolved in ultrapure water to a concentration of 2 mg/ml and deposited as a first layer acting as a uniform anchoring network for the formation of consecutive layers.

Preparation of PEM films. PEM films PEI(HA/Chi)_{3.5-12.0} (sub indexes denote the number of the deposited bilayers) were prepared by layer-by-layer (LbL) technique using the hand dipping method [15] on silicon (100) wafers (10×10 mm, CrysTec GmbH, Germany) preliminarily cleaned by consecutive ultrasonication in acetone and isopropanol (2 min each) and activated by oxygen plasma cleaning. The film build-up was pursued at 25°C by alternating dipping of the silicon wafers into HA and Chi solutions (for 10 min) and three washing steps (2 min each) in the corresponding 250 mM electrolyte solution. The number of deposited HA/Chi bilayers varied from 3.5 to 12. After the last deposition step the samples were washed in water and dried in a nitrogen stream.

Atomic force microscopy (AFM) imaging was performed with NanoScopeV system (Bruker Inc.) in tapping mode in air. Standard silicon nitride (Si₃N₄) probe tips (Budget Sensors, Innovative solutions Ltd., Bulgaria) were used (tip radius <10 nm). The PEM-covered silicon wafers were fixed to the metal pads and scanned with rate 0.5 Hz. Each sample was examined at 5 different locations all over the surface. The images (512×512 pixels) were captured in height and deflection modes and roughness analysis was performed using NanoScope 6.13R1 software. All images are presented with a simple first-order flattening.

The adsorption of bovine serum albumin (BSA) (from Serva Electrophoresis GmbH) on the PE-coated silicon substrates was also investigated by AFM. BSA, at a concentration 0.5 mg/mL, was adsorbed on the outer layer of the PE films. After 60 min the samples were rinsed with water to remove all free and loosely bound albumin molecules, dried with nitrogen and scanned.

Ellipsometry was used to monitor the film growth. Woollam M2000D rotating compensator spectroscopic ellipsometer with wavelength range from 193 to 1000 nm was used. Measurements were performed at an angle of incidence 69°. To obtain the film thickness the raw data were fitted by four-layer model, considering the contribution from the air, PEM, SiO₂, and Si.

RESULTS AND DISCUSSION

The typical ordering of the anions in the Hofmeister series is as follows: SO₄²⁻, HPO₄²⁻, OH⁻, F⁻, HCOO⁻, CH₃COO⁻, Cl⁻, Br⁻, NO₃⁻, I⁻, SCN⁻, ClO₄⁻, but this order can be slightly different, depending on the phenomenon under examination. The species to the left of Cl⁻ (neutral anion) are referred to as kosmotropes (water structure makers), while those to its right are chaotropes (water structure breakers). Sodium salts of F⁻, CH₃COO⁻, Cl⁻, and ClO₄⁻ were selected for HA/Chi multilayers deposition. The specific ion effects on the properties of PE multilayers become important above 100 mM for anions and 250 mM for cations [16]; therefore we used electrolyte concentration of 250 mM.

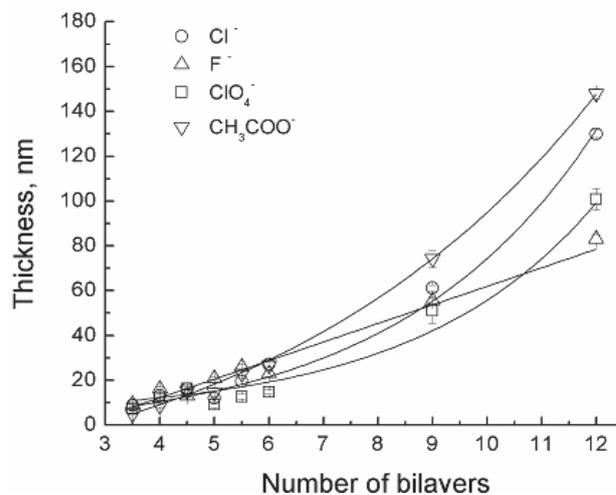


Fig. 1. Thickness of PEM films with different number of HA/Chi bilayers built in the presence of different Hofmeister anions.

(HA/Chi) multilayers growth. The change in films thickness during the deposition of up to 12 bilayers in the presence of different electrolytes is presented in Figure 1. For the thin multilayers composed of less than 6 bilayers the thickness is poorly dependent on the type of the Hofmeister anion involved in the build-up process, and the maximal difference between the thinnest and the thickest film is less than 10 nm, while after deposition of more than 6 bilayers the

thickness difference increases reaching 70 nm at 12 bilayers. The thickness of (HA/Chi)₁₂ films depends strongly on the type of the Hofmeister anion present in the dipping HA and Chi solutions and increases in the order $F^- < ClO_4^- < Cl^- < CH_3COO^-$. Except the films built in the presence of F^- , the thickness of the other HA/Chi films increase in accordance with the arrangement of the anions in the Hofmeister series and the growth of the ion hydration radius. The chaotropic anion ClO_4^- causes thinning of the HA/Chi multilayers, compared to these containing Cl^- . As opposite the kosmotropic anion CH_3COO^- increases the thickness.

The film thickness increases in a nonlinear (exponential) fashion in the presence of the neutral Cl^- , the large kosmotropic CH_3COO^- and chaotropic ClO_4^- anions, whereas the addition of the small kosmotropic F^- anion results in a linear thickness growth. These results corroborate the findings of Picart and co-workers [17], who attributed the exponential growth mechanism to Chi's ability to diffuse in and out of the entire HA/Chi film at each deposition step. These authors also reported that HA is non-diffusing specie in LbL assembly of PEMs. Detailed studies on the film build up of systems comparable to HA/Chi films have also been reported by Porcel et al. [18, 19]. The same linear thickness growth is reported by Salomäki et al. [20] for PSS/PDADMA films built in the presence of F^- , while addition of Cl^- and ClO_4^- leads to exponential growth.

In the case of interaction of ions with strong polyelectrolytes it is generally accepted that the increase in the hydration shell of the ions, which is related to weaker polarizability, leads to weaker interaction between the ions and the polyelectrolytes.

This is associated with weaker coiling of the polyelectrolyte chain, which in turn decreases the thickness and roughness of the multilayers [16, 20-22]. In the case of monatomic ions as in the halide series (F^- , Br^- , and Cl^-) the interaction between anion and synthetic PSS/PDADMAC polyelectrolytes increases with the increase of the ion size and ionic strength [16, 20, 21, 23]. However, we observe clearly expressed opposite effect. Since our experimental data included also polyatomic anions, the use of the anion radius in the characterization of polyelectrolyte binding is not relevant. More accurate interpretation would involve precise consideration of anion hydration shell and the factors affecting it. Several reasons can be suggested for this behavior. Due to its strong binding affinity and low extent of hydration the chaotropic anion ClO_4^- would dehydrate the film, while the kosmotropic anion CH_3COO^- brings along the largest amount of water into the film. It seems that for polymer couples consisting of two or at least one strong polyelectrolyte the interaction between that polyelectrolyte and the counter-ion plays a key role for the final thickness. However for the couple HA/Chi of weak natural polyelectrolytes that are highly hydrated (the water content of the (HA/Chi)₅ multilayer is around 400%, as obtained by thermogravimetry analysis [24]) the degree of hydration of the multilayers is of crucial role.

Films' Morphology. Each step of HA/Chi films build up was imaged by AFM. At the early stages of the deposition small islets are seen but they coalesce and become larger as the number of deposited bilayers increases. After the deposition of approximately 5 bilayers, the surface becomes fully

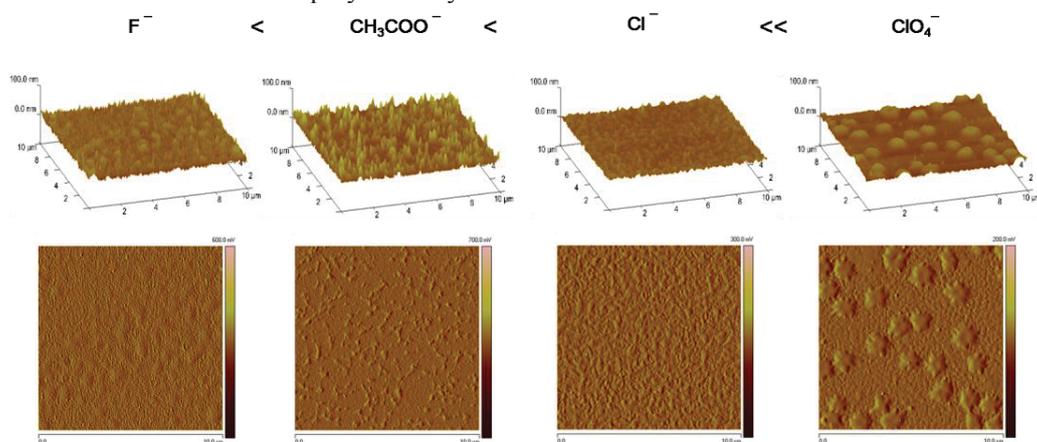


Fig. 2. Typical 3D- (top) and deflection- (bottom) images of (HA/Chi)₅ films on silicon taken by AFM in tapping mode.

coated and a homogeneous film is formed. Figure 2 shows clear difference in the surface morphology of the PEMs triggered by the introduction of different anions into the dipping HA and Chi solutions. Analogously to previous studies, in the presence of Cl^- wormlike or vermiculate pattern emerges [17]. The bumps are about 15 nm in depth and 350-400 nm wide, although these numbers may be an overestimate because of AFM tip convolution effects [25]. Addition of the kosmotropic CH_3COO^- anion results in formation of tread-like interlaces, much smaller but twice higher than the vermiculate pattern. The other kosmotropic anion, F^- , favors the formation of multiple and even smaller, needle-like structures, whereas the chaotropic anion, ClO_4^- , induces formation of star-like islands with uniform shape and diameter of 1 μm . The size of the surface structures formed during the deposition follows the Hofmeister series $\text{F}^- < \text{CH}_3\text{COO}^- < \text{Cl}^- < \text{ClO}_4^-$.

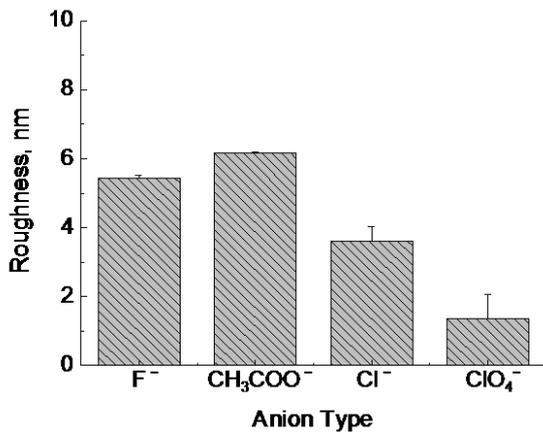


Fig. 3. Root-mean-square roughness of (HA/Chi)₅ films built in the presence of Hofmeister anions.

As anticipated, the observed changes in the films' morphology are reflected in the root-mean-square roughness (Rrms) of the surface. Figure 3 illustrates

that the surface roughness of HA/Chi films (derived from five images with scanned area 10×10 μm , including the images in Fig. 2) grows in the order $\text{ClO}_4^- < \text{Cl}^- < \text{F}^- < \text{CH}_3\text{COO}^-$, thus following the Hofmeister series. It has been found that in the case of polyelectrolyte multilayers built of strong synthetic PE [16, 20-22, 26], chaotropic ions screen stronger the charge of the oppositely charged PE than the kosmotropic, which in turn leads to folding of the PE chain and to a greater thickness and higher roughness of the resultant multilayer. Our results show a pronounced opposite effect - the addition of the chaotropic ClO_4^- anion to the weak polysaccharide solutions results in the construction of very smooth films with surface roughness about 1.4, that is in the range of the substrate roughness, and about 2.6 times lower than the roughness of the films built with Cl^- -addition, which amounts to ca. 3.6 nm. At the same time the roughness of the coatings built in the presence of the kosmotropic anions F^- and CH_3COO^- is 1.5 and 1.7 times higher than that of the reference films, reaching 5.5 and 6.2 nm, respectively.

Biocompatibility. To test the biological response of the resulting PEM films we made a qualitative assessment of the degree of adsorption of the protein albumin. In our case the negatively charged BSA mainly adsorbs on the Chi-ending multilayers. The surface morphology of the multilayer films before and after albumin adsorption is presented in Figure 4. The albumin adsorption depends on the surface properties, the most pronounced one being observed on coatings prepared in the presence of chaotropic anion, which are smoother and albumin accumulation on them strongly increases the roughness thereof (the Rrms in Fig. 4 is derived from five images with scanned area 1.5×1.5 μm , including the images in Fig. 4).

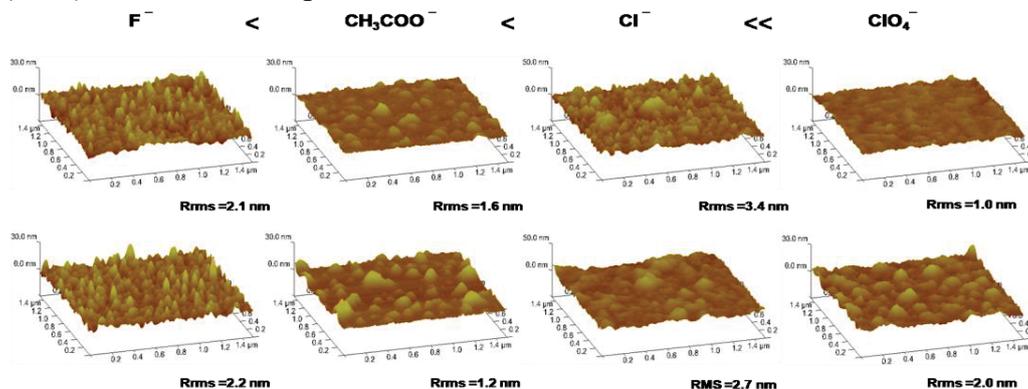


Fig. 4. 3D-topography images of (HA/Chi)₅ films, before (top) and after (bottom) BSA adsorption, taken by AFM in tapping mode at magnification 1.5 × 1.5 μm .

In the case of HA/Chi films deposited in the presence of Cl^- , characterized with highly furrowed and rough surface, the albumin molecules accumulate in the indentations and fill them, and as a result the roughness is reduced. Very weak or completely absent adsorption of albumin is observed on the surface of HA/Chi multilayers prepared in the presence of kosmotropic anions, the surface roughness in the presence of CH_3COO^- is slightly decreased, while it remains the same in the presence of F^- .

CONCLUSIONS

We demonstrate that the physicochemical properties of polyelectrolyte multilayer films, built from the natural linear polysaccharides hyaluronan and chitosan, can be successfully modified by addition of chaotropic and kosmotropic anions belonging to the Hofmeister series. We also proved that the introduction of kosmotropic anions improves the PEMs' biocompatibility contributing to increase of the surface roughness and decrease of the albumin adsorption. Although the adsorption of proteins is very important for the accommodation of medical devices such as surgical tools and implants inserted in a human body, in some cases, like cardiovascular stents implantation, protein adsorption on biomaterials can be an extremely unfavorable event; and the adhesion of clotting factors may induce thrombosis, which may lead to stroke or other blockages [27].

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ЕФЕКТ НА ХОФМАЙСТЕРОВИ АНИОНИ ВЪРХУ ДЕБЕЛИНАТА И МОРФОЛОГИЯТА НА ПОЛИЕЛЕКТРОЛИТНИ МУЛТИСЛОЕВЕ ЗА БИОФУНКЦИОНАЛИЗИРАНЕ НА КАРДИОВАСКУЛАРНИ СТЕНТОВЕ

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(Резюме)

Ефектът на хофмайстерови аниони върху повърхностните свойства на полиелектролитни мултислоеви изградени от хиалуронан и хитозан, е изследван посредством елипсометрия и атомно силова микроскопия. Установено е, че дебелината, грапавината и морфологията на получените покрития зависят от типа на аниона. Посредством оценка на степента на адсорбция на протеин (албумин) е дефинирана зависимостта между повърхностните свойства на полиелектролитните мултислоеви и биологичния им отговор.