

Fabrication of alternating polycation and albumin multilayer coating by electrostatic layer-by-layer adsorption

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The key role of surface for cell-biomaterials interaction has been recognized now. New strategies aim at the tailoring of biomaterial's surface only to render biomaterials biocompatibility, while preserving the bulk properties of the underlying support [1]. The self-organization of polymers has been increasingly explored for the preparation of well-defined surfaces and interfaces in recent years [2, 3]. "Electrostatic self-assembly" (ESA) method, which based on the alternating physisorption of oppositely charged polyelectrolytes, represents a new, alternative solution for biomaterial coating [4, 5].

Albumin, which is the most abundant protein present in plasma, was found to be "passive" to both platelets and bacteria [6]. Many techniques have been employed for the preparation of surfaces covered albumin, including covalent binding of albumin, covalent binding of albumin trigger ligand and the more simple methods of absorbing albumin directly onto the substrate surface [7, 8]. However, most of these techniques require special equipment and/or have severe limitations with respects to substrate size and topology as well as the film quality and stability.

The current research will explore construction of albumin multilayers via ESA method, with the goal of developing a fast, easy processing and shape-independent method for nonthrombogenic coating.

A gold plate (10 × 10 mm) was first cleaned by immersing it in "piranha" solution (3:7 volume ratio of 30% H₂O₂ and concentrated H₂SO₄ for 30 s and thoroughly rinsed in distilled water, followed by immersing into a solution of 2 mM mercaptopropionic acid in absolute ethanol to form a monolayer of mercaptopropionic acid (MPA). The plate was taken out from the solution after 12 h, thoroughly rinsed in absolute ethanol, and allowed to dry. The self-assembled monolayer plate was then immersed into 1 mg/mL PEI solution in PBS (PH = 7.4) for 15 min, and was then washed thoroughly by PBS. Thereafter, this PEI coated substrate was im-

mersed into a 1 mg/mL albumin solution in PBS for 15 min and washed it by PBS. Repeating this cycle gave self-organized multilayer films.

The process of electrostatic self-assembly of PEI/albumin was monitored by electrochemical impedance spectroscopy (EIS) described previously [9].

Fig. 1 shows the results of impedance spectroscopy on modified electrodes with various PEI-albumin layer numbers in the presence of equimolar 2.5 mM [Fe(CN)₆]^{4- / 3-} + 0.5 M Na₂SO₄, which are measured at the formal potential of [Fe(CN)₆]^{4- / 3-}. From Fig. 1, significant differences in the impedance spectra are observed upon the stepwise formation of the multilayers. The impedance spectra present a semicircle line that corresponds to the electron transfer limited process.

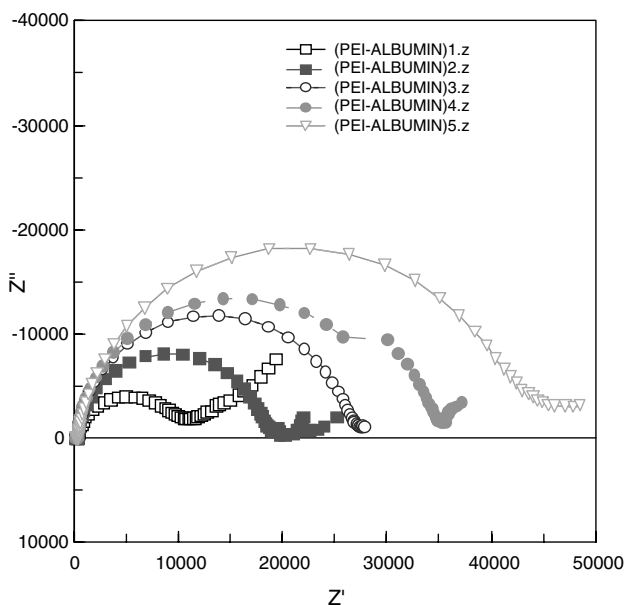


Figure 1 Complex plane impedance plots at a (PEI/albumin)_n multilayers.

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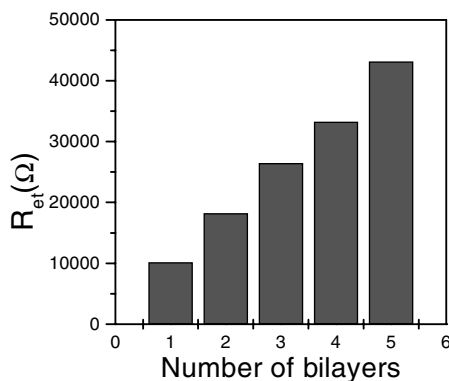


Figure 2 Relationship of electron-transfer resistance with the number of multilayers.

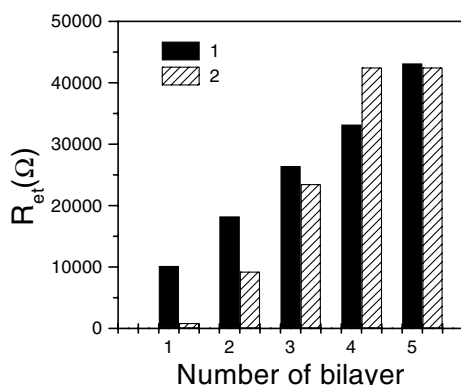


Figure 3 The R_{et} of PEI/albumin multilayers before (1) and after (2) immersion into the tris-HCl buffer for 21 days.

The diameters of the semicircle parts increased significantly with increasing layer numbers. This increase of the diameters indicates that the charge-transfer rate of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ becomes reduced gradually. It is attributed to the hindrance effect to the redox couples, which is caused by the deposition of PEI-albumin insulating multilayers on electrode surface. Upon the stepwise multilayer formation, it becomes more and more difficult for $[\text{Fe}(\text{CN})_6]^{4-/3-}$ to access the electrode surface to react.

The respective semicircle diameters correspond to the interfacial electron-transfer resistance R_{et} . In this

paper, R_{et} reflects the electron-transfer kinetics of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ at the electrode interface. The value of R_{et} depends on the dielectric and insulating features at the electrode/electrolyte interface. Fig. 2 shows the R_{et} changes with stepwise deposition of PEI and albumin layers. Because PEI and albumin are both nonconductive, their multilayer films will block the electron-transfer of $[\text{Fe}(\text{CN})_6]^{4-/3-}$ and R_{et} should increase gradually. The good linear relationship between R_{et} and layer numbers reveals the layer-by-layer assembly of PEI and heparin.

The surface composition was determined by reflection absorption spectra (RAS). The RAS of both 1 bilayer and 3 bilayer of PEI/albumin present the characteristic of amide I and II bands of protein peptide groups at 1656 cm^{-1} and 1548 cm^{-1} . Albumin was successfully deposited onto surface.

In order to assess the stability of the multilayer coating, different deposited layers of PEI and albumin onto plates were dipped into the tris-HCl buffer solution (pH 7.35) for 14 days. The different bilayers of PEI/heparin were then determined by EIS.

The significant decrease of R_{et} in 2 bilayer of PEI/albumin modified surface indicates the 2 bilayer of PEI/heparin was eluted by the tris-HCl buffer solution. With increasing number of bilayers from 4 to 10, the R_{et} of PEI/albumin modified surface was almost the same after tris-HCl buffer elution for 14 days, the multilayer coating was stable and keep the same the hindrance effect to the redox couples.

Static platelet adhesion experiments were used to investigate the blood-compatibility of the control plates and 5 bilayer of PEI/albumin deposited plates. Compared with the control, the PEI/albumin deposited surface significantly reduced the platelet adhesion (Fig. 4).

All above results demonstrated that construction of a stable albumin covered multilayer coating on substrate via ESA of PEI/albumin is possible. The buildup is easy and the procedure can be adapted to almost any type of surface as long as surface charges are present. Moreover, the method is valid whatever the shape of the solid. The researches of constructing albumin covered surface onto true biomaterial, i.e., stainless steel and

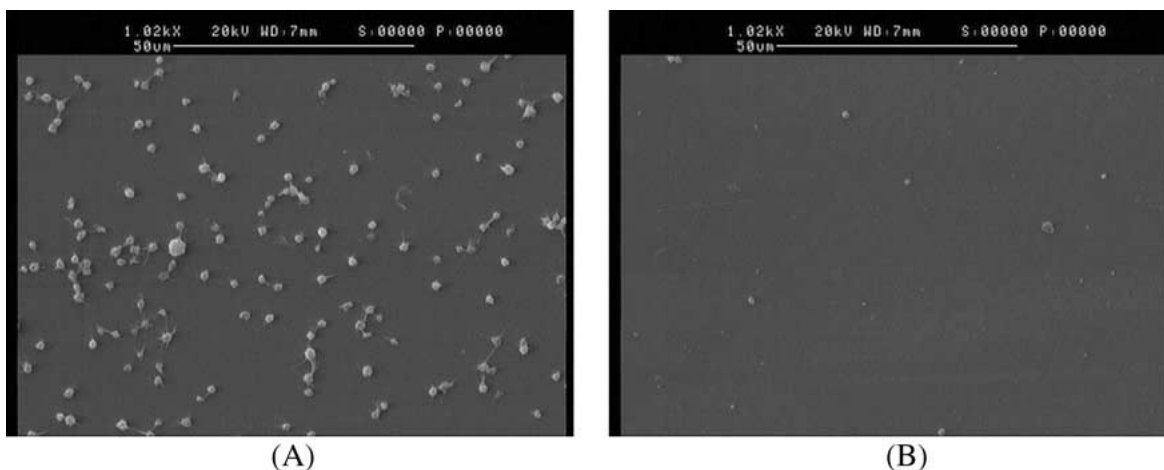


Figure 4 SEM photomicrographs of platelet adhesion onto PEI/albumin unmodified (A) and modified surface (B).

poly(vinyl chloride), are ongoing and will be reported elsewhere.

Acknowledgments

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