High Performance Interface Based on Polyelectrolyte Multilayers for Conventional Immunoasssay.

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Statement of Purpose: Polyelectrolyte multilayers (PEMs) have shown excellent potential in various biomaterial applications. Using given oppositely charged polyelectrolytes, one can easily prepare PEMs by controlling their structure at the nanometer level using layer-by-layer (LbL) technology. Based on the diverse behavior of protein adsorption, we fabricated a heterofunctional interface from differently charged PEMs on both sides of a substrate by a novel alternate drop coating process [1)], which could simultaneously regulate the amount of protein adsorption on each side of the substrate by electrostatic forces [2)]. Moreover, the heterofunctional interface could achieve selectively different charged protein adsorption on each side of the substrate in the protein mixture [3)]. Taking this into account, we considered that the PEMs could also control the protein adsorption during each process in the enzyme-linked immunosorbent assay (ELISA) system, thus leading to an improved conventional immunoassay. In this study, we investigated that the behaviors of protein adsorption in the ELISA system and the relationship between the detection sensitivity and the number of layers of the PEMs [4)].

Methods: Poly(diallyldimethylammonium chloride) (PDDA) and poly(sodium 4-styrenesulfonate) (PSS) were used as strong polyelectrolytes. We dropped PDDA aqueous solution in the wells of a polystyrene (PS) microplate for 1 min at room temperature; subsequently, the plate was rinsed with 1 mmol/L Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, pH 7.4) for 20 sec and then removed. Next, PSS aqueous solution was dropped as described above. This process was repeated for preparation of 1-step to 10-step PEMs.

The primary antibody (rabbit anti-mouse IgG) was adsorbed onto PEMs at 4°C overnight. Next, ovalbumin (OVA) was adsorbed at 37°C for 1 h. Mouse IgG and goat anti-mouse IgG-HRP were used as the antigen and secondary antibody, respectively. The antigen-antibody reaction was carried out at 37°C for 1 h.

Results: On the negative PEMs, the amount of primary antibody adsorption was greater than that of positive PEMs and PS plate. On the other hand, the amount of OVA adsorption on the positive PEMs was greater than that of negative PEMs and PS plate due to the strong electrostatic interaction between the PEMs and OVA molecules. The surface coverage of OVA on the positive PEMs was over 100%. Moreover, the ratio of molecules (OVA/IgG) for the positive PEMs that was 11.5 times and 2.5 times higher than that of negative PEMs and PS plate, respectively (**Fig. 1**). This result indicated that positive PEMs have excellent potential to improve the conventional ELISA system due to the efficient blocking effect.

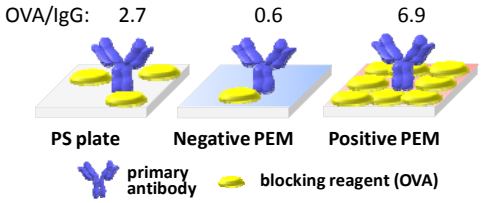

Fig. 1. Illustration of blocking effect per one primary antibody molecule on PS plate, negative, and positive multilayers.

We investigated the sensitivity of antigen detection (specific signal to noise ratio; S/N) of the PEMs and PS plate in order to estimate the PEMs performance in the ELISA system. We obtained that regularly oscillating behaviors in the signal and noise absorbance, and the S/N ratio from 2-step to 10-step assembly. The values of specific signal (S) by subtracting the noise from the signal on the positive PEMs were larger than that of the negative PEMs and PS plate, since the noise was efficiently suppressed by the significant blocking effect of the positive PEMs. Hence, the S/N ratio values took the sharp turn from the PS plate to the 10-step assembly of PEMs (**Fig. 2**). Moreover, the calibration curves for antigen detection on the positive PEMs had wide range of concentration from 0.002 to 5 μg/mL and largest change in signal as compared with the negative PEMs and the PS plate.

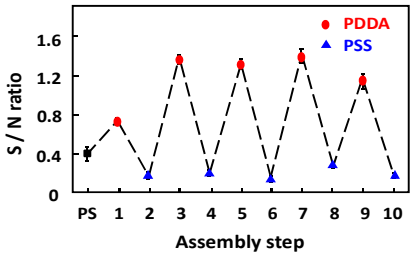

Fig. 2. The specific signal (S) to noise (N) ratio on the PS plate and each layer of the PDDA/PSS multilayers. (n=5)

Conclusions: We discovered that the positively charged polyelectrolyte multilayers obtained highest sensitivity of antigen detection due to the efficient inhibition of nonspecific adsorption by the excellent blocking effect, as compared to the conventional PS plate in the ELISA system. We suggest that positively charged polyelectrolyte multilayers could modify a given substrate to easily create various high performance devices.

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