Nanostructured Phospholipid Polymer Surface for Making a High Sensitive Microdiagnostic Device

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Statement of Purpose: Recently, miniaturized biosensors for microdiagnostic devices have been designed to allow bedside monitoring of chemicals and biomolecules. It will enable to shorten the assay time, to decrease the sample consumption, and to realize automated assay. However, the significant decrease in sample volume results in low specific signals under the detection limit. Furthermore, in the microscale environment, the high surface area/volume ratio magnifies the influence of nonspecific binding of an analyte or a labeled antibody to the solid surface, arising high background or noise level. To develop a highly sensitive assay in a microscale environment, both enhancement of specific signals and reduction of nonspecific signals should be achieved. In this study, we developed a new solid biointerface with a high sensitive enzyme-linked immunosorbent assay (ELISA) by integrating a novel phospholipid polymer with a nanoscale surface modification process known as the electrospray deposition (ESD) method[1].

Methods: Poly [2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA)-co-p-nitrophenyloxycarbonyl diethylene glycol methacrylate (MEONP)] (PMBN) was synthesized[2] (Figure 1(A)). The MPC unit shows a high resistivity to protein adsorption and effectively suppresses the denaturation of biomolecules[3]. The MEONP possesses active ester group, which can conjugate the antibody via dioxyethylene chain to enhance the antigen-antibody reaction efficiency. 5 wt% ethanol solution of PMBN was sprayed by the ESD devise (ES-1000, Fuence, Tokyo, Japan) at a voltage of 20 kV. Au surface was used as a conductive substrate to impress the electric field between surface and polymer solution.

ELISA was carried out using the PMBN surface. First, anti-[human thyroid stimulating hormone (TSH)] mouse IgG was immobilized on the PMBN surface. Using human TSH as the antigen performed the antigen-antibody reaction. Biotinylated anti-TSH mouse IgG and streptavidin-conjugated horseradish peroxidase (HRP) was used to enhance the signal. Then, tetramethylbenzidine was applied as a substrate for HRP. Finally, the increase in absorbance at 450 nm based on the enzyme-substrate reaction was measured using a multilabel counter (Wallac ARVOsx1420, Perkin Elmer).

Results/Discussion: The surface image of the PMBN sprayed by ESD method is shown in Figure 1(B). The nanosphere-shaped polymer network was observed on the surface sprayed by ESD. The highly uneven surface contributes to an increase in the surface area. Therefore, the large number of antibodies can be immobilized.

ELISA was carried out using PMBN. The results are shown in Figure 2. To evaluate the surface modification by ESD method, Au surface was just dipped by 0.2 wt% ethanol solution of PMBN and dried-up, then the surface was used in this assay. The PMBN surface sprayed by ESD showed high specific signal comparing dip coated surface because of a large number of conjugated antibodies by increasing surface area. Nevertheless, its back ground level kept low. It is suggested that MPC unit prevented nonspecific adsorption effectively. The Schematic illustration of the PMBN surface is shown in Figure 1(C). Thus, the high signal/background (S/B) ratio assay was realized using the PMBN surface sprayed by ESD. Additionally, antibodies conjugated to PMBN kept its stability for a several days in a dry condition at 37 ºC. The PMBN surface sprayed by ESD can produce the low detection limit, high sensitive, stable and reliable assay.

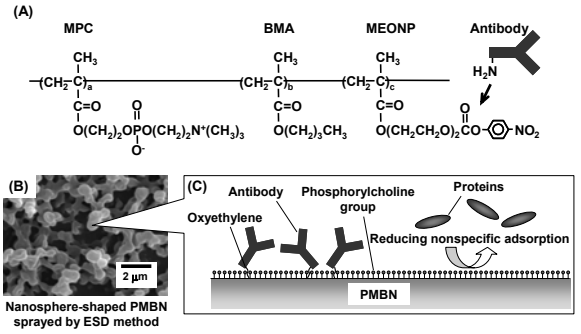

Figure 1. (A) Chemical structure of PMBN. (B) Scanning electron microscope image of the PMBN surface sprayed by ESD at 20 kV on Au surface. (C) Schematic illustration of the PMBN surface.

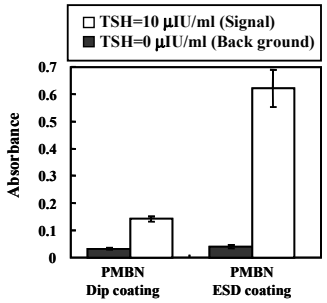

Figure 2. Absorbance values in the ELISA test.

Conclusions: We achieved highly sensitive, stable assay by the novel method of increasing the surface area for the immobilized antibody using ESD method and by decreasing the nonspecific binding applying the phospholipid polymer. Therefore, the PMBN surface prepared by ESD can be useful for the surface on microdiagnostic devices.

References:

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3. Ishihara K. J Biomat Appl. 1998;13;111-127.

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