Electronic Supplementary Information

A fluorometric microarray with ZnO substrate-enhanced fluorescence and suppressed "coffee-ring" effects for fluorescence immunoassays

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Experimental Section

Materials and reagents

Zinc acetate dihydrate, LiOH hydrate, rhodamine B (RB), ethanol, and toluene were purchased from Sigma-Aldrich (Beijing,China). Hexadecyltrimethoxysilane (HDS), aminopropyltriethoxysilane (APS), glutaraldehyde, phosphate buffered saline (PBS), glycerol, triton X-100, bovine serum albumin (BSA), human immunoglobulin G (IgG), anti-IgG antibody, and fluorescein isothiocyanate (FITC) labeled anti-IgG antibody were purchased from Sinopharm Chemical Reagent Co. (China). The human serum samples were kind provided by the local hospital. All other reagents were of analytical grade. Deionized water (>18 MΩ) was supplied from an Ultra-pure water system (Pall, USA).

Apparatus

Hydrophobic analysis of the different microarray surfaces was conducted using the contact-angle measurement machine (Jinhe, Jiangsu, China). Field emission scanning electron microscope (SEM, JSM-6700F, Japan) was employed to characterize the resulting surface of HDS-ZnO-APS microarray. Fluorescence spectrometer (Horiba, Fluoro Max-4, Japan) with microarray read holder was employed for microarray-based fluorometric analysis. Research fluorescent inverted microscope (Olympus, IX73-DP80, Japan) was utilized to image the microarray in the light and dark fields.

Fabrication of HDS-ZnO-APS microarray

Glass slides (72 x 24 mm^2) were cleaned by fresh piranha solution (H_2SO_4 : H_2O_2 = 7 : 3) to activate the substrate surface, thoroughly washed in water, and then dried in nitrogen. Those substrates were immersed separately into 0.5 %, 1.0 %, 3.0 %, 5.0 %, 9.0 % HDS in toluene to yield the hydrophobic HDS-patterned substrates. Next, ZnO nanoparticles with the particle size of about 4.0 nm in diameter were prepared as previously reported.[1] Moreover, aliquots of ZnO nanoparticles were separately mixed with different percents (0.10 %，0.30 %，1.0 %，3.0 %，6.0 %，12 %) of amine-modified APS in ethanol to form ZnO-APS nanocomposites containing 0.050 M ZnO. Meantime, ZnO-APS droplets were separately introduced on HDS-patterned or blank glass slides as the comparable controls. The contact angles were monitored for all substrates or surfaces so constructed. In addition, the ZnO substrate-enhanced fluorescence and the hydrophilic or hydrophobic properties of HDS-ZnO-APS microarray surfaces were comparably investigated by using RB solution (0.10 mmol L^{-1}). Furthermore, aliquots of ZnO nanoparticles with different concentrations (0, 0.010, 0.025, 0.050, 0.100, 0.200 mol L^{-1}) were mixed separately with 1.0 % APS in ethanol. The resulting ZnO-APS suspensions were subsequently spotted onto the HDS-patterned hydrophobic substrates to be dried in vacuum, forming the HDS-ZnO-APS microarray.

HDS-ZnO-APS microarray-based immunoassays

The HDS-ZnO-APS microarray was employed for the sandwiched immunoassays for IgG as a target model, with the fluorometric analysis procedure schematically illustrated in **Scheme 1B**. The amine-derivatized testing microspots of ZnO-APS on the HDS-ZnO-APS microarray were separately activated by 2.5 % glutaraldehyde in 0.10 M PBS (pH 7.4) for 1 h at room temperature. After being washed twice, aliquots of anti-IgG antibody (0.25 g mL^{-1}) containing 2.5 % glycerol and 0.0040 % triton X-100 were separately dropped on the testing microspots to be incubated for 8 h at room temperature. After the rinse of HDS-ZnO-APS microarray thoroughly by PBS, 0.10 % BSA was used to be incubated for 1.0 h to block any active points that might conduct the nonspecific protein binding afterwards. Furthermore, various concentrations of human IgG in serum were separately added to the testing microspots on the microarray to be incubated for 1 h at 37 ^{o}C. After being rinsed with 0.010 M PBS twice, aliquots of FITC labeled anti-IgG (2.0 μg mL^{-1}) were separately introduced to the testing microspots of microarray to be further incubated for 1 h at 37 ^{o}C, and then washed twice. Subsequently, the fluorescence measurements were conducted for the HDS-ZnO-APS microarray-based fluorometric immunoassays.

References

1. S. Li, Z. Sun, R. Li, M. Dong, L. Zhang, W. Qi, X. Zhang and H. Wang, *Sci. Rep.*, 2015, **5**, 8475.