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SERS-based multiplex immunoassay of tumor markers using double SiO_2@Ag immune probes and gold-film hemisphere array immune substrate

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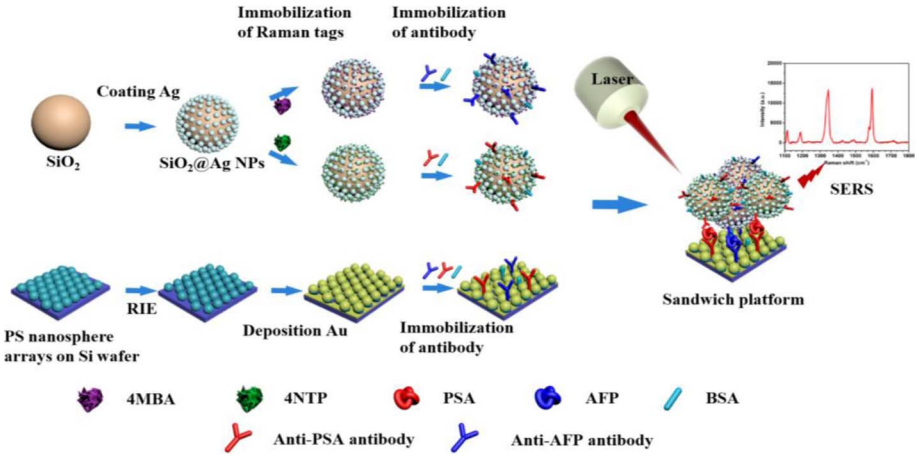
GRAPHICAL ABSTRACT

An ultrasensitive SERS-based multiplex immunoassay is proposed for detecting of multiple tumor markers. The sandwich immunoassay platform is constructed by two kinds of SiO_2@Ag immune probes and the gold-film hemisphere array (Au-FHA) immune substrate, which has been used to simultaneously detect PSA and AFP in the clinical samples with a low limit of detections.



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ABSTRACT

Highly sensitive and specific detection of tumor markers is extremely significant for the early diagnosis and treatment of cancer. We proposed a novel surface-enhanced Raman scattering (SERS)-based multiplex immunoassay proposal to implement the ultrasensitive detection of multiple tumor markers. In our work, the prostate specific antigen (PSA) and α-fetoprotein (AFP) as two kinds of target analytes were simultaneously detected by using the sandwich immune complex consisted of 4MBA-labeled SiO_2@Ag immune probes, 4NTP-labeled SiO_2@Ag immune probes and the gold-film hemisphere array (Au-FHA) immune substrate. And the selection of Raman molecules and the regulation of SERS signals among the probes are the technical keys to realize the effective multiplex immunoassay. The experiment results demonstrate that the constructed

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immunoassay platform exhibits a wider dynamic linear range from 10 fg mL^{-1} to 400 ng mL^{-1} and the limit of detection of 3.38 and 4.87 fg mL^{-1} for PSA and AFP, respectively. And more, the CA-125 was selected as an unspecific antigen to verify the high-specificity of the multiplex immunoassay. In addition, the PSA and AFP in human serum samples had been detected by the proposed immunoassay proposal and the test data present a good consistent with that of chemiluminescent immunoassay (CLIA). It is expected that the SERS-based multiplex immunoassay proposal could be applied in the practical clinical diagnoses of cancer.

1. Introduction

Nowadays, a variety of proteins have been found as tumor markers to screen and diagnose cancer, such as α-fetoprotein (AFP), prostate specific antigen (PSA), carcinoembryonic antigen (CEA) and carbohydrate antigen 125 (CA-125) [1]. And, the enormous attentions have been paid to develop the immunoassay techniques for detecting the tumor markers in blood, urine, or body tissues. For example, the enzyme-linked immunosorbent assay (ELISA), the fluorescence-based immunoassay (FBIA) and chemiluminescent immunoassay (CLIA) are widely applied in clinical quantitative detection of tumor markers [2–4]. Although there are many advantages, the above techniques suffer some deficiencies, e.g., high cost, sophisticated instrumentation and complex operation [5,6]. It is necessary to develop new immunoassay techniques for satisfying the requirements of high-sensitive and high-throughput immunoassay of tumor markers.

Fortunately, Surface-enhanced Raman scattering (SERS) spectroscopy has been considered as an attractive alternate technology to qualitatively and quantitatively measure and analyze the tumor markers due to its excellent characteristics with nondestructive chemical or biochemical analysis, unique spectroscopic molecular fingerprint and high sensitivity [7,8,9]. Based on the electromagnetic and chemical enhance mechanism, the localized surface plasmon resonance (LSPR) of metal nanostructure and the charge transfer between molecular and substrate generates giant enhancement of Raman signal of analytes absorbed on the surface of noble metal nanostructure, which result in the ultrasensitive analysis ability of SESR spectroscopy [10,11]. Recently, the extensive SERS researches have been focused on developing of the Raman molecule-labeled immunoassay of biomarkers, owing to the advantage of high sensitivity at the distinct SERS characteristics of Raman tags [12,13]. For example, the highly sensitive immunoassay of human-IgG had been performed by Raman molecule-labeled immune Au-aggregates on the SERS-active Ag-aggregates immune substrate [14]. A typical target antigen of cardiovascular disease, apolipoprotein B (apoB), had been detected at an extra low detection limit of 2 fg mL^{-1} by the sandwich immunoassay structure of the 4MBA-labeled nano-Au immune probes and nano-Ag immune substrate [15]. And a sandwich-type immunoassay structure consisted of the SiC@Ag immune substrate and the nano-Si immune probes had also been developed to test the contents of PSA, AFP and CA19-9 in the clinic serum samples [16]. On the other hand, the detections of multiple tumor markers are necessary not only for the cancer screening but also for providing of adequate information to improve the diagnostic accuracy of cancer [17,18]. Some techniques have been developed to realize the simultaneous detection of multiple tumor markers. Gao et al. proposed a multiplex electrical detection of microRNA (miRNA-126) and CEA to monitor lung cancer based on silicon nanowire field-effect devices [19]. Xu et al. chose the SERS encoded sliver nanoparticles (NPs) pyramids to realize the multiplex detection of biomarkers (PSA, thrombin and mucin-1) by the specific biorecognition between the biomarker and the given aptamer [20]. By using magnetic beads and SERS nanotags, Cheng et al. developed a SERS-based multiplex immunoassay to simultaneously detect the free PSA (f-PSA) and complexed PSA (c-PSA) for the accurate diagnosis of prostate cancer [21]. Obviously, the multiplex detection enables two or more biomarkers to be recognized simultaneously with high sample throughout, improved assay efficiency and low sample consumption [22,23]. Therefore, it is worth to develop the multiplex detection technique for improving the diagnostic accuracy of cancer.

In this study, the SERS-based multiplex immunoassay was developed for ultrasensitive detection of PSA and AFP by using the two kinds of SiO_2@Ag immune probes and the gold-film hemisphere array (Au-FHA) immune substrate. As for the preparation of the SiO_2@Ag nanoparticles with Raman tags, 4MBA and 4NTP were selected as Raman molecules because their specific Raman peaks can be clearly distinguished, and their concentrations were regulated to achieve an approximate level of the SERS peak intensity for the two kinds of SiO_2@Ag immune probes. In the fabricating process of Au-FHA SERS-active substrate, the hexagonally close-packed polystyrene (PS) nanospheres structure were arranged by Langmuir-Blodgett (L-B) method, the size and gap of PS nanospheres were adjusted by reactive ion etching (RIE) and Au film was deposited on the well-ordered PS nanospheres by electron beam evaporation (EBE). After the anti-PSA and anti-AFP antibodies were linked with the SiO_2@Ag NPs and the Au-FHA SERS-active substrate, the concentration of PSA and AFP were detected by the sandwich immune complex consisted of the double SiO_2@Ag immune probes and Au-FHA immune substrate. It demonstrates an ultra-sensitivity with the low limits of detection. Moreover, the detection of PSA and AFP in the clinical serum samples were also practically operated and achieved a result of consistent with that of CLIA.

2. Experimental

2.1. Chemicals

Polystyrene (PS) nanospheres suspensions (10 wt % in water, surfactant-free) with diameters of 500 nm was bought from Shanghai huge biotechnology Co., Ltd. Sodium dodecyl sulfate (SDS, 98%), tetraethoxysilane (TEOS, 99%), polyvinyl pyrrolidone (PVP, K30), silver nitrate ($AgNO_3$, 99.5%), aqueous ammonia (28%), tetrahydrofuran (THF, 99.9%), acetone (99%) and ethanol (99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd. 4-mercaptobenzoic acid (4MBA) was obtained from Tokyo Chemical Industry Co., Ltd. Phosphate buffer solution (PBS, pH = 7.0, recipe: 0.137 M NaCl, 0.0027 M KCl, 0.0043 M Na_2HPO_4 and 0.0014 M KH_2PO_4), TBS/0.05% Tween 20 buffer solution (TBS, pH = 8.0, recipe: 0.05 M Tris, 0.138 M NaCl, 0.0027 M KCl and 0.05% Tweens20) and 4-nitrothiophenol (4NTP) were purchased from Sigma-Aldrich. Prostate specific antigen (PSA), anti-PSA antibody (detecting and captured), a-fetoprotein (AFP) and anti-AFP antibody (detecting and captured) were obtained from Beijing Key-Bio Biotech Co., Ltd. Bovine serum albumin (BSA) was purchased from Nanjing Sunshine Biotechnology Co., Ltd. The human serum samples were obtained from the Affiliated Hospital, School of Medicine, Ningbo University. Deionized water (resistivity of 18.2 MΩ cm^{-1}) was used to prepare all solutions.

2.2. Preparation of Raman molecular-labeled SiO_2@Ag immune probes

Two kinds of Raman molecular-labeled SiO_2@Ag immune probes, i.e., 4MBA-labeled SiO_2@Ag immune probes and 4NTP-labeled SiO_2@Ag immune probes, were prepared for the immunoassay of PSA and AFP, respectively. Herein, the preparation process of 4MBA-labeled SiO_2@Ag immune probes is taken as an example to be described as follow.

First, the SiO_2 nanospheres were synthesized by the hydrolysis and