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Facile SERS-active chip (PS@Ag/SiO2/Ag) for the determination of HCC biomarker

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ABSTRACT

Hepatocellular carcinoma (HCC), which is one of the deadliest diseases worldwide, has drawn significant attention in recent research. Early diagnosis is important for the quick control and prevention of the disease. Until now, among the various detection methods, the strategies based on surface-enhanced Raman scattering (SERS) are considered the most attractive owing to the high selectivity and sensitivity. In this investigation, we designed a novel type of SERS-active chip for detecting the HCC biomarker *Lens culinaris agglutinin*-reactive Alpha-fetoprotein, AFP-L3, which utilizes a two-dimensional non-close-packed polystyrene colloid sphere arrays@Ag/SiO_2/Ag shell (PS@Ag/SiO_2/Ag) composite structure with different sizes as the SERS-active chip and an antibody-conjugated Ag/SiO_2/Ag surface labeled with 5,5-Dithiobis (succinimidyl-2-nitrobenzoate) (DSNB) as the SERS probe. A good linear relationship between the concentration of AFP-L3 and the Raman intensity ratio I_{1390}/I_{1074} of DSNB was obtained, with the correlation coefficient $R^2 = 0.997$. Moreover, when the proposed SERS-active chip was utilized over the range of AFP-L3 concentrations 0–8 ng/mL, the limit of detection (3σ) was determined to be 0.078 ng/mL. The developed method was applied to the determination of AFP-L3 in three serum samples collected from patients diagnosed with liver disease, demonstrating its potential for application in clinical diagnosis.

1. Introduction

Alpha-fetoprotein (AFP) has been well-known as a disease biomarker for the most common form of hepatocellular carcinoma (HCC) since the 1970s [1]. Although AFP has been deemed a tumor marker of HCC for several decades, its sensitivity and specificity are inadequate to diagnose HCC, especially when the tumor is small in the early stages [2]. Furthermore, high levels of AFP can be present in liver cirrhosis and hepatitis patients, consequently, more attention has been paid to exploring new biomarkers with higher sensitivity. The *Lens culinaris* agglutinin (LCA)–reactive fraction of alpha-fetoprotein (AFP-L3), a fucosylated variant of AFP that reacts with the LCA fraction, is known to be more specific to HCC than AFP [3–6]. AFP-L3, which is an important biomarker for HCC has, high specificity (> 95%) and sensitivity (56%), and hence, can be used in early diagnosis, differential diagnosis, evaluations of therapeutic effects, and prognosis monitoring [3].

To date, there have been many methods developed for the detection of cancer biomarkers, such as enzyme linked immunosorbent assay (ELISA) [7], electrochemical immunosensor [8], chemiluminescence immunoassay [9], fluorescence immunoassay, 10 light-scattering immunoassay [11], and radioimmunoassay [12]. However, all these reported methods are time-consuming and very elaborate. To ensure fast and accurate detection, the establishment of a simple, fast, highly selective, and sensitive detection method is vital for treating the disease successfully.

Surface-enhanced Raman scattering (SERS) is one of the most sensitive techniques for detecting a single analyte in the mixture [13]. Until now, SERS-based assays have been widely applied in biomedical and life sciences [14–16], clinical diagnoses [17], environmental sensing applications [18,19], and food safety controls [20]. For the SERS phenomenon, there are two widely accepted enhancement mechanisms, electromagnetic enhancement (EM) and chemical enhancement (CM), in which CM depends on the charge transfer of an electron from the Fermi level of a metal to a high-energy unoccupied orbital of the molecule [21]. It is almost universally accepted that EM is the most important contribution to the SERS phenomenon observed in electrically

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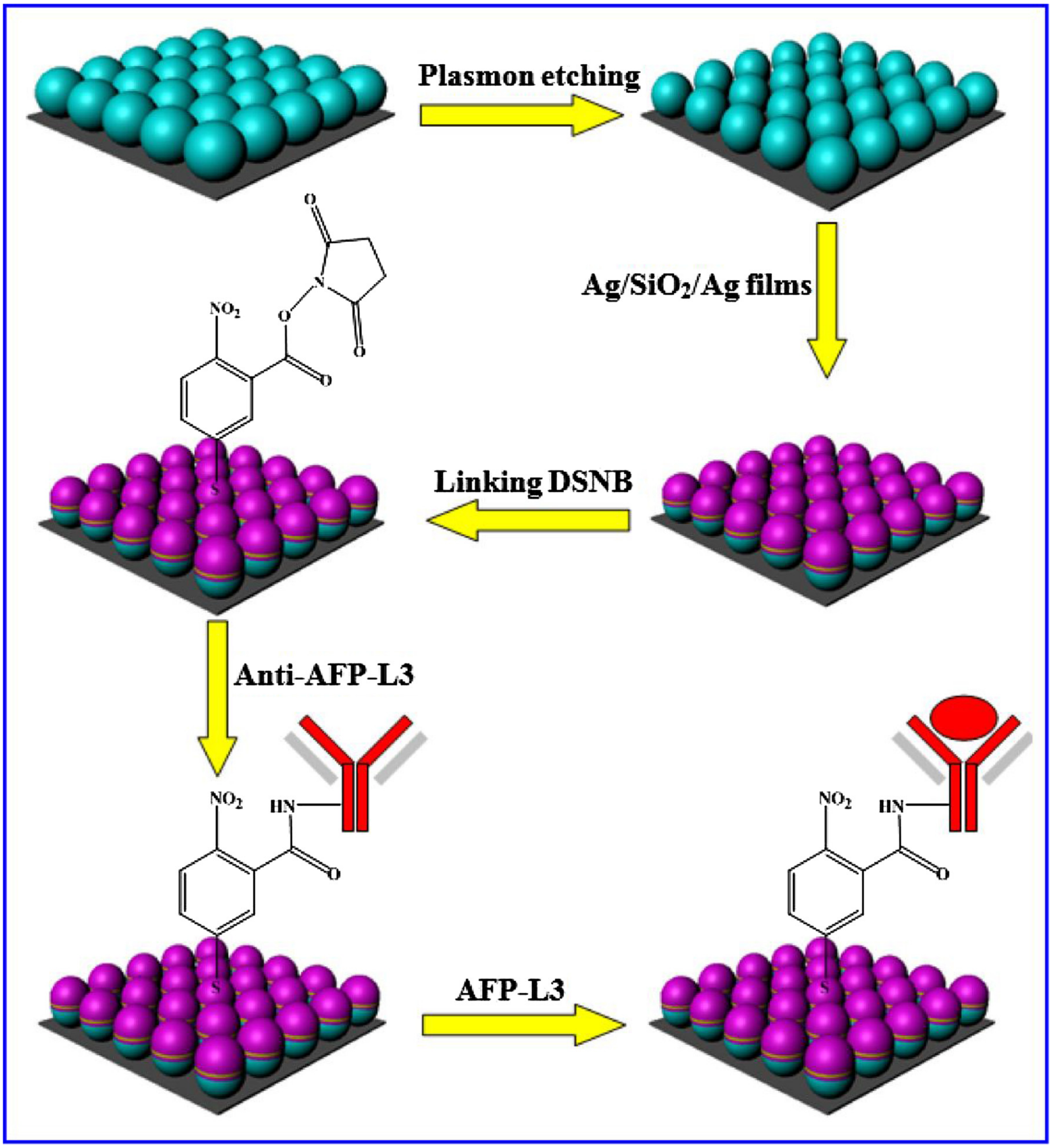

Scheme 1. Fabrication progress of the PS@Ag/SiO_2/Ag chips and chemisorption of the DSNB molecules on the SERS substrates, followed by the covalent binding of anti-AFP-L3 and the subsequent capture of the corresponding antigen AFP-L3.

conducting materials [22]. Recently, the development of ultra-sensitive SERS-active chips has been studied based on the fact that the SERS signal of the Raman reporter adsorbed on the nanoparticles can be significantly enhanced by the interparticle plasmonic coupling induced by the aggregation of nanoparticles. It allows the fast and specific detection of the molecular markers of serious or deadly illnesses. For instance, to implement enrichment and separation simultaneously, many magnetic nanoparticles have been used to develop a sandwich immunoassay to detect cancer biomarkers [23,24]. Another study reported multiplex immunochips for the high-accuracy detection of AFP-L3% based on SERS, which has implications for early liver cancer diagnosis [25]. In addition, gold nanodot array chips were used to detect the AFP antigen quantitatively over a wide concentration range 1 fg/mL–100 ng/ mL in the human serum [7].

It is well known that metallic materials have a significant influence on plasmonic properties. Noble metals, such as Ag, Au, and Cu, are widely accepted as the ideal plasma materials [26,27], especially Ag, which can generate active plasmonic properties. The surface plasma resonance (SPR) of the noble metals depend on the shape, size, and medium, which are the main contribution of EM enhancement near the metallic surface [28]. Besides, the multilayered nature of the nanostructure is beneficial for producing plasmonic enhancement [29]. The monolayer, bilayer (metal/dielectric or metal/metal), and trilayer (metal/dielectric/metal) of the SERS-active substrate always have different contributions. The trilayer materials coated on the nanosphere are more significant because of their additional degree of freedom in the plasmonic band, which can be utilized more effectively to enhance the Raman scattering [30–32].

In this study, SiO_2 was introduced as an ultrathin dielectric layer to form a composite with Ag plasmonic material in order to enhance the SERS performance. Polystyrene sphere@Ag/SiO_2/Ag chips were fabricated by sputtering an ultrathin SiO_2 layer (approximately 2.5 nm thick) between the two Ag layers on the PS templates to form Ag and SiO_2 composite nanosphere arrays. Here, SiO_2 dielectric materials can