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Graphene oxide enabled tandem signal amplification for sensitive SPRi immunoassay in serum†

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A tandem signal amplification using bioconjugated graphene oxide and subsequent silver catalytic deposition is innovated for surface plasmon resonance imaging (SPRi) to sensitively and specifically immunoassay tumor biomarkers in serum, achieving a limit of detection down to 100 pg mL−1 with a broad dynamic range for α-Fetoprotein (AFP).

Surface plasmon resonance imaging (SPRi) is a powerful tool for high-throughput and multiplexed analysis of biomolecules and biomolecular interactions with advantages of label-free and real-time.1,2 Limited sensitivity, however, is a bottleneck of SPRi technique to severely restrict its practical applications in a wide variety of fields. In particular, quantitative detection of specific biomarkers in blood or serum is efficient and inexpensive method in early diagnosis and screening of fatal malignant tumors,3–9 the usually used cutoff values of most of biomarkers for clinic diagnosis are around a concentration level of several or tens nanograms per milliliter, which is very difficult for the current SPRi techniques to achieve.

Several signal amplification strategies such as surface-initiated polymerization, enzyme-catalyzed deposition and bioconjugated nanoparticles have been developed to improve the SPR sensitivity.10,11 All these works are mainly to introduce nanoparticles or polymer/precipitation on sensing surface to intensify the local dielectric constant change for enhancing SPR signals and thus improving the sensitivity. Nevertheless, the ubiquitous nonspecific protein adsorption on the sensing surface seriously impedes specific signal amplification, and thus the bio/chemical reactions used to amplify the SPR signal often result in false positive.12,13 Further, the amplified signal is not proportional to the target concentration in some cases, leading to difficult practical applications due to a narrow detection dynamic range.

Therefore, a reliable signal amplification with high specificity and large dynamic range for quantitative SPRi immunoassay is highly demanded.

We have developed a nonfouling SPRi microarray chip built on poly(oligo(ethylene glycol) methacrylate-co-glycidyl methacrylate) (POEGMA-co-GMA) brush for label-free immunoassay of multiple cancer biomarkers with a detection limit of 20–100 ng mL−1 for various biomarkers.5 The polymer brush is able to load dense probes in a dry environment while ultimately suppress nonspecific protein adsorption in aqueous solution. In this work, we report on an innovative tandem SPRi signal amplification, in which reduced graphene oxide (rGO) plays a key role, as shown in Fig. 1. The second antibody-conjugated rGO sheet interacts with the target (α-Fetoprotein, APF) to form a sandwich immunocomplex for generating the first SPRi signal amplification, followed by catalyzing the silver reductive deposition in a silver enhancement solution containing silver nitrate and hydroquinone18 to produce the secondary SPRi signal amplification. The immunosensing surface is constructed by covalently immobilizing monoclonal biomarker antibody on the POEGMA-co-GMA brush film with a thickness of ca. 30 nm (See AFM image in Fig. S1). The nonspecific adsorption of both protein and rGO bioconjugate on the chip surface is ultimately suppressed due to the nonfouling nature of the polymer brush. The silver reductive deposition is triggered exclusively by the rGO and resulting silver particles are controllably deposited on the rGO surface. With this scheme, highly sensitive and specific immunoassay of biomarkers is accomplished in a wide concentration range.

Fig. 1 Scheme showing the tandem SPRi signal amplification strategy. The capture of target AFP, the detection signal is first amplified by using bioconjugated rGO to form immunocomplexes on the sensing surface; second signal amplification is generated by subsequent silver reductive deposited on rGO surface.

GO sheets are synthesized from natural graphite by modified Hummers method19 with lateral dimension of 1–2 μm and thickness of ~1.3 nm according to the AFM image in Fig.
the attachment of antibodies on the GO is achieved with a mild one-step decoration strategy using the spontaneous affinity of proteins to the basal planes of GO in slightly alkaline solution. Fourier transform infrared (FTIR) spectrum (Fig. 2b) shows the pristine GO characteristic peaks located at 3430, 1735, 1627, 1407, 1228, and 1055 cm\(^{-1}\), corresponding to the vibrations of O-H, C=O, C=C and C-O bonds, respectively. After the bioconjugation, the peak at 1735 cm\(^{-1}\) disappears, indicating partial reduction of GO (rGO) by the reductive residues such as Tyrosine (Tyr) in the protein, and new peaks at 1533, 1450, 1393, 1255, 1160 cm\(^{-1}\) can be assigned to the amide II and amide III bonds of the proteins, confirming successful attachment of antibody on the rGO sheets.

We validate the tandem amplification strategy by detecting AFP as a model biomarker in human serum. After capturing AFP target in 10% human serum with a specific concentration, polyclonal antibody (Rabbit anti-AFP) is introduced to form sandwich structure on the sensing surface, followed by anti-Rabbit IgG conjugated rGO to generate the first signal amplification. Fig. 3 shows the in situ SPRi responses on various sensing spots, where all the reflectivity curves remain stable on the spots of 100 ng mL\(^{-1}\) and below when flowing Rabbit anti-AFP, indicating that the AFP concentrations (\(\leq 100\) ng mL\(^{-1}\)) are too low to evoke detectable signals. However, when the bioconjugated rGO solution is subsequently flowed through the sensing spots, evident increases in reflectivity signals are observed due to the capture of rGO conjugates driving by the affinity between the conjugated anti-Rabbit IgG and Rabbit anti-AFP. It is noted that the signal intensities are positively related to the AFP concentrations and the negative control spot keeps low noise signal, suggesting a AFP-specific nonfouling SPRi chip and good specificity of the first signal amplification. Signal of as low as 5.0 ng mL\(^{-1}\) could be differentiated from the negative control. As the first amplification tag, the rGO sheets load huge amount of antibodies and the rGO-antibody conjugate is able to specifically bind on the sensing surface and efficiently intensify the SPRi signal.

To further amplify the detection signal and decrease the detection limit, silver catalytic deposition on rGO is utilized, where the carbon lattice of rGO catalyzes the silver reductive deposition from the silver solution, as demonstrated by the control experiment (see Fig. S3 for TEM image in ESI and previous work). As shown in Fig. 4, the SPRi responses on various spots increase greatly when the silver growing solution flows through the chip surface for 5 min. In this process, rGO sheets catalyze the deposit of elemental silver (Fig. S3), which is capable of largely amplifying the SPR response due to its high mass-density and localized coupling with the gold thin film of SPRi chip. On the negative control spots where no rGO is captured, the SPRi signal change remains negligible upon flowing silver growing solution, indicating good amplification specificity.

A gold surface can also catalyze the silver reductive deposition, which could result in nonspecific signal amplification. However, in this work, the gold surface is completely covered by a dense layer of polymer brush, which may efficiently block the access of either silver ion or hydroquinone to the underlying gold surface, therefore the catalytic deposition of silver on the gold surface is ultimately suppressed, as proved by curve f in Fig. 4. It is further confirmed by the observation shown in Fig. S4, in which the SPR angle remains stable on polymer brush modified gold chip when exposing to silver growth solution, implying no silver deposition, which distinctly differs from that on clean gold surface. As the silver deposition exclusively occurs on the rGO surface, the second signal amplification possesses excellent specificity.

The biomarker dose responses are plotted with its
concentrations using the tandem signal amplification, as shown in Fig. 5, which is a typical immunoassay curve as experimental and theoretical results reported. Each data point represents the average value obtained from three parallel spots and the corresponding standard deviation is shown on the point as well. According to the $M_0 + 3S_0$ definition, the detection limit (produces signal higher than $M_0 + 3S_0$, where $M_0$ is the mean signal for negative control and $S_0$ is the standard deviation) of 5.0 ng mL$^{-1}$ is achieved for AFP in human serum when using rGO conjugates alone for signal amplification, and is further improved to 100 pg mL$^{-1}$ by subsequent silver catalytic deposition-enabled amplification. The dynamic range covers 100 pg mL$^{-1}$ to 10 ng mL$^{-1}$. The performance is better than previously reported results in terms of detection limit and dynamic range, as summarized in Table S1 in ESI. AFP is a specific indicative of hepatocellular carcinoma and other chronic liver diseases. Its usual cut-off value for clinic diagnosis is 20 ng mL$^{-1}$, therefore, the tandem signal amplification strategy reported here provides good sensitivity for SPRi immunoassay of AFP and other biomarkers in serum even after 100-fold dilution for practical clinic applications.

In brief, we develop a tandem signal amplification method to significantly improve the performance of SPRi chip immunoassay in serum for high sensitivity, good specificity and wide dynamic range, thus providing great potential for high-throughput and accurate SPRi immunoassay in clinic applications.

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