

DOCTORAL THESIS

Nanoparticles assisted disease biomarkers sensing by microscopic and spectrometric methods

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Date of Award:
2016

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ABSTRACT

Nanoparticles has drawn attention in the past few decades for their large surface area-to-volume ratio, unique optical property, fast mass transportation and etc.. They are widely applied in biomedical field as they are excellent signal transducers.

Among all detection approaches, fluorescence detection, especially fluorescence resonance energy transfer (FRET), is one of the most popular approaches for their great convenience. In the first detection scheme, a well-designed nanoprobe was utilized for direct trypsin quantification. Herein, a graphene quantum dot (GQD) applied as a donor while a coumarin derivative, CMR2, acted as an acceptor. Moreover, bovine serum albumin (BSA), as a protein model, was not only considered as a linker for the donor-acceptor pair, but also a fluorescence enhancer of the quantum dots and CMR2. In the presence of trypsin, BSA was digested, thus, the FRET system was destroyed. Consequently, the emission peak of the donor was regenerated while the emission of the acceptor was reduced. The trypsin was quantified by a ratiometric measurement for two emission peaks. The detection limit of trypsin was 0.7 $\mu\text{g/mL}$, which is 0.008-fold of the average trypsin level in acute pancreatitis patient's urine. Moreover, the approach was proved to be highly selective, suggesting a high potential for fast and low cost clinical screening.

On the other hand, the optical property of nanoparticle has captured a great attention as its light scattering is highly sensitive to local dielectric environment. Two light scattering based detection approaches were demonstrated, including simple counting method and plasmonic scattering enhancement method. For simple counting method, antibody modified nanoparticle was applied to target antigen, providing a sensitive but direct approach for cancer biomarkers quantification. As a proof of concept, prostate-specific antigen (PSA) was chosen as an example. Antibody-conjugated silver nanoparticles (AgNP-Ab) were served as the probe to capture PSA, forming AgNP-Ab-PSA complexes. Since the number of complexes was corresponding to the amount of PSA, the antigen was quantified by counting the number of silver nanoparticle under dark field microscopy (DFM) coupled with charge-coupled device (CCD) camera. The detection limit of 9 pM of this assay was well below the PSA threshold of prostate cancer patient, suggested the feasibility of our assay in diagnosis application.

Besides counting of nanoparticle, the scattering intensity of nanoparticle is also informative. In the third assay, immobilized capture antibody-conjugated gold nanoparticles ($\text{AuNP-Ab}_{\text{capture}}$) were firstly utilized in capturing the target analyte, followed by the introduction of strong scattering detection antibody-conjugated

silver nanoparticles (AgNP-Ab_{detection}). In the presence of the corresponding antigen, the two metallic probes sandwiched the antigen and stayed at close proximity, resulting a strong plasmonic coupling effect of those nanoparticles. Consequently, the scattering intensity of gold nanoprobe was greatly enhanced. The antigen was quantified by measuring the intensity change before and after the immunoreaction. To demonstrate the high flexibility of this assay, several antigens including carcinoembryonic antigen (CEA), PSA and alpha fetoprotein (AFP) were quantified with this method, giving detection limit at 1.7 pM, 3.3 pM and 5.9 pM respectively, which were much lower than their cut-off levels of corresponding diseases. Detections of CEA, PSA and AFP in real sample were demonstrated, suggesting a high potential in clinical application.

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