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A photoacoustic immunoassay for biomarker detection

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ABSTRACT

Challenges in protein biomarker analysis include insufficient sensitivity for detecting low-abundance biomarkers, poor measurement reproducibility, and the high costs and large footprints of detection systems. To address these issues, a new detection modality was developed for analyzing protein biomarkers based on the plasmon-enhanced photoacoustic (PA) effect. The detection modality employed a heterogeneous immunoassay scheme and used gold nanoparticles (AuNPs) as the signal reporter. Due to their localized plasmon resonance, AuNPs can strongly interact with intensity-modulated laser excitation and generate strong PA signals, which are subsequently sensed and quantified using a microphone. As an example, the performance of the PA immunoassay was evaluated by detecting the human interleukin 8 chemokine. The PA immunoassay provided approximately 143 × lower limit of detection (LOD) than observed with the gold standard enzyme-linked immunosorbent assay – a decrease from 23 pg/mL to 0.16 pg/mL. In addition to the significant performance improvement in terms of the LOD, the PA immunoassay also offers advantages in terms of compatibility with low-cost instruments and the long-term stability of assay results.

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1. Introduction

The ability to detect and quantify disease-related biomarkers at trace concentrations enables diagnosis of the corresponding disease at an early stage (Fan et al., 2008; Hanash et al., 2011; Ludwig and Weinstein, 2005; Rhea and Molinaro, 2011). The predominant methods used for biomarker detection rely on immunoassays, which enable the measurement of protein-biomarker concentrations in a complex solution with antibodies. Immunoassays can target specific biomarkers and measure their concentrations with very high sensitivity and specificity. Most immunoassays employ some type of labeling materials, such as enzymes, fluorescent dyes, magnetorotational labels, or radioactive isotopes, to generate a detectable signal (Gan and Patel, 2013; Hecht et al., 2011; Huang et al., 2011; Lequin, 2005; Voller et al., 1978; Yingyongnarongkul et al., 2003). Among the various types of immunoassays, enzyme-linked immunosorbent assays (ELISAs) are considered the gold standard, where enzymatic tags are used to generate colorimetric signals in liquid substrates. Chemiluminescence- and fluorescence-based immunoassays have become popular for protein analysis, owing to their capabilities of achieving improved sensitivities (Dodeigne et al., 2000; Mathias et al., 2008). However, their drawbacks include the need for catalysts or enhancers, a long incubation time before reaching a stable output signal, the degradation of labels, and the use of expensive optical detectors and filters (Goldys, 2009; Lakowicz, 1999). An ideal biomarker-detection assay should offer high sensitivity, high specificity, low-cost instrumentation with high throughput, and long-term stability of the labels for future analysis.

The development of nanomaterials and nanoparticles during the past decade provides tremendous opportunities to address existing challenges in biomarker detection on a nanoscale (de la Escosura-Muniz et al., 2010; Huang et al., 2009; Segev-Bar and Haick, 2013). In particular, the interaction of light with a metal nanoparticle results in the collective oscillation of free electrons within the nanoparticle, which is known as localized surface plasmon resonance (LSPR). (Sagle et al., 2011; Willets and Van Duyne, 2007). LSPR is associated with a strongly localized and greatly enhanced evanescent field, which has been utilized in several areas of research, including photovoltaics, photocatalysis, biomolecule sensing, cell imaging, and photothermal therapy (Awazu et al., 2008; Cao et al., 2002; Govorov et al., 2006; Nam et al., 2003; Stratakis and Kymakis, 2013). Here, we utilized the photothermal effect of gold nanoparticles (AuNPs) to develop a new type of immunoassay, referred as the photoacoustic (PA)-

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immunoassay. AuNPs were chosen for three main reasons. First, AuNPs can effectively convert photons into heat owing to non-radiative resonant absorption. The strong photothermal effect of AuNPs has been successfully utilized to selectively kill target cells and to serve as a signal enhancer for photoacoustic tomography (Jaque et al., 2014; Yang et al., 2009). Second, surface AuNPs can be conjugated with various types of ligands that recognize target biomarkers with high specificity (Sperling and Parak, 2013). Third, owing to their excellent physical and chemical stabilities, AuNPs can generate reproducible signals. By quantifying the PA signal, which is directly proportional to the amount of AuNPs, the nanoparticle-based immunoassay enables a sensitive, repeatable, and inexpensive approach for analyzing biomarkers.

Since the mid-1970s, the PA detection has attracted considerable interest in the analytical community due to its capability of measuring optical absorption of samples in gaseous, liquid, and solid phases (McClelland and Kniseley, 1976; Rosencwaig, 1990). The PA detection method offers several compelling features. For example, PA measurements can be performed without difficulties in collecting and detecting photons, common to fluorescence and Raman spectroscopies; as a result, no expensive photodetectors or optical filters are required. The PA technique generates quantitative signals based on a 3-step process involving AuNP optical absorption, conversion of the absorbed energy into heat, and the subsequent heat-induced thermal expansion of the adjacent media, which produces pressure oscillations or propagating acoustic waves when the incident beam is modulated at an acoustic frequency. As illustrated in Fig. 1(a), the PA-based detection of an AuNP-labeled biomarker takes place inside a sealed sample chamber, which is relatively small compared to typical acoustic wavelengths used for PA measurements. A modulated laser beam is used as the excitation source to generate pressure oscillations, and a microphone is installed inside the sample chamber to measure the signal.

Here, we present the first demonstration of a PA-based immunoassay and a detection instrument devised for the assay. By investigating the PA signal with bare AuNPs coated on a plastic substrate, we validate PA measurements as a quantitative approach for detecting AuNPs. Next, a simple test protein (streptavidin) was used to shown the correlation between the PA signal and its concentration. As an example of a PA immunoassay, the PA-based approach was evaluated by analyzing detection of a chemokine biomarker (human interleukin 8 [IL-8]). The PA immunoassay improved the limit of detection (LOD) by approximately a factor of 143 compared with the ELISA method – a decrease from 23 pg/mL to 0.16 pg/mL.

The following sections of this report are as follows. Section 2 describes the experimental details of the PA immunoassay and the detection setup for the PA experiment. Section 3 presents the results of the PA detection of AuNPs, as well as the PA immunoassay used for detecting IL-8. Section 4 concludes this study and discusses future avenues of investigation with the PA immunoassay.

2. Material and methods

2.1. Materials and reagents

Biotin-conjugated gold nanorods and streptavidin-conjugated gold nanorods were purchased from Nanopartz, Inc. (Loveland, CO, USA). Polyvinylamine (PVAm; MW=340 kDa) was obtained from BASF (New Jersey, USA) and diluted in water to obtain a 5% solid solution, which was used as the surface-functionalization material. Glutaraldehyde (GA), bovine serum albumin (BSA), streptavidin (SA) protein, Tween-20, and phosphate buffered saline (PBS, 10 mM, pH=7.4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Human IL-8 ELISA Kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The kit contained a monoclonal mouse anti-human IL-8 antibody (capture antibody), a recombinant human IL-8 protein standard, a human IL-8 biotinylated antibody (detection antibody), horseradish peroxidase-conjugated SA (SA-HRP), tetramethylbenzidine (TMB) substrate, and stop solution (0.16 M sulfuric acid).

2.2. PA detection setup

The PA detection setup consisted of a frequency doubled Nd:YAG laser (λ=532 nm; P_{out}=50 mW), an optical chopper (SRS540, Stanford Research Systems), a PA detector (PAC 200, MTEC Photoacoustics, Inc.), and a data-acquisition device, as shown in Fig. 1 (b). The acrylic substrates were placed inside the PA chamber one at a time for PA measurements. After loading the sample, the chamber was sealed to eliminate ambient noises. For all PA measurements, the chopping frequency of the optical chopper was set at 13 Hz. The laser beam from the Nd:YAG laser passed though the optical chopper and a glass window in the PA chamber. The intensity-modulated laser beam was absorbed by the AuNPs on the acrylic substrate and caused periodic heating of the air inside the

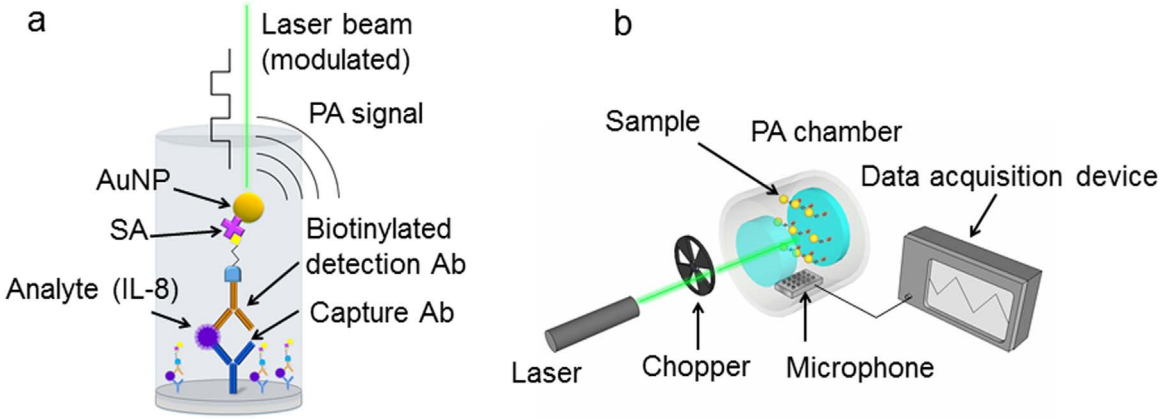

Fig. 1. (a) PA immunoassay format and (b) PA setup. During a PA measurement, a chopper is used as the modulator, which periodically blocks the laser at 13 Hz. The sample placed in a sealed chamber is periodically heated and, therefore, periodically expands and contracts, generating a pressure oscillation that is detected by the microphone and displayed as a waveform on an oscilloscope. A sandwich format is used for the immunoassay, wherein the analyte is immobilized by a capture antibody and tagged with a detection antibody, which is further tagged with a conjugated AuNP. The AuNPs generate a PA signal in the aforementioned setup, and the signal intensity reflects the concentration of the analyte.

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