Photoacoustic Immunoassay Using Plasmonic Nanoparticles

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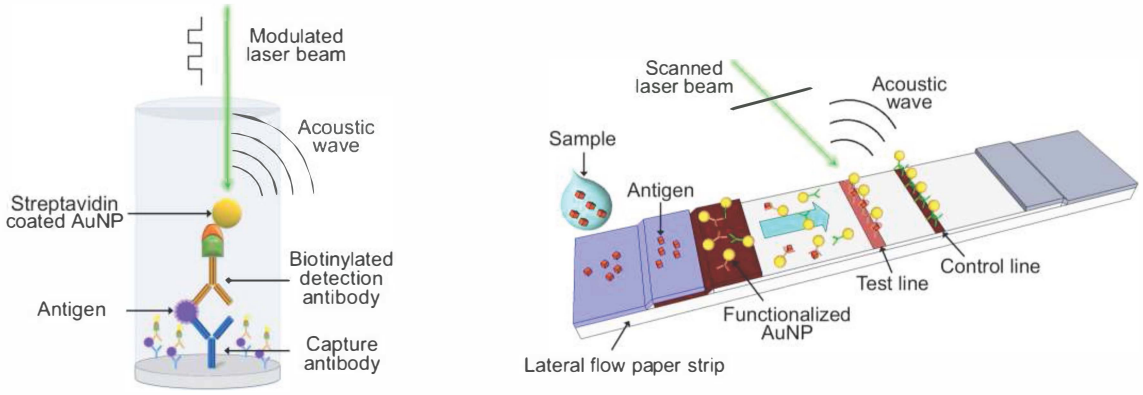
Abstract: Immunoassay based on laser-induced photoacoustic (PA) effect is demonstrated for the detection of disease biomarkers with a significantly enhanced sensitivity. The PA immunoassay employs metal nanoparticles as photoacoustically detectable labels. Compared to the colorimetric method, the PA approach offers sensitivity improvements over 60-fold and 100-fold for the microtiter plate-based immunoassay and the lateral flow immunoassay, respectively.

1. Introduction

Photoacoustic (PA) detections have been used to analyze light absorbing substances over substantial ranges of concentrations [1]. For a common PA analysis, the laser-induced heating of analyte is modulated and results in the thermal expansion and contraction of the surrounding medium, which generates acoustic signals. In this work, we present the PA immunoassay as a novel detection modality for biochemical assays based on the specific binding of a target antigen to its antibodies. The PA immunoassay employs plasmonic nanoparticles, such as gold nanoparticles (AuNPs), as labels. Due to the localized plasmonic resonance (LSPR), AuNPs interact strongly with an incident light [2]. Illuminated by a modulated laser beam near the LSPR wavelength, the AuNPs can generate strong PA signals [3] and thus function as a labeling material for PA immunoassays. The PA approach is highly compatible with many existing formats of immunoassays, such as the microtiter plate-based sandwich immunoassay and the paper-based lateral flow immunoassay (LFA) [4-5]. The PA immunoassay offers the advantages of an improved sensitivity and a low-cost detection instrument.

2. Laser-induced PA immunoassay

We implemented the PA approach in two immunoassay formats: the sandwich immunoassay using microtiter plates and the LFA. Fig. 1 (a) illustrates the principle of the PA immunoassay performed inside a single well of a microtiter plate. The assay was designed based on the standard enzyme-linked immunosorbent assay (ELISA). Instead of using an ezymatic label, the AuNPs were exploited as the label. The resulting PA signals were measured from the bottom of the well using a PA detector (PAC200, MTEC Photoacoustics, Inc.) with an intensity modulated Nd:YAG laser (λ = 532 nm, P = 50 mW, and modulation frequency = 13 Hz). The PA-based LFA assay is schematically shown in Fig. 1 (b). In order to eliminate the background signal, the laser beam was scanned across the test line on the LFA paper strip at the speed of 1.35 mm/sec and the PA signals were measured.



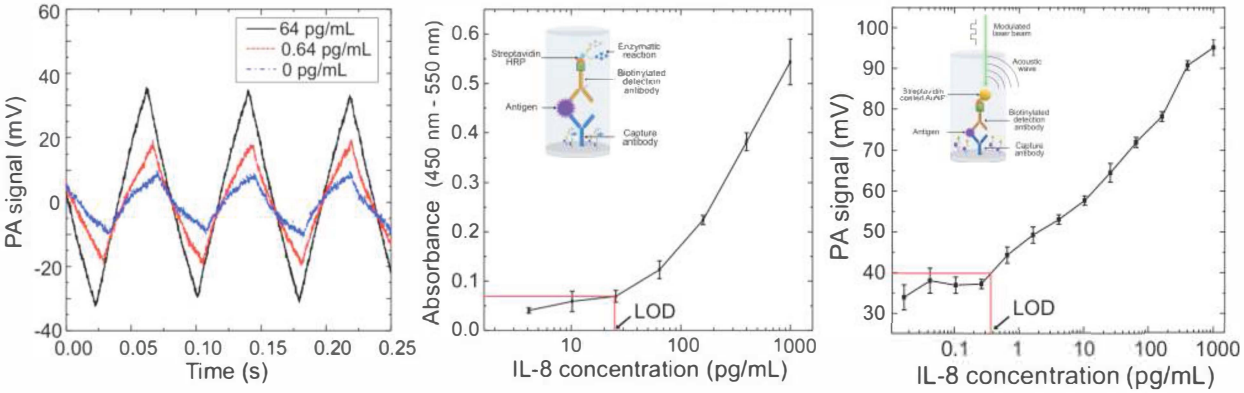
(a) (b)

Figure 1. (a) Sandwich PA immunoassay in a single well of a microtiter plate, where the detection antibodies are tagged by AuNPs. (b) LFA test strip showing that AuNP labeled antibody-antigen pairs bind with antibodies at the test line.

3. Results and Discussion

To characterize the performance of the PA immunoassay, we measured a cytokine protein, human interleukin-8 (IL-8), using the sandwich assay as shown in Fig. 1 (a). A concentration series of the IL-8 at 2.5-fold dilutions was measured. Fig. 2 (a) shows the PA waveforms generated from the IL-8 assays at three different concentrations.

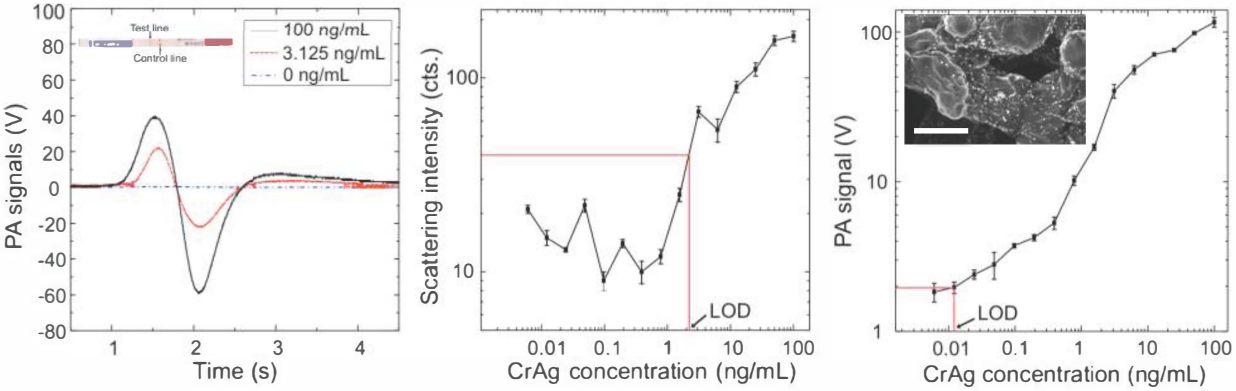
Within a cycle, the rising edge corresponds to the phase of thermal expansion and the falling edge represents the contraction phase. The average peak-to-peak value of the PA waveform taken in one second was calculated as the PA signal for each measurement. The dose-response curves shown in Fig. 2 (b) and (c) were used to determine that the limit of detection (LOD) of the PA approach was 0.38 pg/mL, a significant improvement compared to conventional ELISA method that exhibited the LOD of 25.6 pg/mL. The LODs were calculated using the fitted standard curves.



(a) (b) (c)

Figure 2. PA immunoassay for human IL-8. (a) Measured PA waveforms at three different IL-8 concentrations, (b) a dose-response curve of optical absorption measured using the ELISA method, and (c) PA signal as a function of IL-8 concentration.

Commercial LFA strips (IMMY, Inc.) for cryptococcal antigen (CrAg) were used to characterize the performance of the PA immunoassay on paper strips. For the LFA tests, the green laser beam was scanned across the test line (Fig. 1 (b)), resulting in the laser-induced heating/cooling of the AuNPs in paper strips. Fig. 3 (a) shows the measured PA waveforms of CrAg samples at three different concentrations. Using the peak-to-peak value as the PA signal, the dose-response curve measured by the PA method (Fig. 3 (c)) shows the LOD of 0.012 ng/mL, an improvement of two orders of magnitude compared to that of the visual contrast analysis (Fig. 3 (b)) using Image J. The SEM image taken at the test line on a paper strip is shown as inset of Fig. 3 (c), in which the bright spots represent the AuNPs immobilized upon the nitrocellulose membrane.



(a) (b) (c)

Figure 3. PA-based LFA analysis of CrAg. (a) Measured PA waveforms using LFA paper strips at three different CrAg concentrations. Inset: Photography of a LFA strip showing the test and control lines. (b) Dose response curve of the LFA measured using a colorimetric method. (c) Dose-response curve of PA-based LFA. Inset: SEM of the test line (Scale bar: 300 nm)

References:

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