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Streptavidin-biotin-peroxidase nanocomplex-amplified microfluidics immunoassays for simultaneous detection of inflammatory biomarkers

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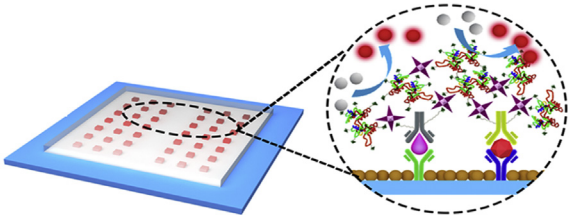
HIGHLIGHTS

- The SA-B-HRP nanocomplex is an effective signal amplification system in MIS.
- MIS has been developed for detection of PCT and IL-6 simultaneously.
- The LOD for PCT and IL-6 by MIS are 48.9 pg mL^{-1} and 1.0 pg mL^{-1}, respectively.
- MIS has successfully detected PCT and IL-6 simultaneously in serum samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous, sensitive and quantitative detection of biomarkers in infectious disease is crucial for guiding antimicrobial treatment and predicting prognosis. This work reported an ultrasensitive and quantitative microfluidic immunoassay combined with the streptavidin-biotin-peroxidase (SA-B-HRP) nanocomplex-signal amplification system (MIS) to detect two inflammatory biomarkers, procalcitonin (PCT, for discriminating bacterial infections from nonbacterial infections) and interleukin-6 (IL-6, for monitoring the kinetics of infectious disease) simultaneously. The amplification system was based on the one step self-assembly of SA and B-HRP to form the SA-B-HRP nanocomplex, which effectively amplified the chemiluminescent signals. The linear ranges for PCT and IL-6 detections by MIS were 250 -1.28×10^5 pg mL^{-1} and 5–1280 pg mL^{-1}, and the limit of detection (LOD) were 48.9 pg mL^{-1} and 1.0 pg mL^{-1}, respectively, both of which were significantly improved compared with microfluidic immunoassays without amplification system (MI). More importantly, PCT and IL-6 in human serum could be simultaneously detected in the same run by MIS, which could greatly improve the detection efficiency

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and reduce the cost. Given the advantages of high sensitivity, multiplex and quantitative detection, MIS could be potentially applied for detection of biomarkers at low concentration in clinical diagnosis.
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1. Introduction

One of the biggest challenges for the clinician is to diagnose the infectious disease in a timely manner. To make a concrete determination of infectious disease, several clinical biomarkers need to be examined simultaneously, including procalcitonin (PCT), interleukin-6 (IL-6), and so forth [1]. PCT is a polypeptide consisting of 116 amino acids and is the precursor of calcitonin [2]. Normally, the level of PCT in serum is under 10 pg mL^{-1} for healthy adult. The PCT level starts to rise after bacterial infection for 3–4 h, and reaches a maximum after 24 h [3]. Previous studies suggest that PCT shows high specificity and sensitivity in discriminating bacterial infections from nonbacterial infections [4]. IL-6 is a multifunctional cytokine consisting of 184 amino acids and regulates the immune response, haemopoiesis, the acute phase response and inflammation. Inflammatory or autoimmune diseases could influence the concentration of IL-6 in human serum [5]. After the infection of pathogen, the concentration of IL-6 rises to a peak value at 3 h, and decreases to the baseline concentration after 8 h [6]. In addition, IL-6 exhibits kinetics for monitoring the efficacy of antibiotic treatment. If the clinician only detects one biomarker from patient samples, the difference in time of abnormal levels of PCT and IL-6 could lead to the misdiagnosis of infectious disease. It is thus necessary to detect PCT and IL-6 simultaneously, which could not only discriminate bacterial infections from nonbacterial infections, but also guide the antibiotic therapy and improve the efficacy of clinical therapy.

Many efforts have been made to develop sensitive and rapid methods for detection of both PCT and IL-6. The most widely used method for rapid detection of biomarkers is the gold lateral flow test with the advantages of simple operation, fast response and effective cost. However, the low sensitivity and qualitative analysis of the method have limited its further application for detection of trace targets in complex samples such as IL-6. Some other approaches with high sensitivity include chemiluminesence immunoassay (CLIA) [7,8], fluorescence immunoassay [9–11], enzyme-linked immunosorbent assay (ELISA) [12], and electrochemical immunoassay [13–19]. Although these methods have been used for measuring the levels of PCT and IL-6 in human serum, most of them utilize bulky instruments and cannot detect two biomarkers simultaneously in the same run, which could cause a misdiagnosis and considerable delay for treatment. The development of a new strategy for multiplexed detection of PCT and IL-6 with sensitivity and rapidity is urgently required for diagnosis and prognosis of infectious diseases. Recently, microfluidic chips have been widely developed as the platforms for chemical analysis [20], bioanalysis [21–24], and clinical diagnostics [25,26], because of the capability for multiplexation [27], portable instrumentation and short time of analysis [28–31]. The micro-sized channels of the chip greatly reduce the consumption of the reagent [32], and are especially suitable for analysis of small volumes of available sample [23]. In addition, with the feature of short distance of molecular diffusion in microchannels, the time of analysis could be reduced from hours to minutes [33,34]. In the previous work, various assays based on microfluidic chip have been designed for the detection of biomarkers [25,31,35–37]. However, most of them still lack sufficient sensitivity, which prevent the real applications of microfluidic assay toward the determination of trace amount of biomarkers such as PCT and IL-6. To address these issues, it is important to develop a strategy that combines the microfluidic chip and effective signal amplification system. The streptavidin-biotin (SA-B) system has been extensively applied to improve the sensitivity of immunoassays due to its extremely high binding affinity. The dissociation constant between B and SA is 10^{-15} M [38], while that between a typical antigen and its antibody is 10^{-7} M [39]. Moreover, SA has four binding sites for B, leading to the high efficiency for the conjugations of B-enzyme, antibodies, dyes and others materials [40]. Although the previous study has demonstrated the biotinylated protein networks for signal amplification [41–46], the strategies either relied on the layer by layer assembly of SA and B-BSA, or only based on the reaction between B-Ab and SA-HRP. In addition, to produce the optimal sized surface polymer, tens of cycles of layer by layer assembly is required in the previous study [41], which is time-consuming and high-cost.

In this work, we proposed a signal amplification system based on the one step self-assembly of SA and B-HRP to form the SA-B-HRP nanocomplex, which is rapid and easy to operate. The combination of SA-B-HRP amplification system with the microfluidic immunoassays (MIS) was adapted for detection of PCT and IL-6 simultaneously in real samples. The mechanism of SA-B-HRP amplification system, limit of detection (LOD), linear detection range, specificity, and recovery of MIS were systemically evaluated. The detection results by MIS were compared with enzyme-linked immunosorbent assay (ELISA) using the chemiluminescent readout. To the best of our knowledge, it is the first report that introduced the SA-B-HRP nanocomplex into the microfluidic chip for high sensitive and quantitative diagnosis of infectious disease.

2. Experimental

2.1. Materials

Procalcitonin (PCT, 1.4 mg mL^{-1}), capture antibody of PCT (Ab_1, 5 mg mL^{-1}), detection antibody of PCT (Ab_2, 5 mg mL^{-1}), Ab_2-HRP of PCT (5 mg mL^{-1}), interleukin-6 (IL-6, 2.4 mg mL^{-1}), Ab_1 of IL-6 (1.11 mg mL^{-1}), Ab_2 of IL-6 (2.4 mg mL^{-1}), Ab_2-HRP of IL-6 (5 mg mL^{-1}), C-reactive protein (5 mg mL^{-1}), human IgG (10 mg mL^{-1}) and horse radish peroxidase (HRP) were purchased from Sino Biological Inc. (Beijing, China). Streptavidin (SA) was obtained from Abcam (England). Sulfo-NHS-LC-Biotin was from Thermo Fisher Scientific (USA). Tablets of phosphates, which were used to prepare 0.01 M phosphate buffered saline (PBS, pH 7.4), were purchased from Amresco (USA). Tween-20 was obtained from Amresco (USA). Bovine serum albumin (BSA) was from Solarbio (Beijing, China). Chemiluminescent (CL) substrate reagent kits (consisting of H_2O_2 and luminol) and centrifugal ultra-filtration units were obtained from Millipore (USA). PCT and IL-6 ELISA kits were purchased from Sino Biological Inc. (Beijing, China). Dimethyl formamide (DMF), $NaHCO_3$ and other solvents were obtained from Beijing Chemical Reagents Co. (Beijing, China). The water used in the experiments was deionized by water purification system (Millipore, USA). Polydimethylsiloxane (PDMS) and curing agent (Sylgard 184) were purchased from Dow Corning Inc. (USA).

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