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Enhanced diffusometric immunosensing with grafted gold nanoparticles for detection of diabetic retinopathy biomarker tumor necrosis factor-α

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ABSTRACT

Diffusometry is sensitive to geometric changes of particles. Target antigens can be detected through diffusivity changes resulting from their immunoreactions by functionalizing particle surface with a specific antibody. Considering that Brownian motion is a self-driven phenomenon, diffusometric immunosensing features several characteristics, such as no-washing steps, rapid detection, high flexibility, and high sensitivity. Until recently, this technique has been applied to many biomedical fields, such as monitoring of microorganism motility and diagnosis of diseases with biomarkers. Despite the abovementioned advantages, diffusivity changes in conventional diffusometry can be compromised at low-abundance antigens because proteins are much smaller than capture particles. To overcome such restriction, we present an improved diffusometric immunosensing technique by grafting additional gold nanoparticles (AuNPs) to capture particles to enhance size changes. A diabetic retinopathy (DR) biomarker, tumor necrosis factor-α was selected to evaluate the proposed immunosensing technique. Spherical AuNPs showed better enhancement than rod-like AuNPs during measurement. Limit of detection was improved by at least 100-fold down to 10 pg/mL. A dichotomous method was also developed to enable rapid detection and avoid tedious calibration. The relationship of concentrations between the two solutions used can be explicitly determined by comparing diffusivity of an unknown concentration of target molecules with that of a reference solution. Minimum discernible concentration reached as low as twofold higher or lower than basal concentration. Tear samples were collected from four volunteers, including three healthy subjects and one proliferative DR patient to prove the concept in diagnosis of the disease. All data showed good agreement with preset conditions. The technique eventually provides an insight into rapid diagnoses of diseases in the early stage.

1. Introduction

Early medical invention is a key to treating diseases. However, conventional diagnostic procedures usually deter people from receiving regular health checks because of tedious and uncomfortable processes. Alternatively, biomarkers have been widely adopted to aid diagnoses of diseases in all aspects in recent years. Biomarkers are proteins associated with specific diseases. After being released from sources, biomarkers possibly spread in body fluids, such as tears, saliva, blood, and urine. Considerable biosensors based on electrochemistry (Esteves-Villanueva et al., 2014; Jarocka et al., 2016), optics (Li et al., 2015; Singh et al., 2017; Soler et al., 2016), mechanics (Peduru Hewa et al., 2009), or electronics (Luo and Davis, 2013; Villamizar et al., 2009) have been developed to detect a wide variety of diseases with unique biomarkers. Recently, Sánchez-Tirado et al. (2017) built an amperometric immunosensor for quantification of tumor growth factor-β1. They claimed a range of measurement linearity between 15 and 300 pg/mL and an LOD of 10 pg/mL. Moreover, ion-sensitive field-effect transistors under dual-gate operation proposed by Lee et al. (2015) even reached a trace amount of hepatitis B surface antigens down to 22.5 fg/mL. Despite high precision, all the mentioned techniques still require a driving instrument to convert signals from one form to another. These techniques limit researchers to rely on stability of instruments or readjust ingredients of buffer media used. For simplicity, paper-based lateral flow diagnostics have been immensely studied in point-of-care testing (Lin et al., 2016; Park et al., 2017). However, sensitivity and LOD of the current paper-based diagnostics remain incomparable to their electrochemical, electrical, and optical counterparts.

Unlike conventional enzyme-linked immunosorbent assay modifications on fixed surfaces, bead-based biosensors receive increasing attention because of their high flexibility. A rapidly increasing number

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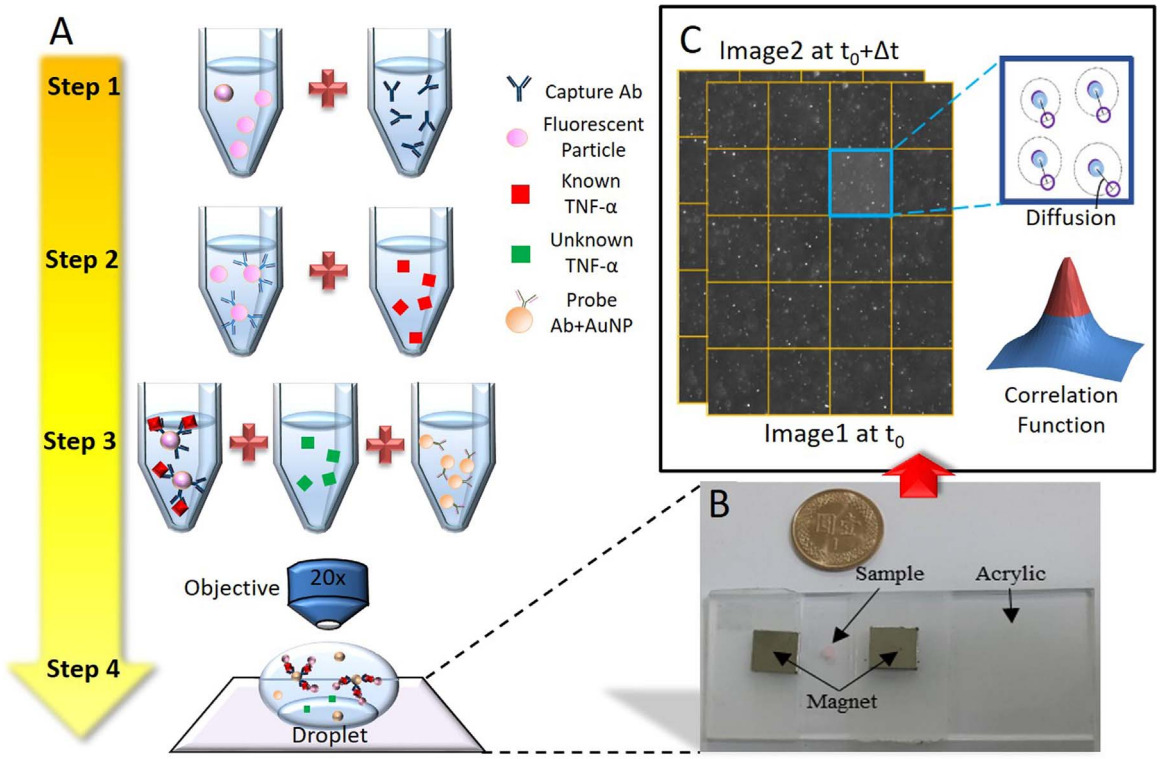

Fig. 1. (A) Flow chart of enhanced diffusometric immunosensing grafted with AuNPs. (B) Top view of acrylic microchip. (C) Schematic of particle diffusivity derived from the spatial cross-correlation algorithm.

of bead-based biosensors has been reported recently (Chung et al., 2017; Jiang et al., 2015; Liu et al., 2009; Wang et al., 2017, 2016). Yu et al. (2011) used bead-based competitive fluorescence immunoassay for sensitive and rapid diagnosis of cyanotoxin risk in drinking water. LOD of 30 ng/L was achieved in their study. A further sophisticated biosensor for quantification of bacteria was accomplished by rotating functionalized magnetic beads (MBs) in a magnetic field (Kinnunen et al., 2011). With 80 nm MBs, a response to as little as single *E. coli* cells was observed. Compared with the abovementioned bead-based biosensors, however, the driving mechanism of diffusometry, which depends mainly on Brownian motion, is relatively reliable and free from instrumental failure. Gorti et al. (2008) first proposed the Brownian motion-enabled detection of M13 viruses. A sensitivity of 500 viruses per nanoliter was achieved by analyzing diffusion change in particles. However, particle size becomes significant especially when nanometer-sized objects should be measured. For instance, 40 nm particles will be necessary to detect small biological molecules with size of 1 nm.

To enable simple bead-based detection of small molecules for early disease diagnosis or low-abundance biomarkers, gold nanoparticles (AuNPs) were grafted on capture particles to enhance diffusometric immunosensing in this study. Therefore, sensitivity of measurement based on Brownian motion was further improved. Consequently, small biological molecules, such as proteins, were detected using optically resolvable particle size down to 200 nm. For proof of concept, a DR biomarker, tumor necrosis factor (TNF)-α, was used in measurement. Compared with conventional bead-based immunosensing, the proposed technique increased LOD by nearly 100-fold, reaching 10 pg/mL. To avoid difficulty of calibration in every diagnosis, we also developed a dichotomous method based on competitive immunoassay to simplify practical use. By analyzing diffusivity of an unknown concentration of biomarker TNF-α against basal concentration, the relationship between these two subjects can be determined. In principle, diffusivity increases when concentration of TNF-α is higher than the basal level and vice-versa. Results showed that unknown concentration of TNF-α remained discernible when its concentration was only twofold higher or lower than the basal level. However, when an unrelated biomarker, LCN1, was present, no certain diffusivity changes can be determined. Repeatability evaluation at different concentrations of TNF-α showed maximum standard deviation of 7.3% in diffusivity. The device was eventually applied in actual diagnoses with three healthy subjects and a proliferative DR (PDR) patient. Basal concentration of TNF-α was set to 10 pg/mL according to a previous study (Costagliola et al., 2013). All measured data from sample tears were consistent with preset conditions. The innovation of this work focuses on (1) the use of a self-driving biosensor based on Brownian motion, (2) improving the sensitivity of bead-based biosensor for detection of small molecules with grafted AuNPs, and (3) a dichotomous method for quick clinical use without the necessity of calibration. The successful attempt provides an insight into quantitative and sensitive diagnosis for disease screening in the early stage.

2. Methods and materials

2.1. Principle of diffusometric immunosensing

Brownian motion is a self-driven and random movement of small particles. Mathematical expression for this phenomenon has been well described in the Stoke–Einstein relation (Einstein, 1956). In the formula, diffusivity is subjected to particle diameter, fluid viscosity, and environmental temperature. A slight change in particle diameter can be obtained from diffusivity by controlling fluid viscosity and environmental temperature. In general, diffusivity increases with decreased particle diameter and vice-versa (see Supporting information). Considering this relationship, surface-modified particles can be used as numerous sensors to detect the presence of target biomarkers. When particles are suspended in high concentration of target biomarkers, more target biomarkers will bind to particle surfaces, resulting in decreased diffusivity. However, the change becomes insignificant due to negligible size of biomarkers compared with capture particles. To enhance the effect of Brownian diffusion, additional AuNPs were used to