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Fluorescent-free detection on nanobiochips based on wavelength-dependent single plasmonic nanoparticles by differential interference contrast microscopy

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ABSTRACT

A selective fluorescent-free detection method of biomolecules on nanoarray biochips was developed based on wavelength-dependent single plasmonic nanoparticles by differential interference contrast (DIC) microscopy. As a model target protein molecule, human cardiac troponin I (cTnI) was bound between primary anti-cTnI oriented on gold-nanopatterned chips (GNCs) and secondary silver nanoparticle (SNP) anti-cTnI. SNPs were employed as non-fluorescent probes to determine the immuno-reaction. Gold and silver exhibited clear peaks with DIC contrast at different wavelengths because plasmonic noble metals display extraordinarily large apparent refractive indices near their plasmon resonance wavelengths. Therefore, when the SNPs-cTnI antibody was bound to GNC, the normalized maximum intensity of the DIC contrast was blue-shifted. A linear relationship of the calibration curve was obtained between the DIC intensity and cTnI concentration in the range of 85 aM–10 fM without spectroscopic interference. This combination method of wavelength-dependent DIC microscopy and plasmonic nanoparticles could overcome the limitations of the potential for nonspecific labeling by promiscuous antibodies and the lack of reagents or biomarkers for disease-specific applications.

1. Introduction

The array chip, a solid phase assay method to detect molecular interactions, is becoming an attractive tool for diagnostics, therapeutics, and basic research (Abbott, 1999). The use of microarrays for parallel screening of biomolecule profiles has been became an industry standard for drug discovery and biomarker identification. However, microarrays require a relatively large sample volume, elongated incubation time, and have a detection limit (Chen and Li, 2007). To overcome these limitations in microarray techniques, nanochip arrays have been fabricated by reducing the spot size of the traditional microarray to the nanometer scale using nanolithography techniques (Lee et al., 2002; Demers et al., 2002; Ginger et al., 2004) or carbon nanotubes (CNT) nanoelectrode arrays using CNTs (Li et al., 2003; Koehne, et al., 2003). Recently, a plasmonic nanoarray biochip was applied to a fluorescent-free detection method without a sophisticated labeling procedure using fluorescent dyes and enzymes (Endo et al., 2011; Lee et al., 2013). However, non-fluorescent detection on nanoarray biochips is not well established.

Chen et al., announced that the future of biosensors will be greatly influenced by the inclusion of nanomaterials, which provides new tools to improve the performance of immunosensors (Chen et al., 2009). A growing number of nanomaterials like metallic nanoparticles (Shim et al., 2008; Cao et al., 2002), CNTs (Jacobs et al., 2010), and superparamagnetic nanoparticles (Lloret et al., 2010) have been employed to replace conventional tags. The unique characteristics of nanomaterials, such as ultra-large surface area, superior electronic and optical properties, and readily tailored surface chemistry, have greatly increased the sensitivity and simplicity of molecular detection (Penn et al., 2003). Specifically, fluorescent-free biosensors, such as dark field scattering (Ling et al., 2009; Lee et al., 2013) and surface plasmon resonance (Hall et al., 2011) techniques, were developed on the basis of plasmonic properties of noble metal nanoparticles. In dark-field microscopy, illumination is provided by a center-blocked, high numerical aperture (N.A.) condenser (typically N.A. is 1.2–1.4). The objective N.A. must be smaller than the condenser N.A. to reject the transmitted illumination beam, resulting in lower spatial resolution

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(Gu et al., 2012). Alternatively, DIC microscopy allows for the use of the full N.A. of the microscope objective and condenser, resulting in the highest possible lateral and axial resolutions when compared to the image of a nanoparticle in dark-field microscopy (Li et al., 2007; Tsunoda et al., 2008).

DIC, also called the Nomarski-DIC theory, and potential applications were described by Allen et al. (1969), and several different versions have since been incorporated (Mehta and Sheppard, 2008). DIC is a polarizing microscopy in which the illumination technique is used to enhance the contrast in free-labeled specimens (Salmon and Tran, 1998). DIC can detect nanometer-sized objects if the optical properties are distinct from the surroundings, and the background signal can be minimized because the homogeneous media cannot produce a measurable optical-path difference. Specifically, plasmonic nanoparticles (i.e., gold and silver) can be used with the DIC technique due to unique high DIC contrast as a function of illumination wavelength and particle size (Sun et al., 2009). However, ultra-sensitive non-fluorescent detection with single plasmonic nanoparticles on biochips by DIC microscopy based on wavelength dependence has not yet been reported.

In this study, we developed a sensitive immunoassay-based biosensor for non-fluorescent detection on nanobiochips based on wavelength-dependent single plasmonic nanoparticles by DIC. DIC optical microscopy was capable of resolving the non-fluorescent immunoassay with noble metals (i.e., silver and gold) because DIC contrast images of the gold spot and SNPs were distinguished by wavelength-dependent microscopy with DIC illumination. The results confirmed whether immuno-reaction occurred based on the selective wavelength. In addition, utilization of the nanopatterned chip can overcome the limitation of non-specific binding and the multiplexing capability for disease diagnosis.

2. Materials and methods

2.1. Surface modification of silver nanoparticles

Colloidal 80 nm silver nanoparticles (SNPs, 1.10×10^9 particles/mL) were purchased from BBI Life Sciences (Cardiff, UK). Before applying SNPs to the sandwich immunoassay, SNP colloids were bioconjugated to monoclonal mouse anti-cardiac troponin I antibody (cTnI, clone 16A11, HyTest, Turku, Finland). To prepare the bioconjugation of the SNPs to the antibody, 10 mM 11-mercaptoundecanoic acid (95% MUA, Sigma-Aldrich Inc., St. Louis, MO, USA) and 30 mM 6-mercapto-1-hexanol (97% MCH, Sigma-Aldrich) dissolved in ethanol were added to a SNP solution suspended in water, followed by sonication for 10 min and reaction for 2 h 30 min to form a self-assembled monolayer (SAM) of MUA-MCH on the SNP surface, which was then washed with ultrapure water by centrifugation (12,000 rpm, 90 min, 4 °C). The SNP-MUA-MCH was resuspended in 50 mM 2-(morphozlino)ethanesulfonic acid (MES, Sigma-Aldrich) and 0.1 M NaCl (pH 6.0).

Conjugation of SNPs with the antibody was performed using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC, Sigma-Aldrich) and *N*-hydroxysulfosuccinimide (NHSS, Pierce, IL, USA). Forty μg of EDC (2 mg/mL in 50 mM MES pH 6.0) and 196 μg of NHSS (2 mg/mL in 1 × PBS) were added to the SNPs-MUA-MCH solution. After stirring at room temperature for 40 min, SNP-MUA-MCH-NHSS was washed by centrifugation (17,000 rpm, 30 min, 4 °C) and then re-dissolved in 1 × phosphate buffered saline (PBS, pH 7.4, Sigma-Aldrich). Ten μg/mL of the monoclonal mouse anti-cTnI (clone 16A11) solution in PBS (pH 7.4) was added to the activated SNPs, allowed to react using a rotary shaker for 4 h at room temperature, and stored for 12 h at 4 °C. Ten mM Tris (pH 7.5, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) and 1 M glycine (Sigma-Aldrich) were added and incubated for 15 min to quench excess hydroxylamine. The final SNP-antibody

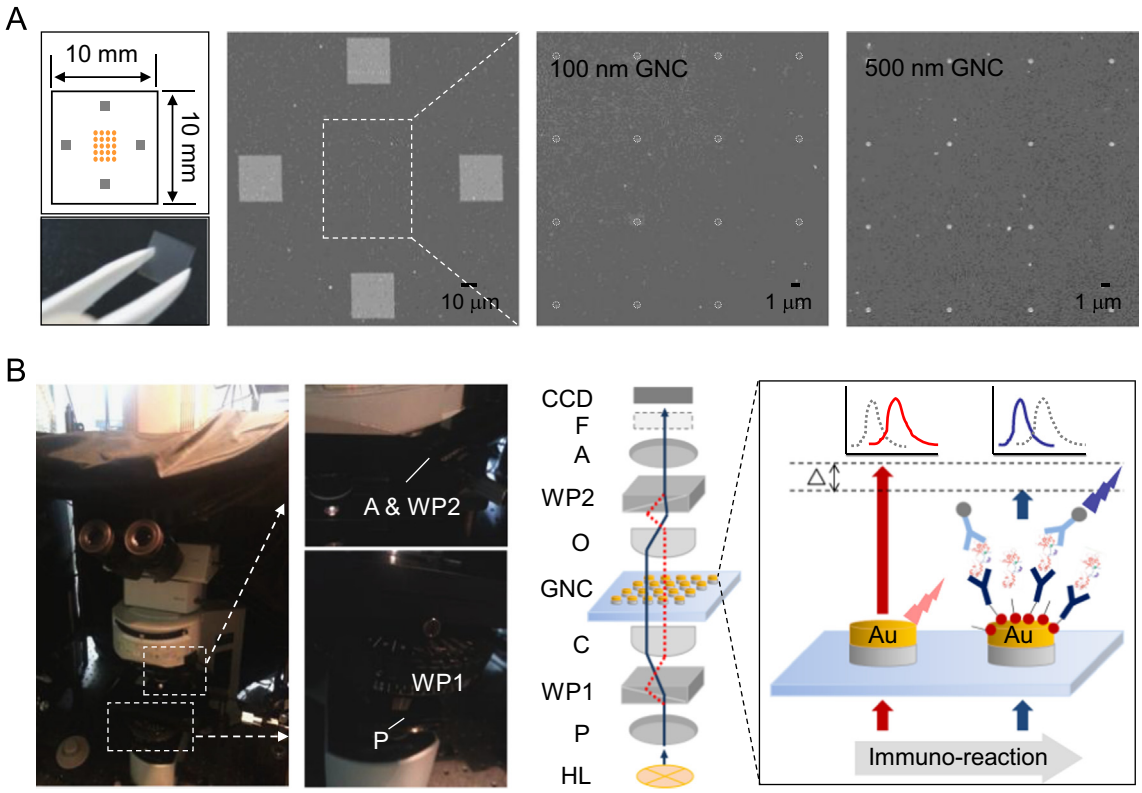

Fig. 1. (A) SEM images of GNCs. (B) Physical layout and schematic diagram of wavelength-dependent DIC for fluorescent-free sandwich immunoassay on GNC. The following acronyms are used: HL, halogen lamp; P, polarizer; WP, wollaston prism; C, condenser; GNC, gold-nanopatterned chip; O, objective lens; A, analyzer; F, filter cube; CCD, charge coupled device; SNP, silver nanoparticle.