Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Single gold nanoplasmonic sensor for clinical cancer diagnosis based on specific interaction between nucleic acids and protein

Xingyi Ma [a], Phuoc Long Truong [b], Nguyen Hung Anh [a], Sang Jun Sim [a,*]

[a] Department of Chemical and Biological Engineering, Korea University, Seoul 136-701, Korea
[b] School of Chemical Engineering, Sungkyunkwan University, Suwon 440-746, Korea

ARTICLE INFO

Article history:
Received 5 April 2014
Received in revised form
18 June 2014
Accepted 19 June 2014

Keywords:
Nanoplasmonic sensor
Gold nanoparticle (AuNP)
Localized surface plasmon resonance (LSPR)
Telomerase activity
DNA mutation
Cancer cell

ABSTRACT

Plasmonic nanomaterials reveal noble optical properties for next-generation biosensors. Nanoplasmonic biosensors have become simple, sensitive, smart, and consistent with advanced healthcare programs requirements. Notably, an individual nanoparticle analysis can yield unique target information, based on which the next-generation biosensor is revolutionary for end-point detection (single or multiplex), and can be functionally extended to biological phenomena monitoring. Here, we present a single nanoplasmonic sensing technology based on localized surface plasmon resonance for label-free and real-time detection of highly reliable cancer markers (mutant gene and telomerase) in clinical samples. The sensor specifically detects mutant DNA, and can detect telomerase from as few as 10 HeLa cells. This approach can be easily translated to detect other pathological targets with high sensitivity and specificity, and monitor key interactions between biomolecules such as nucleic acids and proteins during disease development in real time. This system has great potential to be further developed for on-chip and simultaneous analysis of multiple targets and interactions.

1. Introduction

A collective coherent oscillation of the free electrons surrounding gold nanoparticles (AuNPs) under the electromagnetic field of light results in powerful Rayleigh light scattering. The amplitude of the oscillation reaches maximum at a specific frequency, called localized surface plasmon resonance (LSPR). This phenomenon corresponds to the Mie theory and has been widely applied in (1) nanoplasmonic imaging by dark-field microscopy (Fang and Zhu, 2013; Kauranen and Zayats, 2012; Ozbay, 2006); and (2) optical sensor design and spectroscopic analysis (Estevez et al., 2014; Ma and Sim, 2013). According to the theory, the LSPR λ_{max} shift due to changes in the local refractive index by biochemical binding to the nanoparticle surface can be used to detect biomolecules such as DNA or proteins in a label-free and real-time manner. We have demonstrated an individual nanoplasmonic platform for detecting biological molecules in our previous studies (Cao and Sim, 2007, 2009; Hwang et al., 2012; Song et al., 2013; Truong et al., 2014). In this platform, every individual nanoparticle can be utilized as an independent probe by dark-field microscopy in combination with spectrophotometry. Single nanoparticle probes offer absolute detection limits due to the sensor's miniaturization to the nanoscale level. Using single-nanoparticle spectroscopic techniques, the detection limit can approach a few molecules, and perhaps even a single molecule per nanoparticle sensor (Haes and Van Duyne, 2002; McFarland and Van Duyne, 2003). Moreover, the detection volume of the single nanoplasmonic platform is comparable to that of portable electrical sensors (Kim et al., 2009; Ma and Sim, 2012; Zheng et al., 2005). This platform supports many nanosensor applications and will play a key role in next-generation healthcare systems.

Clinical cancer diagnosis is still a field to be developed. The diagnostic approaches should be rapid, sensitive and reliable (Landercasper et al., 2010; Xue et al., 2013; Zhan et al., 2012; Zhao et al., 2013). We focused on the detection of cancer markers based on the LSPR responses of single gold nanoparticles (AuNPs). Strong evidence in oncology highlights the reliability of DNA mismatch repair protein (MutS) and human telomerase as sensitive cancer markers (Acharya et al., 2003, 1996; Agaram et al., 2010; Blagoev, 2009; Xu et al., 2013; Yi et al., 2014). Both are selectively produced and quantitatively changed inside cells, and originally can be used as tools for biomedical researches. MutS is a mismatch repair protein (diameter ~10 nm) responsible for repairing errors in DNA replication (Lamers et al., 2000). It binds in warped DNA grooves resulting from mismatched base pairs, and increases the fidelity of DNA replication 100–1000 times. It has a close correlation with gene mutation-induced cancer (Agaram et al., 2010). Telomerase is a eukaryotic ribonucleoprotein enzyme (diameter ~12.5 nm) that functions as a telomere terminal

* Corresponding author. Tel.: +82 31 3290 4853; fax: +82 2 926 6102.
E-mail address: simsj@korea.ac.kr (S.J. Sim).

http://dx.doi.org/10.1016/j.bios.2014.06.038
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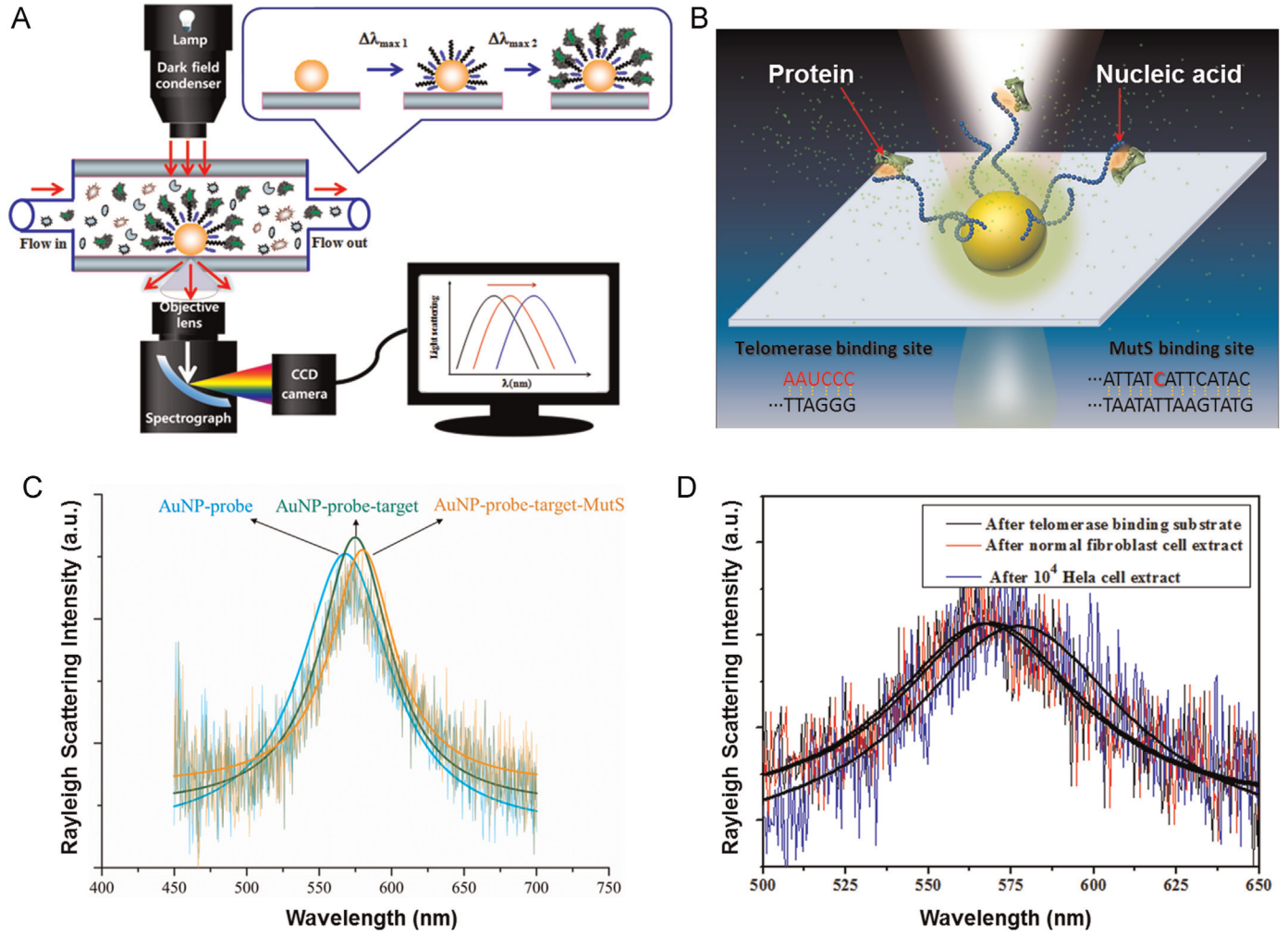

Fig. 1. Illustrations for (A) the system of dark-field microscope in combination with Rayleigh light scattering spectroscope, and (B) the interactions between nucleic acids and proteins in the sensor. The spectra for the detection of (C) mutant gene and (D) telomerase extracted from clinical cells were recorded by measuring the scattering spectra of an individual nanoparticle and fitted by Lorentzian algorithm in the same wavelength range to eliminate the noise from the system.

transferase by adding the telomeric repeats $(TTAGGG)_n$ onto the 3′-end of chromosomes in human stem cells, reproductive cells, and cancer cells using reverse transcription with its intrinsic RNA as a template (Sauerwald et al., 2013; Shay et al., 2001). It is overexpressed in >85% of all known human tumors but not detected in normal cells (Blagoev, 2009; Xu et al., 2013; Yi et al., 2014).

The stable binding of the two proteins with their nucleic acid substrates enables the fabrication of the reliable and sensitive LSPR-based biosensor (Fig. 1A and B). The sensor can detect clinical samples with complex biological medium that contains thousands of high-concentration molecules. The great advantages of a detection method based on LSPR are that biomolecular reactions can be monitored in real time; the detection is simple, rapid, and label-free. However, its major disadvantage is that it is difficult to probe an analyte at low concentrations or with a low molecular mass. Therefore, we combined dark-field microscope with Rayleigh scattering spectrophotometer, so that every single nanoparticle could be utilized as an individual sensing probe with ultrahigh sensitivity. The most notable feature of this study is the first demonstration of analyzing cancer cells *via* a direct assay of interactions between nucleic acids and protein. The concept exploited here can be further developed for multiple molecular interactions. This approach can be easily translated to deciphering key biomolecular interactions during disease development.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), trisodium citrate, magnesium chloride ($MgCl_2$), Tween 20, potassium chloride, ethylene glycol tetraacetic acid (EGTA), Trizma hydrochloride, diethyl pyrocarbonate (DEPC), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), washing buffer, and 3-aminopropyltriethoxysilane (APTES) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). The oligonucleotide sequence was obtained from Bioneer Corp. (Daejeon, South Korea). The first 10T was used as the pacer. The RNase Inhibitor was purchased from Promega Corp. (Madison, WI, USA). TRAPeze® 1× CHAPS lysis buffer and TRAPeze® telomerase positive control cell pellet (HeLa cells) were purchased from Chemicon International, Inc. (Millipore, Milford, MA, USA). Normal lung fibroblasts were purchased from ATCC (Rockville, MD, USA). Ethanol was purchased from Samchun Chemical Co. (Gyeonggi-Do, South Korea). Cover glass slides (22 × 40 × 0.1 mm) were obtained from Deckglaser (Germany). HS $(CH_2)_{11}(OCH_2CH_2)_3OH$ (OEG_3) was purchased from Cos Biotech (Seoul, Korea). Other reagents were supplied by Sigma-Aldrich. Ultrapure water (18.2 mΩ cm^{-1}), purified by a reverse-osmosis filtration system, was used to prepare all solutions. All glassware was cleaned using freshly-prepared aqua regia

Please cite this article as: Ma, X., et al., Biosensors and Bioelectronics (2014), http://dx.doi.org/10.1016/j.bios.2014.06.038