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Long-range surface plasmon resonance immunosensor based on water-stable electrospun poly(acrylic acid) fibers

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ABSTRACT

Water-stable poly(acrylic acid) (PAA) fibers were fabricated on flat gold thin films via an electrospinning technique. The obtained fibers were then used to construct long-range surface plasmon resonance (LR-SPR) biosensors. Because LR-SPR spectroscopy has a greater evanescent field intensity and penetration depth than conventional surface plasmon resonance (SPR) spectroscopy, the increased surface area of the PAA fibers within the surface plasmon evanescent field was efficiently utilized for biosensor applications. The water-stable electrospun PAA fibers were obtained by adding β-cyclodextrin as a crosslinker, followed by thermal treatment at 150 °C for 40 min. In addition, the layer-by-layer deposition of poly(diallyldimethylammonium chloride) and PAA ultrathin films on the electrospun PAA fibers functionalized their surfaces and further increased their water-stability by increasing the number of active carboxylic acid groups. Fiber surfaces were then successfully activated for the construction of immunosensors for the detection of human immunoglobulin G. Therefore, the present study demonstrates the potential of electrospun fibers for LR-SPR biosensor applications.

1. Introduction

Surface plasmon resonance (SPR) spectroscopy is a technique that has been used in biosensors for decades [1–10]. Liedberg et al. [1] reported that SPR enables the real time analysis for biomolecules featuring no labelling needed. To increase the sensitivity of SPR-based sensors, nanoscale thin films have been employed on the metal surfaces of SPR chips, because the penetration depth of surface plasmon fields for SPR-based sensors is approximately 100–200 nm [11]. Kretschmann-type SPR sensors based on attenuated total reflection (ATR) method can be categorized as conventional SPR sensors [12]. Long-range surface plasmon resonance (LR-SPR) involves SPs that propagate along a thin metallic film embedded between two dielectric materials with similar refractive indices, for which both the evanescent field intensity and the penetration depth are greater than those for conventional short-range (SR-SPs) [13–21]. Hence, it is expected that the sensitivity at longer distances from the metal surface will increase with LR-SPR spectroscopy. Recently, Homola et al. [22] reported that the penetration depth of long-range SPs was 1400 nm in a device that detected large analytes such as the bacteria *Escherichia coli* (0.7–1.0 μm) [16,22–24]. In addition, the LR-SPs enhanced the optical field wave at the metal-dielectric interface, leading to a higher sensor sensitivity and increased penetration into the analyte solution than observed for conventional SPR. As a result, a thicker sensor coating with a significantly larger number of binding analyte molecules on the surface could be used [18].

Electrospinning is a technique that produces nanosized polymer fibers by forming an electrically charged jet of polymer solution discharged from the tip of a needle using a high voltage supply. Electrospinning is a simple, convenient, reproducible, and cost-effective method for the production of long, continuous nanofibers [25–30]. Li et al. [26] reported that the use of aqueous poly(acrylic acid) (PAA) solutions with lower concentrations of PAA in combination with lower voltages enabled the continuous formation of thinner fibers with average sizes ranging from 80 to 500 nm. Furthermore, when β-cyclodextrin was added to the aqueous PAA solution and the generated PAA fibers were subjected to thermal treatment at 140 °C for 20 min, the fibers were found to be insoluble in water. For sensor applications, the electrospun nanofibers are of particular interest because their large surface area can enhance

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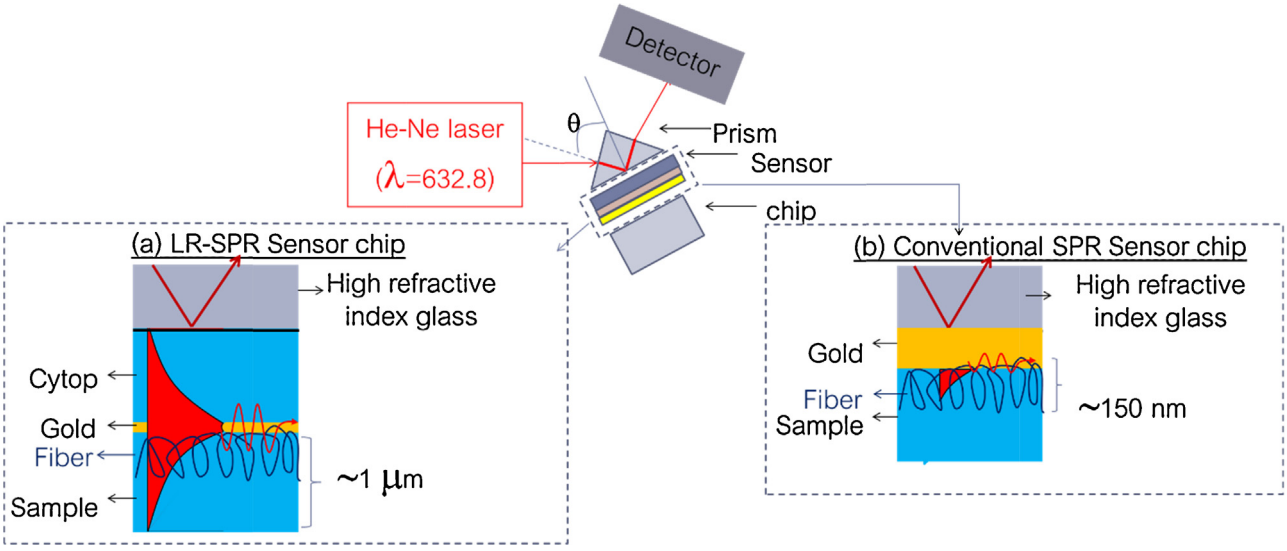

Fig. 1. Schematic of the instrument setup and sensor chip/prism for (a) LR-SPR and (b) conventional SPR at the gold-water interface.

the reactivity and sensitivity of sensors. In addition, the large surface area allows for greater analyte adsorption than is possible with film-based sensors [31–37]. For biomedical applications, the surfaces of electrospun fibers are chemically and physically modified with bioactive molecules and cell-recognizable ligands after the electrospinning process [38]. However, because the diameter of the single fibers ranges from 80 to 500 nm, it is difficult to apply these materials to SPR biosensors because of the limited evanescent field of ca. 150 nm from the metal surface.

In this study, we describe the application of electrospun PAA fibers in LR-SPR biosensors for the detection of human immunoglobulin G (IgG). Water-stable interconnected electrospun PAA fibers were fabricated on a gold surface by adding β-cyclodextrin as a crosslinker to the aqueous PAA solution, followed by heat treatment. Specifically, a mixture of PAA and β-cyclodextrin was electrospun onto a high refractive index glass substrate previously coated with gold and Cytop a highly transparent amorphous fluoropolymer (so called LR-SPR sensor chip). The number of sensing sites and the water resistance of the electrospun PAA fibers were subsequently increased by fabricating multilayers of negatively charged PAA and positively charged poly(diallyldimethylammonium chloride) (PDADMAC) films on the fibers using a layer-by-layer (LbL) deposition technique [39,40], which improved the adhesion of the fibers to the positively charged gold surface. LR-SPR spectroscopy was employed to monitor the deposition of the ultrathin PAA/PDADMAC LbL film. An LR-SPR immunosensor was then constructed using the water-stable electrospun PAA fibers wrapped in the multilayer PAA/PDADMAC ultrathin film with monoclonal anti-human (IgG) as a model antibody, and its ability to analyze the binding of various concentrations of human IgG antigen was evaluated. The electrospun PAA fibers were observed to enhance the signal of the LR-SPR-based immunosensor and bind with human IgG within the penetration depth of the surface plasmon field (approximately 1 μm). Notably, the deposition of the high surface area electrospun PAA fibers on the sensor chip increased the surface area of the substrate, increasing the number of sensing sites (active carboxylic groups).

2. Experimental

2.1. Chemicals and materials

PAA (viscosity-average molecular weight ~450,000), PDADMAC, β-cyclodextrin, phosphate-buffered saline (PBS) tablets, 3-mercapto-1-propanesulfonic acid sodium salt (MPS), monoclonal anti-human IgG (Fab specific) produced in goats, and reagent grade IgG from human serum were purchased from Sigma-Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and ethanolamine hydrochloride (EA-HCl) were purchased from TCI MARK. CTL-809M (Cytop) and CTL-180 (Cytop solvent) were purchased from Asahi Glass. All chemicals were used as received without further purification.

2.2. Conventional SPR and LR-SPR instruments

The ATR setup used for the excitation of SPs in the classical Kretschmann configuration is shown in Fig. 1. SPs were excited using a He-Ne laser at λ = 632.8 nm [3]. Two dielectric layers with similar refractive indices on the opposite sides of a thin metal film were required for LR-SPR, as shown in Fig. 1a. The LR-SPR sensor chip consisted of a Cytop film (ca. 800-nm-thick) with a refractive index of 1.34 (similar to water, which has a refractive index of 1.33) spin-coated on a high refractive index glass. A 30-nm-thick gold film was then deposited via vacuum evaporation onto the Cytop film. Fig. 1b presents a schematic illustration of a conventional SR-SPR sensor chip with a gold film (ca. 47-nm-thick) deposited on high refractive index glass for the optimum excitation of the SPs. For both chips, a 1-nm-thick chromium layer was deposited prior to gold deposition to promote the adhesion of the gold layer. To clearly demonstrate the power of LR-SPR using the electrospun fiber sensor system, angular scan measurements were performed and the results were compared to those obtained using conventional SPR.

2.3. LR-SPR substrate fabrication

A 7% Cytop solution (9% Cytop (CTL-809M; M-grade)) dissolved in Cytop solvent (CTL-180 solvent) was spin-coated on a cleaned, high refractive index glass substrate. A Cytop layer thickness of 800 nm was obtained using an initial spin rate of 500 rpm for 10 s followed by a spin rate of 1300 rpm for 20 s. The Cytop solvent was removed by placing the coated substrate in a 180 °C oven for 1 h. A 30 nm-thick gold film was then deposited onto the Cytop-coated glass substrate via vacuum evaporation.