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Integration of a microfluidic polymerase chain reaction device and surface plasmon resonance fiber sensor into an inline all-in-one platform for pathogenic bacteria detection

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ABSTRACT

We presented the integration of polymerase chain reaction (PCR) microdevice and surface plasmon resonance (SPR) optical fiber sensor into an inline all-in-one device for fluorophore-free detection of PCR amplification. The proposed integrated device consisted of the microfluidic PCR reactor that used channel position-dependent temperature change and the optical fiber SPR sensor with bimetallic (Ag/Al) coating. This all-in-one device performed not only the amplification of DNA of *Salmonella* spp. (injected volume of 20 microlitter) within 30 min, but also the immediately subsequent measurement of the DNA amplicon by the SPR fiber sensor part.

Results showed that the SPR sensor signal increased with the cycle number due to the amplicon refractive index smaller than the sum of its constituent dNTP molecules and its minimum resolvable DNA amplicon was that generated at the 15th cycle. The integrated device could serve as a DNA amplification-to-detection instrument. Both the label-free nature that could allow reusability of the presented device and further miniaturization of the whole system can find use in a point-of-care testing with early diagnosis genetic analysis.

1. Introduction

Nowadays, there are abundant sorts of viruses and bacterial pathogens that infect human to deaths in the world [1–3]. Prevention of their infection and the subsequent transmission basically requires to detect such infectious elements. The detection techniques currently available include those by interrogating a cell, by means of flow cytometry [4] and by immunohistochemistry [5]. For this purpose, small concentration of viruses and bacterial pathogens present in environmental water, food and peripheral blood needs to be identified and detected with high sensitivity and specificity [6–8].

In the past few decades, technologies have been developed for amplification of genes, and DNA/mRNA molecules of viruses/bacterial pathogens for disease diagnosis, such as the polymerase chain reaction (PCR) which has further developed into the formats including the droplet digital PCR and the fluorescence based real-time PCR [9–13]. A conventional PCR method amplified DNA molecules through temperature dependent cyclic steps, the amplified product of which was put to a device for gel electrophoresis based detection. This detection method used electric fields to move charged DNA molecules through a porous gel matrix [14] for fluorescence-aided detection of amplified molecules (DNA amplicons) [15–17].

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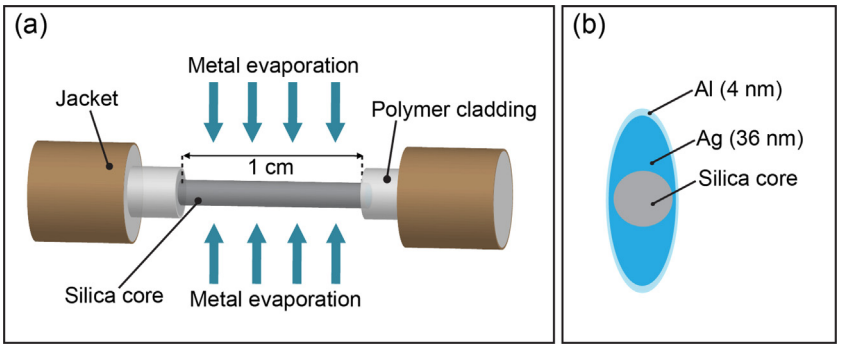

Fig. 1. (a) Metal coating on two sides of the fiber core (b) Expected cross-section profile of metal coated [24].

However, this method has some inherent drawbacks such as considerable time consumption, and the problems with reproducibility and reliability, caused by various user expertise levels. For example, incomplete conjugation of labels with DNAs induces false detection of background fluorescence and this becomes evident particularly in cases of using small volume of sample which produces fluorescence intensity weak enough to suffer from small signal-to-noise ratio.

More than three decades ago, a great deal of efforts have been devoted to developing label-free biosensors that need no labels such as fluorophores. One of the label-free ones is the surface plasmon resonance (SPR) based optical biosensors for detection of chemical or biological elements [18–28]. SPR arises from an optical excitation of normal modes of electron density fluctuations at the interface between two different media that have the relative permittivity of opposite signs, for instance, at an interface between a metal and dielectric. The resonance condition requires that the propagation constant of evanescent wave of incident light from a high index medium (e.g. prism) matches that of surface plasmon at the interface. The resonance condition allows the properties of the sensor output light to be sensitive enough to be detected as a result of change in the refractive index of the dielectric medium (this dielectric medium including injected analyte). A typical SPR based biosensor has been reported to have the detection limit of about 10^{-4}–10^{-5} refractive index unit (RIU) [19]. The SPR biosensor needs no labels such as fluorophores and enabled a real-time detection. Recently, our group has demonstrated the fiber-optic sensor with bimetallic SPR coating, showing minimum detectable refractive index of about 10^{-6} RIU [27,28]. This high sensitivity and ease with which such fiber platform can be fabricated have led us to apply the sensor to label-free biomolecular detection such as proteins and DNAs.

Salmonella spp. has been considered as one of the most foodborne infections worldwide, leading to poisoning of food for both human and animals [29–31]. For early detection of the pathogens, a number of methods have been developed such as those by PCR, culture, immunoassays based biosensor, nucleic acid analysis [32–34]. Of these methods, a PCR method has been widely adopted for bacterial detection with high sensitivity and specificity. In particular, with the development of Micro Total Analysis System (μTAS) [35,36], the on-chip PCR method has shown more advantages such as simple and cost effective operation as compared with the conventional PCR [37].

In this work, we presented the integration of a fiber-optic SPR sensor and a PCR chip into a compact format (integrated device) for generation and detection of DNA amplicon of *Salmonella* spp., as a DNA amplification-to-detection instrument. The SPR fiber sensor was made out of multimode optical fiber with the bimetallic SPR coating, while the PCR chip comprising polymethyl methacrylate (PMMA) substrate in a form of microchannel. The DNA amplicon generated by the PCR chip that ran temperature-dependent cyclic steps was subsequently detected by the in-line SPR fiber sensors without fluorophores. The presented integrated device which required no need of fluorophore labeling but detected refractive index changes around the sensing surface due to the DNA amplicon injection, was free both of labeling relevant parameters including background signals and of volume size of the sample, provided that the DNA sample is in close proximity of the sensing surface. Results showed that the SPR sensor signal increased with the cycle number due to the amplicon refractive index smaller than the sum of its constituent dNTP molecules and its minimum resolvable DNA amplicon was that generated at the 15th cycle. The label-free nature of the device may ensure its reusability unlike fluorophore based assay where fluorophores cannot be reused. The presented device may lead to further miniaturization, finding applications for a point-of-care testing with genetic analysis where human access is limited such as extraterrestrial space and deep underground.

2. Materials and methods

2.1. Materials

Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) and curing agent were purchased from Dow Corning (Midland, MI, USA). *Taq* polymerase, PCR buffer solutions, and dNTPs were purchased from Promega. The DNA ladder (100 bp) was purchased from Takara and agarose powder was purchased from BioShop. Bovine serum albumin (BSA, V fraction) was purchased from Sigma. Loading star was purchased from DYNE Bio. PMMA substrates with thickness of 2 mm were purchased from Goodfellow, and a multimode optical fiber with the core diameter of 200 μm was purchased from JTFLH-Polymicro technologies (Lisle, IL, USA). He-Ne laser was purchased from Thorlabs. Silver pellet and aluminum pellet were purchased from iTASCO (Seoul, Korea).

2.2. Fabrication of the SPR fiber sensor head

A polymer cladding multimode fiber with the core diameter of 200 μm was used to make a SPR sensor head [27]. The cladding of 1 cm length was removed by a soldering machine at temperature of 350 °C. The exposed core part of the fiber was then cleaned by solutions of acetone mixed with methyl alcohol with the volume ratio of 3:1. The bimetallic layer, comprising silver (Ag) of 36 nm thickness (inner layer) and aluminum (Al) of 4 nm thickness (outer layer), was deposited on both side of the fiber core for SPR excitation by using a thermal evaporator (see Fig. 1(a)), leading to the expectation of an asymmetrical coating profile as shown in Fig. 1(b).