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Long-period fiber grating sensor for detection of viruses

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ABSTRACT

Fast and reliable detection of viruses, including those specific to bacteria – bacteriophages – is crucial in clinical and veterinary practice, during biotechnological processes, and in basic research. In this paper, a highly sensitive long-period fiber grating (LPFG) label-free immunosensor is described for the detection of T7 bacteriophages. The LPFGs were tuned up to their highest refractive index sensitivity at the dispersion turning point of higher order cladding modes. The simple and reliable fiber surface functionalization was performed using 3-(triethoxysilyl)propylsuccinic anhydride to covalently bind anti-T7 antibodies. The T7 phage detection was possible by tracing the shift of the resonance wavelength in the LPFG transmission spectrum caused by the increase of the thickness and density of the bio-overlay, in particular induced by antigen-antibody interactions. The obtained sensor was selective and the limit of detection was lower than 5×10^3 PFU/mL. Moreover, regeneration of the sensor surface by removing all organic layers was demonstrated. The regeneration procedure can reduce the costs of production and give the possibility for performing other measurements on the same LPFG structure. The applied procedure enabled real-time measurements and can be easily extended for the detection of other viruses.

1. Introduction

Quick and sensitive detection of viruses is crucial in clinical and veterinary practice in order to begin proper medical treatment as fast as possible. However, these are not the only fields where rapid virus detection is needed. Problems with virus infections appear also in basic research and are related to infections of different laboratory organisms (from bacteria to animals), and during biotechnological and biopharmaceutical processes (infections of bacterial cultures with bacteriophages and eukaryotic cell cultures with specific viruses).

Various methods for the detection of viruses are currently in use or under investigation. The most commonly used are biological assays. In case of bacteriophage detection the double layer agar method is typically applied [1]. Specifically, the layer of host bacteria is infected with the phage at varying dilutions. The virus infected bacterium lyses and spread the infection to adjacent bacteria where the cycle is repeated. The infected area creates a plaque which can be seen with the naked eye and the number of plaques can be correlated with the number of active phages in the sample. Diagnostics methods for viral animal diseases involve virus culturing and isolation followed with serological or antibody tests. These methods are laborious and time-consuming, require well-trained personnel and are prone to give false negative results. Among other typically used techniques are polymerase chain reaction (PCR) and its derivatives [2,3] and enzyme-linked immunosorbent assay (ELISA) [4,5], which are faster and in most cases sensitive, but require expensive equipment and have limited applicability in the field.

Recently, a lot of work has been focused on biosensors development, due to their capability for low cost fabrication, possibilities

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of miniaturization, real-time measurements, and wide availability of different bio-recognition elements, such as antibodies, nucleic acids, peptides, and whole cells. Moreover, label-free sensing mechanism is often used for biosensing, especially in the case of application of optical devices. In those cases, no fluorescence or enzymatic labeling is required, so the whole procedure is faster and easier to implement. One example of a label-free optical biosensor for virus detection was presented by Lee et al. [6] Gold nanoparticles (AuNPs) modified with sialic acid were used for colorimetric detection of influenza B virus. Virions formed complexes with the nanoparticles and caused them to aggregate which could be observed as a sample color change from red to purple. A similar approach was demonstrated for T7 bacteriophage detection with the utilization of specific antibodies instead of sialic acid as a receptor layer on the AuNPs surface [7]. The presented method was rapid and label-free with a limit of detection (LOD) reaching 1.08×10^{10} PFU/mL (plaque-forming units per milliliter). By changing the receptor layer from antibodies to carboxymethyl chitosan the LOD was decreased to 1.2×10^{6} PFU/mL [8]. Label-free virus detection can also be done by surface plasmon resonance (SPR) measurements. For example, gold surface modified with *Escherichia coli* bacteria was utilized for T4 phage detection using SPR [9]. The limit of detection of this method was 1×10^{7} PFU/mL and the whole measurement took only 10 min. A similar sensor was presented for adenovirus detection [10]. In this study the sensor surface was modified with plastic (molecularly imprinted polymers) or natural antibodies and the obtained LODs were 8.08×10^{6} and 1.2×10^{7} PFU/mL, respectively. An interesting SPR immunosensor was also shown for selective, multiplexed detection of MS2 phage and Influenza A virus in aerosol [11]. SPR microscopy were used for the imaging, detection, and mass/size measurement of single particles of Influenza A virus [12]. There have been also label-free sensors utilizing interferometry-based techniques, such as Young interferometry for herpes simplex virus type 1 detection [13] and dark-field interferometry for HIV and λ phage detection [14]. Novel interferometry-based imaging approach was also demonstrated for visualization and counting of recombinant vesicular stomatitis virus Ebola model [15]. Interesting label-free optical device was also presented by Shafiee et al. [16]. Nanostructured photonic crystals modified with specific antibodies were used for capturing and quantifying HIV viruses. This surface resonantly reflected a narrow wavelength band during illumination with a broadband light source. Observed band changed its position after virus capture. Significant shift was observed for concentrations higher than 10^{5} copies/mL. Detection of single virions was done for Influenza A virus by monitoring adsorption of individual particles as a changes in the resonance frequency/wavelength of a whispering-gallery mode excited in a microspherical cavity [17]. Unfortunately, in this study no specificity is provided, because of the lack of surface modification.

Recently, in the field of optical sensors and biosensors, there has been much interest in application of optical fibers with induced long-period gratings, known as long-period fiber gratings (LPFGs). The application of optical fibers instead of planar optical devices makes possible miniaturization of the sensing device, as well as multi-parameter or remote sensing. The LPFG is a periodic modulation of the refractive index of the core of a single-mode optical fiber with a period in the order of hundreds of micrometers [18]. Under specific conditions, the modulation induces coupling of the fundamental core mode with discrete cladding modes. Due to their adsorption and scattering a series of resonances appear in the LPFG transmission spectrum. Since the LPFG couples light into the cladding, the resonance wavelength depends on the refractive index (RI) of the external medium, as well as the thickness and optical properties of the film/overlay formed on the LPFG surface. Therefore, the LPFGs can be used for the real-time and label-free

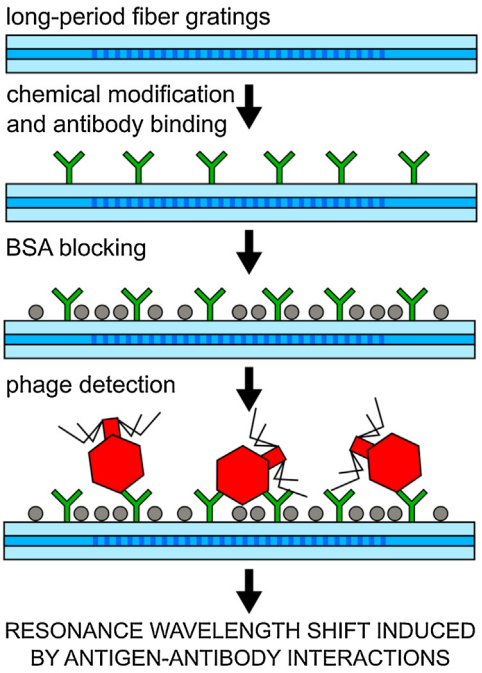

Fig. 1. Schematic representation of the fiber surface modification steps leading to phage detection. The modification takes place on whole cylindrical surface of the fiber, where for sake of clarity only a fragment is shown.

monitoring of the interactions between biomolecules at the grating surface. LPFG-based biosensors were demonstrated for detection of bacteria [19–22], bacterial toxins [23], nucleic acids [24,25], proteins [26–28] and triacylglycerides [29]. The RI sensitivity, which refers to label-free biosensing capabilities of the LPFG, may be highly improved by optimizing the working point of the device up to dispersion turning point (DTP) of the cladding modes [21] or/and by deposition of high RI nano-overlays [30].

Here, we demonstrate for the first time an LPFG-based immunosensor for label-free detection of viruses, in particular bacteriophages. The T7 bacteriophage was chosen as a model virus due to its similarity to pathogenic adenoviruses (both with icosahedral, non-enveloped capsids, similar in size) [31,32] and capability for production in large quantities. The surface of the LPFGs working at DTP was modified with anti-T7 antibodies. The binding reaction of the T7 phage with the sensor surface was monitored in real-time. A simplified scheme of the fiber surface modification and phage detection was shown in Fig. 1. The described approach enables sensitive and selective detection, does not require any additional signal amplification, and it is faster than conventional biological methods [1]. Additionally, the sensing device is simple and the method of surface modification enables re-use of the LPFG after proper regeneration [33]. Moreover, the detection method is applicable to many other bacteriophages and viruses, as long as specific antibodies are available.

2. Materials and methods

2.1. Chemicals and materials

Bovine serum albumin (BSA), sodium hydroxide, hydrofluoric acid, acetone, ethanol, and phosphate buffered saline (PBS) tablets were purchased from Sigma Aldrich. PBS consists of 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4 at 25 °C. LB broth and LB-agar were bought from Carl Roth as instant mixes ready to dissolve in deionized water. LB medium consisted of 10 g/L of tryptone, 5 g/L of