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A novel immunosensor based on excessively tilted fiber grating coated with gold nanospheres improves the detection limit of Newcastle disease virus

Binbin Luo[a,c], Yangfei Xu[a], Shengxi Wu[b,*], Mingfu Zhao[a], Pengjun Jiang[b], Shenghui Shi[a], Zhonghao Zhang[b], Yin Wang[a], Linlin Wang[b], Yong Liu[c]

[a] Chongqing Key Laboratory of Optical Fiber Sensor and Photoelectric Detection, Chongqing University of Technology, Chongqing 400054, China
[b] School of Pharmacy and Bioengineering, Chongqing University of Technology, Chongqing 400054, China
[c] School of Opto-electronic Information, University of Electronic Science and Technology of China, Chengdu 610054, China

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ABSTRACT

A novel immunosensor for detecting Newcastle disease virus (NDV) was developed using excessively tilted fiber grating (Ex-TFG) coated with gold nanospheres (AuNs). AuNs were coated on the Ex-TFG surface via Au–S bonds using 3-mercaptopropyltrimethoxysilane (MPTMS), and the activated staphylococcal protein A (SPA) was linked to AuNs by covalent bonds via cysteamine. AuNs greatly enhanced the impact of the analyte on the fiber cladding mode through the local surface Plasmon resonance (LSPR) effect, thus improving the detection limit and sensitivity of the immunosensor. Meanwhile, SPA enhanced the bioactivity of anti-NDV monoclonal antibodies (MAbs), thus promoting the effectiveness of specific binding events on the fiber surface. Immunoassays were performed by monitoring the resonance wavelength shift of the proposed sensor under NDV samples containing different particle amounts. Specificity was assessed, and clinical tests for NDV were performed by contrast experiments. Experimental results showed that the detection limit for NDV was about 5~10 times improved compared to that of reference Ex-TFG without AuN treatment. Moreover, the novel biosensor was reusable and could potentially be applied in clinic.

1. Introduction

Thanks to advantages such as compact structure, wavelength modulation, fast detection, and remote sensing, many optical fiber grating types, including cladding-etched fiber Bragg grating (FBG), long period fiber grating (LPFG), and tilted fiber Bragg grating (TFBG), are widely applied in biochemical fields, e.g. drug characterization, food security, medical detection and diagnosis, environmental monitoring and virus detection (Krishnendu et al., 2016; Jörg et al., 2015; Albert et al., 2013a, 2013b). Meanwhile, with the biomolecular affinity of gold/gold nanoparticles and unique optical property of supporting surface Plasmon resonance (SPR) or localized SPR (LSPR), optical fiber (OF) based SPR/LSPR biosensors attract increasing attention (Kim et al., 2013; Ahmmed et al., 2016). Shao et al. (2010) reported that TFBG sensors with a tilted angle of ~10° excite SPR at a wavelength of ~1550 nm by coating the fiber surface with a ~50 nm thick gold film, thus greatly improving the refractive index (RI) sensitivity. From then on, Au/Ag coated TFBG-SPR sensors have been developed for many bio-sensing applications, such as high resolution aptasensor (Albert et al., 2013a, 2013b), real-time analysis of cellular behavior (Shevchenko et al., 2014), small biomolecule immunosensing (Ribaut et al., 2016), cancer biomarker detection (Ribaut et al., 2017) and highly sensitive detection of urinary protein (Guo et al., 2016) etc. However, the best detection limits of them for the corresponding targets could only reach the magnitude of sub-nM scale, e.g. 0.4 nM for cytokeratin 7 peptide detection (Ribaut et al., 2016).

Compared to OF-SPR biosensors, OF-LSPR variants are much more localized, allowing for probing at the platform interface with spatial sensitivities well within the nanometer scale (Piliarik et al., 2012). Bialiayeu et al. (2012) showed that TFBG modified with metal nanoparticles can also excite LSPR at a wavelength of ~1.55 μm. Lepinay et al. (2014) analyzed TFBG modified with several types of gold nanoparticles for protein detection, with greatly improved detection limit to be 8 pM for the case of gold nanocages.

Previously, we developed label-free immunosensors based on excessively tilted fiber gratings (Ex-TFGs) immobilized with specific monoclonal antibodies (MAbs) via staphylococcal protein A (SPA), for specific and fast detection of porcine circovirus type 2 (PCV2) (Luo et al., 2016) and heart failure (HF) biomarkers (Luo et al., 2017). Unlike TFBGs with small tilted angles (≤ ~20°), the excessively tilted

* Corresponding author.
E-mail address: sxwu2004@cqut.edu.cn (S. Wu).

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finings (~81°) in Ex-TFGs can couple the core mode to higher-order co-propagating cladding modes, making it resemble LPFG (Shu et al., 2002) but with a relatively smaller grating period (Zhou et al., 2006). Combined with the large birefringent effect induced by the excessively tilted structure, Ex-TFGs have the advantages of negligible thermal cross-talk effect, higher RI sensitivity, and higher Q-factor compared with conventional LPFG based biosensors (Yan et al., 2016). However, in our previous studies, the Ex-TFG based PCV2 immunosensor could only achieve a detection limit of 9.371 $TCID_{50}$/mL (Luo et al., 2016), and the Ex-TFG based HF biomarker one only ~0.5 ng/mL (Luo et al., 2017). Therefore, the detection limit of Ex-TFGs, as biochemical platforms, for viruses or biomarkers remains not satisfactory.

In this work, based on the previous SPA-modified method, a gold nanosphere (AuN) coated Ex-TFG to construct a novel type of immunosensor, which to be our best knowledge has not been reported, for the label-free, high specificity, ultra-trace and fast detection of Newcastle disease virus (NDV). NDV, a highly infectious chook disease induced by the NDV strain (Alexander and Gordon, 2001; Sanchez et al., 2016), is wildly distributed in countries around the world, due to high transmission rate, with high mortality. Currently, with the application of several methods for NDV detection, e.g. chicken embryo inoculation(Chen et al., 2015), serological assays such as hemagglutination inhibition (HA-HI) test, serum neutralization and enzyme linked immunosorbent assay (ELISA) (Swain et al., 1999), as well as molecular diagnostic techniques, including reverse transcription polymerase chain reaction (RT-PCR) (Qiu et al., 2014), monoclonal antibody method (Sun et al., 2010) and gene chip (Zhao et al., 2015), the spread of NDV has been effectively controlled to some extent.

However, these purely biochemical methods are somewhat limited. Chicken embryo inoculation is sensitive and specific (Roy et al., 2000), but the whole process requires about 3 weeks, and the operation is complex, which is not conducive for on-site detection. The HA-HI test is also sensitive, but the result might be significantly affected by external factors, which reduces the practical value of this method. ELISA is suitable for large-scale serological studies (Das and Kumar, 2015), but it also has disadvantages including long time consumption, low specificity, complex process, and inconvenient on-site operation. Although the PCR test possesses high sensitivity as it directly identifies viral genes (Desingu et al., 2015), the high cost in equipment still restricts the use of this method in clinical practice. Therefore, developing detection alternatives is very important for the effective prevention and control of NDV.

The NDV immunosensor proposed in this study was constructed through several surface modified steps in a sequence that include silanization of the Ex-TFG by 3-mercaptopropyltrimethoxysilane (MPTMS), linkage of AuNs to MPTMS, SPA absorption, and immobilization of home-made highly purified anti-NDV MAbs. NDV immunoassay experiments were conducted *in vitro* by monitoring the resonance wavelength shift of the AuN modified Ex-TFG under NDV samples containing different particle amounts. For comparison, NDV detection by a reference Ex-TFG without AuN treatment was also performed. Reusability of the proposed Ex-TFG immunosensor was realized by a designed elution mechanism; finally, specificity assessment and clinical tests for NDV were performed by contrast experiments.

2. Materials and methods

2.1. Reagents

Analytical grade reagents and sterile deionized water were used for all working solutions. 3-Aminopropyltriethoxysilane (APTES), highly purified SPA, fluorescein isothiocyanate (FITC), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), Tris, glycine, cysteamine, polyethylene glycol (PEG-1500), hypoxanthine-aminopterin-thymidine (HAT) and hypoxanthine-thymidine (HT) supplemented medium were purchased from Sigma-Aldrich, China. Phosphate buffered solution (PBS) (0.01 M, pH7.4) and 4-morpholineethanesulfonic acid hydrate (MES) (0.1 M, pH5.5~6.7) buffered solution were obtained from Wuhan Boster Biological Technology, China. AuNs (~80 nm) were purchased from Nanjing Nanoeast Biological Technology, China, and MPTMS was from Damas-beta, Switzerland. Protein A and G chromatography media were purchased from GE, USA. The NDV-AV29 strain was purchased from China Institute of Veterinary Drug Control; the H5 avian influenza virus strain was a kind gift from the Department of Veterinary, Nanjing Agricultural University. To prepare the NDV allantoic fluid, SPF chick embryos (aged 9 d) were inoculated with diluted NDV-AV29 (0.2 mL) into the allantoic cavity. The eggs were hatched at 37 °C for 48 h, followed by allantoic fluid collection. The avian influenza virus (AIV) allantoic fluid was prepared in the same way as the NDV allantoic fluid.

2.2. Fabrication of Ex-TFGs and experimental setup

The scanning amplitude mask technique and doubled frequency Ar + laser (244 nm) were used for the fabrication of Ex-TFGs, as comprehensively described by Zhou et al. (2006). The fabricated Ex-TFGs had a tilted angle of ~81° in the fiber core with a grating period of ~32.0 μm along the fiber axis (Fig. S1(a) and (b)) and a grating length of 10 mm. Since the fabrication process is highly standardized, Ex-TFGs are cost effective, easily made and reproducible. The transmission principle and sensing properties of Ex-TFGs have been specifically elaborated by Yan, et al. (2016). Usually speaking, the angle of Ex-TFG could be design and fabricated between 78.5°~83°, in which impaction of the tilt angle on the RI and temperature sensitivity of the sensor could be neglected. While the main factor to influence the RI and temperature sensitivity of the sensor is the cladding-mode order. Therefore, in order to guarantee the high-order cladding mode to appear in C/L-band to achieve a relatively high RI sensitivity by the constraint condition of grating period ~32 μm, we choose the tilt angle to be 81°. In this case, the corresponding cladding-mode order of the grating is 32nd and 31st in the C and L band, respectively (Yan et al., 2016). The RI and temperature sensitivities of the cladding-mode resonance of the Ex-TFGs used in this study were ~180 nm/RIU (RI 1.33~1.38) and ~5 pm/°C, respectively.

Fig. 1 shows the experimental setup for monitoring the transmission spectrum of the Ex-TFG sensor. Broadband light from one output of the fiber optical grating demodulation system (FOGDS) (MOI-SM125, wavelength accuracy ± 1 pm), integrated with a sweep laser light source (1510~1590 nm, 1 Hz), was launched into the SM28 fiber. The isolator was used to cut off the backscattering and reflected light. The inline polarizer and polarization controller (PC) were used to excite and maintain light launching into the Ex-TFG to allow function in TM mode resonance (Fig. S2), since the SPR/LSPR effect can only be excited by the fiber's TM mode (Allsop et al., 2007). The Ex-TFG sensor was fixed in the biochemical reaction vessel when we carried out the immunoassay. Finally, the transmission spectrum was recorded by another channel of the FOGDS.

2.3. Preparation and identification of the anti-NDV MAbs

BALB/c mice were immunized four times with the inactivated purified subtype AV29 NDV, and spleen cells and SP2/0 cells were fused with PEG-1500 to prepare murine anti-NDV MAbs. A cell fusion rate of 94.0% was obtained, an indirect ELISA was developed to detect the MAbs secreted by hybridoma cell lines. A positive rate of 30.5% was obtained. One hybridoma cell line secreting MAbs to NDV, named 13E7, was developed into two subclones. After purification by Protein A affinity chromatography (Fig. S3(a)), 16 mg anti-NDV MAbs with 96.8% purity at a concentration of 1.938 mg/mL were obtained (Fig. S3(b)). The specificity of anti-NDV MAbs was assessed by Western-blot (Fig. S3(c)). The prepared anti-NDV MAbs would be very useful for the diagnosis and control of Newcastle disease.