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Highly sensitive label-free antibody detection using a long period fibre grating sensor

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ABSTRACT

An optical fibre long period grating (LPG) biosensor is appealing in the detection of biomolecules because of the high sensitivity, label-free and real-time measurement. The miniaturized size, ability of remote sensing and immunity to electromagnetic interference of the LPG biosensor provide various possibility of single-point sensing in situations such as point of care diagnostics and *in vivo* measurement. Two optical fibre LPG based biosensors are reported for detection of streptavidin (SV) and immunoglobulin M (IgM) respectively. The LPG is coated with a film containing three layers of Poly(allylamine hydrochloride)/gold coated silica nanoparticles *via* the layer-by-layer method. Biotin is covalently bonded to the surface of the gold shell by means of the formation of an amide bonds for detection of streptavidin. The concentration of SV in water for detection varied from 1.25 nM to 2.7 μM. The LPG sensor, operating close to the phase matching condition shows a high sensitivity of 3.88 $(ng/mm^2)^{-1}$ and a detection limit of 0.86 pg/mm^2 for the detection of SV. The limit of detection is 22 times lower than previously demonstrated with this type of sensor. The developed IgM sensor has the same configuration of film but has anti-IgM embedded on the LPG instead of biotin, demonstrating versatility of the sensing platform. This was used for the detection of human IgM with concentrations from 15.6 μg/ml to 1 mg/ml. The LPG sensor exhibits a sensitivity of 11 nm $(ng/mm^2)^{-1}$ for the detection of IgM with a detection limit of 15 pg/mm^2. The developed highly sensitive IgM sensor shows the potential application of clinical point of care for detection of lower concentration of IgM *in vitro*. The proposed biosensor exhibits high sensitivity and rapid detection of low concentrations biomolecules from the small size of SV to the large size of IgM.

1. Introduction

Antibodies/antigens are important biomarkers in human body fluids. They participate in the humoral immune response and their levels increases during the immune system responses to pathogen infection. The level of antibody/antigen can be used as an indicator of infectious disease [1]. Immunoglobulin M (IgM), as the earliest antibody to appear in the course of an infection [2], is responsible for agglutination and activating cytolytic responses. The pentameric structure of IgM theoretically provides 10 free antigen-binding sites and high avidity binding. IgM is mainly found in blood and lymph fluid and it is a very effective neutralizing agent in the early stage of disease. The level of IgM has significant clinical value in diagnosis of certain diseases. For example, because IgM antibody does not cross placenta, it can indicate a recent infection or intrauterine infection when it appears in a neonate's serum [3,4]. IgM also plays a vital role in rapid diagnosis of Dengue virus infection as it appears as the initial immune response to a primary infection a few days after the onset of Dengue fever [5]. In addition, IgM regulates autoimmunity and atherosclerosis and deficiency of IgM indicates increased susceptibility [6]. Selective IgM deficiency (SIgMD) is an immune disorder that is associated with serious infection. The deficiency of IgM in blood, usually < 20 mg/dl, can develop to prolonged or life-threatening infection to the patients (often infants and small children). The common method used in clinical practice for detection of antigens or antibodies is based on the enzyme-linked immunosorbent assay (ELISA) [7]. Dependent on the exact method used, the technique usually involves multiple sequential incubations for long periods (60–90 min each) and multiple wash steps, resulting ultimately in a colorimetric readout which is proportional to the level of the antigen or antibody present in the original sample. The readout requires a dedicated optical plate reader for translating the readings. Moreover, the multi-step nature of the procedure and

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requirement for enzyme-labelled antibodies is a significant weakness and does not allow real time detection of target molecules. Fast and accurate detection of IgM in patients' serum would enable early screening of SIgMD and early intervention may help prevent some of the complications seen later in life. Some infections due to the deficiency of IgM in infants and small children such as bacteremia can be life-threatening.

To satisfy the need for real time point-of-care (POC) testing, researchers have focused on the development of different protein measurement methods in order to obtain accurate, fast sensors that would be able to replace the current ELISA method. Surface Plasmon resonance (SPR) has been extensively used for quantitative measurement with sensitivity of the order of μg/ml [8]. However, a drawback is the relatively high price and bulky optical system that usually limits its application to the clinical laboratory. Quartz crystal microbalance (QCM) [9] is also an important label-free method for the detection of antibodies. The shift of resonance frequency is induced by the mass change of crystal resonator after binding of antibodies. However, the viscosity of analysed liquid also contributes to the frequency shift. In order to distinguish the mass-affected frequency shift with the contribution of liquid, a more expensive impedance analyser is required. A hybrid whispering gallery mode sensor [10] has been used for detection and characterization of small molecule such as RNA viruses, however, the lack of a bio-receptor makes the sensor non-specific in terms of biomolecule detection. Flow cytometry [11,12] has been used for the detection of antibodies. The techniques can be used for detection of antibodies which are not be detected by ELISA (*e.g.* glycospecific antibodies). However, the technique is not widely used due to the high cost compared with other alternatives. Planar waveguide sensors are also widely applied in detection of biomolecules [13]. The performance of this type of sensor strongly relies on the choice of substrate material and the quality of the deposited film. Fluorescence has been used for detection of nucleic acid on planar optical waveguides [14]. The use of fluorescent labels in the context of planar optical waveguide allows better sensitivity and specificity. The disadvantages of fluorescence detection include the rapid photo bleaching of fluorescent dyes conjugated to the biomolecules of interest and the potential loss of activity of the biomolecules when chemically conjugated with fluorescent dyes.

To satisfy the requirements of POC devices, it is not only sensitivity and response time that have to be taken into account, but also the size and simplicity of the sensor. In this regard, an optical fibre based sensor, due to its small size, provides an advantage in terms of application in point of care devices used in the clinic or community. In addition, the optical fibre is immune to the interaction of electromagnetic field providing a robust response in environments containing electronic devices.

A long period fibre grating (LPG) is a refractive index grating inscribed in the fibre's core. The theory of operation of LPG and biosensor parameters used in this work can be found in Supporting information (SI). This enables light to be coupled into the cladding region and produces characteristic attenuation bands in the transmission spectrum of the optical fibre [15]. A LPG is sensitive to the refractive index (RI) of the cladding region and therefore bio-sensing can be achieved by modifying the outside of the cladding with a functional material whose RI changes in the presence of an analyte of interest. Sensors based on LPGs have been reported for measurement of pH [16], detection of bacteria [17] and virus [18] after modifying the LPG with corresponding functional materials. The RI sensitivity of LPGs has been reported as high as 10^{-6} [19] offering the potential for bio-molecule detection. This removes the need for enzyme labelling and provides label free, low cost, rapid, point of care detection of bio-molecules.

The optimum functional coating combines high sensitivity and high specificity. Silica nanoparticles, due to their high surface to volume ratio can be incorporated into the coating film of LPG and significantly raise the RI sensitivity of the LPG [20]. Gold nanoparticles, due to their unique optical and electrical property, are commonly applied in the sensor development [21–23]. The localized surface plasmons are generated near the gold nanoparticles surface and enhance the overall electromagnetic field which leads to an increase in sensitivity. In our previous work we have developed [22] a LPG bio-sensor modified with poly(allylamine hydrochloride)/silica core gold shell nanoparticles for detection of the streptavidin with a high sensitivity (6.9 nm/(ng/mm^2)) and the minimum detectable concentration of 19 nM. However, the sensor performance was not optimal due to the poor surface coverage and uniformity of the coating. In addition, selected grating period of the LPG sensor was near rather than at the phase matching turning point (PMTP) which also did not lead to the optimal performance.

In this work, we address these points in order to fully realise the optimum performance of these sensors. In particular, a new approach of functionally coating the LPG is reported in order to provide significantly improved sensitivity (minimum detectable concentration of 1.25 nM for streptavidin compared to 19 nM previously [22]). Silica nanoparticles are first deposited onto the LPG using the layer-by-layer method in order to address the surface coverage problem providing a more uniform surface coverage. The surface of the silica particles is then further modified with 3-aminopropyltriethoxhysilane (APTES) *via* a silanization process in order to covalently attach gold nanoparticles (diameter of 2–5 nm) to the silica nanoparticles, forming a shell structure *via* a chemisorption process. In addition, chemical linkers are applied to covalently link the gold nanoparticles and the bio-receptor in order to minimize unspecific binding to the gold surface. Crucially the LPG sensor is fabricated close to the phase matching condition and, at the deposition of sensing film, the coupling conditions are modified such that sensors operate at the highly sensitive PMTP. The transmission changes at a specific wavelength, corresponding to the resonance band at the PMTP, are detected which has been reported to be more sensitive than the wavelength shift [24]. After optimising the LPG sensor performance using SV, it was then used to detect human IgM by functionalizing the coated LPG with anti-human IgM antibody as a proof of concept for application in clinical point of care.

2. Method

2.1. LPG modification

2.1.1. Modification for streptavidin detection

A list of materials used in this work can be found in SI section 1.3. The LPG was coated with three layers of silica nanoparticles (SiNPs) using the layer-by-layer method [19]. A positively charged polymer, poly(allylamine hydrochloride) (PAH) and negative charged SiNPs were subsequently deposited on the surface of the LPG *via* electrostatic interaction for three cycles as illustrated in Fig. 1a. The SiNPs coated on the LPG were then further decorated with an amine group using APTES to covalently attach gold nanoparticles and detailed description of this procedure is described in SI, section 1.4.

2.1.2. Modification for IgM detection

A three layer silica-Au coated LPG was fabricated using the assembly method by immersion into 20 mM 11-Mercaptoundecanoic acid (11-MUA) solution in ethanol for 30 min. The self-assembled monolayer of the thiol linkers in 11-MUA solution made a continuous binding to the gold surface leading to a termination of −COOH group. The LPG was then immersed into 25 mM EDC solution in MES buffer (0.1 M pH = 5.6, with 0.9% NaCl) for 20 min followed by immediately immersion in 60 mM NHS solution (0.1 M PBS buffer, pH = 7.4) for another 20 min. After brief washing with deionized water, the LPG was immersed into a solution that contains 1 mg/ml anti-IgM in PBS buffer for 1 h at room temperature (temperature regulated at 26 °C). The schematic of the modification is illustrated in Fig. 2.

After washing with deionized water, the anti-IgM functionalized sensor was then immersed into 1 mg/ml BSA for incubation of 1 h in room temperature followed by washing with deionized water to remove