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Detection of dengue NS1 antigen using long-range surface plasmon waveguides

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ABSTRACT

The non-structural 1 (NS1) protein of the dengue virus circulates in infected patients' blood samples and can be used for early diagnosis of dengue infection. In this paper, we present the detection of naturally-occurring dengue NS1 antigen in infected patient blood plasma using straight long-range surface plasmon waveguides. Three commercially-available anti-NS1 monoclonal antibodies were used for recognition and their performance was compared and discussed. A similar figure of merit to the one used in conventional dengue NS1 capture using an enzyme-linked immunosorbent assay (ELISA) was applied to our results. In general, the positive patient samples can be clearly differentiated from the negative ones and the results agree with those obtained using ELISA. The largest signal-to-noise ratio observed during the experiments was 356 and the best detection limit observed is estimated as 5.73 pg/mm^2.

1. Introduction

Dengue is a tropical mosquito borne disease affecting over half of the world population (Beatty et al., 2010) with about 390 million cases annually (Bhatt et al., 2013). The diagnosis of dengue can be difficult because its symptoms are nonspecific and current laboratory techniques are expensive, time consuming, and require highly skilled lab personnel. Current laboratory dengue diagnosis techniques include virus isolation, detection of virus components (RNA or antigen), and detection of dengue-specific antibodies (IgM or IgG) (Peeling et al., 2010; Vorndam et al., 1997; World Health Organization, 2009). Over the past years, much research has been done on a dengue diagnostic tool through the detection of nucleic acid (Baeumner et al., 2002; Zaytseva et al., 2005; Zhang et al., 2006), antigen (Camara et al., 2013; Linares et al., 2013; Silva et al., 2014b; Tai et al., 2005) or antibodies (Kumbhat et al., 2010; Lee et al., 2009; Wong et al., 2014a). However, none of the studies are able to fulfill the requirement of an "ideal" dengue diagnostic test which should be sensitive regardless of the stage of infection (Peeling et al., 2010). We previously argued that not all patients seek medical attention during early onset of symptoms and therefore presented a dengue biosensor which is able to detect dengue-specific antibodies in blood plasma (Wong et al., 2014a). Recent studies suggest that the combined detection of dengue non-structural 1 (NS1) antigen and dengue-specific antibodies improves the diagnostic sensitivity (Blacksell et al., 2011; Fry et al., 2011).

Non-structural 1 (NS1) protein, which is approximately 45 kDa in molecular weight (Allonso et al., 2011; Zhao et al., 1987), is secreted from dengue virus infected cells. Dengue NS1 antigen is an important diagnostic biomarker found circulating in patient blood samples up to 9 days after the onset of symptoms (Alcon et al., 2002). Furthermore, dengue serotypes can be identified by using serotype-specific anti-NS1 monoclonal antibody (Ding et al., 2011). Much of the research on biosensors has been done on the detection of purified dengue NS1 antigen in buffer (Camara et al., 2013; Figueiredo et al., 2015; Hu et al., 2013; Mishra et al., 2014; Silva et al., 2014a; Singh, 2012; Su et al., 2003; Tai et al., 2005) or on spiked NS1 antigen in serum samples (Cecchetto et al., 2015; Dias et al., 2013; Silva et al., 2014b; Yen et al., 2015). The detection of naturally occurring dengue NS1 antigen in serum was reported using electrochemical (Cavalcanti et al., 2012; Parkash et al., 2014), fluorescence (Linares et al., 2013) and quartz crystal microbalance (Wu et al., 2005) biosensors. In this paper we present the detection of purified dengue NS1 antigen in buffer, and naturally-occurring dengue NS1 antigen in patient blood plasma, using straight long-range surface plasmon polariton (LRSPP) waveguides. Five

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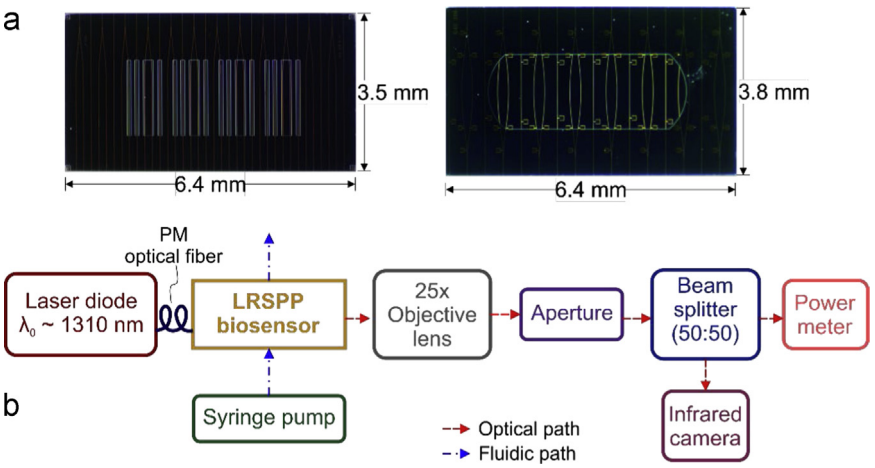

Fig. 1. (a) Microscope images of the sensor die used. (b) Schematic diagram of the experimental set-up.

clinical plasma samples were tested for dengue NS1 antigen. We also compare the performance of three commercially-available anti-NS1 monoclonal antibodies to detect dengue NS1 antigen. This is the first biosensor to demonstrate the detection of naturally occurring dengue NS1 antigen in blood plasma. The detection of dengue NS1 in blood plasma is more challenging than the detection of dengue-specific antibodies (Wong et al., 2014a) because NS1 has a smaller molecular weight and occurs in lower concentration in blood (0.01–2 μg/ml (Alcon et al., 2002)).

LRSPPs are transverse magnetic polarized optical surface waves propagating along a thin metal slab or stripe bounded by dielectrics of similar refractive index (Berini, 2009). The excitation of LRSPPs can be easily achieved by an optical fiber butt-coupled to the metal waveguide. The ease of LRSPP excitation enables compact and miniaturized biosensors. LRSPPs have reduced confinement and lower modal sensitivity than single-interface SPPs but its greater propagation length provides better overall sensitivity (Berini, 2008). For biosensing applications, low-index claddings are used to match the refractive index of biologically compatible sensing fluids ($\sim$1.32) which then maintains the optical symmetry of the mode. Fluoropolymers such as Cytop (Asahi) and Teflon (Dupont) are the most common materials used as low index claddings (Joo et al., 2010; Slavík and Homola, 2007; Wark et al., 2005). The sensor used throughout this paper consists of a straight gold (Au) stripe embedded in Cytop claddings with an etched microfluidic channel for sensing. The sensitivity of the straight waveguide as a biosensor was discussed previously (Wong et al., 2015a).

2. Materials and methods

2.1. Chemicals and reagents

16-Mercaptohexadecanioc acid (16-MHA), phosphate buffered saline (PBS) 0.01 M, pH 7.4, N-Hydroxysuccinimide sodium salt (NHS), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), human IgG kappa antibody, sodium dodecyl sulfate (SDS), 2-Isopropanol semiconductor grade (IPA), acetone HPLC grade $\geq$99.9%, heptane and glycerol (electrophoresis grade) were obtained from Sigma-Aldrich. Distilled water was deionized using Millipore filtering membranes (Millipore, Milli-Q water system at 16 MΩ cm).

Dengue virus mouse anti-NS1 monoclonal antibody (clone D2N3) supplied as cell culture supernatant was purchased from Vivantis Technologies. Purified mouse anti-dengue virus NS1 glycoprotein antibodies were purchased from Abcam (ab138696) and ViroStat (#5162). Dengue virus NS1 full length glycoprotein (ab64456) was purchased from Abcam. All patient plasma samples (POS 1_3, POS 2_1, POS 2_2 and POS 2_3), laboratory-confirmed as dengue NS1-positive, were obtained from the Department of Medical Microbiology, Faculty of Medicine, University of Malaya, Malaysia (Ethical Clearance No. 782.90 from the University Malaya Medical Centre). Normal human plasma in K3 EDTA (lot no. PLE050412) was purchased from Zen-Bio, Inc. to serve as a negative control. The dengue ELISA kit used is SD Dengue NS1 Ag ELISA from Standard Diagnostics Inc., South Korea.

In previous work (Wong et al., 2015a), it was shown that a sensing buffer (PBS/Gly) with a slightly higher refractive index than Cytop will result in a higher response for protein binding. Therefore, a sensing buffer with a refractive index of 1.338 was chosen and used throughout the experiments; this value is high enough to produce a sensitive response to protein binding but also low enough for the LRSPP mode to be below cut-off. The sensing buffer PBS/Gly having an index of 1.338 was prepared by mixing biological buffer (PBS, pH 7.4) with glycerol. The sensing buffer and the deionized water were filtered through Millex-GP filters (PES membrane 0.22 μm).

2.2. Sensing device and instrumentation

The sensor die consists of thin ($\sim$35 nm) narrow (width=5 μm) gold stripes embedded in Cytop claddings, with a fluidic channel of sensing length L=1.65 mm etched into the top cladding. Sensor dies with two different fluidic designs were used in the experiments, as shown in the microscope images of Fig. 1a (left and right). Although Y-junctions and Mach-Zehnder interferometers (MZIs) are also present on the sensor dies, only straight Au waveguides were used throughout.

A schematic of the experimental set-up is sketched in Fig. 1b. A polarization-maintaining (PM) optical fiber with a core diameter of 7 μm (PMJ-3AX-1300-7/125-1-1-1, OZ Optics) which carries the light generated by a semiconductor laser diode (NLK1356STG, λ_0 =1315.89 nm, NTT Electronics) was butt-coupled to the input facet of the LRSPP biosensor. Two multi-axis positioning stages (Thorlabs Inc.) were used to align the optical fiber to the input waveguide. The output signal from the sensing waveguide was magnified and collimated by a 25× objective lens (Melles Griot). Background light in the output signal was minimized using a pinhole aperture before it was sent to a 50:50 beam splitter (BSW12, Thorlabs Inc.). One portion of the output beam was sent to an infrared camera to visually monitor the emerging mode for ease of alignment while the other portion was sent to a power meter (81618A, Hewlett Packard) to record real-time changes in