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Novel Four Layer Metal Sensing in Portable SPR Sensor Platform for Viral Particles Quantification †

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Abstract: Label-free, direct, rapid and real-time quantification method for human enterovirus 71 (EV71) using surface plasmon resonance (SPR) sensor is presented in this study. The performance of the four layer sensor chip was compared with the conventional Au monolayer sensor chip. Four layer sensor chip gave optimum resonance wavelength around 610 nm, which was same with the OLED light source peak wavelength and gave better quality factor (Q = DIP/FWHM). The result showed that the structure with 3 nm Al, 10 nm Au, 20 nm Ag, 10 nm Au layer sensor chip gave better limit of detection than the 3 nm Cr, and 47 nm Au layer sensor chip. The detection limit of direct quantification of EV71 particles is improved to 43 vp/mL of EV71 in DMEM medium. The results proved that the SPR biosensor with four layer sensor chip structure demonstrated great performance in the quantification of EV71 virus species.

Keywords: SPR sensor; four layer sensor chip; EV71 virus; quantification; OLED

1. Introduction

The quantification method of viral particle has been proposed by several groups to replace the viral plaque assay (VPA) quantification method, which is high labor and time consuming up to 7 days for plaque visualization [1]. Another method such as Transmission Electron Microscopy (TEM) viral particle visualization also was introduced following with the conventional counting to estimate the viral particle number [2]. Currently, there is much concern with the Hand, Foot and Mouth Disease (HFMD) caused by Enterovirus 71 (EV71) due to its neurovirulence and high fatalities in some recent outbreaks in the Asia-Pacific region including Taiwan [3–5]. Therefore, an in-depth study on EV71 viral detection is required. Surface plasmon resonance (SPR) biosensor is an emerging technology for the real-time, label-free detection of the EV71 virus. The major contribution of this proposed method is direct, rapid and less-labor intensive for the viral quantification of EV71. SPR phenomena largely depend upon the shape of the resonance curve due to refractive index change in the sensor surface. SPR sensor chip metallic structure play an important rule for the narrower FWHM resonance curve. Ag and Al produce narrower FWHM and larger dip in the SPR curve. Due to narrower FWHM and larger dip, Ag and Al sensor chip produce better quality factor, which gives the better limit of detection. There have been very few reports noticed related to EV71 virus quantification by SPR biosensor. Our group first quantified the EV71 virus by using four layer sensor chip through the portable SPR biosensor platform.

In this study, we presented rapid and real-time quantification of EV71 virus by four layer SPR sensor chip. Whole quantification procedure is simple and less time consuming. We compared the quantification result of four layer sensor chip with conventional Au monolayer sensor chip. The limit of detection of the multi-metallic sensor chip was six fold better than the conventional Au monolayer sensor chip.

2. Materials and Methods

The SPR measurement system in this study is based on the Kretschmann prism configuration. The SPR measuring system utilizing an OLED light source with peak wavelength at 610 nm as an active light source, which is incident to the prism where the sensor chip is placed. The reflected light is detected by spectrometer which is connected to the computer for further data analyzing. OLED light source is integrated with the BEF and DBEF film. BEF is used to send the light to collimator up-to 70 degree angle and DBEF is used as polarizer, which sends only p-polarized light to the sensor surface. Basic portable SPR biosensor platform design in this experiment was described in our previous study [6]. The Essential Macleod simulation tool (Thin Film Center Inc., Tucson, AZ, USA) is used to simulate the SPR reflectivity profile. In our group, we developed the direct, rapid, and simple labor intensive for viral detection using the organic light-emitting diode (OLED) based portable surface plasmon resonance (SPR) sensor for several years [7], as the platform is depicted in Figure 1.

We directly used our sensor chip for the quantification of EV71 virus without any chemical treatment because the amino acid can be attached to gold particle due to binding capability of the amino group and the gold with covalent interaction [8,9]. Concentration ranging from 5×10^1 vp/mL to 1×10^6 vp/mL of EV71 virus under DMEM medium was taken for series measurement.

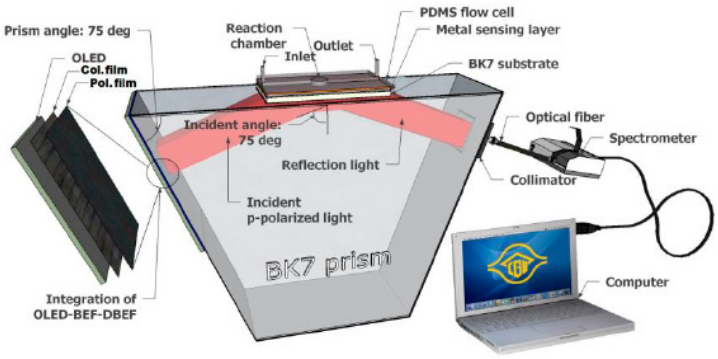

Figure 1. Configuration of portable SPR biosensor instrument using tunable color OLED integrated by brightness enhancement and reflective polarizer microstructure.

3. Results and Discussion

SPR sensor protocols offer high sensitivity, real-time detection, also free assays and fluorescence. It saves quantification time compared to VPA method. Four layer metal structure is introduced to the SPR sensor platform, by Essential McLeod tools for the metal layer optimization. The optimum configuration of the metal structures of Al(3 nm)/Au(10 nm)/Ag(20 nm)/Au(10 nm). In this sensing structure, we utilize Al to replace the current Cr for adhesion layer, due to the Al properties which have the stronger plasmonic behavior compared to Cr. 10 nm of Au over the Al is introduced to cover the Al nano-island, due to the thin film of Al is not yet fully constructed. The void between the nano-islands can be compensated by the 10 nm of Au. By this configuration, the adhesion layer has a strong plasmonic field. Next, the stronger plasmonic properties material of Ag was deposited for 20 nm. Finally, again 20 nm of Au is deposited to protect the sensing metal of Ag from oxidation.

The simulation results of our sensing construction in SPR system is depicted in Figure 2a, which is the reflectivity dip is fully matched with the OLED light source in our experimental. Subsequently, we presented the TEM image of the EV71 viral particles in the TEM substrate (Figure 2b).

Subsequently, it was followed by different samples as concentration increased, for 5 min respectively. VPA obtained for the number of the EV71 viral particles concentrations. The number of EV71 viral particles were from 5×10^1 vp/mL to 1×10^6 vp/mL.

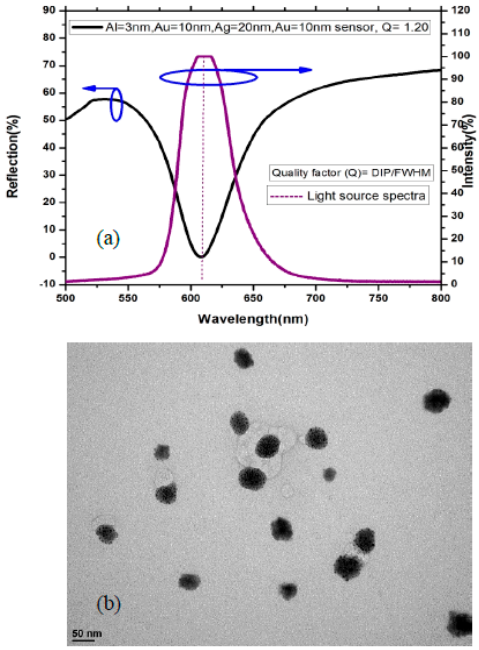

Figure 2. (**a**) Simulation results of novel four layer SPR sensing in this experiment; (**b**) TEM image of EV71 viral particle, after dropcast and washing protocol.

Real-time signal processing we use in this study is depicted in Figure 3a. Finally, calculated SPR signal processed were presented in Figure 3b. The standard deviation of the blank sample (σ) is around 0.1 and resulting the LOD is calculated around 43 vp/mL.

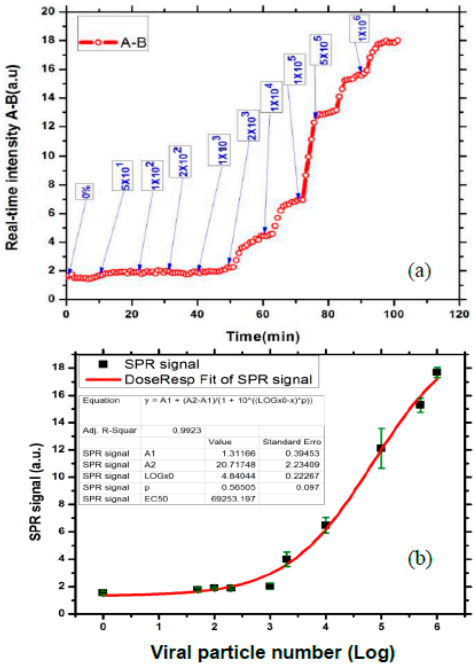

Figure 3. (**a**) Real-time normalized SPR signal with different concentration EV71 viral particles; (**b**) Curve fitting of measured series concentrations of EV71 virus particle sample as standard curve using variable slope dose-response model.

4. Conclusions

We successfully quantified the EV71 virus by using four layer sensor chip with SPR biosensor. The novel sensing structure contains four metal layer, Al/Au/Ag/Au is proposed to enhance the measured SPR signal compared to the conventional Cr/Au sensing metal structure. The detection limit of direct quantification of EV71 particles is improved to 43 vp/mL of EV71 in DMEM medium.

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Author Contributions: R.Y.L.W and K.C.L design the concept, B.A.P manuscript writing and data analysis, M.K.S, P.P, A.A, did the sample preparations, experimental and measurement.

Conflicts of Interest: The authors declare no conflict of interest.

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