DIRECT DETECTION OF ROTAVIRUS USING LABEL-FREE 3D PHOTONIC CRYSTAL BIOSENSOR

B. Maeng[1], Y. Park[2] and J. Park[1*]

[1]*Department of Mechanical Engineering, Sogang University, Republic of Korea*

[2]*Agency for Defense Development, Republic of Korea*

ABSTRACT

In this study, a direct label free biosensor based on 3D photonic crystal structures for Rotavirus has been demonstrated. The proposed sensor can detect the target without any pretreatment and the sensitivity and selectivity performance are analyzed quantitatively by measuring peak wavelength value. This process is critical for enhancing the virus sensing performance. In similar viral loading condition, our PWV shift range is larger than 2D photonic crystal based virus sensor, because the larger surface area in 3D inverse opal structures was realized. These results show that the proposed method useful for developing direct virus detection in the near future.

KEYWORDS: Rotavirus, 3D Photonic crystal, Label-free detection, Biosensor

INTRODUCTION

Today, photonic crystals (PC) have been utilized in many applications, and especially, attracted more and more attention in the field of sensing technology. Various type of PC sensors have been reported for measuring solvents, vapors, temperature, ionic strength, pH, biomolecules, mechanical force etc.. PC possess a periodic arrangement of dielectric material and due to this periodicity, may exhibit a Peak Wavelength Value (PWV). The PWV shifts positively when biological molecules, such as proteins, viruses or cells bind to the PC surface [1], so that direct label-free sensing is possible with high sensitivity, low cost and ease of use. In this study, we present a direct label-free detection method for Rotavirus using 3-dimensional photonic crystal biosensor as shown in figure 1.

Rotavirus is viral agents of gastroenteritis in animals and humans and is distributed worldwide [2, 3]. It is one of the most common enteric agents and mortality associated with infection can be very significant in infants and children under 5 year old, especially affecting developing countries [4].

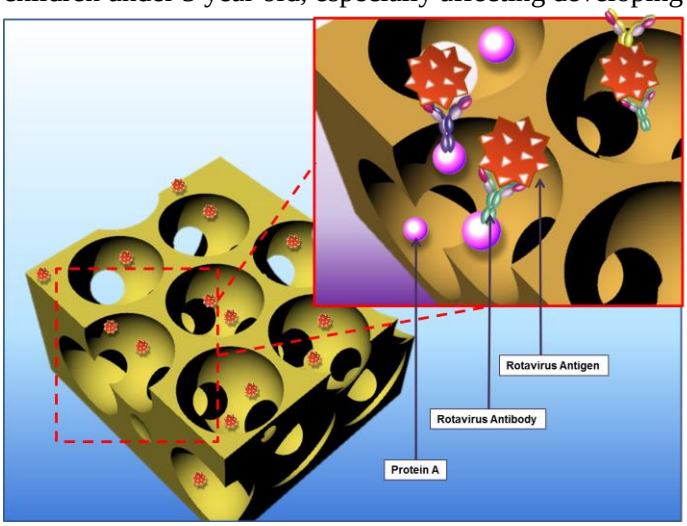

Figure 1: Working principle of label-free 3D photonic crystal virus sensor. The sensor surface was functionalized with protein A and anti-rotavirus IgG in sequence. If the target virus captured by antibody on the sensor surface once, the reflected peak wavelength value (PWV) is changed due to physiochemical change.

Here, an hydrogel based inverse opal structure is implemented, because the larger void space can accommodate more intensive variation in refractive index compared to an opal structure [1, 5]. In addition, the presence of nanopores can not only provide greater surface area and more interaction sites, but also offer easier access for the analytes to the recognition sites [1, 6]. Additionally, in order to facilitate the permeability of target analytes into nanoporous structures in the sensor, the hydrogel backbones in the inverse were treated with reactive ion etching (RIE).

EXPERIMENTAL

Homogenous silica nanoparticles were self-assembled using dip-coating method and the inverse opal structures made from photocurable PEG-DA were constructed by wet etching the silica nanoparticles. Additionally, in order to facilitate the permeability of target virus into nanoporous structures in the sensor, the backbones were treated with RIE etching. This result in enhancing the size of interconnection windows between nanopores, the target virus can access deeply into the sensor and finally the high sensing performance can be achieved. For pathogen specificity, the surface of inverse opal structures was modified with protein A and specific monoclonal Rotavirus antibody. The immobilization of surface modified molecules and the target virus detection were confirmed by fluorescent labelling and PWV shift measured by an optical spectrometer. Capturing the target viruses were confirmed again by scanning electron microscopy (SEM).

RESULTS AND DISCUSSION

As depicted in the figure 2(A), our biosensor was successfully detected the Rotavirus. All of virus sensing experiment was carried out without any chemical modification of the sample just except the dilution. The PWV shift at the highest concentration (3×10^6 FFU) was 3.25±0.31 nm (n=5, P<0.05) and the shift value was changed depending on the virus concentration which was diluted by 10, 100, 200 and 500 times with PBS. The shift values 2.36±0.32 nm (n=5, P<0.05), 1.48±0.24 nm (n=5, P<0.05), 0.8±0.31 nm (n=5, P<0.05) were measured after direct reaction with 10, 100 and 200 times diluted virus solution, which correspond 3×10^5, 3×10^4 and 1.5×10^4 FFU, respectively. In case of 1.5×10^4 FFU virus concentration, the PWV shift was not clearly discriminated with the shift when only PBS buffer was reacted with the sensor surface. Figure 2(B) shows the SEM images for representing the captured target virus on the surface. Gold nanoparticles were tagged on the virus for easy observation in SEM. Figure 3 shows the fluorescent intensities to confirm the pathogen specificity.

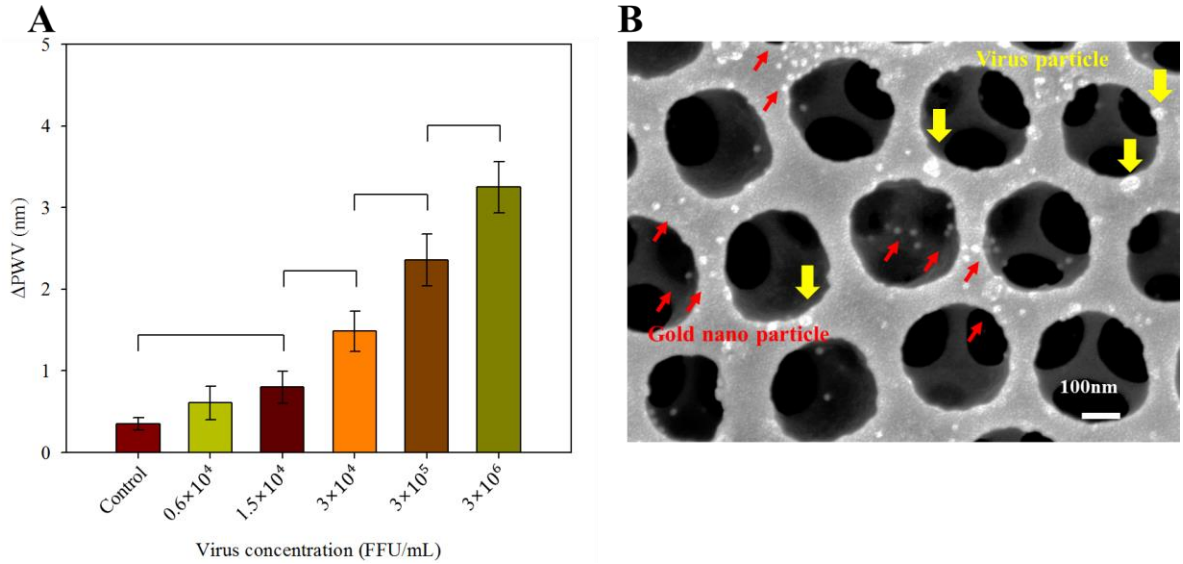

Figure 2. A) Shift of the Peak Wavelength Value (PWV) according to the virus concentration. B) SEM image of Inverse opal sensor surface. Rotavirus and conjugated with gold nanoparticles were attached on structure.

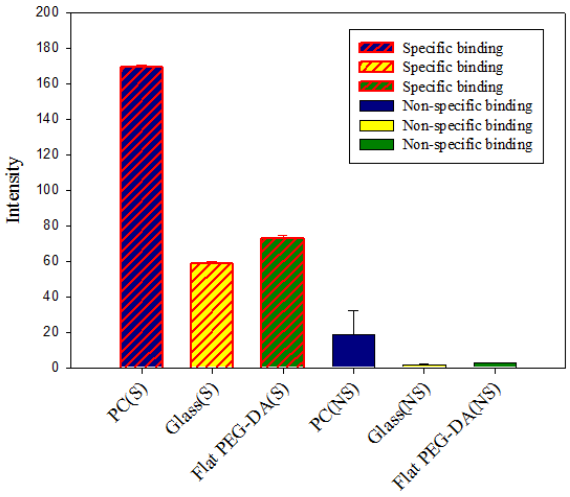

Figure 3. Fluorescent intensity to confirm the specific virus detection when the proposed surface modification is used. Each bar(S) demonstrates fluorescent signal from the specific binding reaction for Rotavirus and bar(NS) from the nonspecific binding on photonic crystal, glass and flat PEG-DA respectively.

CONCLUSION

Compared to PWV shift range (pico meter change) in the previous 2D photonic crystal based virus sensor [4], our PWV shift range (nano meter change) was more larger, because the larger surface area in 3D inverse opal structures was realized. Also, our results supported that the proposed system can be used for quantitative measurement for virus interaction according to the variation of concentration.

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CONTACT

* Jungyul Park ; phone: +82-2-705-8642, E-mail:sortpark@sogang.ac.kr