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Electrochemical Detection of Airborne Influenza Virus Using Air Sampling System

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ABSTRACT

Sampling and pre-detecting infectious diseases are effective ways of preventing them from widely spreading. Among the conventionally used sampling methods, cyclone-based air sampling is considered the simplest and most effective. In this study, we developed a new cyclone-type air sampler device by modifying the size and shape of a commercially available product, Coriolis μ (Bertin Technology). The newly built air sampler's collection efficiency was measured using polystyrene particles by comparing the amounts before and after sampling. Additionally, to test the feasibility of sampling airborne viruses, recombinant influenza virus antigen (H1N1) was injected into the custom-built air chamber where the air sampler's inlet was connected so that the antigen was collected through the sampling process. The solution was measured using a custom-made electrochemical platform that consisted of antibody conjugated Au electrodes on printed circuit board (PCB) and a small size reading system. Our findings showed that the influenza antigen was collected with good efficiency as well as detected with high sensitivity.

Keywords: Airborne; Virus; Sampler; Cyclone; Influenza.

INTRODUCTION

Microorganisms and viruses can become airborne or aerosolized in various ways such as sneezing, talking and other mechanical processes. Airborne transmission of those aerosols can contain infectious pathogens and can spread widely with long survival lifetime. If those infectious particles are transmitted through humans or animals, they can cause infectious diseases. Airborne influenza virus can pose major health and economic risks to the human and animal populations. Influenza is an infectious disease of birds and mammals caused by a virus. Influenza A, B, and C viruses can be differentiated by the nucleoprotein and matrix proteins and influenza viruses are further categorized into subtypes by their surface glycoproteins: hemagglutinin (H) and neuraminidase (N). The influenza A, to date, has been known to contain a total of 16 subtypes of H and nine subtypes of N. Among those influenza subtypes, H1N1 is well known as pandemic to humankind (WHO Pandemic Influenza A (H1N1) Vaccine Deployment Initiative, World Health Organization, 2012). Their effect on economy and health were significant especially in populous countries such as China and South Korea. Recently, the flu has been widespread in most of the United States so 700,000 people were hospitalized and 56,000 people lost their lives (CDC reports, 2018.03). Preventive actions should be taken and appropriate methods should be proposed such that various airborne viruses should be sampled simultaneously. Many types of samplers have been used, such as cyclone samplers (Sung *et al.*, 2017), liquid impingers (May and Harper, 1957; Lin *et al.*, 1999), slit samplers (Bourdillon *et al.*, 1941; Decker and Wilson, 1954; Walls *et al.*, 2016), electrostatic precipitators (Morita and Sakai, 1994; Zhuang *et al.*, 2000; Hong *et al.*, 2016), and filters (Wang *et al.*, 2001). However, effective air sampling with low limit of detection of collected virus is still needed. The liquid impingers can be avoid to lose microorganism if they are collected onto dry solid surface or filters because of impact damage and desiccation. However, the collection efficiency curves tend to be slightly sharper than impactor or cyclones. The electrostatic precipitation works by using a strong electric field to create a high concentration of unipolar ions and these ions cause to collide with charged airborne particles. It can collect bioaerosol particles such as allergens, bacteria, and viruses but it is more expensive than other types of

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sampler and needs to wipe settle dust from surface. It should be stop for wiping time during collection. The most aerosol filter media was classified as fibrous, membrane, and capillary pore and it commonly used to collect bioaerosol particles with simple and low cost. However, it needed to often changed for collecting bioaerosol particles. Therefore, the cyclone is one of alternative sampler because it provides sharper collection efficiency curves and can collect microorganism. Moreover, it can be used continuously without changing or cleaning the cyclone. Herein, we demonstrate a useful tool for collecting airborne virus from a newly modified air -sampler which is based on cyclone and custom-made electrochemical immunoassay platform through systematic collection of airborne viruses.

METHODS

Alkaline phosphatase (ALP)-conjugated goat anti-mouse IgG (A3562), (3-aminopropyl)triethoxysilane (3-APTES), and BlockerTM BSA (10X) in PBS were purchased from Sigma Aldrich and Thermo-Scientific. Ascorbic acid-2-phosphate (AAP) magnesium salt hydrate was obtained from Wako Pure Chemical Industries, Ltd. (013-12061, Japan). Immunogen influenza type A (H1N1) antigen was obtained from Meridian Life Science (R86380). 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP) was purchased from Pierce Chemical Company. The deionized water (DI water, > 18 MΩ cm) used to prepare all solutions in this study was obtained from a water purification system.

Design of Cyclone Air Sampler

The modified air sampler was based on a cyclone, which was divided either tangentially or axially depending on the airflow. A general cyclone has air inflowing tangentially to generate a rotational force using a vortex finder and collects particles on rotational inertia. The axial cyclone has air inflowing axially to generate rotational force using vanes inside the cyclone and collects particles. While sufficient research has been conducted on general cyclones in terms of design equations and computational fluid dynamics (CFD), the study on axial wet cyclones is scarce. The cyclone has been used for various purposes, such as collecting aerosol, bioaerosol and fine particles (Pant *et al.*, 2002; McFarland *et al.*, 2010). It can be applied in various fields since its simple design renders compactness, and it requires low operating power with high efficiency. Therefore, a cyclone air sampler was designed based on the Stokes number and Reynolds number to collect and concentrate airborne particles in a liquid. CFD techniques were used to study the performance of an axial-flow bioaerosol sampling cyclone that continuously collects particles onto a flowing liquid film. As shown in Fig. 1, the design of the vane was based on a previous study (Sung *et al.*, 2017). The cyclone air sampler was designed using Eq. (1) and the dimensions for each part are indicated: The inner size of the air inlet is 22 mm × 32 mm and the inner diameter of the air outlet is 19 mm. The total length of the cyclone is 180 mm and shaped such that the bottom area was tapered to encourage flow to hit the floor and move smoothly toward the outlet.

$$\eta = 1 - \exp[-\frac{\rho_p}{9\mu_g}\left(\frac{2d_p v_t}{D}\right)^2 t_{res} \quad (1)$$

where η is the theoretical collection efficiency in the entire cyclone, ρ_p is particle density, μ_g is dynamic viscosity of gas, d_p is particle diameter, v_t is inlet tangential velocity, D is diameter of cyclone and t_{res} is the residence time of the particle in the cyclone (Clift *et al.*, 1991).

Numerical Analysis Method

The commercial code FLUENT 16.1 was used for numerical analysis, and the analytical condition was a normal turbulent flow model using the RNG k-epsilon and

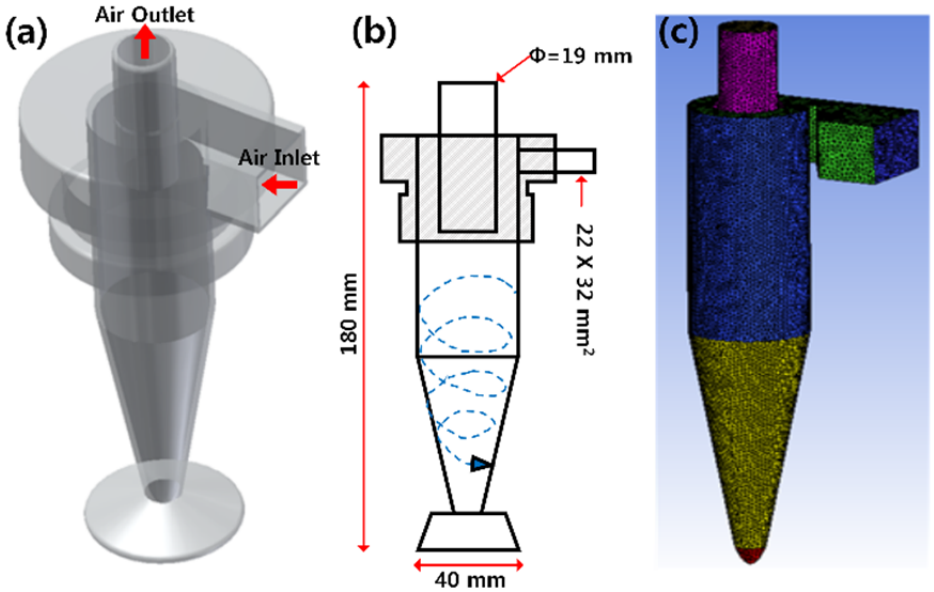

Fig. 1. (a) Simplified model for numerical simulation, (b) design of air sampler, and (c) mesh grid model.

RSM. The boundary condition was maintained at 600 L min^{-1} using the velocity inlet and pressure outlet conditions. To satisfy grid convergence, approximately 0.6 million grids were used. In particular, small grid sizes were used for the air inlet, bottom and air outlet, as shown in Fig. 1(c). After the calculations converged, the numerical computation was performed using the Reynolds Stress Model (RSM), which was the most complicated turbulent flow model and secondary order tension. The model is calculated using Eq. (2) and the FLUENT.

$$\frac{\partial}{\partial t}\left(\rho\overline{u'_i u'_j}\right)+\frac{\partial}{\partial x_k}\left(\rho u_k\overline{u'_i u'_j}\right)=D_{ij}+P_{ij}+\Pi_{ij}-\epsilon_{ij} \quad (2)$$

In this equation, the two mathematical expressions on the left are the partial derivatives for stress and convection with respect to time. D_{ij} is a tensor related to the spread of stress, P_{ij} is a tensor related to generation, Π_{ij} is a tensor related to pressure strain, and ϵ_{ij} is a tensor related to the loss rate.

To identify the particle trajectory, the discrete phase model was used to solve Eq. (3):

$$m_p\frac{dv}{dt}=F_d \quad (3)$$

where m_p is particle mass, v is particle velocity, and F_d is particle drag. Approximately 5000 particles with aerodynamic diameters in the 0.7–2 μm range were injected into the inlet at a constant dispersion. To compute numerical collection efficiency, trap conditions were set in the wall of the body. Collection efficiency was calculated using Eq. (4):

$$\eta_n=\frac{C_{trap}}{C_{injection}} \quad (4)$$

where η_n is the collection efficiency using the numerical method, C_{trap} is the number of trapped particles computed from numerical analysis, and $C_{injection}$ is the number of injected particles.

A cyclone air sampler was designed and built based on a commercially available product (Coriolis μ, Berin; Montigny le Bretonneux). We modified the overall shape and built an installation stand to provide further stability during the experiment and the sampler container was well matched with computational simulation modeling.

Airborne Virus Collecting Setup

The sampling container was connected with the main body that includes a PCB-based controller module, an operating pump, and a display module. A cyclone air sampler container was designed based on the Stokes number and Reynolds number to collect and concentrate the particles. The system was constructed for the collection of airborne virus using the cyclone air sampler and electrochemical immunoassay platform system for the detection of the presence of the sampled viruses in the solution. The influenza virus recombinant proteins were spiked into the solution and then injected into the air chamber by a jet nebulizer. These aerosols containing these antigen proteins were collected using an air sampler which was rebuilt for evaluation. The collected solution contained airborne recombinant proteins and then measured with electrochemical biosensor system. For the prevention of outward spreading during the virus antigen test, an air chamber was constructed with a high efficiency particulate air (HEPA) filter on top of the wall of the ventilation. The air chamber has two air inlets: one for connecting the jet nebulizer and the other for sampling air circulating inside, as shown in Fig. 2. A photograph of the prototype is shown in Fig. S1.

The cyclone air sampler (18 × 4 cm^2) was connected with the main body for control and air chamber (50 × 50 × 20 cm^3) as shown in Fig. S1. Its detailed drawing of design from different views are shown in Fig. S2. Aerosol containing antigen protein was generated by the jet nebulizer (Philips AG classic, 0.2 mL min^{-1}) and injected into the air chamber.

Quantification by Electrochemical Immunoassay

To increase the efficiency and multiplexing properties, we have also built a PCB with gold (Au)-based electrodes and custom-made a miniaturized electrochemical sensor platform. The Au electrode on PCBs were fabricated using the method as in previous studies (Kim *et al.*, 2014; Kim *et al.*, 2015, 2016) and its prototype is shown in Fig. S3 along with other parts. The Au electrode on PCB as working was conjugated on which antibody against target antigen. The readout platform used in this experiment can quantify the concentration based on the electrical signal and display the obtained results. This readout system consists of three slots into which working Au-PCBs could be placed and the Pt counter electrode and Ag/AgCl reference electrode (in 3M KCl) were inserted into two slots located in the center of this platform. Cyclic Voltammetry (CV) measurements were performed with 100 mV s^{-1} between –0.2 V to 0.8 V. The antibody conjugation on Au electrode for this experiment follows the protocols used in other studies. Immobilization of antibodies onto the gold surface was achieved using the homobifunctional linker, 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP), which contains thiol and *N*-hydroxysulfosuccinimide groups for gold and amine reactive units, respectively. The Au surface was pretreated with an oxygen plasma of pressure 5 × 10^{-2} torr, a working pressure of 7.8 × 10^{-1} torr, a power of 50 watts, and an oxygen flow rate of 45 cm^3 min^{-1} for two minutes. After the reaction between the antigen and captured antibody for 30 min, secondary antibodies with signaling label enzyme (alkaline phosphatase, ALP) were then attached. Measurement of the electrical signals from the reaction between the substrate (AAP) and enzyme (ALP) gives the corresponding concentration of antigens present in solution.

RESULTS AND DISCUSSION

Air Sampler Characteristic Depending on Flow Rate

To evaluate the performance of the air sampler, this study investigated various flow rates from 100–600 L min^{-1}

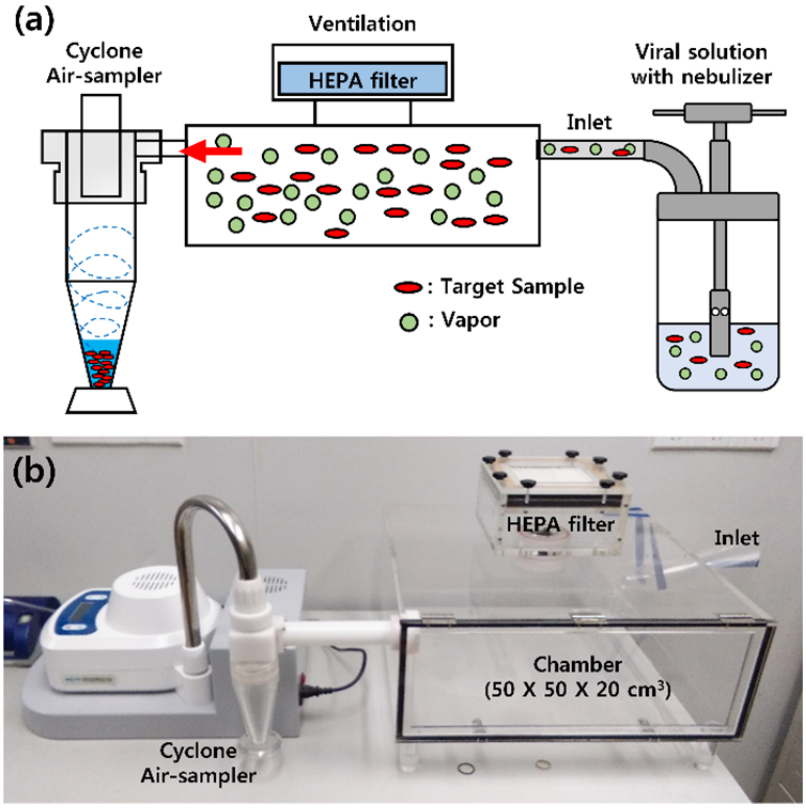

Fig. 2. (a) Schematic of experiment setup and (b) photograph of setup for the detection of target sample using a cyclone air-sampler.

while all other parameter was not changed as shown in Fig. 3. The streamlines indicated a change in velocity occurring in the area where the air flowing in the inlet first entered the vane and the maximum velocity was observed only for 400 L min^{-1} condition. The other condition results showed in Table 1.

At the same time, the pressure change was the largest at the area where the air entered the inlet. As the flow rate was increased from 100–600 L min^{-1}, the maximum velocity and pressure were increased proportionally from 1.61 m s^{-1} to 15.4 m s^{-1} and 342 Pa to 27,980 Pa, respectively, in Table 1. Specifically, using a flow rate of 100 L min^{-1} in the simulations showed a maximum velocity of 16.1 m s^{-1} and pressure drop of 342 Pa as shown in Table 1. The collection efficiency of air sampler was calculated using Eq. (5):

$$E_a = 1 - C_e/C_i \quad (5)$$

E_a is aerosol to aerosol. C_e is the number of particles near outlet. C_i is the number of particles from inlet part. The collection efficiency for 10 μm particles was 31% under this condition. The 200 L min^{-1} condition showed 27.9 m s^{-1} and 768 Pa of maximum velocity and pressure drop, respectively.

Its collection efficiency for 10 μm particles was 39%. The 400 L min^{-1} condition showed 61.0 m s^{-1} and 4,275 Pa of maximum velocity and pressure drop, respectively. Moreover, the collection efficiency for 10 μm particles

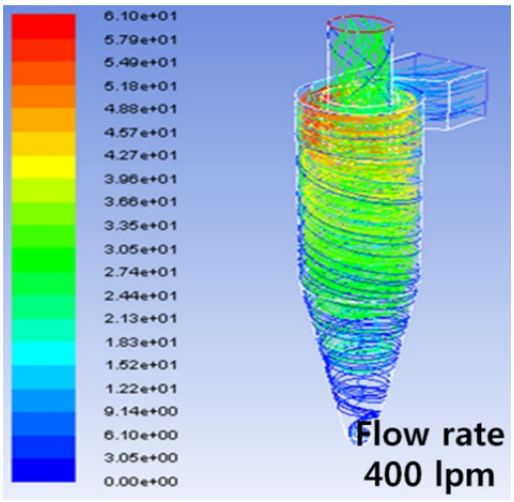

Fig. 3. Simulated result of air flow field at 400 L min^{-1} of flow rate.

was 90%, but the survivability of virus had to be checked. The last 600 L min^{-1} condition showed 154 m s^{-1} and 27,980 Pa in Table 1. The collection efficiency for 10 μm particles was 95%. Based on the simulated results, the 400 L min^{-1} and 600 L min^{-1} conditions caused too high pressure drop; therefore, the protein structure would have been destroyed upon impact at higher flows even if the collection efficiency was high. However, the 200 L min^{-1} condition had low collection efficiency for 1 μm particles;

Table 1. The simulated results of maximum velocity and pressure drop at different sampling flow rate conditions.

Conditions (L min^{-1})	Maximum velocity (m s^{-1})	Pressure drop (Pa)
100	16.1	342
200	27.9	768
400	61.0	4,275
600	154.0	27,980

hence, the optimized condition was expected to be between 200 L min^{-1} and 400 L min^{-1}. The air sampling is much dependent on the flow speed and size of airborne samples. For experimental verification, we have performed collection experiments using airborne polystyrene beads (0.1–10 μm) with flow rate of 100–800 L min^{-1}. Our results showed that the collection efficiency are proportional to both flow rate and particle size increase as shown in Fig. 4.

The collection efficiency was measured using 10 μm particles because the overall size of airborne hydrosols containing the cluster of virus ranges from 1 to 10 μm (Lindsley *et al.*, 2010). Although the size of single virus varies between 0.08–0.12 μm, flu viruses are normally transferred by cough. The 10 μm size of polystyrene beads showed sufficient collection efficiency for 400–600 L min^{-1} range and also this ranges were matched with simulated results. After air sampling and collection of airborne antigen have been completed, the developed electrochemical biosensor platform was inserted into the collected sample solution, and the relative current levels were measured. Three different Au-PCB chips were inserted to measure the reproducibility.

In the electrochemistry based immunoassay, signal of antibodies which was labelled with enzymes were needed for producing the electron during reacting with substrates. Therefore, electrical signals depend on the concentration of an enzymatic product at the sensing electrode surface upon antigen-antibody reaction. In this experiment, we prepared one subtype antigen for injection into the air chamber for each case of H1N1 and three Au-PCBs with antibodies for measuring the electrical responses of the corresponding antigens after sampling.

Fig. 5 shows the electrochemical responses of Au-PCBs against the target H1N1 proteins. Fig. 5(a) shows the electrochemical responses and their variations against the different concentrations of H1N1 proteins upon the insertion of Au-PCBs conjugated with H1N1 antibodies. The standard deviation of each concentration was measured to be within 1–14% range. As expected in overall cases, the Au-PCB conjugated with pair antibodies showed good responses. To compare the electrochemical signal changes or collection efficiencies of proteins, we have compared the signal obtained before and after samplings. The measurement of each H1N1 protein-spiked solution was performed using the same sensor platform. Although the overall response in each case after sampling was decreased to approximately the ranges between 5–37% (Table 2), we observed that this platform could measure small amounts of H1N1 proteins left in the solution, as shown in Fig. 5(b). In real field and in situ measurements, a small and simple platform that provides fast response and qualitative results are more significant; therefore, our platform combinations are expected to provide real field oriented measurements.

CONCLUSIONS

We described a quantitative approach to measuring airborne viruses using a custom-made air sampler system

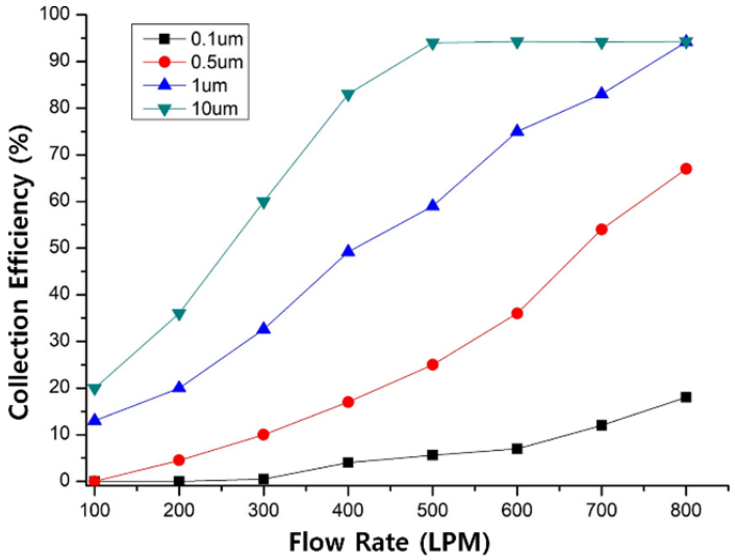

Fig. 4. Experiment result for collection efficiency of airborne polystyrene beads based on flow rate (100–800 L min^{-1}) and particle size (0.1–10 μm).

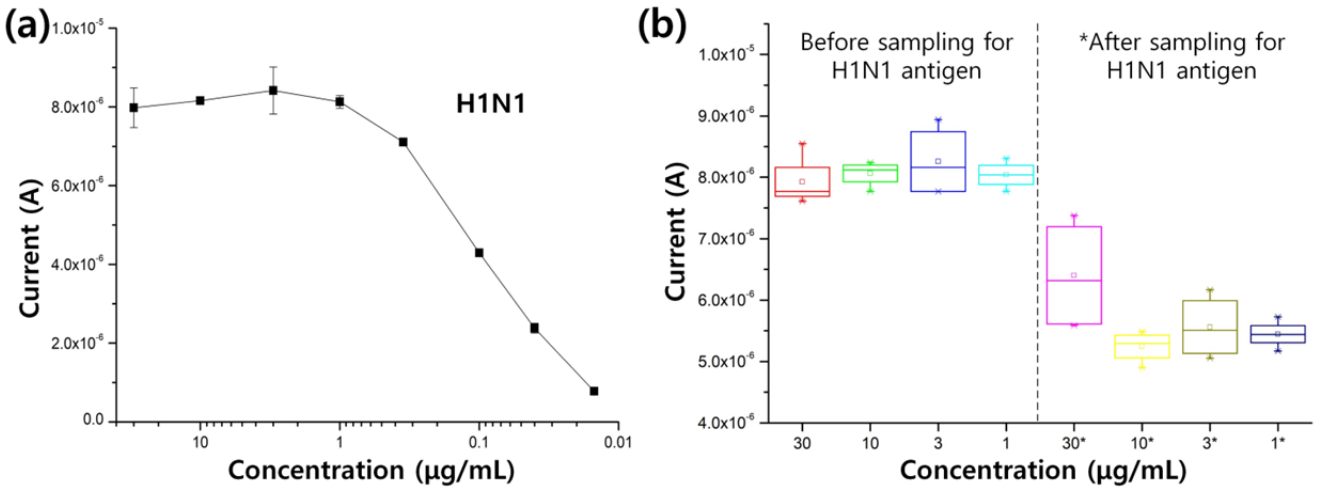

Fig. 5. Electrochemical responses of Au-PCB. The electrodes conjugated with antibodies against H1N1 (black): (a) Electrochemical responses against the different concentrations of H1N1 in solution and (b) the changes in signal before and after sampling of H1N1 spiked solution injected.

Table 2. The change of electrochemical signal ratio between before and after samplings in Fig. 5(b) (unit: %).

No.	30/30*	10/10*	3/3*	1/1*
1	28.0	37.0	35.0	30.0
2	5.0	36.0	32.0	31.0
3	18.0	34.8	31.0	36.0
4	26.0	32.0	33.0	32.0

and a customized electrochemistry readout device. This study experimentally verified the performance of an air sampler whose design was based on analytical and numerical predictions under various flow rate conditions. The simulated results accounted for the maximum velocity, pressure drop, and collection efficiency. Following the numerical simulations, the air sampler system, comprising a wet sampler, an electrochemical probe, and a readout device, was evaluated using spiked injections of influenza protein. The collection efficiency was also evaluated with the controlled injection of target antigens. The experimental results indicated that the sampler was able to collect airborne virus protein with 26–37% efficiency at sampling rate of 400 L min^{-1}. We believe that the system can be easily applied to the collection of viruses suspended in ambient air with a relatively high collection efficiency and high sensitivity to target proteins. In addition, our newly designed electrochemically based biosensor device shows a highly quantifiable sensitivity with a relatively fast response.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at http://www.aaqr.org.

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