Conductive Thread-Based Immunosensor for Pandemic Influenza A (H1N1) Virus Detection

Seong Uk Son, Soojin Jang, Jaewoo Lim, Seung Beom Seo, Taejun Kang, Juyeon Jung, Seo Yeong Oh, Sun-Woo Yoon, Dongeun Yong, Jaejong Lee, and Eun-Kyung Lim*

ABSTRACT: Infectious agents such as viruses pose significant threats to human health, being transmitted via direct contact as well as airborne transmission without direct contact, thus requiring rapid detection to prevent the spread of infectious diseases. In this study, we developed a conductive thread-based immunosensor (CT-IS), a biosensor to easily detect the presence of airborne viruses. CT-IS utilizes an antibody that specifically recognizes the HA protein of the pandemic influenza A (pH1N1) virus, which is incorporated into the conductive thread. The antigen–antibody interaction results in increased strain on the conductive thread in the presence of the pH1N1 virus, resulting in increased electrical resistance of the CT-IS. We evaluated the performance of this sensor using the HA protein and the pH1N1 virus, in addition to samples from patients infected with the pH1N1 virus. We observed a significant change in resistance in the pH1N1-infected patient samples (positive: \( n = 11 \), negative: \( n = 9 \)), whereas negligible change was observed in the control samples (patients not infected with the pH1N1 virus; negative). Hence, the CT-IS is a lightweight fiber-type sensor that can be used as a wearable biosensor by combining it with textiles, to detect the pH1N1 virus in a person’s vicinity.

KEYWORDS: respiratory infection, pH1N1 virus, conductive thread, electric immunosensor, point-of-care detection

INTRODUCTION
Airborne particulate matter is closely related to human health and contains various infectious agents such as viruses and bacteria. These bioaerosols, with diameters ranging from 0.001 to 100 \( \mu \)m, are ubiquitously dispersed in ambient air. Infectious agents such as the influenza virus, severe acute respiratory syndrome coronavirus (SARS-CoV), and SARS-CoV-2, can be transmitted via direct contact as well as cough droplets. Moreover, the Centers for Disease Control and Prevention has also recognized the risk of infection through airborne infectious agents and stated that infections can be caused by exposure to respiratory fluid bioaerosols carrying the infectious virus, without direct contact.5,6 There have been multiple viral epidemics with severe fatalities in the 21st century. The pH1N1 virus emerged in the United States in 2009 and infected approximately 60.8 million people between April 12th, 2009 and April 10th, 2010, with 274,304 hospitalizations and 12,468 deaths.7 The SARS-CoV-2, which emerged in late 2019, causes a severe respiratory syndrome named coronavirus disease (COVID-19). Human-to-human transmission of the infection was first reported on January 22nd, 2020, and the World Health Organization (WHO) declared the SARS-CoV-2 infection to be a pandemic on March 11th.8 The COVID-19 pandemic is still ongoing, as of 4:54 pm CET, December 23rd 2022, with more than 651 million confirmed cases and more than 6.6 million deaths worldwide reported to WHO, despite the development of vaccines and therapeutic agents.9 In addition, several agencies have predicted a continued risk of both the emergence and re-emergence of viral diseases.8 Thus, it is critical to develop preemptive quarantine strategies for the rapid and easy diagnosis of potentially infected people to prevent and control future epidemics.9,10 Recent studies have reported the development of various technologies to capture,11 measure,12 and detect13 bioaerosol-mediated infections. Specifically, biosensors for bioaerosol detection are being developed using various technologies to increase detection sensitivity, shorten analysis time, and ease of use including gold nanoparticles modified carbon electrode-based sensors,14 DNA-antibody complex-base electrochemical sensors,15 and gold nanoparticles-based lateral flow immunosassay (LFIA).16

The miniaturization of various biosensors is also under active investigation. These include portable fluorometer-based
biosensors for on-site disease detection,\textsuperscript{17,18} colorimetric sensors to detect infectious pathogens by color change,\textsuperscript{19–25} and smartphone-based biosensors for point-of-care detection of infectious disease.\textsuperscript{26–28} Efforts are also underway to develop a user-friendly biosensor that can detect bioaerosols in the user’s surroundings in real-time.\textsuperscript{29} In this study, we developed a conductive thread-based immunosensor (CT-IS) for the detection of pH1N1 virus, which is one of the viruses transmitted via bioaerosols. The conductive thread (CT)-based textile sensor is one of the most promising next-generation point-of-care testing devices because it is easy to manufacture, lightweight, low cost, highly electronically integrated, and unobtrusive.\textsuperscript{30–33} It can also be easily integrated into existing garment manufacturing processes and can be produced on an industrial scale using currently available technologies and infrastructure, allowing for efficient scalability. These advantages have led to increased research on its utilization for detecting either chemical or biological compounds.\textsuperscript{34} In 2021, Pasquali et al. developed carbon nanotube thread (CNTT)-based washable, sewable electrodes and signal transmission wires for electronic clothing.\textsuperscript{35} This textile sensor could be easily integrated into traditional clothing and performed comparable to commercial electrodes in measuring electrocardiograms. Liu et al. developed a novel wearable electrochemical sweat biosensor based on CTs decorated with zinc-oxide nano-wires (ZnO NWs) and applied it for the detection of lactate and sodium in perspiration during physical exercise (Biosensors and Bioelectronics, 2021, 188, 113270).\textsuperscript{36} Poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT/PSS)-modified cotton CT (PECOTEX) was developed to enable the mass-production of low-cost, textile-based wearable sensors.\textsuperscript{37}

Here, we developed the CT-based electric immuno SENSOR by first attaching protein G to a CT coated with stainless steel using the highly adhesive polydopamine (PDA).\textsuperscript{38–40} Thereafter, the antibody was treated and bound to protein G attached to this CT.\textsuperscript{38–40} An antibody to detect the hemagglutinin (HA) protein of the pH1N1 virus was applied to detect the pH1N1 virus. As the target protein of the virus binds to the sensor via antigen–antibody interactions, the magnitude of the strain applied to the CT increases, leading to enhanced electrical resistance (Figure 1).\textsuperscript{41–44} Consequently, the target virus is detected by measuring the change in the resistance value of this sensor.

### EXPERIMENTAL SECTION

**Materials.** CT was purchased from Sparkfun (Boulder, CO, USA). Dopamine hydrochloride and Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1 M Tris–HCl buffer, pH 8.5, was purchased from Biosesang (Daejeon, Korea). Protein G was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The anti-influenza A virus HA antibody, influenza A H1N1 HA protein, and human serum albumin (HSA) protein were purchased from Abcam (Cambridge, UK). Pooled human nasal fluid was purchased from Innovative research (Novi, MI, USA). The influenza A (California/04/2009) (pH1N1) and influenza B (Brisbane/60/2008) viruses were provided by the Korea Research Institute of Bioscience and Biotechnology (KRIBB). In particular, patient samples were provided by the Severance Hospital, Seoul, Korea (IRB approval number: 4-2020-0465), to verify the applicability of the biosensor in real clinical samples.

**Preparation of the CT-IS.** The CT-IS was prepared by twisting 12 strands of CTs in one direction and then further treated with O\textsubscript{2} plasma to induce hydrophilicity on the surface of the previously hydrophobic CTs. Next, the CT was coated with PDA and protein G, as previously described.\textsuperscript{35,36} Briefly, CT was coated with PDA and protein G by treating a total of 1 μL of a mixture of dopamine (4 μg/μL) solution in Tris–HCl buffer and protein G (60 μg/μL) solution in 1:1 ratio. Dopamine was polymerized in alkaline solution to form PDA (Figure S1). After resting for 2 h at room temperature, the coated CT was washed three times using phosphate-buffered saline (PBS) with 0.1% Tween 20 detergent (0.1% T-PBS buffer). Next, for CT-IS preparation, 1 μL of antibody (1 μg/μL) was dropped onto the surface of PDA/protein G-coated CT. After 2 h, unbound reactants were removed by washing three times with 0.1% T-PBS buffer. The concentration of the antibody used was selected by screening conditions with stable sensor performance when detecting HA protein at the same concentration compared to various antibody concentrations (Figure S4).

**Characterization of CT-IS.** After dropping 1 μL of water onto the CT-IS for each modification step, its hydrophobicity/hydrophilicity was assessed using a Phoenix 10 apparatus (Gyeonggi, Korea) equipped with a video camera device. The morphology of the CT-IS was analyzed using focused ion beam–scanning electron microscopy (FIB-SEM) with energy dispersive spectroscopy (EDS) using the Carl Zeiss Crossbeam 550 (Jena, Germany). The characteristic bands of bare CT and CT-IS were confirmed using Fourier-transformation infrared spectroscopy (Alpha FTIR, Bruker Optics). Furthermore, the electrical resistance in the CT-IS was measured at each step using a FLUKE287 multimeter (FLUKE, USA) (Figure S5).

**Detection of pH1N1 Virus HA Protein Using CT-IS.** First, the performance (detectability) of the CT-IS was evaluated at the protein level before exposing the CT-IS to the virus. The HA protein was diluted with PBS to prepare solutions of varying concentrations. The HA protein solutions (1 μL) were dropped onto the CT-IS and after 1 h, the CT-IS was washed with 0.1% T-PBS buffer and the resistance for each solution was measured using a multimeter. The HSA was used as a control, and all tests were repeated in triplicates. The detection of the HA protein by the CT-IS was evaluated through the change in the electrical resistance (ΔR), which was calculated using eq 1. R\textsubscript{0} and R indicate the resistance values of CT-IS before and after protein treatment, respectively.

![Figure 1. Schematic of the CT-IS for pH1N1 virus detection.](image-url)
Change in electrical resistance \( (\Delta R) \) (%) = \( \left( \frac{R - R_0}{R_0} \right) \times 100 \) \( (1) \)

\( R \): the electrical resistance after target treatment; \( R_0 \): the electrical resistance before target treatment.

Detection of the pH1N1 Virus Using CT-IS. Both the influenza A (pH1N1) (California/04/2009) and influenza B (Brisbane/60/2008) viruses were provided by the KRIBB. The virus titers were determined using the 50% tissue culture infective dose (TCID\(_{50} \)) in confluent cells in 96-well microplates. First, the ability of the CT-IS to detect the virus was assessed using a series of dilutions of the influenza A (pH1N1) virus prepared in PBS. The viral solution (1 \( \mu \)L) was dropped onto the CT-IS, rested for 1 h, and further washed with 0.1% T-PBS buffer. The electrical resistance was then measured using a multimeter. Viral detection in CT-IS was assessed using clinical samples (nasopharyngeal and sputum samples) collected from 20 patients at the Yonsei University Severance Hospital, who were diagnosed as influenza A (pH1N1) virus-positive \( (n = 11) \) and negative \( (n = 9) \), through a qRT-PCR test of the samples. The change in \( \Delta R \) of the CT-IS before and after the treatment with the clinical samples was measured.

**RESULTS AND DISCUSSION**

Characterization of CT-IS. A CT of uniform thickness was fabricated by unidirectionally twisting 12 strands of CTs for the CT-IS (thickness = 1 mm). As shown in Figure 2ai, based on the contact angle with water, the surface of the bare thread was hydrophobic, making it difficult for hydrophilic biomolecules (e.g., antibodies) to bind to it directly. To increase the hydrophilicity of the CT surface, we coated the surface with PDA, an adhesive molecule, and protein G, an antibody-binding protein, followed by the antibody. A contact angle of 41° or less signifies hydrophilicity, and the contact angle with water decreased post-coating, confirming that the hydrophilicity of the thread was increased (Figure 2aii: 36° and Figure 2aiii: 34°). The hydrophilic surface of the CT enables the detection of viruses in bioaerosols. In addition, the surface morphology of the CT-IS during each fabrication step was characterized using FIB–SEM. As shown in Figure 2b, the bare thread had a smooth surface, whereas aggregates were observed on the CT after PDA/protein G and antibody coatings, respectively. As the coating progressed, aggregates corresponding to biomaterials (proteins and antibodies) were clearly identified. After analyzing the surface elements of the bare CT and antibody-coated CT (CT-IS), we observed that the intensity of the C peak had increased because of the PDA coating, and elements that were not detected on the CT, such as oxygen (O), were detected on the CT-IS (Figure 2c). The peaks corresponding to sodium (Na) and chlorine (Cl) were
increased with increasing HA protein concentration. The RC was 1.9 ± 0.8% at low protein concentration (0.13 μg/μL) and increased to 6.16 ± 1% at high protein concentration (1 μg/μL). In contrast, when HSA was used as the control, ΔR was less than 0.87%, and negligible difference was observed at varying concentrations. Moreover, diagnosis of infectious respiratory diseases mostly uses nasal fluid specimens. Therefore, we evaluated the performance of the CT-IS using specimens containing 1% nasal fluid, because non-specific signals can be generated by floating substances in the nasal fluid. As shown in Figure 4, ΔR gradually increased from 0.3% at 0.13 μg/μL to 7.6% at 1 μg/μL, indicating that ΔR increased with increasing HA protein concentration. Furthermore, we observed that the electrical resistance changed based on the HA protein concentration, regardless of the presence or absence of the nasal fluid.

Detection of pH1N1 Virus Using CT-IS. To confirm that the CT-IS selectively detects the pH1N1 virus, we exposed it to different concentrations of the pH1N1 virus and assessed the ΔR. The influenza B virus was used as a control. Figure 5 shows the ΔR of CT-IS treated with pH1N1 virus. A clear increase in RC with an increase in the pH1N1 virus concentration was observed. Even at a low concentration of 2 × 10^3 TCID_{50}, a slight change in the electrical resistance was observed (Figure 5). However, the CT-IS exposed to the influenza B virus showed little change in the electrical resistance for the applied concentration range, confirming that the CT-IS specifically detects the influenza A (pH1N1) virus, based on antibody–antigen interactions. Additionally, when the CT-IS was exposed to both the viruses spiked onto a viral transport medium (VTM), ΔR was observed only in the CT-IS exposed to the pH1N1 virus. VTM are typically used to collect and preserve the virus from the patient and contain various substances required to culture the virus. Thus, we evaluated the performance of the CT-IS using the virus spiked onto the VTM during patient sample collection. The concentration of 2 × 10^3 TCID_{50} showed 3.4% ΔR, which increased with increasing concentration of the spiked pH1N1 virus. The control influenza B virus spiked onto the VTM did not significantly change the average ΔR (%) at the tested virus concentrations, remaining at a level of about 2%. However, large standard deviations from the mean (average) were measured in some conditions, especially 2 × 10^3 TCID_{50} or less, due to VTM (Figure 6). Based on the virus tests, it was judged that the CT-IS could reliably detect target pH1N1 virus at concentrations above 4 × 10^3 TCID_{50}. Lastly, the performance of the CT-IS was evaluated using clinical samples from patients with pH1N1 infection. A total of 20 clinical samples were tested: 11 samples from patients infected with the pH1N1 virus (positive) and 9 samples from patients infected with the influenza B virus as the control (negative). Each clinical sample (1 μL) was dropped onto the CT-IS, and the corresponding resistance was measured using a multimeter. The 11 pH1N1 virus-infected (positive) samples showed a significant change in the electrical resistance along the cycle threshold (Ct) value of the virus (Figure 7). The Ct is a semi-quantitative value that signifies the concentration of the viral genetic material in a sample assessed using qRT-PCR. The Ct value is inversely related to the virus concentration. A low Ct usually indicates a high concentration of viral genetic material, which indicates a high viral load, whereas a high Ct indicates a low concentration of the virus in the sample. As shown in Figure 7, the pH1N1-infected patient samples with a low Ct value showed a large ΔR in the CT-IS due to high virus concentrations. In the control samples, ΔR was not significant for concentrations in the range of 0 to 1.2%. This indicates that
Figure 5. Resistance change in the CT-IS by influenza A (pH1N1) virus (red circle) and influenza B virus (blue square). Error bars represent the standard deviation of the three determinations.

Figure 6. Resistance change in the CT-IS by influenza A (pH1N1) virus (dark red square) and influenza B virus (dark blue circle), with VTM. Error bars represent the standard deviation of the three determinations.

Figure 7. Resistance change in the CT-IS using patient samples (Ct value: gray bars, ΔR (%): blue and red dots). Error bars represent the standard deviation of the three determinations.
the CT-IS developed in this study can selectively detect the pH1N1 virus.

■ CONCLUSIONS

We developed a CT-IS that can detect the pH1N1 virus by detecting the changes in the resistance. We incorporated antibodies that specifically bind to the HA protein of the pH1N1 virus into the CT-IS, thus ensuring that it directly detects the pH1N1 virus without any specific pretreatment. When the sample containing the target virus was dropped onto the CT-IS, the magnitude of strain applied to the CT-IS increased because the antibody on the CT-IS bound to the target virus (or protein), leading to a change in the electrical resistance. We confirmed that the electrical resistance changed in proportion to the virus concentration due to the piezoresistive effect. Although it is necessary to improve the detection sensitivity of this CT-IS, compared to other existing sensor technologies, its practical applicability has been established, as it can discriminate between pH1N1-infected and non-infected patient samples. Furthermore, based on the targeted virus to be detected, the CT-IS can be selectively changed by changing the bio-receptor applied to it. Therefore, the CT-IS developed in this study provides a simple and low-cost tool for virus detection, compared to different virus detection platforms (Table S1).48–53 Further, it can be applied to textiles or clothing to create a clothing-based biosensor that can selectively detect biohazardous infectious agents including viruses and bacteria.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.2c19403.

Structure of dopamine and polydopamine, FT-IR spectra of CT and CT-IS, CT-IS measurement method, ΔR data according to antibody concentration, and setup image of CT-IS (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Eun-Kyung Lim — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; Department of Nanobiotechnology, KIRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon 34113, Republic of Korea; School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea; orcid.org/0000-0003-2793-3700; Email: eklim1112@kriib.re.kr

Authors

Seong Uk Son — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; Department of Nanobiotechnology, KIRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon 34113, Republic of Korea

Soojin Jang — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; Department of Nanobiotechnology, KIRIBB School of Biotechnology, University of Science and Technology (UST), Daejeon 34113, Republic of Korea

Jaewoo Lim — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea

Seung Beom Seo — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; Department of Cognito-Mechatronics Engineering, Pusan National University, Busan 46241, Republic of Korea

Taejoon Kang — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Juyeon Jung — Bionanotechnology Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea; School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Sun-Woo Yoon — Department of Biological Sciences and Biotechnology, Andong National University, Andong 36729, Republic of Korea

Dongeun Yong — Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul 03722, Republic of Korea

Jaecjong Lee — Department of Nano Manufacturing Technology, Korea Institute of Machinery and Materials (KIMM), Daejeon 34103, Republic of Korea

Sungkyunkwan University, Suwon 16419, Republic of Korea; School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea; orcid.org/0000-0002-5387-6458

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acsami.2c19403

Author Contributions

S.U.S.: CT-IS fabrication research, data analysis, and manuscript writing. S.J.J.: CT-IS fabrication research. J.L.: data analysis. S.B.S.: data analysis. T.K. and J.J. contributed to the discussion section of the manuscript. S.Y.O.: data analysis. S.W.Y.: resources (virus samples). D.Y.: resources (clinical samples). J.-J.L.: manuscript revision. E.-K.L.: study design and manuscript writing. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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