Combination of Carbon Nanofiber-Based Electrochemical Biosensor and Cotton Fiber: A Device for the Detection of the Middle-East Respiratory Syndrome Coronavirus

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ABSTRACT: The miniaturization of biosensors for point-of-care diagnosis is highly important in infection control. Electrochemical biosensors offer several advantages in diagnosis in terms of cost, disposability, portability, and sensitivity. Here, a miniaturized electrochemical immunosensor combined with cotton fiber for the detection of the Middle-East respiratory syndrome coronavirus (MERS-CoV) is described. Taking advantage of the absorption capability of cotton, the nasal and saliva samples can be collected and directly transferred to the immunosensor surface for detection using a single tool. The immunosensor was fabricated on a disposable screen-printed electrode precoated with carbon nanofibers. The electrodes were functionalized with carboxyphenyl groups that were used for the immobilization of the spike protein of the MERS-CoV. A competitive detection scheme was employed using the antibody for the MERS-CoV spike protein, and the square-wave voltammetry technique was used for measurements. The biosensor tested after the cotton coating of the electrode exhibited excellent performance. The biosensor was capable of detecting the MERS-CoV spike protein within a concentration range from 0.1 pg·mL⁻¹ to 1 μg·mL⁻¹ with a limit of detection of 0.07 pg·mL⁻¹, implying the high sensitivity of the method. The immunosensor did not exhibit any cross-reactivity against proteins from HCoV and Influenza A, indicating the excellent selectivity of this approach. Testing of the biosensor in nasal samples showed very high recovery percentages. This disposable biosensor can be used as a miniaturized device for the collection of samples and detection of the virus using a portable potentiostat connected to a smartphone.

1. INTRODUCTION

The Middle-East respiratory syndrome coronavirus (MERS-CoV) is a type of coronavirus that can cause severe infections, resulting in high mortality. According to the World Health Organization (WHO), there are a total of 2566 laboratory-confirmed cases of MERS-CoV and 882 associated deaths globally from 2012 to December 2020. Four additional MERS-CoV cases with one death were reported between June and December 2020 in Saudi Arabia. The infection with MERS-CoV in humans mainly occurs through direct or indirect contact with dromedaries. However, MERS-CoV has also the capability to transmit between humans, particularly in healthcare settings. MERS-CoV symptoms vary from mild illness to severe pneumonia, which can end up with multiorgan failure. Some nonspecific symptoms such as diarrhea, vomiting, fever, anorexia, and abdominal pains can be also seen with MERS-CoV infection. MERS-CoV appears to cause more severe illness in people with renal failure, diabetes, chronic lung disease, and immunocompromised individuals.

As the WHO is expecting more cases of MERS-CoV infection to appear in the Middle East, and to continue to be transferred to other countries, the early identification and isolation of this virus is crucial for disease management. However, with the ongoing COVID-19 pandemic, the testing capacity for MERS-CoV has been severely impacted in many countries, as most of the resources have been redirected towards SARS-CoV-2. Thus, the WHO has been encouraging increased testing of MERS-CoV infections to prevent any possible outbreaks.

Until now, the suspected cases of MERS-CoV infection had to be confirmed by two real-time reverse transcription polymerase chain reactions (rRT-PCR) at two specific genome regions. However, PCR testing is not widely available, particularly in countries with limited resources. Thus, several other methods for the virus detection have been developed, such as reverse transcription loop-mediated isothermal amplification assay (RT-LAMP), immunofluorescence assay, hemagglutination assay, and enzyme-linked immuno-sorbent assay (ELISA). These methods are relatively
SARS-CoV-2 using lateral flow has been drawn towards the development of biosensors for infectious diseases. Some nanobiosensors have been reported previously for the detection of various types of coronaviruses such as SARS-CoV using localized surface plasmon resonance11 and a piezoelectric immunosensor.12 Much attention has been drawn towards the development of biosensors for SARS-CoV-2 using lateral flow assay (LFA),13 field effect transistors,14 and plasmonic photothermal and electrochemical biosensors.15–18 MERS-CoV has been detected using the bio-optical sensor and isothermal rolling circle amplification (RCA) method.19 We have previously developed a multiplexed voltammetric sensor for the detection of MERS-CoV and HCoV, which showed good sensitivity and selectivity.20 However, these methods require the biological samples to be collected using swabbing. The samples are then recovered into an elution buffer, which can be then applied on the sensor surface. Thus, we believe that the fabrication of an integrated biosensor platform that can perform the collection and detection steps in a short time with minimized risk of sample handing is crucial for developing point-of-care diagnostic devices.

The development of miniaturized electrochemical biosensors using low-cost materials for point-of-care testing represents a rapidly developing field of research.21 The integration of electrochemical detection with flexible materials such as plastic, textile, and paper to develop disposable low-cost biosensors has been widely studied.22 The use of flexible materials in biosensors offers several advantages in terms of cost, disposability, weight, and portability. Several electrochemical biosensors were previously fabricated on flexible substrates for different diagnostic applications, such as the wearable and tattoo-based biosensing devices23,24 as well as paper-based platforms.25,26 The combination of a cotton thread and microfluid-based electrochemical sensors has been also reported.27,28 The thread allowed the liquid to flow by capillary forces without external pumping and avoided the need for constructing microchannels.

Recently, cotton swabs have been utilized to develop assays for the detection of various pathogens such as Salmonella typhimurium,29 Methicillin-resistant Staphylococcus aureus,30 and Campylobacter jejuni.31 These detection methods were based on the sandwich immunoassay format using colored beads that lead to color change upon binding with the specific pathogen. These cotton swab assays are low-cost and simple; however, they provide only semiquantitative results and have relatively low sensitivity. Since electrochemical methods can provide highly sensitive and accurate detection, they are perfectly suited to develop integrated biosensing devices for pathogens.32,33 The integration of cotton fiber into electrochemical biosensors to fabricate a miniaturized device for point-of-care testing is a new area. Here, we report the construction and testing of a disposable cotton-coated voltammetric biosensor for MERS-CoV detection. The biosensor can collect the clinical sample by swabbing and detect the electrochemical response via a small potentiostat connected to a smartphone. This integrated MERS-CoV biosensor appears as a promising option to overcome the need of multiple steps for handling and extraction, making it ideal for point-of-care diagnosis.

2. RESULTS AND DISCUSSION

2.1. Functionalization of the Carbon Nanofiber (CNF)-Modified Electrode. The carbon screen-printed electrodes were modified with CNF to increase the electrochemical surface area, which enables the immobilization of more proteins and enhances the electron transfer. CNF dispersion was drop casted on the carbon electrodes. Figure 1A,B show the high-resolution scanning electron microscopy (SEM) images of the carbon surface of the screen-printed electrodes and the CNF-modified electrode, respectively. The
morphological structure of the modified electrode was quite different from the typical graphitic structure of the carbon electrode. The modified electrode exhibited a high density of CNF rods with an average diameter of 40−80 nm and an average length varying from 0.5 to 1.5 μm. Figure 1C represents the cyclic voltammetry (CV) of the electroreduction of the in situ generated diazonium salt. An irreversible cathodic peak is observed at 0.3 V due to the single-electron transfer process, which leads to the removal of the N₂ molecule from the salt and the attachment of the carboxyphenyl moieties to the CNF surface via a covalent bond. It is highly important to monitor the formation of the functionalized layer, as it is well established that the use of multiple CV scans can lead to a progressive buildup of multiple layers, which can have a negative influence on the biosensor construction. As shown in the second CV scan (blue curve, Figure 1C), the reduction peak disappeared because of shielding of the CNF surface with the negatively charged carboxyphenyl moieties. This leads to repulsion of the redox anions from the electrode surface and retardation of the electron transfer. Therefore, only two CV cycles were utilized for the electrografting procedure. It is worth noting that the control of the film thickness and surface coverage is crucial in the diazonium electrografting process and thus, it was extensively studied and optimized in our previous work to ensure proper coverage.34−38

To characterize the surface functionalization of the CNF electrode, XPS was used. The high-resolution C 1s spectra of the bare CNF-modified electrode and the electrodes after the electroreduction of the diazonium salt were recorded. Figure 1D shows a typical XPS C 1s spectra of the CNF-modified electrode. However, the electroreduction leads to the appearance of another peak at 289 eV, which corresponds to the electrografted carboxyphenyl groups. This confirms the successful functionalization of the CNF electrode.

2.2. Characterization of the Various Steps of the Modification of the Electrodes to Construct the MERS-CoV Biosensor. Figure 2A shows a schematic diagram illustrating the process of modifying the screen-printed electrodes to prepare the biosensor. The carbon electrode is first modified with CNF and then functionalized with carboxyphenyl groups, which were then activated using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) chemistry and used for the immobilization of the MERS-CoV spike protein. Figure 2B shows the square-wave voltammetry (SWV) of the electrodes in ferro/ferricyanide redox couple solution. A well-defined single peak was observed for the reduction of the redox couple.
at the different electrodes. The modification of the carbon electrode with CNF leads to a significant increase in the peak current, indicating the increase in the electrochemical surface area of the electrode. On the other hand, the functionalization of the CNF-modified electrode resulted in a dramatic decrease in the peak current, likely due to the shielding of the surface with the aryl groups, as well as the negative charge of the carboxy groups that repels the redox anions. An increase in the peak current was then observed when the spike protein was covalently attached on the surface, presumably due to the neutralization of some of the negatively charged carboxylic groups.

2.3. Design of the Cotton-Coated Electrochemical Biosensor for MERS-CoV.

Prior works have demonstrated the use of a electrochemical biosensor to detect the MERS-CoV spike protein on a gold nanoparticles-modified multi-plexed electrode chip. While this biosensor could perform ultrasensitive measurements, it still requires the use of swabs to collect the nasal and saliva fluids, followed by elution in transport media before applying the solution on the biosensor surface. In this work, we designed a single tool for collection and detection of MERS-CoV by combining the spike protein-modified electrode with cotton fiber. Scheme 1 shows the fabrication of the cotton-coated biosensor and the competitive detection of the MERS-CoV using the SWV displayed on a smartphone. The modified electrodes were blocked with bovine serum albumin (BSA) and dried. As shown in Figure 3A, a piece of sterile cotton was wrapped around the tip of the blocked electrode gently to mimic the function of the Q-tip. This cotton-coated biosensor is then used for swabbing followed by detection after immersing it into the redox solution in a PCR tube. Due to the high absorption characteristic of the cotton, the body fluid collected by swabbing will be absorbed into the cotton fiber and the immersion in the antibody solution will facilitate the flow of the solution to the biosensor surface through capillary action. The detection of the MERS-CoV is based on a competition between the free spike protein on the sample and the immobilized protein on the sensor surface for a fixed concentration of the antibody added. Thus, when the concentration of the virus in the sample is high, it will bind to most of the antibody molecules in the solution and less antibody will be available to bind to the biosensor surface, which will lead to a lower biosensor response. However, a low concentration of the virus will lead to a higher biosensor response. The biosensor response is monitored by measuring the SWV of the cotton-coated immunosensor after binding in a ferro/ferricyanide redox solution.

To explore the feasibility of using the cotton-coated electrochemical biosensor for the detection, it was highly important to first assess to what extent the coating with cotton can affect the measurements as well as the binding. For this purpose, the SWVs of the electrodes were compared with and without the cotton coating (Figure 3B). As shown in the figure, the coating of the bare electrode with cotton did not have any remarkable effect on the SWV current (3% difference). Similarly, the MERS-CoV biosensor (the spike protein-modified electrode) reduction signal did not change after the cotton coating. These results indicate that the cotton fibers were capable of efficiently transporting the redox solution to

Figure 3. (A) Photographic image of the screen-printed electrode and size comparison of the cotton-coated electrode with a standard Q-tip. (B) Comparison between the SWV reduction current of the CNF electrode and the spike antigen-modified electrode with and without the cotton coating. (C) SWV of the spike protein-modified electrode before and after binding with 10 μg·mL⁻¹ of the anti-spike antibody. (D) Comparison of the percentage of change in the SWV current at 0.12 V (the biosensor response) towards the binding with the anti-spike antibody for the electrodes before and after coating with cotton fiber. The measurements were recorded in a 10 mM ferro/ferricyanide solution in PBS buffer, pH 7.4.
the electrode surface and did not impact the diffusion of the redox molecules. Next, we investigated the binding capability of the cotton-coated biosensor in comparison to the uncoated biosensor. To this end, the uncoated and cotton-coated MERS-CoV biosensors were dipped into a solution of the anti-spike antibody. Figure 3C reveals the SWVs of the MERS-CoV immunosensor before and after incubation with 10 μg·mL⁻¹ of the anti-spike antibody. It can be observed that the binding of the antibody to the biosensor surface caused a drastic increase in the peak current. This could be attributed to the positive charge of the antibody that caused attraction of the redox anions to the surface of the electrode, which in turn facilitates the electron transfer. The typical isoelectric point (pI) values for antibodies fall in the range of 8.0–9.3.⁴⁰ Thus, at pH 7.0, the antibody is supposed to carry a net positive charge. Moreover, the conformational change of the antibody–antigen complex may also play a role in the signal-on mechanism of the sensor, as it can give more access to the redox molecules to the surface, enhancing the electron transfer. Tunable signal-off and signal-on electrochemical sensors have been previously reported.⁴¹,⁴²

Figure 3D shows the response (the % of the current change) of the two MERS-CoV biosensors towards the binding to the antibody. The results revealed that the responses of the two biosensors were almost similar. It is worth mentioning that the amount of cotton fibers was optimized to ensure enough coverage. More layers of cotton fiber would need longer time for the diffusion of the solutions to the electrode surface during the incubation and measurements. Thus, 30 mg of cotton was used.

Considering that there was no significant influence of the cotton on the electrochemical measurements or the biosensor response signal, this biosensor can be used to accomplish a double role in collecting the fluid sample as well as detecting the electrochemical signal. It can be considered a much simpler and easier detection method, which reduces the handlining and pipetting steps, making it more suitable for point-of-care diagnosis.

2.4. Detection of MERS-CoV Spike Protein on the Cotton-Coated Biosensor. To assess the analytical range of the biosensor, it was used to detect different concentrations of the spike protein, ranging from 0.1 pg·mL⁻¹ to 1 μg·mL⁻¹. A concentration of 10 μg·mL⁻¹ of the anti-spike antibody solution was used in all of the experiments. Figure 4A exhibits the SWVs of the biosensor at various concentrations of the spike protein mixed with the antibody. A gradual change in the SWV reduction current was observed at different concentrations. The calibration curve of the MERS-CoV biosensor was drawn by plotting the response of the biosensor (the % of

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Figure 4. (A) SWVs on the MERS-CoV biosensor and the biosensor after binding with 10 μg·mL⁻¹ spike antibody mixed with varying concentrations of the spike protein. (B) Calibration curve of the MERS-CoV biosensor (the biosensor response signal ((I – I°)/I°) was plotted against the logarithm of the MERS-CoV spike protein concentration). The error bars represent the standard deviations of the measurements (N = 3). The measurements were recorded in a 10 mM ferro/ferricyanide solution in PBS buffer, pH 7.4.

Figure 5. (A) SWVs of the MERS-CoV biosensor and the biosensor after binding with 1 μg·mL⁻¹ spike antibody mixed with zero concentration of virus (blank sample), and 1 ng·mL⁻¹ of Influenza A, HCoV or MERS-CoV proteins. (B) Response signal of the MERS-CoV biosensor to the binding with 1 ng·mL⁻¹ of MERS-CoV spike protein, Influenza A, and HCoV. The error bars represent the standard deviation of the measurements (N = 3). The measurements were recorded in 10 mM of the ferro/ferricyanide solution in PBS buffer, pH 7.4.
change in the current) versus the logarithm of the spike protein concentration (Figure 4B). A linear response was observed within the concentration range of 0.1 pg·mL⁻¹ to 1 μg·mL⁻¹ with a linear regression equation of \((i - i°)/i°% = 447.8 + -124.8 \log C (\text{ng·mL}^{-1}), R = 0.992,\) and a limit of detection (LOD) of 0.07 pg·mL⁻¹. The limit of detection was calculated as the concentration that corresponds to three times the average response of the blank signal. This very low LOD is an indicator of the high sensitivity of the cotton-coated MERS-CoV biosensor. It is worth noting that this LOD is much less than ELISA, which has a LOD of 1 ng·mL⁻¹, as well as the previously reported electrochemical biosensors using gold nanoparticles-modified electrodes. Triplicate measurements were done for each experiment and the relative standard deviations (RSD %) were calculated. All of the experiments showed an RSD % from 2.7 to 6.6%, demonstrating the excellent reproducibility of the biosensor.

It was important to assess the stability of the immunosensor. Therefore, the biosensor was kept in the fridge for 7 days and then used for the detection. There was no significant change (less than 2%) of the biosensor response after storage for a week, indicating the very good stability of the sensors.

2.5. Evaluating the Cross-Reactivity of the MERS-CoV Biosensor. Cross-reactivity experiments were carried out to explore any possible cross-reaction of the biosensor with different viruses. HCoV and Influenza A proteins were tested after mixing with the MERS-CoV spike antibody, and the experiments were performed as described previously. Figure 5A shows the change in SWVs of the MERS-CoV biosensor after binding with the same concentrations of MERS-CoV spike protein, Influenza A, and HCoV and without adding any virus (blank sample). A significant difference between the biosensor response towards the MERS-CoV spike protein and the other nonspecific proteins was observed, implying the ability of the sensor to discriminate between the target and other proteins. Thus, it can be concluded that the MERS-CoV biosensor did not cross-react with the nonspecific proteins, indicating the excellent selectivity of the biosensor.

2.6. Testing of the MER-CoV Biosensor in Spiked Samples. To explore the practicality of using the cotton-coated sensors for the determination of MERS-CoV in real samples, they were tested in spiked nasal fluid. In this study, the cotton-coated biosensor was employed to swab and collect the nasal sample from a healthy person (PCR-negative sample). Then, the biosensor was incubated in the spike antibody solution, and the detection was realized as described in the Experimental Section after washing the biosensor and immersion in the redox solution. The samples were spiked with 0.001 and 100 ng·mL⁻¹ of the spike protein. The results showed excellent recovery percentages of 96 and 91.5%, respectively. The RSD % of the measurements was between 4.1 and 6.2%. These results demonstrate the potential applicability of the cotton-coated biosensor for the sensitive and accurate detection of the MERS-CoV spike protein using a single tool.

3. CONCLUSIONS

We present a miniaturized platform for the detection of MERS-CoV by combining an electrochemical biosensor with cotton fiber to collect and detect the virus using a single tool. The electrochemical biosensor was fabricated on a carbon nanofiber-modified screen-printed electrode. The integration of the electrodes with the cotton was investigated, exhibiting excellent performance. The carbon nanofiber electrodes were functionalized with carboxyphenyl groups, which were used to immobilize the MERS-CoV spike protein. A competitive assay format was employed for the detection, and the biosensor response was determined using square-wave voltammetry. The cotton-coated MERS-CoV biosensor showed excellent performance in terms of sensitivity, accuracy, reproducivity, and selectivity. High recovery percentages were obtained when the biosensor was tested in spiked nasal samples, revealing the feasibility of applying the biosensor in real samples. Thus, the simplicity, low cost, portability, simplicity, reduced pipetting steps, and high sensitivity of this immunosensor make it a promising tool for the point-of-care testing of MERS-CoV.

4. EXPERIMENTAL SECTION

4.1. Reagents and Materials. The antigen 725 Spike Protein S1 for MERS-CoV and its antibody were obtained from Sino Biological (Beijing, China). The protein N1H1 No. J8034 for Influenza A was obtained from BiosPacific (California). The protein HK41N for HCoV virus was purchased from Medix Biochemica (Finland). Sodium nitrite, potassium ferricyanide (K₃Fe(CN)₆), bovine serum albumin (BSA), potassium ferrocyanide (K₄Fe(CN)₆), hydrochloric acid, 4-aminobenzoic acid, and phosphate-buffered saline (PBS) were purchased from Sigma (Ontario, Canada). PCR tubes, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were obtained from Fisher Scientific (Ontario, Canada). sterile cotton balls were purchased from a local pharmacy. The preparation of the solutions of the virus antigens, antibody, and BSA was done in 1x PBS buffer, pH 7.4. The EDC/NHS solution for the activation step was prepared in 1x PBS buffer, pH 5.5. The powder of carbon nanofibers (CNF) was purchased from Metrom DropSens, Inc. (Asturias, Spain). The CNF dispersion was done by sonicating the CNF powder in DMF for 30 min (1 mg) until a homogeneous mixture was observed. Milli-Q water was used in the preparation of all of the solutions throughout this study.

4.2. Equipment. 4.2.1. Autolab Potentiostat, Model: PGSTAT302N (from Metrohm, Switzerland) Was Utilized to Carry out the Electrochemical Measurements [Square-Wave (SWV) and Cyclic (CV) Voltammetry]. The disposable screen-printed carbon electrodes utilized to fabricate the biosensor were obtained from BioDevice Technology (Nomri, Japan). The electrodes (PCR P01) are adapted to fit into PCR tubes (30 × 4 × 0.3 mm³). Three electrode configurations were used: a central silver/silver chloride (Ag/AgCl) reference electrode and rectangle-shaped carbon working and counter electrodes. The electrode end could be attached to the potentiostat for the measurements via a special connector purchased from BioDevice Technology. The final measurements were tested using a Sensit smart potentistat obtained from PalmSens (Netherlands) that was inserted directly into a smartphone and controlled through the Android application PStouch. The scanning electron microscopy (SEM) images for the electrodes were acquired at a magnification of 50 000× using an acceleration voltage of 3 kV and a working distance of 2.1 mm.

4.3. Methods. 4.3.1. Fabrication of Functionalized Carbon Nanoﬁber Electrodes. First the rectangular carbon working zone of the screen-printed electrodes was coated with the carbon nanoﬁber by drop casting 0.5 μL of the 1 mg·mL⁻¹ CNF dispersion. Different amounts of CNF (0.5, 1, and 2 mg mL⁻¹) were used, and the results of the voltametric signal
showed better electrochemical performance for the electrodes coated with 1 mg·mL\(^{-1}\) of CNF dispersion. A low concentration of CNF can lead to insufficient coverage, while a very high concentration can lead to aggregation of the fiber rods and inhomogeneity of the surface. Thus, 1 mg·mL\(^{-1}\) was used for all the experiments. The electrodes were kept overnight to dry at room temperature and then rinsed with distilled water to get rid of any excess CNF residues. The morphology of the CNF-coated surface was characterized via SEM. The CNF-modified electrodes were then attached to the potentiostat and placed into a PCR tube containing the diazonium mixture (2 mM of sodium nitrite mixed with 100 \(\mu\)L of 2 mM 4-aminobenzoic acid in 0.5 M HCl solution), which was pre-stirred for 10 min to form the diazonium salt. Electrografting of the carboxyphenyl groups was then performed using 2 CV cycles from +0.1 to −0.7 V at a scan rate of 50 mV·s\(^{-1}\) as reported previously.\(^5\) The success of the functionalization step of the CNF electrodes was confirmed using X-ray photoelectron spectroscopy (XPS) measurements.

4.3.2. Attachment of the MERS-CoV Spike Protein on the Functionalized CNF Electrodes. After the modification of the electrodes with carboxyphenyl groups, the extreme carboxylic groups on the surfaces were activated using 100 mM EDC and 20 mM NHS in PBS buffer, pH 5.5. The electrodes were incubated in the activation mixture at room temperature for 1 h, then rinsed with PBS buffer, pH 7.4. Then, 10 \(\mu\)g·mL\(^{-1}\) of the spike protein solution was then added to the activated electrode surface and kept for 3 h in a water-saturated atmosphere. Following this, the spike protein-modified electrodes were washed and blocked with 0.1% BSA solution for 30 min to fill the available sites on the sensor surface.

4.3.3. Fabrication of the Cotton-Coated Electrochemical Biosensor for MERS-CoV. To prepare the sensor, a piece of sterile cotton fiber (around 30 mg) was wrapped gently around the narrower end of the screen-printed electrode (the area that contains the spike antigen-modified electrode). The sterile cotton-tipped biosensor is now ready to be used for swabbing. It can be also stored dry at 4 °C until the next use.

4.3.4. Application of the MERS-CoV Immunosensor for the Detection of Spike Protein. The cotton-coated immunosensors were used to detect different concentrations of the spike protein in buffer. First, different concentrations of the spike protein solution (from 0.1 pg·mL\(^{-1}\) to 1 \(\mu\)g·mL\(^{-1}\)) were mixed with 10 \(\mu\)g·mL\(^{-1}\) of spike protein antibody solution in PBS buffer. The biosensor was then immersed into the mixture solutions in PCR tubes and incubated at room temperature for 20 min for binding. After that, the biosensor was washed by immersing it into a tube containing 500 \(\mu\)L of PBS buffer, pH 7.4, for 3 times. The MERS-CoV biosensor was then immersed in 100 \(\mu\)L of the 10 mM redox couple solution (ferro/ferrocyanide) in PBS buffer, pH 7.4, in a PCR tube, and the electrochemical measurements were carried out. The detection was realized by measuring the difference between the SWV reduction current of the blank biosensor (before any incubation) and the biosensor after incubation with the antibody–antigen mixture. The biosensor response is calculated as \((i) − (i^*)/i^*\%), where \(i^*\) represents the peak current of the blank sensor and \(i\) is the peak current of the biosensor after binding.

4.3.5. Voltammetric Measurements. The diazonium electrografting was performed using two consecutive CVs at a scan rate of 50 mV·s\(^{-1}\) from +0.1 to −0.7 V. The measurements of the SWV were carried out in 10 mM 1:1 ferro/ferrocyanide redox couple solution in PBS buffer, pH 7.4, from 0.3 to −0.5 V. The SWV measurements were recorded using a frequency of 25 Hz, a step potential of −5 mV, and an amplitude of 20 mV. The SWV signals were baseline-corrected for better presentation of the results.

4.3.6. Selectivity Study of the Biosensor for MERS-CoV against Other Viruses. To investigate the cross-reactivity of the biosensor with other viruses, the immunosensors were incubated with different mixtures of spike MERS-CoV antibody (10 \(\mu\)g·mL\(^{-1}\)) with either MERS-CoV spike S1 protein, HCoV or Flu A proteins. The biosensors were then washed as explained above and SWV measurements were recorded. The biosensor response was then calculated in each case and compared.

4.3.7. Biosensor Application in Spiked Nasal Fluid with MERS-CoV S Protein. A nasopharyngeal sample was collected from healthy individuals (PCR negative) using the cotton-coated biosensor. The biosensor was then dipped into a solution of the anti-spike antibody (10 \(\mu\)g·mL\(^{-1}\)) spiked with different concentrations (1 pg·mL\(^{-1}\) and 100 ng·mL\(^{-1}\)) of the spike antigen for 20 min. The biosensor is then washed in PBS buffer, pH 7.4, and then immersed into the measuring redox solution for the SWV measurements using the parameters described above.

Author Contributions

S.E. and M.Z. conceived the idea of this work. S.E. planned the work, designed and performed the experiments, and wrote the manuscript. All authors reviewed the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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