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ABSTRACT

This study investigated a SARS-CoV-2 virus detection mechanism using dry-stored disposable strips. The accuracy of this sensing platform is as good as polymerase chain reaction (PCR) with a detection time of fewer than 30 s. SARS-CoV-2 antibodies were biofunctionalized on disposable strips similar to glucose detection strips to detect the presence and concentrations of SARS-CoV-2 in saliva samples. Eight 1 ms electric pulses were sent through the sensor strip with a saliva sample in its microfluidic chamber. A circuit board embedded with MOSFET was also employed to amplify the detected signals and convert the signal to digital readings displayed on an LCD screen. The COV-antibody functionalized disposable strips were stored in a dry condition for at least one day before analyzing clinical human saliva samples with known cycling threshold (Ct) values confirmed with conventional PCR tests. Results demonstrate our system is capable of showing qualitative positive or negative results within 30 s and providing quantitative SARS-CoV-2 concentrations in terms of Ct values in 5 min.

I. INTRODUCTION

The global pandemic caused by the SARS-CoV-2 virus has claimed the lives of 6.3×10^6 people worldwide and resulted in widespread economic and social chaos. The mutation of different variants of the virus is an ongoing process and makes treating the disease more and more challenging. Variants of Omicron with the lineages BA.2, BA.4, and BA.5 are currently the most popular ones. According to the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO), 300×10^6 confirmed cases have been recorded since the Omicron variant emerged in late 2021, contributing to half of all cases accumulated in history. To address skyrocketing infection rates and costly mitigation policies, such as vaccinations, quarantines, and routine testing, rapid, accurate, and cost-effective detection solutions are needed to reduce the burden on federal and state agencies.

During the global pandemic, various virus detection methods have been developed for clinical use. The most commonly used are polymerase chain reaction (PCR) and lateral flow antigen tester.[1] Although PCR can provide quantitative results with high specificity and sensitivity, the required processing time and the need for high-end lab equipment restrained this method from being applied to meet the rapid, daily, point-of-care demands.[2,3] In contrast, lateral flow testers provide simple binary results with fast detection time from cost-effective commercial kits. However, due to the fact that a clear safe threshold of virus concentration has not been firmly established,[4] the low limit of detection feature of the lateral flow testers increases the risk of being exposed to the asymptomatic but contagious patients, and largely raises the danger to the public.

To design a new procedure and overcome the disadvantages of both PCR and lateral flow testers, BioFET technology is ahead in the development of rapid detection of the virus with precision.

Replacing the traditional external signal delivered to the gate electrode by probes of biomolecules allowed for variation in the virus concentration, which could then be amplified. This created a system with an extra-low response time, a miniature design, and a low limit of detection.[5] Seo *et al.* designed a graphene-based BioFET, using PBASE (1-pyrenebutyric acid *N*-hydroxysuccinimide ester) as the functionalization group and achieved real-time results along with a limit of detection of 16 pfu/ml on the cultured virus and 2.42×10^2 copies/ml on clinical samples.[6] Other liquid probes of functionalization have also been implemented as portable, low background noise, and PCR-comparable diagnostic systems.[7–9] However, the fact that the BioFET device has to be replaced after each test casts doubt on the reusability of the device, making the cost of replacement prohibitive, and minimizing the flexibility of the device in detecting various microorganisms and its variants.

In our previous work, an alternative idea was proposed. The separation of the detection mechanism and the amplification from the technology of BioFET was achieved by utilizing disposable testing strips as an extension of the functionalized biomolecule probe. An inexpensive, rapid, reconfigurable, and high-sensitivity detection platform was developed with a printed circuit board (PCB).[10] Furthermore, to eliminate the undesirable charge accumulation phenomenon and screening effect, a double-pulse gating method was also implemented in the system.[11] Detection methods of several common targets such as the Zika virus, cerebrospinal fluid, and cardiac troponin I protein have all been realized with the same approach.[12–14] The goal of this study was to examine the possibility of storing the disposable sensor strips under dry and room temperature conditions. Selected clinical human saliva samples were tested for a relatively long time period to evaluate their performance after a moderate storage time.

II. EXPERIMENT

Inspired by the commercially available glucose testing strips, disposable strips printed with carbon electrodes were designed in this study. An illustration of the example strip is shown in Fig. 1(a). At the tip of the strip, a microfluidic channel was opened and the surface of an exposed electrode was plated with gold particles, followed by the bio-functionalization of the SARS-CoV-2 antibodies. The functionalization process involved preparing a 10 mM thioglycolic acid (TGA) solution in de-ionized water, which was then applied to the microfluidic channel. On the surface, the gold particles combined with the thiol functional groups of TGA and created Au–S bonds, which stand as the pillars of the following attached antibodies. The sensor strips were rinsed with de-ionized water and

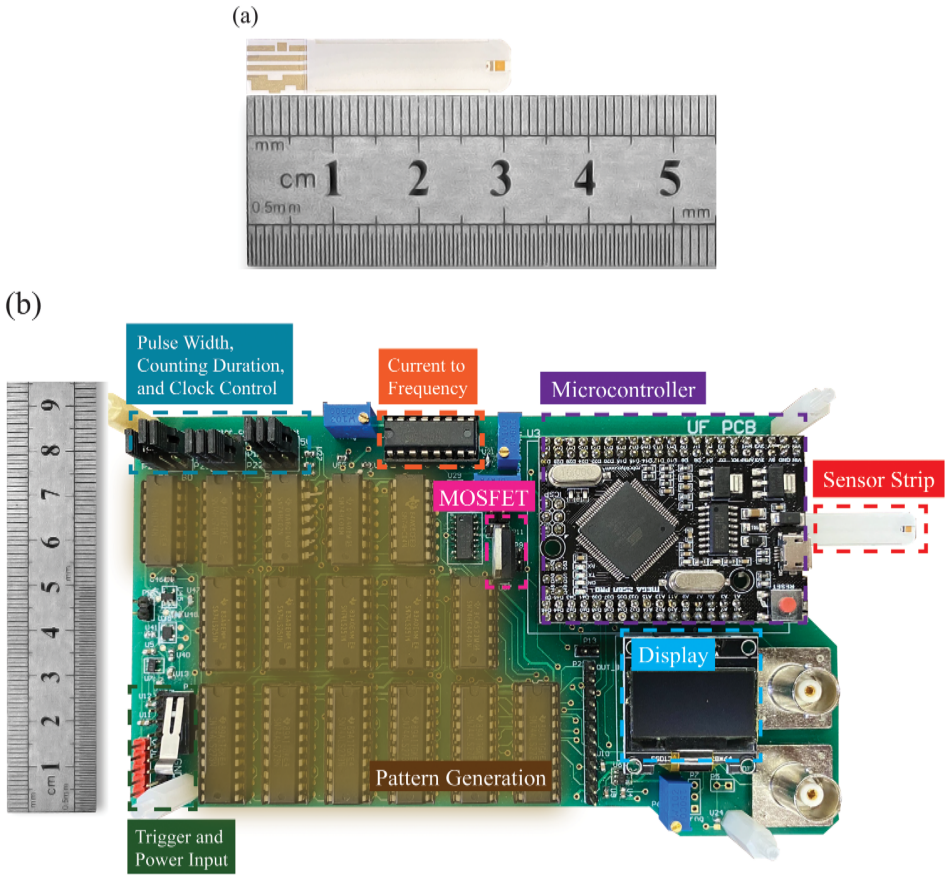

FIG. 1. Schematic and photograph of the (a) testing strip and (b) testing platform including a PCB board and a test strip inserted on to the board.

dried with nitrogen after 2 h of reaction time. Subsequently, 0.1 mM *N*,*N*′-dicyclohexylcarbodiimide (DCC) was made in 25% acetonitrile and 75% de-ionized water and applied for 1 h. Another hour of 0.1 mM *N*-hydroxysuccinimide (NHS) in de-ionized water was soaked afterward. De-ionized water, isopropyl alcohol, and nitrogen gas were then applied in sequence to remove any residual liquid in the microfluidic channel. In the next step, 20 μg/ml of anti-SARS-CoV-2 spike glycoprotein RBD antibody 5g8 (Abcam, Waltham, MA) prepared in 1% phosphate-buffered saline (PBS) solution was injected into the channel, followed by 18 h of incubation under 4 °C. These completely functionalized strips were rinsed again with the 1% PBS solution, blow-dried with nitrogen, and stored in sealed test strip containers at room temperature.

In addition to the testing strips, a PCB shown in Fig. 1(b) was also developed in our previous study[10] with the ability to perform a detection method using the functionalized disposable strips. Two synchronous patterns, one with the voltage of 1.5 V pulsed to the electrode functionalized by antibodies and the other with the voltage of 5 V pulsed to the drain electrode of the embedded silicon MOSFET STP200N3LL (STMicroelectronics, Geneva, Switzerland) on the PCB, were formed. The variation of the induced charges in the microfluidic channel then outputs to the gate electrode and is amplified by the MOSFET. The analog output waveform from the drain electrode of the transistor corresponds to the real-time concentration of the SARS-CoV-2 virus captured by the functionalized antibodies on the microfluidic channel, which was then integrated with an Arduino microchip. Ten pulses with two-second time gaps in between were sent upon manually triggering the device. The results were then averaged and displayed on the onboard LCD with four-digit digital readings.

Sixteen (16) clinical human saliva samples were previously tested using the gold standard PCR test. Eight (8) samples were positive for SARS-CoV-2 with PCR Ct values ranging from 25 to 36 and the remaining eight were negative for SARS-CoV-2 with PCR Ct values over 45. Ct values refer to the number of duplications or cycles needed to produce the detectable amount of RNA in PCR tests. These saliva samples were tested using our system. Functionalized testing strips were stored in a dry condition at room temperature for at least one day before the experiment. On the PCB platform, the pulses inputted to the strips were triggered continuously within 5 min. The average of the resultant digital readings was recorded with an interval of 30 s.

III. RESULTS AND DISCUSSION

The ability of our strip-based sensor technology to detect SARS-CoV-2 was reported previously. For these previous studies, the microfluidic channel on the strips with the functionalized electrodes was filled with PBS for storage. The sensitivity and performance of these strips were comparable with the current gold standard PCR test.[15] In the microfluidic channel of the sensor strip, S-proteins, which are part of the SARS-CoV-2 structure, of the tested sample were captured by antibodies on the surface of the functionalized electrode. With higher virus concentrations, more virus-antibody bonds could be found on the surface. By applying an electrical pulse to the functionalized electrode, the antibody, as well virus, will be moved as a double spring due to the charges on the antibody and virus. The gate electrode of the MOSFET is connected to the unfunctionalized electrode on the sensor strip. These charge changes on the functionalized electrode and leads to charge changes on the gate electrode of the MOSFET. This induced charge change is amplified with the operation of the MOSFET. The output voltage of the MOSFET amplifier is connected to a voltage-controlled oscillator (VCO) to convert the detected analog signal into a digital signal and exhibit a digital display. In our circuit board design, higher digital numbers in the display indicate fewer detected virus concentrations.

Besides the virus concentration, the configurations of the antibody-virus complex also play a significant role in the resulting process. For the dry-stored strips, antibody molecules are flattened on the functionalized electrode. These flattened antibodies require some time to swell into 3D structures after the strips are exposed to the liquid samples, as shown in Fig. 2(a). This figure illustrates the 3D structure of a functionalized electrode where the antibody-virus complexes lie flat on the surface of the electrode. The second figure illustrates the antibody-virus complexes after the strip is exposed to the human saliva sample. This configuration change is expected to occur if the strips are stored in a dry condition. Since the distance between the antibody-virus complex and the electrode are closer in this situation, the charges on the antibody-virus complex are closer to the electrode. Thus, the induced charges on the gate of the MOSFET would be higher, and the corresponding digital output of the circuit board would be similar to the sample for the higher virus load. After the strips are exposed to the saliva sample for 4–5 min, as shown in Fig. 2(b), the antibody-antigen complexes rise gradually due to buoyancy, which leads to the complexes becoming normal 3D steady-state configuration. During this dynamic configuration change of the complexes, the distance of the charges on the complexes would change correspondingly. The COV virus concentration detection would vary during this period. A steady-state level will eventually be reached once the 3D configuration of the antibody-virus complex stops changing. This steady-state level is reached after about 5 min of strip exposure to the test samples. The digital readings obtained after 5 min represent the real virus concentration.

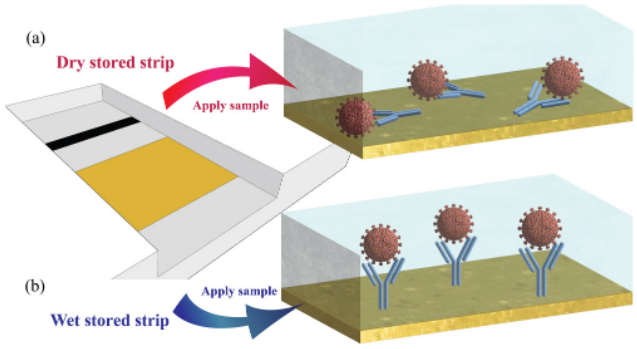

FIG. 2. Illustration of the surface of the functionalized electrode under (a) dry and (b) wet storage conditions.

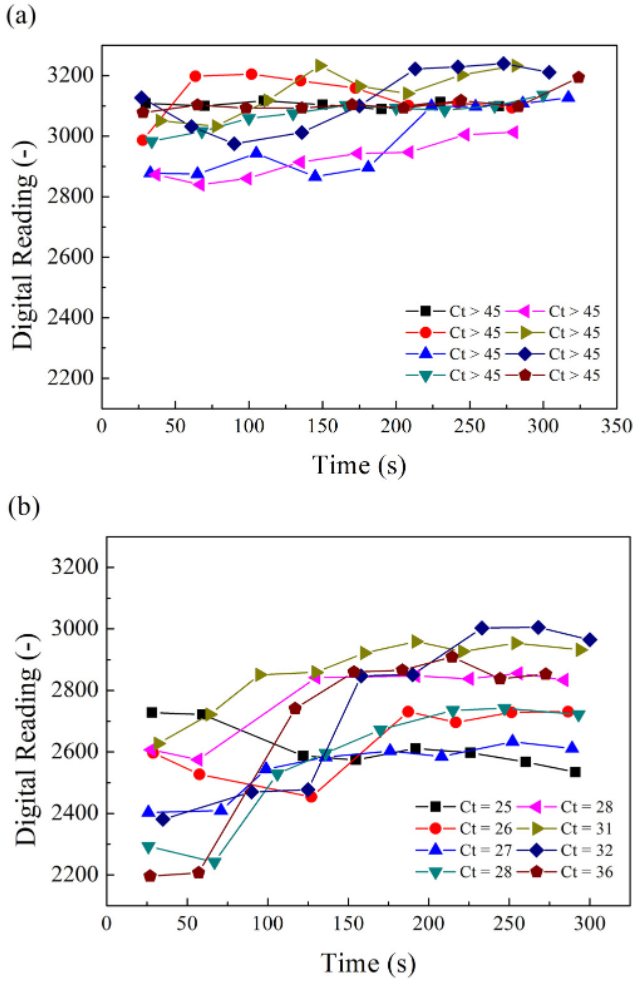

FIG. 3. Correlation of digital readings and time after applying (a) negative and (b) positive clinical human saliva samples with various SARS-CoV-2 virus concentrations and Ct values.

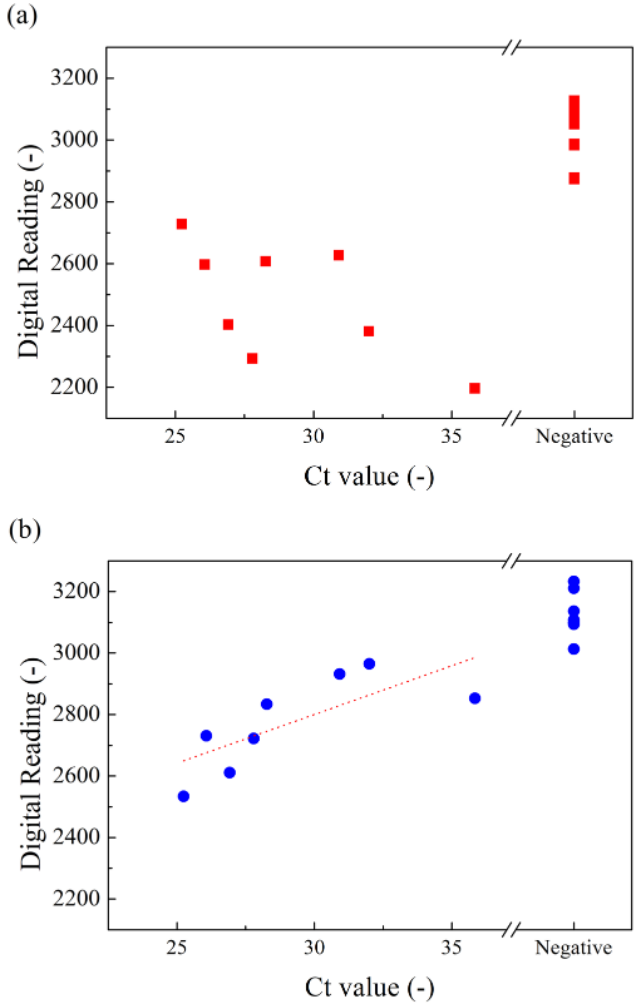

FIG. 4. Correlation of digital readings and Ct values of the testing results after (a) 30 s and (b) 5 min.

To further validate this phenomenon, the correlation between the digital readings and time after applying negative [Fig. 3(a)] or positive [Fig. 3(b)] clinical saliva samples with various Ct values (confirmed with RT-PCR) were plotted. For the samples with negative SARS-CoV-2 PCR test results, the digital readings demonstrated no significant changes or trends, even after prolonged exposure of the strips to the saliva sample. This indicates that no virus was captured by the antibody on the surface of the electrodes. However, the samples with positive PCR results (Ct value <35) had clear trends of rising digital readings following the longer testing times. The digital readings of all eight positive clinical samples reach a steady state in 5 min.

Although the precise quantitative virus concentrations could not be detected within the first minute of testing for the dry storage testing strips, qualitative results could still be obtained at first glance. Figures 4(a) and 4(b) illustrate the digital output readings of the first and last measurements within the 5 min testing timespan, respectively. In the first 30 s, we can already distinguish the positive clinical samples from the negative ones with 2800 digital readings as the threshold. Given a longer time for the reaction to saturate, a linear fitting regression line could be established, which shares the same conclusion with our previous study of wet stored testing strips.[15]

IV. CONCLUSIONS

The surge and continued transmission of SARS-CoV-2 wreak havoc on global economies. The current approach to finding viral coexistence leads to a growing need for new detection methods. The results of this study demonstrate the versatility of our detection

system. For the average person, qualitative results can be obtained rapidly in real-time, much faster than the popular lateral flow testers. For clinical applications, quantitative results can be obtained with precise sensitivity, comparable with PCR tests within a reasonable timeframe. The elucidation of the mechanism behind the antibody-COV virus complex orientation also proves the feasibility of the dry storage condition of our disposable sensor strips and marks a leap forward toward the commercialization of this technology.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Chao-Ching Chiang: Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Writing – original draft (equal). **Chan-Wen Chiu:** Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal). **Fan Ren:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Writing – original draft (equal); Writing – review & editing (equal). **Cheng-Tse Tsai:** Data curation (equal); Formal analysis (equal); Investigation (equal). **Yu-Te Liao:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal). **Josephine F. Esquivel-Upshaw:** Conceptualization (equal); Funding acquisition (equal); Supervision (equal); Writing – review & editing (equal). **Stephen J. Pearton:** Conceptualization (equal); Supervision (equal); Writing – original draft (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available within the article.

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