Electrochemical Detection of a Few Copies of Unamplified SARS-CoV-2 Nucleic Acids by a Self-Actuated Molecular System

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ABSTRACT: The existing electrochemical biosensors lack controllable and intelligent merit to modulate the sensing process upon external stimulus, leading to challenges in analyzing a few copies of biomarkers in unamplified samples. Here, we present a self-actuated molecular-electrochemical system that consists of a tentacle and a trunk modification on a graphene microelectrode. The tentacle that contains a probe and an electrochemical label keeps an upright orientation, which increases recognition efficiency while decreasing the pseudosignal. Once the nucleic acids are recognized, the tentacles nearby along with the labels are spontaneously actuated downward, generating electrochemical responses under square wave voltammetry. Thus, it detects unamplified SARS-CoV-2 RNAs within 1 min down to 4 copies in 80 μL, 2−6 orders of magnitude lower than those of other electrochemical assays. Double-blind testing and 10-in-1 pooled testing of nasopharyngeal samples yield high overall agreement with reverse transcription-polymerase chain reaction results. We fabricate a portable prototype based on this system, showing great potential for future applications.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) has infected over 515 million people including 6,255,965 deaths through the middle of 2022. Multiple variants have emerged, such as Beta, Delta, and Omicron.1−4 Nucleic acid testing by a reverse transcription-polymerase chain reaction (RT-PCR) is acknowledged as the golden standard to diagnose COVID-19 as well as other infectious diseases such as Zika and Ebola.5−8 However, it requires time-consuming nucleic acid extraction and amplification processes performed in specialized laboratories.9−14 To curb the virus outspreading, it is necessary to simplify the testing procedure while guaranteeing accuracy.15−18 Until now, various technologies have been developed to solve the above issues. Among these technologies, electrochemical biosensing, especially electrochemical biosensing based on microelectrodes, has been widely studied because of its rapid response, high current density, simple operation, low cost, and portability.19,20 These advantages make the electrochemical biosensor a great candidate for nucleic acid testing.21−24

The electrochemical sensors face a long-standing challenge to ultrasensitively detect biomarkers at a level of several copies.25−27 It is attributed to the uncontrollability in the sensing operation as follows. First, the missing acting force orients the probes upward making the probes prone to entangle or adsorb to the electrode surface, which inactivates the probes and decreases the biorecognition efficiency. Second, there exist possibilities that the electrochemical labels move close to the electrode, leading to pseudosignal and low accuracy when detecting trace nucleic acids. Third, the sensing process lacks spontaneity upon biorecognition; thus, the signal transduction has low efficiency and the signals are easily drowned out by the background noises.19,28 At present, due to their insufficient sensitivity, nucleic acid testing by electrochemistry relies on amplification of genetic species. After amplification, the limit of detection (LoD) is 10−15−10−18 M, approximately 60−60000 copies in 80 μL of testing solution.29−32 When testing unamplified SARS-CoV-2 RNAs, the lowest LoD is only 147 to 552 copies in 80 μL of testing solution.33−35

Organisms on the earth have undergone a long-term evolutionary process, which has thus been the source of all kinds of technical ideas, engineering principles, and great inventions.36−41 Hydras can keep their tentacles upright afloat voluntarily, featuring efficient responses and accurate self-motion when recognizing external stimuli.42−44 Here, we demonstrate a self-actuated molecular-electrochemical system (MECS) modified on a graphene microelectrode. MECS consists of a tentacle and a trunk constructed by DNA nanostructure. The probe and electrochemical label on the
tentacle maintain an upright orientation, which improves biorecognition and avoids pseudosignal. After recognizing targeted nucleic acids, the configuration of the tentacles nearby changes spontaneously, pushing the electrochemical labels downward. The configuration change can be further modulated by electrical excitations of square wave voltammetry (SWV) or differential pulse voltammetry (DPV). Owing to the self-actuated sensing process at the molecular scale, MECSs overcome the aforementioned limitations, enabling electrochemical detection of SARS-CoV-2 in vitro transcribed (IVT) RNA down to $\sim 80$ zM ($1 \text{ zM} = 10^{-21} \text{ M}$), equivalent to four copies in 80 $\mu$L of saliva. Without nucleic acid amplification, double-blind testing and 10-in-1 pooled testing of clinical samples are performed within 1 min and exhibit high consistency with RT-PCR results. Finally, a portable MECS-based prototype is developed for point-of-care SARS-CoV-2 testing.

### RESULTS AND DISCUSSION

**MECS and MECS-Modified Graphene Microelectrodes.** As shown in Figure 1a, the hydra consists of a tentacle and an upstanding trunk. Inspired by the hydra, the trunk of MECS as a prop unit is a regular tetrahedral structure with four double-stranded DNAs (ds-DNA) that intertwine to form edges. A flexible single-stranded DNA (ss-DNA) extends from the top vertex of the tetrahedral trunk as the tentacle. The tentacle contains a probe targeting ORF1ab genes of SARS-CoV-2 RNA as a recognition unit, 5 thymine nucleotides as swivel bearing for free-movement of tentacle after target recognition, and an electrochemical label of methylene blue as signal generator at the tip of tentacle (Table S1). In the testing, a negative potential was applied on the graphene microelectrode. Owing to negative charges on the ss-DNA backbones, the tentacle remains upright spontaneously in the local electrical field on the graphene surface (Figure 1b). Once nucleic acids are recognized, the steric effect as well as the electrostatic force generated by the negative charges of the targets actuate the tentacle downward to the electrode surface automatically. The bottom of the trunk carries the amino groups to imitate the basal disk, which are used as a modification unit to link to 1-pyrenebutanoic acid succinimidyl ester (PASE) on the working electrode of graphene electrode (Figure 1c and Figures S1 and S2). Finally, a polydimethylsiloxane (PDMS) chamber is attached to the electrodes to hold the testing solution.

As shown in an atomic force microscope (AFM) image measured in fluids, MECSs maintain their three-dimensional structures and are uniformly anchored on the graphene surface (Figure 1d). The theoretic height of a trunk is around 5.3 nm, which approximates the theoretic length of the tentacle (6.8 nm). MECS consists of a tentacle and a trunk with a height around 12 nm in AFM, which is similar to the designed structure. X-ray photoelectron spectroscopy (XPS) and Raman spectra also confirm that the graphene was functionalized with PASE and modified with MECS. Figure 1e and Figure S3a show the XPS of the pristine graphene, PASE-treated graphene, and MECS/PASE-modified graphene. The increased intensity of the P 2p peak indicates that the MECS is successfully modified on the surface of PASE-treated graphene.

As shown in Figure 1f, Figure S3b, and Figure S3c, the Raman spectra of pristine graphene (red line) and PASE-treated graphene (blue line). Fluorescence intensity images of the graphene immobilized with MECS before (g) and after (h) addition of SARS-CoV-2 IVT RNA, respectively.
Cyclic voltammetry (CV, Figure S5) is performed by using graphene microelectrodes with different modifications. The pristine graphene microelectrode has a peak current of $8.87 \times 10^{-7}$ A, which is more significant than others. This result indicates the successful modification of MECSs and a dominant edge effect on the microelectrode. To examine the spatial configuration change of the tentacle after capturing pristine graphene and PASE-treated graphene surfaces show two prominent peaks (the G and 2D peaks). After PASE treatment, the D peak and D′ peak obviously appeared due to the relative resonance of sp³ bonding, orbital hybridization, and pyrene group binding on the surface of pristine graphene. After PASE treatment, the average roughness of graphene obtained by AFM increases from 0.231 to 0.483 nm (Figure S4).

Cyclic voltammetry (CV, Figure S5) is performed by using graphene microelectrodes with different modifications. The pristine graphene microelectrode has a peak current of $8.87 \times 10^{-7}$ A, which is more significant than others. This result indicates the successful modification of MECSs and a dominant edge effect on the microelectrode. To examine the spatial configuration change of the tentacle after capturing pristine graphene microelectrode, a fluorescent dye cyanine3 (Cy3), instead of methylene blue, is conjugated at the top of the SARS-CoV-2 RNA sample is added, the redox current as the baseline over operation and over time (Figure S7). After the SARS-CoV-2 RNA sample is added, the redox current as the electrochemical response appears in the DPV and SWV curves. Over the next 30 min, the electrochemical response also remains almost unchanged. The results show that SARS-CoV-2 RNA leads to an electrochemical response, not the disturbance of the adding sample procedure. As shown in Figure 2a,b, the concentration of SARS-CoV-2 IVT RNA increased from 0.05 to 500 copies μL⁻¹, while the peak current of electrochemical response enhanced to approximately 3.5 $\times$ 10⁻⁷ A and 1.8 $\times$ 10⁻⁷ A using SWV and DPV, respectively. The testing is repeated three times to investigate the relationship between the concentration of SARS-CoV-2 IVT RNA and normalized electrochemical current of DPV and SWV. The normalized current signal yielded by both SWV and DPV is plotted as a function of SARS-CoV-2 RNA concentration on a logarithm scale in Figure 2c, showing linear correlations that can be described as

$$I_{DPV} = 0.244 \log C_{RNA} + 0.883$$  \hspace{1cm} (1)

$$I_{SWV} = 0.52 \log C_{RNA} + 0.223$$  \hspace{1cm} (2)

where $C_{RNA}$ (copies μL⁻¹) is the concentration of SARS-CoV-2 IVT RNA. $I_{SWV}$ and $I_{DPV}$ ($10^{-7}$ A) represent the normalized electrochemical current response on the sensors measured by SWV and DPV, respectively. The LoD of SARS-CoV-2 IVT RNA testing was estimated as 0.035 and 0.025 copies μL⁻¹ for DPV and SWV measurements, respectively, with 3δ/slope calculation using the dose-dependent fitting curve. The results showed that SWV on MECS had higher sensitivity (slope = 0.52) than DPV (slope = 0.244). The relative standard

![Figure 2. Ultrasensitive electrochemical detection of SARS-CoV-2 IVT RNA using MECS. (a) DPV and (b) SWV response of MECS-modified graphene microelectrode upon addition of SARS-CoV-2 IVT RNA (from 0.05 copies μL⁻¹ to 500 copies μL⁻¹) in artificial saliva. (c) Normalized current of DPV (red line) and SWV (blue line) at different concentrations of SARS-CoV-2 IVT RNA in logarithm scale, and error bars represent the standard deviations ($n = 3$). (d) Statistic for specific testing of SARS-CoV-2 RNA, and error bars represent the standard deviations ($n = 3$). The concentrations of sample 1 (human ssDNA), sample 2 (MERS-CoV IVT RNA), and sample 3 (SARS-CoV IVT RNA) are all fixed on 5 copies μL⁻¹, while the concentration of sample 4 (SARS-CoV-2 IVT RNA) is 0.5 copies μL⁻¹.](https://doi.org/10.1021/jacs.2c02884)
deviation (RSD) can quantify the uniformity of the sensing performances. As shown in Table S2, the RSD of MECS-modified sensor for detecting SARS-CoV-2 RNA by DPV is 11.414%, while that for detecting SARS-CoV-2 RNA by SWV is 4.667%. Thus, the sensors have high uniformity. To demonstrate the testing stability, we repeat DPV and SWV testing every 10 s (Figure S8). All results have no redox current response when testing blank samples, while stable electrochemical signals are obtained when testing the SARS-CoV-2 RNA sample after incubation for 30 min, indicating high testing stability. Figure 2d displayed the specificity of SARS-CoV-2 IVT RNA using MECS-modified graphene microelectrode with both SWV and DPV. The concentrations of MERS IVT RNA, SARS-CoV IVT RNA, and human IVT ssDNA are 5 copies μL⁻¹, while the concentration of SARS-CoV-2 IVT RNA is just 0.5 copies μL⁻¹. As shown in Figure S9, MERS IVT RNA, SARS-CoV IVT RNA, and human IVT ssDNA have no electrochemical current response in both SWV and DPV, only changes in the base current, indicating that the tentacles of MECS capture none of these nucleic acids. In contrast, SARS-CoV-2 IVT RNA with only a tenth of the concentration of SARS-CoV-2 RNA had electrochemical current response in both SWV and DPV. The current responses of SARS-CoV-2 IVT RNA (9.36 × 10⁻⁸A for DPV and 1.39 × 10⁻⁷A for SWV) exceed three times these above-mentioned nucleic acids in repeat testing, showing a statistical difference (P < 0.001). The results suggested that MECS had a good specificity toward SARS-CoV-2 RNA without the interference of other nucleic acids. Long-term stability is important for the application. Both DPV and SWV curves of the blank sample show no redox current, while the redox current occurs and increases with concentration of SARS-CoV-2 RNA (Figure S10). The electrochemical response maintains a certain value after 3 weeks. Thus, MECS-modified sensors maintain high sensitivity after long-term storage.

**Sensing Mechanism.** Self-actuated biorecognition and signal transduction are keys to the sensitivity of the MECS biosensor. As shown in Figure 3a and Figure S11, inspired by hydra, the tetrahedron trunk of MECS orients upright so as to prevent the aggregation of adjacent tentacles and their nonspecific adsorption to the graphene. Meanwhile, the free tentacle maintains away from the electrode automatically under an electrostatic repulsive force, minimizing a possible pseudosignal. Upon target recognition, the steric effect and the heavily charged nucleic acid cloud result in a significant conformation change of tentacles. Thus, the tentacle as well as nearby labels move downward spontaneously via the repulsive force to the vicinity of the interface. Then due to the modulation of the repulsion force caused by the electric field of SWV or DPV, the tentacle can be controllably actuated closer to an electrode (Figure S12). Such a self-actuated mechanism yields an appreciable electrochemical response. In previous work, we demonstrated graphene field-effect transistors (g-FETs) for rapid COVID-19 diagnosis.17,18,48,49 In the case of g-FETs, DNA nanostructures with probes are modified on the graphene surface, and the current signal is associated with potential perturbations when charged analytes are recognized by the probes. Here, MECS has a different principle from that of g-FET. The current signal is associated with the electro-
chemical reaction when the labels are actuated downward to the graphene surface. Compared with g-FETs, the MECS-modified sensors have advantages such as simple architecture, high signal readability, and low cost.

Different radii of electrodes are first fabricated to study the edge effect for the sensitive detection and acceptable detection range. The selected electrode radius range was determined by theoretical calculations. The calculation process of the formula is listed in the Supporting Information.

\[
\frac{1}{D} \frac{dc}{dt} = \frac{1}{r^2} \frac{dc}{dr} + \frac{\partial^2 c}{\partial r^2} + \frac{\partial^2 c}{\partial z^2}
\]  

(3)

According to eq 3, the different radii of electrodes are fabricated from 2 μm to 1 mm. As shown in Figure 3b and Figure S13, although the initial current of the 1 mm electrode is largest in all kinds of electrodes, the slope of the 1 mm electrode is smaller than that of the 200 μm electrode. When the concentration of SARS-CoV-2 RNA is larger than 5 copies μL−1, the current does not increase significantly and even decreases. The results indicated that the edge effect does not dominate in the millimeter-scale electrode. Although the electrode with the radius of 20 μm is more sensitive in the first few concentrations, its detection range is much narrower, just from 0.05 to 5 copies μL−1.

The recognized events lead to the tentacle’s position and configuration change with the electrochemical group on the graphene electrochemical electrode, which induces electrochemical response changes associated with SARS-CoV-2 RNA in real time. Different deposition voltages for 1 min are applied before DPV and SWV detection (Figure 3c and Figure S14). The tentacles are adsorbed near the electrode surface after 0.7 V deposition to produce an electrochemical response in DPV and SWV, even though there is no target RNA in the artificial saliva. In contrast, there is only a weak electrochemical response in the absence of deposition voltage or −0.7 V. The results indicated that under the repulsive force of the negative changed local electric field the tentacle maintains an upright orientation away from the electrode, which is more suitable for target recognition and detection. Then the role of distance regulation between tentacle and electrode for efficient electrochemical signal transduction under modulation of the repulsive force is studied by detecting different concentrations of SARS-CoV-2 RNA using MECS with different trunk heights. The trunk height of 17bp (17 base pairs for tetrahedral edges) for the regular tetrahedral structure is around 5.3 nm, approximate to the tentacle length (6.8 nm), while the trunk heights of 7 and 37bp are 2.2 and 11.1 nm, respectively. The 17bp of MECS shows a more comprehensive detection range and higher sensitivity than the 7bp and the 37bp (Figure 3d). Hence, when applying the excitation voltage of SWV and DPV, the change of force balance actuates the tentacle on the trunk of the 17bp regular tetrahedral structure together with the electrochemical group and SARS-CoV-2 RNA downward, being much more controllable and coming closer to the graphene surface than other height of the trunk.

MECS not only simulates the appearance characteristics of hydra to keep the tentacle’s activation but also imitates the predation behavior of hydra by controlled force balance of the tentacle to improve signal transduction during SARS-CoV-2 RNA detection. As shown in Figure 3e and Figure S15, different amplitudes imitating the predation behavior of hydra are performed to verify the controlled force balance effects for distance regulation between the tentacle and electrode to enhance the sensitivity. When the amplitude increased from 5 to 50 mV, the slope of the concentration-dependent curve, namely sensitivity, is significantly improved in both DPV and SWV. The slope is almost unchanged when the amplitude is greater than 50 mV. The results indicated that the amplitude of 50 mV can actuate almost all the tentacles that capture the...
SARS-CoV-2 RNA to the electrode surface and the redox reaction occurs to generate the signal response. As shown in Figure S16, the excitation voltages of SWV and DPV are used to imitate the different tension of the tentacle. The amplitude of SWV is much greater than that of DPV. In summary, the reduction of repulsive force by different electrical fields effects and electrical field forms can control the force balance to regulate the distance between the tentacle and electrode.

Direct Testing of Clinical Samples. Traditional RT-PCR detection requires nucleic acid amplification and purification after RNA extraction (Figure 4a). Here, we simply heat the clinical sample to release RNA. Then the clinical samples are directly detected using a MECS-modified graphene microelectrode. A portable smartphone-based electrochemical system has been developed (Figure S17). It can be used for on-site and point-of-care testing (POCT) outside of laboratories, such as in airports, clinics, local emergency departments, and even at home (Figure S18). We measured the response of the MECS-modified graphene microelectrode to serially diluted clinical samples to estimate the sensing capability for accurate samples. As shown in Figure 4b and Figure S19, an appreciable average current response up to 4.95 × 10^−8 and 9.68x 10^−8 A for DPV and SWV, respectively, is observed even with 10^3 dilutions (~0.04 copies μL^−1) in repeat testing, indicating the high sensitivity of MECS. Compared with commercial COVID-19 detection kits and reported diagnosis methodologies, MECS shows the rapid detection time (less than 1 min) and lower LoD (0.025 copies μL^−1 for SWV and 0.035 copies μL^−1 for DPV) to SARS-CoV-2 RNA, even in a clinical sample (Table 1).

Then the clinical samples are out of order and tested without nucleic acid amplification using MECS for double-blind testing to verify validity. Although the viral transport medium (VTM) contains a variety of biomolecules, there is no electrochemical signal on MECS in the blank sample (VTM solution) and health sample after 30 min for incubation (Figure S20a,c). The unmistakable electrochemical signal generated after the samples of SARS-CoV-2 patients were added into the PDMS groove and incubated for 30 min (Figure S20b,d). The normalized current responses of SARS-CoV-2 patient’s samples are distinctly more significant than those of healthy samples. The results show high overall agreement (~100%) with the RT-PCR results, which indicates that MECS can be used for the accurate diagnosis of COVID-19 (Figure 4c).

It is well-known that pooled sample testing is one of the most effective methods for large-scale screening to prevent an outbreak of a pandemic. MECS is also employed for 10-in-1 pooled clinical sample testing. Isometric nasopharyngeal swab samples from 10 healthy volunteers’ samples were mixed as negative samples (M1), while isometric 9 healthy volunteers’ samples and 1 SARS-CoV-2 patient’s sample were mixed as positive sample (M2). As shown in Figure 4d, electrochemical currents of 1 × 10^−7 and 3 × 10^−7 A in SWV and DPV are observed, respectively, in M2, while no redox peak current was observed in M1, which is similar to the blank sample. The statistical data shows that the normalized current of positive pooled samples is larger than that of negative pooled samples, showing a statistical difference (P < 0.001) (Figure 4e). The detection times, defined as the electrochemical testing time, for COVID-19 diagnosis are 15 s in SWV and 1 min in DPV, respectively. Hence, the MECS-modified graphene microelectrode could be used for broad COVID-19 screening.

### CONCLUSION

In summary, this work demonstrates a MECS constructed by DNA nanostructure for ultrasensitive SARS-CoV-2 RNA testing. The MECS modulates the electrochemical sensing process at molecular scale, solving a challenging issue of electrochemical assay in detection sensitivity. We demonstrate that automatically controlling the position of labels enables the most sensitive electrochemical assay so far for nucleic acid testing. The detection of SARS-CoV-2 RNAs approaches the physical limit of 4 copies in 80 μL artificial saliva, 2–6 orders of magnitude lower than other electrochemical biosensors and commercial kits. The idea of MECS also has broad application prospects to construct electrochemical biosensors for other infectious diseases like Ebola, Zika, and variants of SARS-CoV-2, such as Beta, Delta, and Omicron, by replacing the nucleotide sequence of the tentacle.

In the application, MECS enables direct testing of unamplified clinical samples. The results of double-blind testing are highly consistent with the RT-PCR results, demonstrating the merit in the ultraprecise diagnoses of COVID-19. More importantly, the MECS can identify positive 10-in-1 pooled clinical samples within 1 min. Pooled testing reduces the cost per test, increases testing efficiency, and has been considered as an effective strategy for population-scale disease screening to alleviate the burden on public healthcare services. Benefitting from the advantages such as rapid detection speed, low cost, and easy operation, MECS sensors hold great promise to solve the current dilemma in SARS-CoV-2 RNA testing and realize worldwide reopening. Combined with smartphone-based electrochemical system, MECS enables on-site and POCT for nucleic acid detection, such as in airports, clinics, local emergency departments, customs and even at home. As we know, antigen testing, mainly gold immunochromatography, is the primary commercial method of COVID-19 home testing. However, it can identify only 70% of positive cases. Individuals with low viral load could be ignored. In contrast, benefitting from the self-actuated sensing process at the molecular scale, MECSs overcome the limitations toward monomolecular electrochemical detection, which might be the missing piece of the puzzle enabling electrochemical testing to be a comprehensive tool in future epidemic prevention and control.

### METHODS

Fabrication of MECS-Modified Graphene Microelectrodes. The graphene electrochemical microelectrode was fabricated via a thermally assisted bilayer lift-off process based on semiconductor IC processing technology. As shown in Figure S21, fabrication of the graphene electrochemical microelectrode included electrode preparation and graphene transfer. Graphene with poly(methyl methacrylate) (PMMA) transferred from Cu foil by the electrochemical bubbling method was carried out onto the working electrodes. PMMA was subsequently removed by acetone. In electrode preparation, two layers of resist (sacrificial layer LOR 3A and photoresist S1813) were sequentially spin-coated on the SiO_2/Si wafer. The wafer was heat-treated at 170 °C for 5 min and 115 °C for 1 min, respectively. The ultraviolet lithography (Microwriter ML3, Durham Magneto Optics Ltd., UK) was used to pattern the electrode form (working electrode of radius 1 mm, 200 μm, 20 μm, 2 μm). After that, 5/45 nm Cr/Au were deposited on the wafer by using a thermal evaporator.
| detection method                  | disease diagnosis | sample type | target analyte | amplification | LoD         | time        | ref/company               |
|----------------------------------|-------------------|-------------|----------------|---------------|-------------|-------------|---------------------------|
| RT-PCR                           | COVID-19          | Viral RNA   | ORF1ab, N, E   | Yes           | 1000 copy mL⁻¹ | 90 min      | Beijing Applied           |
| RT-PCR                           | COVID-19          | Viral RNA   | ORF1ab, N, E   | Yes           | 330 copy mL⁻¹  | 50 min      | BioFire Defense, LLC      |
| RT-PCR                           | COVID-19          | Viral RNA   | N             | Yes           | 50 copy mL⁻¹    | 1−1.5 h     | DiaSorin Molecular LLC    |
| RT-PCR                           | COVID-19          | Viral RNA   | N, ORF1ab     | Yes           | 50 copy mL⁻¹    | 90 min      | Shanghai GeneoDx Biotech Co., Ltd. |
| RT-PCR                           | COVID-19          | Viral RNA   | E, N, ORF1ab  | Yes           | 1 copy µL⁻¹    | 2h          | Maccura Biotechnology (USA) LLC |
| RT-PCR                           | COVID-19          | Viral RNA   | ORF1ab, N     | Yes           | 200 copy mL⁻¹  | 90 min      | Sansure BioTech Inc.      |
| RT-LAMP                          | COVID-19          | Viral RNA   | ORF1ab/E/N gene | Yes          | 1000 copies mL⁻¹ | 30 min      |                          |
| iLACO (RT-LAMP)                  | COVID-19          | Synthetic RNA | ORF1ab gene   | Yes           | 10 copies/reaction | 15−40 min   |                          |
| SHERLOCK assay (LAMP)            | COVID-19          | Viral RNA   | N gene        | Yes           | 100 copies/reaction | 40−70 min   |                          |
| qRT-PCR (US CDC)                 | COVID-19          | Viral RNA   | N1, N2, N3    | Yes           | 1−3.2 copies µL⁻¹ | >120 min    |                          |
| CRISPR/SHERLOCK/assay           | COVID-19          | Viral RNA   | S, N, ORF1ab  | Yes           | 42 copies µL⁻¹   | >60 min     |                          |
| CRISPR-nCoV Assay               | COVID-19          | Viral RNA   | ORF1ab gene   | Yes           | 1.25 copies µL⁻¹ | 40 min      |                          |
| AID -CRISPR-Cas12a assay         | COVID-19          | Viral RNA   | N gene        | Yes           | 5 copies µL⁻¹    | 20−40 min for extraction; 20 |                          |
| Thermoplasmonic-Assisted Cyclic Cleavage Amplification (LSPR) | COVID-19          | Complementary DNA | ORF1ab gene   | Yes           | 0.1 ± 0.04 pM or >10⁹ copies µL⁻¹ | <30 min |                          |
| Fluorescent and colorimetric closed-tube (Penn-RAMP) | COVID-19          | Synthetic DNA | ORF1ab gene   | Yes           | 7 copies/reaction | 75 min      |                          |
| RPA/SHERLOCK/assay              | COVID-19          | Synthetic RNA | S and ORF1ab gene | Yes          | 10 copies µL⁻¹  | 1 h         |                          |
| Electrochemical DPV             | COVID-19          | Viral RNA   | ORF1ab, N gene | No            | 3 aM          | 190 min     |                          |
| Electrochemical DPV             | COVID-19          | Viral RNA   | S, N gene     | Yes           | 1 copy µL⁻¹    | <120 min    |                          |
| Electrochemical DPV             | COVID-19          | Viral RNA   | ORF1ab gene   | Yes           | 26 fM          | 2 h for incubating and 5 min |                          |
| Electrochemical DPV             | COVID-19          | Antigen     | SARS-CoV-2 S-protein | No          | 90 fM          | 10−30 s     |                          |
| Electrochemical DPV             | COVID-19          | oncogenesis | Cell mRNA     | microRNA-21    | Yes           | 43.3 aM     | 50 min incubation time   |
| Electrochemical DPV             | COVID-19          | oncogenesis | Synthetic DNA | microRNA-21    | Yes           | 63.1 aM     | 9 h for incubating        |
| Electrochemical DPV             | COVID-19          | oncogenesis | Synthetic DNA | microRNA-141   | Yes           | 11 aM       |                          |
| Electrochemical open circuit voltage | oncogenesis          | Synthetic DNA | microRNA-141   | Yes           | 1.4 aM       | 120 min     |                          |
| current−voltage electrochemical assay | COVID-19          | Viral RNA   | N gene        | No            | 6.9 copies µL⁻¹ | 30 min for extraction, 5 min |                          |
| Electrochemical SWV             | COVID-19          | Viral RNA   | S and ORF1ab gene | No          | 2 and 3 copies µL⁻¹ | 1 h         |                          |
| Electrochemical amperometry     | Cancer susceptibility and housekeeping | Synthetic DNA | TP53 and GAPDH | No            | 50 aM        | 30 min for incubating     |                          |
| Chronoamperometry               | Ebola             | Viral nucleic acid | EBOV cDNA      | Yes           | 100 fM        | 7.5 min for incubating 1 min |                          |
| Chronoamperometry               | Zika              | Viral nucleic acid | ZIKV and DENV | Yes           | 0.7 pM        | 69 |                          |
| MECS modified graphene microelectrode using SWV and DPV | COVID-19          | Viral RNA   | ORF1ab gene   | No            | 0.025 copies µL⁻¹ SWV, 0.035 copies µL⁻¹ DPV | 30 min for incubating <1 min for detection | This work |
Graphene was grown by chemical vapor deposition according to reported literature. First, Cu foil (25 μm) was placed in the tube furnace (GSL 1200×), and then it was heated to 1030 °C under H$_2$ (10 sccm, 99.999%) for 30 min. Then the Cu foil was annealed at 1030 °C in H$_2$ (7 sccm, 99.999%) in next 30 min. After that, CH$_4$ (16 sccm, 99.999%) was used for the grown process of graphene lasted for 20 min in the atmosphere of H$_2$ (7 sccm, 99.999%). Finally, the tube furnace was rapidly cooled to room temperature under the atmosphere of H$_2$. The graphene film on the Cu foil was stored under indoor temperature and dry.

The MECS included an upstanding regular tetrahedral base and bioinspired tentacles. The regular tetrahedral base was a tetrahedral structure with four double-stranded DNA (Table S1). The vertex of the tentacles was modified with methylene blue as the label to generate an electrochemical response. All the DNA were prepared and purified by Sangon Biotechnology Inc. Equimolar quantities (1 μM) of four strands for the assemblage of the MECS were mixed in 1×TM buffer at 95 °C for 10 min, and then were cooled to 4 °C immediately using a thermal cycler (SimplAmp, Thermo Fisher Scientific). The device was immersed in an acetone solution of 5 mM PASE for 2 h at room temperature, then washed with acetone, ethanol, and DI water three times in a turn, and dried with nitrogen in the air ambient. MECS (1 μM, 50 μL) was added to the PDMS chamber for 12 h at room temperature. After these steps, MECS was immobilized onto the graphene electrochemical microelectrodes successfully.

Characterization of MECS-Modified Graphene Microelectrodes. The morphologies of the MECS on graphene microelectrode in fluids (1×TM) was measured by AFM (Fastscan, Bruke). As for the height variation between the bare graphene and after treatment with PASE, we took the AFM measurement operated in ScanAsyst mode using a 20−25 nm radius tip (Scanasyst air) in the air to analyze the height difference among the measured results. After being treated by PASE, the graphene was measured by a Raman spectrometer (LabRam HR Evolution, Horiba Jobin Yvon, 532 nm Ar ion laser) with the point and the mapping measurement. The fluorescence intensities were imaged using a confocal fluorescence microscope (C2+, Nikon) and measured before and after 100 μL SARS-CoV-2 IVT RNA (10^8 copies μL$^{-1}$) was added. XPS (Thermo Scientific K-Alpha) was used to study the modification of electrodes. COMSOL Multiphysics performed simulations of electric field distribution. The primary and secondary current distribution module simulated the electric field distribution when voltages of −0.3, −0.35, and −0.4 V were applied at the working electrode, respectively.

Preparation of Standard Samples and Clinical Samples. The samples of SARS-CoV-2, SARS-CoV, MERS-CoV IVT RNA, and human ssDNA (nt 13321−15540, GenBank Nos. MT027064.1, NC004718.3, and NC019843.3) with a titer of 10^8 copies μL$^{-1}$ were provided by Shanghai Institute of Measurement and Testing Technology (SIMT). The samples were centrifuged at 3000 rpm at 4 °C for 5 min and diluted into 10000 copies μL$^{-1}$ first in full artificial saliva (Solarbio, China) and 2% of RNase inhibitor.
saliva to concentrations of 1000, 100, 10, 1, and 0.1 copies μL⁻¹.

The clinical samples used in this study were obtained from the Department of Laboratory Medicine, Shanghai Public Health Clinical Center. Seven nasopharyngeal swab samples were from RT-PCR-positive COVID-19 patients. Seven nasopharyngeal swab samples were from healthy volunteers. Viral transport medium (500 μL, Yocun, China) was used to store the swab and was heated at 56 °C for 30 min to release the RNA. Then the 14 clinical samples were randomly divided into 42 equal-volume samples (100 μL) titled S1–S42 for double-blind testing by other experimenters who do not perform electrochemical detection of SARS-CoV-2 RNA. The mediums were directly used for SARS-CoV-2 RNA testing by MECS-modified graphene microelectrodes, without the requirement of the extraction procedure. This research was approved by the Shanghai Public Health Clinical Center Ethics Committee (approval ID No. 2020-Y114-01) with informed consent from the participants.

**Electrochemical Measurement.** Buffer as the blank sample (80 μL) was added into the PDMS chamber for electrochemical detection. Then, 40 μL of buffer in the PDMS chamber was taken out, and 40 μL of RNA samples were added. Each sample of different concentrations was measured after 30 min of incubation. An Ag/AgCl electrode was used as the reference electrode in the solution. For human ssDNA, SARS-CoV-2 IVT RNA, SARS-CoV IVT RNA, and MECS-modified graphene microelectrodes, without the requirement of the extraction procedure. This research was approved by the Shanghai Public Health Clinical Center Ethics Committee (approval ID No. 2020-Y114-01) with informed consent from the participants.

buffer as the blank sample (80 μL) was added into the PDMS chamber for electrochemical detection. Then, 40 μL of buffer in the PDMS chamber was taken out, and 40 μL of blank samples were added again. During this process, the SWV and DPV results of the sensor signal can be used to show the stability of the baseline. After this, 40 μL of solution was taken out, and 40 μL of SARS-CoV-2 RNA samples were added into the PDMS chamber for electrochemical detection after 30 min of incubation time. Finally, the electrochemical detections were repeated again after the next 30 min. The process was used to make sure the response is a result of the SARS-CoV-2 RNA.

**Construction of the Electrochemical Portable Prototype.** The electrochemical portable prototype was divided into the microcontroller unit (MCU, C8051, Silicon Laboratories), the digital analog converter (DAC, DAC8552, TI, USA), the potentiostat (AD8606, ADI), the power management chip (TPS61085 and TPS79933), and the Bluetooth (HC-06). The prototype was programmed by KEIL. The application (APP) installed in the smartphone connected to the prototype by Bluetooth and sent commands to it. The APP was programmed by Android Studio and featured some functions including communication, data processing, data filtering, and real-time plotting.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.2c02884.

Calculation equation of edge effect, figures of Raman observation of graphene, graphene modified with MECS, and AFM observation of graphene, and graphene modified with MECS added SARS-CoV-2 IVT RNA (PDF)

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