Supplementary Information

Detection of SARS-CoV-2 Virus Amplification Using a Crumpled Graphene Field-effect Transistor Biosensor

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Contents

Materials and Methods

Table S1. RT-LAMP primer sequences

Figure S1. Raman spectra of Adenine absorbed on crumpled graphene

Figure S2. 2D images and cross-section profiles of AFM height and phase of flat and crumpled graphene surfaces

Figure S3. I-V curves of crumpled graphene FET sensors of 3 known positive clinical samples with shortened amplification time (RT-LAMP; 65 °C, 30 min) on cgFET

Figure S4. Detection of SARS-CoV-2 virus in 25% VTM clinical samples using the off-chip RT-LAMP assay performed with the EvaGreen dye

Table S2. Summary of the RT-PCR Ct values of the VTM clinical samples tested. RT-PCR tests performed by OSF Healthcare.

Figure S5. End-point measurement of Dirac point shift of primers adsorption on cgFET with different concentrations

Figure S6. Fabrication of the crumpled graphene on polystyrene substrate

Figure S7. Geometry and dimension of chamber and gate electrode of detection cell for virus test

Figure S8. Charge transfer characteristics of the flat and crumpled graphene FET devices


**Materials and Methods**

**Materials**

Single-layer graphene was purchased from Haesung DS (Seoul, South Korea). Polymethyl methacrylate (PMMA), sodium persulfate, and acetic acid were obtained from Sigma Aldrich (St. Louis, MO). Gamma-Irradiated SARS-Related Coronavirus 2 (Isolate USA-WA1/2020), NR-52287, was acquired from BEI resources. RT-LAMP primer sequences for the N gene detection of SARS-CoV-2 virus (Supplementary Information, Table S1) were obtained from (Ganguli et al. 2020b) and synthesized by Integrated DNA Technology (IDT). CDC-compliant VTM was obtained from Redoxica (VTM-500ML). The RT-LAMP assay components were acquired from New England Biolabs and Sigma-Aldrich. TE buffer is IDTE pH 8.0 (1X TE Solution) from IDT.

**Viral Transport Media (VTM) clinical samples**

We used discarded patient VTM samples prior to the RNA purification step. Ten samples are from patients who were tested positive for COVID-19 and ten additional samples are from patients who were tested negative for COVID-19 at OSF Healthcare (Peoria, IL) by a RT-PCR test performed at OSF Healthcare. The samples we received were deidentified, frozen, and obtained through an approved institutional review board (OSF Peoria IRB # 1602513 via the University of Illinois College of Medicine with waiver for consent).

**Fabrication of flat gFET and crumpled gFET devices**

The graphene/Cu foil was spin-coated with PMMA at 1500 RPM (45 seconds) and soft-baked on a hotplate (110°C, 90 s.). The PMMA coat was used as a supporting layer for graphene. Using a razor blade, the graphene sheet was cut into 2 x 20 mm² sections. The Cu foil was removed by etching overnight (0.1 M sodium persulfate) and then rinsed with deionized water. Then, the PMMA/graphene was transferred onto a polystyrene substrate using a general wet graphene transfer method. After dehydration (RT, 2 h.), the PMMA layer was removed (acetic acid, 10 min.) and then the sample was rinsed with deionized water (10 min.). Subsequently, the graphene/polystyrene substrate was perforated in the shape of a circle (crumpled graphene: diameter = ~50 mm., flat graphene: diameter = ~25 mm.). The sample was then annealed (120°C, 1 h. in an oven), resulting in a shrinkage of the polystyrene substrate to half the original diameter (~25 mm.) and graphene to a section of 1 x 10 mm² (50% crumpled graphene). After, the polystyrene substrate was
flattened, it was placed between two metal plates, pressed with clips, and then annealed (120°C, 15 min.). Finally, conductive silver paint was used to fabricate the source and drain electrodes at both ends of the graphene ribbon. Details of the fabrication of the crumpled graphene and sensing chamber can be found in the Supplementary Information (Fig. S6 and S7).

**Preparation of SARS-CoV-2 virus samples**

For the inactivated virus samples, the gamma-irradiated SARS-CoV-2 virus was first aliquoted and stored at -80°C. Before use, the inactivated virus aliquot was thawed at room temperature and spiked in the VTM (25% in TE buffer) at the desired concentration. In the case of VTM clinical samples, they were also thawed at room temperature before use. Before testing, both types of samples were then thermally lysed (95°C, 10 min.), followed by mixing with TE buffer (1:1 ratio).

**RT-LAMP reaction**

The RT-LAMP assay was designed to amplify the target RNA sequence within the N-gene region in SARS-CoV-2 virus (Fig. 1A). The RT-LAMP reaction mix was comprised of the following components: 4 mM of MgSO₄ (New England Biolab), 1x final concentration of the isothermal amplification buffer (New England Biolab), 0.29 M Betaine (Sigma-Aldrich), 1.025 mM each of deoxy-ribonucleoside triphosphates (dNTPs), 0.3 U/μL WarmStart Reverse Transcriptase (New England Biolabs), 0.47 U/μL Bst 2.0 WarmStart DNA Polymerase (New England Biolabs), 1 mg/ml BSA (New England Biolabs), and 0.735X EvaGreen (Biotium). 1X primer concentration in each reaction consisted of 0.2 μM of F3 and B3, 1.6 μM FIP and BIP, and 0.8 μM of LoopB primers. EvaGreen dye was not added for combined experiments of RT-LAMP reaction and graphene FET measurements. A total volume of 16 μL, which consisted of 2 μL of RNA template concentrations and 14 μL of RT-LAMP reaction mix, was added into 0.2 ml PCR tubes. The RT-LAMP reaction was performed in a QuanStudio 3 Real-Time PCR System (Applied Biosciences) at an isothermal condition (65°C) for the desired duration. Fluorescence data was recorded every 1 minute to create the amplification curve. The amplification threshold time was calculated at 20% of amplification from the normalized fluorescence amplification curve.
**Electrical measurements**

A semiconductor parameter analyzer was used to measure I-V curves of the graphene FET (Agilent 4155B and Keithley 4200-SCS). Before electrical measurements, the graphene FET devices were incubated overnight (0.1X PBS). Before analysis of samples, stabilization of the electrical signal of the gFET devices was achieved. Stabilization was accomplished by repeating the blank measurements (0.1X PBS) every 5 minutes, changing the buffer every time, until the Dirac point shift was negligible (< 3mV). After reaching Dirac point stabilization, the sample (70 μL) was loaded into the device, incubated (20 min.), and the device was then washed to remove non-specific bonded residues (0.1X PBS, for 5 times). To perform the electrical measurements, the gate voltage was swept from 0.2 to 0.8 V and the drain–source current (Ids) was measured at Vds = 30 mV. Charge transfer characteristics and stabilization procedure of the graphene FET device can be found in the Supplementary Information (Fig. S8).

**Raman spectroscopy and Atomic Force microscopy (AFM) characterization**

Raman spectroscopy for obtaining spectra of primers on crumpled graphene was performed using a Nanophoton Raman 11 laser confocal microscope (Nanophoton, Osaka, Japan) with an excitation wavelength of 532 nm. The Raman signals were detected by a Peltier cooled CCD camera at −70°C. The excitation power was set at 0.1 mW with 3 seconds exposure time and 5 times averaging for mapping. In Raman mapping mode, the defined region of interest was 20 by 75 μm in X, and the Y axis with 200 nm/pixel resolution with NA 0.9 100X Plan Fluor objective lens with 600 g/nm grating. The wavenumber range covered was 500 – 2900 cm⁻¹. The wavenumber shift compensation was -8.6 cm⁻¹ after calibration using a standard, low-pressure Neon lamp. AFM images were measured using an Asylum research MFP-3D AFM system (Asylum Research, Santa Barbara, CA) with tapping mode tip.

Before Raman spectroscopy and AFM characterization, all samples were electrically tested as explained in Section 2.6.
**RT-LAMP primer sequences**

|    | Primer Sequence                                      |
|----|------------------------------------------------------|
| F3 | GTTCCTCATCACGTAGTCG                                   |
| B3 | GTTTGGCCTTGTTGTGTT                                  |
| FIP| GCCAGCCATTCTAGCAGGAG-CAACAGTTAAGAAATTCAACTCC        |
| BIP| GATGCTGCTTTGCTTTGCT-ACCAGACATTTTGCTCTCAA           |
| Loop B | GCTGCTTGACAGATTGAACCAG |
Figure S1. Raman spectra of Adenine absorbed on crumpled graphene. 1 µM Deoxyadenosine triphosphates (dATP, obtained from Sigma Aldrich (St. Louis, MO)) in 0.1X PBS was incubated on cgFET chamber for 3 hours and then washed by 0.1X PBS for 5 times. The excitation power of Raman spectroscopy was set at 0.1 mW with 3 seconds exposure time and 5 times averaging for mapping. The wavenumber shift compensation was -8.7 cm\(^{-1}\) after calibration using a standard, low-pressure Neon lamp.
Figure S2. 2D images and cross-section profiles of AFM (A) height and (B) phase of flat and crumpled graphene surfaces showing primer adsorption on graphene after amplification of inactivated SARS-CoV-2 virus spiked in 25% VTM.
Figure S3. I-V curves of crumpled graphene FET sensors of 3 known positive clinical samples with shortened amplification time (RT-LAMP; 65 °C, 30 min) on cgFET.
Figure S4. Detection of SARS-CoV-2 virus in 25% VTM clinical samples using the off-chip RT-LAMP assay performed with the EvaGreen® dye. (A) Normalized fluorescence data (n=3) of 20 clinical samples (10 positive and 10 negative) for 50 minutes of reaction time at 65°C. RT-LAMP amplification threshold is determined at 20% of normalized fluorescence signal. Three known positive clinical samples with the latest threshold time (lowest viral load) were highlighted with different colors. (B) Threshold time of the clinical samples analyzed from the normalized fluorescence signal.
Table S2. Summary of the RT-PCR Ct values of the VTM clinical samples tested. RT-PCR tests performed by OSF Healthcare.

| SAMPLE # | Test result (Positive / Negative) | Diasorin CT Values | Cophold CT Values |
|----------|-----------------------------------|--------------------|-------------------|
|          |                                   | FAM                | JOE               | E     | N2    |
| N1-N10   | N                                 | 0                  | 0                 | 0     | 0     |
| P1       | P                                 | 12.7               | 13.6              |       |       |
| P2       | P                                 |                    | 22.8              | 24.7  |       |
| P3       | P                                 | 11                 | 11.4              |       |       |
| P4       | P                                 |                    | 23.6              | 25.2  |       |
| P5       | P                                 | 28.6               | 29.1              |       |       |
| P6       | P                                 | 18                 | 19.5              |       |       |
| P7       | P                                 | 15.1               | 15.9              |       |       |
| P8       | P                                 | 24.3               | 25.1              |       |       |
| P9       | P                                 | 16.6               | 18.5              |       |       |
| P10      | P                                 | 18                 | 19.3              |       |       |
Figure S5. End-point measurement of Dirac point shift of primers adsorption on cgFET with different concentrations. The primers were incubated for 20 min and washed by 0.1X PBS buffer 2-times until the Dirac point was saturated. As a result, Dirac point shifted -17.31 mV, -26.94 mV, and -78.50 mV at 44 fM, 44 pM, and 44 nM, respectively. Dirac point of 44 nM primers was constant (-78.63 mV) when the device was incubated with 0.1X PBS for 1 h. Light blue arrows represent washing step.
Figure S6. Fabrication of the flat and crumpled graphene on polystyrene substrate. (A) Polystyrene substrate was punched into a 50 mm-diameter circle shape and washed by IPA and DI water. Oxygen plasma was used via 2 min exposure in low-pressure to activate hydrophilic surface on polystyrene substrate. 2 mm × 25 mm graphene was transferred on polystyrene substrate and dried for 3 h in room temperature. (B) To fabricate the flat graphene, the graphene/polystyrene substrate was perforated in the shape of a circle (diameter = ~25 mm.). (C) To fabricate the crumpled graphene, graphene-polystyrene substrate shrunk ~50 % after annealing at 120°C for 1 h. in oven. (D) Two clips and stainless washers were used to flatten bended graphene-polystyrene substate. All scale bars are 1 mm.
Figure S7. Geometry and dimension of chamber and gate electrode of detection cell for virus test. (A) Chemical-resistant Teflon sheet (McMaster-Carr) was used to fabricate the chamber and the detection cell cover by laser cutting. Two clips were assembled on the bottom substrate that connected to the source and drain electrodes on the flat and crumpled gFET devices. A chemical resistant O-ring was placed in the center of the graphene used as a reservoir. (B) Two platinum wires were used as the counter and reference electrodes. (C) Potentiostat connected with the gate electrodes was used to apply a stable linear gate voltage sweep. Buffer (PBS 0.1X) was used as the electrolyte in the gate electrode. After experiments with viral samples, all components were disinfected with 99% acetone and isopropyl alcohol.
**Figure S8.** Charge transfer characteristics of the (A) flat and (B) crumpled graphene FET devices. Gate voltage was swept from -0.5 V to 1 V and drain–source voltage was picked 0.05 V for virus test. (C) Before virus sample loading, stabilization was achieved by repeating measurements at least 5 times every five minutes until the difference of the last two Dirac points was less than 3 mV.
Reference

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