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Materials Today: Proceedings 49 (2022) 2546–2549

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

Selective and electronic detection of COVID-19 (Coronavirus) using carbon nanotube field effect transistor-based biosensor: A proof-of-concept study

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ARTICLE INFO

Article history:
Available online 9 May 2021

Keywords:
SARS-CoV-2
COVID-19
Biosensor
Rapid detection
Coronavirus
Carbon nanotube field-effect transistor

ABSTRACT

In this work, we propose and demonstrate a carbon nanotube (CNT) field-effect transistor (FET) based biosensor for selective detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). CNT FETs were fabricated on a flexible Kapton substrate and the sensor was fabricated by immobilizing the reverse sequence of the RNA-dependent RNA polymerase gene of SARS-CoV-2 onto the CNT channel. The biosensors were tested for the synthetic positive and control target sequences. The biosensor showed a selective sensing response to the positive target sequence with a limit of detection of 10 fM. The promising results from our study suggest that the CNT FET based biosensors can be used as a diagnostic tool for the detection of SARS-CoV-2.

Selection and peer-review under responsibility of the scientific committee of the Indo-UK International Virtual Conference on Advanced Nanomaterials for Energy and Environmental Applications (ICANEE-2020).

1. Introduction

As of today, the COVID-19 pandemic is a global challenge and scientists are working on therapeutics, vaccines and fast diagnostic techniques. Since, therapeutics being delayed and vaccines are under way, fast diagnostic techniques could help to control the spread and to open the borders without affecting the economy [1]. Rapid detection techniques have been developed and commercialized using antibody and antigen sensors [2]. However, the antibody test is not a reliable test for detecting SARS-CoV-2. As per WHO, Real time polymerase chain reaction (qPCR) is the recommended test for detecting SARS-CoV-2. However, expensive equipment, trained personals and longer processing time make qPCR as a non-effective method for point-of-care application. Longer time taken for qPCR test is due to longer amplification step before detecting the positive sequence. The time taken for the amplification step can be saved by employing a highly sensitive and selective detection mechanism instead of the optical labeling method used in qPCR test. CNT network FETs are known as one of the best platforms for electronic biosensors [3–5] that can selectively detect metal ions [6] and biomolecules including hormones [7], viruses and whole cells. Recently, a graphene FET based biosensor is reported using a specific antibody against SARS-CoV-2 spike protein as the primary sensing element for detecting SARS-CoV-2 [2].

In this work, we report a proof of concept electronic biosensor for rapid detection of SARS-CoV-2 based on liquid gated CNT network FETs. The sensors were successfully fabricated and selective detection of SARS-CoV-2 was demonstrated.

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https://doi.org/10.1016/j.matpr.2021.05.011
2214-7853/
Selection and peer-review under responsibility of the scientific committee of the Indo-UK International Virtual Conference on Advanced Nanomaterials for Energy and Environmental Applications (ICANEE-2020).

2. Materials and methods

2.1. CNT FET and sensor fabrication

The carbon nanotube field effect transistors on a flexible Kapton substrates with encapsulated electrodes were fabricated as reported earlier [6,8]. 99% semiconducting CNTs with an average diameter of 1.5 nm was used to fabricate the CNT network. The CNT thin film on the Kapton substrate were deposited a simple solution process technique [9,10]. The CNT suspension was made by sonicating a tweezer tip amount of CNT bucky paper. Then the Kapton substrate was functionalized with a monolayer of 2-mercaptopyridine using polydimethylsiloxane (PDMS) (Sylgard 184) stamping method. The functionalized Kapton film was immersed into the CNT suspension for 20 min. The CNT deposited Kapton surface was cleaned by immersing it into absolute ethanol for 10 min and dried in nitrogen. The CNTs in the unwanted area were removed by plasma cleaning at 600 mTorr pressure and 10 SCCM flow of 99.99% oxygen gas. The electrodes were then deposited using successive evaporation of 5 nm Cr and 50 nm Au after defining the electrodes using photolithography. The electrodes were encapsulated using AZ1518 photoresist to avoid gate leakage and hard baked at 200 °C for 10 min [11,12]. The probe sequences were immobilized onto the CNT sidewalls as described in refs [6,13] using pyrenebutanoicacid, succinimidyl ester as the molecular linker.

All the sequences used in this study was purchased from Integrated DNA Technologies, USA. The probe sequence that was immobilized onto the CNT sidewalls is the reverse sequence of the RNA-dependent RNA polymerase gene of the SARS-CoV-2 [14]. Before functionalization, the probe suspension in 20 mM Tris buffer was annealed at 70 °C in an electric oven (BioBase).

2.2. CNT FET and sensor measurements

The transfer characteristic curves of the pristine and probe immobilized CNT FETs were measured under liquid gated conditions using 2 mM Tris buffer solution and a Ag/AgCl standard electrode as the gate. The schematic of the fabricated device along with the measurement setup is illustrated in Fig. 1. A well made up of PDMS was mounted onto the channel to hold the electrolyte and target solution in the channel area. A computer interfaced Kiethley 2602 source measure unit was used for electrical measurements. The device current (I_{ds}) was measured by sweeping the gate voltage (V_{lg}) from −0.5 V to 1 V with a step size of 0.02 V. The sensor measurements were done by continuously monitoring I_{ds} with a sample time interval of 1 S. The sensors were tested for both positive and negative target sequence as given in table 1. Both positive and control sequence solution was prepared by dissolving the purchased sequences in 2 mM tris buffer.

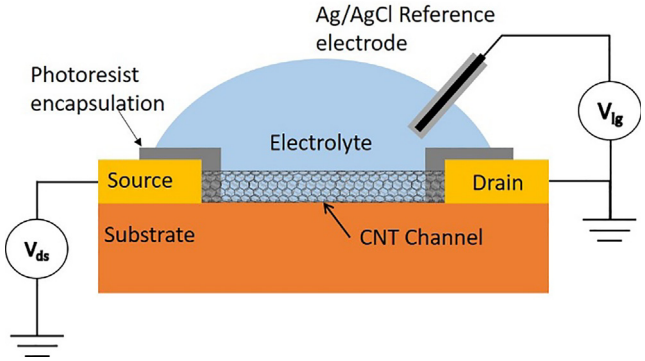

Fig. 1. Schematic of the CNT FET fabricated on a flexible Kapton substrate with encapsulated electrodes with external electrical circuit for FET and sensor measurements.

Table 1
Sequences of probes and positive and negative targets used in this study.

	Sequence of the primers and probes
Probe	Amine-5′-GCATC TCCTG ATGAG GTTCC ACCTG-3
Positive target sequence (SARS-CoV-2 RdRP)	CAGGT GGAAC CTCAT CAGGA GATGC
Control target sequence	C CAGGT GGAAC ATCAT CCGGT GATGC

3. Results and discussion

We fabricated a CNT FET based biosensor to selectively detect a portion of the SARS-CoV-2 RNA. The positive target sequence was the RNA-dependent RNA polymerase gene (RdRP) of the SARS-CoV-2 as reported previously [14]. The probe for selective detection used was the reverse sequence of the positive target sequence with an amino modification at the 5′ end for immobilization purpose. The negative control sequence was the RNA-dependent RNA polymerase gene of the SARS virus. The negative sequence has four mismatches with the reverse sequence of the RNA-dependent RNA polymerase gene of the SARS-CoV-2. We chose the control sequence for differentiate the closely related RNA sequence of the severe acute respiratory syndrome (SARS) viral RNA from the RNA of SARS-CoV-2.

3.1. Immobilization of the probe sequence

The CNT FETs were electrically tested to ensure the immobilization of the probe sequence. Fig. 2 shows the transfer characteristic curves of the pristine and probe immobilized CNT FETs. The pristine CNT FET showed ambipolar characteristics with a positive threshold voltage (V_{th}) which indicate the hole dominated electrical conduction at a gate bias of 0 V. A clear positive shift in threshold voltage is observed after immobilization of probe sequence. This is consistent with our previous works and ensures the tethering of probes on the sidewalls of CNTs [6,7]. The Debye length of the 2 mM tris buffer solution used was about 10 nm. The length

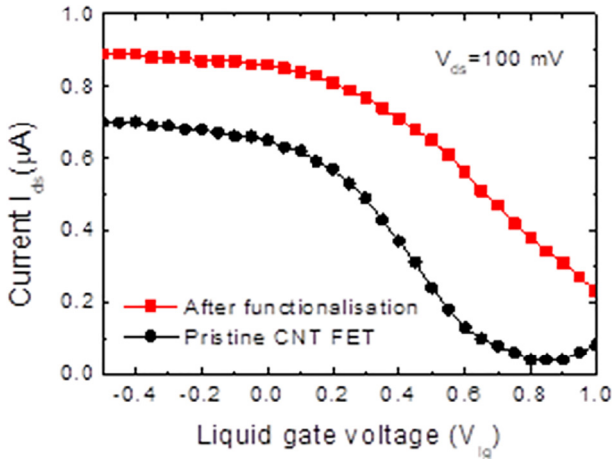

Fig. 2. Transfer characteristic curves of a pristine (black circle) and probe immobilized (red square) CNT network FET under liquid gated conditions with 2 mM Tris buffer as an electrolyte and Ag/AgCl as the standard electrode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

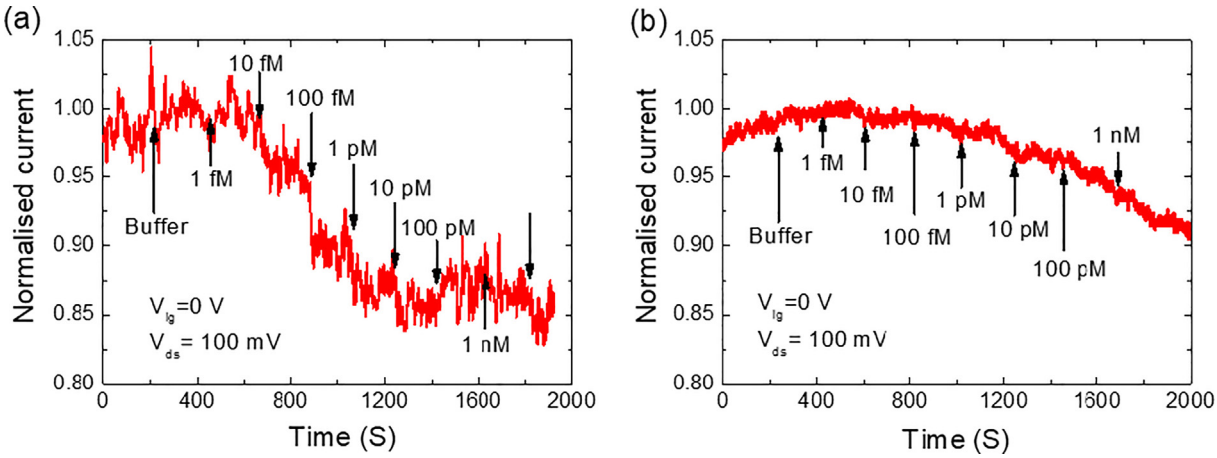

Fig. 3. Normalized real time sensing response of the probe immobilized CNT FET biosensor for (a) positive target sequence and (b) control target sequence with increasing concentrations.

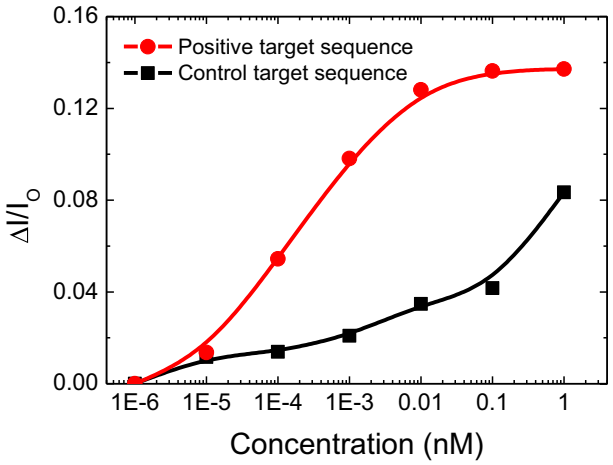

Fig. 4. Normalized concentration depended current response of the probe immobilized CNT FET biosensors for both positive and control target sequence.

of the probe sequence is also about 10 nm and any changes in the probe sequence will be in the vicinity of the CNT channel.

3.2. Sensor measurements

The target sequences were prepared with different concentrations in 2 mM Tris buffer. The initial stock solution was prepared at 10 μM as given in the suppliers information and diluted further to obtain target sequences with concentrations of 10 pM. The target sequences were added into the PDMS well by monitoring the current I_{ds} with an interval of 1 S. Fig. 3 shows the response of the biosensors for both positive and control targets. The addition of positive target shows a clear concentration depended drop in device current. The drop in the device current could be attributed to the change of band realignments [11] and electrostatic gating [6] at the metallic-semiconductor carbon nanotube junction due to the hybridization of the DNA.

The concentration depended normalized current response is given in Fig. 4. The deviation between the response of the positive and control sequences is prominent and this could be a clear indication of the selective response of the tested biosensor. As discussed earlier, the control sequence is selected to differentiate SARS-COV-2 and SARS virus. Hence, we can say that the relatively higher response of the biosensor for the control sequence at concentration of 1 nM is due to the aggregation of the negative sequence within the electrical double layer of the CNT channel.

4. Conclusion

We demonstrate a proof-of-concept electronic detection technique for selectively diagnosing SARS-COV-2. The RNA hybridization was used as the primary signal generator and the liquid gated CNT network FET was used as the signal transducer. The sensors produced a promising sensing signal at 10 fM concentrations of the positive target sequences at real time sensing conditions. The proposed CNT FET based biosensor can be developed as a reliable, fast and cheap alternative for existing diagnostic techniques.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors sincerely acknowledge Dr. Natalie O V Plank for allowing to use the devices fabrication facilities in clean room at Victoria University of Wellington. Dr. M. Thanihaichelvan acknowledges National Science Foundation, Sri Lanka for a research grant (NSF/COVID/2020/HS/02).

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