Extended Abstracts of the 2018 International Conference on Solid State Devices and Materials, Tokyo, 2018, pp721-722

Detection of Human-Infectious Influenza Virus Using Sialoglycan-Modified Graphene Field-Effect Transistor

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Abstract

For early detection of emerging human-infectious avian influenza virus, sialoglycan-modified graphene field-effect transistor was developed. Utilizing infection mechanism of influenza virus, this novel device specifically detected human-infectious influenza virus of 2.56 HAU concentration, which is comparable to human saliva, for around 20 minutes. The speedy and high-sensitive detection will prevent oncoming pandemic caused by human-infectious avian influenza virus.

1. Introduction

Graphene has high carrier mobility and two-dimensional structure. Owing to these features, graphene field-effect transistor (G-FET) keenly responds to target molecules bound to its graphene channel [1]. In this study, G-FET was applied to detection of specific influenza virus (IFV). Highly pathogenic avian influenza has attracted global attention in poultry farming. Although it hardly affects human species, it has high mortality rate about 60%, once affecting humans. In 2012, it was found that highly pathogenic avian IFV achieves strong human infectivity by only four mutation in its membrane protein hemagglutinin [2]. Now there are global concerns about emergence of human-infective avian IFV, which potentially cause severe pandemic like Spanish flu in 1918. For the detection of human infectivity of IFV, the authors focused on its infection mechanism. IFV infection starts from binding of hemagglutinin to sialoglycan on human or avian cell surfaces. Hemagglutinin recognizes slight difference in structure of human and avian sialoglycan, and specifically binds to either of them. Therefore, if one can detect binding specificity of IFV to sialoglycan in high sensitivity, it leads to early detection of the pandemic potentials and prevention of the oncoming pandemic. Here, the authors modified human-type sialoglycan on graphene field-effect transistor (G-FET) and electrically detected the binding of human-infectious IFV to sialoglycan (Fig. 1(a)).

2. Experimental procedure

G-FET was fabricated from exfoliated graphene. After transferred to Si/SiO_2 substrate, graphene was connected to Au/Ni electrodes (Fig. 1(b)). Human-type α2.6 sialoglycan was then modified on graphene via π-stacking linker 1-pyrenebutyric acid *N*-hydroxysuccinimide ester (PBASE). The modification was confirmed by atomic force microscopy (Fig. 2(a)). Sialoglycan increased graphene thickness from its original value (0.32 nm) to 2.3 nm. The modification was also confirmed by the shift in ambipolar transfer characteristics of G-FET toward positive voltage direction. π electron of PBASE and carboxyl group of sialoglycan induced hole carrier to graphene [3].

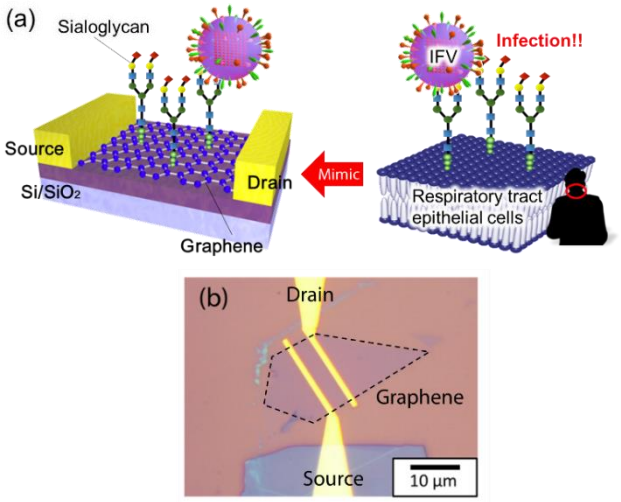

Fig. 1: (a) Schematic illustration of IFV detection using G-FET. (b) Optical micrograph of G-FET.

Nonspecific binding of IFV was reduced by surface blocking using 1 % polyvinylpyrrolidone and 0.05 % polysorbate 20. All measurements were carried out in pH 7.4 phosphate buffer.

3. Results and discussions

Transfer characteristics of G-FET modified with human-type α2.6 sialoglycan were shifted toward positive voltage direction after adding human-infectious IFV (H1N1 subtype, A/Suita/117/2011, Fig. 3(a)). The shift direction is consistent

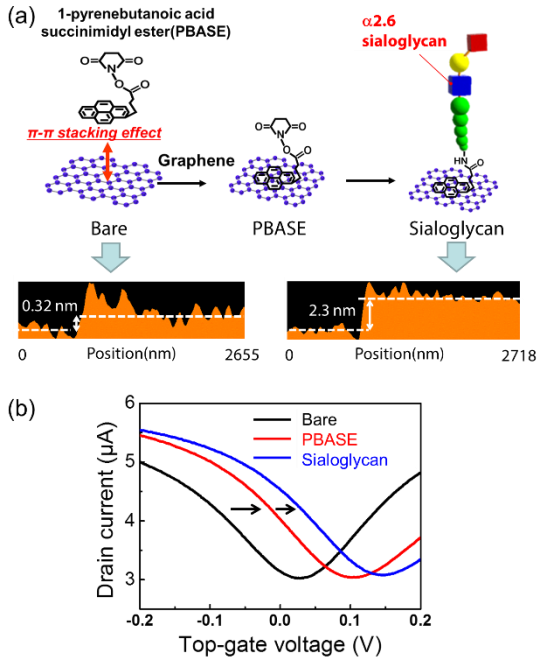

Fig. 2: Sialoglycan modification of G-FET. (a) Modification scheme and cross sectional profile at graphene edge before and after modification. (b) G-FET transfer characteristics in modification process.

with the negative surface charge of the virus. In contrast, unmodified G-FET showed little change in transfer characteristics after the adding (Fig. 3(b)). These results show G-FET detected sialoglycan-specific binding of IFV. The shift width monotonically increased with the virus concentration (Fig. 4). Moreover, the G-FET hardly respond to avian influenza virus (H9N2 subtype, A/Turkey/Wisconsin/1/1966). Between human and avian IFV, significant difference was observed in electrical response even under virus concentration of 2.56 HAU, which is almost comparable to that in human saliva. The whole measurement took around 20 minutes, while the conventional infectivity discrimination takes a week so far, due to its lack of sensitivity.

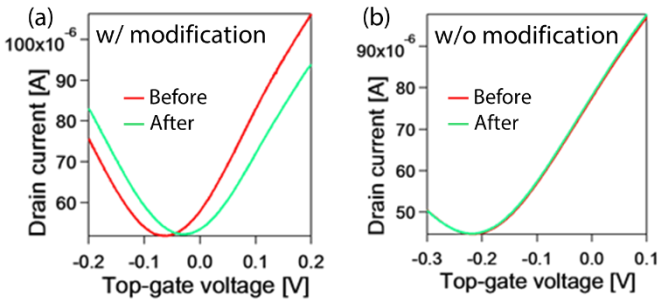

Fig. 3: Transfer characteristics before and after IFV injection on G-FET with (a) and without (b) sialoglycan modification. Horizontal shift width was 32.6 mV in (a) and 1.4 mV in (b).

These results from electrical measurements were supported by AFM and fluorescent microscope images, that is, after fluorescent staining of viral lipids, particles of several tens of nm diameter (close to diameter of the virus) were observed in AFM images and the particles were found to contain viral lipids by fluorescent images (Fig. 4). These particles were observed only on G-FET where human-infections IFV was introduced.

4. Conclusion

Sialoglycan-modified G-FET was developed and successfully detected human-infectious IFV selectively. This novel method achieved sensitive and quick detection potentially applicable to on-site diagnosis of influenza for early detection of emerging pandemic potentials.

Acknowledgements

This work was supported by JST-CREST (JPMJCR15F4) and Kakenhi 18K14107, Japan.

References

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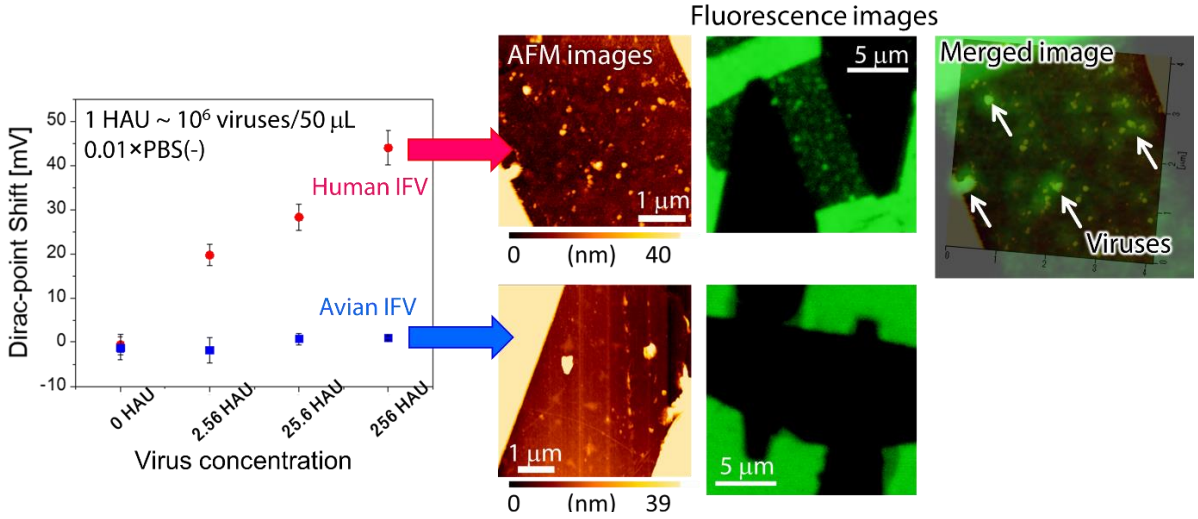

Fig. 4: Discrimination of human-infectious IFV. (Left graph) Shift width of transfer characteristics of G-FET under variety of IFV concentration. Error bars: one standard deviation of current noise. (Right images) AFM and fluorescent images of G-FETs after introduction of human IFV (upper images) and avian IFV (lower images). Virus particles in merged image are indicated by arrows.