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Novel graphene-based biosensor for early detection of Zika virus infection

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ABSTRACT

We have developed a cost-effective and portable graphene-enabled biosensor to detect Zika virus with a highly specific immobilized monoclonal antibody. Field Effect Biosensing (FEB) with monoclonal antibodies covalently linked to graphene enables real-time, quantitative detection of native Zika viral (ZIKV) antigens. The percent change in capacitance in response to doses of antigen (ZIKV NS1) coincides with levels of clinical significance with detection of antigen in buffer at concentrations as low as 450 pM. Potential diagnostic applications were demonstrated by measuring Zika antigen in a simulated human serum. Selectivity was validated using Japanese Encephalitis NS1, a homologous and potentially cross-reactive viral antigen. Further, the graphene platform can simultaneously provide the advanced quantitative data of nonclinical biophysical kinetics tools, making it adaptable to both clinical research and possible diagnostic applications. The speed, sensitivity, and selectivity of this first-of-its-kind graphene-enabled Zika biosensor make it an ideal candidate for development as a medical diagnostic test.

1. Introduction

Zika is a vector borne viral infection originating in the Zika Forest of Uganda in the mid-20th century (Sikka et al., 2016). Since its discovery, there have been several significant outbreaks of Zika, most recently in 2015 in North and South America including the United States (Peterson et al., 2016). The long-term effects of Zika include severe brain defects in fetuses (Rasmussen et al., 2016) and Guillain-Barre Syndrome in adults (Ladhani et al., 2016). As a result, the Zika virus epidemic has been a growing concern for public health in the United States. Over 41,000 cases of Zika virus infection have been reported since January 2015 within the United States and its territories, of which 3461 cases are pregnant women (Centers for Disease Control, 2017). The current diagnostic standards for Zika detection are RNA Nucleic Acid Testing (RNA NAT), Trioplex rRT-PCR, or the Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) in urine, serum, or cerebrospinal fluid (Huzly et al., 2016; Lanciotti et al., 2008). However, due to cross-reactivity with co-circulating viruses, such as dengue and Japanese encephalitis (JEV) (Sironi et al., 2016; Xu et al., 2016), the confirmation rate of presumptive positive results is less than 50% for the commercial IgM capture ELISA (Food and Drug Administration, 2016). Both timely and accurate diagnostic capabilities from emerging technologies are needed to combat this public health threat.

Highly sensitive nanomaterials show promise as biosensors by affording lower limits of detection with better selectivity than traditional assays. A promising biosensor candidate, graphene, is a single layer, two-dimensional sheet of hexagonally arranged carbon atoms first experimentally isolated in 2004 (Geim and Novoselov, 2007; Novoselov et al., 2004). As a conductive, two-dimensional material, every atom in a graphene sheet is in direct contact with its environment and responds to electrostatic fluctuations, making it an ideal candidate for sensing applications (Zhu et al., 2013). Indeed, graphene has been incorporated into sensors of all varieties, including pressure sensors (Zhu et al., 2013), chemical vapor sensors (Esfandiar et al., 2013; Lu et al., 2010), optical sensors, (Lu et al., 2012) and biomolecular sensors (Lerner et al., 2014).

We have recently demonstrated use of a commercially available graphene chip as a biosensor, with read out by portable electronic hardware (Lerner et al., 2016). Graphene is selected as the base material for our platform over other nanomaterials because of this extensive publication history of sensor devices coupled with recent

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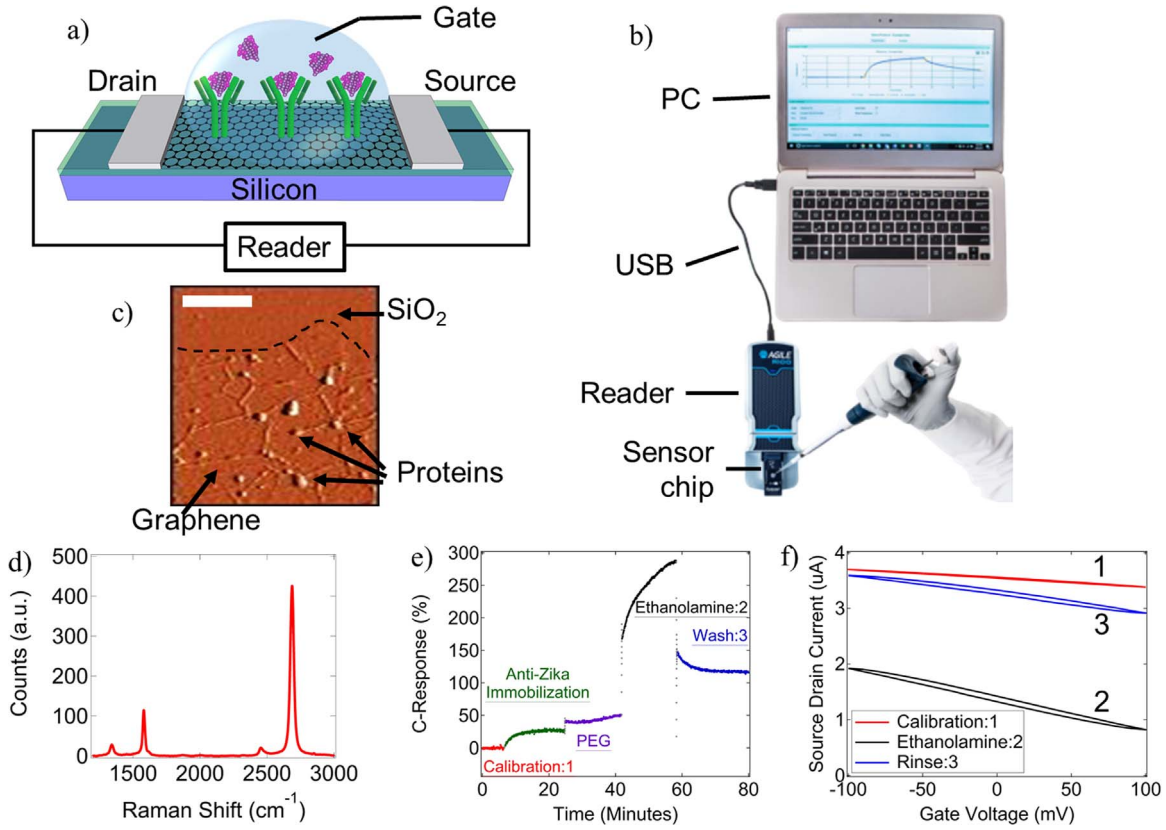

Fig. 1. (a) Diagram of the sensor element of the graphene biosensor chip. Antibodies are immobilized on pristine graphene using a zero-length linker. Along with the PEG block, these antibodies form the dielectric in a liquid gated transistor with a graphene channel. (b) Illustration of the entire sensor chip system, incorporating the sensor chip, reader electronics and digital control, and PC running control and data presentation software. (c) AFM image of the graphene after successful protein attachment, scale bar is 1 μm and Z height is 10 nm. (d) Raman spectrum after device fabrication demonstrating low D/G ratio, which indicates high quality graphene. (e) Percent change in capacitance during target immobilization and quenching on the graphene biosensor chip surface. (f) I-Vg curves at different immobilization steps. The dramatic steepening of the I-Vg slope indicates substantial change in the surface chemistry and increased sensitivity of the biosensor.

availability of graphene biosensor chips that have been processed through commercial fab, packaging and QA processes (Lerner et al., 2016). Use of commercially available biosensor chips rather than lab built biosensor chips allows us to evaluate the material and device characteristics using scaled production, closer to what is needed for a potential fielded diagnostic device compared to a laboratory constructed device. These chips are modified here to demonstrate sensitive and specific detection of Zika viral nonstructural protein 1 (ZIKV NS1).

2. Materials and methods

2.1. Graphene biosensor fabrication

Biosensor chips are fabricated at a commercial foundry as described previously (Lerner et al., 2016). Briefly, photolithography and plasma-enhanced CVD is used to pattern and passivate graphene with Ti/Pt leads on 6" silicon wafers (Lerner et al., 2016). High quality, single layer graphene films are grown via CVD on copper foil (Alfa Aesar) and transferred via bubbing transfer (Gao et al., 2012; Kybert et al., 2014). Individual dies are packaged using a chip-on-board process and encapsulated with epoxy to form an exposed well above the graphene biosensor surface.

2.2. Graphene biosensor measurement

Graphene biosensor chips are read using the commercial Agile R100 system (Nanomedical Diagnostics), previously described in detail (Lerner et al., 2016). With this system, graphene biosensor chips are inserted into an electronic reader that applies a source-drain voltage across the graphene channel and a gate voltage between the applied liquid and the drain electrode of the graphene. There are 2 read-out channels available: the I-Response (current through the channel) and the C-Response (capacitance of biosensor to the liquid). In this study, the C-Response channel is used. Control of the system, as well as data readout is performed via the Agile Plus software, which is run on a PC attached to the system via USB, as shown in Fig. 1b.

2.3. Protein Immobilization

Our previously described NHS surface chemistry was used to build sensor chips ready for protein attachment (Afsahi et al., 2017).

The biosensor chips were then functionalized with the target, anti-Zika NS1 mouse mAb 6B1 developed by the Centers for Disease Control (CDC). Anti-Zika NS1 was diluted to a working concentration of 14.6 nM in 1X phosphate buffered saline (PBS) pH 7.4. During immobilization and measurement, 75 μL of all solutions were used on the biosensor chips and incubation steps took place at room temperature. The software was calibrated to baseline in 1X PBS pH 7.4 for 60 s. Anti-Zika NS1 was immobilized on the surface by incubating for 15 min. Polyethylene glycol (PEG) has previously been shown to be an effective block against non-specific interactions in general and specifically when covalently attached to graphene devices (Gao et al., 2016; Liu et al., 2013). Thus, residual active NHS groups were first quenched using 3 mM amino-PEG_5-alcohol (amino-PEG) pH 7.4 (BroadPharm) before a final quench with 1 M Ethanolamine pH 8.5. Each quench step was done for 15 min each, followed by several washes in 1X PBS pH 7.4. The high concentration of PEG used during blocking leads to both covalently linked PEG to the graphene surface and