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# Impedimetric Dengue Biosensor based on Functionalized Graphene Oxide Wrapped Silica Particles

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ABSTRACT

A composite of 3-Aminopropyltriethoxysilane (APTES) functionalized graphene oxide (APTES-GO) wrapped on $SiO_2$ particles ($SiO_2$@APTES-GO) was prepared via self-assembly. Transmission electron microscopy (TEM) and ATR-Fourier Transform Infrared spectroscopy (ATR-FTIR) confirmed wrapping of the $SiO_2$ particles by the APTES-GO sheets. An impedimetric biosensor was constructed and used to sensitively detect dengue DNA and dengue RNA via primer hybridization using different oligonucleotide sequences. The results demonstrated that the $SiO_2$@APTES-GO electrode material led to enhanced dengue RNA detection sensitivity with selectivity and detection limit (1 femto-Molar), compared to both APTES-GO and APTES-$SiO_2$. The three-dimensional structure, higher contact area, electrical properties and the ability for rapid hybridization offered by the $SiO_2$@APTES-GO led to the successful design of a dengue biosensor with the lowest detection limit reported to date.



## 1. Introduction

Dengue fever is one of the most important arthropod-borne viral diseases that can lead to complications such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), and has the capacity to rapidly spread once a viral outbreak is established [1]. Tropical and subtropical areas in South America, Africa and South-East Asia are especially affected by dengue virus (DENV), which is spread by mosquitoes. Moreover, global warming, intercontinental transportation, and international travel are transforming DENV from a once limited regional problem into a global one [2]. According to the WHO report (World Health Organization), about 40% of world's populations is at risk of dengue and it is presently endemic in over 100 countries. The CDC (Center of Disease Control) estimates that as many as 400 million people are infected yearly.

Early diagnosis of dengue is crucial to decreasing medical conditions [3], such as hemorrhage and shock from DHF and DSS, because there is no effective vaccine to prevent the dengue virus infection. A recent large test trial on the CYD-TDV vaccine for dengue did not provide the anticipated results. The vaccine proved to be ineffective, especially for the dengue serotype 2, which was the predominant serotype in the test [4].

Current DENV detection methods rely on complex polymerase chain reaction (PCR) and enzyme-linked immune-sorbent assay (ELISA). However, performing these tests require a high time investment, and meticulous specimen preparation [5,6]. Although the ELISA method is less complicated than PCR, it requires several days between fever symptom emergence and diagnosis because it is based on the detection of immunoglobulin (Ig) in blood. Thus, the test cannot be conducted until either antibodies such as IgM [7] or IgG are produced in response to infection.

Biosensors are bioanalytical tools that measure the presence of analytes by combining the sensitivity of biomolecular recognition elements with a physical transduction mechanism. They play a major role in the development of time-effective, low-cost and easy-to-use analytical tools and are particularly suitable for miniaturization and portability. Their advantages include their high sensitivity and specificity provided by the biocatalytic or biorecognition sensing elements. Various kinds of biosensors (enzyme-based, immunosensors, DNA-sensors) have been broadly studied but only few of them have been successfully commercialized [8].

The biosensors market is expected to grow from $6.72 billion in 2009 to $14.42 billion in 2016. Most of the developed biosensors address medical needs and are used for diagnostics purposes [8,9]. Applications in environmental and agricultural fields, and particularly for anti-terrorist activity and homeland security, are also rapidly increasing [10,11]. For example, optical biosensors have now the highest sensitivity, approaching theoretical limits of

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interface sensitivity, which is critical for detection of drug candidates, viruses or pathogens [12–16].

Electrochemical biosensors function on the basis of correlating the electronic signal given off upon interaction of the biological recognition element with the analyte. There are different types of electrochemical biosensors, which measure the electrical properties of an electrode surface and the binding kinetics of molecules. In particular, electrochemical impedance spectroscopy (EIS) can measure the changes of the electrical properties of a surface arising from the interaction with the captured analyte [17], while minimizing sample damage during measurements.

Graphene has emerged as a promising candidate material for electrical applications due to its high electrical conductivity, chemical stability, and high mechanical strength [18,19]. These characteristics make graphene an attractive material platform for electrochemical biosensor development. Incorporating graphene-based materials in biosensing configurations led to enhanced sensitivity, low detection limits, and long-term stabilities for various types of biosensors [18,20]. In particular, graphene has been used for integration in DNA sensing platforms. Graphene sheets functionalized with polyaniline and Au nano-particles were developed to further enhance electrical conductivity and surface characteristics [21]. CVD-grown graphene–Pt (MPA) nanocomposites were also reported as successful biosensing platforms for the detection of human C-reactive protein [22]. The chemical reactivity and high surface area of graphene allowed for a high loading of Pt particles, which, in turn, resulted in the attachment of a large number of antibodies, and therefore enhanced sensor performance in the detection of the human C-reactive protein.

Graphene wrapped nanoparticles, on the other hand, exhibited excellent electrochemical performance in batteries and capacitors [23,24], but were rarely explored as a biosensor material. A graphene-encapsulated nanoparticle FET biosensor for cancer biomarker detection was among the few such reports. The detection limit for this sensor was 100 pM target breast cancer biomarkers (HER2) [25].

Here we report on the fabrication of positively charged graphene wrapped particles and their use as sensitive biosensor material platform for dengue DNA and RNA detection. Graphene oxide (GO) sheets were functionalized with 3-Aminopropyltriethoxysilane (APTES) to induce a positive surface charge before coating silica nanoparticles ($SiO_2$). Next, the particles were incorporated into an impedimetric biosensor and functionalized with specific primers for the detection of dengue DNA and RNA separately. Sensitivity, selectivity, and detection limits are here reported for the first time for a Dengue biosensor employing this type of electrode material.

## 2. Experimental

### 2.1. Biological elements preparation

The oligonucleotide primers, dengue complementary DNA, and non-complementary DNA, were purchased from Integrated DNA Technologies, Inc. Serotype 2 Dengue DNA was used as complementary DNA and West Nile virus DNA was used for the source of non-complementary DNA. An oligonucleotide primer was designed and used as a probe for DNA hybridization and its sequence is 5′-GGT-TGG-ATG-CGC-GCA-TCT-ATT-CTG-ACC-CAC-TGG-3′.

The primer for RNA was designed as this sequence 5′-ATA-CAA-TGT-GGC-ATG-TCA-CAC-GTG-GCG-3′ and Integrated DNA Technologies, Inc. prepared the sequence of primer. The Serotype 2 Dengue RNA was prepared using infected mosquito cell lines by extraction and precipitation. (See supplementary information)

### 2.2. Materials synthesis

Silica particles were synthesized by the modified Stober method [26]: 9.01 ml of DI water, 50 ml of ethanol (100%, KOPTEC) and 1.37 ml of ammonium hydroxide ($NH_3$ 28 ~ 30%, Sigma-Aldrich) were mixed together and 3.2 ml of tetraethyl orthosilicate (TEOS, 99%, Fluka) was added drop-wise into the mixed solution. After 1 hour, the synthesized particles were separated from the mixed solution using an ultracentrifuge (Eppendorf AG 22331, Hamburg, Germany) spinning at 14.5 krpm, and then repeatedly washed using ethanol at least six times. The washed particles were first dried at 80 °C for 6 hours and then were grinded into fine particles. Subsequently, they were heat-treated in air at 110 °C for 24 hrs. The end product was finally grinded again.

The graphene oxide (GO) sheets were prepared through chemical oxidation of graphite particles by a modified Hummer's method [27]. The oxidized graphene was washed and dried under vacuum environment then finally exfoliated via ultra-sonication.

Positively charged graphene oxide was prepared using 3-Aminopropyltriethoxysilane (APTES, 99%, Sigma-Aldrich) by the reflux method. 20 mg of GO was first dispersed in 100 ml of toluene (99.8%, Sigma-Aldrich). The GO dispersed solution was degassed using nitrogen gas (99.995%) for 15 min to remove oxygen within the solution, then 0.6 ml of APTES was injected into the mixed solution. The solution was stirred for 3 hrs at 30 °C in a nitrogen atmosphere and then refluxed at 110 °C for 10 hrs under an inert nitrogen gas environment. The APTES grafted-GO (APTES-GO) was rinsed several times with toluene, ethanol and DI water, using an ultracentrifuge. APTES-grafted $SiO_2$ particles were prepared through the reflux method, similarly to the preparation of APTES-GO. (See supplementary information)

Each material was dispersed in aqueous solution using 12 mg of APTES-GO, 4 mg of $SiO_2$ particles and DI water separately. The APTES-GO solution was dropped into the $SiO_2$ dispersed solution under ultra-sonication, then stored for 24 hrs. The coagulated $SiO_2$@APTES-GO composite was rinsed using an ultracentrifuge with DI water several times.

### 2.3. Materials characterization

Microstructural observations were performed using a FEI-Tecnai Transmission Electron Microscope (TEM), to confirm the morphology and size of $SiO_2$ particles, as well as the microstructure of the $SiO_2$@APTES-GO composite. The surface charge was measured using a Malvern Zetasizer (Nano Z, Malvern, UK). We employed 0.02 wt% of material dispersed DI water solution to check Zeta (ζ) potential. The Smoluchowski model was used in order to convert from the electrophoretic mobility to ζ potential. The functional groups present before and after self-assembly synthesis of the composite were confirmed via ATR-FTIR spectroscopy using a Spectrum 100 FTIR spectrometer (Perkin Elmer, Waltham, MA).

### 2.4. Electrochemical characterization

Biosensor platform was fabricated on a 5 mm platinum electrode. Functionalized material was dispersed on the Pt electrode than primer was immobilized on the material coated electrode that finally incubated in DNA or RNA solution for hybridization. A platinum electrode was first cleaned by polishing with alumina paste and washing by sonication with DI and ethanol solution, and subsequently used for biosensor platform fabrication. A concentration of 0.2 wt.% of the positively functionalized material (APTES-$SiO_2$, APTES-GO, and $SiO_2$@APTES-GO) solution was prepared using DI water. A volume of 20 ul of the mixture was dropped on the Pt electrode, and then unadsorbed materials were