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# Microscale loop-mediated isothermal amplification of viral DNA with real-time monitoring on solution-gated graphene FET microchip

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ABSTRACT

Rapid and reliable molecular analysis of DNA for disease diagnosis is highly sought-after. FET-based sensors fulfill the demands of future point-of-care devices due to its sensitive charge sensing and possibility of integration with electronic instruments. However, most of the FETs are unstable in aqueous conditions, less sensitive and requires conventional Ag/AgCl electrode for gating. In this work, we propose a solution-gated graphene FET (SG-FET) for real-time monitoring of microscale loop-mediated isothermal amplification of DNA. The SG-FET was fabricated effortlessly with graphene as an active layer, on-chip co-planar electrodes, and polydimethylsiloxane-based microfluidic reservoir. A linear response of about 0.23 V/pH was seen when the buffers from pH 5–9 were analyzed on the SG-FET. To evaluate the performance of SG-FET, we monitored the amplification of Lambda phage gene as a proof-of-concept. During amplification, protons are released, which gradually alters the Dirac point voltage ($V_{Dirac}$) of SG-FET. The resulting device was highly sensitive with a femto-level limit of detection. The SG-FET could easily produce a positive signal within 16.5 min of amplification. An amplification of 10 ng/μl DNA for 1 h produced a $\Delta V_{Dirac}$ of 0.27 V. The sensor was tested within a range of $2 \times 10^2$ copies/μl (10 fg/μl) to $2 \times 10^8$ copies/μl (10 ng/μl) of target DNA. Development of this sensing technology could significantly lower the time, cost, and complications of DNA detection.



## 1. Introduction

Even with the tremendous development in bio-sensing technologies, the diagnosis of viral diseases still follows enzyme-linked immunosorbent assay (ELISA) and blotting based analysis. These conventional methods to detect biomarkers require a high-cost labeling process and have a complex detection sequence with long analysis time. Thus, a much of valuable time is lost in diagnosis rather than for the treatment. Likewise, most of the reported biosensors require an additional reporter molecule and intricate sensor components. Therefore, rapid, simple, and accurate diagnostic devices are needed to diagnose the disease efficiently before its advancement.

Recently, loop-mediated isothermal amplification (LAMP) is gaining attention for its outstanding amplification and rapid diagnostic ability (Notomi et al., 2000; Parida et al., 2004; Shirato et al., 2007; Thai et al., 2004). LAMP is a gene amplification technique that uses a *Bst* DNA polymerase and multiple primers to amplify the template DNA in the isothermal condition and in a short time with high selectivity. In earlier days, monitoring of LAMP relied on optical methods, due to the change in turbidity by the released magnesium pyrophosphate precipitate as a byproduct during amplification (Mori et al., 2004). Lately, a number of miniaturized LAMP sensors with electrical analysis of amplicon have been reported. The single temperature condition for the gene amplification during LAMP is advantageous for developing a point-of-care (POC) system (Hsieh et al., 2012; Sayad et al., 2016; Sun et al., 2015; Veigas et al., 2014).

Graphene, a single carbon layer of graphite structure, has also emerged as a material of interest for fabricating biosensors. It has a large surface area, high electrical conductivity, excellent mechanical strength, and can be functionalized easily. Its outstanding properties and biocompatibility have influenced applications in numerous biosensors (He et al., 2012; Lv et al., 2010; Reiner-Rozman et al., 2015). Among various kinds of graphene-based sensors, a solution-gated field effect transistor (SG-FET), in which the gate and graphene active layer are separated by electrolyte instead of the dielectric insulator is a promising sensing platform. SG-FET is a better biosensor because it works on the electrical field based high transconductance (Hess et al., 2011). SG-FET based biosensors have proved to be highly sensitive, selective, and stable. The electrons in carbons of graphene active layer are all exposed outside the plane, which makes it very sensitive to subtle changes such as a change in pH or DNA attachment (Dong et al., 2010; Ohno et al., 2009; Yan

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et al., 2014; Zhang et al., 2014). The other major FET and pH based sensing platform is ion-sensitive FET (IS-FET) and was first reported 40 years ago. Bergveld et al. have extensively discussed the advancement of IS-FETs as sensors (Bergveld, 2003). Generally, IS-FETs are fabricated on a silicon substrate using CMOS process (Huang et al., 2015; Moser et al., 2016). Although IS-FETs have proven their worth, it is laborious to fabricate silicon and CMOS processed IS-FETs. Consequently, with silicon as a substrate, flexible IS-FET sensors are quite not possible. The usual structures of IS-FET and SG-FET embody a gate (reference) electrode immersed in the electrolyte from outside. This external electrode hinders the miniaturization of the device for portable use. Additionally, an internal fluid network is essential for creating a multiplexed lab-on-a-chip.

Several groups have attempted electrical detection of DNA amplification, in the past. Most of these reports achieved success in terms of DNA detection, but the presented techniques are expensive and complex to implement. Pourmand et al. reported a charge perturbation based detection of DNA synthesis. However, it requires immobilization of DNA on gold, which is tedious (Pourmand et al., 2006). Similarly, an electrochemical-based study of LAMP suggested conversion of the released pyrophosphate into electrochemically active molybdophosphate (Xie et al., 2015). However, a real-time monitoring of amplification is not possible following this study. Additionally, numerous other works on DNA detection through hybridization or covalent bonding on graphene transistors have been done (Chen et al., 2013; Dong et al., 2010; Green and Norton, 2015; Guo et al., 2011; Yin et al., 2012), but the real-time monitoring of DNA amplification on solution-gated graphene transistor has seldom been attempted.

Therefore, in this work, a monolithic solution-gated graphene FET is presented for the real-time monitoring of LAMP of viral DNA. The FET was fabricated on a glass substrate containing thin-film coplanar electrodes instead of silicon substrate and conventional Ag/AgCl electrode. The label-free sensing of DNA was depended on the change in Dirac point voltage ($V_{Dirac}$) of FET, due to the release of protons during LAMP (Toumazou et al., 2013). We demonstrate the potential of SG-FET to quantify Lambda phage gene in real-time. Development of this sensing technology could significantly lower the time, cost, and complications of DNA detection in the future.

## 2. Experimental

### 2.1. Fabrication of solution-gated graphene FET

The overall scheme for graphene transfer, microchannel fabrication, and SG-FET fabrication is shown in Fig. S1. We fabricated two FETs with common gate electrode on one substrate for multiplexed analysis; however, in this study, each FET was independently used (Fig. 1(a) and (b)). Coplanar gold electrodes for source, drain, and gate were patterned through standard photolithography and thermal evaporator on a glass substrate (Han et al., 2013). In summary, the AZ-1512 photoresist was spin-coated on glass and patterned using UV-exposure. After patterning, titanium (supporting layer) followed by gold was deposited on the glass in a vacuum thermal evaporator. The additional photoresist was removed by ultra-sonicating the glass in an acetone bath. The width of the patterned electrodes was 1 mm with a separation of 1 mm between each of them. The gate electrode exposed inside the fluid channel provided gate voltage through the electrolyte.

Graphene layer grown by chemical vapor deposition was purchased from Graphenea (Spain). The copper (Cu) foil containing graphene layer was cut in the size of $3 \times 3$ mm. A thin layer of polydimethylsiloxane (PDMS) (sylgard 184, Dow Corning, USA, A: B=9:1) was spin coated on the graphene/Cu sheet and cured in a dry oven for 30 min, at 75 °C. After curing, the PDMS/graphene/Cu sheet was floated on $FeCl_3$ (0.25 mg/ml) solution for 30 min, at room temperature to etch the Cu. After Cu etching, the transparent PDMS/graphene sheet was washed with copious amount of DI water. The cleaned PDMS/graphene was later placed on glass substrate containing patterned gold electrodes. The PDMS/graphene sheet was aligned on the source and drain electrode under an optical microscope. The graphene sheet was allowed to bond with the glass substrate by incubating at 75 °C for 3 min. Finally, the PDMS thin film was removed from the graphene using tweezers. A negatively molded PDMS-based microfluidic channel was

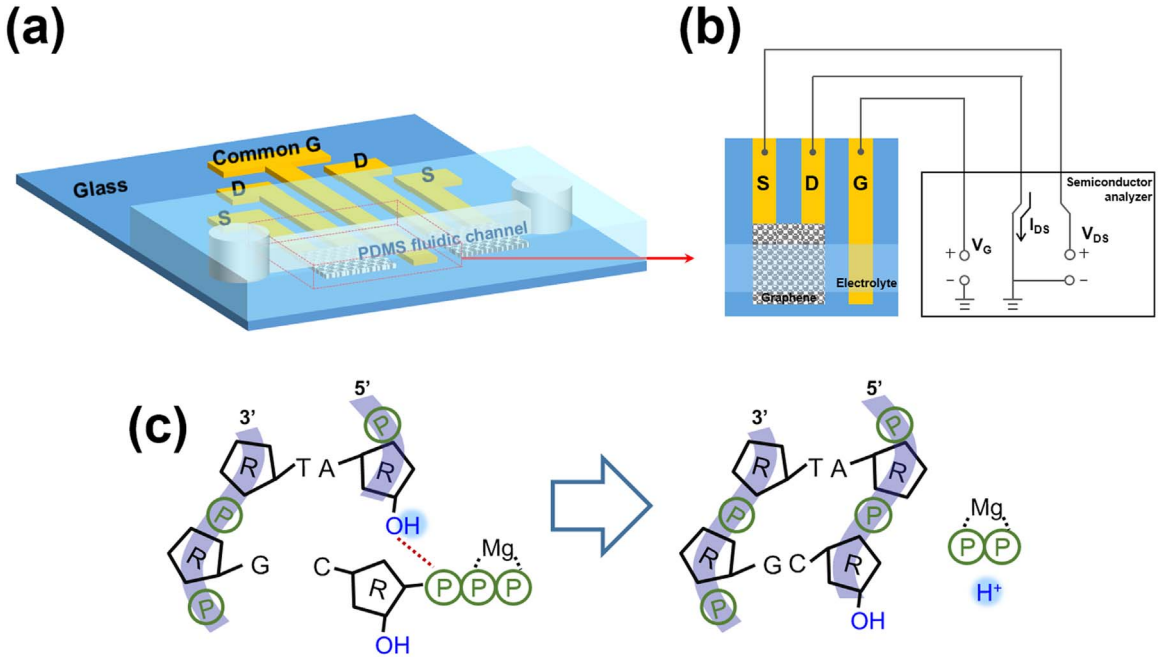


**Fig. 1. Solution-gated graphene FET (SG-FET)**: (a) schematic of SG-FET couple with common gate; where S, D, and G are source, drain, and gate electrodes respectively, (b) measurement setup of a single SG-FET, and (C) mechanism of proton release during DNA synthesis.