Biological and Medical Applications of Materials and Interfaces

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ACS Appl. Mater. Interfaces, **Just Accepted Manuscript** • DOI: 10.1021/acsami.8b04260 • Publication Date (Web): 25 Jun 2018

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The detection of bisphenol A using DNA-functionalized graphene field effect transistors integrated in microfluidic systems

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KEYWORDS: Solution-gated graphene transistor, graphene, Bisphenol A detection, Biosensor, DNA aptamer

ABSTRACT

Bisphenol A (BPA) detection has attracted much attention recently for its importance to food safety and environment. DNA-functionalized solution-gated graphene transistors are integrated in microfluidic systems and used for recycling detections of BPA for the first time. In the presence of BPA, both single- and double-stranded DNA molecules are detached and released from graphene surface in aqueous solutions, leading to the change of device electrical performance. The channel currents of the devices change monotonically with the concentration of BPA. Moreover, the devices modified with double-stranded DNA are more sensitive to BPA

and show the detection limit down to 10 ng/mL. The highly sensitive label-free BPA sensors are expected to be used for convenient BPA detections in many applications.

1. INTRODUCTION

The contamination of bisphenol A (BPA) in many commercial products has attracted tremendous attention for its adverse impact on animal, human health and environment[1-3]. As one of the widely used chemical and raw materials for producing epoxy resins and some polycarbonate polymers, BPA is exposed through plastic and paper industries in many respects in the daily lives of human beings. According to the regulations in European Union, BPA residue is allowed to be less than 3 mg/kg in a food container or a packing material made by polycarbonate[4]. Thus, rapid and low-cost techniques for the determination of BPA with high sensitivity are urgently needed. Although many methods have been exploited fort BPA detection, including gas chromatography coupled with mass spectrometry (GC-MS)[5], high-performance liquid chromatography (HPLC)[6, 7], fluorimetry[8] and enzyme-linked immunosorbent assay (ELISA)[9], these techniques are unportable, expensive and time-consuming.

Nowadays, another technique based on electrochemical characterization has been widely applied into BPA determination. Due to their fast responses and real-time detections, electrochemical BPA sensors have been reported by many groups[10-19]. Most of the devices are based on the construction or modification of electrodes with nanoparticles (NPs), carbon nanotubes (CNTs), graphene, and other nanomaterials to increase their surface area, conductivity and electrochemical activity. These techniques can improve the sensitivity of BPA detection and decrease the operational voltage of the devices. Specifically, Zheng et al. presented glassy carbon electrodes functionalized with Pt NPs-modified poly(diallyl dimethyl ammonium chloride)-

diamond composite for electrochemical sensing of BPA and bisphenol S with the sensitivity range of 5-30 μM (1.1 - 6.8 μg/mL) and 10-60 μM (2.3-14 μg/mL), respectively[17]. Another highly sensitive electrochemical BPA sensor was realized with the immobilization of tyrosinase onto a diamond electrode that was also modified with diazonium, and CNTs[18], which demonstrated a large linear detection range between 10 pM and 100 nM (2.3pg/ml to 23 ng/mL). In 2017, an electrochemical sensor based on a highly conductive graphite NP-modified electrode was reported by Piao et al., showing a detection limit of 35 nM (10 ng/mL)[19]. Besides, a simple and convenient way of using a label-free sensor has been reported to have a detection limit of 0.1 ng/mL, in which aggregation-induced color change can be visualized even by naked eyes[20].

Recently, thin film transistors based on graphene have obtained much attention in the applications of various chemical and biological sensors[21-30]. As a two-dimensional material, graphene is quite sensitive to the charge environment variations due to the reaction or adsorption of an analyte on the surface of it. Hence, a graphene transistor is promising in the detection of various molecules, such as BPA. Herein, we have developed a solution-gated graphene transistor (SGGT) modified with DNA molecules in a microfluidic system for a recycling detection of BPA. The device shows a high sensitivity and a low detection limit of 10 ng/mL, which is sensitive enough for characterizing BPA levels in many applications. As the recognition element of sulphur, both single stranded probe DNA (ssDNA) and double stranded complementary DNA (dsDNA) molecules are utilized to functionalize graphene surface with the assistance of Au NPs. In the presence of BPA in the microfluidic system, DNA will be destroyed and detached from the graphene surface, leading to the change of the electrical performance of the devices. More important, the device is reusable and stable. This technique provides a novel approach for the repeated characterization of BPA concentration in aqueous solutions.

2. EXPERIMENTAL

2.1 Graphene fabrication and transfer

Single-layer Graphene was grown on copper foils by a chemical vapor deposition (CVD) method. Graphene layers were then transferred to target substrates by the following method reported in our previous work[31, 32]. A thin layer (100 nm) of poly(methyl methacrylate) (PMMA) was spin-cast on the surface of graphene on a Cu foil. After the sample was annealed at 90□ for around 30 min, it was transferred into an aqueous solution of iron chloride. Several hours later, Cu foil underneath the graphene/PMMA was etched, leaving a semitransparent thin film floated on the solution surface. Then the graphene/PMMA film was moved to deionized (DI) water. The sample was taken out by using a glass substrate, followed by a treatment of acetone that can remove the PMMA from graphene. According to our previous report[26], to have Au doping, the graphene film was placed in a $HAuCl_4$ aqueous solution (Concentration: 2 mM) at 80 □ for around 30 min. The sample was then taken out and rinsed by DI water to remove the unattached Au NPs. After that, lots of Au NPs can be immobilized onto the graphene surface.

2.2 Device fabrication

The source and drain electrodes (Cr/Au, 10/100 nm) were fabricated on top of patterned graphene films (4 mm×2 mm) by magnetic sputtering through a shadow mask. The microfluidic chips were fabricated by patterning channels in poly(dimethylsiloxane) (PDMS) (Sylgard 184, Midland, MI, USA) using soft lithograph as reported before[21]. The channel width of a graphene transistor between the source and drain electrode is 1 mm, while the length and width of the microfluidic channel is 10 mm and 0.8 mm, respectively. An Ag/AgCl wire (Diameter: 100 μm) was placed into the PDMS channel as a gate electrode. The distance between the gate and the

graphene channel is about 2.0 mm. PDMS chip was treated by oxygen plasma and then bonded onto the glass substrate. The flow velocity in the microchannel was controlled by a syringe pump (Baoding Longer Precision Pump Co., Ltd.).

2.3 DNA immobilization

According to the previous reports, an anti-BPA ssDNA aptamer (sequence: 5'-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA -3') can bind specifically to BPA molecule with high affinity[20, 33].We chose this highly sensitive and selective anti-BPA ssDNA for BPA detection, which was synthesized by Sangon Biotech (Shanghai) Co., Ltd. Phosphate-buffered saline (PBS, pH 7.0) solution containing ssDNA probes with a concentration of 10 μM was injected into the microfluidic channel and incubated for 12 hours to ensure the immobilization of ssDNA on the Au NPs on the graphene layer. Then, the channel was rinsed with pure PBS solution, which can remove the residual and weakly-bonded DNA molecules. Complementary DNA (sequence: 5'-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA -3') were chosen to form dsDNA with DNA probes, which was also synthesized by Sangon Biotech (Shanghai) Co., Ltd. After the immobilization of ssDNA probes on the graphene channel, 10 μM complementary DNA PBS solution was injected into the channel for incubation. After 6 hours, the channel was rinsed with a fresh PBS solution to remove redundant and physically adsorbed DNA molecules.

2.4 BPA detection

The device with ssDNA and dsDNA were then used for the detection of BPA, respectively. BPA powder (M=228.3) was first dissolved into PBS solution with the concentration ranging from 10

ng/mL to 100 μg/mL. Then, the various concentrations of BPA solutions from low to high concentration were injected into the microfluidic channel sequentially and waited for 30 minutes at each concentration. For every measurement, the channel was rinsed with pure PBS solution to remove the residual and weakly-bonded DNA molecules and the SGGTs were measured in pure PBS solution.

2.5 Characterization

Graphene films modified with Au NPs were characterized under a transmission electron microscopy (TEM, JEOL JEM-2010). The SGGTs were measured by using a semiconductor parameter analyzer (Agilent 4156C). For transfer curves of the devices, the channel currents (I_d) between the source and drain electrodes were measured as functions of the gate voltages (V_g) under a drain voltage (V_{ds}) of 0.05 V. For the measurement of time-dependent I_d, the V_g and V_{ds} were kept at 0 and 0.05 V, respectively.

3. RESULTS AND DISCUSSION

Figure 1a displays the schematic diagram of a SGGT fabricated on a glass substrate with a cover of PDMS channel. The graphene channel was modified with Au NPs, as shown in the TEM image in **figure 1b**. Lots of Au NPs with a rather uniform diameter of around 30 nm can be observed on the surface of graphene. The probe DNA molecules were then immobilized onto the Au NPs on the graphene surface. **Figure 1c** shows the transfer characteristics (I_d versus V_g at the drain voltage of V_{ds} =0.05 V) of the device before and after the immobilization of DNA. A typical ambipolar behavior of the device can be observed[21]. The Dirac point of the transfer curve of the device before the immobilization of DNA is around 0.22 V, indicating a p-type doping effect induced by the Au NPs. A significant left-shift (n-doping) for 70 mV of the transfer curve

was observed after the immobilization of probe ssDNA onto Au NPs. The n-type doping effect on graphene caused by DNA can be attributed to the negative charge of DNA molecules in the PBS solution, which is in agreement with the previous reports[34-36].

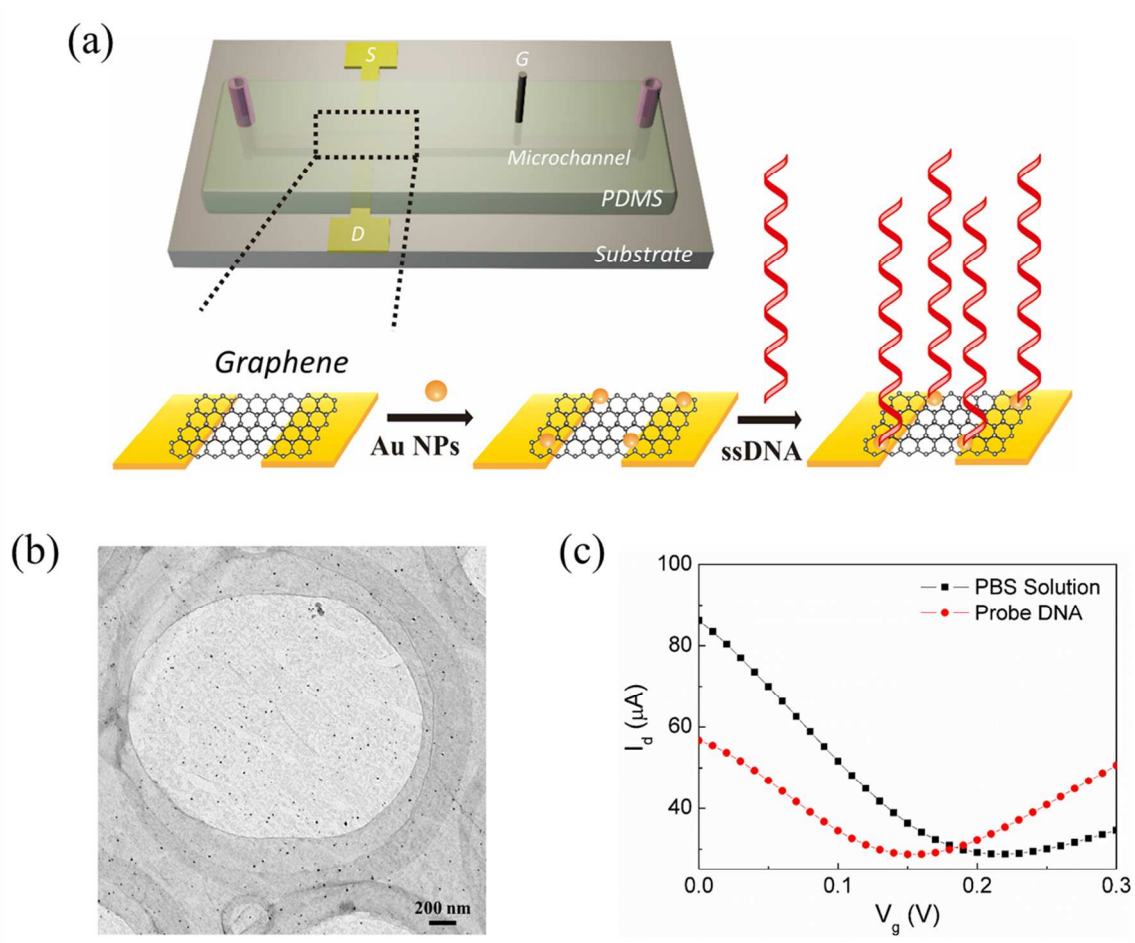

Figure 1. (a) Schematic illustration of the graphene transistor functionalized with Au NPs and ssDNA molecules. (b) TEM image of a single-layer graphene film doped with Au NPs, (c) Transfer characteristics of the SGGT modified by Au NPs and probe DNA.

The device with probe DNA was then used for the detection of BPA dissolved in PBS solution. The BPA solutions from low to high concentration were injected into the microfluidic channel sequentially. **Figure 2a** shows the transfer curves of the device before and after the BPA

treatment. It can be observed that the transfer characteristics of the device modified with probe DNA shift from left to right with the increase of BPA concentration. The device can detect BPA with the concentration as low as 1 μg/mL, which causes an obvious shift of the Dirac point from 0.15 V (without BPA treatment) to 0.16 V. Further increases of the BPA concentration lead to more obvious shifts of the Dirac point, indicating that BPA can induce DNA release from graphene surface and reduce the n-doping effect from probe DNA, which is opposite to the effect of DNA probes on the graphene channel. When the concentration of the BPA increased to 100 μg/mL, the Dirac point of the transfer characteristic can shift back to 0.21 V, which is almost the same as the pristine device before DNA modification.

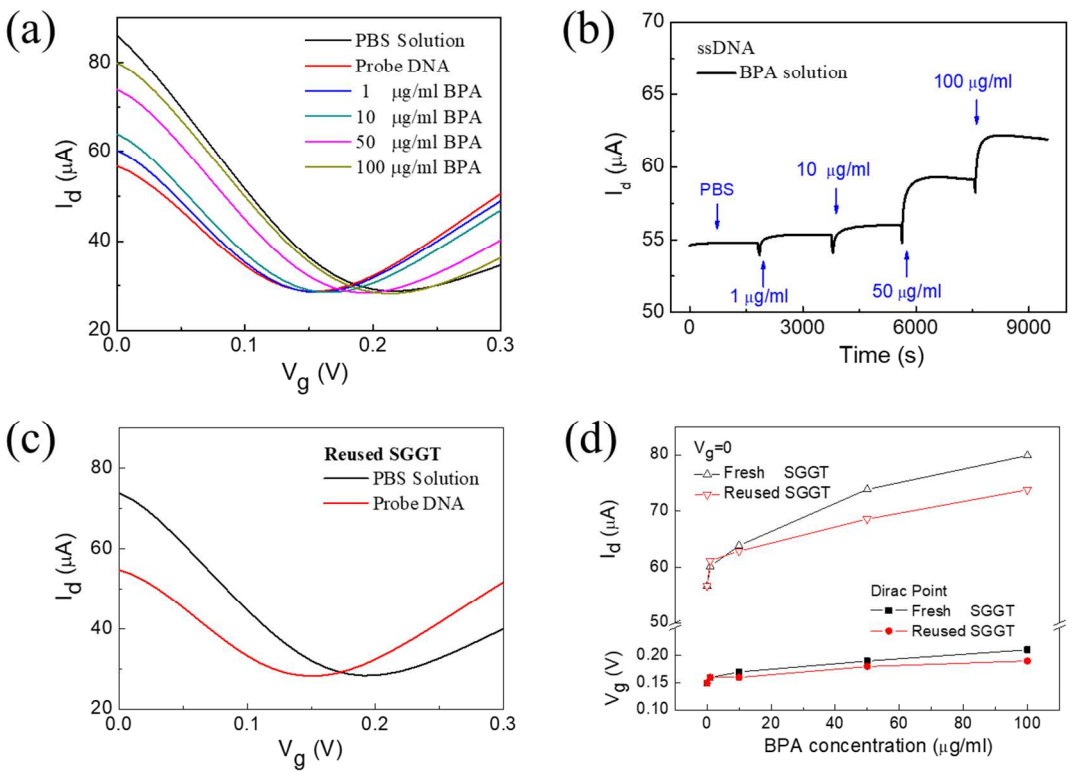

Figure 2. (a) Transfer characteristics of a SGGT modified by probe DNA for the detection of different concentrations of BPA, (b) Time-dependent I_d of a ssDNA -modified SGGT characterized with the injection of BPA solution with different concentrations (V_{ds}=50 mV,

V_{gs}=0), (c) Transfer characteristics of the reused SGGT modified by probe DNA, (d) The variation of I_d (when V_g=0) and V_g of the Dirac point for the transfer curves of the fresh and reused SGGT modified by probe DNA as a function of different concentrations of BPA.

Next, the real-time responses of the device with DNA functionalized to the additions of various concentrations of BPA in PBS solution were monitored. **Figure 2b** shows the time-dependent I_d upon the additions of BPA with different concentrations ranging from 1 to 100 μg/mL measured at the fixed gate and drain voltage (V_g = 0, V_{ds} = 0.05 V). Notably, the value of I_d increases with the increase of BPA concentration. We can observe an obvious current response to BPA injection even at a concentration as low as 1 μg/mL, which is consistent with the response of the transfer curve shown in figure 2a. After every 30 min, along with the concentration of the BPA increased gradually from 1 μg/mL to 100 μg/mL, each upward step of I_d from around 55 μA to 62 μA can be observed correspondingly.

The effect of BPA on the ssDNA molecules modified on graphene surfaces was also observed under an inverted fluorescence microscope. The aptamer (sequence: 5' HS-C_6-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA -FAM-3') was immobilized on the Au NPs of graphene surface, pristine PBS and BPA contained PBS buffer were used to treat the samples, respectively. The variation of the aptamer on the surface of the graphene before and after the treatment was characterized by the inverted fluorescence microscope. As shown in figure S2, comparing the images of the same sample, it can be observed that with only 1 μg/mL BPA solution treatment, the amount of the aptamer on the graphene surface decreases significantly (see figure S2e). When the concentration of BPA in PBS buffer was increased to 10 μg/ml and used to treat the samples, as depicted in figure S2f, the

aptamer cannot be clearly observed, which indicates that the BPA sensitive aptamer is detached from the surface of graphene after the BPA treatment. While on another sample treated with pristine PBS solution without BPA, most of the aptamer were remained on the graphene surface (see figure S2b and S2c). Therefore, the BPA treatment can induce the detachment of ssDNA from graphene surface.

The sensing mechanism can be attributed to the interaction between BPA and the ssDNA probes and the DNA damage resulting from BPA treatment is irreversible[37-42]. In the previous report, Qiu et al. investigated that the BPA radicals resulted from the electro-oxidation of BPA led to the damage of DNA molecules[38]. In this process, phenoxenium ions (II) as one type of phenoxy radicals were generated from BPA molecules, which could react with some kinds of nucleophiles. Thanks to the nucleophilic sites on the DNA probes, the DNA molecular structure could be changed by the electrophilic reagents, resulting in the break of the S-Au bond. Therefore, some of the DNA probes could be released from the graphene surface and removed out of the channel by PBS solution. It can be observed that the Dirac point of the graphene transistor shifts to a more positive gate voltage with the increase of BPA concentration due to the decreased amount of ssDNA on the graphene channel. When the BPA concentration is about 100 μg/mL, the transfer curve is recovered to the original position of the device before the modification of DNA, indicating that most of the anchored ssDNA probes are detached from the graphene channel. Therefore, the n-type doping on the graphene channel by ssDNA is alleviated by BPA.

Since ssDNA probes can be detached completely by BPA at a high concentration and without any damage to the graphene template, we attempted to reuse the device for BPA determination in the second round. After the immobilization of ssDNA probes on the graphene channel with the

same condition used in the first round, the device was used to detect BPA from low to high concentration again. As shown in Figure 2c, the transfer curve shifts to a negative gate voltage due to the n-type doping of the ssDNA probes. The responses of the device to different BPA concentrations are shown in figure S1 in the supporting information. Similar to the result observed in the first round, the transfer curve shifts horizontally to a higher gate voltage with the increase of BPA concentration. Figure 2d shows the device responses characterized in the first and second rounds. The responses of I_d (V_g=0) and V_g (at the Dirac point) increase monotonically with the increase of BPA concentration. These results obtained in the two rounds indicate that the SGGT device is sensitive and reusable, which can significantly decrease the cost for repeated BPA detection.

In the previous report from Qiu et al., they studied the interaction of BPA with ssDNA and dsDNA on modified electrodes and demonstrated that the current of BPA oxidation at a ssDNA-modified electrode was smaller than that obtained at a dsDNA-modified electrode due to the lack of the double helix in ssDNA and the weaker interaction between the ssDNA and BPA molecules in comparison with the case of dsDNA. Since dsDNA can be more sensitive to BPA molecules[33], in order to further improve the BPA detection sensitivity of our device, we used dsDNA to modified the surface of graphene template. Complementary target DNA (sequence: 5'-CCG GTG GGT GGT CAG GTG GGA TAG CGT TCC GCG TAT GGC CCA GCG CAT CAC GGG TTC GCA CCA -3') were hybridized with DNA probes to form dsDNA on the graphene channel. **Figure 3a** shows the transfer curves of the device characterized in a PBS solution before, and after the immobilization of probe ssDNA, and the hybridization of complementary dsDNA. It can be observed that, after the immobilization of ssDNA, the Dirac point of the device shifts to a negative gate voltage, and a more negative shift is induced by the DNA hybridization,

indicating that dsDNA can induce a more pronounced n-type doping effect on graphene[34, 43]. Next, the device was used for the detection of BPA with different concentrations. As shown in **figure 3b**, after the BPA treatment, the transfer curve of the device shifts to a more positive gate voltage with the increase of BPA concentration. Notably, the dsDNA -modified SGGT can show an obvious response to the lowest BPA concentration of 10 ng/mL, which is two orders of magnitude lower than the detection limit of the device with ssDNA probes. We can find that 10 ng/mL of BPA can induce a right shift of the Dirac point of the device for about 10 mV. When the BPA concentration is 100 μg/mL, the transfer curve of the device almost shifts back to the original position before the DNA modification.

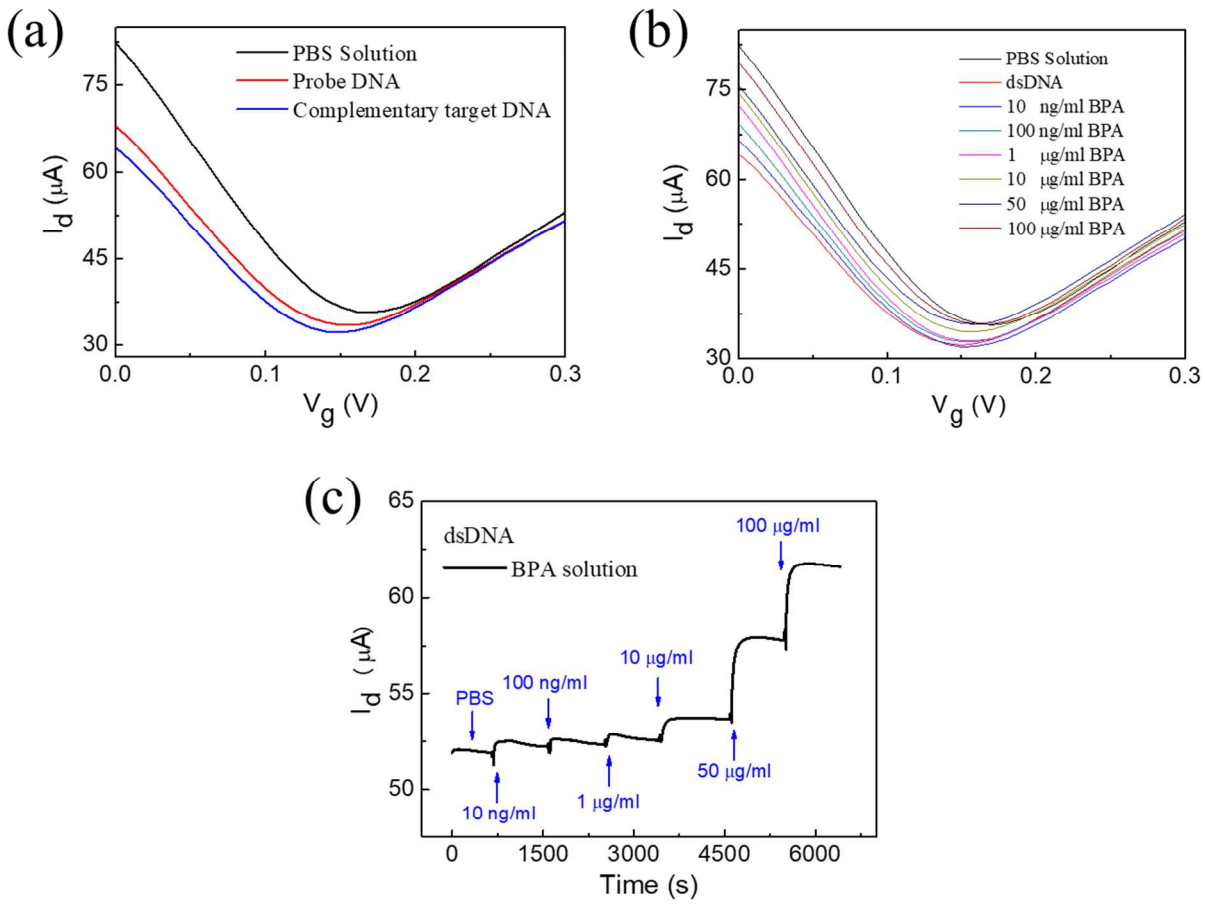

Figure 3. (a) Transfer characteristics of SGGT before and after the modification of ssDNA and dsDNA molecules, respectively; (b) Transfer characteristics of a SGGT modified by dsDNA for the detection of different concentration of BPA; (c) Time-dependent I_d of a dsDNA -modified SGGT characterized with the injection of BPA solutions with different concentrations (V_{ds}=0.05 V, V_{gs}=0).

Time-dependent channel current response of the dsDNA -modified device with the increase of BPA concentration is illustrated in **figure 3c**. An obvious increase of channel current from 51.9 μA to 52.5 μA can be observed when the BPA concentration is only 10 ng/mL. The increase of the channel current can be attributed to the release of dsDNA by BPA, which is consistent with results shown in **figure 3b**. Notably, the SGGT modified with dsDNA shows a much higher sensitivity to BPA than the device modified with ssDNA. The detection limit of the device is extended for two orders of magnitude by the dsDNA. It is noteworthy that, although our presented detection limit is not as high as those previously reported, a broad linear range of 10 ng/mL to 5×10^4 ng/mL for dsDNA-modified SGGT can be obtained (see supporting information, Table S1 and Figure S3). And, the detection limit (10 ng/mL) of our device, which can be repeatedly used, is much lower than the requirements of the standards of United States Federal Drug Administration (50 ng/mL)[20].

The effects of BPA on ssDNA and dsDNA molecules immobilized on graphene surface are presented in **figure 4**. For the device functionalized with ssDNA, due to the interaction between BPA and DNA, the BPA molecules can form covalent adducts with DNA molecules, which lead to the damage of the DNA structure and interruption of the chemical bonds of Au-S[39-41]. As a result, the ssDNA molecules could be detached from the graphene surface, as shown in **figure 4a**. After treatment of a high-concentration BPA solution (up to 100 μg/mL), most of the ssDNA

will be released from the graphene platform and the intact graphene template can be reused again for repeated BPA characterization.

For the device modified with dsDNA, BPA molecules can intercalate into the dsDNA pairs more easily due to the double helix of dsDNA as mentioned before. Hence, the higher sensitivity of the dsDNA modified sensor can detect the concentration of BPA as low as 1 μg/mL. As depicted in figure 4b, due to the break of the Au-S bond after BPA treatment, the dsDNA could also be detached from the graphene surface. Based on the intact graphene template, the SGGT as a recyclable BPA sensor can be modified by DNA molecules for the next time detection.

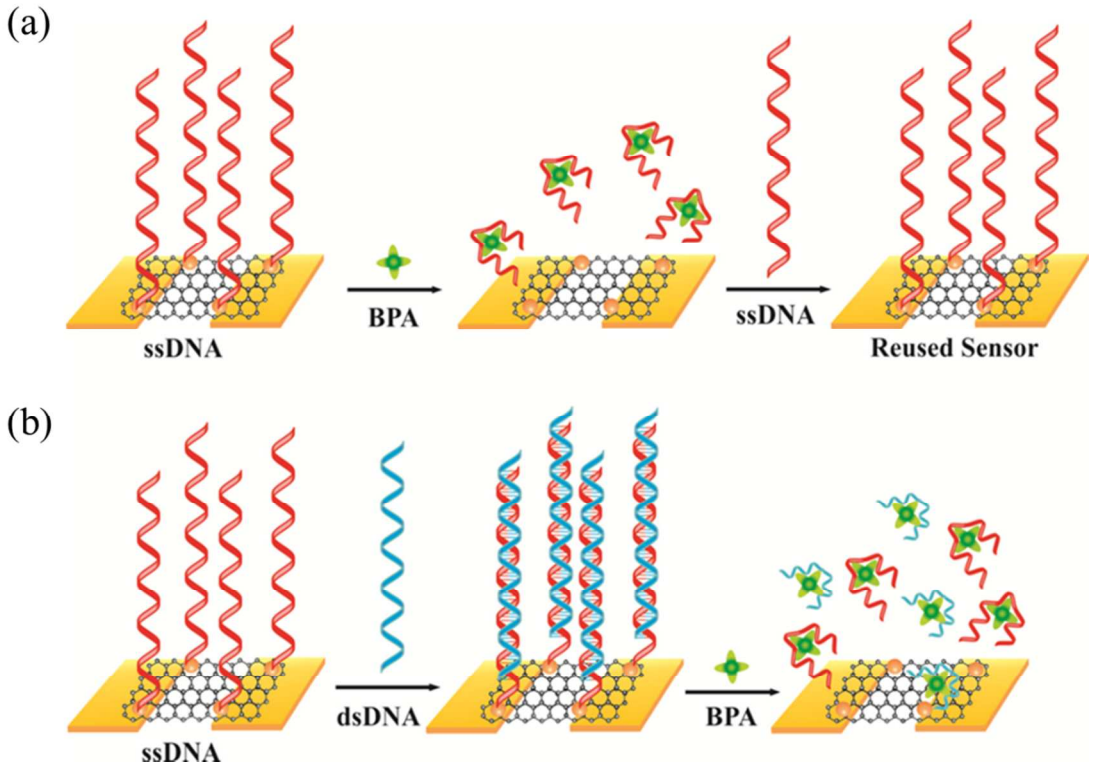

Figure 4. Schematic of the recycled device with BPA-induced self-release of (a) ssDNA and (b) dsDNA molecules from the Au NP incorporated graphene surface.

4. CONCLUSION

In summary, SGGTs integrated in microfluidic systems are used for BPA detection for the first time. ssDNA and dsDNA are immobilized onto graphene channels with the assistance of Au NPs. The device modified with dsDNA shows a lower detection limit of 10 ng/mL due to a stronger interaction of BPA with dsDNAs than ssDNAs. The sensing mechanism is attributed to the intercalation of BPA into the DNA molecules, leading to the damage of the DNA structure and the detachment of DNA from the graphene surface. Since the performance of a SGGT is quite sensitive to the variations of charge environment in the channel, such as the amount of DNA on the graphene surface, different concentrations of BPA can result in different device performance. After a treatment of BPA with a high concentration, most of the DNA molecules could be released from the graphene surface and the SGGT can be reused after the modification of fresh DNA. This technique provides a cost-effective approach for the detection of BPA with a high sensitivity.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information is available free of charge on the ACS Publications website. Transfer characteristics of the reused SGGT modified by probe DNA for detection of different concentration of BPA. Fluorescence images of the probe DNA anchored on the surface of Au-doped graphene before and after the treatment of pristine PBS or BPA contained PBS solution.

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ACKNOWLEDGMENTS

This work is financially supported by the Research Grants Council (RGC) of Hong Kong, China (Project No. C5015-15G) and The Hong Kong Polytechnic University (Project No. 1-ZVGH, G-YBJ0 and 1-ZVK1).

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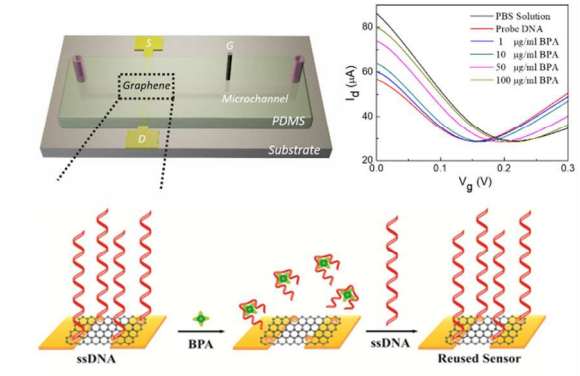
TOC figure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