Monolayer graphene chemiresistive biosensor for rapid bacteria detection in a microchannel

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ABSTRACT

Bacteria, such as Escherichia coli (E. coli), can cause food poisoning and serious diseases. In fact, E. coli has emerged multiple times as a severe public health concern in recent years. Therefore, it is important to detect bacteria like E. coli in simple, rapid and sensitive approaches. In this study, a gate-free chemiresistive biosensor based on monolayer graphene (MG) is proposed to detect E. coli, thanks to the high sensitivity stemming from the anti-E. coli antibody-coated graphene. The immobilization of the antibodies is performed via streptavidin and biotin conjugation on the graphene surface. Compared to conventional bacterial biosensors, the proposed chemiresistive biosensor captures the E. coli bacteria on the surface of the sensor and performs the detection through electric readouts, other than optical signals. That is the resistance measured in the biosensor increases along with the increase of concentration of the bacteria. To further extend the capabilities of this biosensor and provide a controllable test flow, a microchannel is integrated on the graphene surface. The results show that the developed chemiresistive biosensor is able to detect E. coli in trace concentration down to 12 cfu mL⁻¹, indicating an excellent sensing performance. The proposed monolayer graphene based chemiresistive biosensor clearly manifest its advantages of low-cost, ease of fabrication, portability and good sensitivity, as well as the potential for rapid in-situ bacterial detection.

1. Introduction

Bacteria accompany our lives, but sometimes they cause problems in our health. For example, Escherichia coli (E. coli), which usually colonizes human intestines right after birth [1], inevitably affects human beings throughout their entire life spans. The discovery of E. coli can be dated back to 1885 by Theodor Escherich, and it was originally considered as a healthy mutualism bacterium helping human beings with synthesis of vitamin K2 [2]. In 1996, scientists found that E. coli can cause food poisoning in human beings, as evidenced by a worldwide outbreak of food poisoning occurred in Scotland [3]. In addition, E. coli infections can cause diarrhea and lead to life-threatening conditions, such as kidney failure, high blood pressure, chronic kidney disease, and neurologic problems. To minimize the impact of E. coli on human society, efficient diagnosis of the bacteria is of utmost importance. At present, a variety of methods have been developed to detect and determine the concentration of E. coli. For instance, one of the most straightforward methods is through cell culture, in which E. coli is cultured on a petri dish for 24 h at 37 °C in an incubator. By means of counting the number of E. coli colonies, the concentration of E. coli can be determined [4]. Nonetheless, this method usually requires long preparation time. Therefore it has been gradually substituted by other approaches. For example, nucleic acid-based polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) technology could give rise to a much more accurate result in a shorter amount of time [5–7]. Nevertheless, they are still not satisfactory for rapid detection as they are costly, time consuming and require immobile laboratory settings.

Recently, various electrochemical based biosensors for the detection of E. coli pathogen has been developed [8]. For example, anodic particle coulometry technique has been combined with cyclic voltammetry (CV), and the absorption spectra mean decreases as the concentration of the E. coli decreases [9]. Moreover, nanomaterials such as gold nanorods and gold nanoparticles (AuNPs) have also been incorporated into sensing assays to further enhance the specificity and sensitivity of detection [10]. For example, Bhalla and coworkers have shown that gold nanoparticles can increase the sensitivity of biosensors significantly. Specifically, the gold nanoparticles helped the detection of cardiac troponin I (cTnl) at a concentration as low as 0.2 ng mL⁻¹, which is much better than ELISA tests of 4.3 ng mL⁻¹ [11]. Depending on the function of gold nanoparticles, they can be linked to aptamer for capturing target bacteria. In order to find the adaptor on the E. coli membrane for aptamer, the amplified single-strand DNA need to be translated based on the DNA array of E. coli, in which the concentration of E. coli can be obtained [12]. However, the DNA screening of aptamer is relatively difficult, and the speci-
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Fig. 1. (a) The structure of the PDMS microchannel. The PDMS microchannel was fixed on the monolayer-graphene with electrodes. Under the PDMS microchannel is the structure of the biosensor in the center of the microchannel. (b) The sputter coater was used to coat two sides of electrodes. The mask was covered on the upper surface of the graphene.

Fig. 2. A photo of the proposed microfluidic chemiresistive biosensor.

2. Materials and methods

2.1. Materials

Monolayer graphene coated on a square substrate of Si/SiO₂ was purchased from Graphenea, Inc. Gold/palladium nano-particle target was purchased from Ted Pella, Inc. Phosphate buffered saline (PBS, 0.1 mol L⁻¹, pH 7.4) with 0.1% Tween 20 was purchased from Sigma Aldrich Inc. Stereptavidin was purchased from AnaSpec, Inc. E. coli K12 ER2925 were purchased from New England Biolab, Inc. Biotinylated anti-E. coli (from rabbit) antibody was purchased from abcam, Inc. Goat anti-rabbit IgG (Heavy & Light Chain) antibody (Atto 488) was purchased from Antibody-Online, Inc. The tryptic soy broth and tryptic soy agar for medium were purchased from Thermo Fisher Scientific, Inc.

2.2. Device fabrication

The overall detection system is illustrated in Fig. 1(a). A monolayer-graphene substrate with a size of 1 cm × 1 cm was first scrutinized to ensure good quality. Afterwards, two electrodes were coated on the surface of the graphene substrate with gold/palladium alloy target (99.99% Au/Pd, 60:40 ratio) supported by a low pressure argon gas chamber (0.05 mbar) using a Au/Pd Polaron sputter coater ES5100 series II. As shown in Fig. 1(b), a predesigned mask was fixed on the upper surface of the substrate, after which the sputter coater was used to deposit two 15 nm thick Au/Pd continuous films as electrodes (for 10 min). After stripping the mask, the sputtering coater was used for a second time,
but the second time coating was on the entire surface of the substrate and was only for 10 s. This way, the center of the graphene substrate had been coated with nanoparticle stratum granulosum as Au particle linker.

After preparation of the electrodes, two PDMS layers were added onto the substrate to create a microchannel. The first layer was the microchannel layer of PDMS which was used to cover the top of graphene piece. The width, length, and the depth of the microchannel are 800 μm, 8 mm, and 1 mm, respectively. The dimensions are chosen because they can be made conveniently using low-cost technology. Specifically, the PDMS layers were created via standard soft lithography [23,24]. The mold of the microchannel was fabricated on a polymethyl methacrylate (PMMA) plate by a micro-milling machine (CNC Mini-Mill/3 PRO). The PDMS pre-mixture was then mixed, degassed, and poured onto the mold, and kept in an oven for 8 min at 75 °C. While the PDMS was still a little bit sticky, it was adhered onto the graphene, and punched for the injection and extraction holes on the two sides of the channel. After that, the graphene was placed with the PDMS layer down on the plate. Then the second layer PDMS liquid was poured onto the substrate and sealed the graphene substrate into the PDMS. The whole device was placed in the oven for 20 min at 75 °C. The two grooves were cut on the two sides of the electrodes, which were used to gain electric contact on the electrodes for electric measurements (Fig. 2). Finally, the microchannel was rinsed with deionized water (DI water), in order to remove impurities in the microchannel. Afterwards, the microchannel was injected and incubated overnight with 15 μL streptavidin (1 mg mL⁻¹) at 4 °C. After rinsing with buffer (PBS containing 0.005% Tween 20) and DI water thoroughly, the graphene was treated by 15 μL of 0.5 mg mL⁻¹ biotinylated anti-E. coli antibodies for 2 h, followed by rinsing with DI water. After the fabrication of devices, they can be used immediately for the detection of bacteria. In our experiment, a series of E. coli K12 solutions with different concentrations were injected into the microchannel for 5 min consecutively for E. coli detection and measurements. If the devices are not to be used immediately, it is recommended to seal an antibody stabilizer PBS buffer in the microchannels for preserving the proteins and antibodies for a relatively long time, and the devices should be kept in a refrigerator (2–8 °C).

2.3. Bacteria preparation

The original E. coli K12 was cultured in tryptic soy broth solution and incubated in an incubator for 16 h at 37 °C with 246 RPM stir
3. Results and discussion

3.1. Characterisation of monolayer graphene

The SEM image of the MG is depicted in Fig. 3(a), which shows an area in between the two electrodes of the sensor. As observed, the graphene film is continuous, uniform, and dominantly single-layered structure across the entire surface. Raman spectra measurements were performed to further confirm the presence of the high-quality MG layer. As shown in Fig. 3(b), the MG displays at two prominent peaks at 1580 cm\(^{-1}\) and 2676 cm\(^{-1}\), corresponding to the well-documented G and 2D peaks, respectively [25,26]. The fact that the 2D peak is greater than the G peak indicates the presence of a defect-free MG layer on the chemiresistor sensor, which is also in accordance with the evidence from the SEM image (Fig. 3(a)).

3.2. Characterisation of linker

The linkers were applied on the graphene substrate to capture the E. coli, which then causes a change in resistance in MG for detection. The primary linkers are Au nanoparticle, streptavidin and antibody on...
the graphene. Streptavidin is the most widely used analogue of avidin [27]. The avidin-biotin affinity interaction was approximately 10^3 to 10^6 times higher than an antibody-antigen interaction [28]. Therefore, application of streptavidin in this biosensor enables very stable linking (compared to antibody-antigen). Moreover, streptavidin can protect the biotinyl esters from hydrolysis, while avidin augments this hydrolysis [29]. Au nanoparticles and streptavidin are biologically specific binding. These functional groups of water-soluble nanoparticles are usually carboxylic acids, which stabilize nanoparticles by electrostatic repulsion and can be used to conjugate other molecules to particles [30].

In order to verify that these linkers (Au nanoparticle, streptavidin and anti-E. coli antibody) can be linked together very well, we used labelled secondary antibody to verify that the anti-E. coli had fixed on the surface. The Au nanoparticles were coated on a glass slide. Meanwhile a circle was drawn on the glass slide, and the streptavidin and anti-E. coli solution were dropped on this area respectively. After that, PBS solution was applied to rinse the slide surface three times. The secondary antibody with fluorescence under a laser microscope was observed in the circled area only. Therefore, it was confirmed that these linkers had been joined together and the other linkers which were not linked had been rinsed out.

3.3. Sensing results and discussion

In the bacteria-antibody binding system, the amount of the bacteria-antibody bonds being produced during the sensing process directly affects the resistance of the MG. It is reported that the membrane of natural biological cells shows a resistance of 10^2–10^3 Ω cm^{-1} [18,31]. When the bacteria cells attach to the surface of the graphene film, the formation of bacteria-antibody conjugation could produce a barrier for the electron transfer process, which may induce more distinct changes in the carrier hole density in graphene film through polarization of cell-surface charges, intracellular bioactivity. In addition, more mobile electron clouds were caused by steric hindrance, which restricts the passage of electrons to the electrode surface, resulting in the resistance increase of the chemiresistive sensors. Therefore, the resistance change of the system can be directly used to indicate the concentration of E. coli in the sample solution. In order to determine the kinetics of bacteria-antibody binding event, the MG-based chemiresistive sensor was made in contact with 10^7 cfu mL^{-1} of E. coli bacteria solution. As shown in Fig. 4, the graphene resistance was measured in every 5 min from 0 to 50 min, which shows a trend of resistance increase in time. This is attributed to the gradual increase in the number of E. coli captured by the antibodies on the graphene surface. The resistance values increased sharply and reached a plateau as the incubation time increases. These results suggest that the binding reaction finished in about 10–15 min due to the saturation of bacteria-antibody binding. In order to avoid the exhaustion of antibodies for sensing purpose, we decided to choose 5 min for the time lag in all the following sensing experiments.

The resistance spectra was obtained in Fig. 5(a) for the MG-based chemiresistive sensor when bacteria solution was injected into the microchannel and let sit for 5 min. The tested concentrations of the E. coli K12 solutions ranged from as low as 2.4 x 5 to 2.4 x 5^2 cfu mL^{-1}. In Fig. 5, ΔR is defined as the absolute change of resistance

\[ ΔR = R_f - R_0 \]  

where \( R_0 \) is the initial resistance of the non-bacteria chemiresistive sensor and \( R_f \) is the actual resistance value of the chemiresistor after 5 min of injection and sitting of E. coli K12 samples with various concentrations of 2.4 x 5^3 cfu mL^{-1} (\( x = 0–8 \)). The result shows that the resistance of the MG-based chemiresistive sensors tend to increase with logaritmically increasing adsorption of E. coli on the sensor surface. The (ΔR) was finally plateaued at about 260 ohms, which means that the antibody was probably completely consumed by E. coli in 5 min at high concentrations. These results confirm that the combination of MG-based chemiresistive sensor with the real-time measurement of the resistance change is appropriate for the development of highly sensitive pathogen bacteria detection sensors for a wide range of concentration.

We also explored another sampling mode, where the sample flow rate was maintained at 2 μL min^{-1} after a series of E. coli K12 solutions with different concentrations were injected in the microchannel. The results are shown in Fig. 5(b), and the increasing trend is very similar to the injection-and-stop sampling method. This means that the MG-based chemiresistive biosensor is suitable for being integrated into continuous-flow microfluidic systems for more sophisticated sensing and diagnostics tasks.

4. Conclusions

In this work, we developed a novel microfluidic approach to detect E. coli K12 using a monolayer graphene chemiresistive biosensor. The microchannel was fabricated using PDMS, and Au nanoparticles and streptavidin combination on the graphene were applied to conjugate the antibody. The solution samples were injected into the microchannel for testing, which was achieved by monitoring the graphene resistance changes during the adsorption of E. coli by the antibody. A linear increasing trend of ΔR was observed with logarithmically increasing E. coli concentrations from 2.4 x 5 to 2.4 x 5^2 cfu mL^{-1}. Both injection-and-stop and constant-injection modes of the samples were tested and the results indicate a similar trend. Overall, the developed microfluidic sensor demonstrates rapid and sensitive bacterial detection. One major limitation here is that the graphene sensors cannot be recycled for reuse. Therefore, these sensors will have to be disposable in real-life applications. But the portability, low-cost, and ease of fabrication of the sensor provides a promising approach for future development of multiplexed microfluidic biosensor platforms for a wide variety of applications.

Declaration of Competing Interest

The authors declare no conflict of interest.

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