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Research Paper

Biofunctionalized graphene oxide wrapped carbon nanotubes enabled microfluidic immunochip for bacterial cells detection

Chandan Singh[a,b], Md. Azahar Ali[a], Venu Reddy[c], Dinesh Singh[d], Cheol Gi Kim[e], G. Sumana[a,*], B.D. Malhotra[f]

[a] Biomedical Instrumentation Section, CSIR-National Physical Laboratory, Dr. K.S. Krishnan Marg, New Delhi, 110012, India
[b] Academy of Scientific and Innovative Research (AcSIR), CSIR-National Physical Laboratory Campus, Dr. K.S. Krishnan Marg, New Delhi, 110012, India
[c] Nanotechnology Research Center, SRKR Engineering College, China Amiram, Bhimavaram, 534204, India
[d] Sophisticated Analytical Equipment Division, CSIR-National Physical Laboratory, Dr. K.S. Krishnan Marg, New Delhi, 110012, India
[e] Department of Emerging Materials Science, DGIST, Daegu, 711873, South Korea
[f] Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi, 110042, India

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ABSTRACT

A sensitive and selective microfluidic immunochip was fabricated for detection of *Salmonella typhimurium* (*S. typhimurium*) bacterial cells. In this sensor, graphene oxide (GO) nano sheets wrapped carboxylated multiwalled carbon nanotubes (cMWCNTs) composite acted as a transducer material. The colloidal solution of GO-cMWCNTs composite was selectively deposited onto patterned indium tin oxide (ITO) electrode and sealed with polydimethylsiloxane (PDMS) micro channels. The *S. typhimurium* antibodies (StAb) were *in situ* biofunctionalized followed by EDC-NHS covalent chemistry *via* amidation reaction. The presence of abundant functional groups at the GO-cMWCNTs composite improved the loading of antibodies (StAb) against *S. typhimurium* leading to improved biosensing characteristics. Wrapping of cMWCNTs with GO resulted in superior electron transfer behavior enhancing the sensitivity ($162.47\ \mu A/CFU^{-1}/mLcm^{-2}$) almost two folds as compared to that based on GO ($89.16\ \mu A/CFU^{-1}/mLcm^{-2}$) sheets for bacterial cells detection. Besides this, GO wrapped cMWCNTs integrated microfluidics biosensor offered low detection limit as 0.376 CFU/mL and negligible interference due to presence of *Escherichia coli* (*E. coli* (O157:H7).

1. Introduction

Salmonella, a Gram-negative facultative intracellular pathogen causes a wide range of illness in humans and animals, depending on the host susceptibility and the bacterial reservoir [1]. Some reservoirs may cause localized intestinal infection (gastroenteritis),while others are known to be responsible for systematic infection of a healthy host (typhoid) and systematic infection in susceptible host (non-typhoid salmonellosis) [1]. Among these, non-typhoid salmonellosis is considered to be the main cause for blood stream infection among children suffering from malaria, malnutrition and adults infected with human immunodeficiency virus [2,3]. This has been found to result in annual incidence of 175–388/100,000 among children (<5yr) generating high fatality rate of 22–25% [2,3]. Salmonellosis can be transferred from animals to humans due to consumption of infected foods. *S. Enteritidis*, *S. Heidelberg* and *S. typhimurium* are the most common reservoirs that are known to be responsible for salmonellosis infection. Among these, *S. typhimurium* is considered to be more fatal. *S. typhimurium* takes 12–36 h after the consumption of infected food resulting in symptoms like diarrhea, abdominal pain, nausea and vomiting. Conventional methods for *S. typhimurium* detection include selective enrichment and plating followed by biochemical tests that require 3–4 days to obtain results and another 6–7 days for the confirmation [4]. For the last two decades, many optical and electrochemical methods have been developed for rapid and reliable detection of *S. typhimurium*. Enzyme-linked immune magnetic electrochemical technique has been developed for *S. typhimurium* detection [5]. Similarly, a magneto-electrode has been designed for label free detection of *S. typhimurium* [6]. However, these techniques suffer from poor sensitivity, limited detection range and require a high volume of sample for the precise measurement.

* Corresponding author.
E-mail addresses: sumanagajjala@gmail.com (G. Sumana), bansi.malhotra@gmail.com (B.D. Malhotra).

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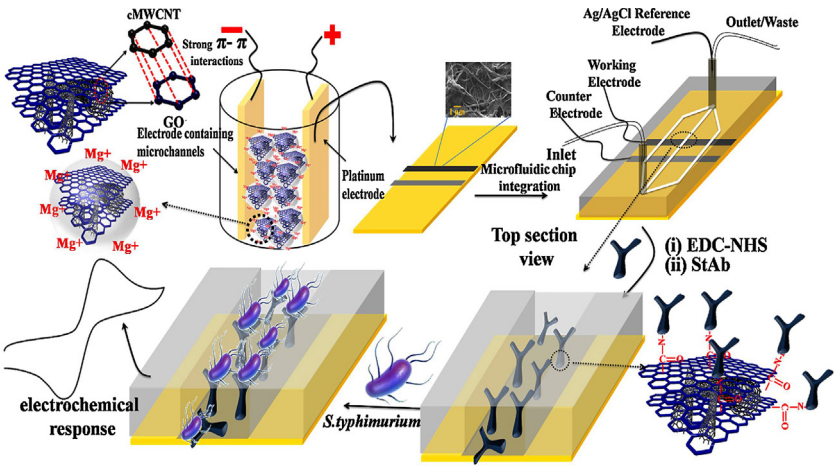

Fig. 1. Schematic for the fabrication of graphene oxide wrapped multiwalled carbon nanotubes integrated microfluidic chip, immobilization of StAb antibodies and detection of *S. typhimurium* cells using CV technique.

The application of microfluidics in biosensing may lead to significant improvements such as minimizing the sample volume, reducing the consumption of costly chemicals and the reduced processing time [7,8]. Combination of microfluidics with biosensing offers advantages of high throughput analysis, and portability resulting in the smart point-of-care diagnostics (POC). Due to comparable size of biomolecules, engineered nanomaterials can be used for functionalization and detection of different bioanalytes [9,10]. With larger surface area and signal amplification capability of nanomaterials, the high aspect ratio of microfluidic devices may result in increased sensitivity and low limit of detection [11–14]. Zhou et al. reported gold nanoparticles integrated microfluidic chamber to detect cardiac biomarkers [11]. Ali et al. reported a multiwalled carbon nanotubes integrated biosensor for the cholesterol detection with improved biosensing features [15].

GO is considered to be a potential candidate for sensing/diagnostic applications due to its excellent electron transport properties, very high specific surface area and availability of different functional groups on the edge and basal plane, [16,17]. It is a derivative of carbon nanotubes (CNTs) cut along the axis and contains many conduction pathways per unit mass compared to the CNTs resulting in higher conductivity. However, the difficulties associated with the mechanical cleavage method and drawbacks in the reduction methods of GO (*e.g.* hydrazine works only on the basal plane, while $NaBH_4$ results in incomplete reduction with aggregates) and is known to have poor conductivity [18,19]. However, wrapping of GO nanosheets on CNTs may yield enhanced conductivity [20]. For diagnostics applications, solution process for GO is preferred as it allows easy fabrication of films using Langmuir–Blodgett technique, solution casting and filtration. However, these techniques suffer from lack of film architecture due to aggregation of GO, resulting in loss of the surface area [18]. The incorporation of CNTs may perhaps result in physical separation of two-dimensional GO sheets and decreased resistance, as electrical conductivity of the composite is known to depend on the percolated network of the CNTs formed in the composite film whereas GO acts as a carrier [21]. Additionally, intertwined GO with CNTs may result in improved electrochemical sensing of the target analyte [22]. Romano et al. reported that GO-CNTs nanocomposite offered controlled porosity with maximized electroactive surface area that may provide enhanced surface for the immobilization of the biomolecules [23]. Many reports are available on the synergistic effect of GO and CNTs resulting in improved electrochemical and mechanical properties as compared to that of the individual components [24]. Cheng et al. reported several fold increase in the electrochemical performance of the GO-CNTs composite mainly due to the synergistic effect [25].

We report results of the studies relating to fabrication of a sensitive, selective microfluidic immunosensor for detection of *S. typhimurium via* antigen-antibody interactions. The GO-cMWCNTs composite was electrophoretically deposited onto the hydrolyzed ITO microelectrode integrated with PDMS microchannels. Antibodies of *S. typhimurium* were immobilized using EDC-NHS coupling chemistry and the label-free detection of *S. typhimurium* was achieved using electrochemical technique. The schematic of fabrication of GO-cMWCNTs integrated microfluidic chip is shown in Fig. 1.

2. Experimental section

2.1. Chemicals

Graphite powder flakes (45 μm, >99.99 wt%) were procured from Sigma Aldrich, USA. *S. typhimurium* antibody and antigen (heat killed S. *typhimurium* cells) were purchased from KPL Laboratory, USA. Bovine serum albumin (BSA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were procured from Sigma, Aldrich, USA. The SU8-100 negative photoresist and SU-8 developer were purchased from Microchem (Newton, MA, USA). All other chemicals were of analytical grade and were used without further purification. During the entire experiments, Millipore water purification system was utilized for de-ionized water.

2.2. Characterization

The UV–vis spectroscopy (Perkin-Elmer, Lambda 950), Fourier transform infra-red spectroscopic (FT-IR, Perkin-Elmer, Model 2000) were used for characterization of the synthesized nanocomposite. The structural and morphological studies were carried out using scanning electron microscopy (SEM, LEO 440), transmission electron microscopy (HRTEM, Tecnaii-G2F30 STWIN). Electrochemical studies were performed on cyclic voltammetry (CV) conducted using Autolab, Potentiostat/Galvanostat (Eco Chemie, the Netherlands, Model AUT84275), Harvard syringe pumps and ILS microsyringes (Germany) were utilized to control the fluid flow into the microfluidic channels.