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Applying graphene oxide nano-film over a polycarbonate nanoporous membrane to monitor *E. coli* by infrared spectroscopy

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ABSTRACT

Nano-biosensors are excellent monitoring tools for rapid, specific, sensitive, inexpensive, in-field, on-line, and/or real-time detection of pathogens in foods, soil, air, and water samples. A variety of nano-materials (metallic, polymeric, and/or carbon-based) were employed to enhance the efficacy, efficiency, and sensitivity of these nano-biosensors, including graphene-based materials, especially graphene oxide (GO)-based materials. GO bears many oxygen-bearing groups, enabling ligand conjugation at the high density critical for sensitive detection. We have fabricated GO-modified nano-porous polycarbonate track-etched (PCTE) membranes that were conjugated to an *Escherichia coli*-specific antibody (Ab) and used to detect *E. coli*. The random distribution of nanopores on the PCTE membrane surface and the bright coating of the GO onto the membrane were confirmed by scanning electron microscope. Anti-*E. coli* β-gal Abs were conjugated to the GO surface via 1-ethyl-3,3-dimethylaminopropyl carbodiimide hydrochloride–*N*-hydroxysuccinimide chemistry; antibody coating was confirmed by the presence of a characteristic IR peak near 1600 cm^{-1}. A non-corresponding Ab (anti-*Pseudomonas*) was used as a negative control under identical conditions. When *E. coli* interacted anti-*E.coli* β-gal with Ab-coated GO-nano-biosensor units, we observed a clear shift in the IR peak from 3373.14 to 3315 cm^{-1}; in contrast, we did not observe any shift in IR peaks when the GO unit was coated with the non-corresponding Ab (anti-*Pseudomonas)*. Therefore, the detection of *E. coli* using the described GO-nano-sensor unit is highly specific, is highly selective and can be applied for real-time monitoring of *E. coli* with a detection limit between 100 μg/mL and 10 μg/mL, similar to existing detection systems.

1. Introduction

Infections caused by pathogenic bacteria pose serious health problems worldwide and are often borne by water, soil, and food [1–5]. Conventional bacterial detection methods [6,7] are mostly based on (i) culture and colony counting methods (which involves counting of bacteria) [8], (ii) immunology-based methods (which involve antigen–antibody (Ag-Ab) interactions) [9], and (iii) the polymerase chain reaction (PCR) method, which involves DNA analysis and requires highly skilled personnel or a long span of time, up to 7 or 8 days, to yield an answer [10]; however, currently available methods are limited by the time required to perform them and assay sensitivity, which led scientists to invent detection devices for the rapid screening of pathogens, including biosensors. Biosensors (also called immunosensors) are based on the principle of highly specific Ag-Ab recognition, and have been widely used for the sensitive and quantitative detection of disease-related proteins, which is critical for biomedical research and diagnostics [11–16].

In recent years, highly-sensitive biosensing materials have been explored to increase biosensor detection limits for pathogens in samples [17]. Among these, graphene and graphene-based nanomaterials (such as GO) are attractive candidates for biosensing [18]. GO is a chemically-modified form of graphene that contains oxygen functional groups, such as epoxides, alcohols, and carboxylic acids; these functional groups make GO more reactive than inert graphite and easier to intercalate with other partner groups [17]. The availability of several types of oxygen-containing functional groups on the basal plane and sheet edge allows GO to interact with a wide range of organic and inorganic materials in non-covalent, covalent, and/or ionic manners so that functional hybrids and composites with unusual properties can be readily synthesized [15,16,19–21], making graphene-based materials ideal for biosensing [18]. By exploiting abundant carboxylic groups (—COOH) on GO, covalent conjugation of amines on the protein molecules can be achieved by using well-known EDC–NHS (1-ethyl-3,3-dimethylaminopropyl carbodiimide hydrochloride–*N*-hydroxysuccinimide) chemistry.

Real-time detection of microorganisms is possible by monitoring the changes in the IR patterns of samples. Hence, FT-IR spectroscopy can advance our basic knowledge of molecular interactions by characterizing the bonding pattern of the target sample structures at high spatial

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resolution [22,23]. In this report, we coupled the unique characteristics of GO with the FT-IR spectroscopy to test *Escherichia coli* binding and aimed to generate a highly sensitive method of *E.coli* detection. We have demonstrated that GO surfaces are suitable platforms for the conjugation of ligands, such as the *E. coli*-specific antibody (anti-*E. coli* β-galactosidase (β-gal) Ab) used in this study. Hence, GO can be considered the ideal material and component of the nano-biosensor system for pathogen detection. The interaction of *E. coli* with a surface-conjugated anti-*E.coli* β-gal Ab resulted in a clear shift in the FT-IR spectra, demonstrating the specific reaction of *E. coli* with the GO nano-sensor. The GO-coated nano-sensor system described here offers several novel features: (a) PCTE membranes contain well-defined cylindrical nanopores with a narrow pore size distribution, (b) deposition of GO (bearing carboxylic group) on the porous membrane offers the potential to further modify membrane characteristics, (c) ligand attachment can be directly achieved via the functionally active GO surface, and (d) the IR band analysis of the GO-coated PCTE membrane provides a direct and simple method to monitor the specific antibody-antigen interaction of the analyte being investigated. To our knowledge, we have described the first application of a GO-based nano-porous membrane surface for pathogen detection, presenting a novel platform for *E. coli* detection.

2. Experimental details

2.1. Chemicals, materials, and reagents

The solvents and chemicals were of laboratory and analytical grade, and procured from SRL, Merck, and Molychem (India). Sodium chloride (NaCl, RANKEM), potassium chloride (KCl, ANALAR), potassium dihydrogen phosphate (KH_2PO4, SRL), and sodium hydrogen phosphate ($NaHPO_4$, ACROS) were prepare phosphate-buffered saline (PBS). PBS (pH 7.2) was prepared by mixing NaCl (0.8 g), KCl (0.02 g), KH_2PO_4 (0.024 g), and $NaHPO_4$ (0.144 g) in 100 ml of double distilled water. GO was kindly provided by Dr. Rakesh K. Joshi, Marie Curie Fellow, University of Manchester, UK and 1.6 mg/ml GO was prepared by dilution in distilled water. The anti-*E.coli* β-gal antibody and the anti-*Pseudomonas* antibody were purchased from Bangalore Ge Nie. The gold-coated PCTE membrane was purchased from Whatman (USA).

2.2. Instruments

The GO coating on the PCTE membrane was initially confirmed by acquiring images using an inverted microscope (MAGNUS, Olympus, Chennai, India); for further detailed and in-depth analysis, the GO-coated membranes were examined by the scanning electron microscopy (SEM; LEO 438VP SEM equipped with an Energy Dispersive Spectroscopy (EDS/EDX) System, BRUKER, Germany). EDS/EDX which detects the X-rays emitted from a sample during electron imaging was used for the elemental analysis. Thermo Gravimetric Analysis (TGA) of the GO-coated PCTE membrane was carried out under N_2 flow using a Thermo Gravimetric Analyser Q50 (USA). The FT-IR study was done using a Nicolet 6700 spectrometer (Thermo Scientific, USA). The membrane holder was purchased from Thermo Scientific.

2.3. Modification and characterization of the PCTE membrane

A nano-porous polycarbonate track-etched (PCTE) membrane (100 nm) was used for surface modification. The drop coating method was used to add GO to the PCTE membrane surface. The round membrane was arranged on the circular planar base of a Petri dish and the surface of the membrane was coated by the pipetting method. The membrane to be coated was arranged with the help of forceps and GO was uniformly dropped onto the surface of the membrane by a micropipette; the coated membrane was kept overnight at room temperature. In this time period, the surface of membrane was covered by a thin layer of GO. The GO-functionalized membrane was characterized by inverted microscopy, SEM, EDC/EDX, and TGA (see Section 2.2) and used for the FT-IR, Ag-Ab interaction studies (described below, Section 2.4).

2.4. Antibody immobilization

An antibody solution was prepared from a 1.7 mg/ml stock by dissolving 100 μl of anti-*E. coli* (β-gal) in 900 μl of PBS to achieve a final concentration of 170 μg/ml, after which the GO-coated membrane was covered with this solution and incubated for 15 h at 4 °C.

3. Results and discussion

3.1. Characterization of GO-coated membranes

The normal and GO-coated PCTE membranes were placed on a Petri dish (Borosil) one-by-one and the micrograph was recorded using inverted microscopy (40×), SEM, and TGA (see Section 2.2, Methods section). The results are presented in Figs. 1–5. Fig. 1 is a pictorial depiction of micrographical representation of GO-PCTE membrane fitted in membrane holder cell with different micrographs wherein Fig. 1a shows an image of the liquid cell used to hold the PCTE membrane. The micrograph in Fig. 1b depicts the grey surface structure of the normal membrane; whereas the micrograph of GO-coated membrane surface shows the golden brown colour of GO on the surface of the polycarbonate membrane as shown in Fig. 1c. Therefore, bright-field image analysis clearly demonstrates that the GO is uniformly coated over the surface of the membrane. The GO coating onto the PCTE membrane was further confirmed by SEM showed Fig. 1d. The SEM micrograph of GO-coated PCTE membrane is represented at 15.00 kX magnifications with a SE1 detector and EHT at 20.00 kV. The SEM micrograph of the normal PCTE membrane represented in Fig. 1e, at 1.00 kX magnification with a SE1 detector and EHT at 20.00 kV, shows a black uniform layer of GO over the surface of the nano-porous membrane and the absence of nanopores due to the healing of the surface by the

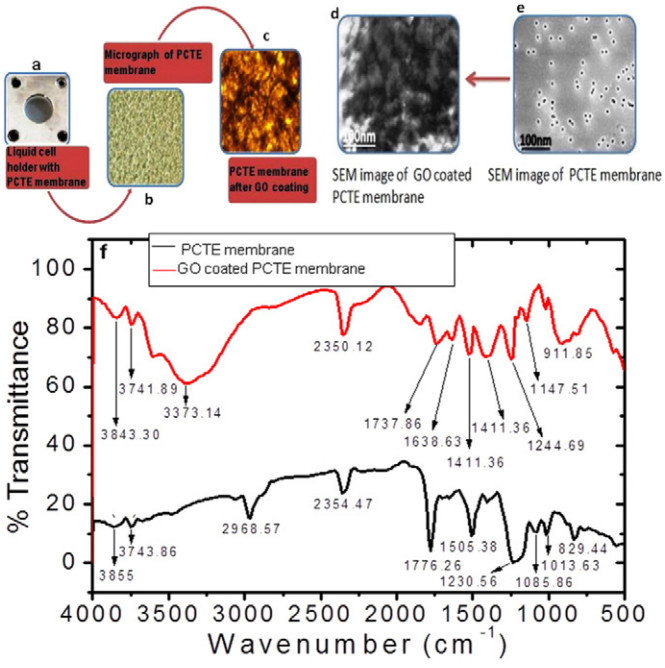

Fig. 1. (a) Liquid cell holder with mounted PCTE membrane. (b) Inverted microscopic micrograph (40×) of PCTE membrane. (c) Inverted microscopic micrograph (40×) of PCTE membrane after GO coating. (d) SEM micrograph of GO coated membrane (Mag = 1 kX). (e) SEM micrograph of normal PCTE membrane (Mag = 15 kX). (f) FT-IR spectra of GO coated PCTE membrane.