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Rapid Detection of *Staphylococcus aureus* Using Graphene Oxide Coated Screen-Printed Sensor Strips

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Staphylococcus aureus (*S. aureus*) is the most common reason behind bacterial infections in humans. They cause a wide array of infections on skin, bone and joint medical implants and blood stream. In order to mitigate these infections, detecting the presence of this bacterium is of prime importance. This paper explores a new rapid detection technique to identify *S aureus* bacteria. A screen-printed sensor coated with graphene oxide was used as an identification platform. Graphene oxide was synthesized using modified Hummer's method and was characterized using X-ray Diffractometer and Scanning Electron Microscope. The developed sensors showed significant decrease in the bulk resistance with the increase in bacterial concentrations. The developed sensors were subjected to selectivity, repeatability and reusability tests and showed good results.

Keywords: Rapid detection, Graphene Oxide, Strip sensors

Introduction

Staphylococcus aureus is a Gram-positive bacterium (stain purple by Gram stain) that are cocci-shaped and tend to be arranged in clusters. *Staphylococcus aureus* (including drug-resistant strains such as MRSA) are found on the skin and mucous membranes, and humans are the major reservoir for these organisms. *S. aureus* are one the most common infectious bacteria in humans and are the causative agents of multiple human infections, including bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections, gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. [1]

Detection Methods of *S. aureus*

The development of suitable analytical methods for bacterial identification with speed and specificity is vital. One of the most commonly used methods for detecting *S. aureus* is using PCR (Polymerized chain reaction) amplification. There are many readily available test kits for rapid detection of *S. aureus* which means the detection time is less than a minute. Laboratories also resort to deoxyribonuclease (DNase) and thermonuclease (TNase) tests for definitive identification.[2]

Phenotic detection methods which include: Oxacillin disc diffusion method and cefoxitin diffusion method, Oxacillin screen agar test, CHROMagar, Latex agglutination test for detection of PBP2a and Antimicrobial susceptibility testing are some other detection methods.[3]

Another study presents an analytical method based on the affinity nanoprobe-based mass spectrometry that enables detection of *S. aureus* in aqueous samples.[4] Cepheid's on-demand Xpert MRSA/ SA SSTI test detects the presence of *S. aureus* (SA) or methicillin-resistant *S. aureus* (MRSA) in skin and soft tissue infections in less than one hour. A novel technique which utilizes the bulk resistance of the sensor as a parameter to detect the presence of *Staphylococcus aureus* is explored in this paper.

Material and Methods

Synthesis of Graphene Oxide

Graphene oxide was synthesized using Modified Hummer's method through oxidation of graphite.[5] The synthesized graphene oxide was characterized using XRD, FTIR spectroscopy, SEM and UV-Spectrophotometry.

Preparation of GO solution

GO solution was prepared by dissolving 10mg of GO powder in 100ml distilled water. This solution was sonicated for 60 minutes to ensure completed dissolution of GO powder in water and was used as the GO solution for coating on the screen printed strips.

Fabrication of Screen-printed sensors

Conductive screen-printing silver paste was used to screen print 2cm x 2cm square conductive patches on

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flexible substrates. These tracks were coated with an insulating polymer to ensure there is no shorting of current. The overall dimensions of the strip was taken to 7.5cm x 2cm.

Coating of Graphene Oxide over screen printed sensor base

Using a 1ml micropipette, 500 µL of GO solution was drop casted on the silver patches. This was allowed to dry in the hot air over at temperature 60 ºC.

The graphene oxide coated silver patches were fitted with a square acrylic 1ml volume sample holder with the dimensions to match the square silver patch. The sample holder was used to circumscribe the *S. aureus* culture when it was dropped on the patch.

Results and Discussions

X-ray Diffraction

For the XRD analysis of the GO sample as shown in Fig. 1 (a), Bruker advance D8 Eco diffractometer was used. The step size and the range of 2θ was taken as 0.020432 and 5.0001-79.5 respectively. Advance x-ray diffractometer with a CuKα source was used.

By Debye Scherer formula it was seen that the particle size of the synthesized nano particles was found to be in the range of graphene oxide 4–6 nanometers. The inter-planar distance calculated from Debroglie's formula was found to be around 0.76nm.[6]

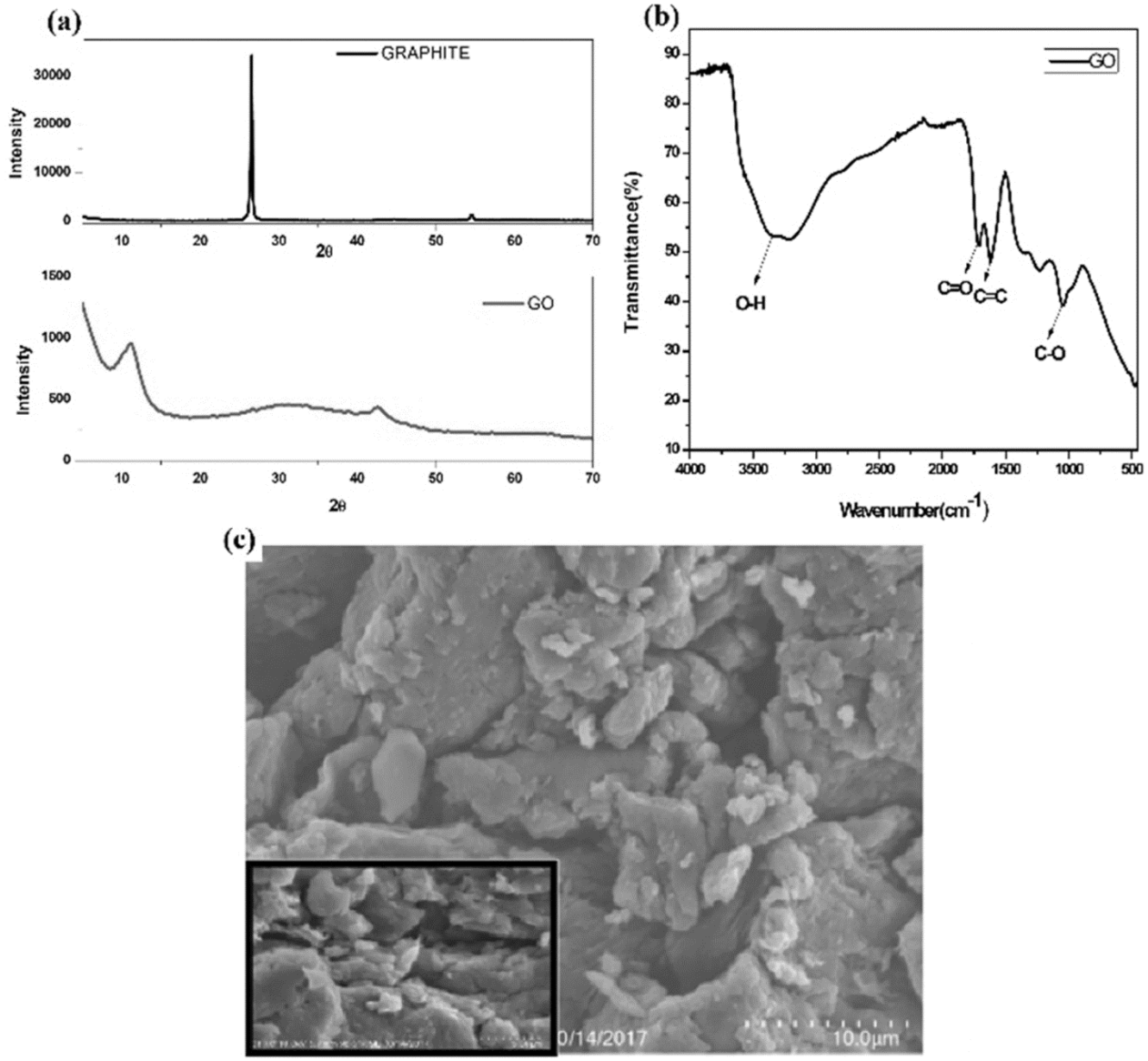

Fig. 1 — (a) XRD pattern, (b) FTIR spectrum, (c) SEM image of the synthesized Graphene Oxide

FT-IR spectrum

The FT-IR spectrum of the prepared sample was recorded in a 1S Fourier Transform Infrared spectrophotometer, Shimadzu in the range of 4600–400 cm^{-1} at a resolution of 2 cm^{-1}. FTIR peaks are shown in Fig. 1(b). Peak at 3371 indicates O-H group stretching vibration. Peak at 1702 indicates C=O, ketones stretchy vibrations. Peak at 1610, indicates C=C Alkene presence or the skeletal remains of the un-oxidised graphene. Peak at 1043 indicates C-O bond confirmation, presence of oxidized functional group

Scanning Electron Microscope Image

GO sample imaging as shown is Fig. 1(c), was carried out using Hitachi SU3500 instrumentation. From these imaging results it can be concluded that the material synthesized from Modified Hummers method is irregularly shaped Graphene Oxidemicro and nanoparticles maybe due to partial agglomeration.

Culture Preparation

Staphylococcus aureus broth in glycerol stock form along with Tryptic Soy Agar /MSA/blood agar[7] was obtained from Sigma Aldrich. The serial dilution method was followed where sterilized phosphate-buffered saline was used for dilution of the stock solution. For bacteria detection, the bacteria S*taphylococcus aureus* was prepared in inoculum and further diluted to get various reduced concentrations. The bacteria were cultured in agar plates for 24 hours. The number of colony forming units (cfu) per ml of culture was determined by quantitative colony counts after serial dilution in sterile phosphate-buffered saline (PBS).[8]

Placement of Probe

Bacterial culture was dropped onto the sensor using a micropipette and the bulk resistance variation with respect to different dilution levels was noted down using a digital multi-meter probe.

Testing of Sensors

Sensor response

The sensor response with increasing bacterial serial dilution is shown in Fig. 2. Six sensors samples with concentration of 2ml of GO were used as S1 to S6. The *S aureus* culture was dropped on the sensor and the resistance variation was noted down for different bacterial dilution. It can be observed that all the six sensors exhibited similar characteristic behavior that is, the decrease in bulk resistance with increase in bacterial count indicating repeatability of the sensor strips.

In order to test the selectivity of the sensors, Escherichia Coli (e coli) bacterial sample was dropped onto the sensor strips. It was observed that there was no significant variation in resistance for different bacterial dilution as shown in Fig. 3(a). This indicates that the sensor is not selective to *e-coli* bacteria.

Reusability studies for the developed sensors are shown in Fig. 3(b). It was found that up to 3 times of re-usage of the sensor, the resistance was constant, after which the resistance varied continuously. From this, it was concluded that the sensor can be re-used only thrice. The sensitivity is affected due to multiple usage.

Discussions and Conclusion

The interaction of the bacterial cells with the graphene oxide particles is described. The outer membrane of bacteria maintains their morphology and acts as a barrier of protection from external environments. When GO comes in contact with the bacterial cells then it induces oxidative stress. GO has the ability to induce oxidative stress by Reactive Oxygen Species (ROS) and lipid peroxidation.[9] Oxidative stress refers to elevated intracellular levels of reactive oxygen species (ROS) that cause damage to lipids, proteins and DNA. Due to oxidative stress the cell homeostasis is disrupted and the enzymes responsible for reducing ROS (superoxide dismutase and glutathione peroxidase) fail, the macromolecules, such as proteins, DNA, and lipids, can be damaged, which greatly influence the cell metabolism and signalling.[10] The exposure of cells to GO induced the production of superoxide radical anion and loss of cell viability. The

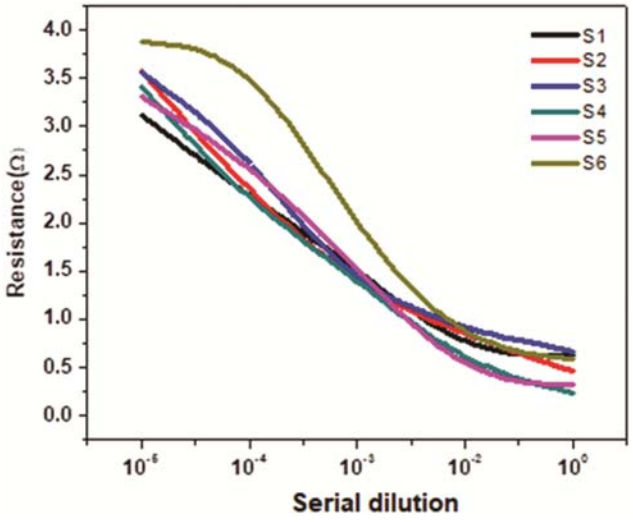

Fig. 2 — Graph indicating the sensor response to the bacteria

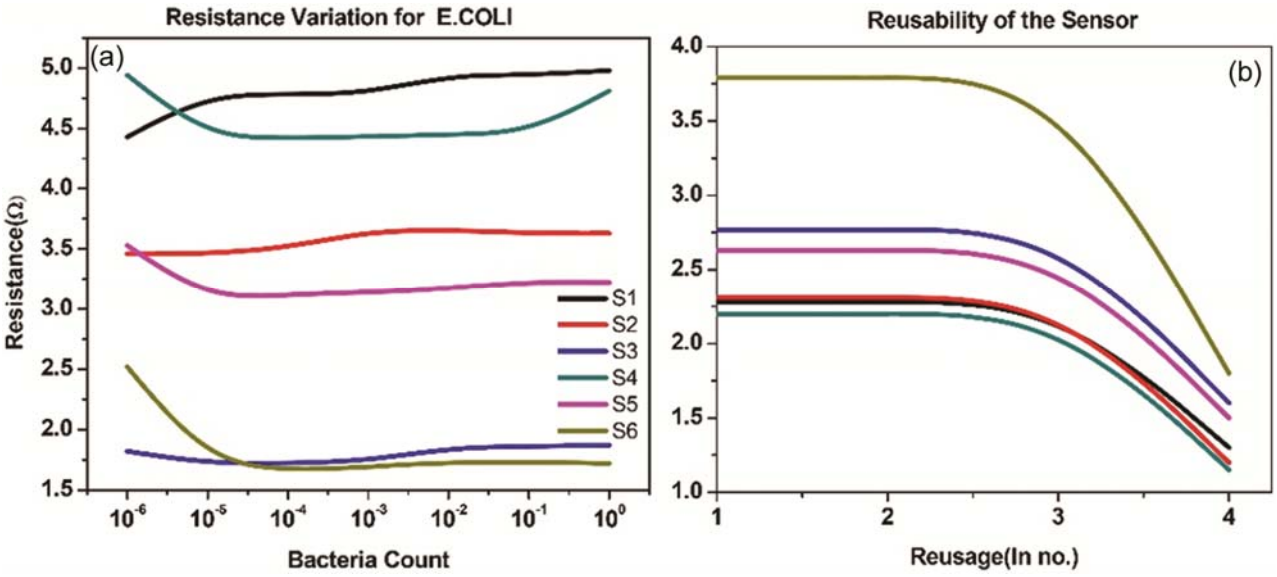

Fig. 3 — a. Graph indicating selectivity of the sensor, b. Graph indicating reusability of the sensor

decrease in the resistance observed is indicative of the graphene oxide being reduced by the removal of oxygen groups in the above-mentioned process.[5]

A novel type of sensor was developed for rapid detection of *Staphylococcus aureus* bacteria. The novelty of the sensor lies in the detection technique which is dependent on the interaction of graphene oxide particles with the bacterial cells and this was characterized with the change in the resistance. The sensors responded rapidly to the *S aureus* cells which signified instantaneous reaction. Other repeatability, re-usability and selectivity tests done on the sensors were found to be effective. The major advantage of such sensors is the rapid detection ability compared to the other detection techniques which involves culture.

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