ABSTRACT

Nowadays, one of the greatest health challenges worldwide is an infectious disease caused by pathogens. Antibiotics play a significant role in decreasing mortality and morbidity. However, current antibiotics therapies are not effective due to the rise of multiple drug-resistant (MDR) bacteria which is an increasing threat to the human race. To address this problem there is an urgent need to discover a novel antimicrobial agent for killing and early-stage screening methods for the detection of bacteria. Metal-antibiotic hybrid can play an important role in achieving these twin objectives. Based on the antibiotic-specific interaction with the cell wall of bacteria, detection of pathogens becomes highly specific. In this study, the metal-antibiotic hybrid has been used for the detection of bacteria using electrochemical impedance spectroscopy (EIS) and surface-enhanced Raman spectroscopy (SERS) technique and the killing of bacteria.

The impedance sensor was fabricated using gold interdigitated electrodes onto a tungsten oxide thin film. Tungsten oxide was functionalized with vancomycin, a glycopeptide antibiotic known to have a specific interaction with the peptidoglycan layer of Gram-positive bacteria. The functionalized tungsten oxide (metal-antibiotic hybrid) sensor was highly efficient in the capture of Gram-positive bacteria with a limit of detection of 10^2 colony forming units/mL. Label-free detection under physiological conditions was tested and achieved. The impedance measurement was also sensitive to differentiate between viable and non-viable Gram-positive bacteria.

For the detection of bacteria using SERS, vancomycin functionalized bimetallic magnetic/silver core-shell nanoparticles specifically capturing and concentrating Gram-positive pathogen was used. The capture efficiency and calibration graphs of these van-coated Fe@Ag nanoparticles were obtained and examined over different bacterial concentrations of *S. aureus* (10^1 -10^7 CFU/mL). These captured bacteria were detected with a low concentration of up to 10 CFU/mL using SERS. These nanoparticles were able to capture specifically Gram-positive bacteria which were separated by using an external magnetic field and were rapidly detected (~ 20 min).

For specific detection of Gram-negative bacteria, ampicillin was used to functionalize on the silver nanorods (AgNRs) prepared using glancing angle deposition technique (GLAD). Firstly, ampicillin concentration was optimized for the specific capturing of Gram-negative bacteria.

Ampicillin coated AgNRs SERS substrates selectively detected Gram-negative bacteria with a LOD of 10 CFU/mL. It was also able to differentiate viable cells from non-viable Gram-negative bacteria. Ampicillin coated AgNRs SERS substrates provide a rapid (~ 3 min) detection of bacteria having a simple operating procedure and high sensitivity.

In addition, the metal-antibiotic hybrid has been fabricated for developing a new class of metal-antibiotic nanoformulation to kill bacteria. Antibiotic coated AgNPs with highly efficient antibacterial and antibiofilm activity were synthesized by the coating of AgNPs with bacitracin (BA) and allyl isothiocyanate. BA is a cyclic peptide that disrupts bacterial cell wall synthesis whereas isothiocyanate (ITC), a natural plant product known for antibacterial effect. The ITC and BA were coated on the AgNPs surface and characterized by FTIR, UV-spectrometer, and TEM. ICP-MS results showed a very low silver content present in antibiotic coated AgNPs. The antibiotic coated silver nanoparticle efficiently inhibited the growth of both Gram-negative and Gram-positive bacteria. This fact was confirmed by MIC study, cell viability assay and time-kill curve. In addition, they also showed the activity of inhibition of biofilm, and the selective cytotoxicity against bacterial cells over the mammalian cells.

Keywords: Vancomycin, ampicillin, electrochemical impedance sensor, surface enhanced Raman spectroscopy, bacitracin, allyl isothiocyanate, antibiotic coated silver nanoparticles