Nanomaterial Biocompatibility and Antimicrobial Effects on Escherichia coli

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Abstract. We studied the effects of cultivating E. coli with nanomaterials involved in biosensor development. E. coli were cultured separately with each of these nanomaterials in Luria-Bertani broth medium. Bacterial growth curves were plotted by measuring optical density over a period of 12 hours, after which time bacterial cell dry weight was measured. After 12 hours of incubation, optical density was 0.588, 0.983, and 0.633 and cell dry weight was $4.6 \times 10^{-3}$ g/ml, $4.9 \times 10^{-3}$ g/ml, and $4.1 \times 10^{-3}$ g/ml for bacteria cultivated in the presence of CNTs, AgNPs, and AuNPs respectively, suggesting that these nanomaterials have bactericidal or antimicrobial properties. On the other hand, graphene oxide (GO) does not have intrinsic bactericidal, cytotoxic, and/or antibacterial properties and greatly enhances bacterial growth. These findings advance our knowledge concerning the properties of nanomaterials and can guide the selection of appropriate nanomaterials for the fabrication of the transducer layer of a biosensor.

Keywords: Escherichia coli, biosensors, nanomaterials, antimicrobial, biocompatibility.

INTRODUCTION

Nanotechnology and biotechnology are two interesting fields of science that have experienced tremendous growth (Chatterjee et al., 2011). Nanobiotechnology is an interdisciplinary subject that combines physical and chemical properties with biological characteristics (Amin et al., 2011). One can modify a nanostructure by modifying the nanoscale dimensions according to the need for better integration of biological element into nanomaterials; one possible result of surface-layer modification is enhanced biocompatibility (West & Halas, 2000). The combination of biology and physical science leads to the creation of biosensors with better specificity, functionality, and sensitivity. Employing nanomaterials for the fabrication of a biosensor boosts its performance, owing to unique properties such as quantum effect, surface effect, mini-size effect, and macro-quantum tunnel effect (Amin et al., 2011). Moreover, in a hybrid system, nanoparticles and most biomolecules are in the same size range, making them natural companions. Nanomaterial-based biosensors are highly versatile, small, easy to handle, portable, low cost, and easily integrated into a small biochip (Jianrong et al., 2004).

Utilization of carbon nanomaterials, e.g., carbon nanotubes (CNTs) and graphene oxide (GO), and metal nanoparticles, e.g., gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs), has increased greatly in the fabrication of third-generation biosensors (Amin et al., 2011). Carbon nanomaterials and metal nanoparticles have been used in transducer layers to provide fast electron transfer, and better electrocatalytic performance, enhancing biosensor sensitivity. Although nanomaterials have immense potential to improve many aspects of biosensor performance, the bactericidal effect of nanomaterials is still an important factor that cannot be ignored. Studies have shown that different nanomaterials exhibit cytotoxicity to different mammalian cells as well as to bacteria (Shvedova et al., 2003; Manna et al., 2005; Ghosh et al., 2010). The effects of nanomaterials in terms of biocompatibility and antimicrobial properties on cells are crucial to avoid signal...
artifacts arising from both the recognition element and the transducer layer. The interaction between transducer layer and responding cells needs to be understood. Employing nanomaterials on the biosensor transducer layer can significantly affect cell viability, owing to cytotoxic and bactericidal properties.

We studied *Escherichia coli* growth in the presence of carbon nanomaterials and metal nanoparticles as part of our attempt to develop a portable biosensor for *E. coli* detection. Moreover, *E. coli* is a single-cell microorganism, and therefore an ideal model for investigating the bactericidal activity of nanomaterials (Akhavan *et al.*, 2010; Chatterjee *et al.*, 2011).

**MATERIALS AND METHODS**

**MATERIALS**

*E. coli* was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at -80 °C until preparation of the culture stock solution. The bacteria samples were prepared under laminar flow; the work place was disinfected with 70 % ethanol before and after work to minimize contamination. The samples were placed in closed tubes for further measurements. All measurements were made on three separate sample sets and averaged to get a statistically correct result with 5% standard error. All biohazardous waste was autoclaved.

LB broth medium, ultra-highly-concentrated graphene oxide (GO), multi-wall carbon nanotubes (CNTs), gold nanoparticle (AuNPs) 15 nm in diameter, and silver nanoparticle (AgNPs) 10 nm in diameter were all obtained from Sigma-Aldrich (St. Louis, MO, USA).

**Bactericidal test.** Three sets of samples were prepared to observe the bactericidal effect of these carbon nanomaterials and metal nanoparticles on *E. coli*. In the first set, two types of sample were prepared: 1% v/v *E. coli* was inoculated into 100 mL of LB broth medium and supplemented with 1% CNTs or GO. In the second set, two types of sample were again prepared: 1% *E. coli* was inoculated into 100 mL of LB broth medium and supplemented with 1% AgNPs or AuNPs. The third set was prepared in the absence of nanomaterials: 1% *E. coli* was inoculated into 100 mL of LB broth medium. All samples were cultured in a shaker incubator for 12 hours at 37 °C and 150 rpm.

The optical density (OD) was measured every two hours to determine the bacterial growth curve. After 12 hours of incubation, the cell dry weight of *E. coli* was measured as follows: 1 mL cultured bacteria was centrifuged at 5,000 rpm for 15 min. Next, 1 mL distilled water was added to the pellet and vortexed for 30 seconds. The mixture of bacterial cells in distilled water was dried in an oven at 80°C for 12 hours and the dried cell biomass measured. Comparisons were conducted between control (cells only) and cells with nanomaterials.

**RESULTS AND DISCUSSION**

Figure 1 shows the growth curves of *E. coli* and corresponding bar graph. The growth curves of bacteria under the influence of nanomaterials display
distinct effects of nanomaterials, compared to the growth curve of the control. In the absence of nanomaterials, the growth curve of *E. coli* clearly represents all growth phases: lag, log, stationary, and death. After 8 hours in the presence of CNTs, AgNPs, and AuNPs, the bacteria have proliferated more and reached absorbance OD 600 levels of 0.951, 1.034, and 0.908, respectively, slightly higher than the control (0.901), and GO with absorbance OD 600 levels of 0.774. Subsequently, the bactericidal activity of CNTs, AgNPs, and AuNPs became obvious when the death phase suddenly appeared in the middle of the log phase and bacterial growth rate decreased rapidly. After 12 hours of incubation, the bacterial growth absorbance under the influence of CNTs, AgNPs, and AuNPs was 0.588, 0.983, and 0.633, respectively. Interestingly, it can also be seen from the growth curve that the sample with GO achieved a higher absorbance of 1.77 at 12 hours of incubation, compared to the control with absorbance of 1.44.

Measurements of bacterial cell dry weight after 12 hours of incubation followed by 12 hours drying at 80 °C confirm the bacterial growth results. From Figure 2 we observed that after 12 hours the bacterial biomass in the samples containing CNTs, AgNPs, and AuNPs was much lower than in the control: 4.6 × 10⁻³ g/ml, 4.9 × 10⁻³ g/ml, 4.1 × 10⁻³ g/ml, and 10.4 × 10⁻³ g/ml respectively. In the presence of these nanomaterials, bacterial growth was 60% inhibited. On the other hand, comparing the bacterial biomass of the control and the GO-containing sample, it can be seen that GO promoted bacterial growth over that of the control, as shown by the bacterial biomasses of 12.7 × 10⁻³ g/mL. The mechanism of CNT bactericidal activity is that single-wall carbon nanotubes physically puncture the bacterial cell membrane and bacterial death is due to degradation of bacterial cell integrity (Liu *et al.*, 2009). In the case of metal nanoparticles, the bactericidal mechanism has been explained in previous studies by the fact that metal oxides are

**Figure 2:** Cell dry weight (CDW) of *E. coli* grown in the presence of carbon-based nanomaterials and metal-based nanoparticles compared to the normal CDW of *E. coli* after 12 hours of incubation followed by 12 hours of drying.
positively charged, whereas *E. coli* are gram-negative bacteria with an outer covering of phospholipids and lipopolysaccharides imparting a strong negative charge. The electromagnetic attraction between the negative surface charge of the bacteria and the positive metal nanoparticles can result in oxidation of bacterial cell followed by bacterial death (Zhang & Chen, 2009).

**CONCLUSION**

In terms of biocompatibility and antimicrobial properties, the results of this experiment clearly prove that CNTs, AgNPs, and AuNPs have similar bactericidal or antimicrobial properties. Furthermore, based on the results, CNTs, AgNPs, and AuNPs are not biocompatible, as they inhibit bacterial cell proliferation; this is clear from the growth study. On the other hand, this study demonstrates that GO does not have intrinsic bactericidal, cytotoxic, or antibacterial properties. GO enhances bacterial growth by increasing the proliferation and attachment of the bacterial cell. This preliminary knowledge concerning the biological properties of carbon nanomaterials and metal nanoparticles can guide the selection of biocompatible nanomaterials for fabricating the transducer layer of an electrochemical biosensor.

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