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Cells-on-chip based transducer platform for probing toxicity of metal nanoparticles

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ABSTRACT

In this work, we developed a cells-on-chip based transducer (CoCT) platform for probing toxicity of silver nanoparticles (Ag NPs) using non-Faradaic electrochemical impedance spectroscopy (nFEIS). This transducing platform was consists of arrays of capacitor on chip in which each capacitor was functionalized with living *Escherichia coli* cells. These cells were capable of responding to exposure of different size and concentration of Ag NPs. The capacitive response of CoCT was dependent on size and concentration of NPs. *E. coli* cells-on-chip response exhibited dramatic loss of capacitance and showed that maximum toxicity to cells-on-chip occurred with smaller 10 nm sized Ag NPs compared to larger size of 100 nm NPs. The cells tend to resist to the larger 100 nm size of NPs that did not affect the cells-on-chip. Our results demonstrated that whole-cell biosensor chip response at a particular frequency enabled determining the severity of the stress imposed by smaller size of Ag NPs. Further, our results were validated through fourier transform infrared spectroscopy (FTIR) and growth of cell/cellular debris and also determined the NPs stress induced toxicity in cells as a proof-of-concept. The methodology developed in this study potentially be extended to other nanomaterials (NMs) for classifying toxic from non-toxic NMs.

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1. Introduction

The nanotechnology industry is rapidly growing with promises of substantial benefits that will have significant economic and scientific impacts. It is also applicable to whole host of areas ranging from aerospace engineering and nano-electronics to environmental remediation and medical healthcare [1]. It is estimated that over 500 consumer goods that are already available consist of variety of nanomaterials (NMs). Nanoparticles (NPs) are present in some sunscreens, cosmetics, toothpastes, sanitary-ware coatings, silicon chips and even in food products. Worldwide investment on nanotechnology is on the rise [2,3] and the trend is expected to continue over the next decade. Unusual physicochemical properties of engineered NMs are attributable to their small size, chemical composition, surface structure, solubility, shape, and aggregation [4]. Yet, concerns have been raised due to the unique properties of nanostructured materials could potentially lead to unforeseen health and environmental hazards. Due to expanding use of NMs and commercialization of nanotechnology products, their exposure in the environment and to humans tend to increase with time. Colloidal silver, including formulations now known to contain silver nanoparticles (Ag NPs), has been used commercially for almost 100 years [5]. However, registration of nanosilver products has increased dramatically over the last decades [5,6], most likely as a result of improved capabilities in nanoscience and engineering that allow Ag NPs to be formulated to confer increased durability and/or sustained antibacterial action, even under harsh environmental conditions [7,8]. As is the case for many other types of nanoparticles, a controversy has arisen about whether the Ag NPs should be subjected to increased regulatory scrutiny compared to macroscale or "bulk" silver. Indeed, Ag NPs have been demonstrated to exhibit toxic effects in plants and bacteria at environmentally relevant concentrations [9]. To date, most traditional biological methods for *in vitro* and *in vivo* toxicological studies of Ag NPs and other engineered NMs on microbial cells are based on cellular activity and proliferations. These methods include growth and viability assays [10–12], proteomic assays, reactive oxygen species (ROS) detection tests [13–15] and molecular-level evaluations based on genetic responses [5,16,17]. Recntly, size dependent effect of Ag NPs against aerobic bacteria *Escherichia coli* and anaerobic oral pathogenic bacteria *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nuceatum*, *Streptococcus mitis*, *Streptococcus mutans and Streptococcus sanguis* have been studied through cell growth

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method [18]. Among all of the above methods, *in vitro* cytotoxicity methods are currently employed, which required labeling with fluorescent molecules for detection. These methods are used as markers for cell-viability and consist of procedures that provide results only at a final time-point [19]. The existing conventional analytical techniques reported in the literature usually requires a lengthy and time-consuming process and often produce false positives, and often cannot be implemented for studying cell behavior without interference from its surrounding environment. Hence, there is a demand for a rapid, sensitive and accurate method for assessing toxicity in cells.

Recently, due to the advantages of automation of fluids and minimization of human errors, integration of a cells-on-a-chip system is gaining importance for nanotoxicity assessments [20]. In recent studies, chip-based electrochemical approach was used to test the toxic effect of NMs. The measurements were recorded based on differential pulse voltammetry and or electrical impedance methods [21–23]. All the above cells-on-chip approaches for nanotoxicity assessment were based on Faradaic-electrochemical measurements that require a redox mediator/chemical reagent to generate detectable signal which often leads to undesirable quenching effects with NPs. Therefore, the present study aims to develop toxicity assay using electrical sensing platform that provides a new tool to understand the toxicological impacts of Ag NPs as model for other NMs using bacterial cell-functionalized biosensor chip.

In this study, we designed a CoCT using electrical sensing platform that provides a new tool to understand the toxicological impacts of Ag NPs as model for other NMs. This CoCT measures the capacitance from immobilized cells over electrodes as a function of applied AC frequency and its measuring principle is based on non-faradiac method. Changes in capacitance can be detected that occurred as a result of changes in the cellular activity after their interaction with NPs. The results obtained from the CoCT studies were confirmed through optical imaging, FTIR as well as probing cellular interactions at the cell-membrane after exposure of NPs by growth assay. The fact that the present study is different from the previous reports in an important consideration that include the deleoped biochip was tested for metal oxide NPs and it is important to test metal NPs response as well to extend the applicability of developed CoCT to other NMs [24].

2. Material and methods

2.1. Reagents

Silicon wafers of 4 inch size, (100) oriented, p-type with the resistivity of 9–12 Ω cm and thicknesses of 500 ± 25 μm with 1 μm thick SiO_2 layer on top were obtained from University Wafers, USA. Wild-type *E. coli* DH5α strain was used as model living bacterial cells in this study. Luria-Bertani broth (LB-broth) and Luria-Bertani agar (LB-agar) were obtained from Difco (MI, USA). Phosphate-buffered saline (PBS), 3-mercaptopropionic acid (MPA), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS), Ag NPs (with sizes of 10, 20 and 100 nm) were purchased from Sigma-Aldrich, Germany. All other reagents used in this study were of analytical grade and filtered through 0.22 μm sterile filters.

2.2. Characterization of Ag NPs

The shape and size of Ag NPs used in this study were characterized using transmission electron microscopy (TEM) technique and dynamic light scattering (DLS,Malvern Zetasizer). For TEM characterization sample preparation, 5% solution of each size of Ag NPs were suspended in aqueous medium and then placed on fromvar coated copper grids. The grids were then allowed to dry at room temperature. The images were captured using 2000 SX JEOL-TEM at 160 kV EHT voltage with a magnification of 250Kx.

2.3. Fabrication of capacitor arrays

Gold interdigitated electrode based capacitor sensor arrays were patterned on SiO_2 surface using image reversal technique. In this process, the metal layers were patterned using the dual tone photoresist AZ5214E. A 2 μm thick AZ5214E photoresist was patterned with the help of a mask for a lift-off process in pure acetone as a solvent. Following this step, a very thin tungsten layer of 50–60 nm size was layered to improve the adhesion of gold on the SiO_2 film by DC sputter deposition and about 200–210 nm thick gold layer was deposited. The dimension of each electrode was 800 μm in length, 40 μm in width with a distance between two electrodes of 40 μm. Each capacitor sensor contained 24-interdigitated gold electrodes within a total area of 3 mm^2.

2.4. Immobilization of E. coli cells on capacitor array chip and exposure of NPs

The bacterial strain used in this study was *E. coli* DH5α. Actively growing *E. coli* cells were inoculated into fresh Luria-Bertani (LB) medium and allowed to grow till mid-logarithmic growth phase. The cells were then harvested by centrifugation at 1000*g* for 3 min and washed thrice with phosphate buffered saline (PBS) pH 7.2, and resuspended in same buffer. The cell concentration was determined by colony counting after serial dilution followed by plating on LB-agar plates. The chip was first rinsed with sterile distilled water and dried with pure nitrogen followed by immersing the chip in 20 mM of mercaptopropionic acid (MPA) and incubated overnight at room temperature. The chips were then incubated with a mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 50 mM of *N*-hydroxysuccinimide (NHS) for 2 h. The chip was then removed, thoroughly washed with distilled water and each capacitor in an array was incubated with 5 μl of bacterial suspension containing 8×10^7 colony forming units (CFU) in PBS solution for 2 h. This sensing area was incubated with a series of Ag NPs concentrations (0.2–2 μg/ml PBS containing 0.01% Tween 20) in 5 μl volumes containing three different sizes 10, 20 and 100 nm, respectively for 2 h at 25 °C. The chip was quickly washed with PBS and dried using nitrogen gas. The dried chips were then subjected dielectric measurements.

2.5. Optical microscopy imaging of immobilized E. coli cells on chip

Optical images of the sensors after immobilization of *E. coli* cells on chip were taken using Carl Zeiss Axio Scope A1 MAT.

2.6. Impedance/capacitance measurement

The impedance/capacitance responses were measured sequentially to ensure that chips remain active after every step that includes; (a) bare capacitor sensor, (b) after *E. coli* immobilization and (c) after NPs exposure. A negative control experiment was conducted using capacitor chip containing heat-killed *E. coli* cells. For this, capacitor chips containing immobilized *E. coli* cells (8×10^7) were subjected to heat treatment in an air-tight pre-heated humid chamber at 95 °C for 5 min followed by quickly freezing at −70 °C for 5 min, and thrice at 25 °C for 15 min. The above treatment process was repeated thrice and finally the capacitive chip was dried under N_2 gas. The capacitance response in between the gold interdigitated electrodes of capacitor sensor surface was measured in the frequency range 50 MHz to 1 GHz using a Network Analyzer (Karl-Suss

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