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Label-free capacitive *E. coli* biochip for determining chemicals that induce cellular toxicity

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Abstract

A novel method for the detection of toxicity of chemicals on living cells was developed using carboxy-CNT activated gold interdigitated (GID) capacitor arrays immobilized with viable wild-type *E. coli* (biochip). The toxicity detection is based on label-free non-Faradaic electrochemical impedance spectroscopy (nFEIS). The technique enabled monitoring the stress/toxicity imposed by chemicals on viable bacteria. Here, model stress inducing chemicals such as, acetic acid (metabolic), H_2O_2 (oxidative) and sodium chloride (salt) were tested with the developed biochip. The resulting response of biochip determined the concentration dependent toxicity levels imposed by chemicals on the cells. Interesting feature of the developed biochip sensor is the characteristic dispersion peaks at 463 and 582 MHz that determined the toxic nature of the chemicals. The biochip developed finds advantages in labeling chemicals that are potentially harmful to humans and the ecosystem.

1. Main text

Microorganisms such as bacteria can be ideal choice as biological sensing elements to determine the toxicity nature of a variety of chemicals [1]. Chemicals derived of pharmaceutical preparations, drugs, defense agents, environmental and food samples may exhibit detrimental effects by causing cellular damages, such as oxidative, genotoxic, and metabolic stresses that are harmful to living organisms [2]. Live cells can be utilized to assess toxicological risks and to determine the toxic nature of chemicals has on humans. The toxicity response of bacterial cells is often determined in terms of various stress

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responses. The stress responses in bacteria are classified into different types based on the nature of the chemical compound to induce toxicity [3].

Bacteria can respond to various cellular stresses under the AC electric field and the changes in electrical responses of bacteria to external stress can be captured or monitored by nFEIS. This can be accomplished by tethering live bacterial cells on GID as sensing surface (biochip). When toxic chemicals were exposed to these bacterial cells on sensor surface, the cells respond to the external stimuli and gives rise to surface charge distribution, which can be measured by nFEIS against the AC frequency (Fig. 1a-b). As a result, the total charges present on the sensor surface polarize and relaxes that dependents on a specific frequency. The change in response of bacterial cells on sensor surface against the toxic/stress chemicals can then be monitored.

Here, we attempted to develop a new method to determine toxicity of chemicals, which is label-free and non-invasive that utilizes carboxy-CNT activated capacitors immobilized with *E. coli* bacteria as biosensing elements that did not require any mediators by nFEIS. The proposed detection methodology is based on nFEIS, which can be used to screen various chemicals, toxic gases, pharmaceuticals, drugs, defense agents, environmental and food samples for the determination of chemicals' potential to cause cytotoxicity.

1.1. Electrical response of E. coli bacterial cells

E. coli cells were first freshly grown until the cells reached to mid-exponential growth phase. Active cells were then immobilized on the GID surface of biochips that were previously activated with carboxy-CNTs through covalent coupling. The biochip containing immobilized cells were then subjected to capacitance measurements after applying AC electrical frequency from 50 to 600 MHz range. Active dispersion peaks was observed at 463 and 582 MHz frequencies that were characteristics of viable *E. coli* bacteria under electrical environment. The response of cells at 463 MHz showed that the increase in capacitance response was observed with increasing the number of cells from 8.7×10^6 to 1.74×10^7 cells on an area of 3×3 mm^2 biochip surface. Additionally, heat killed cells (1.74×10^7 cells) showed significant loss in capacitance compared with same number of viable cells (Fig. 1c).
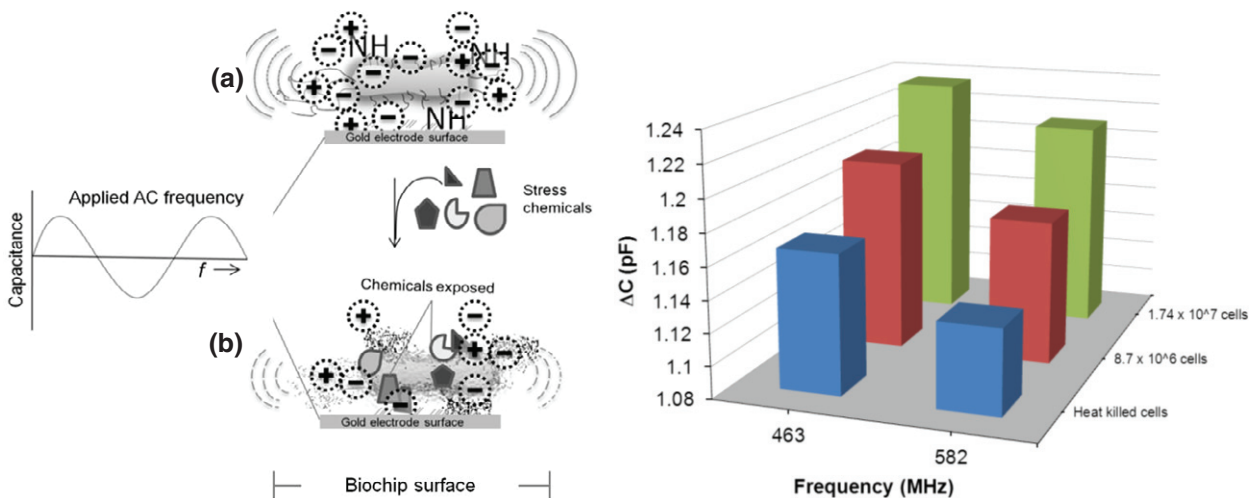
Fig. 1. Schematic diagram of an *E. coli* cell immobilized on gold electrode surface of biochip showing the electrical response: (a) before; (b) after the chemical treatment; and (c) change in capacitance (ΔC) response of heat-killed 1.74×10^7 cells as well as two concentrations of viable cells (8.7×10^6 and 1.74×10^7) under AC frequency at 463 MHz.

1.2. Capacitance response of E. coli cells to stress inducing chemicals

Chemicals that induce cellular stress responses were tested using capacitive biochip developed in this study by nFEIS technique. The detection technique is based on the change in surface charge distribution after the interaction of toxic chemicals on the outer surface of viable *E. coil* cells. For this, the biochip surface was first activated with carboxy-CNTs to enhance the sensitivity and this was followed by immobilization of *E. coil* cells as biological sensing elements. The biochip was then used to probe the cellular stress responses against model chemicals.

Three model stress inducing chemicals were employed in this study, such as (a) acetic acid, which induces acid or metabolic stress, (b) H_2O_2 induces hydroxyl radical stress that is responsible for oxidative toxicity and (c) NaCl induce salt or osmotic stress. In addition, these chemicals were exposed at two different time intervals (for 1 and 3 h) to study the impact of chemical exposure time. The resulting response of cells to stress chemicals was distinct that was dependent on the nature of chemical to induce toxicity. For example, acetic acid was more toxic than H_2O_2 and this was evidenced by consistent decline in capacitance response, irrespective of exposure time. This result suggests that the cells were severely affected by the acid stress (Fig. 2a) while H_2O_2 at initial 1 h exposure did not affected the cells. However, at prolonged exposure (3 h) with 2% H_2O_2, the cells exhibited adaptive behavior against the oxidative toxicity occurred with 2% H_2O_2 (Fig. 2b). Further increase in concentration of H_2O_2 (3%) exposure only yielded detrimental effects with declined capacitance indicating that 3% H_2O_2 was toxic to cells (Fig. 2b).
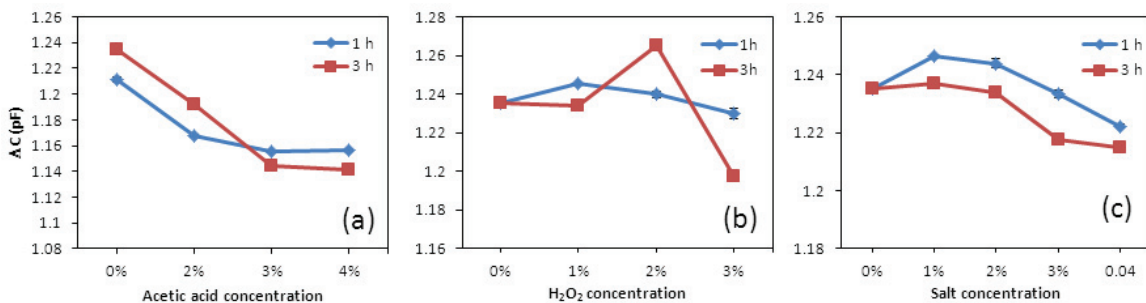

Fig. 2. Change in capacitance (ΔC) response of *E. coli* cells (immobilized on CNT activated sensor chip) as a function of different concentrations of (a) acetic acid (acid stress), (b) H_2O_2 (oxidative stress) and (c) NaCl (salt stress) at a constant AC frequency (463 MHz) for 1 and 3 h treatment times.

The biochip was further tested by treating with yet another chemical, which is not toxic to cells, but at its higher levels induces salt/osmotic stress. To test this, various concentrations of NaCl (0-4%) was incubated on biochip to study the salt stress responses. It was observed that the cells experienced mild adaptive responses to 0-3% concentration at the initial 1 h of treatment and the cells tend to experience the salt stress with 4% of NaCl concentration (Fig. 2c). It should be noted that the salt concentration used to test the salt stress was well above the normal physiological levels (above 1%). At prolonged exposure

of cells from 1 to 3 h, the salt concentration above 2% found to induce salt stress while the cells were not affected by at lower salt concentrations (1-2%) (Fig. 2c). This result is in agreement with previous studies where salt tolerance levels has been reported up to 1.1 M (~6.5%) NaCl for *E. coli* [4] and the cells could adapt to high salt concentrations for osmotolerance [5, 6]. Our results demonstrated that the biochip response at a particular frequency enabled determining the severity of the stress imposed by chemicals and the methodology described here served as a novel means to determine cytotoxicity.

   In conclusion, the developed new method to determine toxicity of chemicals, which is label-free and non-invasive that utilizes carboxy-CNT activated capacitors immobilized with *E. coli* bacteria as biosensing elements that did not require any mediators by nFEIS. The proposed detection methodology is based on nFEIS, which can be used to screen various chemicals, toxic gases, pharmaceuticals, drugs, defense agents, environmental and food samples for the determination of chemicals' potential to cause cytotoxicity.

Acknowledgements

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