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Probing synergistic toxicity effects on living cells by combination of two different sized nanoparticles by a whole–cell based biochip

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Abstract

In this study, a whole-cell based capacitive sensor chip was employed to determine the toxicity effects caused by pure and mixed combinations of TiO_2 and Fe_3O_4 nanoparticles (NPs). Our results demonstrated that the cells-on-chip severely affected by the exposure of pure forms of NPs, while a synergistic effect occurred with significantly less toxicity when TiO_2:Fe_3O_4 combinations were used. The toxic effects with pure forms of NPs are attributed to disorganization of cell-surface charges dominated as compared to exposure of mixed combinations of NPs. The developed whole-cell chip offered real-time, label-free and non-invasive detection tool for probing cellular responses against NPs.

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Keywords: Nanoparticles; cells-on-chip; toxicity; living cells

1. Introduction

Metal oxide nanomaterials (NMs) are promising candidates for many industrial, consumer, and medical applications because of their unique physicochemical properties (i.e., high surface areas and extraordinary electronic properties) [1-4]. Given the widespread applications and their commercialization, there is an increasing potential for humans to be exposed to multiple forms of NMs. While much work in nanotoxicology has been focused upon specific nanoparticles (NPs) effects, little attention has been given to the effects of mixtures, or combinations, of

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various NPs on biological systems. The real-world exposure is very likely to occur in the form of mixture of multiple substances, such as airborne particulate matter or contaminated waste-water. In a nanotoxicology setting, mixed

NMs exposure to environment/human is likely because multiple types of NMs are often used simultaneously such as metal oxide NPs (Fe_3O_4, TiO_2). These mixed NMs are likely to be used in various industrial applications [5-8]. Therefore, it is imperative to understand the toxicological effect of exposure of cells to mixed NMs. In this study, the toxicity assay was tested for multiple metal oxide NPs by mixing two different metal oxides such as <130 nmTiO_2 NPs mixed with varying sizes such as 5 and 100 nm of Fe_3O_4 NPs on developed *E. coli* whole-cell biosensor (WCB) chip.

2. Experimental

2.1. Whole-cell chip and toxicity assay development

The experimental methods related to whole-cell chip fabrication were followed reported in [9]. To test and validate the toxicity assay for multiple metal oxide NPs, TiO_2 (<130 nm) and Fe_3O_4 (5 and 100 nm) NPs were used. Interaction of *E. coli* immobilized cells on chip with or without mixed NPs was carried out by first preparing each type of NPs (<130 nm TiO_2 and 5 and 100 nm of Fe_3O_4) as a homogeneous suspension in a solution containing 5% ethanol + 95% PBS at 2 μg/ml concentration. The NM-suspensions were prepared separately and later mixed at 1:1 ratio for "<130 nm TiO_2:5 nm Fe_3O_4" and "<130 nm TiO_2:100 nm Fe_3O_4" combinations. The mixed and pure NPs suspensions were stirred vigorously and then immediately exposed to *E. coli* immobilized chip. The exposure of four sample types that include (a) single (pure) NP forms of <130 nm TiO_2 NPs, (b) single (pure) NP forms of 5 nm Fe_3O_4 NPs, (c) single (pure) NP forms of 100 nm Fe_3O_4 NPs, (d) mixed NPs combinations of <130 nm TiO_2:5 nm Fe_3O_4 and (e) mixed NPs combinations of <130 nm TiO_2:100 nm Fe_3O_4. All the above 5 different NP suspensions were allowed to interact at the interface of cells present on sensor chips by incubating at 1 and 3 h, respectively. The chips were thoroughly washed with PBS before taking dielectric/capacitance measurements.

2.2. Capacitance measurements

To measure dielectric parameters (impedance/capacitance), Network Analyzer (Karl-Suss (PM-5) RF Probe Station and an Agilent-8720ES S-parameter) were used. The Network Analyzer was calibrated using the short-open-load-through (SOLT) method for scan frequency range 100-250 MHz. All measurements were performed in triplicate for deviation analysis. The measured S11 parameter values were exported to Matlab for analysis. The relative capacitance variations were calculated from the data obtained at 200 MHz frequency under standard assay conditions using the following Eqn. (1) as described previously (10).

$$\frac{C - C_0}{C} X\ 100 \qquad (1)$$

where C is the actual capacitance after the interaction of each sizes of NPs with *E. coli* cells at a particular concentration and C_o is the capacitance before interaction. For control, *E. coli* immobilized chips were treated with only PBS solution in place of NPs (blank/control).

2.3. Whole-cell capacitor characterizations

The fabricated capacitor chip was examined using an optical microscope (Carl Zeiss Axio Scope). The surface of chip immobilized with *E. coli* cells were examined by using scanning electron microscopy (SEM LEO Supra 35VP) at different magnifications to observe the uniformity of cell-layers on microelectrodes.

3. Results and discussion

Initially, capacitor arrays were fabricated using photolithography technique. Surface topology of the bare capacitor chips were shown in Fig.1 (a-b).

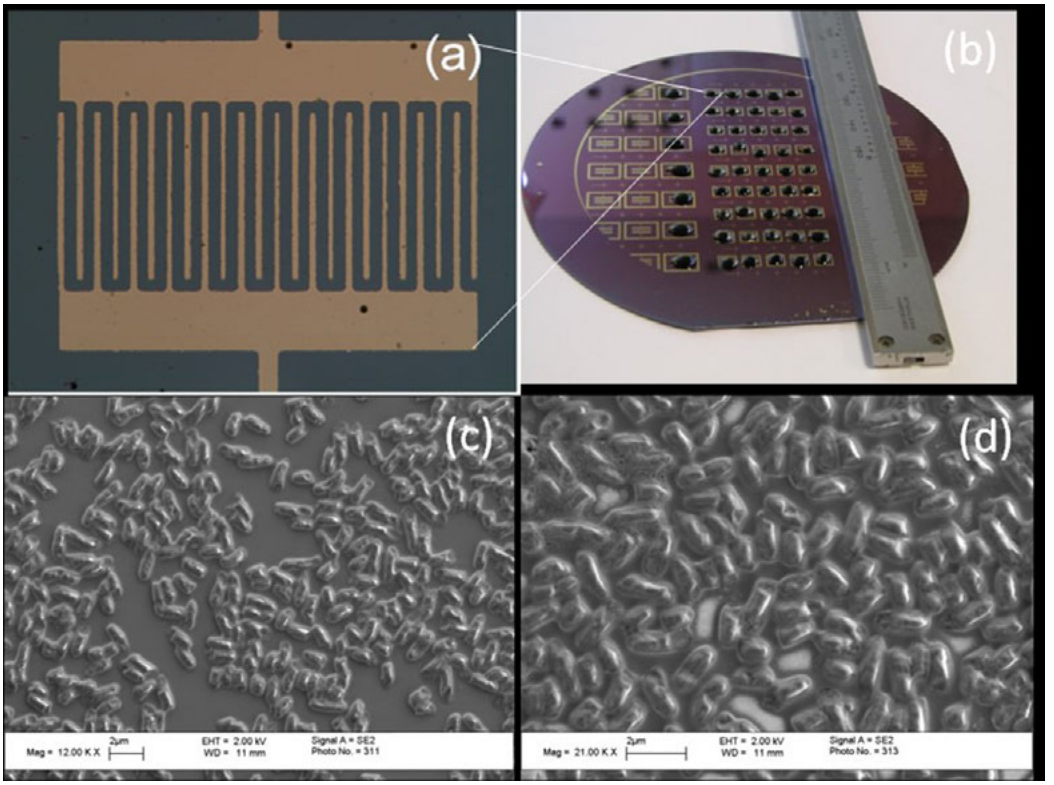

Fig. 1. (a-b) Optical micrograph of a bare capacitor and whole-cell chip; (c-d) SEM images of immobilized cells on chip.

The surface of bare capacitor showed uniformly patterned gold-interdigitated microelectrodes on sensors which was essential for the sensitivity of the sensors. Each wafer contained 45 independent capacitors in arrays each made of 24 gold microelectrodes within a total area of 3 mm^2 that served as individual sensors (Fig.1b). The immobilized cells on developed WCB chip are shown in Fig.1 (c-d). The cells were found to be uniformly distributed on the chip.

The capacitance response profile from whole-cells on chip upon exposure to multiple NPs combination such as <130 nm TiO_2 NPs mixed with two sizes (5 and 100 nm) of Fe_3O_4 exhibited a size dependent response at 1 and 3 h (Fig.2).

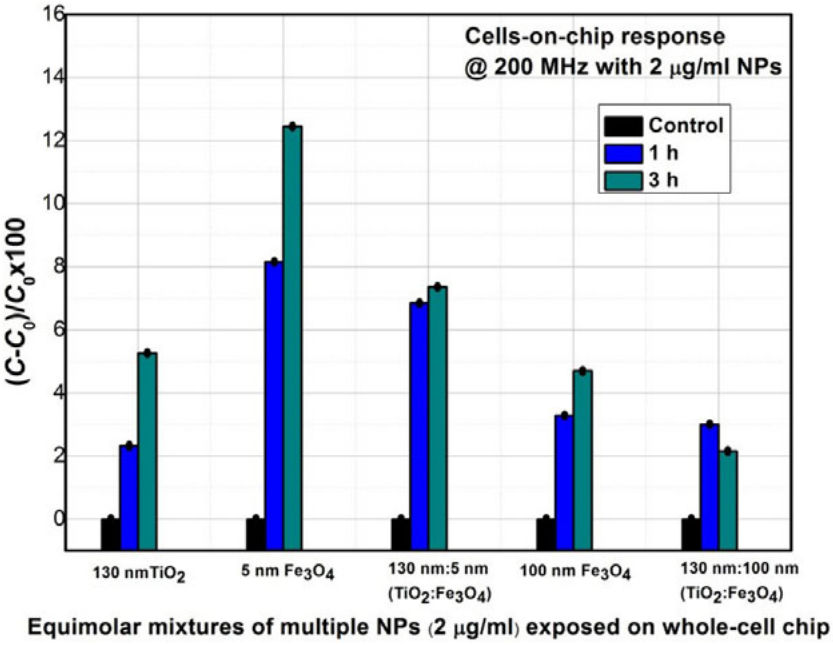

Fig. 2. Relative change in capacitance from whole-cell chip sensors against multiple metal oxide combinations such as TiO_2 NPs (130 nm) mixed with varying size of Fe_3O_4 NPs (5 and 100 nm) at 1 h and 3 h exposure time, respectively.

The mixed NPs first combination (<130 nm TiO_2:5 nm Fe_3O_4) showed increase in capacitance response as compared with its counterpart single/pure TiO_2 NPs response alone. This result implies that the cells on chip interacted and significantly affected by the exposure of mixed forms of NPs. Therefore, the response of cells-on-chip is attributed to disorganization of cell-surface charges likely to have occurred predominantly by 5 nm Fe_3O_4 NPs in the mixture that may have induced toxicity in cells as compared to exposure of single/pure forms TiO_2 NPs. Therefore, we observed that exposure of a mixture of larger+smaller sized NPs (e.g., <130 nm TiO_2:5 nm Fe_3O_4) on sensor chip exhibited a synergistic effect as that of the responses seen with pure forms of smaller sized NPs.

4. Conclusions

In this study, a whole-cell based capacitive sensor chip was developed and probed the toxicity effects caused by pure and mixed combinations of TiO_2 and Fe_3O_4 NPs. The response against pure/single type of 5 nm Fe_3O_4 alone exhibited large capacitance change implying that maximum damage may have occurred in cells-on-chip. The results suggested that the immobilized *E. coli* cells on sensors undergo less deformation when exposed to mixed NPs combination with larger+smaller sized of NPs, resulting in reduced surface charge distribution on sensor surface and therefore reducing the toxicity of smaller sized 5 nm Fe_3O_4 NPs alone. We hypothesized that exposure to different size/type of NPs tend to mitigate the toxic effect when compared to the pure forms of smaller sized NPs responses alone. Further, the validations of these results using biochemical method are under investigation. The developed whole-cell chip made of multiple arrays of capacitor sensors offered real-time, label-free and non-invasive detection tool for probing cellular responses against NPs that can provide useful information on the nature/toxicity of NPs.

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