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Structural morphology and *in vitro* toxicity studies of nano- and micro-sized zinc oxide structures

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ABSTRACT

The structural morphology and *in vitro* toxicity of nano- and micro-sized ZnO structures were investigated. All the ZnO samples were characterized to determine their morphologies, particle sizes, structures and optical bandgaps. Transmission electron microscopy and field-emission scanning electron microscopy results revealed that the morphologies of nano-sized ZnO-N1 and ZnO-N2 samples consisted of spherical and irregularly-shaped particles, respectively. The corresponding particle sizes were 20–40 nm and 50–80 nm, respectively. The morphologies of micro-sized ZnO-M1 and ZnO-M2 samples were found to be mostly rod-structures and plate-structures, respectively. Rod diameters were 40–100 nm whereas plate widths were 50–150 nm. ZnO-N1 had the highest toxicity towards the cells, causing the cell viability to be less than 70% for all concentration ranges. This phenomenon was due to the small particle size and release of zinc ions. Micro-sized ZnO-M1 and ZnO-M2 samples had toxicity limits of 0.3 mM at most.

Introduction

Nanomaterials have specific physicochemical properties that are not evident in bulk samples. Compared to micro-sized materials, most of the unique properties of nanoparticles have been attributed to their high surface-to-volume ratio. Nanoparticles offer a large surface for adsorption, and sometimes a high reactivity in many processes [1,2]. Toxicology has become an important area of research in dealing with the interactions of nanomaterials, nanostructures and nanodevices with biological molecules and organisms [3]. The evaluation of the safety of nanoparticles provides useful information about their undesirable effects, and even contributes to the development of tools to prevent such effects.

ZnO micro/nano-structures have attracted a great deal of attention due to their useful optoelectronic properties and novel applications in catalysis, paints, UV detectors, transparent conductive films, varistors, gas sensors, solar cells and cosmetic products [4–9]. Furthermore, ZnO nanoparticles are frequent constituents or ingredients in many personal healthcare products such as cosmetics and sunscreens, as a result of their superior UV absorption and reflectance properties [10]. The expanding production and use of ZnO has led to the potential for its release into the environment. For instance, Gottschalk et al. [11] reported that ZnO nanoparticles were found with concentration of 10 ng/l in natural surface water and 430 ng/l in treated wastewater in Europe. Moreover, the concentration of ZnO nanoparticles was estimated to be 100 μg/l (water) and few mg/kg (soil) in UK environment [12]. The review by Daughton and Ternes revealed that the level of ZnO particles would increase continually due to the extensive application of these materials [13].

Recently, there were some research works that had been conducted to investigate the toxicity impact of ZnO nanoparticles towards variety of organisms, such as human cell lines [14,15], bacteria [16,17], algae [18,19], nematodes [20], and plants [21]. Nonetheless, data and information about the toxicological effects and mechanisms of ZnO nanoparticles are still limited. The results of many researchers showed different views on the toxicity mechanisms. One or more mechanisms possibly responsible for the toxicity effects in which the major mechanism may depend on the test organism species and the test media. More extensive research is required to reveal a deeper insight into the toxic action

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of this widespread material in order to justify the potential adverse effects on human, animals, microorganisms and the environment. This study evaluates the *in vitro* biocompatibility of micro- and nano-sized ZnO particles using *L929* mouse fibroblast as cell model. Brayner et al. [22] reported that ZnO nanoparticles at a concentration of between 3 and 10 mM could lead to 100% inhibition and damage to bacterial cells. Therefore, the concentration of ZnO samples used in this study was manipulated from 0.1 to 0.5 mM, in order to justify the toxicity limit of the ZnO samples.

In this study, four types of ZnO particles (two micro-sized and two nano-sized) with different morphologies were used in toxicology experiments to determine their effects on *L929* mouse fibroblast cell lines. A comparison of their toxicity levels was performed and their potential mechanisms of toxicity were elucidated.

Experimental details

Two micro-sized ZnO samples and two nano-sized ZnO samples were used as the starting materials in this study. The micro-sized ZnO particles were synthesized through French process according to the oxidation of zinc metal in a factory, which was done in our previous work [23]. The nano-sized ZnO samples were commercial ZnO nanoparticles, purchased from Canada. Micro-sized ZnO particles were named ZnO-M1 and ZnO-M2 while nano-sized ZnO particles were named ZnO-N1 and ZnO-N2. All of the samples were of very high purity (>99%).

The structural morphologies of the ZnO samples were investigated using a Phillips CM12 transmission electron microscope (TEM) and a FEI NovaNanoSEM 450 field-emission scanning electron microscope (FESEM). The percentage composition (atomic percentages) of the ZnO samples was examined through energy-dispersive X-ray spectroscopy (EDS) analysis. The crystalline structures of the samples were characterized using a PANalytical X'Pert PRO MED PW3040 high resolution X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å). Optical absorption properties of ZnO samples were examined using a Shidmazu UV–vis 1800UV spectrophotometer. Optical bandgaps were obtained from the absorption spectra based on the UV-visible spectrum measurements. Besides, zeta potentials of the ZnO samples were studied using Zetasizer Nano-zs at room temperature. In the sample preparation, 0.01 g ZnO powder was suspended in 100 ml distilled water, which was then sonicated for 15 min to fully disperse ZnO agglomeration.

Cytotoxicity tests were conducted to study the toxicity effects of ZnO samples towards *L929* fibroblasts mouse cell lines. Initially, the cell lines were sub-cultured in Dulbecco's modified Eagle medium supplemented with 10% foetal bovine serum. The cells were grown at 37 °C in a 5% CO_2 incubator and the sub-culture process was repeated 3–4 times until the cell lines were confluent in the T-25 cell culture flask. Subsequently, *L929* cells were seeded into 6-wellplates and allowed to attach to the plates for 24 h. Twenty thousand cells were manipulated for seeding into each well. Dispersed ZnO particles with a concentration of 1 mM were used as a stock suspension and were sterilized using an autoclave. Then, the ZnO particles were diluted to 0.1, 0.2, 0.3, 0.4 and 0.5 mM by addition of culture media. The ZnO-media mixture was left in the incubator for 24 h so that the ZnO particles could react with and treat the media. After 24 h incubation, media in the 6-wellplates was discarded. The respective ZnO-media mixture was syringe-filtered to remove the insoluble ZnO particles. Then, the treated media was added to the cells in the 6-well plates. Cells without ZnO particles were also prepared as negative controls in a respective experiment. Besides, positive control was prepared through the treatment of cells with calamine lotion. A calamine lotion contained high amount of ZnO powder, which dominated

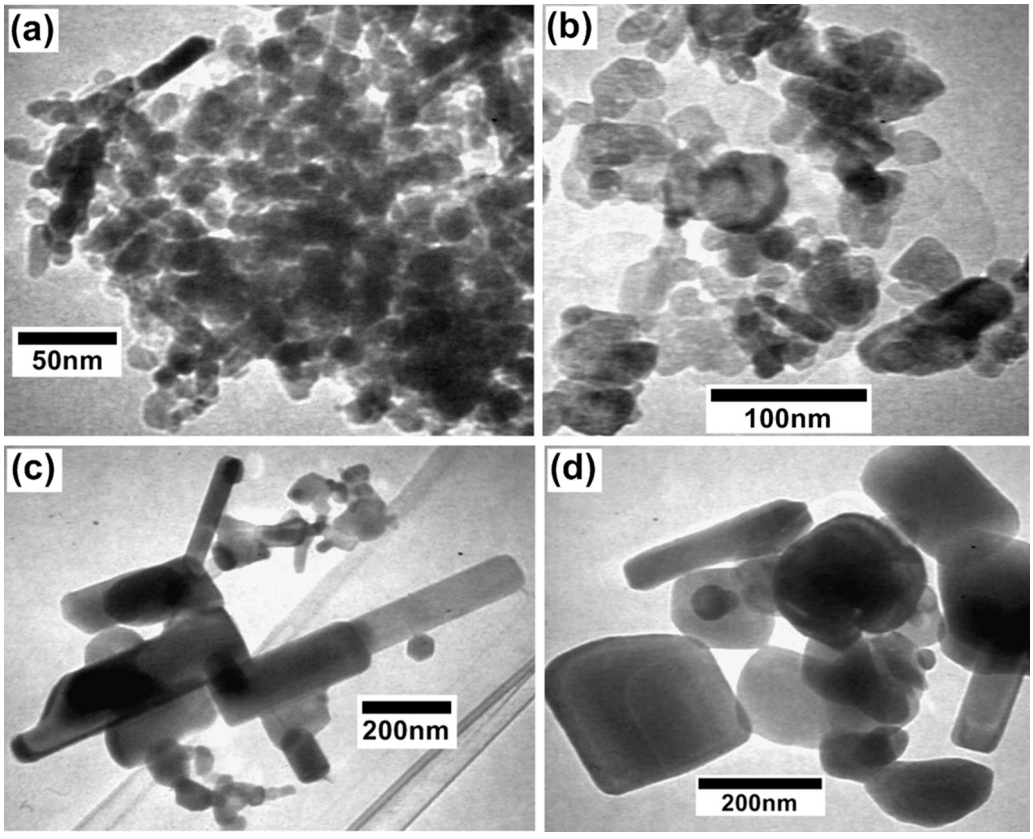

Fig. 1. TEM images of (a) ZnO-N1; (b) ZnO-N2; (c) ZnO-M1 and (d) ZnO-M2.