

Cytotoxic Effects of Engineered Nanoparticles in Human Mesenchymal Stem Cells

Ali A. Alshatwi, Vaiyapuri S. Periasamy, Jegan Athinarayanan

Abstract—Engineered nanoparticles' usage rapidly increased in various applications in the last decade due to their unusual properties. However, there is an ever increasing concern to understand their toxicological effect in human health. Particularly, metal and metal oxide nanoparticles have been used in various sectors including biomedical, food and agriculture. But their impact on human health is yet to be fully understood. In this present investigation, we assessed the toxic effect of engineered nanoparticles (ENPs) including Ag, MgO and Co₃O₄ nanoparticles (NPs) on human mesenchymal stem cells (hMSC) adopting cell viability and cellular morphological changes as tools. The results suggested that silver NPs are more toxic than MgO and Co₃O₄NPs. The ENPs induced cytotoxicity and nuclear morphological changes in hMSC depending on dose. The cell viability decreases with increase in concentration of ENPs. The cellular morphology studies revealed that ENPs damaged the cells. These preliminary findings have implications for the use of these nanoparticles in food industry with systematic regulations.

Keywords—Cobalt oxide, Human mesenchymal stem cells, MgO, Silver.

I. INTRODUCTION

NANOTECHNOLOGY is currently receiving increased attention from researchers, manufacturers and even the general population. Nanomaterials are being used in consumer products due to their unique physical, chemical and biological characters. A number of new consumer products include nanomaterials as an ingredient [1]. The use of engineered nanoparticles including metals, metal oxides and carbon nanotubes in consumer products increases every year [2], [3]. The global market value for nano-based food and food products was estimated at US \$4 million in 2006 and is increased to US \$13 billion in 2012 [4]. Moreover, engineered nanoparticles have been applied in various sectors, including cosmetics, biomedicine, electrical, and textiles [5]. In particular, nanomaterials have rapidly been applied in the food sector to improve the texture, color, flavor, structure, taste and quality of foodstuffs [6].

Several metal and metal oxide-based polymer nanocomposites have been used in food packaging for antimicrobial and UV absorption purposes. The health effects of nanomaterials can be divided into two aspects: the potential

for nanotechnological innovations to have therapeutic applications, and the potential health hazards posed by exposure to nanomaterials [7].

Silver nanoparticles are being used increasingly in wound dressings, catheters, and various household products due to their antimicrobial activity. The assessment of toxicity of silver nanoparticles has been carried out in different cellular models; the studies revealed that silver nanoparticles induced oxidative stress through cell morphological changes [8]. Moreover, MgO nanoparticles were used as food additive (E530), ceramic material, fire retardant and corrosion inhibitor. Ge et al. reported that MgO nanoparticles significantly triggered the NO release of the human umbilical vein endothelial cells [9]. Occupational exposure of cobalt can lead to various lung diseases, such as interstitial pneumonitis, fibrosis, and asthma. Previous studies reported that cobalt nanoparticles induce genotoxicity in humans. In additionally, cobalt/tungsten carbide composite exhibited synergistic effect of reactive oxygen species generation and induced DNA fragmentation [10]. In view of their possible risk for human health as nanotechnology products, the cytotoxic effects Co₃O₄ NPs are of concern. The effects of ENPs including silver, magnesium oxide and cobalt oxide on human health responses are unknown and need to be explored. Hence, we have assessed the potential risks of these particles by assessing their ability to induce cytotoxic effects and morphological changes in human mesenchymal stem cells.

II. MATERIALS AND METHODS

A. Materials

Eagle minimum essential medium, trypsin-EDTA, and antibiotic solutions were purchased from ATCC (USA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, USA) or Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of molecular biology or research grade.

B. Characterization of Engineered Nanoparticles

The particle size distribution was measured by dynamic light scattering (DLS) using a Zetasizer (Nano ZS-90, Malvern). The engineered nanoparticles were dispersed in distilled water under sonication. From the solution thus prepared, 1.5 mL was transferred to a square cuvette for DLS measurements. The average size was calculated from the intensity, volume, and number of distributions as measured, using software. The deionized water or solvent was also tested to ensure that no particulate contamination had occurred.

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C. Cell Culture

Human mesenchymal stem cells were obtained from Thermo Scientific Hyclone (USA). The cells were cultured in DMEM medium supplemented with 10% bovine serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin in 96-well culture plates at 37°C in a humidified atmosphere containing 5% CO₂. All experiments were performed with cells from passage 15 or less.

D. Cell Viability Assay

The cytotoxic properties of engineered nanoparticles were performed by MTT assay. Briefly, hMSC were seeded at a density of 1×10^4 cells per well in 200 µL of fresh culture medium. After overnight incubation, the cells were treated with different concentrations (25–400 µg/mL) of ENPs (Ag, MgO and Co₃O₄) for 24, 48 and 72 h. After incubation, 20 µL of MTT solution [5 mg/mL in phosphate-buffered saline (PBS)] was added to each well. The plates were wrapped with aluminum foil and incubated for 4 h at 37°C. The plates were centrifuged, and the purple formazan product was dissolved by the addition of 100 µL of DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96-well plate reader (Bio-Rad, CA, USA). Data were collected for three replicates of each concentration of ENPs, and these data were used to calculate the mean.

E. Microscopic Studies

Cell morphology was analyzed by bright field microscopy, respectively, in ENPs-treated hMSC after 24 h. The cellular morphology of the hMSC was analyzed after treatment with ENPs for 24 h. Control cells were grown in the same manner in the absence of ENPs. The cells were examined under an inverted bright field microscopy (Carl Zeiss, Jena, Germany). The results are presented as a series of three separate experiments.

III. RESULTS AND DISCUSSION

Nanomaterials are being used in recent times in the different sectors because of their unusual properties. However, the environmental and health impacts of nanomaterials are unknown; the usage of nanomaterials in commercial products has increased, leading to a growing public debate [7]. For instance, nano-based packaging materials, supplements and additives are utilized in the food sector to improve food quality [6]. Recently, numerous studies have evaluated the potential for toxicity of metal-based nanoparticles. In this regard, studying the possible effects of silver, MgO and Co₃O₄ nanoparticles on health is of great importance. In this study, we sought to fill in the knowledge gap regarding the silver, MgO and Co₃O₄ nanoparticles influence on cell viability and cellular morphology in hMSC.

A. Estimation of Particle Size by Dynamic Light Scattering

Dynamic light scattering measurements were performed on a Zetasizer NanoZS 90 (Malvern, USA) at room temperature. The volume-averaged particle size in distilled water and the percentile distribution of ENPs are shown in Fig. 1. The

results show that the average sizes of silver, MgO and Co₃O₄ nanoparticles were 45.2, 163 and 220 nm respectively. The results are the average of three measurements, and the error was expressed using the standard deviation. The obtained results agree with those previously reported in the literature for the same nanoparticles under the same conditions. The particle size analysis results of MgO and Co₃O₄ nanoparticles slightly differ from electron microscopy results, due to the aggregation of small particles.

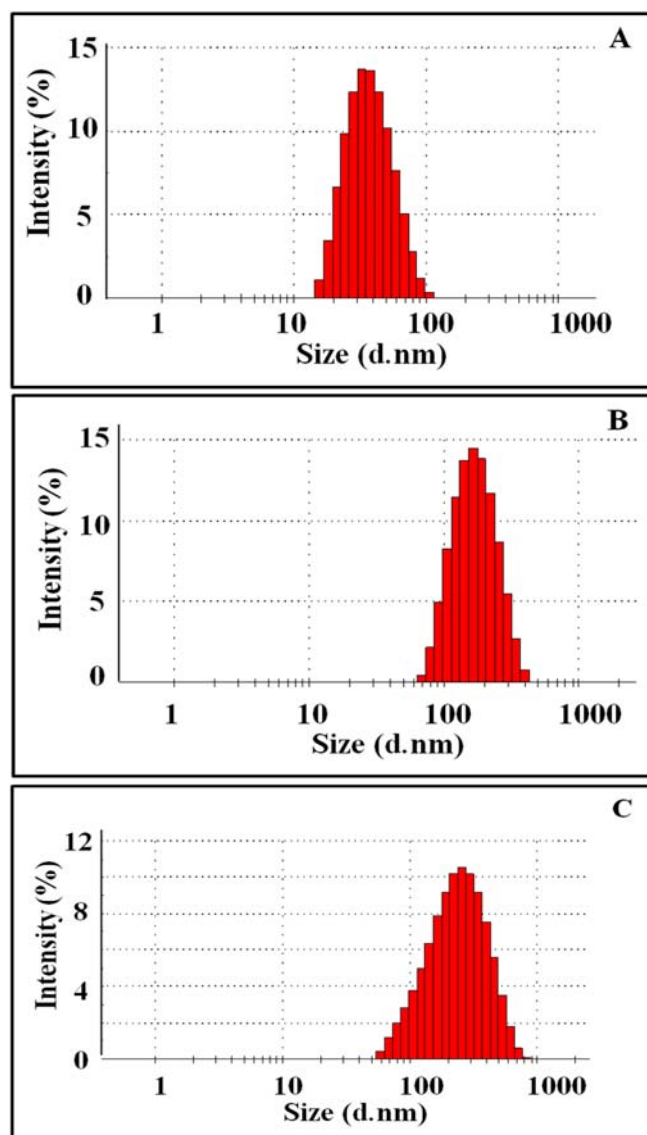


Fig. 1 Particle size distribution of ENPs (A) Ag NPs, (B) MgO NPs (C) Co₃O₄ NPs

B. Cytotoxicity of ENPs in hMSC

Normal human mesenchymal stem cells were treated to silver, MgO and Co₃O₄ nanoparticles at different concentrations (0, 50, 100, 200, or 400 µg/ml) for 24, 48 and 72 h. Cell viability was measured with an MTT assay. After the 24-h exposure to ENPs, we found significant changes in cell viability (Fig. 2). However, when the concentration of ENPs was increased, ENPs significantly affected cell viability.

Thus, ENPs triggered dose-dependent changes in cell viability. At low concentration, MgO and Co₃O₄ NPs are more toxic than Ag NPs; but at high concentration Ag NPs exhibited more cytotoxic effect on hMSC than MgO and Co₃O₄ NPs. The Ag NPs showed time and dose dependent cytotoxicity. The Co₃O₄ NPs shows low toxic effect than MgO NPs. Fig. 2 (A) indicates that low and high concentration of Ag NPs reduce the cell viability to 80% and 13% respectively. The high concentration of MgO and Co₃O₄ NPs reduces the cell viability to 33 and 18% respectively. Figs. 2 (B) and (C) shows the cell viability of 48 and 72 h exposure of ENPs respectively. Moreover, when the ENPs exposure time was increased (48 and 72 h), treatment with 50, 100, 200 and 400 µg/ml ENPs particles caused the cell viability to decrease compared to untreated cells. Our results suggested that ENPs induced toxicity in human mesenchymal cells through mitochondrial membrane potential depletion and ROS generation. Our previous study demonstrated that food additive silica nanoparticles induced dose and time dependent cytotoxicity through ROS generation and cell cycle progression [11].

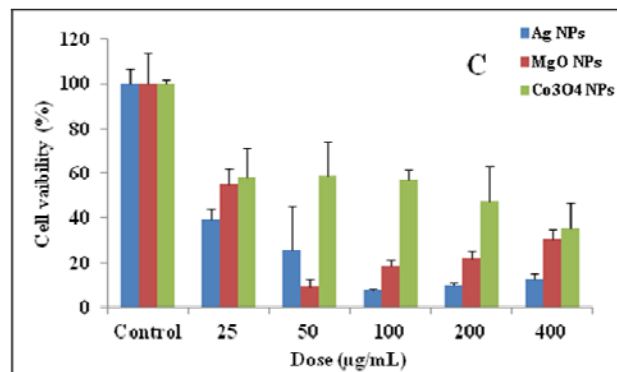
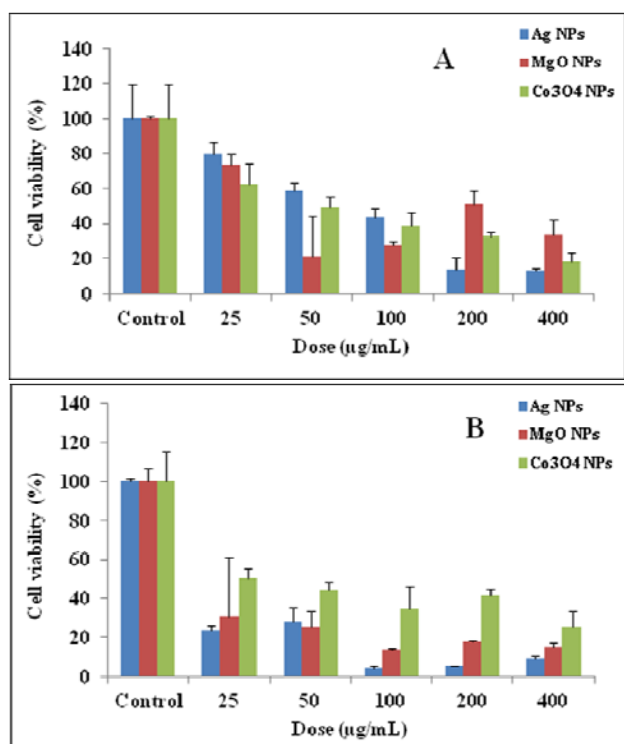


Fig. 2 Cytotoxicity of ENPs in human mesenchymal stem cells, Cells were treated with ENPs at various concentrations for (A) 24 h (B) 48 and (C) 72 h, and cell viability was determined using the MTT assay

C. Microscopic Analysis

The growth-inhibitory effects of the nanoparticles on hMSC were analyzed using bright field microscopy.

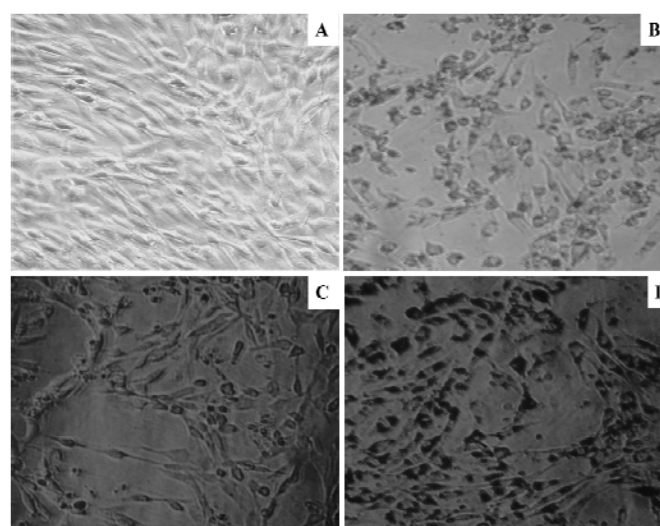


Fig. 3 Morphological changes in human mesenchymal stem cells treated with ENPs for 24 h at 400 x Bright-field microscopic examination of stem cells treated with ENPs. (A) Untreated control cells; (B) Ag NPs (C) MgO NPs and (D) Co₃O₄ NPs treated cells with moderate dose (100 µg/mL); the microscopic images shown abnormal cell morphology

The microscopic images revealed that the untreated cells were morphologically normal. The ENPs treated cells showed significant morphological changes. The morphological observations indicated that ENPs induced cell death in hMSC. The cellular morphology and biochemical changes are associated with apoptosis, ROS generation, DNA fragmentation and mitochondrial membrane potential [12]. The cytotoxic effects of ENPs nanoparticles are enhanced due to propagation through inflammatory or oxidative stress pathways. Excessive level of ROS triggers oxidative stress, which leads to a variety of complex diseases such as respiratory and/ or inflammatory disorders (asthma), neurologic disorders, aging, and cancer. Further studies are underway to investigate the toxicity of ENPs at the molecular

level in human mesenchymal stem cells.

IV. CONCLUSION

Our results suggest that ENPs reduce the cell viability and triggered cellular damage may leads mitochondrial membrane potential depletion, and ROS generation. ENPs may cause cell cycle distribution and modulate the expression of antioxidant enzymes and stress response through cellular morphological damage. Thus, we suggest that ENPs used products should be studied further to provide a better understanding of the potential negative impacts of ENPs on biological systems including *in vitro* and *in vivo* models.

ACKNOWLEDGMENT

We gratefully acknowledge the financial support of the National Program for Science and Technology, King Saud University, Saudi Arabia (Project code: BIO-981-10).

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