Cytotoxicity and DNA Damage Induced by Magnetic Nanoparticle Silica in L5178Y Cell

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Abstract
As recent reports suggest that nanoparticles may penetrate into cell membrane and effect DNA condition, it is necessary to assay possible cytotoxic and genotoxic risk. Three different sizes of magnetic nanoparticle silica (MNP@SiO\(_2\)) (50, 100 and 200 nm diameter) were tested for cytotoxicity and DNA damage using L5178Y cell. MNP@SiO\(_2\) had constant physicochemical characteristics confirmed by transmission electron microscope, electron spin resonance spectrometer and inductively coupled plasma-atomic emission spectrometer for 48 h. Treatment of MNP@SiO\(_2\) induced dose and time dependent cytotoxicity. At 6 h, 50, 100 or 200 nm MNP@SiO\(_2\) decreased significantly cell viability over the concentration of 125 \(\mu\)g/ml compared to vehicle control \((p<0.05\) or \(p<0.01\)). Moreover, at 24 h, 50 or 100 nm MNP@SiO\(_2\) decreased significantly cell viability over the concentration of 125 \(\mu\)g/ml \((p<0.01)\). And treatment of 200 nm MNP@SiO\(_2\) decreased significantly cell viability at the concentration of 62.5 \(\mu\)g/ml \((p<0.05)\) and of 125, 250, 500 \(\mu\)g/ml \((p<0.01,\) respectively). Cellular location detected by confocal microscope represented they were existed in cytoplasm, mainly around cell membrane at 2 h after treatment of MNP@SiO\(_2\). Treatment of 50 nm MNP@SiO\(_2\) significantly increased DNA damage at middle and high dose \((p<0.01)\), and treatment of 100 nm or 200 nm significantly increased DNA damage in all dose compared to control \((p<0.01)\). Taken together, treatment of MNP@SiO\(_2\) induced cytotoxicity and enhanced DNA damage in L5178Y cell.

Key Words: Magnetic nanoparticle silica, Cytotoxicity, Cellular location, Comet assay, DNA damage

INTRODUCTION
It seems that the safety of nanoparticles and the factors that influence their hazards are not fully understood, even in rapid expansion of nanotechnology. As nanoparticles have commercial potential benefit in market, it is needed to evaluate the possible effects on human and environmental health (Hol-sapple and Lehman-McKeeman, 2005; Thomas et al., 2006).

It has been reported that nanoparticles might facilitates their uptake into cells and transcytosis across epithelial and endothelial cells into body (Ober dorster et al., 2005), and some nanoparticles could induce undesirable harmful interactions with biological systems and the environment (Nel et al., 2006). As smaller size nanoparticles increase reactivity in cells (Thomas and Sayre, 2005) and even more, nanoparticles can bind to DNA or amino acid (Nel et al., 2006), it seems that they are often much more reactive than their bulk material counterparts. Despite intensive research efforts, it seems that cellular responses to nanoparticles are often inconsistent and even contradictory in some reports.

Actually, it was reported that no significant toxic effects due to silica nanoparticles at the molecular and cellular levels (Jin et al., 2007). However, recent study reported silica nanoparticles were found to induce oxidative stress indicated by induction of reactive oxygen species generation, and membrane lipid peroxidation (Akhtar et al., 2010), and the toxicity were confirmed in recent studies (Nabeshi et al., 2010; Yang et al., 2010; Ye et al., 2010). It seems that these discrepancy of toxicities may be associated with nano characteristics such as size, shape, surface chemistry and degree of aggregation influenced the production of free radicals and oxidative stress (Aillon et al., 2009).

Magnetic nanoparticle silica (MNP@SiO\(_2\)) is a developing nanoparticle for cell imaging having cobalt-ferrite magnetic...
MNP@SiO₂ was reported as an agent for specific targeting, cell sorting and bioimaging (Yoon et al., 2005; Yoon et al., 2006). Even magnetic silica nanoparticle can have wide applications in diagnosis, imaging and drug delivery, possible cytotoxicity and genotoxicity are not fully assessed. We set a test chemical as MNP@SiO₂, and tested cellular toxicity and examined cellular location and DNA damage in L5178Y cell, which has been used in genetic toxicology for mutagenesis and clastogenesis testing.

MATERIALS AND METHODS

Materials

Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). Methyl methanesulphonate (MMS) was obtained from Sigma (St. Louis, MO).

Cell line, cell culture and nanoparticles treatment

L5178Y was purchased from American Type Culture Collection (ATCC, Manassas, VA), and was cultured in DMEM medium supplemented with 1.5 g/L sodium bicarbonate, 10% FBS and 1% penicillin-streptomycin.
FBS and 1% penicillin at 37°C in a 5% CO₂ atmosphere.

### Synthesis and chemical characteristics of MNP@SiO₂

Three different sizes of MNP@SiO₂ (50, 100 or 200 nm) were purchased from Biterials Co. Ltd. Korea. They were prepared by the method as reported previously (Yoon et al., 2005). Analyses of size, component and inducing radical of synthetic MNP@SiO₂ were carried out from 0 to 48 h by transmission electron microscope (TEM), inductively coupled plasma-atomic emission spectrometer (ICP-AES) and electron spin resonance spectrometer (ESR), respectively.

### Cytotoxicity assay

Cytotoxicity was assessed by direct cell counting. In brief, L5178Y cells (2×10⁵ cells/ml) were treated with three different sizes of MNP@SiO₂ and were incubated for 6, 24 or 48 h at the concentration of 0, 31.25, 62.5, 125, 250 and 500 μg/ml, and cell counting was carried out.

### Cellular location

Confocal laser scanning microscopy was used to determine the localization of the MNP@SiO₂. Cellular images captured by confocal mode were segmented into region of interest using high content screening system from 0 to 6 h after treatment of it using BD Pathway HT (BD Biosciences, San Jose, CA, USA) in manner of real-time imaging.

### Comet assay

Cells (1×10⁶ cells/ml) were cultured in 12-well plate were treated with three different sizes of MNP@SiO₂ as low dose (31.25 μg/ml), middle dose (62.5 μg/ml) and high dose (125 μg/ml) for 2 h.

Cells were mixed with LMAgarose, and these mixture was put into Comet Slide™ (Trevigen, MD) and then into lysis solution for 30-60 min at 4°C, alkaline solution for 30-60 min, and were carried out electrophoresis for 30 min, and were dried out after dipping into 70% alcohol. And then these were stained with ethidium bromide and examined by fluorescence microscope, and were analyzed by Comet assay program (Komet 3.1, Andor Technology, Belfast, UK) to calculate tail moment. MMS (325.75 mg/ml) was used as positive control.

### Statistical analysis

Statistical analyses for cytotoxicity and Comet assay were performed with the Tukey-Kramer method using the JMP program (SAS Institute, Cary, NC). For all comparisons, probability values less than 5% (p<0.05) were considered to be statistically significant.

### RESULTS

#### Chemical characteristics of MNP@SiO₂

There were no alterations of size of MNP@SiO₂ during the time points of 6, 24 and 48 h confirmed by TEM. And there were no inducing radical or alteration of component of MNP@SiO₂ during the time points of 6, 24 and 48 h by ESR and ICP-AES, respectively (Fig. 1).

#### Cytotoxicity at 6, 24 and 48 h after treatment of 50, 100 or 200 nm MNP@SiO₂

L5178Y is cultured in DMEM medium supplemented with 1.5 g/L sodium bicarbonate, 10% FBS and 1% penicillin at 37°C in a 5% CO₂ atmosphere and cytotoxicity is assessed at following time points. (A) 6 h, (B) 24 h, (C) 48 h. *,**Significantly different from vehicle control (p<0.05, p<0.01, respectively).
**Cytotoxicity assay**

Treatment of MNP@SiO$_2$ induced dose and time dependent cytotoxicity. At 6 h after treatment, 50, 100 or 200 nm MNP@SiO$_2$ decreased significantly cell viability over the concentration of 125 μg/ml compared to vehicle control ($p<0.05$ or $p<0.01$) (Fig. 2A).

Moreover, at 24 h after treatment, 50 or 100 nm MNP@SiO$_2$ decreased significantly cell viability over the concentration of 125 μg/ml compared to vehicle control ($p<0.01$). And treatment of 200 nm MNP@SiO$_2$ decreased significantly cell viability at the concentration of 62.5 μg/ml ($p<0.05$) and of 125, 250, 500 μg/ml ($p<0.01$, respectively) compared to vehicle control (Fig. 2B).

Furthermore, at 48 h after treatment, 50, 100 or 200 nm MNP@SiO$_2$ decreased significantly cell viability at the concentration of 62.5 μg/ml ($p<0.05$) and of 125, 250, 500 μg/ml ($p<0.01$, respectively) compared to vehicle control (Fig. 2C).

**Cellular location**

Cells were treated with three different sizes of MNP@SiO$_2$ and the cells were fixed with fixatives, and cellular location was detected by confocal microscope. It showed that these nanoparticles were existed in cytoplasm at 2 h after treatment of MNP@SiO$_2$. Representative figure was shown in Fig. 3, showing the cellular location of MNP@SiO$_2$ in cytoplasm, mainly around cell membrane.

**Comet assay**

Cell were treated with three different sizes of MNP@SiO$_2$ as low dose (31.25 μg/ml), middle dose (62.5 μg/ml) and high dose (125 μg/ml) for 2 h. Treatment of 50 nm MNP@SiO$_2$ significantly increased DNA damage at middle and high dose ($p<0.01$), and treatment of 100 nm or 200 nm MNP@SiO$_2$ significantly increased DNA damage in all dose compared to control ($p<0.01$) (Fig. 4). And treatment of MMS as positive control also significantly increased DNA damage compared to control ($p<0.01$).

**DISCUSSION**

In the present study, treatment of 50, 100 or 200 nm MNP@SiO$_2$ represented cellular toxicity in L5178Y cells. And DNA damage was appeared at the treatment of 50, 100 or 200 nm MNP@SiO$_2$ by Comet assay.

Treatment of 50, 100 or 200 nm MNP@SiO$_2$ induced dose and time dependent cytotoxicity. At 6, 24, 48 h after treatment, 50, 100 or 200 nm MNP@SiO$_2$ decreased cell viability over the concentration of 62.5 μg/ml compared to vehicle control, and this may be related to entrance of these nanoparticles in cytoplasm. And cellular images captured by confocal mode using high content screening system showed that nanoparticles were moved into cytoplasm within a short time.

It was reported that sizes of nanoparticles were critical determinants of degree of cytotoxicity and potential mechanisms of toxicity (Sohaebuddin et al., 2010) and nanoparticles may be more toxic than micron-sized one, as showing that nanosized cobalt-chromium alloy induced more DNA damage than micron-sized particles (Papageorgiou et al., 2007) and the ultrafine particles elicited a persistently high inflammatory reaction in the lungs of the animals compared to the larger-sized particles (Oberdorster et al., 1994). However, in this study, MNP@SiO$_2$ did not show this tendency and 200 nm size of silica nanoparticles also induced cytotoxicity. As confocal microscope findings showed that MNP@SiO$_2$, could enter the cell after treatment, irrespective of size, it may be owing to early cellular entry of MNP@SiO$_2$. Base on recent reports that toxicity of silica nanoparticles was mediated through oxidant generation (Akhtar et al., 2010), and treatment of silica nanoparticles induced inflammation (Hamilton et al., 2008) and enhanced inflammatory cytokines (Nishimori et al., 2009), they could induce cellular toxicity even in larger size as well as small size.

In this study, we do not exclude the possibility they can enter the nuclei. Recent report illustrated that carbon nanotubes were seen to enter the cytoplasm and localize within the cell.
nucleus, causing cell mortality in a dose-dependent manner (Porter et al., 2007). Further studies will be warranted in this possibility with specific method.

Many of nanoparticles assessed were found to cause genotoxic responses (Singh et al., 2009). In fact, titanium dioxide could induce oxidative damage to human bronchial epithelial cells (Gurr et al., 2005) and zinc oxide could enhance genotoxicity in irradiated circumstance (Dufour et al., 2006). Genotoxicity of nanoparticles in cells can be assessed by several methods for determination of gene mutations, cytogenetic assessment of chromosome damage and detection of micronuclei and evaluating DNA strand breaks (Hillegass et al., 2010). Among these, Comet assay was microgel electrophoresis method to find DNA damage directly in cellular level (McNamee et al., 2000). In this study, MNP@SiO2 treatment induced DNA damage detected by Comet assay. Interestingly, we found that there were little variation of oliver tail moment in control and positive control value, in contrast with large variation in the groups of 50, 100 or 200 nm of MNP@SiO2 treatment. It seems that there may be different cellular susceptibility and variable level of cellular damage in treated cells, irrespective of size.

Even some nanoparticles (including metal nanoparticles, metal-oxide nanoparticles, quantum dots, fullerenes) were

Fig. 4. Comet assay for treatment of MNP@SiO2. DNA damage as represented as oliver tail moment. Treatment of 50, 100 or 200 nm MNP@SiO2 increase DNA damage, as equivalent level shown in the treatment of methyl methanesulphonate (MMS) as positive control. Representative figures from control and 50, 100 or 200 nm MNP@SiO2 treatment groups; L, M and H mean low (31.25 μg/ml), middle (62.5 μg/ml) and high dose (125 μg/ml) treatment of three different size of MNP@SiO2, respectively. VC: vehicle control; PC: positive control. **Significantly different from vehicle control (p<0.01).
found to cause genotoxic responses, such as chromosomal fragmentation, DNA strand breakages, point mutations, it is difficult to draw conclusions that nanoparticles might promote genotoxicity, largely due to physicochemical features and study design (Singh et al., 2009). Actually, the MNP@SiO2 did not induce any significant chromosome aberrations (Kim et al., 2006). However, our results clearly suggest that they might induce DNA damage. And recent study reported that silica nanoparticles induce global genomic hypomethylation (Gong et al., 2010). In this time, it will be needed to do more research for assessing toxicity of silica nanoparticles detected by conventional or advanced method(s) in specific condition.

Taken together, treatment of MNP@SiO2 induced cytotoxicity and they were located in cells within a short time, and they might induce DNA damage in L5178Y cell, associated with cellular location within short time after treatment.

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REFERENCES


