Genotoxicity of Copper Oxide Nanoparticles with Different Surface Chemistry on Rat Bone Marrow Mesenchymal Stem Cells

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The surface chemistry of nanoparticles (NPs) is one of the critical factors determining their cellular responses. In this study, the cytotoxicity and genotoxicity of copper oxide (CuO) NPs with a similar size but different surface chemistry to rat bone marrow mesenchymal stem cells (MSCs) were investigated. The morphology, size and surface charge of four types of CuO NPs, i.e., CuO-core, CuO–COOH, CuO–NH2, and CuO-PEG NPs, were characterized by TEM, dynamic light scattering (DLS) and zeta-potential measurement, respectively. All of the four CuO NPs had a negative surface charge around –10 mV and showed a similar tendency to form agglomerates with a size of ~200 nm in cell culture environment. The cytotoxicity of CuO NPs to MSCs at various concentrations and incubation periods were firstly evaluated. The CuO NPs showed dose-dependent and time-dependent toxicity to MSCs, and their surface chemistry had influence on the toxicity to some extent too. The intracellular reactive oxygen species (ROS) level of MSCs was then quantified. Finally, the genotoxicity of the CuO NPs was studied by comet assay. The results suggest that the genotoxicity of CuO NPs was mainly dependent on NPs concentration, and was only slightly influenced by their surface chemistry. The osteogenic and adipogenic differentiation abilities of MSCs exposed to different CuO NPs were studied by Alizarin Res S and Oil Red O staining. The preliminary results showed that the exposure to 10 μg/mL CuO NPs will not lead to significant impact on the differentiation potential of the MSCs.

Keywords: Copper Oxide, Surface Coating, MSCs, Cytotoxicity, Genotoxicity.

1. INTRODUCTION

Metal and metal oxide nanoparticles (NPs) are widely applied in personal care products, catalysis, optical and recording devices, and water purification. It is well known that NPs have the ability to enter, translocate and damage living organisms primarily because of their small size, which allows them to penetrate physiological barriers, deposit in several organs, and cause adverse biological reactions. Unfortunately, their potential toxicological impacts are still a matter of investigation, and our actual knowledge on the effects of nano-sized contaminants on biological systems remains incomplete. These effects need to be carefully addressed in order to provide a scientific basis for a safe development of nanotechnologies.

Copper oxide (CuO) NPs possess biocide properties, leading to their applications in antimicrobial textiles, paints and plastics. The CuO NPs are also used in heat transfer fluids, semiconductors and TiO2 NPs to human epithelial cells. Moreover, the CuO NPs are able to induce over-expression of inflammatory factors such as interleukin-8 (IL-8). DNA damage
and even the leakage of blood-brain barrier.\textsuperscript{20} Genotoxicity of CuO NPs is of particular importance since an alteration of the genetic materials may favor cancer development or fertility impairment.\textsuperscript{10}

However, these adverse health effects are not invariably induced by CuO NPs, and their toxicity depends on various factors, including size, aggregation, and surface function-alization, etc. Therefore, the reasonable consideration is to correlate the toxicity of nanomaterials with their physicochemical properties such as size, charge, and chemical compositions, which may lead to classifications of nanomaterials according to their toxicological actions. The correlations are also fundamental on the design of nanomaterials and devices to ensure the safe use for humans and environment.\textsuperscript{8,21–24}

The surface chemistry of NPs is one of the critical factors determining cellular responses \textit{in vitro}.\textsuperscript{25–29} For example, Hoshino et al.\textsuperscript{30} investigated a series of surface-modified CdSe/ZnS quantum dots (QDs) in murine cancer cells, and found that the surface chemistry is an indicator of toxicity rather than the core material. The QDs coated with carboxyl groups are less toxic than QDs with an amine surface coating. We found previously that QDs coated with carboxyl groups are less toxic than QDs coated with anionic polymers, although cells are always viable.\textsuperscript{31} These observations indicate that the toxicity of CuO NPs may be varied by changing their surface chemistry. However, very few studies have been reported on the toxicity of surface-coated CuO NPs.

Mesenchymal stem cells (MSCs) with versatile differentiation capacity to various lineages including osteoblasts, adipocytes and neurons have drawn intensive attention in tissue regeneration.\textsuperscript{32–34} In addition, they are actively proliferated and repetitively expanded in contrast to other primary cells. Therefore, MSC is an important type of stem cells for the study of cytotoxicity, and particular genotoxicity of CuO NPs with different surface chemistry. In this study, four types of CuO NPs, i.e., non-modified CuO (CuO-core), carboxyl groups-capped CuO (CuO-COOH), amino groups-capped CuO (CuO–NH\textsubscript{2}), and poly(ethylene glycol)-modified CuO (CuO-PEG) with a similar size but different surface chemistry were investigated in terms of toxicity on rat MSCs in a time- and dose-dependent manner. Their genotoxicity to MSCs is then studied by a comet assay. Finally, the osteogenic and adipogenic differentiation potential of the MSCs exposed to CuO NPs is studied by Alizarin Red S and Oil Red O staining.

2. MATERIALS AND METHODS

2.1. Materials

Four types of CuO NPs with different surface chemistry were synthesized in house and thoroughly characterized as a part of the Nanosolutions project (www.nanosolutions.eu). Sodium dodecyl sulfate (SDS) was purchased from Haotian Co., Ltd., China. Normal melting point agarose (NMPA) and low melting point agarose (LMPA) were obtained from Amresco, USA. Triton X-100, ethidium bromide (EB), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), trishydroxyl methyl aminomethane (Tris), sodium ethylene diamine tetracetate (EDTA), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), hydrochloric acid (HCl), sodium chloride (NaCl) and methanol were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Alpha minimum essential medium (\textalpha-MEM), fetal bovine serum (FBS) and other cell culture reagents were obtained from Life technology, USA. All chemicals were of analytical grade and used as received if not specifically described. Milli-Q water was used throughout the experiments.

2.2. Characterization of CuO NPs

2.2.1. Morphology

The morphology of the four kinds of CuO NPs was analyzed using a JEM-200 transmission electron microscope (TEM, JEOL, Japan). A drop of the CuO NPs suspension in water was added onto a copper meshwork with a carbon membrane, and dried overnight at ambient condition. The sizes of single particles and agglomerates in dry state were analyzed from TEM images using Image J software.

2.2.2. Preparation of CuO NPs Dispersions for Toxicity Study

Firstly, 10 mg CuO NPs were added into a 20 mL glass bottle, and sterilized under UV light for 15 min. 10 mL water was added into the glass bottle and the solution was sonicated for 5 min or 30 min using a probe sonicator (Sonicator 4000, Misonix, USA, operating at 50 Hz) in an ice bath to form a stock solution of CuO NPs (1 mg/mL). The sonicator probe had a diameter of 3.2 mm, length of 16.5 mm and the maximum peak-to-peak amplitude was 240 \textmu m. The CuO suspension was treated at 50% of the maximum peak-to-peak amplitude. Prior to the experiments the probe sonicator was calibrated using a calorimetric method in order to provide a delivered acoustic power of 13 W (Standard SOP of Nanosolutions). The suspension was diluted with \textalpha-MEM/10\% FBS to a final solution containing 100 \mu g/mL CuO NPs with the assistance of 5 min sonication. All the particles were used immediately as dispersed.

2.2.3. Size and Surface Charge

The size (Z-average mean diameter) and zeta potential of different CuO NPs were determined upon dynamic light scattering and electrophoretic light scattering measurement on a Beckman DelsaN\textsuperscript{TM} Nano (Beckman Coulter).
2.3. Cell Experiments

2.3.1. Cell Culture

The committee on animal experimentation of Zhejiang University approved the animal experiments. Bone mesenchymal stem cells (MSCs) were isolated from bone marrow of Sprague-Dawley rats (6–8 weeks old) as described previously. Briefly, the bone marrow cells were obtained from the femoral shafts of rats by flushing out with 10 mL of α-MEM/10% FBS, supplemented with 100 μg/mL penicillin and 100 U/mL streptomycin. The released cells were collected into a 9 cm cell culture dish (Corning, USA) containing 10 mL of culture medium and incubated in a humidified atmosphere of 95% air and 5% CO2 at 37 °C. After the cells reached about 80% confluence, they were detached and serially sub-cultured. The MSCs at passage 4 were used in this study.

2.3.2. Cell Viability

The MSCs were plated at a density of 5 × 10^3 cells per well on a 96-well plate and cultured for 16 h to allow cell attachment. The medium was replaced with a fresh one containing variable concentrations of CuO NPs, prepared according to the method described in Section 2.2.2.

To determine the cell viability after co-incubation with CuO NPs for predefined time intervals, 10 μL MTT (5 mg/mL) was added to each well, and the cells were continuously cultured at 37 °C for 3 h. The dark blue formazan crystals generated by the mitochondria dehydrogenase in viable cells were dissolved in dimethyl sulphoxide (DMSO). The absorbance was measured at 570 nm by a microplate reader (Model 680, Biorad). Three parallel experiments were conducted, and the data were normalized to that of the particle-free control (100%).

2.3.3. Measurement of Intracellular Reactive Oxygen Species (ROS)

The oxidation-sensitive probe DCFH-DA was employed to determine the intracellular ROS level. DCFH-DA is an amphiphilic non-fluorescent molecule that readily crosses cell membrane, deacetylated by esterases, and then oxidized to highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of intracellular ROS. In this study, MSCs were seeded on 24-well plates at a density of 2.5 × 10^4 cells per well and allowed to adhere for 16 h. Then the cells were incubated with CuO NPs for different time or with different concentrations of CuO NPs for a fixed time interval, respectively. 10 mM H2O2 treated cells for 10 min as Positive control, untreated cells as control. At the predetermined time intervals, the cells were incubated with CuO NPs and washed with PBS and observed under a fluorescence microscope (IX81, Olympus, Japan). The fluorescence intensity of obtained images was further analyzed by Image J software.

2.3.4. The Comet Assay (Single Cell Gel Electrophoresis)

The comet assay was used to study DNA strand breaks and alkaline labile sites in MSCs exposed to CuO NPs. 2.0 × 10^4 cells were plated on 24-well plates per well and allowed to attach for 16 h. The cells were exposed to various CuO NPs at 4 doses: 1, 2, 5, and 10 μg/mL (corresponding to 0.26, 0.52, 1.3, 2.6 μg/cm^2) for 3, 24 and 72 h, respectively. The doses were chosen according to the cytotoxicity results. Particle-free cells and the cells treated with 10 mM H2O2 for 10 min were used as negative and positive controls, respectively.

### Table I. Size and surface charge properties of CuO NPs with different surface coatings under various conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter (TEM, nm)</th>
<th>Diameter by DLS (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single</td>
<td>Aggregates</td>
<td>Water 5 min sonication</td>
</tr>
<tr>
<td>CuO-core</td>
<td>18.1 ± 4.0</td>
<td>309.1 ± 215.1</td>
<td>1278.1 ± 38.4</td>
</tr>
<tr>
<td>CuO-NH2</td>
<td>9.2 ± 2.4</td>
<td>1007.2 ± 606.3</td>
<td>961.4 ± 36.8</td>
</tr>
<tr>
<td>CuO-COOH</td>
<td>8.4 ± 1.2</td>
<td>333 ± 123.9</td>
<td>327.1 ± 23.4</td>
</tr>
<tr>
<td>CuO-PEG</td>
<td>7.3 ± 1.5</td>
<td>248 ± 90.2</td>
<td>586.6 ± 106.3</td>
</tr>
</tbody>
</table>
The comet assay was performed according to the method described previously. Briefly, the particles-exposed cells were trypsinized and collected by centrifugation at 1000 rpm for 5 min. 1–3 × 10⁴ cells were re-suspended in 75 μL 0.7% LMPA at 37 °C. The re-suspended cells in LMPA were put onto glass slides (26 mm × 76 mm) pre-coated with 1.5% NMPA and then covered with a coverslip. The LMPA was allowed to solidify for 10 min on an ice box. The slides were immersed in cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100) for at least 1 h at 4 °C after removal of the coverslip. Then the slides were transferred to an electrophoresis tank containing freshly made cold electrophoresis buffer (1 mM EDTA and 300 mM NaOH, pH > 13), where they were kept for 20 min to allow DNA unwinding. Electrophoresis was performed in the same buffer at room temperature for 15 min at a voltage of 26 V (0.75 V/cm) and 300 mA. The slides were then washed 3 times with 0.4 M Tris buffer (pH 7.5), air-dried, and fixed in methanol. Finally, the slides were stained with ethidium bromide (EB) aqueous solution (2 μg/mL) for 5 min and analyzed using a fluorescence microscope (IX81, Olympus, Japan). At least 70 cells were analyzed per sample by Image J software with OpenComet plugin (v1.3). The average percentage of DNA in the comet tail was used to indicate the amount of DNA damage.

2.3.5. Differentiation Test of the MSCs
The MSCs were plated at a density of 2 × 10⁴ cells per well on a 24-well plate and cultured for 16 h to allow cell attachment. The medium was replaced with a fresh one containing 10 μg/mL of different CuO NPs, respectively. Cells were cultured with NPs-containing medium for 3 days, and then incubated with particle-free osteoinductive and adipoinductive media (Life Technologies, USA), respectively. The medium was changed every 3 days.

Alizarin Red S was first dissolved into 1% (w/v) water solution. After 14 days culture, the MSCs were washed three times with PBS and fixed in 4% paraformaldehyde for 15 min. Then they were stained in Alizarin Red S solution at room temperature for 15 min, washed with water three times, and observed under microscope.

Oil red O was dissolved in isopropanol (1% w/v) to prepare a stock solution. The stock solution was mixed with distilled water (3:2) and then filtered to obtain a work solution. After 14 days culture, the cells were washed three times with PBS and fixed in 4% paraformaldehyde for 15 min. They were then stained in Oil red work solution at room temperature for 15 min, washed with 60% isopropanol for 2 times and PBS for three times, and observed under microscope.

2.4. Statistical Analysis
The data are expressed as mean ± standard deviation (SD). The statistical significance between groups is determined by one-way analysis of variance (ANOVA) in the Origin software. The Tukey Means Comparison method is performed and the statistical significance is set as p < 0.05.

3. RESULTS AND DISCUSSION
3.1. Characterization of CuO NPs
The morphology, size, and surface charge of the CuO NPs were characterized by TEM, DLS and zeta potential.
Figure 3. Relative concentration of intracellular reactive oxygen species (ROS) of MSCs incubated with 10 μg/mL CuO NPs as a function of incubation time. Cells were incubated with DCFH-DA for 30 min and analyzed with fluorescence microscope. The intensity of obtained images was further analyzed by Image J software. * indicates significant difference at \( p < 0.05 \) level.

measurements and the results are shown in Figure 1 and Table I. The TEM analysis showed that the CuO NPs had an irregular morphology and were agglomerated to the order of a few hundred nanometers regardless of the types of their surface coatings. The agglomerates were composed of several constituent primary particles with average diameters of 18.1 ± 4.0, 9.2 ± 2.4, 8.4 ± 1.2, and 7.3 ± 1.5 nm by TEM for the CuO-core, CuO–NH₂, CuO–COOH, and CuO-PEG NPs, respectively (Table I). The obtained sizes concur with the observations by TEM, which show the presence of CuO NPs agglomerates of several hundred of nanometers. In order to achieve higher de-agglomeration CuO nanopowders, we decided to prolong the sonication time to 30 min. As a result, the measured hydrodynamic diameters were 148.7 ± 3.0, 180.4 ± 3.9, 281.5 ± 5.1, and 223.3 ± 20.7 nm, respectively, indicating that agglomerates were still present (Table I). The difference in surface chemistry resulted in the different surface charge: positive for the CuO-core and CuO–NH₂ NPs and negative for the CuO–COOH and CuO-PEG NPs. However, the difference of size and surface charge of the four types of CuO NPs became neglectable when the NPs were dispersed in cell culture medium (α-MEM/10% FBS), with a hydrodynamic diameter in the range of 170–200 nm and a zeta potential about −10 mV, respectively. The alternation of the size and surface charge of the NPs is attributed to the protein adsorption in the serum-containing medium. Nonetheless, the similar size and zeta potential are beneficial to investigate the influence of surface chemistry on the toxicity to MSCs.

3.2. Cytotoxicity

Incubation with CuO NPs may bring a series of adverse influences on the cell viability and functions. Firstly, the cell viability was assessed by MTT assay. As shown in Figure 2(a), in general all types of the CuO NPs had similar dose-dependent toxicity to MSCs after 3 h incubation. The NPs did not have obvious impact on cell viability by probe-sonication at a specific calibrated acoustic power (see methods). The hydrodynamic diameter measured for the obtained of the CuO-core, CuO–NH₂, CuO–COOH, and CuO-PEG NPs suspensions was 1278.1, 327.1, 961.4, and 586.6 nm, respectively (Table I). The obtained sizes concur with the observations by TEM, which show the presence of CuO NPs agglomerates of several hundred of nanometers. In order to achieve higher de-agglomeration CuO nanopowders, we decided to prolong the sonication time to 30 min. As a result, the measured hydrodynamic diameters were 148.7 ± 3.0, 180.4 ± 3.9, 281.5 ± 5.1, and 223.3 ± 20.7 nm, respectively, indicating that agglomerates were still present (Table I). The difference in surface chemistry resulted in the different surface charge: positive for the CuO-core and CuO–NH₂ NPs and negative for the CuO–COOH and CuO-PEG NPs. However, the difference of size and surface charge of the four types of CuO NPs became neglectable when the NPs were dispersed in cell culture medium (α-MEM/10% FBS), with a hydrodynamic diameter in the range of 170–200 nm and a zeta potential about −10 mV, respectively. The alternation of the size and surface charge of the NPs is attributed to the protein adsorption in the serum-containing medium. Nonetheless, the similar size and zeta potential are beneficial to investigate the influence of surface chemistry on the toxicity to MSCs.

Figure 4. Representative images of comet assay of (a) untreated cells, (b) cells treated with 10 mM H₂O₂ for 10 min, and (c–f) cells treated with (c) 1, (d) 2, (e) 5, and (f) 10 μg/mL CuO-core NPs for 24 h, respectively. Scale bar = 10 μm.
when the concentration was below 5 μg/mL. The cell viability decreased along with the increase of NPs’ concentration, and reached 50–60% of that of the particle-free control when the concentration was 50 μg/mL. The difference of toxicity between different NPs at the same concentration was insignificant at this stage.

When the exposure time was prolonged to 24 h, the cell viability decreased significantly in the whole range of CuO NPs concentrations. Most of the NPs exhibited statistically significant cytotoxicity even at a very low concentration of 1 μg/mL, and the cell viability was reduced to about 60% of that of the particle-free control except of the CuO-core NPs (Fig. 2(b)). The toxicity of CuO-core NPs to MSCs remained relatively low up to 10 μg/mL, got comparable with the toxicity of the –NH₂ and –PEG modified NPs at 20 μg/mL, and then became significantly larger than that at 50 μg/mL. At this concentration, the cell viability remained over 40% of the particle-free control for the CuO-PEG and CuO–NH₂ NPs. Generally, the CuO–COOH NPs showed the highest toxicity, and almost all the cells were dead at the highest concentration (50 μg/mL). The lower toxicity of CuO-core NPs at the lower concentration might be attributed to the lower cellular uptake and/or lower solubility of the CuO-core NPs compared to their surface-functionalized counterparts. Literature data indicate that polymer-coated CuO NPs are internalized at a larger quantity by algae cell after co-culture for 6 h, resulting in a higher toxicity compared to the bare CuO NPs. Similar to CuO NPs, other types of transition metals NPs such as ZnO are less toxic at the lower concentration (<20 μg/mL) compared with ZnO NPs coated with oleic acid (OA) and poly(meth acryl acid) (PMAA).27

When the exposure time was further prolonged to 72 h, all the NPs showed significant cytotoxicity, and its extent increased along with the NPs’ concentration (Fig. 2(c)). The most pronounced drop of viability levels of MSCs over time was observed for uncoated and –COOH modified CuO NPs.

All the results indicate that the CuO NPs have dose- and surface chemistry dependent toxicity to MSCs. Comparatively, the CuO–COOH NPs led to the most severe toxicity towards MSCs especially at concentrations higher than 20 μg/mL. The cytotoxicity of CuO NPs depends strongly on intracellular solubility. Studer et al., compared the stabilized carbon-coated CuO NPs and the degradable pristine CuO NPs, and found that the soluble NPs are taken up by cells, and digested and dissolved in the acidic pH environment of lysosomes. Therefore, the different uptake amount and intracellular solubility may be the reason of different degree of toxicity of the CuO NPs.27 According to the cytotoxicity results, we denoted 10 μg/mL as the critical concentration, because at this concentration the cytoviability was reduced to about 50% of that of the particle-free control (IC₅₀) for all the CuO NPs. Therefore, the concentrations lower than 10 μg/mL were used for the further genotoxicity and intracellular ROS studies.

### 3.3. Intracellular ROS Level

The reactive oxygen species (ROS) are essential intermediates in oxidative stress. High intracellular ROS level and subsequent oxidative stress is known as a common cause...
for cellular damage induced by metal and metal oxide NPs.\textsuperscript{41} In order to determine the intracellular level of ROS, a cell-permeable oxidation-sensitive probe DCFH-DA was used. As shown in Figure 3, in general the incubation of all kinds of CuO NPs caused significant elevation of the intracellular ROS level (at least 10 times), which was slightly reduced along with the prolongation of incubation time. In particular, the pristine and –COOH modified CuO NPs induced the highest level of intracellular ROS after being incubated with MSCs for 3 h and 6 h, respectively. Other CuO NPs induced similar ROS expression. The significantly higher level of ROS induced by the CuO-core and CuO–COOH is consistent with cytotoxicity results, conveying the ROS production is at least one of the important reasons for the higher cytotoxicity induced by these two types of CuO NPs.

### 3.4. Genotoxicity of CuO NPs

Single cell gel electrophoresis (SCGE) was introduced by Ostling et al.\textsuperscript{42} in 1984 and was modified by Singh et al.\textsuperscript{43} in 1988, which can be used for sensitive and fast detection of single cell DNA strand breaks and alkaline labile sites, and for the quantitative evaluation of genotoxicity. The SCGE is also known as comet assay because the image of stained single cell DNA resembles a “comet” with a distinct head and tail. The formation of “comet” is based on the different molecular weight of DNAs, which have different migration distance in agarose gel when undergo electrophoresis in alkaline electrophoresis liquid (pH > 13). The damaged or broken pieces of DNAs migrate away from the nucleus to the anode direction, forming the “tail,” whereas the intact DNAs stay in the nuclear skeleton, forming the “head.” The DNA damage percentage can be quantitatively analyzed after the DNAs are stained with fluorescent dye, enabling the analysis of optical density and migration distance. Besides double strand breaks,\textsuperscript{44} and single strand breaks,\textsuperscript{45} the comet assay can also be used to study the alkali labile sites,\textsuperscript{46} oxidative base damage,\textsuperscript{47} and DNA repair monitoring by living cells.\textsuperscript{48}

The representative fluorescent images of EB-stained DNA patterns from the cells treated with different CuO NPs are shown in Figure 4. The sample from the
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particle-free cells showed a very short tail (Fig. 4(a)), indicating the intact DNA structure. In contrast, the sample from the cells treated with 10 mM H₂O₂ showed a very long tail, suggesting significant degradation of DNA. In general, the tails of the samples were elongated along with the increase of NPs’ concentration. In order to quantitatively analyze the results, over 70 cells from each sample were analyzed and the average percentage of the tail was calculated. As shown in Figure 5, all of the four CuO NPs induced dose-dependent but time-independent DNA damage (genotoxicity). For example, the percentage of DNA fragment (tails) from the cells treated with CuO-core NPs for 3 h increased along with the NPs’ concentration, reached over 40% of the total DNA when the NPs’ concentration reached 10 μg/mL, which was 8.9 times of that of the negative control. The CuO-PEG NPs had the smallest genotoxicity, especially at the lower concentrations (1 and 2 μg/mL). The genotoxicity increased at the relatively high concentration (10 μg/mL), with a tail ratio of 15%. It has been reported that PEG-decorated NPs can resist protein adsorption and prevent cell uptake, leading to a smaller impact on the genotoxicity and cell viability.39-51 The CuO-COOH and CuO-NH₂ NPs had a similar impact on the DNA integrity of MSCs at the same concentrations as CuO-PEG NPs. The results suggest that the genotoxicity of CuO NPs is mainly dominated by particle concentration, and is only slightly influenced by their surface concentration. Moreover, the ratio of DNA fragment (tail) did not increase significantly along with the particle incubation time, indicating that the majority of DNA damage takes place at a relatively early stage.

3.5. Differentiation Potential of MSCs

Furthermore, the differentiation abilities of the MSCs exposed to 10 μg/mL CuO NPs for three days were studied. Under the osteogenic condition, it was found that the presence of CuO NPs did not have significant influence on cell viability (Fig. 6(a)). Similarly, lipid formation inside MSCs under adipogenic condition was also not influenced by the presence of CuO NPs (Fig. 6(b)). This impact is understandable since the toxicity of nanoparticles is usually dose and time dependent.52 For example, low concentration of Ag nanoparticles have no obvious influence on stem cell differentiation, but high concentration of Ag NPs can inhibit the cell differentiation because of their high toxicity.52-53 Therefore, we can conclude that although 10 μg/mL CuO NPs can induce certain extent genotoxicity on the MSCs, but they have no significant influence on the differentiation behavior of the MSCs within limited exposure time. In future, more quantitative studies shall be carried out to strengthen this conclusion.

4. CONCLUSION

Four kinds of CuO NPs with different surface chemistry showed a similar aggregation behavior and surface charge in cell culture medium, minimizing the impact of surface functionality. The CuO NPs showed dose-dependent toxicity to MSCs, and their surface chemistry had an impact to a smaller extent. The genotoxicity of the CuO NPs was mainly determined by the particles’ concentration, and was only slightly influenced by their surface chemistry and exposure time. The osteogenic and adipogenic differentiation ability of MSCs was not significantly influenced by 10 μg/mL CuO NPs within three days exposure.

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References and Notes

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