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Genotoxic effects of zinc oxide and titanium dioxide nanoparticles on root meristem cells of *Allium cepa* by comet assay

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Abstract: Nanogenotoxicity is an emergent field, relevant for estimating the potential genotoxic risk of nanomaterials. In this study we investigated the genotoxic potential of zinc oxide (ZnO, ≤35 and 50 nm) and titanium dioxide (TiO_2, 21 and 50 nm) nanoparticles (NPs) in the nuclei of *Allium cepa* root meristem cells by using a modified alkaline comet assay. ZnO and TiO_2 NPs were supplied to cells treated in vitro at 3 different concentrations (10, 100, and 1000 µg/mL). For the comet assay, our results indicate a dose-dependent increment in DNA damage in cells exposed to 100 and 1000 µg/mL of ZnO NPs (≤35 and 50 nm) for percentage of DNA tail (% DNA tail). On the other hand, the results from TiO_2 NPs indicated that the DNA damage in the cells exposed to only the highest concentration (1000 µg/mL) of TiO_2 NPs (21 nm) is statistically significant in comparison to the control cells, while all concentrations of TiO_2 NPs (50 nm) are stat

Key words: Zinc oxide nanoparticles, titanium dioxide nanoparticles, *Allium cepa*, comet assay, genotoxicity, DNA damage

1. Introduction

Industrial applications of nanotechnology are rapidly increasing due to the new and interesting physicochemical properties of manufactured nanomaterials. Wide expansion of this market implies the growing existence of such materials around us. Today, although there are some people who are exposed to nanoparticles (NPs), a very dramatic increase in the number is expected in the near future (Lux Report, 2008).

Nanomaterials are defined by their small size (<100 nm) and their novel physicochemical properties, which are increasingly imposed on different research and economical fields. Among nanomaterials, different metal NPs are the most widely used. The high biological reactivity of nanometals in comparison with their corresponding bulk materials may suggest an increased toxicity, and include genetic material as a target. For these reasons nanotoxicology and nanogenotoxicology are expanding as novel fields that are looking for the potential risk of nanomaterials as well as their possible mechanisms of action (Landsiedel et al., 2009; Singh et al., 2009; Ai et al., 2011).

Titanium dioxide (TiO_2) NPs are widely used in food products, dyes, plastics, paper, drugs, and cosmetics, e.g., in sunscreens to effectively attenuate UV radiation (Gurr et al., 2005; Baan, 2007). Zinc oxide (ZnO) NPs are widely used in industrial products including cosmetics, sunscreens, UV light blockers, paints, and medical materials (Yeber et al., 2000; Hackenberg et al., 2011). In spite of the intensive use of TiO_2 and ZnO NPs in biology and medicine, in terms of human health and environmental safety, the information on TiO_2 and ZnO NPs is still inadequate or incomplete. The data from several in vitro studies demonstrate that TiO_2 NPs reveal various adverse effects at the cellular level, such as oxidative stress and DNA damage (Wang et al., 2007; Falck et al., 2009; Shukla et al., 2011). In mammalian cells, several in vitro studies have demonstrated the genotoxic potential of ZnO NPs, as shown in Chinese hamster ovary (CHO) cells by chromosome aberration test (Dufour et al., 2006), in a human epidermal cell line (A431) (Sharma et al., 2009), in primary human epidermal keratinocytes (Sharma et al., 2011a) and in human liver cells (HepG2) (Sharma et al., 2011b) by comet assay, and in the human carcinoma (Hep-2) cell line by comet and micronucleus assays (Osman et al., 2010).

Single cell gel electrophoresis (SCGE) assay or comet test is a very simple, rapid, and sensitive technique for measuring DNA damage (Singh et al., 1988; Fairbairn et al., 1995; Demir et al., 2011; Demir, 2012; Demir and Kaya, 2013). This technique can be used to test if NPs, as well as other genotoxic agents, generate DNA damage (Kumar et al., 2011a; Shukla et al., 2011). In this study we selected ZnO and TiO_2 NPs because of their industrial

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uses. In spite of their use in biology and medicine, data on the potential genotoxic effects of ZnO and TiO_2 NPs are missing or sparse. Therefore, to elucidate the possible genotoxic risk associated with ZnO and TiO_2 NP exposure we evaluated genotoxicity in the nuclei of *Allium cepa* root meristem cells in an in vitro comet assay by detecting the induction of DNA damage in these cells.

2. Materials and methods

2.1. Chemicals

ZnO NPs with ≤35 nm average particle size (APS) (CAS No. 1314-13-2) and ZnO NPs of 50–80 nm (CAS No. 1314-13-2) were provided by Sigma Chemical Co. (St Louis, MO, USA) and Ziploc (US Research Nanomaterials, Inc.), respectively. Nano-TiO_2 (21 nm, CAS No: 13463-67-7) and Nano-TiO_2 (50 nm, CAS No: 13463-67-7) were provided by Sigma Chemical Co. (St Louis, MO, USA) and MK Impex Corp. (Mississauga, ON, Canada), respectively. Both nano-TiO_2 were in anatase crystalline form. All the other compounds used in the comet assay were provided by Sigma.

2.2. Nanoparticle characterization

Nano-ZnO and nano-TiO_2 characterization was carried out by using transmission electron microscopy (TEM), dynamic light scattering (DLS), and laser Doppler velocimetry (LDV) methodologies. TEM methodologies were carried on a JEOL JEM-2011 instrument to determine size and morphology. DLS and LDV were performed on a Malvern Zetasizer Nano-ZS ZEN3600 instrument for the characterization of hydrodynamic size and zeta potential. For dispersion, nano-ZnO and nano-TiO_2 were submitted to ultrasonication (S-250D, Branson Sonifier, USA) at 20 kHz for 16 min in an ice-cooled bath.

2.3. Plant material and mutagenic treatment

Among the several higher plants used as test organisms, the species *A. cepa* has been an efficient genetic standard for environmental monitoring (Levan, 1938; Royal Swedish Academy of Sciences, 1973; Seth et al., 2008; Kumari et al., 2009; Yıldız et al., 2009; Kumari et al., 2011; Liman et al., 2011; Akyıl et al., 2012; Sharma et al., 2012). *A. cepa* (2n = 16) onion bulbs (5–10 g, 25–30 mm diameter), without treatment, were purchased from a local supermarket. Healthy onion bulbs were grown in the dark in a cylindrical glass beaker at room temperature (26 ± 2 °C) and given a renewed water supply every 24 h. When the roots reached 2–3 cm in length, the 4 best growing bulbs were treated with different concentrations (10, 100, and 1000 µg/mL) of ZnO and TiO_2 NP suspensions dissolved in distilled water for 18 h at 26 °C in the dark. Selection of the concentrations of tested chemicals was based on previously published data (Ghosh et al., 2010; Kumari et al., 2011). Distilled water and EMS (4 mM) were used as negative and positive controls, respectively.

2.4. Cell viability assay

After exposure, cell viability was evaluated as quickly as possible with a mix of fluorescein diacetate (FDA) and ethidium bromide (EtBr) (Strauss, 1991). Cells were stained with a 1:1 solution of 80 µg/mL FDA plus 50 µg/mL EtBr and observed under a fluorescence microscope (Nikon Eclipse E200) with an excitation filter of 488 nm (blue light). Living cells were stained green, while dead cells exhibited their nuclei stained orange. Two hundred cells were scored for viability in each treatment. More than 70% of cells were viable in the applied treatments, and this agrees with the conditions required for the comet assay (Henderson et al., 1998).

2.5. Isolation of nuclei of *Allium cepa* root meristem cells and comet assay

The comet assay was carried out on isolated nuclei of *A. cepa* as previously described (Seth et al., 2008; Yıldız et al., 2009; Ghosh et al., 2010; Liman et al., 2011), with slight modifications. The nuclei of the root cells were isolated as described by Pfosser et al. (1995). After the treatments with ZnO NPs, TiO_2 NPs, the negative control, and the EMS, we isolated the nuclei of the excised root meristem cells separately for each treatment in order to prepare the slides. Twenty milligrams of root tips (from 15 to 30 seedlings) were placed in a 60-mm petri dish kept on ice and spread with 1 mL of ice-cold Tris-$MgCl_2$ buffer (0.2 M Tris, pH 7.5; 4 mM $MgCl_2$-$6H_2O$; 0.5% w/v Triton X-100). The roots were immediately and gently chopped using a fresh razor blade, and isolated root nuclei were collected in the buffer. The plate was kept tilted on the ice so that the isolated nuclei would collect in the buffer. The nuclei were filtered through a 60 µm nylon net filter and precipitated by centrifugation at 200 g (~1000 rpm) for 5 min (4 °C) in a swinging bucket rotor. The pellet was then resuspended in 200 µL of Tris-$MgCl_2$ buffer. The integrity of the nuclei was checked under a microscope (100× magnification) after staining with ethidium bromide (60 µg/mL). Two comet slides were prepared for isolated nuclei from each treatment. One hundred microliters of the nuclear suspension were mixed with 100 µL of 0.8% LMA prepared in PBS. The nuclei and agarose were gently mixed by repeated pipetting using a cut micropipette tip and then layered onto microscope slides precoated with 1% NMA (dried for 25 min). The slides were immediately covered with cover slips and kept on ice for 5 min to solidify the agarose. After solidification, the cover slips were removed, and 100 µL of molten 8% LMA prepared in PBS was spread on the slides. The slides were again covered with cover slips and kept on ice for 5 min. Then the cover slips were removed, and the slides were placed in a horizontal gel electrophoresis tank filled with freshly prepared, chilled electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH ≥13) and incubated for 30 min

to allow DNA unwinding. Electrophoresis was conducted in the same buffer for 30 min at 25 V (1 V/cm) and 300 mA. To avoid additional DNA damage due to stray light, all these steps were performed under dimmed light. Unwinding and electrophoresis were done in an ice bath. After electrophoresis, the slides were gently rinsed 3 times at intervals of 5 min in fresh chilled 400 mM Tris buffer (pH 7.5) stained with 50 µL of ethidium bromide (EtBr) solution (60 µg/mL) for 10 min and covered with a cover slip. For visualizing DNA damage, slides were examined at 400× magnification using a fluorescence microscope (Nikon Eclipse E200) connected to a CCD camera and an image analysis system (Comet assay IV version 4.11, Kinetic Imaging, UK). In this study, 2 independent experiments were conducted, and 100 randomly selected cells (50 cells on each of the 2 replicate slides) were analyzed for each experiment. All the values were obtained from the mean ± standard error of 2 independent experiments: in total 200 cells for each concentration. The cells with no head or a dispersed head were regarded as apoptotic cells and were not included in the analysis.

2.6. Statistical analysis

The statistical analysis was performed using SPSS (version 15.0). The percentage of DNA in the tail (% DNA tail) and tail moment (µm) were measured as comet parameters. All data were presented as arithmetic mean ± standard error. The statistical approach was Student's t-test, which was used to evaluate the significance of the difference in DNA damage between the control and treated cells. Results were considered statistically significant at $P < 0.05$.

Each compound was tested in 2 independent experiments, and a good concordance was observed between these.

3. Results

3.1. ZnO and TiO_2 NP characterization

To better characterize the selected NPs we used TEM to determine the size and the morphology of ZnO and TiO_2 NPs. Figures 1–4 show the TEM figures, and, as observed, the majority of NPs were spherical, and no important agglomerations were observed following the dispersion protocol used. The average hydrodynamic

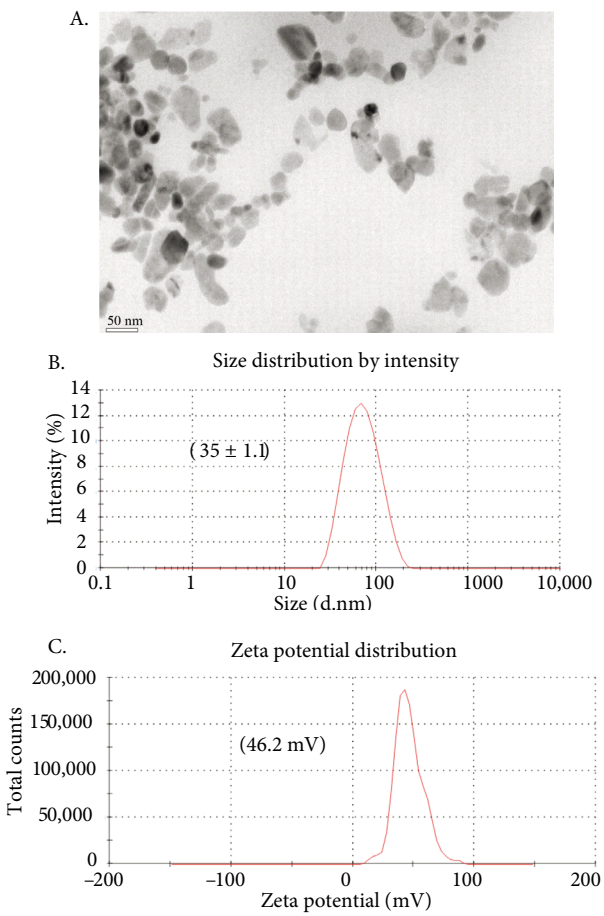


Figure 1. Characterization of ZnO NPs (≤35 nm). (A) typical TEM images; (B) and (C) represent size distribution and zeta potential, by DLS and LDV characterization.

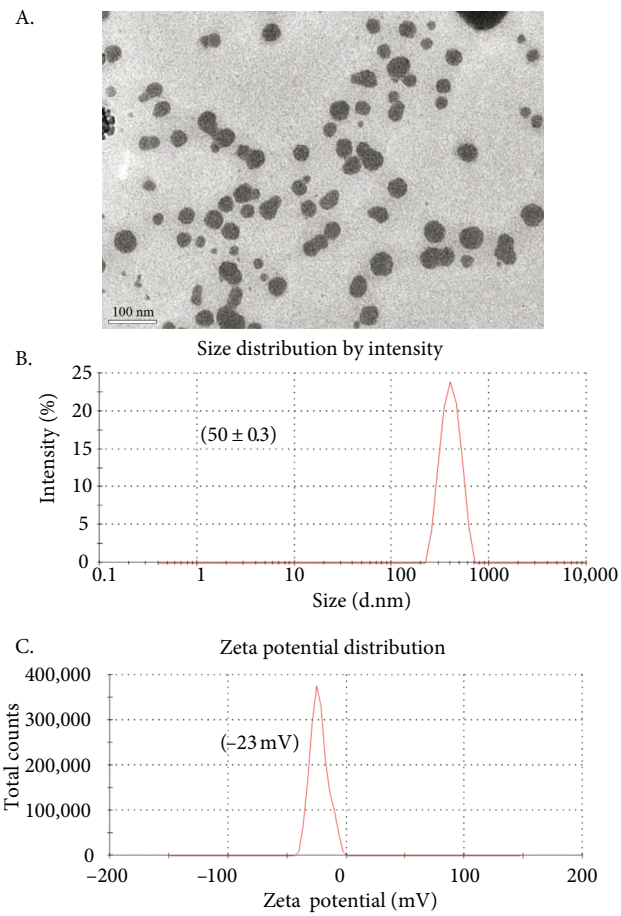


Figure 2. Characterization of ZnO NPs (50 nm). (A) typical TEM images; (B) and (C) represent size distribution and zeta potential, by DLS and LDV characterization.

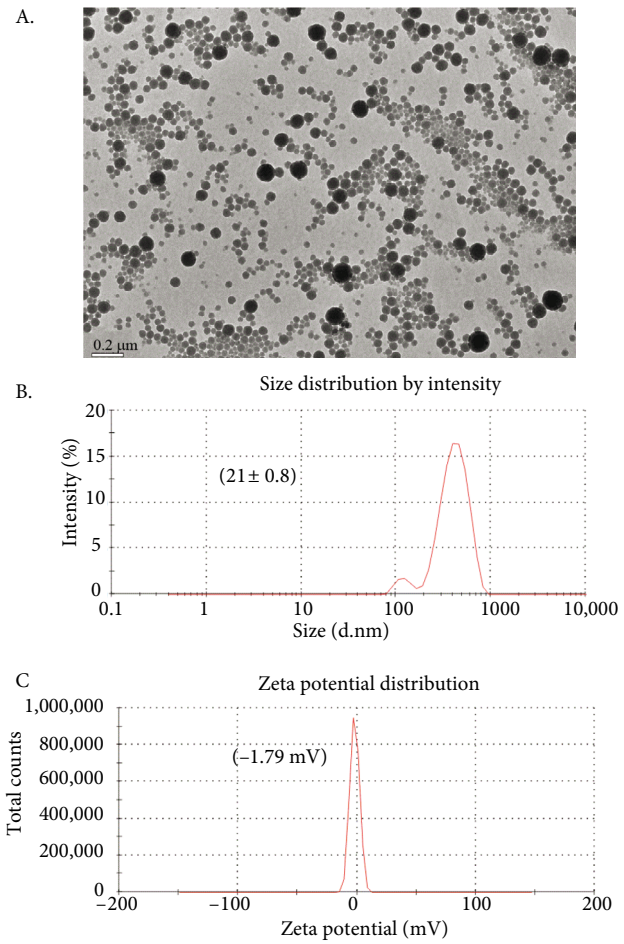


Figure 3. Characterization of TiO_2 NPs (21 nm). (A) typical TEM images; (B) and (C) represent size distribution and zeta potential, by DLS and LDV characterization.

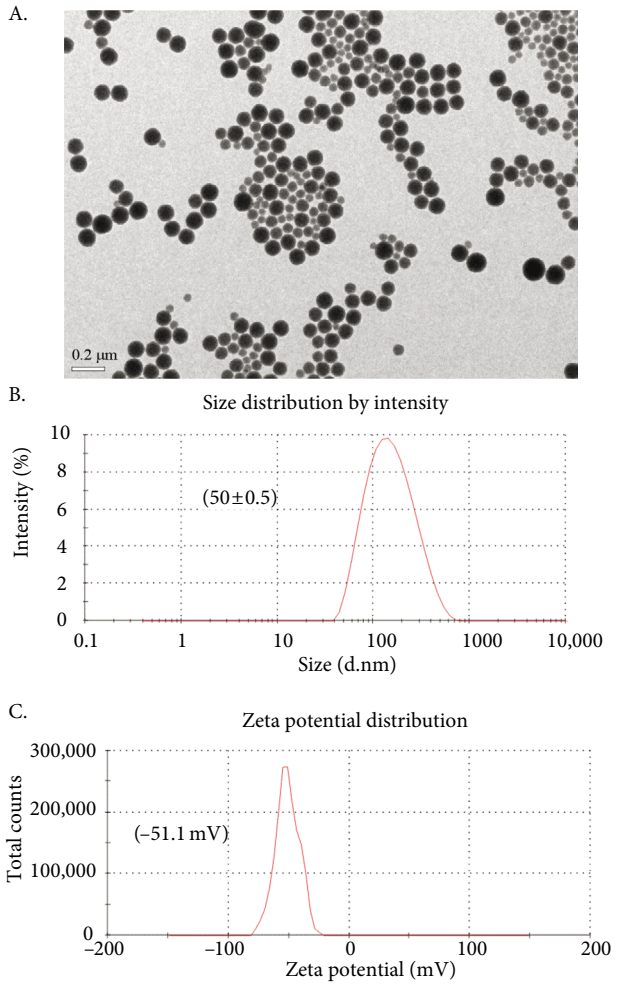


Figure 4. Characterization of TiO_2 NPs (50 nm). (A) typical TEM images; (B) and (C) represent size distribution and zeta potential, by DLS and LDV characterization.

diameter and zeta potential of the ZnO NP suspensions were determined by DLS and LVD, respectively. The average diameters in suspension obtained using DLS were 35 ± 1.1 nm for ZnO NPs (≤35 nm) and 50 ± 0.3 nm for ZnO 50 nm NPs. Zeta potential was 46.2 mV for ZnO NPs (≤35 nm) and –23 mV for ZnO NPs (50 nm). The average diameters in suspension obtained using DLS were 21 ± 0.8 nm for nano-TiO_2 (21 nm) and 50 ± 0.5 nm for nano-TiO_2 (50 nm) nanomaterials. Zeta potential was –1.79 mV for nano-TiO_2 (21 nm) and –51.1 mV for nano-TiO_2 (50 nm).

3.2. ZnO and TiO_2 NP genotoxicity

Before testing the genotoxicity of the selected doses of ZnO and TiO_2 NPs using the comet assay, we evaluated the toxicity of the selected doses using the FDA/EtBr viability assay. FDA permits detection of living cells (stained green) only, while EtBr detects only dead cells (stained red). Two hundred cells were scored for viability in each treatment. Our results indicate that the selected doses (up to 1000 µg/mL for treatments lasting 1 h) of ZnO and TiO_2 NPs were not cytotoxic in the root meristem cells of *A. cepa*, at the concentration range selected, always producing viability values higher than 70%.

The results obtained in the comet assay show that both tested ZnO NPs are genotoxic in the root meristem cells of *A. cepa* in terms of both the percentage of DNA in tail and tail moment. The induced genotoxicity followed a direct dose-response effect with positive induction at 100 and 1000 µg/mL doses for the percentage of DNA in the tail (% DNA tail) while demonstrating positive induction exposure to all concentrations of both tested ZnO NPs for tail moment (Table 1).

For TiO_2 NPs the results obtained from root meristem cells of *A. cepa* demonstrated that only the highest

Table 1. The genotoxic effects of 2 different sizes (≤35 and 50 nm) of zinc oxide nanoparticles (ZnO NPs) in nuclei of *Allium cepa* root meristem cells using the comet assay.

Compound, concentration (μg/mL)	% DNA tail [a]	Tail moment (arbitrary units) [a]
Control (water)	26.55 ± 2.91	14.88 ± 2.23
EMS (4 mM)	39.34 ± 4.40 **	43.11 ± 5.44 **
ZnO NPs (≤35 nm)		
10	33.06 ± 4.06	23.70 ± 3.60 *
100	43.38 ± 4.57 **	31.46 ± 3.84 **
1000	71.25 ± 4.12 **	79.26 ± 5.38 **
ZnO NPs (50 nm)		
10	33.32 ± 4.35	30.30 ± 4.57 **
100	40.02 ± 4.28 *	29.70 ± 3.63 **
1000	40.68 ± 4.39 **	44.10 ± 5.54 **

EMS: ethyl methanesulfonate.
[a]Mean ± standard error from 2 independent experiments; 100 cells were counted for each experiment.
*P < 0.05 versus control (water) using Student's t-test.
**P < 0.01 versus control (water) using Student's t-test.

concentration (1000 μg/mL) of TiO_2 NPs (21 nm) was statistically significant in comparison to the control, while all concentrations of TiO_2 NPs (50 nm) were significant for % DNA tail (Table 2). On the other hand, TiO_2 NPs tested did show a dose-dependent increment for tail moment (Table 2).

Table 2. The genotoxic effects of 2 different sizes (21 and 50 nm) of titanium dioxide nanoparticles (TiO_2 NPs) in nuclei of *Allium cepa* root meristem cells using the comet assay.

Compound, concentration (μg/mL)	% DNA tail [a]	Tail moment (arbitrary units) [a]
Control (water)	26.55 ± 2.91	14.88 ± 2.23
EMS (4 mM)	39.34 ± 4.40 **	43.11 ± 5.44 **
TiO_2 NPs (21 nm)		
10	29.36 ± 2.82	27.81 ± 3.32 **
100	31.92 ± 3.30	30.42 ± 3.87 **
1000	37.57 ± 3.60 *	35.57 ± 4.28 **
TiO_2 NPs (50 nm)		
10	37.52 ± 3.33 *	32.16 ± 3.49 **
100	41.07 ± 3.72 **	32.61 ± 4.11 **
1000	56.54 ± 3.82 **	56.90 ± 5.24 **

EMS: ethyl methanesulfonate.
[a]Mean ± standard error from 2 independent experiments; 100 cells were counted for each experiment.
* P < 0.05 versus control water using Student's t-test.
** P < 0.01 versus control water using Student's t-test.

4. Discussion

Studies conducted to increase information on genotoxic risks related to exposure to emerging nanomaterials are of increasing interest (Landsiedel et al., 2009), mainly those regarding compounds with limited information regarding potential genotoxic risks. The results reported in the current study add new and interesting information on the genotoxic effects of ZnO and TiO_2 NPs in isolated nuclei of *A. cepa* root meristem cells, mainly demonstrating that they pose genotoxic potential. Our results show that different sizes of ZnO and TiO_2 NPs can generate damage to DNA, producing primary DNA damage as measured by the comet assay.

Although ZnO NPs can be internalized into bacteria, weak or no genotoxic potential has been reported in these organisms (Kumar et al., 2011a; Nam et al., 2012). The lipid peroxidation reaction subsequently causes DNA damage, glutathione depletion, and disruption of membrane morphology and the electron transport chain, which leads to cell death (Kumar et al., 2011b). Nevertheless, the in vitro results obtained using the comet assay in this study produced positive responses independent of the cell type used. This occurred when this nanomaterial was tested using different mammalian cell line models such as primary mouse embryo fibroblasts (Yang et al., 2009), human epidermal (A431) cells (Sharma et al., 2009), human negroid cervix carcinoma Hep-2 cells (Osman et al., 2010), human bronchial epithelial cells BEAS-2B (Ng et al., 2011), human epidermal keratinocytes (HEK) (Sharma et al., 2011a), human liver HepG2 cells (Sharma et al., 2011b), human hepatocytes (L02), and human embryonic kidney (HEK293) cells (Guan et al., 2012). On the other hand, Kumari et al. (2011) studied the cytotoxicity and genotoxicity of ZnO NPs (<100 nm) on root cells of *A. cepa*. The results demonstrated that ZnO NPs can be a clastogenic/genotoxic and cytotoxic agent. All of these studies, compatible with the results obtained from *A. cepa* root meristem cells, show that genetic damage observed via the comet assay increased significantly with dose.

The International Agency for Research on Cancer (IARC) recently classified TiO_2 as possibly carcinogenic to humans (class 2B), based on sufficient evidence in experimental animals (Baan, 2007). A number of studies have produced conflicting results related to the genotoxicity of TiO_2 NPs in different test systems. Bhattacharya et al. (2009) and Hackenberg et al. (2010) reported that TiO_2 NPs did not cause any genotoxic activity in lung epithelial (BEAS-2B) cells, lung fibroblast (IMR-90) cells, or human nasal epithelial cells using the comet assay. Nakagawa et al. (1997) reported no effect of ultra-fine (25 nm) TiO_2 on a series of genotoxicity parameters measured in mouse lymphoma and Chinese hamster cells by comet assay, microbial and cell mutation assays, and chromosomal aberration. TiO_2 NPs also had no cytotoxic or genotoxic effect on cultured rat liver epithelial cells (Linnainmaa et al., 1997). Furthermore, Theogaraj et al. (2007) did not find that TiO_2 NPs were genotoxic for CHO cells using the chromosomal aberration test as an indicator of DNA damage. Ghosh et al. (2010) have shown that TiO_2 NPs (~100 nm) are able to induce significant increases in genetic damage in *A. cepa* and *Nicotiana tabacum* when they used the comet assay for testing genotoxic effects. Our results showed that the highest concentrations (1000 µg/mL) of the TiO_2 NPs (21 nm) and all concentrations of TiO_2 NPs (50 nm) have a genotoxic effect. Similarly, recent studies have shown that TiO_2 NPs induce genotoxicity in various lines of cultured cells. For example, TiO_2 (<20 nm) causes a significant dose-dependent increase in the induction of micronuclei and apoptosis in Syrian hamster embryo cells (Rahman et al., 2002). Additionally, anatase (10 and 20 nm) TiO_2 particles, in the absence of photo-activation, induce oxidative DNA damage, lipid peroxidation, micronuclei formation, and increased hydrogen peroxide and nitric oxide production in a human bronchial epithelial cell line (Gurr et al., 2005). Wang et al. (2007) also found that TiO_2 NPs are cytotoxic and genotoxic towards cultured WIL2-NS human lymphoblastoid cells. Long et al. (2006) found that TiO_2 causes oxidative stress in brain microglia cells under in vitro conditions.

In vivo toxicity studies have demonstrated that inhalation of TiO_2 NPs causes pulmonary inflammation in rats and mice (Bermudez et al., 2004) and TiO_2 NPs induce DNA damage and genetic instability in mice (Trouiller et al., 2009). Furthermore, Reeves et al. (2008) showed oxidative-stress–related effects, including inflammation, cytotoxicity, and genomic instability, either alone or in the presence of UVA irradiation, in mammalian studies. Similarly, Türkez and Geyikoğlu (2007) demonstrated that TiO_2 NPs were capable of causing genotoxicity by inducing sister chromatid exchange and micronucleus formation in human white blood cells. Gurr et al. (2005) have shown that TiO_2 NPs induce mainly the generation of hydrogen peroxide and nitric oxide, leading to lipid peroxidation and oxidative DNA damage in lung epithelial cells. Kang et al. (2008) observed an activation of DNA damage check-points and an up-regulation of P53 along with DNA damage caused by the ROS generation of these NPs in peripheral blood lymphocytes. Vevers and Jha (2008) demonstrated the genotoxic and cytotoxic potential of TiO_2 NPs on fish cells in vitro. The possible mechanism of TiO_2-NP–induced cellular toxicity in human epidermal cells under in vitro conditions has been shown (Shukla et al., 2011).

Based on the current findings we hypothesize that the different sizes of the TiO_2 and ZnO NPs may cause the significant genotoxicity. In our study, % DNA tail and tail

moment values for 50 nm (except 10 µg/mL) of ZnO NPs are lower than 35 nm. Conversely, % DNA tail and tail moment values for 50 nm of TiO_2 NPs are higher than 21 nm. Potential genotoxicity of NPs shows some differences related to their sizes, doses, surface topology, and physical properties (Rahman et al., 2002; Balasubramanyam et al., 2009). We conclude that based on the results of the comet assay, ZnO and TiO_2 NPs have high potential to interact with DNA and to cause primary DNA damage in *A. cepa* root meristem cells as an in vitro model. The results indicate that the 4 selected NPs have genotoxic effects in *A. cepa* root meristem cells, showing a direct dose-effect relationship. The relative genotoxic effects according to the highest concentrations are as follows: ZnO NPs (≤35 nm) (% DNA tail = 71.25 ± 4.12), TiO_2 NPs (50 nm) (% DNA tail = 56.54 ± 3.82), ZnO NPs (50 nm) (% DNA tail = 40.68 ± 4.39), and TiO_2 NPs (21 nm) (% DNA tail = 37.57 ± 3.60). These results are of interest when dealing with the potential risk associated with exposure to materials containing such chemicals. However, further studies should be carried out with different test systems (in vivo and in vitro) and model organisms related to their genotoxic profiles.

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References

Ai J, Biazar E, Jafarpour M, Montazeri M, Majdi A, Aminifard S, Zafari M, Akbari HR, Rad HG (2011). Nanotoxicology and nanoparticle safety in biomedical designs. Int J Nanomed 6: 1117–1127.

Akyıl D, Oktay S, Liman R, Eren Y, Konuk M (2012). Genotoxic and mutagenic effects of aqueous extract from aerial parts of *Achillea teretifolia*. Turk J Biol 36: 441–448.

Baan RA (2007). Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. Inhal Toxicol 19: 213–228.

Balasubramanyam A, Sailaja N, Mahboob M, Rahman MF, Hussain SM, Grover P (2009). In vivo genotoxicity assessment of aluminium oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. Mutagenesis 24: 245–251.

Bermudez E, Mangum JB, Wong BA, Asgharian B, Hext PM, Warheit DB, Everitt JI (2004). Pulmonary responses of mice, rats, and hamsters to subchronic inhalation of ultrafine titanium dioxide particles. Toxicol Sci 77: 347–357.

Bhattacharya K, Davoren M, Boertz J, Schins RPF, Hoffmann E, Dopp E (2009). Titanium dioxide nanoparticles induce oxidative stress and DNA adduct formation but not DNA breakage in human lung cells. Part Fibre Toxicol 21: 6–17.

Demir E, Kaya N, Kaya B (2011). Evaluation of DNA damage in *Capsicum annuum* L. exposed to spinosad in the comet assay. Fresen Environ Bull 20: 1926–1930.

Demir E (2012). In vivo genotoxicity assessment of diflubenzuron and spinosad in *Drosophila melanogaster* with the comet assay using haemocytes and the SMART assay. Fresen Environ Bull 21: 3894–3900.

Demir E, Kaya B (2013). Studies on the genotoxic properties of four benzyl derivatives in the in vivo comet assay using haemocytes of *Drosophila melanogaster*. Fresen Environ Bull 22: 1590–1596.

Dufour EK, Kumaravel T, Nohynek GJ, Kirkland D, Toutain H (2006). Clastogenicity, photo-clastogenicity or pseudo-photo-clastogenicity: genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells. Mutat Res 607: 215–224.

Fairbairn DW, Olive PL, O'Neill KL (1995). The comet assay: a comprehensive review. Mutat Res 339: 37–59.

Falck GC, Lindberg HK, Suhonen S, Vippola M, Vanhala E, Catalan J, Savolainen K, Norppa H (2009). Genotoxic effects of nanosized and fine TiO_2. Hum Exp Toxicol 28: 339–352.

Ghosh M, Bandyopadhyay M, Mukherjee A (2010). Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: plant and human lymphocytes. Chemosphere 81: 1253–1262.

Guan R, Kang T, Lu F, Zhang Z, Shen H, Liu M (2012). Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles. Nanoscale Res Lett 7: 602.

Gurr JR, Wang AS, Chen CH, Jan KY (2005). Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology 213: 66–73.

Hackenberg S, Friehs G, Froelich K, Ginzkey C, Koehler C, Scherzed A, Burghartz M, Hagen R, Kleinsasser N (2010). Intracellular distribution, geno- and cytotoxic effects of nanosized titanium dioxide particles in the anatase crystal phase on human nasal mucosa cells. Toxicol Lett 195: 9–14.

Hackenberg S, Zimmermann FZ, Scherzed A, Friehs G, Froelich K, Ginzkey C, Koehler C, Burghartz M, Hagen R, Kleinsasser N (2011). Repetitive exposure to zinc oxide nanoparticles induces DNA damage in human nasal mucosa mini organ cultures. Environ Mol Mutagen 52: 582–589.

Henderson L, Wolfreys A, Fedyk J, Bourner C, Windebank S (1998). The ability of the comet assay to discriminate between genotoxins and cytotoxins. Mutagenesis 13: 89–94.

Kang SJ, Kim BM, Lee YJ, Chung HW (2008). Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. Environ Mol Mutagen 49: 399–405.

Kumari M, Mukherjee A, Chandrasekaran N (2009). Genotoxicity of silver nanoparticles in *Allium cepa*. Sci Total Environ 407: 5243–5246.

Kumari M, Khan SS, Pakrashi S, Mukherjee A, Chandrasekaran N (2011). Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. J Hazard Mater 190: 613–621.

Kumar A, Pandey AK, Singh SS, Shanker R, Dhawan A (2011a). Cellular uptake and mutagenic potential of metal oxide nanoparticles in bacterial cells. Chemosphere 83: 1124–1132.

Kumar A, Pandey AK, Singh SS, Shanker R, Dhawan A (2011b). Engineered ZnO and TiO_2 nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*. Free Radical Bio Med 51: 1872–1881.

Landsiedel R, Kapp MD, Schulz M, Wiench K, Oesch F (2009). Genotoxicity investigations on nanomaterials: methods, preparation and characterization of test material, potential artifacts and limitations-many questions, some answers. Mutat Res 681: 241–258.

Levan A (1938). The effect of colchicine on root mitosis in *Allium*. Hereditas 24: 471–486.

Liman R, Ciğerci İH, Akyıl D, Eren Y, Konuk M (2011). Determination of genotoxicity of Fenaminosulf by *Allium* and comet tests. Pestic Biochem Phys 99: 61–64.

Linnainmaa K, Kiveipensa P, Vainio H (1997). Toxicity and cytogenetic studies of ultrafine titanium dioxide in cultured rat liver epithelial cells. Toxicol In Vitro 11: 329–335.

Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B (2006). Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol 40: 4346–4352.

Lux Report (2008). Nanomaterials state of the market: stealth success, broad impact. Available from the website: http://portal.luxresearchinc.com/research/document/3735.

Nakagawa Y, Wakuri S, Sakamoto K, Tanaka N (1997). The photogenotoxicity of titanium dioxide particles. Mutat Res 394: 125–32.

Nam SH, Kim SW, An YJ (2012). No evidence of the genotoxic potential of gold, silver, zinc oxide and titanium dioxide nanoparticles in the SOS chromotest. J Appl Toxicol doi: 10.1002/jat.2830.

Ng KW, Khoo SP, Heng BC, Setyawati MI, Tan EC, Zhao X, Xiong S, Fang W, Leong DT, Loo JS (2011). The role of the tumor suppressor p53 pathway in the cellular DNA damage response to zinc oxide nanoparticles. Biomaterials 32: 8218–8225.

Osman IF, Baumgartner A, Cemeli E, Fletcher JN, Anderson D (2010). Genotoxicity and cytotoxicity of zinc oxide and titanium dioxide in HEp-2 cells. Nanomedicine-UK 5: 1193–1203.

Pfosser A, Amon A, Lelley T, Heberle-Bors E (1995). Evaluation of sensitivity of flow cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. Cytom Part A 21: 387–393.

Rahman Q, Lohani M, Dopp E, Pemsel H, Jonas L, Weiss DG, Schiffmann D (2002). Evidence that ultrafine titanium dioxide induces micronuclei and apoptosis in Syrian hamster embryo fibroblasts. Environ Health Persp 110: 797–800.

Reeves JF, Davies SJ, Dodd NJF, Jha AN (2008). Hydroxyl radicals (·OH) are associated with titanium dioxide (TiO_2) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. Mutat Res 640: 113–22.

Royal Swedish Academy of Sciences (1973). Evaluation of genetic risks of environmental mutagens. Environ Health Persp 27: 3–6.

Seth CS, Misra V, Chauhan LKS, Singh RR (2008). Genotoxicity of cadmium on root meristem cells of *Allium cepa*: cytogenetic and comet assay approach. Ecotox Environ Safe 71: 711–716.

Sharma V, Anderson D, Dhawan A (2011b). Zinc oxide nanoparticles induce oxidative stress and genotoxicity in human liver cells (HepG2). J Biomed Nanotechnol 7: 98–99.

Sharma S, Nagpal A, Vig AP (2012). Genoprotective potential of *Brassica juncea* (L.) Czern. against mercury-induced genotoxicity in *Allium cepa* L. Turk J Biol 36: 622–629.

Sharma V, Shukla RK, Saxena N, Parmar D, Das M, Dhawan A (2009). DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol Lett 185: 211–218.

Sharma V, Singh SK, Anderson D, Tobin DJ, Dhawan A (2011a). Zinc oxide nanoparticle induced genotoxicity in primary human epidermal keratinocytes. J Nanosci Nanotechno 11: 3782–3788.

Shukla RK, Sharma V, Pandey AK, Singh S, Sultana S, Dhawan A (2011). ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicol In Vitro 25: 231–241.

Singh N, Manshian B, Jenkins GJ, Griffiths SM, Williams PM, Maffeis TG, Wright CJ, Doak SH (2009). NanoGenotoxicology: the DNA damaging potential of engineered nanomaterials. Biomaterials 30: 3891–3914.

Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175: 184–191.

Strauss GH (1991). Non-random cell killing in cryopreservation: implications for performance of the battery of leukocytes tests (BLT). I. Toxic and immunotoxic effects. Mutat Res 252: 1–15.

Theogaraj E, Riley S, Hughes L, Maier M, Kirkland D (2007). An investigation of the photo-clastogenic potential of ultrafine titanium dioxide particles. Mutat Res 634: 205–219.

Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiesti RH (2009). Titanium dioxide nanoparticles induce DNA damage and genetic instability *in vivo* in mice. Cancer Res 69: 8784–8789.

Türkez H, Geyikoğlu F (2007). An in vitro blood culture for evaluating the genotoxicity of titanium dioxide: the responses of antioxidant enzymes. Toxicol Ind Health 23: 19–23.

Vevers WF, Jha AN (2008). Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro. Ecotoxicology 17: 410–420.

Wang JJ, Sanderson BJ, Wang H (2007). Cyto- and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells. Mutat Res 628: 99–106.

Yang H, Liu C, Yang D, Zhang H, Xi Z (2009). Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J Appl Toxicol 29: 69–78.

Yeber MC, Rodriguez J, Freer J, Durán N, Mansilla HD (2000). Photocatalytic degradation of cellulose bleaching effluent by supported TiO_2 and ZnO. Chemosphere 41: 1193–1197.

Yıldız M, Ciğerci IH, Konuk M, Fidan AF, Terzi H (2009). Determination of genotoxic effects of copper sulphate and cobalt chloride in *Allium cepa* root cells by chromosome aberration and comet assays. Chemosphere 75: 934–938.