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Evaluation of cytotoxicity of biphasic TiO_2 nanoparticles with organic surface coatings

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Abstract: Titanium dioxide nanoparticles (TiO_2 NPs) are used in lots of human applications because of their extraordinary nano scaled properties. Particularly, due to their photoprotective properties, they are used in topical dermatologic preparation and also as white pigment. Due to these properties their use in human life is more and more frequent. Despite the fact that nano dimension brings various beneficial properties, it could bring also bad features. Therefore, in this study, we evaluated a cytotoxicity of two types of biphasic TiO_2 NPs using distinct cells of epithelial origin. We found that TiO_2 NPs can induce cytotoxic stress resulting in fragmentation of DNA.

Key Words: nanotoxicology, TiO_2 nanoparticles, *in vitro* tests

INTRODUCTION

Nanotechnologies are developing very rapidly, and the production of different types of nanomaterials promises a wide range of uses, mainly thanks to the new acquired properties (Grumezescu 2016). But the living biological systems are more easily transduced due to nano dimension material, so the physiological effects of penetrated nanoparticles could have a greater impact on the whole organism (Hamblin et al. 2016).

TiO_2 nanoparticles (NPs) have been widely used in industry since 1990. One of the extensive branches of application is cosmetics industry (Johnson et al. 2011). TiO_2 NPs have photoprotective properties, they reflect and scatter UV radiation, but no visible light, thus they act as effective UV filters (Hamblin et al. 2016). TiO_2 NPs can exist in three crystalline forms: anatase, rutile and brookite (Horie et al. 2016). In our study we used two types of biphasic TiO_2 NPs containing brookite and anatase crystalline forms. The TiO_2 NPs were surface–capped with dichloroacetic acid (DCAA) and monochloracetic acid (MCAA) to increase their stability and enhance their photocatalytic activity. Overall, the study shows that upon administration to epithelial cells, both types of TiO_2 NPs can induce cytoxicity through DNA fragmentation.

MATERIALS AND METHODS

Cell cultures

The human prostatic cell lines used in this study were: i) the PNT1A established by immortalization of normal adult prostatic epithelial cells by transfection with a plasmid containing SV40 genome with a defective replication origin; ii) the A375 human melanoma cell line, originally established from a lymph node metastasis of a melanoma patient; and iii) SH–SY5Y human cell line established from a bone marrow metastasis of a 4–y–old female neuroblastoma patient. All cell lines were purchased from Health Protection Agency Culture Collections (Salisbury, UK). Cells were cultured in a RPMI–1640 medium with 10% FBS. Media were supplemented with penicillin (100 U/mL) and streptomycin (0.1 mg/mL), and the cells were maintained at 37 °C in a humidified incubator

(Eppendorf, Hamburg, Germany) with 5% CO_2. The treatment with sarcosine was initiated after cells reached 60–80% confluence.

Synthesis and characterization of TiO_2 NPs

TiO_2 NPs were prepared by using reductive colloidal synthesis and using the sintering at extreme conditions. TiO_2 NPs was capped by dichloroacetic acid (DCAA) and monochloroaceitc acid (MCAA). The morphology of NPs was investigated using transmission electron microscope (TEM) Tecnai F20 (FEI, Eindhoven, Netherlands). The crystallinity of TiO_2 NPs was examined by X–ray diffraction (XRD, Smart Lab diffractometer Rigaku, Rigaku, Tokyo, Japan) using the Cu lamp and the Bragg–Brentano geometry.

Investigation of internalization of TiO_2 NPs

TiO_2 NPs labeling was performed using recombinantly produced TdTomato that was cloned into BamHI and XhoI sites of pRSET–B plasmid (Thermo Fisher, Waltham, MA, USA) in frame with 6xHis, T7 and Xpress tag. The integrity of open reading frame confirmed with Sanger sequencing. The TdTomato was produced in BL21(DE3)pLysS *E. coli* and isolated and precipitated in ammonium sulphate. For labeling, 0.1 µL of TdTomato was mixed with 10 µL of TiO_2 NPs. Unbound TdTomato was removed by repeated centrifugation (10 000×g, 5 min). Internalization was investigated using EVOS FL Auto Cell Imaging System (Thermo Fisher) upon 3 h incubation of NPs with cells and washing with phosphate buffered saline (PBS, pH 7.4).

Screening of effect of biphasic TiO_2 NPs on cellular viability

Screening of cytotoxicity (MTT verified by trypan blue exclusion) was performed to identify antiproliferative and cytotoxic effects of TiO_2 NPs. Treatments were carried out for 24, 48 and 72 h. Then, 10 µL of MTT [5 mg/mL in phosphate buffered saline (PBS)] was added to the cells and the mixture was incubated for 4 h at 37 °C. After that, MTT–containing medium was replaced by 100 µL of 99.9% dimethyl sulfoxide (DMSO) and after 5 min incubation the absorbance of the samples was determined at 570 nm using Infinite 200 PRO (Tecan, Männedorf, Switzerland).

Single–cell gel electrophoresis for analysis of DNA fragmentation

The cells were plated at a density of 106 cells/well in six–well dishes and treated with TiO_2 NPs (100 µg/mL) for 24 h. As positive control, 150 µM H_2O_2 was employed. After harvesting, about 15 µL of the cell suspension was mixed with 75 µL of 0.5% low melting point agarose (CLP, San Diego, CA, USA) and layered on one end of a frosted plain glass slide. Then, it was covered with a layer of the low melting agarose (100 µL). After solidification of the gel, the slides were immersed in a lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10) containing 1% Triton X–100 and 10% DMSO), with an overnight incubation at 4 °C. A cold alkaline electrophoresis buffer was poured into the chamber and incubated for 20 min at 4 °C. The electrophoresis was carried at 4 °C, (1.25 V/cm, 300 mA) for 30 min. The slides were neutralized (0.4 M Tris, pH 7.5) and then stained with ethidium bromide (2 µg/mL). The cells were analysed under fluorescence microscope EVOS FL Auto Cell Imaging System (Thermo Fisher Scientific) and classified according to the shape of the fluorescence of the comet tail [0 (no visible tail) to 4 (significant DNA in tail)].

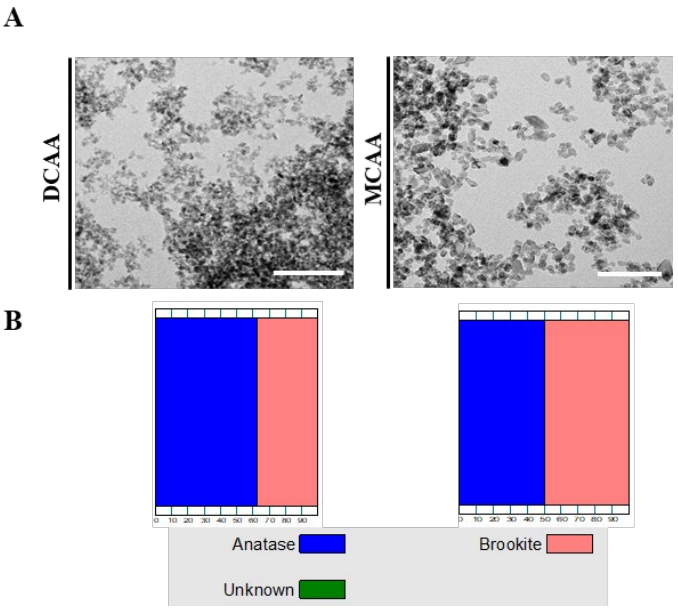
Descriptive statistics and applied bioinformatic tools

For the statistical evaluation of the results, the median was taken as the measurement of the main tendency, while the standard deviation was taken as the dispersion measurement. Differences between groups were analysed using a paired t–test. For analyses, Software Statistica 12 (StatSoft, Tulsa, OK, USA) was employed.

RESULTS AND DISCUSSION

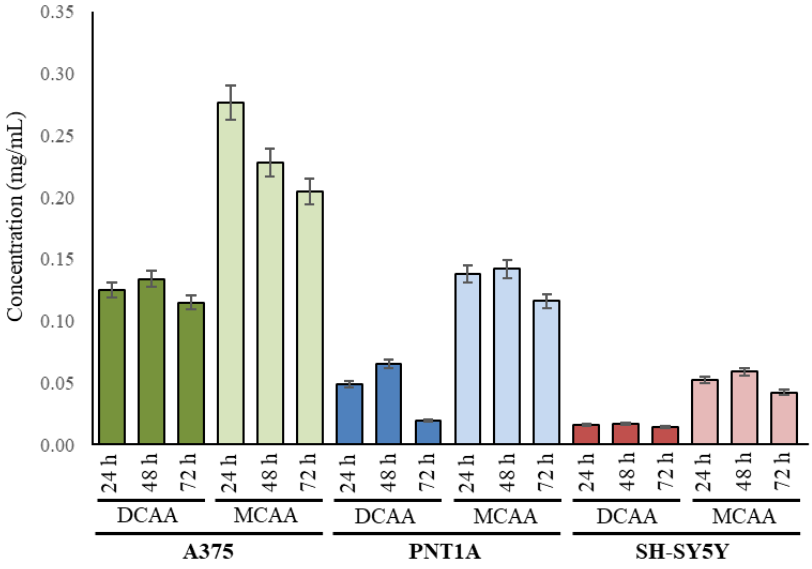
Both types of synthesized TiO_2 NPs had oval–to–spherical morphology with diameter ranging between 15–20 nm (Figure 1A). Moreover, XRD analyses confirmed that both types of TiO_2 NPs had a biphasic crystallinity with anatase prevailing for DCAA–capped NPs and with an equal distribution of brookite and anatase for MCAA–capped NPs (Figure 1B). Overall, these analyses validated the suitability of TiO_2 NPs for further cellular testing of their potential cytotoxic effects.

Figure 1 (A) TEM micrographs of synthesized TiO_2 NPs, scale bar, 50 nm. (B) Distribution patters of anatase and brookite in prepared NPs



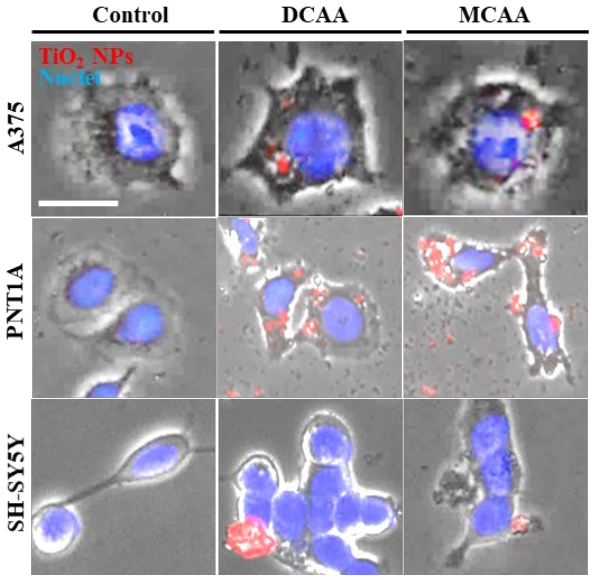
We further studied the effect of TiO_2 NPs on viability of three distinct cell lines of epithelial origin. Figure 2 depicts that within the tested cell lines, NPs exhibited significantly different IC_{50} values. The highest toxicity was found for neuroblastoma–derived cells SH–SY5Y, while the A375 cells (melanoma) were relatively tolerant to NPs presence. Overall, anatase–prevailing NPs DCAA were more toxic that MCAA NPs.

Figure 2 IC_{50} values obtained from MTT assay and trypan blue exclusion upon 24, 48 and 72 h incubation.



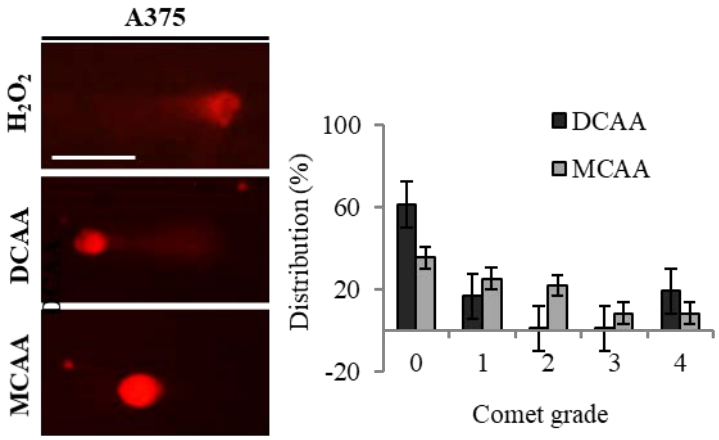
As it was shown in next experiment, both types of TiO_2 NPs were able to be cross the cellular membranes and internalize within intracellular space (Figure 3). Internalization is among the most important prerequisites for inducing further cytotoxic actions by dissolving the NPs in organelles or binding the intracellular metabolites or proteins for further use in signaling.

Figure 3 Fluorescence micrographs of TdTomato–labeled TiO_2 NPs.



To elucidate a mechanism responsible for TiO_2 NPs cytotoxicity, we focused on analysing the DNA fragmentation in A375 cells. Figure 3 demonstrates that both types of NPs were able some extent induce the DNA fragmentation. The highest portion of uncoiled DNA was found for DCAA TiO_2 NPs, which is in line with their cytotoxicity and prevalence of anatase crystallinity.

Figure 3 Comet assay demonstrating the uncoiling of DNA in A375 cells due to the exposure to TiO_2 NPs. Scale bar, 30 µm. Bar graph shows quantitation of comet grades.



CONCLUSION

We successfully prepared two types of biphasic TiO_2 NPs. Using several approaches, we identified that despite both types of NPs are able to internalize to intracellular space, DCAA NPs with a prevailing anatase in their crystallinity planes exhibit higher cytotoxicity to all three types of tested epithelial cell lines. The results demonstrate that despite Ti–based nanomaterials are commonly used in dermatology and industry, during long–term exposures, they can possess a significant health risk. Future studies on a molecular mechanism of cytotoxicity are on the way.

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REFERENCES

Grumezescu, A.M. 2016. Nanobiomaterials in Galenic formulations and cosmetics. Applications of Nanobiomaterials. 1st ed., Oxford UK: Elsevier.

Hamblin, M.R. et al. 2016. Nanoscience in Dermatology. 1st ed., London UK: Academic press.

Horie, M. et al. 2016. Does photocatalytic activity of TiO_2 nanoparticles correspond to photo–cytotoxicity? Cellular uptake of TiO_2 nanoparticles is important in their photo–cytotoxicity. Toxicology Mechanisms and Methods, 26(4): 284–294.

Johnson, A.C. et al. 2011. An assessment of the fate, behaviour and environmental risk associated with sunscreen TiO_2 nanoparticles in UK field scenarios. Science of the Total Environment, 409(13): 2503–2510.

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