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Research Article

Combined *In vitro* Effects of TiO₂ Nanoparticles and Dimethyl Sulfoxide (DMSO) on HepG₂ Hepatocytes

Abstract

Introduction: Professional workers that manufacture or use titanium dioxide (TiO₂)-based paints are exposed to potentially toxic TiO₂ nanomaterials as well as to different paint solvents such as dimethyl sulfoxide (DMSO). In this context, we evaluate the combined cytotoxic effects of TiO₂ nanoparticles and DMSO on HepG₂ human hepatocytes.

Methods: Three types of TiO₂ nanoparticles were used: commercial Degussa P25 and two samples synthesized by a hydrothermal procedure – undoped and Fe³⁺-doped TiO₂. The effects of TiO₂ nanoparticles on HepG2 cells exposed to DMSO before, after or together with the TiO₂ treatment were investigated by viability and intracellular reactive oxygen species (ROS) determinations, performed using the MTT and DCFH-DA(2',7'-dichlorofluorescein-diacetate) methods respectively.

Results: Results indicated that DMSO made HepG2 cells more susceptible to toxic effects induced by nanosized TiO₂. In the absence of DMSO, none of the tested nanoparticles exhibited significant cytotoxic effects. Viability increases were detected after 48 hours of treatment and attributed to possible redox-sensitive proliferation mechanism striggered by the low and moderate amounts of produced ROS. The combined action of TiO₂ and DMSO led to a general viability decrease tendency. Significant effects (viability reductions and ROS generation) were observed in the case of cells first treated with Degussa P25 TiO₂ and afterwards exposed to DMSO. The hydrothermal materials exhibited reduced *in vitro* reactivity on HepG₂ hepatocytes.

Conclusion: The study reveals the enhancement of nanosized TiO₂ toxicity induced by DMSO exposure, its findings having potential to help in the evaluation of professional health risks associated to the combined action of TiO₂ nanomaterials and paint solvents.

Abbreviations

BET: Brunauer-Emmett-Teller; DCFH-DA: 2'-7'-dichlorofluorescein-diacetate; DLS: Dynamic Light Scattering; DMEM-F12: Dulbecco's Modified Eagle Medium; Nutrient Mixture F-12; DMSO: dimethyl sulfoxide; DRIFT: Diffuse Reflectance Infrared Fourier Transform Spectroscopy; EDX: Energy-dispersive X-ray Spectroscopy; FBS: Fetal Bovine Serum; FeHT: Fe³⁺-doped anatase TiO₂ nanoparticles synthesized under hydrothermal conditions; HT: undoped anatase TiO₂ nanoparticles synthesized under hydrothermal conditions; LD50: lethal dose 50%; MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; P25: Degussa P25 TiO₂ nanoparticles; PBS: Phosphate-buffered Saline; ROS: Reactive Oxygen Species; TEM: Transmission Electron Microscopy; XRD: X-ray Diffraction

Introduction

Recent studies regarding the widespread use of paints that contain titanium dioxide (TiO₂, titania) nanoparticles for bacterial decontamination and self-cleaning purposes revealed significant advantages of these new technologies, but also potential health risks induced by the release of nanosized TiO₂ from painted surfaces [1-4]. If the amount of released TiO₂ was shown to be relatively small under the studied conditions [5], significant professional health risks may

be associated to workers that manufacture or use TiO₂-based paints. These workers are exposed to potentially toxic TiO₂ nanomaterials as well as different solvents used during the processing or application of such paints. Among the used solvents, dimethyl sulfoxide (DMSO) was considered to be more convenient due to its relatively reduced toxicity [6,7]. However, DMSO is also known to easily penetrate human skin and, in some cases, to serve as carrier agent, promoting the percutaneous absorption of other compounds (including drugs and toxins) [8,9]. Under these circumstances, DMSO may facilitate the penetration of skin by TiO₂ nanoparticles.

To evaluate the implications and hazards involved by the potential exposure to both, TiO₂ nanoparticles and DMSO (or other paint solvent), it is essential to possess detailed knowledge regarding their combined effects on vital organs, such as brain, liver, heart, kidney, lung or spleen. Among these, the liver represents one of the most important organs involved in the processing of exogenous compounds (including nanomaterials) and detoxification, a significant amount of published studies concerning the hepatotoxic effects of solvents (including DMSO in several cases)[10-13].

The present study gives an insight into the combined *in vitro* effects of TiO₂ nanoparticles and DMSO (seen as a generic paint solvent).

The experiments have been performed on HepG2 cells (human hepatocarcinoma cells-ATCC[®] HB-8065TM), a well characterized cell line, widely used in cytotoxicity studies due to its convenient specific characteristics, such as:

- biosynthetic capabilities similar to those of normal hepatocytes
- retention of cell surface receptors – response capacity similar to normal cells [14,15].

The studied effects concern the cytotoxicity and intracellular reactive oxygen species (ROS) production induced in HepG2 cells under different treatment schemes with nano-TiO₂ and DMSO.

We have used three types of TiO₂ nanoparticles: the commercial Degussa P25 TiO₂ (P25) and other two samples synthesized under hydrothermal conditions in our laboratory – undoped (HT) and Fe³⁺-doped (FeHT) anatase TiO₂. Degussa P25 TiO₂ was often tested (and utilized as reference material) in studies concerning intracellular ROS generation [16] and toxicity induced by titania nanomaterials [17-19]. The hydrothermal TiO₂ samples have similar structural characteristics (crystal structures, shapes, sizes and specific surface areas [20] – the relevance of these factors being frequently considered in TiO₂ nanotoxicology studies [21,22]), but different band gap energies (relative to each other) and colloidal behaviors (compared to Degussa P25) [20].

The obtained results are analyzed with respect to the structural and physicochemical properties of the tested nanomaterials [20], the characteristics of the used cells and the cell penetration and hydroxyl radical scavenger properties of DMSO [23].

Materials and Methods

Materials synthesis

The undoped and iron-doped TiO₂ nanoparticles were prepared starting from TiCl₃ (solution 15 % in HCl 10 %, from Merck) and Fe₂O₃ (RITVERC 95.44 % ⁵⁷Fe Isotopic Enrichment) [20].

To obtain the Fe³⁺ (1 at. %)-doped TiO₂, the titanium and iron precursors were processed as follows: TiCl₃ was oxidized (by air barbotage) to TiCl₄ and Fe₂O₃ was reacted to hydrochloric acid (4N) to form FeCl₃. The resulted solutions were involved in a coprecipitation process, NH₄OH being drop wise added to their mixture up to pH=8. The obtained precipitate was washed with deionised water, resuspended in double-distilled water and exposed to hydrothermal treatment in a 50 cm³ Teflon-lined autoclave at 200 °C for one hour. The undoped TiO₂ was synthesized using the same procedure involving the titanium precursor only.

Material characterization

The structural, morphological, optical and physicochemical characteristics of the used materials have been studied by X-ray diffraction (XRD), transmission electron microscopy (TEM), *Energy-dispersive X-ray spectroscopy* (EDX), Mössbauer spectroscopy, Brunauer-Emmett-Teller (BET) nitrogen adsorption, UV-Vis reflectance spectroscopy, Dynamic Light Scattering (DLS) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) and described in a previous work [20].

Cellular and noncellular experiments regarding the *in vitro* effects of TiO₂ nanoparticles on HepG2 hepatocytes pre-treated, co-treated or post-treated with DMSO

Cell viabilities and intracellular ROS productions in HepG2 hepatocyte cultures exposed to TiO₂ nanoparticles and DMSO have been performed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and the DCFH-DA (2',7'-dichlorfluorescein-diacetate) test respectively.

The experiments were such designed to elucidate the following aspects:

- effects of the studied TiO₂ nanoparticles on hepatocytes that were “already damaged” – the cells were first treated with DMSO and two hours later exposed to the action of nano-TiO₂;
 - viability and intracellular ROS production in case of HepG2 cells simultaneously treated with DMSO and nano-TiO₂;
 - response of hepatocytes to the action of DMSO, administered two hours after the cells were exposed to nano-TiO₂;
1. The working protocol was established based on the following: LD50 for DMSO after 24 h of exposure – the DMSO dose capable of killing 50% of the cells after 24 h of exposure;
 2. the effects of nano-TiO₂ alone (without DMSO) on the studied cells;

Cell treatment

The HepG2 cells were seeded in 24-well culture plates at a density of 10⁵ cells/cm² in volumes of 1 ml of DMEM-F12 culture medium containing 10% FBS. After 24 h required for cell adherence and growth, the culture medium was discarded and replaced with fresh medium (1 ml/well) containing the necessary treatment agents (TiO₂ (2.5, 7.5, 15, 25, 50, 75, 100 µg/ml), DMSO (LD50) or both) according to the stimulation scheme presented above. In case of pre-treatment or post-treatment with DMSO, either TiO₂ or DMSO, were added two hours after the initial stimulation.

Preliminary experiments have established the LD50 for DMSO to be 5 µl DMSO/1 ml of culture medium.

Cell viability assay

After 24, 48 and 72 h of treatment, the culture medium was removed and MTT solution (1 mg/ml MTT in phosphate buffered saline (PBS)) was added to each well (300µl/well). The obtained samples were incubated for 2 hours, the MTT solution being afterwards discarded. To dissolve the produced formazan crystals, DMSO (Sigma-Aldrich) was added to each well (300µl/well). The optical density of the purple formazan solution was determined at 540 nm using a Thermo Multiskan EX spectrophotometer.

The obtained results were quantified with respect to control samples consisting of untreated cells (in case of experiments in which TiO₂ alone was used) or cells treated with DMSO (in case of experiments involving both TiO₂ and DMSO). Cell viability was expressed as percents versus control. Standard deviations were

computed based on three technical replicates corresponding to each sample and three independent biological replicates of each experiment.

The results are represented as average values \pm standard deviations (error bars).

Determination of intracellular ROS production

To quantify the intracellular ROS production, the cells were incubated for 24 hours with the treating agents (TiO_2 (2.5, 25, 50, 100 $\mu\text{g/ml}$), DMSO (LD50) or both). After incubation, the culture medium was removed and replaced with fresh medium containing DCFH-DA (0.2 μl of 2'-7'-dichlorofluorescein-diacetate (DCFH-DA) stock solution (25 mg/mL DCFH-DA in TFS) in 1 ml of culture medium). The obtained samples were incubated again for 30 minutes. The DCFH-DA medium was afterwards removed; the cells were detached with trypsin (0.25% trypsin and 0.53 mM EDTA solution), suspended in PBS and centrifuged for 10 min at 1500 rpm and 4°C , the supernatant being discarded. The excess of fluorescein was removed by washing the cells twice in PBS and the cells were resuspended in 500 μl PBS. The homogenized suspensions were transferred to 96-well plates (100 μl /well). A Fluoroskan FL (Thermo) equipment (excitation wavelength 485 nm/emission wavelength 530 nm) was used to perform the fluorimetric determinations.

The obtained results were quantified with respect to control samples (described above for cell viability experiments) and expressed

as percents versus control. Standard deviations were computed based on three technical replicates corresponding to each sample and three independent biological replicates of each experiment.

The results are represented as average values \pm standard deviations (error bars).

Data analysis and representation

Data statistical analysis and representation were performed using the Sigma Plot-11 software package. Depending on data normality, either one-way ANOVA or one-way ANOVA on ranks tests were performed. The *Student-Newman-Keuls* (SNK) posthoc test was employed in order to complete the analysis. A value of $p < 0.05$ was considered significant. All samples statistically different from controls were marked on figures with a (*).

Results

Materials characterization

The detailed structural and physicochemical characterization of the three TiO_2 nanomaterials used in this study was published in a previous work [20]. Briefly, all three types of titania have similar shapes (no acicular shaped particles) and average particle sizes between 10-30 nm. The hydrothermal, HT and FeHT, samples have anatase structure and Degussa P25 TiO_2 is a mixture of anatase and rutile polymorphs with anatase/rutile weight ratio of 85:15(%). BET specific surface areas are 49 m^2/g for Degussa P25, 130.62 m^2/g for HT and 114.81 m^2/g for FeHT. The band gap energies were approximately

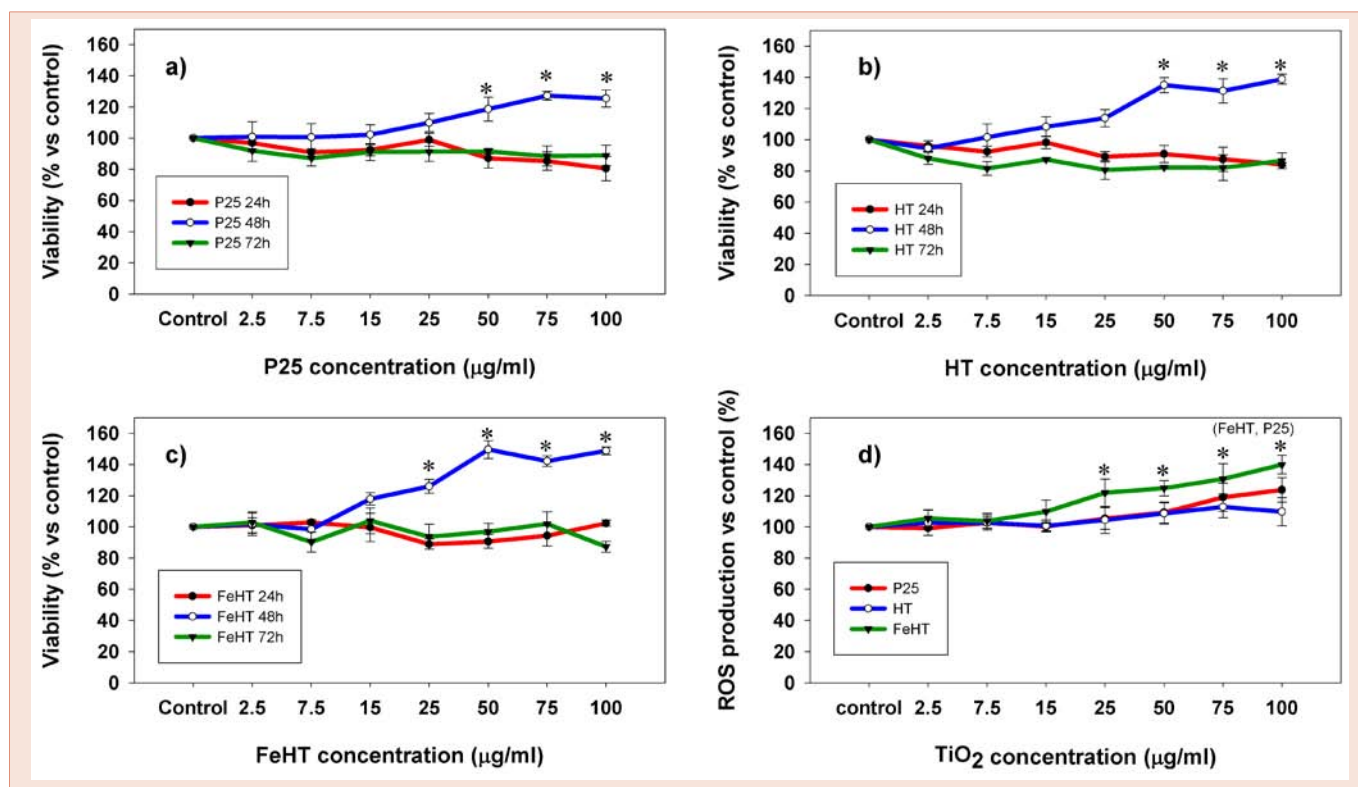


Figure 1: Cell viability (a-c) and intracellular ROS production (d) for HepG2 treated with P25, HT and FeHT; (*) - significant differences with respect to control ($p < 0.05$ - calculated based on three biological replicates).

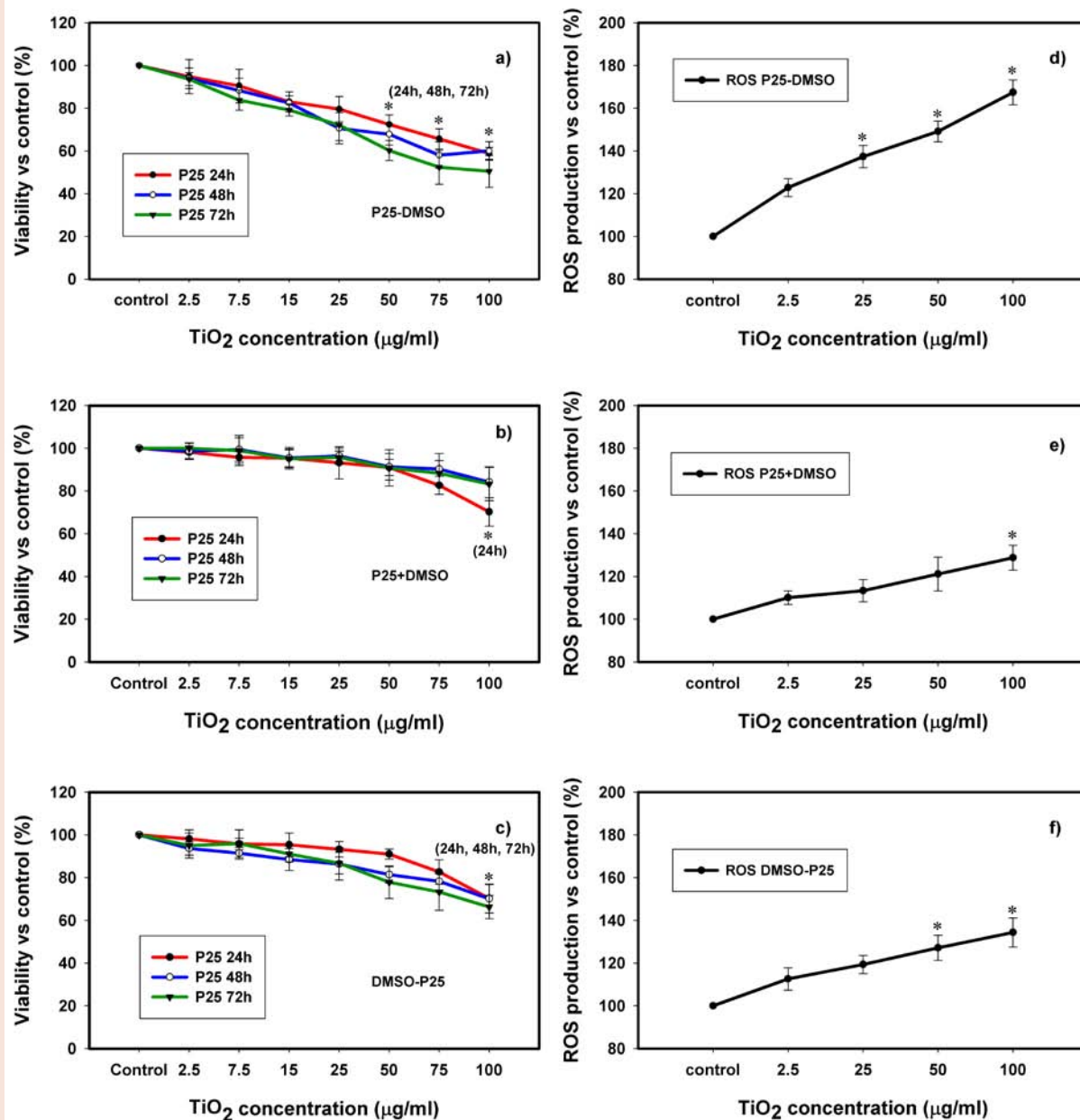


Figure 2: Cell viability (a-c) and intracellular ROS production (d-f) for HepG2 cells treated with Degussa P25 nanoparticles and DMSO; (*) - significant differences with respect to control ($p < 0.05$ - calculated based on three biological replicates). The (*) symbols placed above or below particular data points refer to all cases (treatment times) specified in the brackets.

3 eV for Degussa P25 and HT and 2.848 eV for the iron doped, FeHT, sample. Degussa P25 has considerably higher colloidal stability in aqueous suspensions compared to HT and FeHT. The colloidal stabilization effect of proteins from culture medium was revealed.

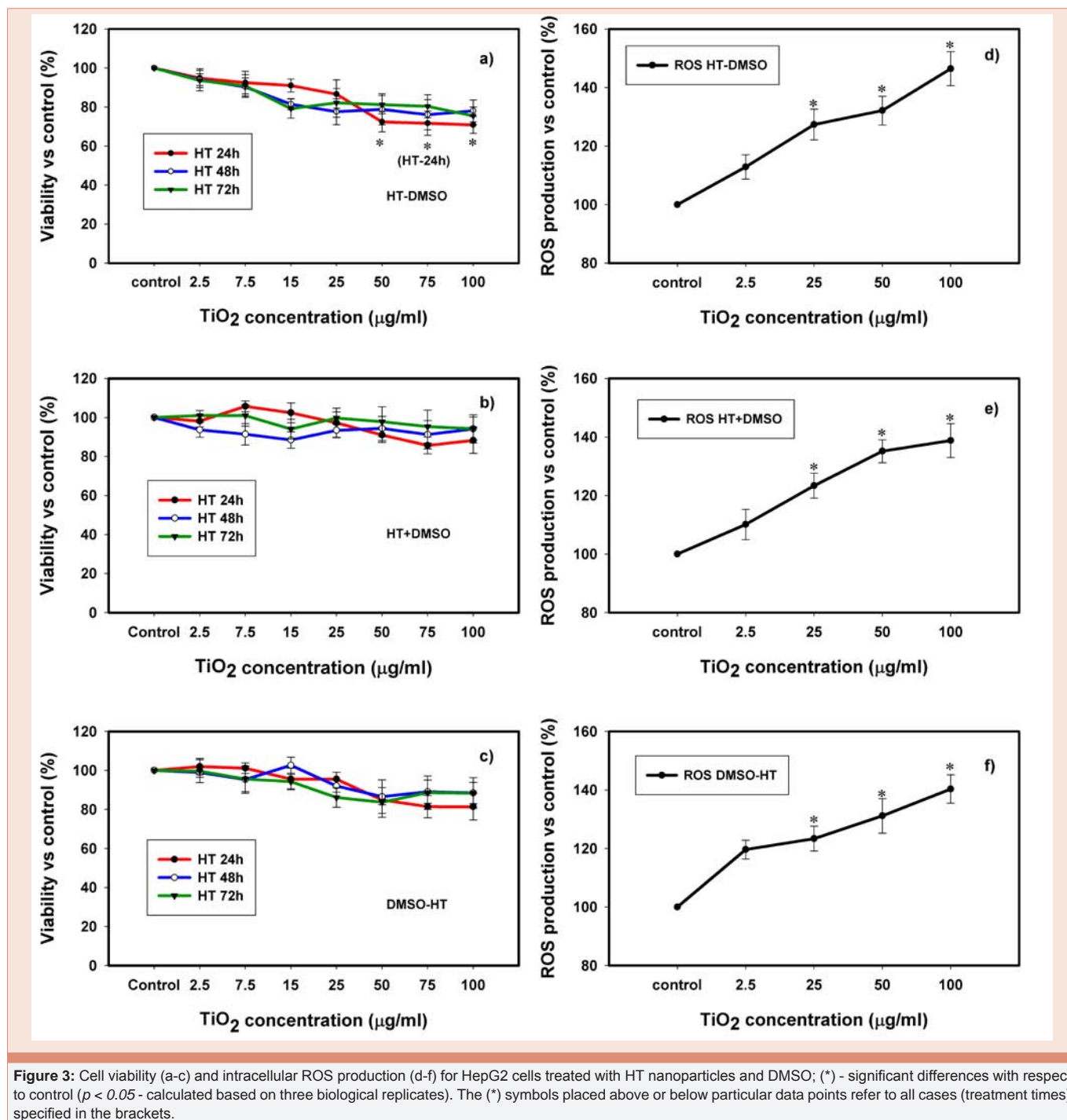
In vitro effects of undoped and Fe³⁺-doped TiO₂ nanoparticles on HepG2 hepatocytes

The results obtained in this study are displayed for each of the tested nanomaterials (P25, HT and FeHT) in Figures 1-4.

Cell viability

TiO₂ alone: Cell viabilities obtained in experiments involving TiO₂ alone (no DMSO) are represented in Figure 1a-1c. No significant viability variations were observed after 24h and 72h of treatment for none of the tested TiO₂ samples. At 48h after exposure, viability increases (up to 49 %) were detected for all nanomaterials, being more pronounced in case of FeHT. The observed increases were proportional to the concentration of TiO₂ in the studied samples.

To better illustrate the specific features of each experimental case



(pre-, co- or post-treatment with DMSO) and each type of tested TiO_2 , the results are described and discussed comparatively below.

TiO_2 and DMSO: While for all the tested materials, the most prominent viability reductions were observed in the case of cells post-treated with DMSO (TiO_2 -DMSO) (Figures 2a-4a), the highest cell killing effect was induced by Degussa P25 nanoparticles (Figure 2a). The hydrothermal materials (HT and FeHT) induced only weak or insignificant cytotoxic effects.

On the other hand, no significant cellular effects were detected in the case of cells simultaneously exposed to TiO_2 and DMSO (TiO_2 +DMSO) (Figures 2b-4b).

The observed viability variations did not depend on the treatment time (24, 48, 72 hours), for none of the tested TiO_2 types.

Intracellular ROS production

TiO_2 alone: Regarding the intracellular ROS production,

significant increases (between 21- 39 %) were induced by the iron-doped sample (Figure 1d). The commercial P25 TiO₂ induced a significant increase only at its maximum concentration (100 µg/ml). The observed increases were concentration-dependent. No variation was detected in case of HT sample (Figure 1d).

TiO₂ and DMSO: Small or moderate increases in the intracellular ROS levels of the treated cells were observed in all experimental cases (Figures 2(d-f)-4(d-f)), being more pronounced in the case of DMSO post-treatment (TiO₂-DMSO) (Figures 2d-4d). Although

the highest ROS production was determined for Degussa P25 TiO₂, corresponding to the TiO₂-DMSO case, no clear distinction can generally be made between the pro-oxidative effects of commercial and hydrothermal samples.

The determined ROS production increases were, in most cases, proportional to the concentration of TiO₂.

Discussion

To ensure the validity of the obtained results, one should

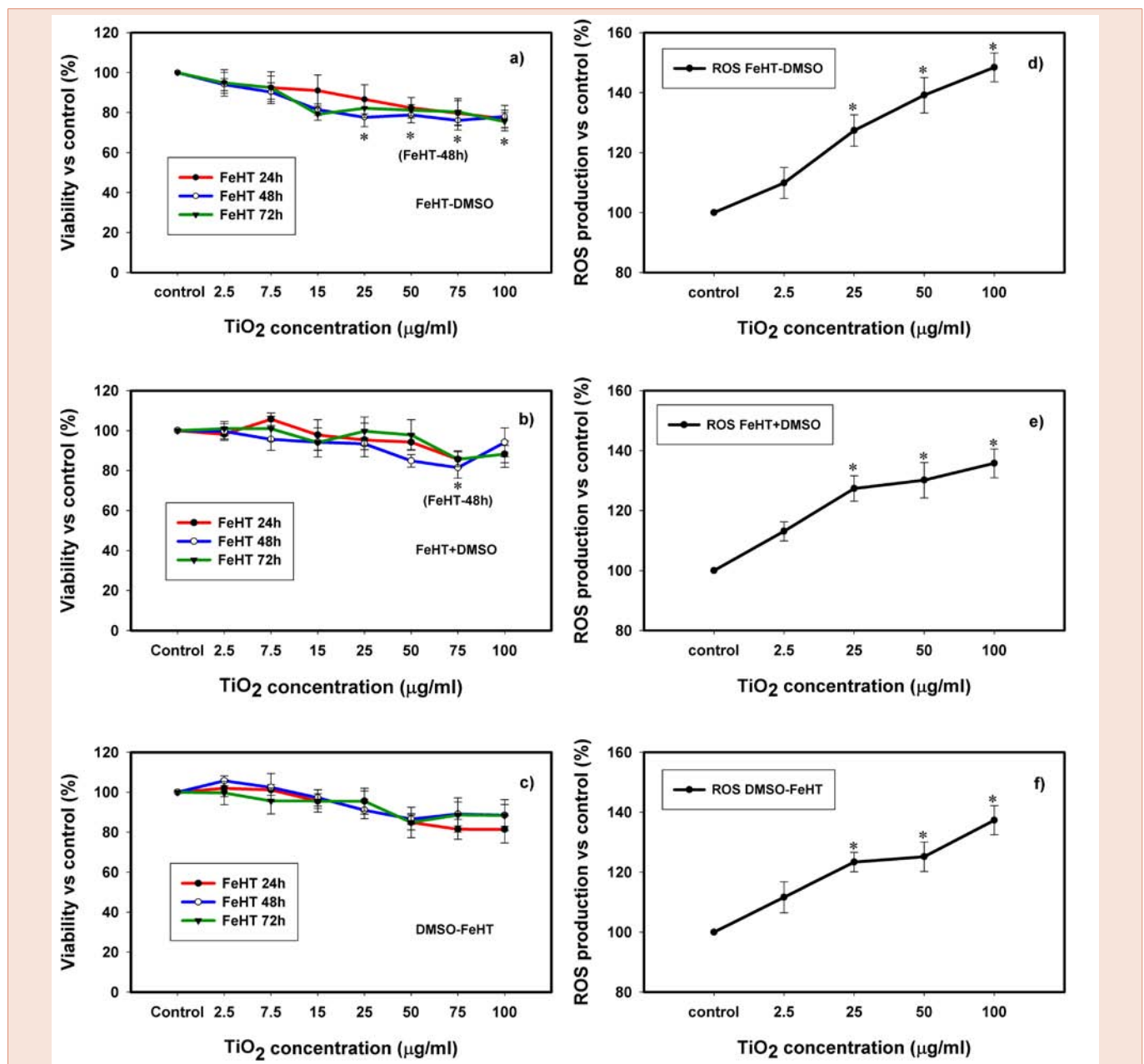


Figure 4: Cell viability (a-c) and intracellular ROS production (d-f) for HepG2 cells treated with FeHT nanoparticles and DMSO; (*) - significant differences with respect to control ($p < 0.05$ - calculated based on three biological replicates). The (*) symbols placed above or below particular data points refer to all cases (treatment times) specified in the brackets.

consider the possible interferences that may occur between the studied nanomaterials and the biochemical methods used through the performed study. Being a known photocatalyst [24-29], TiO₂ may interfere with MTT and induce experimental artifacts, as described by Lupu and Popescu [30]. This hypothesis was tested in noncellular experiments for each of the studied TiO₂ samples. Only one experimental case (Degussa P25, no DMSO) revealed weak TiO₂ (P25)-MTT interferences (data not shown). This effect was considered in data analysis in a manner similar to that described by Lupu and Popescu [30].

The cell viability results obtained in experiments involving TiO₂ alone revealed the lack of toxicity of the three tested nanomaterials on HepG2 cells, under the experimental conditions of the present study. The proliferation effects observed after 48 hours of TiO₂ treatment suggest the possible involvement of redox-sensitive cell proliferation mechanisms [31-37], triggered by the low and moderate levels of intracellular ROS production induced by the action of nano-TiO₂. One such ROS species is H₂O₂, which is known to either promote cell proliferation or induce cell cycle arrest, as a function of its concentration in the studied system [35,38-45]. In this context, it is important to note that H₂O₂ is among the characteristic oxygen species detected by the DCFH-DA method [46-48], its formation and proliferative effects being thus likely to occur in our study. Moreover, the DCFH-DA test was performed after 24h of TiO₂ treatment, the detected ROS generation being a plausible cause for the cell proliferation effect observed after 48h of treatment. After longer times however, cell viability is reduced by the oxidative environment. The onset of ROS effects (either cell proliferation or toxic effects due to oxidative stress) requires more or less time to occur, depending on the type and amount of generated ROS. Under the experimental conditions of our study, the effects of ROS produced during the first 24h of TiO₂ treatment and later became significant after longer times, being determined after 48h and 72h respectively. The increased amounts of ROS generated at later times induced the viability decrease observed after 72h of treatment.

Although the mechanisms by which TiO₂ nanoparticles induce intracellular ROS formation are not well understood, in the case of HepG2 cells, ROS overproduction may be favored by their tumor nature [49,50] and/or their role in detoxification [51,52].

Regarding the combined effects of TiO₂ and DMSO on HepG2 cells, the results presented above (Figures 2-4) are displayed with respect to control samples containing DMSO (LD50). Thus, the observed variations in viability or intracellular ROS production describe only the effects of TiO₂ nanoparticles on cells exposed to LD50 of DMSO. To understand how DMSO exposure influences the action of TiO₂ on the studied cells, these results should be analyzed in comparison to those obtained on cells that were not exposed to DMSO (Figure 1). The comparison indicates that DMSO exposure makes HepG2 cells more susceptible to toxic effects induced by nanosized TiO₂.

In this view, cells exposed to DMSO show no proliferation effects induced by the tested TiO₂. Although a general tendency towards viability reductions can be observed, only Degussa P25 produces consistent (for all treatment times), concentration-dependent toxic effects, mainly visible when cells are first exposed to TiO₂

and afterwards treated with DMSO (the TiO₂-DMSO case (Figure 2a)). Not only the highest viability reduction but also the highest intracellular ROS production was associated to the TiO₂-DMSO experiment, this finding suggesting that the observed effects were dictated by the early action of TiO₂ and associated to oxidative stress.

A possible mechanism to account for the enhanced toxicity of TiO₂ in the case of DMSO treated cells involves autophagy, which may be induced by both TiO₂ [53,54] and DMSO [55]. TiO₂ was also reported to induce lysosome membrane permeabilization, which represents a well known cell death mechanism (including lysosomal-iron mediated oxidative stress) [54].

The attenuated cellular effects observed either in the case of co-treatment (TiO₂+DMSO) or pre-treatment with DMSO (DMSO-TiO₂) may indicate a possible interaction between TiO₂ and DMSO, leading to the attenuation of the damaging effects of TiO₂ (especially of Degussa P25). This hypothesis is supported by two aspects: the known photocatalytic properties of TiO₂ and the known hydroxyl radical scavenger properties of DMSO [56-58]. In principle, by photocatalytic processes, TiO₂ nanoparticles may generate hydroxyl radicals on their surface and induce oxidation reactions. Also, in principle, DMSO may act as scavenger for these radicals and reduce their oxidative action. However, it is unfortunately not possible to accurately test, at least not in a straightforward manner, whether these processes can occur under relevant *in vitro* conditions.

All experiments performed in this study confirm the reduced toxicity of the hydrothermal TiO₂ samples (HT and FeHT) to HepG2 hepatocytes. Besides identifying nanomaterials with low toxicity, our study points towards the risks involved by the combined exposure to DMSO and nano-TiO₂. Results also reveal the importance of material properties (other than chemical composition) for their biological effects. Regarding the hydrothermal TiO₂ samples, although they exhibit larger surface specific area than Degussa P25, their *in vitro* reactivity appears to be reduced. This may be related to their different surface charge properties in culture media as well as ROS photogeneration capacities and hydrophilicity [20].

To clearly establish the relation between the *in vitro* reactivity of the tested TiO₂ nanomaterials and their complex physicochemical properties, further studies are required.

Conclusion

The described study gives an insight into the combined *in vitro* effects of TiO₂ nanoparticles and DMSO (seen as a generic paint solvent) on human hepatocytes. The observed effects were shown to depend on the properties of TiO₂ and the characteristics of the exposure. Results indicated that DMSO makes HepG2 cells more susceptible to toxic effects induced by nanosized TiO₂. These findings may help in the evaluation of professional health risks associated to workers that manufacture or use TiO₂-based paints.

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References

- Kaiser JP, Roesslein M, Diener L, Wick P (2013) Human Health Risk of Ingested Nanoparticles That Are Added as Multifunctional Agents to Paints: an *In vitro* Study. *PLoS ONE* 8: e83215. doi:10.1371/journal.pone.0083215.
- Occupational Exposure to Titanium Dioxide. Current Intelligence Bulletin 63. Department of Health and Human Services. Center for Disease Control and Prevention. National Institute for Occupational Safety and Health. 2011.
- Koponen IK, Jensen KA, Schneider T (2009) Sanding dust from nanoparticle-containing paints: physical characterization. *Journal of Physics. Conference Series* 151: 012048.
- Wang J, Fan Y (2014) Lung Injury Induced by TiO₂ Nanoparticles Depends on Their Structural Features: Size, Shape, Crystal Phases and Surface Coating. *Int J Mol Sci* 15: 22258-22278.
- Al-Kattan A, Wichser A, Vonbank R, Brunner S, Ulrich A, et al. (2013) Release of TiO₂ from paints containing pigment-TiO₂ or nano-TiO₂ by weathering. *Environ Sci Process Impacts* 15: 2186-93. doi:10.1039/c3em00331k.
- Marti M, Molina L, Aleman C, Armelin E (2013) Novel Epoxy Coating Based on DMSO as a Green Solvent, Reducing Drastically the Volatile Organic Compound Content and Using Conductivity Polymers As a Nontoxic Anticorrosive Pigment. *ACS Sustainable Chem Eng* 1: 1609-1618.
- Wu G (2010) Assay Development. Fundamentals and Practices. Chapter 13. High-Throughput Screening Assay Development. Wiley ISBN 978-0-470-19115-6, page 328.
- Capriotti K, Capriotti JA (2012) Dimethyl Sulfoxide. History, Chemistry and Clinical Utility in Dermatology. *J Clin Aesthet Dermatol* 5: 24-26.
- Botana LM (Ed) (2007) Phycotoxins: Chemistry and Biochemistry. Blackwell Publishing ISBN-13: 978-0-8138-2700-1, page 41.
- Malaguamera G, Cataudella E, Giordano M, Nunnari G, Chisari G, et al. (2012) Toxic hepatitis in occupational exposure to solvents. *World J Gastroenterol* 18: 2756-2766.
- Baratti Y, Charles AL, Wolff V, Tahar LB, Smiri L, et al. (2014) Age Modulates Fe₃O₄ Nanoparticles Liver Toxicity: Dose-Dependent Decrease in Mitochondrial Respiratory Chain Complexes Activities and Coupling in Middle-Aged as Compared to Young Rats. *Bio-Med Research International* 474081: 10.
- Kshirsagar A, Vetal Y, Ashok P, Bhosle P, Ingawale D (2008) *Drug Induced Hepatotoxicity: A Comprehensive Review*. The Internet Journal of Pharmacology 7.
- Mohammadi S, Mehrparvar A, Labbafinejad Y, Attarchi MS (2010) The effect of exposure to a mixture of organic solvents on liver enzymes in an auto manufacturing plant. *J Public Health* DOI:10.1007/s10389-010-0340-z.
- Pinti M, Troiano L, Nasi M, Ferraresi R, Dobrucki J, et al. (2003) Hepatoma HepG₂ cells as a model for in vitro studies in mitochondrial toxicity of antiviral drugs: which correlation with the patient? *J Biol Regul Homeost Agents* 17: 166-71.
- Dehn P, White C, Connors D, Shipkey G, Cumbo T (2004) Characterization of the human hepatocellular carcinoma (HEPG2) cell line as an in vitro model for cadmium toxicity studies. *In Vitro Cellular & Developmental Biology – Animal* 40: 172-182.
- Long TC, Tajuba J, Sama P, Saleh N, Swartz C, et al. (2007) Nanosize titaniumdioxide stimulates reactive oxygen species in brainmicroglia and damages neurons in vitro. *Environ Health Perspect* 115: 1631-1637.
- Sayes CM, Wahi R, Kurian PA, Liu Y, West JL, et al. (2006) Correlating nanoscale titania structure with toxicity: a cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. *Toxicol Sci* 92: 174-185.
- Han W, Wang YD, Zheng YF (2008) *In vitro* biocompatibility study of nano TiO₂ materials. *Adv Mater Res* 47-50: 1438-1441.
- Wadhwa S, Rea C, O'Hare P, Mathur A, Roy SS, et al. (2011) Comparative in vitro cytotoxicity study of carbon nanotubes and titania nanostructures on human lung epithelial cells. *J Hazard Mater* 191: 56-61.
- Popescu T, Lupu AR, Diamandescu L, Tarabasanu-Mihaila D, Teodorescu VS, et al. (2013) Effects of TiO₂ nanoparticles on the NO₂- levels in cell culture media analysed by Griesscolorimetric methods. *J Nanopart Res* 15: 1449-1450.
- Jin C, Tang Y, Yang FG, Li XL, Xu S, et al. (2011) Cellular toxicity of TiO₂ nanoparticles in anatase and rutile crystal phase. *Biol Trace Elem Res* 141: 3-15.
- Hsiao IL, Huang YJ (2011) Effects of various physicochemical characteristics on the toxicities of ZnO and TiO₂ nanoparticles toward human lung epithelial cells. *Sci Total Environ* 409: 1219-1228.
- Mokudai T, Nakamura K, Kanno T, Niwano Y (2012) Presence of Hydrogen Peroxide, a Source of Hydroxyl Radicals, in Acid Electrolyzed Water. *PLoS ONE* 7: e46392. doi:10.1371/journal.pone.0046392.
- Linsebigler AL, Lu G, Yates JT Jr., (1995) Photocatalysis on TiO₂ surfaces: principles, mechanisms, and selected results. *Chemical Reviews* 95: 735-758.
- Carp O, Huisman CL, Reller A (2004) Photoinduced reactivity of titanium dioxide. *Progress in Solid State Chemistry* 32: 33-177.
- Chen X, Mao SS (2007) Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. *Chemical Reviews* 107: 2891-2959.
- Fujishima A, Rao TN, Tryk DA (2000) Titanium dioxide photocatalysis. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 1: 1-21.
- Hashimoto K, Irie H, Fujishima A (2005) TiO₂ photocatalysis: a historical overview and future prospects. *Japanese Journal of Applied Physics* 44: 8269-8285.
- Kumar SG, Devi LG (2011) Review on modified TiO₂ photocatalysis under UV/visible light: selected results and related mechanisms on interfacial chargecarrier transfer dynamics. *Journal of Physical Chemistry A* 115: 13211-13241.
- Lupu AR, Popescu T (2013) The noncellular reduction of MTT tetrazolium salt by TiO₂ nanoparticles and its implications for cytotoxicity assay. *Toxicology In Vitro* 27: 1445-1450.
- Alfadda AA, Sallam RM (2012) Reactive oxygen species in health and disease. *J Biomedicine Biotechnol* 2012: 936486.
- Greene EL, Velarde V, Jaffa AA (2000) Role of reactive oxygen species in bradykinin-induced mitogen-activated protein kinase and c-fos induction in vascular cells. *Hypertension* 35: 942-947.
- Ruiz-Gines JA, Lopez-Ongil S, Gonzalez-Rubio M, Gonzalez-Santiago L, Rodriguez-Puyol M, et al. (2000) Reactive oxygen species induce proliferation of bovine aortic endothelial cells. *J Cardiovasc Pharmacol* 35: 109-113.
- Sainz RM, Lombo F, Mayo JC (2012) Radical decisions in cancer: redox control of cell growth and death. *Cancers* 4: 442-474.
- Sarsour EH, Kumar MG, Chaudhuri L, Kalen AL, Goswami PC (2009) Redox control of the cell cycle in health and disease. *Antioxid Redox Signal* 11: 2985-3011.
- Sauer H, Wartenberg M, Hescheler J (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem* 11: 173-186.
- Trachootham D, Lu W, Ogasawara MA, Rivera-Del Valle N, Huang P (2008) Redox regulation of cell survival. *Antioxid Redox Signal* 10: 1343-1374.
- Barnouin K, Dubuisson ML, Child ES, Fernandez de Mattos S, Glassford J, et al. (2002) H₂O₂ induces a transient multiphase cell cycle arrest in mouse fibroblasts through modulating cyclin D and p21Cip1 expression. *J Biol Chem* 277: 13761-13770.

39. Burdon RH (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18: 775-794.
40. Burdon RH, Gill V (1993) Cellularly generated active oxygen species and HeLa cell proliferation. *Free Radic Commun Res* 19: 203-213.
41. Duque I, Rodriguez-Puyol M, Ruiz P, Gonzalez-Rubio M, Diez-Marques ML, et al. (1993) Calcium channel blockers inhibit hydrogen peroxide-induced proliferation of cultured rat mesangial cells. *J Pharmacol Exp Ther* 267: 612-616.
42. Herbert JM, Bono F, Savi P (1996) The mitogenic effect of H₂O₂ for vascular smooth muscle cells is mediated by an increase of the affinity of basic fibroblast growth factor for its receptor. *FEBS Lett* 395: 43-47.
43. Li M, Zhao L, Liu J, Liu AL, Zeng WS, et al. (2009) Hydrogen peroxide induces G2 cell cycle arrest and inhibits cell proliferation in osteoblasts. *Anat Rec* 292: 1107-1113.
44. Los M, Droge W, Stricker K, Baeuerle PA, Schulze-Osthoff K (1995) Hydrogenperoxide as a potent activator of T lymphocyte functions. *Eur J Immunol* 25: 159-165.
45. Timblin CR, Janssen YW, Mossman BT (1995) Transcriptional activation of the proto-oncogene c-jun by asbestos and H₂O₂ is directly related to increased proliferation and transformation of tracheal epithelial cells. *Cancer Res* 55: 2723-2726.
46. McNeil SE (Ed.) (2011) Characterization of Nanoparticles Intended for Drug Delivery. Series: Methods in Molecular Biology 697: 173-179.
47. Poncin S, Colin IM, Gerard AC (2009) Minimal oxidative load: a prerequisite for thyroid cell function. *J Endocrinol* 201: 161-167.
48. Sitharaman B (2011) Nanobiomaterials Handbook. CRC Press 30-38.
49. Afanas'ev I (2011) Reactive oxygen species signaling in cancer: comparison with aging. *Aging Dis* 2: 219-230.
50. Massaoka MH, Matsuo AL, Figueiredo CR, Farias CF, Girola N, et al. (2012) Jacaranone induces apoptosis in melanoma cells via ROS mediated down regulation of Akt and p38 MAPK activation and displays antitumor activity in vivo, *PLoS One* 7: e38698. doi: 10.1371/journal.pone.0038698.
51. Liska DJ (1998) The detoxification enzyme systems. *Altern Med Rev* 3: 187-98.
52. Xu C, Li C, Kong, AT (2005) Induction of Phase I, II and III drug metabolism / transport by xenobiotics. *Arch Pharm Res* 28: 249-268.
53. Kanazoui BH, Bernasconi CC, Guney-Ayra S, Juillerat-Jeanneret L (2012) Induction of oxidative stress, lysosome activation and autophagy by nanoparticles in human brain-derived endothelial cells. *Biochem J* 441: 813-821.
54. Stern ST, Adisheshaiah PP, Crist RM (2012) Autophagy and lysosomal dysfunction as emerging mechanisms of nanomaterial toxicity; *Particle and Fibre Toxicology* 9: 20.
55. Song YM, Song SO, Jung YK, Kang ES, Cha BS, et al. Dimethyl sulfoxide reduces hepatocellular lipid accumulation through autophagy induction. *Autophagy* 8: 1085-1097.
56. Panganamala RV, Sharma HM, Heikkila RE (1976) Role of hydroxyl radical scavengers, dimethyl sulfoxide, alcohols, and methional in the inhibition of prostaglandin synthesis. *Prostaglandins* 11: 599-607.
57. Del Maestro R, Thaw HH, Bjork J, Planker M, Arfors KE (1980) Free radicals as mediators of tissue injury. *Acta Physiol Scand Suppl* 492: 91-119.
58. Ashwood-Smith MJ (1967) Current concepts concerning radio protective and cryro protetive properties or dimethyl sulfoxide in cellular systems. *Ann NY Acad Sci* 141: 41-62.

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