Influence of TiO$_2$ nanoparticles on cellular antioxidant defense and its involvement in genotoxicity in HepG2 cells

Jana Petković, Bojana Žegura and Metka Filipič

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, SI-1000 Ljubljana, Slovenia

E-mail: metka.filipic@nib.si

Abstract. We investigated the effects of two types of TiO$_2$ nanoparticles (<25 nm anatase, TiO$_2$-An; <100 nm rutile, TiO$_2$-Ru) on cellular antioxidant defense in HepG2 cells. We previously showed that in HepG2 cells, TiO$_2$ nanoparticles are not toxic, although they induce oxidative DNA damage, production of intracellular reactive oxygen species, and up-regulation of mRNA expression of DNA-damage-responsive genes (p53, p21, gadd45α and mdm2). In the present study, we measured changes in mRNA expression of several antioxidant enzymes: catalase, superoxide dismutase, glutathione peroxidase, nitric oxide synthase, glutathione reductase and glutamate-cysteine ligase. As reduced glutathione has a central role in cellular antioxidant defense, we determined the effects of TiO$_2$ nanoparticles on changes in the intracellular glutathione content. To confirm a role for glutathione in protection against TiO$_2$-nanoparticle-induced DNA damage, we compared the extent of TiO$_2$-nanoparticle-induced DNA damage in HepG2 cells that were glutathione depleted with buthionine-(S,R)-sulfoximine pretreatment and in nonglutathione-depleted cells. Our data show that both types of TiO$_2$ nanoparticles up-regulate mRNA expression of oxidative-stress-related genes, with TiO$_2$-Ru being a stronger inducer than TiO$_2$-An. Both types of TiO$_2$ nanoparticles also induce dose-dependent increases in intracellular glutathione levels, and in glutathione-depleted cells, TiO$_2$-nanoparticle-induced DNA damage was significantly greater than in nonglutathione-depleted cells. Interestingly, the glutathione content and the extent of DNA damage were significantly higher in TiO$_2$-An- than TiO$_2$-Ru-exposed cells. Thus, we show that TiO$_2$ nanoparticles cause activation of cellular antioxidant processes, and that intracellular glutathione has a critical role in defense against this TiO$_2$-nanoparticle-induced DNA damage.

1. Introduction

The genotoxicity of nanomaterials is believed to be due to oxidative stress as the underlying key mechanism [1,2]. This involves alterations in redox homeostasis, which includes both increased production of reactive oxygen species (ROS) and depletion of antioxidant defenses. ROS are highly cytotoxic and can cause DNA damage, impair protein function, and result in peroxidation of lipids [3] and in apoptosis [4]. Generation of ROS can also be promoted by large surface area, which is one of the main characteristics of nanoparticles (NPs). Consequently, the smaller the NPs, the greater the oxidative stress they can induce. In addition, with their high surface area, transition metal ions that can be released from certain NPs, such as titanium, cadmium, chromium, cobalt, copper, iron, nickel, and zinc, have the potential to cause the conversion of cellular oxygen metabolic products, such as H$_2$O$_2$ and superoxide anions, to hydroxyl radicals (\(\cdot OH\)), which is one of the primary DNA damaging mechanisms.
species in cells [5]. Numerous studies have demonstrated that TiO$_2$ NPs can induce ROS production and promote oxidative DNA damage [6,7]. In our previous study, we showed that in a test system with HepG2 human hepatoma cells, TiO$_2$ NPs are not toxic, although they induce oxidative DNA damage, which is associated with intracellular ROS production. Exposure of these cells to TiO$_2$ NPs also induced changes in mRNA expression of DNA-damage-responsive genes (p53, p21, gadd45$\alpha$ and mdm2), which provides additional evidence that TiO$_2$ NPs are genotoxic [8]. However, although considerable attention has been given to the involvement of ROS in the genotoxicity of TiO$_2$ NPs, their influence on the antioxidant defense systems has been largely overlooked.

Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and nitric oxide synthase (NOS), convert ROS into less damaging compounds, thus providing the first line of antioxidant defense [9]. The second tier of defense is provided by enzymes such as glutathione peroxidase (GPx), glutathione S-transferase, aldo-keto reductase and aldehyde dehydrogenase, which detoxify the reactive intermediates. Glutathione (GSH) has a central role in these intracellular antioxidant defense processes as it is a free-radical scavenger and can conjugate reactive intermediates via glutathione S-transferase and GPx [3]. However, because of its importance, GSH is also a vulnerable target in cell defense, as different agents (environmental and other) can directly deplete or inactivate GSH. For example, depletion of intracellular GSH has been shown to occur by direct conjugation to or reaction with GSH by environmental agents, or indirectly by the activity of GSH-transferases [10]. The consequence is the facilitated accumulation of ROS and their by-products [4]. Despite the importance of GSH in defense against oxidative stress, its role in TiO$_2$-NP-induced genotoxicity has not been investigated yet.

The aim of this study was to determine the effects of two types of TiO$_2$ NPs on cellular antioxidant defense in HepG2 cells: <25 nm anatase (TiO$_2$-An) and <100 nm rutile (TiO$_2$-Ru). We measured changes in mRNA expression of the key antioxidant enzymes: CAT, SOD, GPx and NOS. CAT catalyzes the production of water and molecular oxygen from H$_2$O$_2$ and has a very important role in oxidative-stress tolerance [11]. SOD has a critical role in efficient dismutation of superoxide anions [12], and GPx detoxifies H$_2$O$_2$ or organic hydroperoxides using GSH as the reducing equivalent. NOS catalyzes the production of nitric oxide, which leads to the formation of peroxynitrite and reactive nitrogen species [13]. In addition, we measured expression of glutathione reductase (GSR), which reduces oxidized GSH (glutathione disulfide) to the reduced, sulfhydryl form, and of glutamate-cysteine ligase (GCL), which catalyzes the final step in GSH synthesis [14]. As reduced GSH has a central role in cellular antioxidant defense, we determined the effects of TiO$_2$ NPs on changes in the intracellular content of GSH. The role of GSH in protection against TiO$_2$-NP-induced DNA damage was confirmed by comparing the extent of TiO$_2$-NP-induced DNA damage in HepG2 cells that were GSH depleted with buthionine-(S,R)-sulfoximine (BSO) pretreatment to that of the same cells without GSH depletion.

2. Materials and methods

2.1. Chemicals

Eagle’s minimal essential medium, penicillin/ streptomycin, L-glutamine, phosphate-buffered saline (PBS), trypsin, fetal bovine serum, non-essential amino-acid solution (100x), D,L-buthionine-[S,R]-sulfoximine (BSO), tert-butyl hydroperoxide (t-BOOH), ethidium-bromide solution, dimethyl sulfoxide were obtained from Sigma-Aldrich (St. Louis, USA). Monochlorobimane (mBCl) was from Fluka (Steinheim, Germany). TRIzol was from Invitrogen (Carlsbad, USA), and cDNA High Capacity Archive kits, TaqMan Universal PCR Master Mix, and TaqMan Gene Expression assays were from Applied Biosystems (Foster City, USA). Normal melting-point agarose and low melting-point agarose were from Gibco BRL (Paisley, Scotland).
2.2. Cell culture
HepG2 human hepatoma cells were obtained from the European Collection of Cell Cultures. They were grown in Eagle’s minimal essential medium containing 10% fetal bovine serum, 1% non-essential amino acid solution, 2 mM L-glutamine, and 100 U/ml penicillin plus 100 µg/ml streptomycin, at 37°C in a humidified atmosphere with 5% CO₂.

2.3. Characteristics of TiO₂ nanoparticles
Two grades of TiO₂ powders with different average particle sizes (d <25 nm; d <100 nm) and crystalline structures (anatase; rutile) were used in this study, which were obtained from Sigma-Aldrich (St. Louis, USA). These are abbreviated as TiO₂-An (Cat. No. 637254: anatase; particle size <25 nm; 99.7% trace metals basis) and TiO₂-Ru (Cat. No. 637262: rutile; particle size ~10 × 40 nm; 99.5% trace metals basis). Their size, crystalline structure, specific surface area, and agglomeration/aggregation were confirmed experimentally in our previous study [8].

2.4. Preparation of TiO₂ nanoparticles
Powdered TiO₂ NPs were suspended in PBS at a concentration 10 mg/ml and sonicated for 30 min at a frequency of 30 kHz in an ultrasonic bath (Sonorex, Bandelin Electronic, Berlin, Germany), to ensure uniform suspensions. These stock solutions were subsequently diluted in complete cell-growth medium to yield final concentrations ranging from 1 µg/ml to 250 µg/ml. These samples were then sonicated for 30 min to produce stable, less-aggregated suspensions before exposure of the cells in culture.

2.5. mRNA expression analysis
Cells were seeded at a density of 1 × 10⁶ cells in T-25 flasks (Corning Costar Corporation, Corning, New York, USA) and incubated for 20 h at 37°C in 5% CO₂ for their attachment. The growth medium was then replaced with fresh medium containing 0, 1, 10, or 100 µg/ml TiO₂ NPs, and the cells were incubated for a further 4 h or 24 h. Each experiment included a negative control. Total RNA from these cells was isolated using TRizol reagent, and the cDNA was synthesized using 2 µg total RNA with cDNA High Capacity Archive kits (Applied Biosystems), according to the manufacturer protocol. Gene expression levels of CAT, SOD, GPx, NOS, GSR and GCL were quantified using quantitative real-time PCR (ABI 7900 HT Sequence Detection System, Applied Biosystems). TaqMan Universal PCR Master Mix and the following TaqMan Gene Expression Assays were used (all from Applied Biosystems): CAT (catalase), Hs00937387_m1; SOD (superoxide dismutase 1, soluble), Hs00166575_m1; GPx (glutathione peroxidase 1), Hs01028922_g1; NOS (nitric oxide synthase 2, inducible), Hs01075529_m1; GSR (glutathione reductase), Hs00167317_m1; and GCL (glutamate-cysteine ligase, catalytic subunit), Hs00155249_m. Amplification of a glyceraldehyde 3-phosphate dehydrogenase probe was performed as an internal control. The conditions for the quantitative real-time PCR were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. The data obtained from TaqMan Gene Expression assays were analyzed using the ΔΔCt algorithm. Statistical significance between controls and treated groups was determined using two tailed Student’s t-tests, and P < 0.05 was considered significant. Independent experiments were performed in duplicate, and each was repeated at least three times.

2.6. Determination of intracellular reduced glutathione
The level of GSH in HepG2 cells was quantified using a fluorogenic bimane probe, mBCl, as described by [15], with minor modifications [10]. The nonfluorescent probe mBCl forms a stable, fluorescent adduct with GSH in a reaction catalyzed by GSH S-transferases [15].

The cells were seeded into 96-well, black, tissue-culture-treated microtiter plates (Nunc, Naperville, USA) at a density of 1.25 × 10⁵ cells/ml. After a 20 h incubation at 37°C in 5% CO₂, the cells were treated with 0, 1, 10, 100 or 250 µg/ml TiO₂ NPs in PBS. Negative (nonNP-treated) and
positive (0.5 mM t-BOOH treated) cell-treatment controls were included in each experiment. After 2 h, 4 h and 6 h, 100 µM mBCl in PBS was added directly to the cells in the microtiter plates. After 15 min, the fluorescence of the wells was measured at 485 nm, with excitation at 390 nm, using a microplate-reading spectrofluorimeter (Tecan, Genios, Austria). The data are expressed as percentage fluorescence compared with the relevant negative controls. Statistical significance between control and treated groups was determined by two tailed Student’s t-tests, and \( P < 0.05 \) was considered as statistically significant. Three independent experiments were performed, each with five replicates.

2.7. Comet assay
The HepG2 cells were seeded at a density of \( \approx 6 \times 10^4 \) cells/ml in 12-well microtiter plates (Corning Costar Corporation, Corning, New York, USA). They were then pre-cultured in the absence and presence of 150 µM BSO in complete cell-growth medium for 20 h. The GSH levels in these HepG2 cells pre-treated with BSO were determined using the fluorogenic bimane probe mBCl, as described in the previous subsection. After pretreatment with BSO, the growth medium containing BSO was replaced with fresh medium containing 0, 1, 10, 100 or 250 µg/ml TiO₂ NPs and incubated for 4 h. Each experiment included negative and positive controls (0.25 mM t-BOOH). After 4 h, the comet assays were performed as described previously [8]. Fifty cells were analyzed per experimental point, in each of three independent experiments. The percentages of tail DNA were used to measure the levels of DNA damage. One-way analysis of variance (ANOVA, Kruskal–Wallis) was used to analyze the differences between the treatments within each experiment. Dunnet's tests were used for comparing median values of percentage tail DNA; \( P < 0.05 \) and \( P < 0.001 \) was considered as statistically significant.

3. Results

3.1. Effects of TiO₂ NPs on expression of oxidative-stress-responsive genes
The mRNA expression of selected oxidative-stress-responsive genes (CAT, SOD, GPx, NOS, GSR, GCL) was determined after 4 h and 24 h exposure of HepG2 cells to 0, 1, 10 or 100 µg/ml TiO₂ NPs, using quantitative real-time PCR.

In cells exposed to TiO₂-An for 4 h (Figure 1A) there were significant increases in expression of CAT and GSR at the highest TiO₂-An concentration (100 µg/ml), which after 24 h returned to control levels. After 24 h of exposure to TiO₂-An, the expression of NOS was significantly decreased at the highest TiO₂-An concentration (100 µg/ml) (Figure 1B).

In cells exposed to TiO₂-Ru, the expression of all six measured genes (CAT, SOD, GPx, NOS, GSR, GCL) were significantly increased at the highest TiO₂-Ru concentration (100 µg/ml) (Figure 1C). After 24 h of exposure, only expression of GPx and GCL remained significantly elevated at 100 µg/ml TiO₂-Ru, while that of NOS significantly decreased at 10 µg/ml TiO₂-Ru. Under the same conditions, mRNA expression of the other genes (CAT, SOD, GSR) remained unchanged (Figure 1D).
Figure 1. Quantitative real-time PCR analysis of changes in CAT, SOD, GPx, NOS, GSR, and GCL gene expression after exposure of HepG2 cells to TiO$_2$-An (A, B) and TiO$_2$-Ru (C, D) for 4 h (A, C) and 24 h (B, D). The quantitative real-time PCR was performed as described in Materials and methods. The data are expressed as relative mRNA expression normalized to the untreated controls. Duplicate experiments were repeated at least three times.

*, significant difference of TiO$_2$-NP-treated groups over controls (Student’s t-tests; $P < 0.05$).

3.2. Changes in intracellular GSH

The intracellular levels of reduced GSH in HepG2 cells were quantified using the fluorogenic bimane probe mBCl, after 2 h, 4 h and 6 h exposure to 0, 1, 10, 100 and 250 $\mu$g/ml TiO$_2$ NPs.

Figure 2. Intracellular reduced GSH levels in HepG2 cells after treatment with TiO$_2$-An (A) and TiO$_2$-Ru (B) at 0, 1, 10, 100 and 250 $\mu$g/ml TiO$_2$ NPs for 2 h, 4 h and 6 h. The positive control was 0.5 mM t-BOOH (K+). GSH levels were quantified using the fluorogenic bimane probe mBCl. Each bar represents the mean ($\pm$SD) of three independent experiments.

*, significant difference of TiO$_2$-NP-treated groups over controls (Student’s t-tests; $P < 0.05$).
At 10 µg/ml, 100 µg/ml, and 250 µg/ml, exposure of HepG2 cells to TiO$_2$-An for 2 h, 4 h, and 6 h resulted in significant, dose-dependent increases in intracellular GSH levels. The maximal increase in the GSH levels was observed at the highest TiO$_2$-An concentration (250 µg/ml) after 4 h of exposure (to 2.4-fold the control) (Figure 2A).

In the cells exposed to TiO$_2$-Ru, there were significant increases in GSH levels at 100 µg/ml and 250 µg/ml TiO$_2$-Ru at all times of exposure (2 h, 4 h, 6 h). The maximal GSH level was observed after 4 h at 250 µg/ml TiO$_2$-Ru (to around 1.9-fold the control), which declined slightly after 6 h exposure, but remained significantly greater than the control (at 1.5-fold the control) (Figure 2B).

As the positive control for this test system, 0.5 mM t-BOOH led to a 60% decrease in intracellular GSH levels, compared to control cells.

3.3. The role of GSH in TiO$_2$-NP-induced DNA damage

To explore the role of GSH in TiO$_2$-NP-induced DNA damage, the cells were cultured in the absence and presence of BSO in complete cell-growth medium for 20 h. The intracellular levels of reduced GSH were then quantified using the fluorogenic bimane probe mBChl, after 4 h exposure to 0, 1, 10, 100 and 250 µg/ml TiO$_2$ NPs. The positive control was 0.25 mM t-BOOH. In these BSO-pretreated HepG2 cells, the GSH levels were reduced to 60% compared to the control cells (data not shown).

In the non-BSO-pretreated cells exposed to the increasing concentrations of TiO$_2$-An, there was a small but dose-dependent increase in DNA strand breaks in comparison to the non-TiO$_2$-An-treated control, which was significant only at the highest concentration of TiO$_2$-An (250 µg/ml; $P < 0.05$). On the other hand, in the BSO-pretreated cells, TiO$_2$-An induced a further significant dose-dependent increase in the percentage tail DNA, from TiO$_2$-An concentrations of 10 µg/ml and above ($P < 0.001$; Table 1).

The percentage DNA damage in non-BSO-pretreated cells exposed to the increasing TiO$_2$-Ru concentrations did not significantly differ from the control cells. In the BSO-pretreated cells, there was a small, but not statistically significant, increase in the DNA strand breaks over the control with the 100 µg/ml, and 250 µg/ml exposure to TiO$_2$-Ru (Table 2).

| TiO$_2$-An (µg/ml) | % Tail DNA (mean ±SD) |
|--------------------|------------------------|
|                    | Non-BSO-pretreated     | BSO-pretreated         |
| 0                  | 3.67 ±0.76             | 5.15 ±0.52             |
| 1                  | 4.40 ±1.73             | 5.29 ±0.86             |
| 10                 | 5.24 ±1.19             | 8.90 ±1.51**           |
| 100                | 5.65 ±0.56             | 12.63 ±2.13**          |
| 250                | 6.17 ±1.85*            | 13.75 ±2.32**          |
| K+                 | 44.56 ±9.62**          | 39.87 ±5.67**          |

* $P < 0.05$; ** $P < 0.001$: significant difference between TiO$_2$-NP-treated and control groups using ANOVA, and Kruskal-Wallis with Dunnet’s post test.
Table 2. Levels of TiO$_2$-Ru-induced DNA strand breaks in control (nonBSO-pretreated) and BSO-pretreated HepG2 cells.

| TiO$_2$-Ru (µg/ml) | %Tail DNA (mean ±SD) | NonBSO-pretreated | BSO-pretreated |
|-------------------|-----------------------|--------------------|----------------|
| 0                 | 3.58 ±0.76            | 3.67 ±0.80         |
| 1                 | 3.96 ±0.49            | 3.78 ±0.92         |
| 10                | 3.74 ±0.74            | 3.89 ±1.15         |
| 100               | 4.14 ±1.16            | 4.49 ±0.37         |
| 250               | 3.99 ±0.82            | 5.27 ±1.62         |
| K+                | 41.06 ±7.40**         | 34.32 ±2.50**      |

**, P < 0.001: significant difference between TiO$_2$-NP-treated and control groups using ANOVA, and Kruskal-Wallis with Dunnet’s post test.

4. Discussion and conclusions

In this study, we have shown for the first time that different crystalline forms of TiO$_2$ NPs can differently activate cellular antioxidant processes at the gene-expression level, and that intracellular GSH has a critical role in defense against TiO$_2$-NP-induced DNA damage. From our previous study [8], it appeared that ROS formation and the induction of oxidative DNA damage by TiO$_2$ NPs is a consequence of deregulation of the endogenous antioxidant enzymes, including CAT, SOD and others, that synergize with the GSH redox system.

After short-term exposure (4 h) to TiO$_2$-Ru, we observed up-regulation of all of the genes monitored, while TiO$_2$-An up-regulated only the expression of CAT and GSR. These observations indicate that the cells responded to the exposure to these TiO$_2$ NPs by activation of their antioxidant defense system, which provides indirect evidence that they induce oxidative stress. This finding would also explain our previously reported higher ROS production and oxidative DNA damage induced by TiO$_2$-An compared to TiO$_2$-Ru NPs [8]. After longer exposure to TiO$_2$ NPs (24 h), expression of all of these genes monitored returned to their control levels, and some were even slightly decreased. This pattern of elevated expression after short-term exposure followed by normal levels of expression after longer exposure is probably the normal cellular response to exogenous stress caused by agents like these TiO$_2$ NPs. As during the prolonged exposure the mRNA levels in these cells returned to normal levels even though the TiO$_2$ NPs were not removed, this indicates that the cells adapted to such stressors, and in this case TiO$_2$ NPs.

Another marker of response to oxidative stress that we monitored in the present study was the change in the intracellular GSH levels, which has been often shown to be involved in cellular responses to toxins and xenobiotics [3,10]. Exposure of HepG2 cells to TiO$_2$ NPs resulted in dose-dependent and time-dependent increases in the intracellular GSH levels. Gerloff et al. [16] recently reported that in Caco-2 cells exposure to TiO$_2$ NPs (80% anatase, 20% rutile) for 4 h did not change the cellular GSH content. On the contrary, in human keratinocyte HaCaT cells, Horie et al. [17] reported a significant decrease in intracellular GSH levels after 24 h of exposure to nanosized TiO$_2$, but not to fine-sized TiO$_2$ particles. The reasons for these contradictory results might be because Horie et al. [17] measured the GSH levels after 24 h of exposure, while in the present study, we measured GSH levels only up to 6 h of exposure; at the same time, these different cell types might respond differently to this exposure to TiO$_2$ NPs.

Our demonstration that HepG2 cells responded to exposure to TiO$_2$ NPs by increases in their intracellular GSH levels, and taking into account the evidence that TiO$_2$-NP-induced genotoxicity is ROS mediated, led us to the assumption that GSH is involved in cellular protection against
TiO$_2$-induced DNA damage. To confirm this hypothesis, we used an approach with BSO, as a GSH-depleting agent that inhibits GCL [18]. The data here clearly showed that exposure of the GSH-depleted cells to TiO$_2$-An resulted in significantly higher levels of DNA damage, as compared to nondepleted cells. TiO$_2$-Ru however, did not induce DNA damage, neither in GSH-depleted nor in nondepleted cells.

This study has demonstrated that the cell redox status is disturbed after exposure to TiO$_2$ NPs. Both crystalline types of TiO$_2$-NP induced changes in gene expression of the enzymes involved in the cellular defense against oxidative stress, with TiO$_2$-Ru NPs being significantly more efficient. However, TiO$_2$-An was a more efficient inducer of increases in cellular GSH levels, and only TiO$_2$-An NPs induced DNA damage, with intracellular GSH shown to have a role in protection against TiO$_2$-An-induced genotoxicity. Similar to this study, our previous study showed that TiO$_2$-Ru NPs induce the expression of DNA-damage-responsive genes more efficiently, while TiO$_2$-An induced more intracellular ROS and oxidative DNA damage [8]. The lack of genotoxic effects of TiO$_2$-Ru can therefore be explained, such that in cells exposed to TiO$_2$-Ru, an early defense response triggers the antioxidant enzymes that reduce the formation of ROS, and repair processes that eliminate DNA damage before it can be detected. Our studies thus demonstrate that these anatase and rutile crystalline types of TiO$_2$ NPs have different toxic potentials, while also showing differences in their toxicity mechanisms.

References
[1] Kim S, Choi JE, Choi J, Chung K-H, Park K, Yi J and Ryu D-Y 2009 Toxicol. in Vitro 23 1076-84
[2] Schins RP and Knaapen AM 2007 Inhal. Toxicol. 19 189-98
[3] Hayes JD and McLellan LI 1999 Free Radical Res. 31 273-300
[4] Franco R, Sánchez-Olea R, Reyes-Reyes EM and Panayiotidis M 2009 Mutat. Res.-Gen. Tox. En. 674 3-22
[5] Singh N, Manshian B, Jenkins GJS, Griffiths SM, Williams PM, Maffeis TGG, Wright CJ and Doak SH 2009 Biomaterials 30 3891-914
[6] Karlsson HL, Cronholm P, Gustafsson J and Moller L 2008 Chem. Res. Toxicol. 21 1726-32
[7] Gurr JR, Wang AS, Chen CH and Jan KY 2005 Toxicology 213 66-73
[8] Petković J, Žegura B, Stevanović M, Drnovšek N, Uskoković D, Novak S and Filipič M 2010 Nanotoxicology
[9] McCord JM and Fridovich I 1988 Free. Radic. Biol. Med. 5 363-9
[10] Zegura B, Lah TT and Filipic M 2006 Mutat. Res. 611 25-33
[11] Brown MR, Miller FJ Jr, Li W-G, Ellingson AN, Mozena JD, Chatterjee P, Engelhardt JF, Zwacka RM, Oberley LW, Fang X, Spector AA and Weintraub NL 1999 Circ. Res. 85 524-33
[12] Zelko IN, Mariani TJ and Folz RJ 2002 Free. Radic. Biol. Med. 33 337-49
[13] Halliwell B and Gutteridge JMC 1999 Free Radicals in Biology and Medicine (Oxford: Clarendon Press)
[14] Krohn-Ehrich G, Schirmer RH and Untucht-Grau R 1977 Eur. J. Biochem. 80 65-71
[15] Fernández-Checa J and Kaplowitz N 1990 Anal. Biochem. 190 212-9
[16] Gerloff K, Albrecht C, Boots AW, Förster I and Schins RPF 2009 Nanotoxicology 3 355-64
[17] Horie M, Nishio K, Fujita K, Kato H, Endoh S, Suzuki M, Nakamura A, Miyauchi A, Kinugasa S, Yamamoto K, Iwashashi H, Murayama H, Niki E and Yoshida Y 2010 Toxicol. in Vitro 24 1629-38
[18] Griffith OW and Meister A 1979 J. Biol. Chem. 254 7558-60