Nano-silicon dioxide toxicological characterization on two human kidney cell lines

V Paget, JA Sergent and S Chevillard
Laboratory of Experimental Cancerology, Institute of Cellular and Molecular Radiobiology, CEA, Fontenay-aux-Roses, France
E-mail: sylvie.chevillard@cea.fr

Abstract. Silicon dioxide nanoparticles (n-SiO$_2$) have recently encountered a wide variety of applications in medicine or engineering but their toxicological effects are poorly understood. In this study, we have used SiO$_2$-25 nm and SiO$_2$-100 nm mono-dispersed nanoparticles labeled with Rhodamine B and TMPyP respectively. These two fluorophores were incorporated during synthesis in order to track nanoparticles cell incorporation. Up-to-date, no evaluation of the toxicological effects of these nanoparticles upon human kidney has been published. As kidney is one of the major traditional retention organs, the aim of our study is to evaluate the potential toxicity of these nanoparticles on two human cell lines from proximal tubule (Caki-1 and Hek293). Our results report that the two cell lines do not show similar responses after 24 hours of exposure to SiO$_2$-nanoparticles disregarding a similar origin in the kidney. Interestingly, our results indicate that for both tested SiO$_2$-nanoparticles, Caki-1 cells present a higher sensitivity in terms of cytotoxicity and genotoxicity than Hek293 cells. Furthermore, our results show that for similar concentration of exposure, SiO$_2$-25 nm seems to be more cytotoxic and genotoxic than SiO$_2$-100nm for both tested cell lines.

1. Introduction
Nanotechnology development has grown rapidly in the past decade in various fields of application such as drug delivery [1,2], environmental health [3], electronics or biomedical engineering [4]. Nevertheless, this rapid development might be also associated with unintended hazards. Despite intensive research efforts, reports of cellular responses to nanomaterials are often inconsistent and even contradictory. Furthermore, toxicological data of SiO$_2$ nanoparticles on human renal cells in vitro and their detailed molecular mechanisms still remain unknown. Indeed, to our knowledge, there are no published data reporting the study of such nanoparticles upon biological human material from kidney, which is among the more important retention organs. The aim of our study is to evaluate the potential toxicity of these nanoparticles on two human cell lines from proximal tubule (Hek293 and Caki-1).

Unfortunately, the intrinsic properties of nanomaterials have already shown some unintended interferences with some usual cytotoxic and genotoxic assays, leading to false negative or false positive results [5]. Thus it clearly appears critical to use a wide range of conventional cytotoxicity assays in order to determinate which of them are the most accurate to study a specific nanoparticle. We have decided to study the genotoxicity of two SiO$_2$ nanoparticles (25 and 100 nm) via five conventional cytotoxicity assays: Neutral Red, LDH release, XTT, MTT and SRB. To complete these classical approaches, we have adapted two methods based on flow cytometry and confocal microscopy to study i) DNA ploidy and cell mortality ii) DNA double strand breaks by the detection of phosphorylated γ-H2Ax-foci, together with the intracellular accumulation of nanoparticles. In order to
track the nanoparticles, we have used SiO$_2$-25 nm and SiO$_2$-100 nm dispersed nanoparticles labeled with Rhodamine B and TMPyP respectively, these two fluorophores being incorporated during synthesis.

2. Experimental

2.1. Cell Culture

Human Caki-1 cells (ATCC number: HTB-46™) and human HeK293 (ATCC number: CRL-1573™) were routinely grown at 37°C in a humidified atmosphere of 5% CO$_2$ and 95% air, in Dulbecco's Modified Eagle Medium (DMEM) Glutamax supplemented with 10% (v/v) inactivated fetal bovine serum (FBS) and 1mM Anti-Anti (InVitrogen).

2.2. Characterization of SiO$_2$ nanoparticles

2.2.1. Materials. Triton® X100 (TX-100), Igepal CO-520, 1-hexanol anhydrous ( ), cyclohexane reagent plus® ( ), aqueous ammonia (NH$_4$OH) solution (25%), tetraethylorthosilicate (TEOS, 98%), ethanol, Rhodamine B, 5,10,15,20-tetrakis(1-methyl-4-pyridino)porphyrin tetra(toluene-4-sulfonate) (TMPyP) all purchased from Aldrich, were used without further purification. Water was purified with a Milli-Q system (Millipore, Bedford, MA) including a SynergyPak® unit. The exclusive Jetpore®, ultrapure grade mixed-bed ion-exchange resin, was also used in this unit. Water achieved resistivity above 18.0 MΩcm at 25°C. A C 3.12 centrifuge (Jouan, France), and a Sonorex Digitec sonication water-bath (Roth, France) were used. Dialysis tubes have been purchased from Roth, and possess a nominal filter rating of 3500, and a MWCO of 4000-6000 D.

2.2.2. Synthesis of SiO$_2$ 25 nm. Silica nanoparticles were synthesized using a reverse micro-emulsion method, as described by Boudreau et al. in the literature [6]. To a clean 50 mL erlenmeyer, Igepal CO-520 (8 mL), cyclohexane (20 mL) and an aqueous solution or rhodamine B (650 µL at 0.01M) were mixed and stirred at room temperature to form a homogeneous micro-emulsion. After 10 minutes of equilibration, aqueous ammonia NH$_4$OH (35 µL) was introduced in the micro-emulsion as the catalyst in the synthesis of the silica shell. After another 10 minutes of equilibration, TEOS (60 µL) was added. After a 24 hours aging period, the nanoparticles were centrifuged (8000 g for 15 minutes) and washed several times with ethanol to remove unreacted and untrapped chemical species. Ultrasonification (35 Hz) was used in order to disperse nanoparticles aggregated into the washing solvent and to increase the desorption rate of surfactant from the surface of the synthesized nanoparticles. Then the sample is subjected to dialysis against deionised water for a week.

2.2.3. Synthesis of SiO$_2$ 100 nm. Silica nanoparticles were synthesized using a reverse micro-emulsion method, as described by Tan et al. in the literature [7]. Consequently a quaternary micro-emulsion consisted in mixing Triton X-100 (12.6 mL, 13.5 g, d = 1.07), 1-hexanol (12.3 mL), and cyclohexane (57 mL) under a vigorous stirring at room temperature, followed by additions of a concentrated aqueous solution of TMPyP dye in water (600 µL at 0.1M), water (1.2 mL), aqueous ammonia NH$_4$OH (725 µL at 25%) and, TEOS (725 µL) in that order. The mixture was allowed to stir for 24 h at room temperature and a subsequent addition of ethanol (100 mL) disrupted the inverse micelles. Particles were recovered by centrifugation (8000 g for 15 minutes) and washed thoroughly three times with ethanol and one time with water. Ultrasonification (35 Hz) was used in order to disperse nanoparticles aggregated into the washing solvent and to increase the desorption rate of surfactant from the surface of the synthesized nanoparticles. Then the sample is subjected to dialysis in deionised water for a week.

2.2.4. Transmission Electron Microscopy. The morphologies and sizes of dye-doped silica nanoparticles were characterized using a transmission electron microscope (JEOL 2000 FX). The
sample for transmission electron microscopy (TEM) were prepared by plunging a 200 mesh carbon-coated copper grid, 30-50 nm thickness, (Euromedex, France) in the desired nanoparticle-containing aqueous solution just after dispersion by ultra-sonification. Further to the evaporation of the water, the particles were observed at an operating voltage of 200 kV. Once the samples were imaged, TEM micrographs of dye-doped silica nanoparticles were converted to digitize images using imaging software (IMIX, PGT). Furthermore elemental analysis of the samples could be performed by Energy Dispersion RX Spectroscopy (EDS).

2.2.5. Particle Sizing. The hydrodynamic diameter and dispersivity of the silica nanoparticles were determined by Dynamic Light Scattering (DLS) technique using a Zetasizer Nano ZS from Malvern Instruments. The light scattering measurements were performed using a 633 nm red laser in a back-scattering geometry (θ = 180°). The particle size was analyzed using a dilute suspension of particles in deionized (or ultrapure) water and in culture media.

2.2.6. Fluorescence measurements. All fluorescence measurements were performed at room temperature on a steady-state FS920 spectrofluorimeter (Edinburgh Instruments, UK) with a high spectral resolution (signal to noise ratio > 6000:1), using water as the solvent, and a 1 cm cell, the latter oriented at 90° to the direction of the excitation light beam. The spectrofluorimeter covers the wavelength range from 200 to 1670 nm using two detectors: a photomultiplier R928 for UV-Vis scans (up to 870 nm) and a solid InGas TE G8605-23 detector for IR scans. The excitation source is a continuous Xenon Arc lamp (450 W) coupled to two Czerny-Turner DMX300X 1800 tr/mm monochromators, one for UV excitation (focal length 300 mm) and one for visible wavelength (focal length 500 mm). Fluorescence intensity values were integrated over the wavelength region specified. Data were recorded in a comparative manner, ca. using the same aperture of slits.

2.3. Cytotoxicity
Caki-1 and Hek293 human cells were exposed to SiO$_2$-25 and 100 nm at concentrations of 0; 0.1; 1; 10; 50 and 150 µg/mL for 24 h at 37°C in 96-well micro-plates. Cytotoxicity was evaluated according to the Borenfreund protocol [8] for Neutral Red protocol, with a LDH assay (Roche) according to the Decker protocol [9], for LDH release with a XTT assay (Sigma) according to the Scuderio protocol [10], with a MTT assay (Sigma) according to the Alley protocol [11], and with a SRB assay (Sigma) according to the Skehan protocol [12].

2.4. Flow Cytometry
After 24 h treatments, cells were washed and trypsinised for 5 minutes. Trypsine was inactivated by complete DMEM, cells were centrifuged for 5 minutes at 300 g and then resuspended in 500 µL of DMEM with serum in flow cytometry compatible tubes (BD 352058). Multi-parametric analyses were performed on BD Facsalibur using FlowJo 7.5.5 software. A first analysis was realized on size/granulometry to collect living and dead cells and to remove fragmented cells. Rhodamine-doped SiO$_2$-25 nm nanoparticles events are then collected on FL2 and TMPyP-doped SiO$_2$-100 nm on FL3, respectively corresponding to both optimal fluorescence emission. FL2 and FL3 signals are collected after excitation by Argon laser at 488 nm, between 564-606 nm and superior to 670 nm, respectively. To-Pro3 (Molecular Probes, Invitrogen) signal was collected on FL4 and was used for the analysis of cell viability since this dye requires a 635 nm red laser, which excitation is compatible without interference with equipment and nanoparticles detection. FL4 is collected between 656 and 667 nm after He-Ne laser excitation at 636 nm. The results are reported as the mean distribution of cell fluorescence, obtained on 3 replicas with at least 20 000 events per replica. Measurements are given on a bi-parametric representation divided in quarters and are associated to four different cell populations: living cells without nanoparticles (To-Pro3/Nanoparticles negative cells), dead cells with nanoparticles (To-Pro3 negative/Nanoparticles positive cells), dead cells with nanoparticles...
(ToPro3 positive/Nanoparticles positive cells) and dead cells without nanoparticles (ToPro3/Nanoparticles positive cells).

2.5. Genotoxicity

2.5.1. Phosphorylated-γH2Ax-foci. Thirty thousand cells were seeded on Lab-Tek™ II Chamber Slide™ 8 wells (Nunc) 24 hours before exposure and cells were treated for 24 hours with nanoparticles at doses of 10 and 50 µg/mL. After treatment, cells were washed and fixed for 15 minutes with PFA 4%, washed twice with 200 µL of 1X PBS and then permeabilized for 10 minutes at room temperature in [1X PBS and Triton 0.1%]. Due to the number of different dyes used in this method, we paid attention in the choice of dyes for DNA, actin and γH2Ax foci labelling to avoid any interference. As SiO₂ nanoparticles 25 and 100 nm are respectively labelled with Rhodamine B (λ<sub>ex</sub>: 557 nm, λ<sub>em</sub>: 577 nm) and TMPyP (λ<sub>ex</sub>: 420 nm, λ<sub>em</sub>: 660 nm), we labelled nuclei with Hoechst 33342 (λ<sub>ex</sub>: 345 nm, λ<sub>em</sub>: 480 nm), γH2Ax with Alexa Fluor® 488 (λ<sub>ex</sub>: 495 nm, λ<sub>em</sub>: 519 nm). Cells were blocked in [1X PBS, 0.025%, 10% of goat serum (Jackson ImmunoResearch)] for one hour at room temperature, then incubated for 75 minutes at room temperature with 1:500 of monoclonal γH2Ax antibody (Anti-phospho-Histone H2A.X (Ser139), clone JBW301, 05-636, Upstate Millipore), washed with 3×300 µL of [1X PBS, Triton 0.025%] and then incubated at room temperature for 45 min with 1:500 dilution of Alexa Fluor® 488 goat anti-mouse IgG (H+L) (Molecular Probes, InVitrogen) as a secondary antibody. Samples were washed with 3×300 µL of [1X PBS, Triton 0.025%] and cells were incubated at 37°C for 20 min with 200 µL of [1X PBS and Hoechst 33342 at 0.1 µg/mL (Molecular Probes, InVitrogen)]. Finally, Lab-Tek™ Chamber Slide™ 8 wells were washed with 3×300 µL of [1X PBS, Triton 0.025%] before mounting in ProLong® Gold antifade reagent (Molecular Probes, InVitrogen) in order to proceed for confocal microscopy visualization.

2.5.2. Confocal microscopy. Fixed and labeled cells were photographed with a 40x PlanApo under a fluorescence confocal microscope (Leica TCS SP2, Wetzlar, Germany) equipped with lasers at 364 nm (Hoechst and TMPyP labelled SiO₂-100 nm nanoparticles), 488 nm (Alexa fluor 488) and 543 nm (Rhodamine B labelled SiO₂-25 nm). Hoechst signal was collected between 410-450 nm, γH2Ax foci signal between 500-540 nm while SiO₂-25 nm and 100 nm signals were collected between 540-570 nm and 670-725 nm, respectively. Each analysis was made on at least 100 cells and at least three images of each condition were analyzed. Confocal microscopy optical slice sections of 8 to 20 µm were made from the luminal to the basal pole of the cells, each acquisition containing nine stacks. Cell Profiler software [13] was used for the detection of foci and scoring in Alexa Fluor images.

2.5.3. Statistical analysis. To test whether the basal number of γH2Ax-foci observed in control cells was significantly different from that observed in nanoparticle exposed-cells, a Wilcoxon rank test based on at least 100 observations for each condition was performed.

3. Results

Among the five cytotoxicity assays which were used in this study, no interferences between the reading wavelengths and the intrinsic properties of both nanoparticles were observed. Even though Caki-1 and Hek293 human cell lines derivate from the same localization (proximal tubule), their responses after of SiO₂-nanoparticles exposure are different. The cytotoxicity study assessed on both cell lines has shown that Caki-1 cells are more sensitive than Hek293 cells after a 24 hours of exposure to SiO₂-25 nm nanoparticles (figure 1).
Figure 1. Cytotoxicity of SiO$_2$-25 nm and SiO$_2$-100 nm nanoparticles on Caki-1 and Hek293 human cell lines exposed for 24 hours: Neutral Red (1), LDH release (2), MTT (3), XTT (4) and SRB (5).
A. 24 hours of exposure to SiO$_2$-25 nm

![Flow cytometry analysis of Caki-1 control cells and cells exposed for 24 hours to SiO$_2$-25 nm (A) and SiO$_2$-100 nm (B) nanoparticles. On each series of three images, the first one correspond to the FSC vs SSC signal, the second to FL2 vs number of events for SiO$_2$-25 nm or FL3 vs number of events for SiO$_2$-100 nm, and the last one correspond to FL4 vs FL2 for SiO$_2$-25 nm or FL4 vs FL3 for SiO$_2$-100 nm.](image)

B. 24 hours of exposure to SiO$_2$-100 nm

![Flow cytometry analysis of Caki-1 control cells and cells exposed for 24 hours to SiO$_2$-25 nm (A) and SiO$_2$-100 nm (B) nanoparticles. On each series of three images, the first one correspond to the FSC vs SSC signal, the second to FL2 vs number of events for SiO$_2$-25 nm or FL3 vs number of events for SiO$_2$-100 nm, and the last one correspond to FL4 vs FL2 for SiO$_2$-25 nm or FL4 vs FL3 for SiO$_2$-100 nm.](image)
A. 24 hours of exposure to SiO$_2$-25 nm

B. 24 hours of exposure to SiO$_2$-100 nm

**Figure 3.** Flow cytometry analysis of Hek293 control cells and cells exposed for 24 hours to SiO$_2$-25 nm (A) and SiO$_2$-100 nm (B) nanoparticles. On each series of three images, the first one correspond to the FSC vs SSC signal, the second to FL2 vs number of events for SiO$_2$-25 nm or FL3 vs number of events for SiO$_2$-100 nm, and the last one correspond to FL4 vs FL2 for SiO$_2$-25 nm or FL4 vs FL3 for SiO$_2$-100 nm.
Moreover this result is confirmed with the five other cytotoxicity assays: LDH activity (reported by LDH release), the mitochondrial activity (reported by MTT and XTT) and the metabolic activity (reported by SRB) (figure 1). On the other hand, similar experiments done after a 24 hours of exposure to SiO$_2$-100 nm have not shown a significant increase of cytotoxicity (figure 1) when comparing the two cell lines. This cytotoxicity study was extended by flow cytometry analysis, which allows to distinguish dead cells and living cells. Moreover, flow cytometry is a powerful and very sensitive approach to visualize incorporation of traceable nano-objects. Recent studies [14-16] have suggested that flow cytometry is of interest with regard to incorporation of nanoparticles of environmental particles and with regards to light scattering analysis. We propose an analysis of the nanoparticle incorporation, not based on light scattering but on the direct fluorescence emitted by nanoparticles. Indeed flow cytometry allows bi-parametric analysis using multi-channel detection which permits coupling the viability data to the presence of nanoparticles. Based on that principle, flow cytometry was performed upon Caki-1 and Hek293 human cells exposed to SiO$_2$-25 and 100 nm nanoparticles using a bi-parametric analysis: specific fluorochrome nanoparticles detection (FL2 channel for SiO$_2$-25 nm and FL3 channel for SiO$_2$-100 nm) and mortality evaluation (FL4 channel with ToPro-3).

Figure 2 shows that Caki-1 cells exposed for 24 hours to SiO$_2$-25 or 100 nm do not present the same profile. Caki-1 cells mostly die without detectable intracellular nanoparticles at 24 hours after exposure to 50 µg/mL of SiO$_2$-25 nm (figure 2.A), while at the same time, Hek293 cells are mostly living with detectable intracellular nanoparticles after an exposure to 50 µg/mL of SiO$_2$-100 nm (figure 3.B). These kinetics differences of nanoparticles penetration are depicted on figure 2, which reports an absence of gap in the FL2 channel for Caki-1 cells exposed to 50 µg/mL of SiO$_2$-25 nm nanoparticles compared to the signal obtained in non-exposed cells (figure 2.A. and figure 3.A.). On the other hand, a high gap in the FL3 channel was observed for the two cell lines exposed to 50 µg/mL of SiO$_2$-100 nm (figure 2.B. and figure 3.B.). A second bi-parametric analysis, which split up the signal into FL2 vs FL4 (for SiO$_2$-25 nm) and into FL3 vs FL4 (for SiO$_2$-100 nm), allowed us i) to directly correlate the absence of gap on the FL2 channel compared to control cells to the poor level of SiO$_2$-25 nm incorporation and to the high level of cell mortality, especially for Caki-1 cells ii) to directly correlate the high gap on the FL3 channel compared to control cells to the high level of SiO$_2$-100 nm incorporation in exposed cells, especially for Hek293 (figure 2.B and Figure 3.B.).

In order to investigate the genotoxicity of these two nanoparticles, we have adapted a second method based on phosphorylated γ-H2Ax-foci detection by confocal microscopy. The two tested concentrations for both nanoparticles and cell lines show a highly significant difference between the numbers of γ-H2Ax-foci observed in controls cells compared to those observed in exposed-cells (figure 4; p < 0.001 for all conditions excepted for Hek293 exposed to 10 µg/mL of SiO$_2$-100 nm for which p < 0.05). Furthermore, the two human cell lines used in this study, do not present the same sensitivity in terms of DNA breaks: Caki-1 cell line seems to be once again more sensitive for an exposure to SiO$_2$-nanoparticles (higher Med values observed in Caki-1 cells) than Hek293 cells for the two concentrations and for both sizes of nanoparticles. Contrary to the cytotoxicity data (where no significant differences were observed between the two cell lines for SiO$_2$-100 nm nanoparticles (Figure 4), the number of γ-H2Ax-foci indicates that the sizes of nanoparticles are more genotoxic for Caki-1 cells than for Hek293 cells (figure 4). Moreover, the data obtained for Caki-1 exposed to SiO$_2$-25 nm nanoparticles seem to suggest a dose response (Med = 9 foci per nuclei and Med = 18, for 10 µg/mL and 50 µg/mL, respectively). To confirm this point, it could be interesting to do same experiments using larger range of concentrations.
4. Conclusions

Overall, no interference was detected between labeled SiO$_2$ nanoparticles and conventional toxicological assays used in our study. Furthermore the two adapted confocal microscopy and flow cytometry methods, used to track intracellular nanoparticles together with the analysis of cell survival and genotoxicity, are very useful techniques.

Our results indicated that SiO$_2$-25 nm nanoparticles are more cytotoxic and genotoxic than SiO$_2$-100 nm on both tested cell lines. On the other hand, Caki-1 cells are more sensitive to both nanoparticles than Hek293 cells, which are able to survive with intracellular nanoparticles.

More generally, these approaches could be routinely applied to others cell lines and to all others nanoparticles in mass dye labeled during synthesis.

Figure 4. Number of H2Ax-foci per nuclei in Caki-1 and Hek293 cells exposed for 24 hours to SiO$_2$-25 nm and 100 nm nanoparticles at 10 µg/mL and 50 µg/mL compared to the control.

(Statistical analysis: Wilcoxon rank test: *: p < 0.05; ***: p < 0.001; Max: maximum; Min: minimum; Med: median; 75 per: 75$^{th}$ percentile; 25 per: 25$^{th}$ percentile).

Example of γ-H2Ax-foci in Hek293 treated 24 hours with 50 µg/mL of SiO$_2$-25 nm
Acknowledgements
This work was supported by the NanoSciences CEA-Transverse program and C’Nano Ile-de-France.

References
[1] Vega-Villa KR, Takemoto JK, Yáñez JA, Remsberg CM, Forrest ML, Davies NM. (2008) Clinical toxicities of nanocarrier systems. Adv Drug Deliv Rev. 60(8): 929-38
[2] Sung, J.C., B.L. Pulliam, D.A. Edwards. (2007) Nanoparticles for drug delivery to the lungs. Trends Biotechnol. 25(12): 563-70
[3] Kahru A, Dubourguier HC. (2010) From ecotoxicology to nanocotoxicology. Toxicology 269(2-3): 105-19
[4] Fadeel B, Garcia-Bennett AE. (2010) Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Adv Drug Deliv Rev. 62(3): 362-74
[5] Laaksonen T, Santos H, Vihola H, Salonen J, Riikonen J, Heikkilä T, Peltonen L, Kumar N, Murzin DY, Lehto VP, Hirvonen J. (2007) Failure of MTT as a toxicity testing agent for mesoporous silicon microparticles. Chem Res Toxicol. 20(12): 1913-18
[6] Viger ML, Live LS, Therrien OD, Boudreau D. (2008) Reduction of self-quenching in fluorescent silica-coated silver nanoparticles. Plasmonics 3(1): 33-40
[7] Bagwe RP, Yang C, Hilliard LR, Tan W. (2004) Optimization of Dye-Doped Silica Nanoparticles Prepared Using a Reverse Microemulsion Method. Langmuir 20: 8336-42
[8] Borenfreund E, Puerner JA (1985) Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicol Lett. 24(2-3): 119-24
[9] Decker T, Lohmann-Matthes ML. (1988) A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J Immunol Methods 115(1): 61-69
[10] Scudiero DA, Shoemaker RH, Paull KD, Monks A, Tierney S, Nofziger TH, Currens, Seniff D, Boyd MR. (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines, Cancer Res. 48 (1988) 4827-33
[11] Alley MC, Scudiere DA, Monks A, Czerwinski M, Shoemaker RH, Boyd MR. (1986) Validation of an automated microculture tetrazolium assay (MTA) to assess growth and drug sensitivity of human tumor cell lines, Proc. Am. Assoc. Cancer Res. 27: 389
[12] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82: 1107-12
[13] Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang JH, Lindquist RA, Moffat J, Golland P, Sabatini DM. (2006) CellProfiler: image analysis software for identifying and quantifying cell phenotypes. Genome Biology 7: R100
[14] Zucker RM, Massaro EJ, Sanders KM, Dgn LL, Boyes WK. (2010) Detection of TiO2 nanoparticles in cells by flow cytometry. Cytometry 77(7): 677-85
[15] Stringer B, Inrich A, Kobzik L. (1995) Flow cytometric assay of lung macrophage uptake of environmental particulates. Cytometry 20(1): 23-32
[16] Suzuki H, Toyooka T, Ibuki Y. (2007) Simple and easy method to evaluate uptake potential of nanoparticles in mammalian cells using a flow cytometric light scatter analysis. Environ Sci Technol. 41(8): 3018-24.