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A new synthesis pathway for colloidal silica spheres coated with crystalline titanium oxide and its comparative cyto- and genotoxic study with titanium oxide nanoparticles in rat osteosarcoma (UMR106) cells

A.L. Di Virgilio [a], I. Maisuls [b], F. Kleitz [c], P.M. Arnal [b,*]

[a] CEQUINOR and Cátedra de Bioquímica Patológica, Facultad de Ciencias Exactas, UNLP. 47 y 115 (1900) La Plata, Argentina
[b] CETMIC (Centro de Tecnología de Recursos Minerales y Cerámica), Cno Centenario y 506, CC 49 (B1897ZCA), M.B. Gonnet, Prov. Buenos Aires, Argentina
[c] Department of Chemistry and Centre de Recherche Sur les Matériaux Avancés (CERMA), Université Laval, Quebec City, QC, Canada G1V 0A6

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ABSTRACT

Spherical particles with an amorphous core of silica and a crystalline shell of titanium oxide (SiO_2@TiO_2) formed in a three-step procedure, being the last step a mild chemical treatment. SiO_2@TiO_2 had a shell with pores (micro and mesopores) permeating between TiO_2 nanocrystals (anatase) and a solid core of amorphous silica. The spheres had an outstanding specific surface area (300 $m^2\,g^{-1}$). A cyto- and genotoxic study of SiO_2@TiO_2 and titanium oxide nanoparticles (TiO_2-NP) on UMR106 cells with 24 h exposure showed that SiO_2@TiO_2 colloidal particles were less toxic than TiO_2-NP.

1. Introduction

Fine particles with a characteristic size smaller than 100 nm (i.e., nanoparticles) as well as fine particles with a characteristic size within the range 100 nm–1 μm (i.e., colloidal particles) spread out in nowadays life. They constitute commercially available products such as cosmetics, participate in medical diagnostics and treatments, and are used in agriculture.

Among them stands out a group, which common characteristic is to have both one material at the core and another material at the shell (i.e., core@shell particles). These core@shell particles attracted the interests of the scientific community, because of their potential biomedical applications in drug delivery and bioimaging as well as in bioelectronics and water cleaning, among others [1–5].

Core@shell particles having an amorphous, solid core of SiO_2 and a crystalline, porous shell of TiO_2 (i.e., SiO_2@TiO_2) are interesting candidates for removing organic contaminants from water. One reason is that titanium oxide (TiO_2) is a well-known photo-catalyst for decomposing organic molecules present in water [6]. Many organic compounds polluting water can be converted into CO_2 with TiO_2 and UV light [7]. Furthermore, SiO_2@TiO_2 particles with crystalline shell have been investigated, for instance, as photocatalysts for the photodegradation of Rhodamine B [8] and methylene blue [9]. A second reason is that colloidal SiO_2@TiO_2 particles may be less harmful to biological systems than TiO_2 nanoparticles. At any given composition, nanoparticles may be more harmful to biological systems than granular material with sizes somewhere above the nano-domain up to sands (i.e., particles with diameters somewhere between 100 nm and 100 μm) [10–14]. Moreover, harm caused by nanoparticles may depend on their size as well as on their shape and surface functionality suggesting that the interaction between surfaces of nanomaterials and cells may play a key role regarding their toxic effects [15]. Even titanium dioxide, long considered to be biologically inert [16,17], rose more recently serious concerns, because of its potential risks when present in the form of nanoparticles [18–21].

SiO_2@TiO_2 has been synthesized using SiO_2 spheres as templates by diverse pathways. One synthesis pathway proceeded via heterocoagulation of silica spheres and titania nanosol [8]. Another one built SiO_2@TiO_2 particles via layer-by-layer self-assembly of cationic polyelectrolyte and anionic titania nanosheets [22]. In a third approach, SiO_2@TiO_2 particles formed after mixing $Ti(OCH_2CH_3)_4$ dissolved in ethanol and silica spheres in a solution of water, ethanol, and hydroxipropyl cellulose [23]. In a different procedure, silica

* Corresponding author. Fax: +54 221 471 00 75.
E-mail address: arnal@cetmic.unlp.edu.ar (P.M. Arnal).

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spheres and $Ti(OCH_2CH_2CH_2CH_3)_4$ were first dispersed in an aqueous ethanol solution, then water and ammonium hydroxide were added to the suspension by continuous feeding for about 4 h, the colloid was refluxed for 1.5 h and finally stirred for another 1.5 h to form $SiO_2@TiO_2$ particles. While in the first synthesis pathway crystallization of TiO_2 occurred before the synthesis of core@shell, in all other synthesis pathways crystallization of titanium oxide was brought about by thermal treatment a posteriori.

With regard to their biocompatibility, both nano and colloidal core@shell particles have been evaluated with different biological systems both *in vitro* and *in vivo*. The toxicity of nanoparticles has been analyzed using different cell lines. $AlCl_3$ nanoparticles increased micronuclei [24] and damaged DNA [25]. TiO_2 nanoparticles induced sister chromatid exchanges and micronuclei in Chinese hamster ovary—K1 cells [26]. Silver nanoparticles provoked cyto- and genotoxic damage in human mesenchymal stem cells. Comet assay and chromosomal aberration tests showed DNA damage at concentrations as low as 0.1 ppm [27]. Finally, reduced graphene oxide nanoplatelets produced genotoxic effects through DNA fragmentation and chromosomal aberrations in the same cells [28].

Strict comparison of different *in vitro* and *in vivo* studies of core@shell particles is difficult, because of the formation of a "protein corona" at the particle's surface [29,30]. A protein corona forms by adsorption of biomolecules such as proteins and lipids. It usually contains 10–50 proteins that have the highest affinity for the surface, while several thousand proteins are present in human biological fluids. Its formation is a complex dynamic process that depends not only on physicochemical properties of the solid particles, but also on the composition of the biofluid they are immersed in. Hence, biocompatibility ought to evaluate on a case-by-case basis.

$SiO_2@TiO_2$ particles stand out from other materials as a candidate in photocatalysis. However, $SiO_2@TiO_2$ synthesized so far had relatively low specific surface areas, and their biocompatibility was not reported. Hence, this work presents a new synthesis pathway to obtain silica spheres coated with TiO_2 nanocrystals (anatase) having a specific surface area around $300\ m^2.g^{-1}$. In addition, this work presents a comparative study on the toxicological effects with TiO_2 nanoparticles (anatase) in osteoblast-like cells (UMR106).

2. Experimental

2.1. Materials

This work used following materials and chemicals. *Synthesis of $SiO_2@TiO_2$*. Lutensol AO5 solution was prepared with 11.01 g MQ water and 0.45 g Lutensol AO5. Absolute ethanol, ammonia solution in water, distilled water, tetrabutyl ortotitanate, concentrated HCl, and tetraethyl ortosilicate (*TEOS*). TiO_2-NP (anatase) was purchased from Aldrich (Milwaukee, WI, USA). *Biocompatibility studies*. Tissue culture materials were purchased from Trading New Technologies (Buenos Aires, Argentina). Dulbecco's Modified Eagles Medium (DMEM) was purchased from GBO Argentina, fetal bovine serum (FBS) from Internegocios SA (Buenos Aires, Argentina); trypsin-EDTA was provided by Gibco (Gaithersburg, Md, USA); MTT, Neutral Red dye, Trypan Blue and cytochalasin B from *Dreschslera dematioidea* were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dihydrorhodamine 123 (DHR) was from Molecular Probes (Eugene, OR, USA). Bleomycin (*BLM*) (Blocamycin®) was kindly provided by Gador S.A. (Buenos Aires, Argentina). Syber Green and Low melting point agarose were purchased from Invitrogen Corporation (Buenos Aires, Argentina). Stock suspensions of TiO_2-NP and $SiO_2@TiO_2$ were prepared in phosphate-buffered saline (PBS), vortexed for 10 min, and stored at 4 °C in the dark. After preparation, test dispersions were immediately used by diluting with *Dulbecco's Modified Eagle Medium (DMEM)*.

2.2. Synthesis of $SiO_2@TiO_2$

Spherical particles with an amorphous, solid core of SiO_2 and a porous, polycrystalline shell of TiO_2 formed in a three-step procedure. The first step yielded silica spheres, the second step produced sphere of silica covered with a layer of amorphous titanium oxide, and the last step crystallized the titanium oxide in the shell with a mild chemical treatment. Synthesis of silica spheres with a narrow distribution in size followed as described in an earlier contribution [31]. Formation of an homogenously thick layer of titanium oxide followed likewise as previously described [32]. We adapted a procedure developed for amorphous gels of TiO_2 to crystallize the shell [33]. Thus, we obtained core@shell spheres with an amorphous, solid core of SiO_2 and a crystalline, porous shell of TiO_2 ($SiO_2@TiO_2$).

The shell crystallized with a mild chemical treatment. First, we poured 16.62 g of ethanol and then 0.083 g of HCl 36.5–38.0% from a glass beaker into a clean round-bottom flask (200 mL, one neck) in a fume hood and closed the flask with a septum. Then, we added 0.500 ± 0.001 g of silica spheres covered with amorphous TiO_2, a magnetic stir bar, and closed again the flask with the septum. We immersed the closed flask in an oil bath until the liquid surface in the flask remained below the liquid surface of the oil. After inserting a metallic needle (outer diameter 2 mm) through the septum, we turned on the heating plate. The temperature in the oil bath rose to 100.0 ± 0.1 °C under slow magnetic stirring. After reaching 100.0 ± 0.1 °C, the flask remained at this temperature 12 h. During this time, the liquid evaporated through the needle and a pale yellow solid appeared inside the flask. Then, we removed the flask from the oil bath and let it cool down to room temperature. After opening the flask, we removed the solid inside from the inner glass wall with a metallic spatula, collected it over a paper sheet, and finally swept it into a glass vial, which we closed with a screw lid.

2.3. Physicochemical characterization of nano- and core@shell-particles

We determined crystalline phases in core@shell spheres with X-ray diffraction (XRD), and final shape and mean particle size of core@shell particles, as well as the remaining shells after selectively dissolving the cores from $SiO_2@TiO_2$ with Scanning Electron Microscopy (SEM). We determined shape and mean particle size of TiO_2-NP from TEM-images and obtained nitrogen adsorption–desorption isotherms at 77 K from samples previously activated under vacuum at 423 K for at least 2 h. We calculated specific surface areas with the Brunauer–Emmett–Teller (BET) method.

2.4. Cell culture

Rat osteosarcoma-derived cells (UMR106) were originally obtained from the American Type Culture Collection (ATCC, CRL 1661, Rockville, MD, USA). Cells were grown as monolayers with DMEM culture medium supplemented with 10% inactivated fetal calf serum, 50 UI mL^{-1} of penicillin and 50 ppm of streptomycin sulfate in a humidified incubator at 37 °C and 5% CO_2 atmosphere. Cells were subcultured using 0.25% trypsin, 1 mM EDTA in phosphate-buffered saline.

2.5. Biocompatibility assays

2.5.1. Cell viability assay

After treatment with different concentrations of TiO_2-NP or $SiO_2@TiO_2$ for 24 h, cells were detached with trypsin and counted