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Toxicological assessment of mesoporous silica particles in the nematode *Caenorhabditis elegans*

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ABSTRACT

Here we report the toxicological evaluation of mesoporous silica particles (MSPs) in the nematode *C. elegans*. Specifically, we have investigated the effect of bare micro- (**M0**) and nano-sized (**N0**) MSPs, and their corresponding functionalized particles with a starch derivative (**Glu-N**) (**M1** and **N1**, respectively) on *C. elegans* ageing parameters. The toxicity of MSPs, their impact on *C. elegans* lifespan, movement capacity, progeny and ability to survive upon exposure to acute oxidative stress were assessed. This study demonstrated that both size particles assayed (**M0** and **N0**), labeled with rhodamine and monitored through fluorescence microscopy, are ingested by the nematode. Moreover, toxicity assays indicated that bare nano-sized particles (**N0**) have a negative impact on the *C. elegans* lifespan, reducing mobility and progeny production. By contrast, micro-sized particles (**M0**) proved innocuous for the nematodes. Furthermore, functionalization of nanoparticles with starch derivative reduced their toxicity in *C. elegans*. Thus, oral intake of **N1** comparatively increased the mean lifespan and activity rates as well as resistance to oxidative stress. The overall findings presented here demonstrate the influence of MSP size and surface on their potential toxicity *in vivo* and indicate the silica-based mesoporous particles to be a potential support for encapsulation in oral delivery applications. Furthermore, the good correlation obtained between healthy aging variables and viability (mean lifespan) validates the use of *C. elegans* as a multicellular organism for nanotoxicology studies of MSPs.

1. Introduction

In recent years, inorganic nanomaterials have gained appeal as suitable supports for delivery applications (Mo et al., 2014). Among inorganic supports for encapsulation and controlled release, mesoporous silica particles (MSPs) have received great interest (Valtchev and Tosheva, 2013; Stein, 2003; Soler-Illia and Azzaroni, 2011; Angelos et al., 2007). MSPs have tunable and homogeneous pore size distribution (in the 2–10 nm diameter range), and high specific surface area and volume, which provide a large loading capacity (Salonen and Lehto, 2008; Wight and Davis, 2002). Apart from being a porous structure, MSPs stand out for exhibiting a high concentration of structural defects on their surface in the form of silanol (Si-OH) groups, which can easily react with trialkoxysilane derivatives ((R′O)3-Si-R), enabling the generation of organic−inorganic hybrid supports (Vinu et al., 2005; Kickelbick, 2004). This strategy offers a wide range of new perspectives in the design of on-command release particles to control the delivery of a previously entrapped guest (Angelos et al., 2007; Coll et al., 2013; Aznar et al., 2016; Sancenón et al., 2015). In accordance with this concept, the literature reports examples of MSPs functionalized with a number of different molecules and biomolecules able to deliver the cargo upon the application of various stimuli, such as physical (light, temperature, magnetic fields, ultrasounds) (Mal et al., 2003; Agostini et al., 2012; Fu et al., 2003; Aznar et al., 2011; Giri et al., 2005), chemical (anions, cations, neutral molecules, redox-active species and pH) (Fujiwara et al., 2006; Angelos et al., 2009; Casasús et al., 2008) and biochemical (enzymes, DNA and antibodies) (Oroval et al., 2013; Schlossbauer et al., 2009; Bernardos et al., 2010; Park et al., 2009). However, in spite of the promising applicability of MSPs, their toxic effect after oral administration is still poorly understood.

Among the biological models available, the nematode *Caenorhabditis elegans* has emerged as a well-suited *in vivo* model for

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toxicological studies owing to its established biology and readily scorable life traits. *C. elegans* is a multicellular organism with a short lifespan (21 days). In addition, experiments with *C. elegans* are less expensive than those carried out with vertebrate models and allow for a wide set of tests under different conditions in a short time span (The *C. elegans* Sequencing Consortium, 1998). Moreover, research reports that results obtained with *C. elegans* can be predictive of those in higher eukaryotes because many physiological processes, signal transduction pathways and genes are conserved (Leung et al., 2008). In addition, quantitative parameters of toxic effects on *C. elegans* can be easily determined through progeny production, mortality (lifespan), sensitivity to oxidative stress and changes in movement capacity (healthy aging evaluation). These features have led to an increase in the use of *C. elegans* as a suitable model in toxicological studies. Thus, recent toxicological studies with nanomaterials have been carried out in *C. elegans* (Cha et al., 2012; Wang et al., 2009; Gonzalez-Moragas et al., 2015). Nonetheless, very few studies have been reported with *C. elegans* and silica-based particles. In particular, amorphous (non-porous) silica nanoparticles have been evaluated (Pluskota et al., 2009; Scharf et al., 2013). Our results suggest that non-porous silica nanoparticles (smaller than 50 nm) induce premature aging, causing progeny reduction and alterations in phenotypes related to aging. However, studies with *C. elegans* and MSPs are lacking, and there is an absence of correlation studies of lifespan and healthspan of nematodes fed with MSPs.

Taking into account the increasing interest in the design and use of mesoporous silica particles for delivery applications, we report herein the evaluation of toxicity of nano- and micro-sized MSPs based on *C. elegans* lifespan and healthspan analysis (movement capacity, resistance to acute oxidative stress and offspring production). Moreover, we studied the impact of functionalization of particles. The results show that surface functionalization of MSPs is a suitable procedure to significantly reduce the toxicity of nano-sized particles.

2. Materials and methods

2.1. Chemicals

All the chemicals were purchased at the highest possible grade available and were directly used with no further purification. Chemicals tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTABr), sodium hydroxide, triethanolamine (TEAH), (3-aminopropyl) triethoxysilane (APTES) were provided by Aldrich. Hydrolyzed starch Glucidex® 47 (5% glucose, 50% maltose, 45% oligosaccharides and polysaccharides) was provided by Roquette.

2.2. C. elegans strain and maintenance

C. elegans strain Bristol (wild-type) N2 was obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota and was maintained at 20 °C on nematode growth medium (NGM). Also the *Escherichia coli* OP50 strain used as a normal diet for nematodes was obtained from the same culture collection.

2.3. Synthesis of micro-sized mesoporous silica particles (M0)

Micro-sized mesoporous silica particles were synthesized by the "atrane route" (Cabrera et al., 2000) in which 4.68 g of CTABr were added at 118 °C to a TEAH solution (25.79 g) that contained 0.045 mol of a silatrane derivative (TEOS, 11 mL). Next 80 mL of water were slowly added with vigorous stirring at 70 °C. After a few minutes, a white suspension was formed. This mixture was aged at room temperature overnight. The resulting powder (as- synthesized material) was collected by filtration and washed. The solid was dried at 70 °C and was finally calcined at 550 °C for 5 h in an oxidant atmosphere in order to remove the template phase.

2.4. Synthesis of nano-sized mesoporous silica-based particles (N0)

Nano-sized mesoporous silica particles were synthesized by the following procedure. Cetyltrimethylammoniumbromide (CTABr, 1.00 g, 2.74 mmol) was first dissolved in 480 mL of deionized water. Then 3.5 mL of a NaOH 2.00 mol L^{-1} solution were added, followed by an adjustment of temperature to 80 °C. TEOS (5.00 mL, 22.4 mmol) was then added dropwise to the surfactant solution. The mixture was stirred for 2 h to give a white precipitate. Finally, the solid was collected by centrifugation, washed with deionized water and dried at 70 °C overnight (as-synthesized material). To prepare the final mesoporous nanoparticles (**N0**), the as-synthesized solid was calcined at 550 °C in an oxidant atmosphere for 5 h to remove the template phase.

2.5. Synthesis of the starch derivative (Glu-N)

A solution of APTES (5.85 mL, 25 mmol) was added to a suspension of hydrolyzed starch (Glucidex@ 47) in ethanol (Bernardos et al., 2010). The reaction mixture was stirred for 24 h at room temperature and heated at 60 °C for 30 min. The solvent was evaporated under reduced pressure (Bernardos et al., 2010).

2.6. Synthesis of starch-functionalized mesoporous silica particles (M1 and N1)

To prepare the starch-functionalized mesoporous silica particles **M1** and **N1**, **Glu-N** was added to **M0** and **N0** in a 1:1 w/w ratio at aqueous solution. The final mixture was stirred for 5.5 h at room temperature under argon. The solid was filtered, washed with abundant deionized water and dried for 12 h at 35 °C.

2.7. Synthesis of labeled particles (M0-rhd and N0-rhd)

Particles **M0** and **N0** were labeled with rhodamine B using a similar procedure to that reported by Xu and coworkers (Xu et al., 2014). First the solid surface was modified with APTES. For this purpose, **M0** or **N0** nanoparticles were suspended in toluene (30 mL) and 0.19 mL of APTES (0.8 mmol) were added. The final suspension was refluxed at 110 °C for 20 h. Afterwards, 50 mg of the corresponding solid was suspended in ethanol with 50 mg B rhodamine isothiocyanate (RITC) for 20 h to obtain **M0-rhd** and **N0-rhd**.

Finally, ethanol suspensions were filtered and solids were washed with abundant deionized water, and dried for 12 h at 35 °C.

2.8. Material characterization

PXRD measurements were taken on a Seifert 3000TT diffractometer using CuKα radiation. TEM images were obtained under a 100 kV Philips CM10 microscope. Thermogravimetric analyses were carried out on a TGA/SDTA 851e Mettler Toledo balance in an oxidant atmosphere (air, 80 mL min^{-1}) with a heating program that consisted of a heating ramp of 10 °C per minute from 120° to 1000 °C, and an isothermal heating step at this temperature for 30 min.

N_2 adsorption-desorption isotherms were recorded in a Micromeritics ASAP2010 automated sorption analyzer. Samples were degassed at 120 °C in vacuum overnight. The specific surface areas were calculated from the adsorption data within the low pressure range using the BET model (Brunauer et al., 1938).

Dynamic light scattering (DLS) studies for size distribution were conducted at 25 °C using a Malvern Zetasizer Nano ZS and Malvern Mastersizer 2000 (Malvern, U.K.). Data analysis was based on the Mie theory using refractive indices of 1.33 and 1.45 for the dispersant and MSP, respectively. To determine the zeta potential (ζ) of bare and functionalized MSP, a Zetasizer Nano ZS (Malvern Instruments, U.K.) was used. Zeta potential was calculated from the particle mobility values by applying the Smoluchowski model. The average of five