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Silica nanoparticles: Preparation, characterization and *in vitro*/*in vivo* biodistribution studies

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ABSTRACT

Background: The current progress in pharmaceutical nanotechnology field has been exploited in the design of functionalized radiolabelled nanoparticles that are able to deliver radionuclides in a selective manner to improve the outcome of diagnosis and treatment. Silica nanoparticles (SNPs) have been widely developed for biomedical applications due to their high versatility, excellent functional properties and low cost production, with the possibility to control different topological parameters relevant for multidisciplinary applications.
Purpose: The aim of the present study was to characterize and evaluate both *in vitro*, by microscopy techniques, and *in vivo*, by scintigraphic imaging, the biodistribution of silica nanostructures derivatives (Cy5.5 conjugated SNPs and ^{99m}Tc radiolabelled SNPs) to be applied as radiotracers in biomedicine.
Methods: SNPs were synthesized by hydrolysis and condensation of silicon alkoxides, followed by surface functionalization with amino groups available for fluorescent dye and radiolabelling possibility.
Results: Our data showed the particles size distribution (200–350 nm), the surface charge (negative for bare and fluorescent SNPs and positive for amino SNPs), polydispersity index (broad distribution), the qualitative composition and the toxicity assessments (safe material) that made the obtained SNPs candidates for *in vitro*/*in vivo* studies. A high uptake of fluorescent SNPs in all the investigated organs was evidenced by confocal microscopy. The ^{99m}Tc radiolabelled SNPs biodistribution was quantified in the range of 12–100% counts/g organ using the scintigraphic images.
Conclusions: The obtained results reveal improved properties, namely, reduced toxicity with a low level of side effects, an improved biodistribution, high labelling efficiency and stability of the radiolabelled SNPs with potential to be applied in biomedical science, particularly in nuclear medicine as a radiotracer.

1. Introduction

In the last period, the growing interest in molecular therapy is due to the possibilities offered by nanoparticles (NPs) as new tools for the delivery of therapeutic and target-specific drugs (Morales-Avila et al., 2012). Multifunctional radiolabelled nanoparticles provide an ideal platform to combine different approaches such as drug delivery with functional imaging techniques and used to target the site of the disease via both specific and non-specific mechanisms (Hong et al., 2009). Thus, novel strategies were explored for the radiolabelling of NPs in order to investigate the *in vivo* biodistribution in dependence with their architecture, size and structure (Md et al., 2013). In order to investigate the *in vivo* characteristics of NPs it has to be considered how they are interacting with tissues and cells, and especially which time frame allows a suitable visualization of certain effects and functions (Loudos et al., 2011). The necessity of creating radiolabelled nanocarriers is noticeable in all biomedical fields worldwide. Their fundament is anticipated to lead to advancements in understanding biological processes at the molecular level in addition to progress in the development of diagnostic tools and innovative therapies (Peer et al., 2007). Traditional radioisotope agents have certain disadvantages (instability, lack of specificity, low biodistribution, etc.) that must be improved by using new multifunctional radiolabelled structures (Garg et al., 2008; Newman et al., 2003).

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Nanoparticles provide a large surface area and different types of functional groups available for bioactive agents or ligands attachment. Diverse nanomaterials with unique properties can be found in various biomedical applications, including *in vitro* or *in vivo* imaging, separation and purification of cells or biomolecules, and delivery of therapeutic agents. In the last two decades silica-based nanoparticles (SNPs) have gained increasing interest for medical applications because of their biocompatibility, versatility, stability, monodispersity, large surface area, high drug loading efficiency, and potential for hybridization with other materials (Argyo et al., 2014).

The surface of SNPs is usually negatively charged due to the presence of the hydroxyl group, therefore it is convenient to modify the SNPs' surface through the silane chemistry. In order to control the physico-chemical, toxicological and pharmacological properties, various reactive functional groups like amine, carboxyl, phosphate or polyethylene glycol could be easily conjugated to hydroxyl SNPs. Alternatively, the surface chemistry of the SNPs can be fine-tuned for a specific biological application, optimizing the dispersion stability and/or cellular uptake, the covalent attachment of imaging agents and targeting ligands the rational control of drug release rate (Mamaeva et al., 2013).

Numerous studies pointed toward their excellent potential as biomarkers, calibration standards in confocal fluorescence microscopy, drug delivery and targeting systems (tumor imaging and therapy *in vitro* and *in vivo*) in biomedical science (Legrand et al., 2008; Lu et al., 2007; Slowing et al., 2007). The imaging agents such as, fluorescein isothiocyanate, methylene blue, quantum dots, gadolinium chelates, tetramethylrhodamine or the targeting ligands, such as aptamers, antibodies, peptides, and folic acid, can be easily doped into or modified on the surface (Wu et al., 2014). Despite the growing body of papers related to the use of SNPs as therapeutic and imaging tool, today's challenge remains the biodistribution of silica nanostructures for *in vivo* diagnostic and therapeutic applications. This study focuses on the biodistribution of SNPs (fluorescent with Cy5.5 dye and radiolabelled with ^{99m}Tc, respectively) in rodents, in order to determine possible uses in therapeutic and/or diagnostic schemes. Due to the increasing health concerns regarding the use of nanoparticles in medical applications, and more specifically on amorphous silica nanoparticles, we also performed an acute toxicology screening of SNPs used in this study in order to evaluate their safety.

2. Materials and methods

2.1. Synthesis of SNPs

For this part of our study TEOS (tetraethyl orthosilicate), APTES (3-aminopropyltriethoxysilane), aqueous ammonia solution (NH_3, 28–30%), Cy5.5 reactive dye and ethanol (>99.9%) from Sigma Aldrich were used. All solutions were prepared with ultrapure water.

Four types of silica nanoparticles derivatives were synthesized and used for *in vitro*/*in vivo* evaluation, as follows:

- *Batch AA1:* bare SNPs were produced by hydrolysis and condensation of TEOS in ethanol in the presence of ammonia as a catalyst using a modified version of the method described by Stober et al. (1968). Briefly, a solution consisting of ammonia (25%) and water in 100 mL of ethanol was prepared. 0.28 M TEOS solution (in ethanol) was added at room temperature under vigorous stirring for 24 h. Finally, the colloidal solution was separated by centrifugation at 6000 rpm for 5 min and then washed with ethanol and ultrapure water for several times to remove the unreacted species.
- *Batch AA2:* the surface amine functionalization involved a standard procedure to synthesize the NH_2-SNPs. First, ethanol, ultrapure water and TEOS (0.28 M) mixtures were prepared, followed by addition of 0.14 M APTES in ethanol. The hydrolysis and co-condensation of TEOS and APTES was initiated by the addition of 1 mL of ammonia solution (25%) to the reaction mixture and stirred for 24 h at room temperature, resulting in the formation of the core–shell-NH_2-SNPs. Samples were then centrifuged (6000 rpm for 10 min) and washed with ethanol and ultrapure water.
- *Batch AA3:* the terminal amine groups from batch AA2 were used for the conjugation of Cy5.5, a near infrared (NIR) optical probe, using a mixture of Cy5.5 dye (commercially available with an N-hydroxysuccinimide ester group for binding to amine groups), ethanol and buffer solution (1 mg; 0.14 M) added under continuous stirring at room temperature for 6 h.
- *Batch ^{99m}Tc-SNPs:* amine surface-modified SNPs (batch AA2) were used for coupling ^{99m}Tc on the nanoparticle's surface as a radiotracer to study the biodistribution of the so-produced SNPs. Briefly, SNPs were suspended in ultrapure water (5 mg/mL) and dispersed by sonication for 15–20 min. An aqueous solution of $NaBH_4$ (reducing agent) was added under continuous stirring and homogenized for 1 h. Then, to the above mixture $^{99m}TcO_4^-Na$ solution was added quickly under vigorous stirring and left for another 30 min. The obtained product was separated by centrifugation and washed with ultrapure water to remove the uncoupled ^{99m}Tc radionuclide.

$^{99m}TcO_4^-Na$ (sodium pertechnetate) was chosen for labelling because it is the most commonly used emitting radioisotope in nuclear medicine having a convenient half-life of approximately 6 h, appropriate energy (140 keV) for imaging on a standard gamma camera and less attenuation by soft tissue. A 12.5 GBq Drytec Technetium Generator was used for the production of $^{99m}TcO_4^-Na$ supplied by GE Healthcare.

2.2. Characterization of the SNPs

SEM experiments were carried out at an accelerating voltage of 20 kV on a field emission scanning electron microscope (FE-SEM, Zeiss, SUPRA VP 40). Samples suspended in ultrapure water (1 mg/mL) were deposited on freshly cleaved mica surface, dried and gold/palladium coated.

Determinations of nanoparticles size, zeta potential and polydispersity index were performed using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments). The samples were dispersed in ultrapure water and measured at a scattering angle of 90° and 25 °C. Qualitative chemical composition assessment of the nanoparticles was performed by FTIR analysis (Bomem MB 104 spectrometer). The material was finely grounded and dispersed into KBr powder-pressed pellets using a ratio of approximately 1 mg sample/200 mg KBr. IR absorbance data were obtained over a range of wavenumbers from 4000 to 400 cm^{-1}.

2.3. Experimental animals

All animals experimental procedures employed in the present study were strictly in accordance with the European Community Guidelines regarding ethics and approved by "Gr. T. Popa" University of Medicine and Pharmacy animal care and use committee. The animal breeding facility of the Central Drug Testing Laboratory, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, supplied adult male Swiss mice with an average weight of 20 ± 2 g and male guinea pigs with an average weight of 600 ± 50 g. The animals were housed in a temperature-controlled room (21 ± 2 °C) with a 12/12 h light/dark cycle, and given food and water ad libitum.