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Comparative safety evaluation of silica-based particles

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ABSTRACT

Purpose: Silica nanoparticles (SNPs) are increasingly used as drug delivery systems (DDS) and for biomedical imaging. Therapeutic and diagnostic agents can be incorporated into the silica matrix to improve the stability and dissolution of drug substances in biological systems. However, the safety of SNPs as drug carriers remains controversial. To date, no validated and accepted nano-specific tests exist to predict the potentially harmful impact of these materials on the human body.
Methods: We synthesized by a systematic approach 12 different types of SNPs with varying size, surface topology (porous vs non-porous), and surface modifications. We characterized these particles in terms of dry state and hydrodynamic diameter, specific surface area, and net surface charge (ζ-potential). For cellular studies, we exposed non-phagocytic (HepG2) cells, phagocytic (THP-1) cells, and erythrocytes to SNPs. Cellular uptake and stability of fluorescently labeled SNPs were analyzed by confocal microscopy and flow cytometry.
Results: SNPs with a porous surface and negative net surface charge had the strongest impact on cell viability. This is in contrast to non-porous SNPs. None of the studied particles induced oxidative stress in either cell lines. Particles with a negative surface charge induced hemolysis in a concentration-dependent manner.
Conclusions: Physico-chemical properties promoting cytotoxicity and hemolysis were investigated. Our study revealed potential hazards of spherical amorphous SNPs.

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1. Introduction

During the past years, interest in silica nanoparticles (SNPs) has steadily increased. Such nanoparticles are traditionally used as filler, desiccant, thickener for liquid dosage forms, or anticaking agent in powders. An example of such materials is fumed or pyrogenic silica, which was introduced in 1942 under the brand name of Aerosil®. Recently, amorphous SNPs were proposed to be used as drug delivery systems (DDS) (Rosenholm et al., 2009; Wu et al., 2011) or imaging probes (Ow et al., 2004). Hence, humans are increasingly exposed to SNPs.

For DDS, different routes of administration have been proposed, namely oral intake as a suspension or tablet (Ensign et al., 2012), transdermal delivery (Escobar-Chavez et al., 2012), or inhalation (Bailey and Berkland, 2009). In all cases, the DDS has to cross cellular barriers (e.g. the inner surface of the gastrointestinal tract) before reaching its target. Any clinical use raises the question whether these materials are safe and well tolerated in humans (Kettiger et al., 2013; Wu et al., 2011). Nanoparticles can enter target cells via various energy-dependent routes (dos Santos et al., 2011; Tao et al., 2009), and even tight cell layers, such as those found at the level of the blood–brain barrier, can be crossed by small-sized SNPs in vivo (Barandeh et al., 2012).

Several in vitro toxicity studies have been carried out to assess the toxicity of SNPs. Frequently used endpoints include cell viability, membrane leakage, generation of reactive oxygen species (ROS), and genotoxicity (Kroll et al., 2011). The cell type used in each assay has been described to strongly influence the outcome of these endpoints (Sohaebuddin et al., 2010). Additionally, plasma proteins, nutrients, or growth factors can adsorb to the surface of nanoparticles and impact cell viability (Casey et al., 2008). For example, incubations in the presence of serum resulted in a lower toxic potential of nanoparticles (Ge et al., 2011). Shape and crystalline state additionally affect the toxicity profile of SNPs. For crystalline silica, this phenomenon is known as silicosis, a severe chronic inflammation of the lung (Byrne and Baugh, 2008). Furthermore, smaller SNPs have a higher toxic potency as compared to their bigger counterparts (Li et al., 2011; Napierska et al., 2009).

Chemical properties of SNPs may vary and are a function of the chosen synthetic route. The sol–gel synthesis results in exposed silanol (Si–OH) groups on the particle surface, whereas fumed silica is characterized by corresponding siloxane (Si–O–Si) groups (Fig. 1). It was shown that silanol remains intact when the SNPs are dried under vacuum at ambient temperature. Siloxane formation on the particle's surface is observed under vacuum at temperatures above 100 °C (Zhuravlev,

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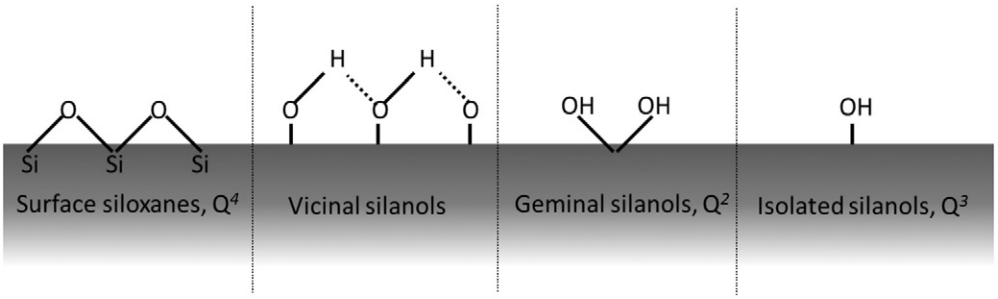

Fig. 1. Schematic representation of amorphous silica surfaces. Siloxane formation on the surface is mainly observed when the SNPs are obtained by flame synthesis. Silanols are abundantly present on the surface of particles prepared by the sol–gel method.

2000). The chosen synthetic route and thus surface properties strongly influence biological outcomes like oxidative stress generation and hemolysis. For example, siloxane rings on the SNPs surface are able to induce oxidative stress (Zhang et al., 2012). In contrast, no oxidative stress was found for SNPs synthesized with the sol–gel route. The different synthetic routes may partially explain conflicting data on SNP toxicity. Suppliers of commercially available SNPs do not necessarily disclose their proprietary protocols for the synthesis of nanoparticles. Consequently, information on surface properties of such nanoparticles is hardly available (Sayes et al., 2007).

The aim of this study was to avoid this uncertainty by using in house synthesized SNPs for a systematic evaluation of their toxicological properties. The synthesized SNPs were carefully purified and characterized. Synthetic impurities and contaminants were removed. In contrast to commercial SNPs, our particles have a known thermal history. We have synthesized 12 types of SNPs and altered their physico-chemical properties in a systematic way. The SNPs vary with respect to size, surface charge, and porosity. We have chosen two cell lines, namely a phagocytic cell line (THP-1) and a non-phagocytic cell line (HepG2), to study cellular interactions with nanoparticles (Kettiger et al., 2013). The physico-chemical properties of the SNPs were related to their cytotoxicity, the generation of oxidative stress, and the hemolysis of erythrocytes.

2. Materials and methods

2.1. Materials

Absolute ethanol (EtOH), tetraethylorthosilicate (TEOS, 98%), (3-aminopropyl)triethoxysilane (APTES), thiazolyl blue tetrazolium bromide (MTT), fluorescein isothiocyanate isomer 1, 2′,7′-dichlorofluorescin diacetate (FITC), hexadecyltrimethylammonium bromide (CTAB), ethylene glycol (EG), dichloro–dihydro-fluorescein diacetate (DCFH-DA), and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (Buchs, Switzerland). Ammonium hydroxide solution (30 wt.%) was obtained from Roth (Arlesheim, Switzerland).

2.2. Synthesis of non-porous silica nanoparticles

According to Stöber et al., we prepared non-porous SNPs of two different sizes (non-porous small SNPs (SS) and non-porous big SNPs (SB)) (Stoeber et al., 1968). In brief, the reaction solution for the small SNPs had a molar composition of TEOS: NH_3: EtOH: H_2O = 1: 0.087: 64.3: 27.8 and for the big SNPs of TEOS: NH_3: EtOH: H_2O = 1: 0.35: 64.3: 27.8. Alteration of net surface charge was carried out by direct incorporation of amine groups by co-condensation of aminosilane (APTES) and by altering the amount of APTES relative to TEOS (2.5 mol%, 5 mol%, or 10 mol%). This resulted in SNPs with a negative (−), a neutral (0), and a positive (+) surface charge. The mixture was stirred under ambient conditions for 3 h (SS) and 4 h (SB). The SNPs were collected by centrifugation, washed three times with EtOH, and dried overnight in a vacuum oven at ambient temperature.

Fluorescent particles (SB(−)) were needed for cellular uptake studies. Fluorescent dye was introduced into the synthesis as described elsewhere (Karaman et al., 2012). Briefly, an excess of FITC was added to pre-conjugate with APTES in ethanol solution and was stirred for 2 h under argon. This mixture was then introduced into the synthesis of the SB(−). Incorporation of FITC had no detectable impact on size and ζ-potential of the particles (data not shown).

2.3. Synthesis of mesoporous silica nanoparticles

Mesoporous SNPs were synthesized as described above, albeit with certain modifications (Karaman et al., 2012). In brief, an ethanol basic aqueous reaction solution was prepared and structure directing agent CTAB was added. Subsequently, TEOS was added as silica source. Briefly, the prepared reaction solution contained the reagents with the molar composition TEOS: CTAB: H_2O: EtOH: NaOH = 1: 0.12: 946: 73: 0.32. After mixing the reagents, the reaction mixture was stirred overnight at room temperature. In order to modify particle surfaces to obtain different net surface charges, APTES was introduced as above. For these synthesis solutions, molar composition was TEOS: APTES: CTAB: H_2O: EtOH: NaOH = 1: 0.04: 0.12: 946: 73: 0.32 and TEOS: APTES: CTAB: H_2O: EtOH: NaOH = 1: 0.1: 0.12: 946: 73: 0.32. For mesoporous small SNPs (MS), a modified synthesis protocol was used (Gu et al., 2007). The synthesis solution for MS consisted of a molar ratio of TEOS: CTAB: NH_3: H_2O: EG = 1: 0.45: 12.9: 1392: 74.7. The synthesis solution was subjected to vigorous stirring for 2 h at 50 °C, and then kept without agitation at 50 °C overnight. APTES was added to the reaction mixture to obtain different net surface charges of SNPs. The resulting particles were collected by centrifugation and washed at least three times with 50 mL extraction solution (ethanol ammonium nitrate solution) to completely remove the CTAB template (Han et al., 2011). FITC was introduced into the synthesis of MB(−) as described above.

2.4. Characterization of silica nanoparticles

For size determination of the SNPs, we used two approaches. First, dynamic light scattering (DLS) was used to measure the hydrodynamic diameter of SNPs in suspensions in two solutions, namely 10 mM HEPES (pH 7.2) and cell medium (Dulbecco's modified eagle medium; DMEM) containing 10% fetal calf serum (FCS) (Table 1). Size distribution of particles is given by the polydispersity index (PDI). Second, diameter of dry SNPs was determined by transmission electron microscopy (TEM). With TEM, we were additionally able to assess the porosity of the SNPs. TEM samples were negative-stained and then analyzed using a Philips CM100 electron microscope, operated in bright field mode at 80 kV. Surface charge (ζ-potential) measurements were performed in 10 mM HEPES or in cell culture medium (DMEM) containing 10% FCS to mimic the SNPs behavior as suspension in cell culture. The amount of APTES incorporated was determined by thermo-gravimetric analysis (TGA) (supplementary information, Fig. S1). The structural parameters

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