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Fluorescent non-porous silica nanoparticles for long-term cell monitoring: Cytotoxicity and particle functionality

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ABSTRACT

Inorganic nanoparticles such as silica particles offer many exciting possibilities for biomedical applications. However, the possible toxicity of these particles remains an issue of debate that seriously impedes their full exploitation. In the present work, commercially available fluorescent silica nanoparticles 25, 45 and 75 nm in diameter optimized for cell labelling (C-Spec® particles) are evaluated with regard to their effects on cultured cells using a novel multiparametric setup. The particles show clear concentration and size-dependent effects, where toxicity is caused by the number and total surface area of cell-associated particles. Cell-associated particles generate a short burst of oxidative stress that, next to inducing cell death, affects cell signalling and impedes cell functionality. For cell labelling purposes, 45 nm diameter silica particles were found to be optimally suited and no adverse effects were noticeable at concentrations of 50 μg ml^{-1} or below. At this safe concentration, the particles were found to still allow fluorescence tracking of cultured cells over longer time periods. In conclusion, the data shown here provide a suitable concentration of silica particles for fluorescent cell labelling and demonstrate that at safe levels, silica particles remain perfectly suitable for fluorescent cell studies.

1. Introduction

Silica nanoparticles (Sil NPs) are being developed rapidly and their already extensive applications are further increasing, aiming more towards biomedical and pharmaceutical use [1–3]. Depending on their physicochemical characteristics, Sil NPs can be subdivided into several groups, such as solid, amorphous, mesoporous and crystalline silica particles. Every type of particle offers different functionalities that make it an interesting system for a wide number of applications. Amorphous silica particles are commonly used as food additives, for instance as carriers of fragrances or flavours, and synthetic amorphous silica is registered within the EU as a food additive (E551) [4]. Mesoporous silica particles are currently a hot topic for the encapsulation and triggered release of pharmaceutical agents and offer many exciting possibilities for drug delivery [2]. Solid silica particles can be loaded with organic fluorophores and offer an interesting tool for fluorescence imaging purposes. Previous work has shown that the photophysical characteristics of the silica-embedded fluorophores differ distinctly from those of fluorophores in solution, rendering the particles much more bright and photostable than their constituent fluorophores [5].

One issue that is impeding the full exploitation of Sil NPs for biomedical applications is their possible toxicity both in vivo as well as in vitro. The toxic effects of Sil NPs remain an issue of debate. While several studies highlight the biocompatible nature of silica and describe no toxic effects [6,7], other studies demonstrate significant toxic effects caused by Sil NPs, such as the induction of reactive oxygen species (ROS) [8,9], inflammation [10], but also hepatotoxicity [11], inhibition of stem cell differentiation [12] or the occurrence of pregnancy complications [13]. These discrepancies are likely caused by the wide variety in different types of silica, differences in size and surface properties and highly variable incubation conditions (time, concentration and administration route) [14,15]. Furthermore, the area of nanotoxicology remains relatively new and is still developing into a more mature scientific discipline. As a result, the first steps towards standardized and optimal protocols have only recently been taken, which have led to large differences in cell types used and parameters studied in currently available literature. Recently, we proposed a multiparametric protocol that allows us to examine the cytotoxic potential of nanomaterials according to a standardized methodology, enabling easy comparison of results obtained for different types of particles [16]. Using murine C17.2 neural progenitor cells, primary human umbilical vein endothelial cells (HUVECs) and rat PC12 pheochro-

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mocytoma cells, multiple parameters are evaluated, allowing us to discern which mechanisms play a role in the cytotoxic profile of the particles and finally defining the "no-observed adverse effects levels" (NOAELs) of the particles as the concentration of particles that can be used for a variety of cell types without resulting in undesired toxic effects. This concentration can then be used as a starting point for in vitro experiments with the particles described.

In terms of Sil NPs for use in biomedical applications, small, PEGylated Sil NPs have recently been approved for a first-in-human clinical trial as cancer-selective probes, allowing the real-time fluorescent detection and imaging of melanoma with associated lymphatic drainage patterns and nodal metastases [17]. These particles are commercially available through Hybrid Silica Technologies, Inc., and branded as C-Spec® particles. Another application for which the C-Spec® particles are likely to become an important tool is fluorescence microscopy of living cells. For that purpose, bare, solid Sil NPs of 25, 45 and 75 nm diameter are available, which contain tetramethylrhodamine-5-(and-6)-isothiocyanate (TRITC) and have been described to exhibit excellent photostability and were found to be ideally suited for in vitro fluorescence imaging [5]. The cytotoxic effects of these particles, however, have not been studied in depth, and therefore caution must be taken when using these particles. Here, the cytotoxic effects will be studied systematically in multiple cell types using the multiparametric protocol described previously [16].

2. Materials and methods

2.1. Nanoparticles

The Sil NPs used were provided by Hybrid Silica Technologies, Inc. (Ithaca, New York, USA) as C-Spec® particles. All Sil NPs used contain tetramethylrhodamine-5-(and-6)-isothiocyanate (TRITC) and are supplied as stable suspensions of the particles at 2 mg ml^{-1} with a diameter of 25, 45 or 75 nm.

2.2. Dynamic light scattering and electrophoretic mobility measurements

The hydrodynamic diameter and ζ-potential of the Sil NPs were measured using a Nanosizer instrument (Malvern, Worcestershire, UK). The particles were suspended in phosphate buffered saline (PBS: 10 mM; pH 7.0), after which the measurements were performed (12 cycles per run) in triplicate. The value for the hydrodynamic diameter of the particles is obtained using the intensity scaling. Data are expressed as mean ± standard deviation ($n = 3$). For analysis in the presence of serum, the particles were suspended in 10% serum-containing cell media (Dulbecco's modified Eagle's medium (DMEM); Gibco, Invitrogen, Merelbeke, Belgium) at 50 μg ml^{-1} and shaken at 200 rpm for 1 h, after which the particles were left to incubate at 37 °C. Dynamic light scattering was performed after 24 h and 48 h of incubation using 12 cycles per run in triplicate. To get the actual hydrodynamic diameter of the particles, the mean value of the second peak was used, where the peak caused by the proteins (at ~10 nm) was excluded.

2.3. Cell culture

C17.2 neural progenitor cells and PC12 cells are maintained in high glucose DMEM, supplemented with 10% fetal bovine serum, 5% horse serum, 2 mM L-glutamine and 1% penicillin/streptomycin (Gibco, Invitrogen, Merelbeke, Belgium). Cells were maintained in a humidified atmosphere at 5% CO_2 and fresh medium was given every other day. C17.2 cells were passaged (1/10) when reaching 90% confluency. PC12 cells were grown in 25 cm^2 cell culture flasks (Corning, Amsterdam, The Netherlands) that were coated with collagen (rat tail collagen type I, Invitrogen, Belgium) and passaged (1/5) when growing in small clumps (~5 cells per clump and reaching 70–80% confluency). Fresh medium was given every other day. To establish non-proliferating cell populations, cells were exposed with 60 μM Apigenin (Sigma–Aldrich, Bornem, Belgium) together with the Sil NP exposure. After removal of the medium, fresh media containing 60 μM Apigenin was used, where media were replaced for 50% every other day with fresh Apigenin-containing medium for the duration of the experiments. Under these conditions, cell death was found to be minimal and cell proliferation was reduced to ~9% of the normal value. Furthermore, removal of the medium with normal cell culture medium not containing any Apigenin resulted in a recovery of cell proliferation to near-control levels after ~3 days.

Primary human umbilical vein endothelial cells (HUVECs) were kindly provided by Dr. Aldo Ferrari (ETH Zurich, Switzerland). For cultivation, cells were kept in 75 cm^2 cell culture flasks (Corning, Amsterdam, the Netherlands) coated with collagen (rat tail collagen type I, Invitrogen, Belgium) prior to cell seeding. The cells were maintained in endothelial cell basal growth medium and growth supplement (Cell Applications, Tebu-Bio, Le Perray en Yvelines, France) and passaged (1/5) when reaching 80–90% confluency. Every other day, fresh medium was given. To establish non-proliferating HUVEC cultures, cells were given endothelial cell serum-free defined medium (Cell Applications, Tebu-Bio, Le Perray en Yvelines, France) when reaching high levels of confluency. Confluent HUVEC monolayers could then be maintained for at least 1 week without any observable signs of cell death or ROS induction.

2.4. Cell–nanoparticle interaction studies

A full methodology can be found in the Supplementary Information that accompanies this paper.

3. Results and discussion

3.1. Nanoparticle characterization

The Sil NPs used have three different diameters, 25, 45 and 75 nm, allowing us to evaluate the effect of size on any observed cytotoxicity. As the particles will be exposed to the cells while present in an aqueous environment, the hydrodynamic diameter of the particles is more significant to relate to the interaction of the particles with cells. Using dynamic light scattering, the particles were found to have hydrodynamic diameters of 32, 45 and 80 nm, respectively, when suspended in phosphate-buffered saline (PBS) (Supplementary Table S.1). The ζ-potential of the particles was ~−15 mV for all three types of Sil NPs, the negative charge due to the presence of surface-located silanol groups. The ζ-potential is, however, only slightly negative, and may not be sufficiently strong to ensure colloidal stability of the particles in more complex media, such as cell culture media. In the literature, it has been described that serum-containing media results in the avid adsorption of serum proteins on Sil NPs, which can lead to agglomeration of the particles and hereby also induce cytotoxic effects by covering the surface of living cells with a layer of Sil NPs [18]. To investigate this, dynamic light scattering was performed on Sil NPs suspended in 10% serum-containing media, a physiologically relevant concentration (Supplementary Table S.1, Supplementary Fig. S.1). The data show that after 1 or 2 days of incubation in serum-containing media, high levels of proteins were adsorbing, seemingly resulting in multiple layers of proteins, hereby increasing the hydrodynamic diameter of the particles. However, although an increase in hydro-