Cytotoxicity of bare and PEGylated silica nanoparticles as potential gene-delivery vectors

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ABSTRACT

Bare and PEGylated silica spheres of 80 nm in diameter have been synthesized and characterized in order to study their potential application as gene-delivery vectors. In a first stage standard protein adsorption, experiments (i.e., immobilization of BSA) were carried out in order to analyze the potential avoidance of the reticuloendothelial system achieved with those nanoparticles after pegylation. *In vitro* biocompatibility of bare and PEGylated silica nanoparticles was assessed in cultures of human tumor cell lines (Hela and Saos-2) and mesenchymal cells derived from human bone marrow of healthy donors (hMSC).

Keywords: Pegylation, silica, nanoparticles, cytotoxicity, transfection

1 INTRODUCTION

Silica and other inorganic carriers (i.e., gold, carbon-based materials, layered double hydroxides, etc.) are one of the four major groups used to transport drugs and bioactive molecules (peptides, proteins, enzymes, DNAs (transfection), etc.) into cells [1]. The other groups are viral carriers, organic cationic compounds (lipids and polymers), and recombinant proteins.

Silica is currently used as a food additive (rheological enhancer, anti-packing agent, generally recognized as safe by the FDA for over 50 years [2]), and is present in many pharmaceutical systems such as tablets, suspensions, etc. [3]. The main advantages of silica compared to organic vectors are the following: no swelling or porosity changes in its structure when changes in pH are observed, it is not vulnerable to microbial attack [4], silica nanoparticles show low polydispersity in the size distribution obtained after synthesis, low cost, and high thermal (it can withstand autoclaving) and chemical resistance (it is unaffected by bile salts and lipase) [5]. The main disadvantage of silica is its non-biodegradability in biological conditions due to its low solubility and resistance to enzymatic attack.

The use of porous silica can provide two advantages in transfection applications: one, that a plasmid can be adsorbed on the external surface of the silica and two, simultaneously, a drug or fluorochrome can be adsorbed or covalently grafted on its mesoporous internal structure. Thus, mesoporous silica possesses a large specific surface area and narrow pore-size distribution, with average pore diameters that can be selected between 15 and 100 Å, by adjusting the synthesis conditions and/or by employing surfactants with different chain lengths in their preparation.

2 EXPERIMENTAL

2.1 Materials synthesis

Bare silica mesoporous nanoparticles (Figure 1) were prepared following the work of Zeng et al. (2005) [6] which describes the hydrothermal synthesis of MCM-41 microparticles using a precursor gel with the following molar composition: 1 TEOS: 0.035CTABr: 0.0175NaOH: 692.5H_2O. The synthesis was optimized in order to obtain nanoparticluated materials. In order to coat the nanoparticles with a PEG layer, approximately 100 mg of surfactant-free nanoparticles were treated with a solution containing 2.0 g of PEG dissolved in 24 mL of methanol and 6 mL of NH_3 (30 wt. %) at room temperature overnight. After this, the nanoparticles were washed repeatedly and the un-reacted PEG was separated by dialysis.

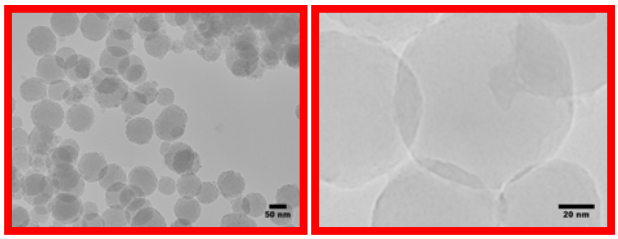

Figure 1: SiO_2 nanoparticles used in this work

2.2 Cell culture

Human tumor cell lines (Hela and Saos-2) and mesenchymal cells derived from human bone marrow of healthy donors (hMSC) were maintained at 37 °C under 5% CO_2 and 95% air in a humidified incubator. Human osteosarcoma Saos-2 cells (ECACC, Salisbury, Wiltshire, UK) were grown in DMEM medium supplemented with

10% (v/v) heat-inactivated fetal bovine serum (FBS), 500 UI/ml of penicillin and 0.1 mg/ml of streptomycin. Human mesenchymal stem cells from bone marrow (hMSC) were purchased from Cambrex Bio Science (Verviers, Belgium). These cells were maintained in growth medium and switched to the osteoblastic phenotype by incubation in osteogenic induction medium (both from Cambrex).

2.3 Cytotoxicity Assay

Actin cytoskeleton reorganization and cell viability were evaluated using the alamar blue cytotoxicity assay by culturing human osteoblastic Saos-2, Hela cells, and mesenchymal cells with the different nanoparticles.

Alamar Blue is a non-fluorescent substrate and is cleaved to a fluorescent product by living cells. This activity is dependent on the cell viability. The amount of fluorescence will thus correlate with the number of living cells. The cellular metabolic activity was measured for increased amounts of nanoparticles (0, 1, 5, 10, 50, 100 ng/cell) added to the cultured cells.

Combined fluorescence and reflection confocal laser scanning microscopy (CLSM) were used to evaluate the cell morphology (the organization of the actin cytoskeleton).

3 RESULTS

Nanoparticle cytotoxicity depends on intrinsic characteristics as surface chemistry, size, shape, surface area, agglomeration state, and solubility.

Combined fluorescence and reflection confocal laser scanning microscopy (CLSM) did not reveal the presence of intracellular particle agglomerates.

After exposure for 24 h to nanoparticles, actin cytoskeleton was well organized for the three cell types examined. When the incubation period was extended to 72 h, PEGylated silica impaired metabolic activity in cells to a higher extent than bare particles. Interestingly, Hela and Saos-2 tumor cells exhibited a significantly higher sensitivity to both types of nanoparticles than did hMSC (Figure 2).

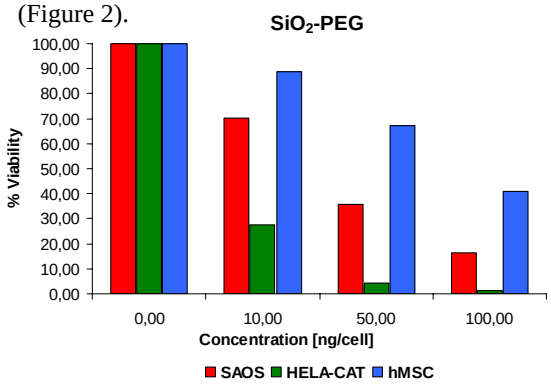

Figure 2a: Cytotoxic activity of the PEGylated silica nanoparticles.

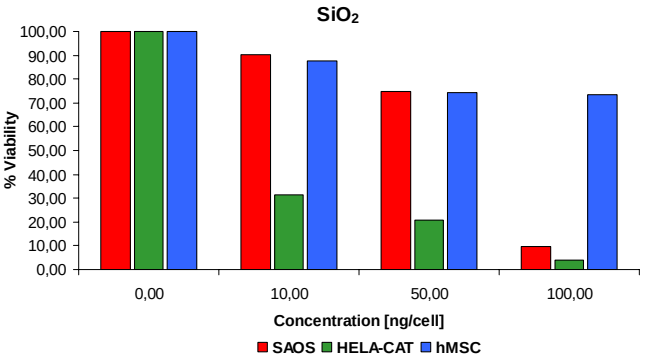

Figure 2b: Cytotoxic activity of the silica nanoparticles.

Amino functionalized bare and PEGylated silica nanoparticles were successfully coupled to a pGL4.13 vector (Promega) by electrostatic interaction. In Figure 3 the adsorption isotherm for the plasmid on the surface of the amino-conjugated silica nanoparticles is shown.

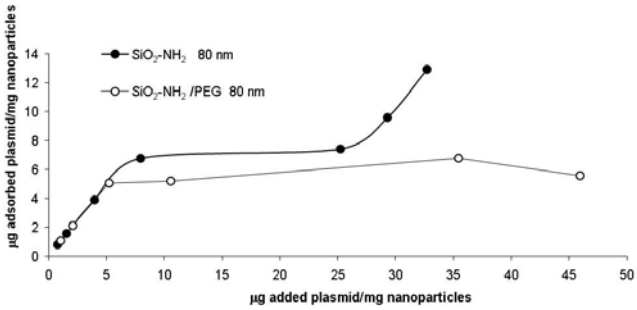

Figure 3. Adsorption isotherm of plasmid (pGL4.13) on silica nanoparticles.

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