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Oxidized silicon nanoparticles for radiosensitization of cancer and tissue cells

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ABSTRACT

The applicability of ultrasmall uncapped and aminosilanized oxidized silicon nanoparticles (SiNPs and NH_2-SiNPs) as radiosensitizer was studied by internalizing these nanoparticles into human breast cancer (MCF-7) and mouse fibroblast cells (3T3) that were exposed to X-rays at a single dose of 3 Gy. While SiNPs did not increase the production of reactive oxygen species (ROS) in X-ray treated cells, the NH_2-SiNPs significantly enhanced the ROS formation. This is due to the amino functionality as providing positive surface charges in aqueous environment. The NH_2-SiNPs were observed to penetrate into the mitochondrial membrane, wherein these nanoparticles provoked oxidative stress. The NH_2-SiNPs induced mitochondrial ROS production was confirmed by the determination of an increased malondialdehyde level as representing a gauge for the extent of membrane lipid peroxidation. X-ray exposure of NH_2-SiNPs incubated MCF-7 and 3T3 cells increased the ROS concentration for 180%, and 120%, respectively. Complementary cytotoxicity studies demonstrate that these silicon nanoparticles are more cytotoxic for MCF-7 than for 3T3 cells.

1. Introduction

In the recent past intense research activities were focused onto the development of silicon nanoparticles as photosensitizer of singlet oxygen generation for photodynamic therapy [1,2]. The applicability of the photodynamic therapy is limited to the treatment of superficial tumors due to the small penetration depth of visible light as well as the short diffusion path and short lifetime of singlet oxygen [3].

In case of ionizing radiation, such as X-rays or gamma rays, the penetration depth can be easily optimized to the range of 8–14 cm. Therefore, approximately half of the patients, who develop cancer, receive radiotherapy as a main component of their treatment. Radiotherapy uses X-rays for targeted destruction of malignant cells. X-rays may act directly on cellular material through either ionizing or exciting or indirectly, via interactions with molecules by generating free radicals that in turn can damage DNA or cellular organelles. However, there are various tumors that are hardly responsive or even resistant to radiotherapy. One goal of radiotherapy is to enhance the radiotherapeutic efficacy for cancer to minimize its harmful effects on normal tissues. Therefore radiosensitizers have been developed which increase the sensitivity of tumor cells to X-ray radiation [3]. A recent *in vitro* study revealed that surface-oxidized silicon nanoparticles may increase the impact of X-radiation on the formation of reactive oxygen species (ROS) for clinically relevant doses [4]. Under ambient conditions silicon nanoparticles form an amorphous SiO_x shell (with $x < 2$). The SiO_x shell is considered to enhance X-ray induced generation of oxygen radicals ($OH^{\cdot}$, $HO_2^{\cdot}$, O_2^{-}) in aqueous solutions. Moreover, singlet oxygen is generated in X-ray treated solutions containing surface-stabilized, oxidized silicon nanoparticles [5].

Silica nanoparticles (SiO_2NPs) were reported to induce oxidative stress in a dose-dependent manner which was proven by the formation of ROS, lipid peroxidation (LPO) and depletion of glutathione (GSH) [5,6]. Molecular oxygen was observed to react with the fractured silica surface, where both, homolytic ($Si^{\cdot}$, $SiO^{\cdot}$) and heterolytic (Si^{+}, SiO^{-}) cleavage of the silicon–oxygen bond may take place [7]. The mechanism of intracellular ROS generation in the presence of SiO_2NPs presumably involves both, the mitochondrial respiration and the NAD(P)H oxidase system [8].

Coating of SiO_2NPs surfaces or those of oxidized silicon nanoparticles with suited organic groups was observed to suppress the SiO_2 or SiO_x induced ROS generation and is hence required for the development of biocompatible and biodegradable silicon

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nanoparticles for medical application [6]. Therefore they may be covered with detergents [9] or phospholipid micelles [10]. Otherwise, silicon nanoparticles can be functionalized with ethenyl derivatives that provide surface charges in aqueous environment due to their terminal amino (NH_2), azide (N_3) and carboxylic acid (COOH) groups [11]. The influence of surface charges on the cytotoxicity of such functionalized silicon nanoparticles was examined by Bhattacharjee et al. [12]. Positively charged silicon nanoparticles were observed to exhibit the highest cytotoxicity, since they significantly increase the intracellular ROS production.

In this contribution, the cellular uptake and influence of ultrasmall amino-silanized oxidized silicon nanoparticles (NH_2-SiNPs) and uncoated oxidized silicon nanoparticles (SiNPs) on the cell viability and oxidative stress were studied. The applicability of NH_2-SiNPs and SiNPs as radiosensitizer for X-rays in tumor cells was examined. Therefore these nanoparticles were internalized in human breast cancer (MCF-7) and mouse fibroblast (3T3) cells, which were exposed to X-radiation at a single dose of 3 Gy. The radioenhancement effect was assessed by measuring the intracellular ROS concentrations.

2. Materials and methods

2.1. Chemicals and instruments

Silicon tetrachloride (Aldrich, 99%), tetraoctylammonium bromide (TOAB, Aldrich, 98%) Aminopropyltriethoxysilane (APTES, Aldrich, ⩾98%,), $LiAlH_4$ (Fluka, >97%), toluene (VWR, 99.5%), cyclohexane (VWR, 100%) and methanol (VWR, p. a.) were used as received. KH_2PO_4 (p. a.) and K_2HPO_4 (99%) were purchased from Merck, NaCl (99.5%) from Fluka and Triton X-100 from Riedel-de Haën. DMEM, L-glutamine, FCS, penicillin–streptomycin-solution, sodium pyruvate, PBS, MEM, trypsin/EDTA, MTT (98%), trypan-blue-solution (0.4%), sodium dodecylsulfate (SDS) (90%), Tris–HCl (99%), disodium EDTA (99%), trichloroacetic acid (TCA) (99%), 2-thiobarbituric acid (TBA) (98%), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (98%), 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (95%) were purchased from Sigma–Aldrich and glutaraldehyde (25%) and potassium pyrophosphate from Roth. DCFH-DA was dissolved in dimethyl sulfoxide (DMSO) (99.7%, Baker) to obtain a stock solution (0.01 M) and was kept frozen at −20 °C. For loading the cells with DCFH-DA the stock solution was mixed with DMEM at a concentration of 100 μM.

The photoluminescence spectra of the NH_2-SiNP and SiNP colloids and DCF assay were recorded on a Horiba Jobin-Yvon Fluoro-Max-3 spectrofluorometer. Transmission electron microscopy (TEM) images of SiNPs were taking using a Zeiss EM 900 instrument that was operated at 80 kV accelerating voltage, and the cells were imaged using a Zeiss 906 transmission electron microscope (LEO, Oberkochen, Germany). The concentrations of the NH_2-SiNP and SiNP sample solutions were determined using ICP-AES, in order to prepare a cell culture medium that contains silicon at the concentration of 0.1 mg/ml. The MTT assay was measured at 590 nm using an Elisa microplate reader (Dynatech Laboratories, Inc.). The different cells experiments were irradiated using a 120 kV X-ray tube (Isovolt, Seifert, Ahrensberg, Germany). UV–Vis measurements were carried out using a Perkin Elmer Lambda 2 in the range of 300–700 nm.

2.2. Syntheses of SiNPs and NH_2-SiNPs

Silicon nanoparticles were synthesized using a reverse-micelle wet-chemistry procedure [13]. 0.63 g of TOAB was dispersed for 20 min in toluene using an ultrasonication bath. 0.63 mL of $SiCl_4$ was added to the solution and the sonication was maintained for another 20 min. Then 0.76 g of $LiAlH_4$ was added to the solution. After 30 min sonication, 30 mL of MeOH was slowly incorporated to the suspension to eliminate the excess of the reductant. In order to break the micelles, the mixture of solvents was evaporated, and the particles were dispersed in 30 mL of cyclohexane. Three liquid–liquid extractions were performed with 30 mL of water. Finally, the organic phase was evaporated and the particles were dispersed in 20 mL of toluene or water for the cell experiments.

The SiNPs were functionalized via silanization. The toluene suspension of the SiNPs was stirred for 24 h at reflux with 1.5 mL of APTES. Afterwards the suspension was evaporated, and 20 mL of water was added. The purification of the functionalized SiNPs was performed via dialysis. The aqueous suspension was put into a Serva Membra-Cell MWCO 7000 membrane, and the dialysis tubing was submerged in distilled water. After 4 h, the water was changed twice and the suspension was left overnight. Finally, the suspension was evaporated and the aminosilanized SiNPs (NH_2-SiNPs) were dispersed in toluene or water.

2.3. Cell culture

The MCF-7 and 3T3 cells were cultured in DMEM containing 4500 mg glucose/L, which was enriched with 10% fetal calf serum (FCS), 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine and 1% MEM nonessential amino acids. In a humidified environment of 5% CO_2 at 37 °C the cells were incubated and subcultivated twice a week.

2.4. Transmission electron microscopy (TEM)

MCF-7 and 3T3 cells were incubated with cell culture medium containing SiNPs or NH_2-SiNPs at a concentration of 0.1 mg Si/mL. Cells were washed with PBS and fixed with 2.5% glutaraldehyde overnight at 4 °C and then postfixed in 1% osmium tetroxide and 3% potassium ferricyanide at room temperature. Through-graded alcohols cells were dehydrated, embedded in Epon and mounted on Epon blocks. Uncontrasted silver-grey ultrathin sections were imaged.

2.5. Cell viability assay

The mitochondrial function and cell viability of the NH_2-SiNPs and SiNPs, TOAB and APTES were evaluated using the 3-(4,5-dimethylthiazol)-2-diphenyltetrazolium bromide (MTT) assay. The two different cell lines were seeded in a 96 well-plate at a density of 10^3 cells per well. After 3 days the cell culture medium was replaced with one containing TOAB (0.1 mg/mL), APTES (0.1 mg/mL), SiNPs or NH_2-SiNPs both at a concentration of 0.1 mg Si/mL. After 24, 48 and 72 h incubation 50 μL of MTT solution (0.5 mg/mL in PBS) was added. The solution was carefully removed after 1 h, and the formazan crystals were solubilized with 100 μL SDS (0.2 mg/mL)-HCl (0.02 M)-solution. The metabolic activity was determined by measuring the absorbance of the formazan solution at 550 nm.

2.6. Intracellular ROS measurement

MCF-7 or 3T3 cells were cultivated in 96 well-plates at a density of 10^3 cells per well and were allowed to grow over 3 days. After removing the medium, the cells were incubated for 24 h with cell culture media that contains SiNPs or NH_2-SiNPs (0.1 mg Si/mL). Afterwards the cells were washed with PBS and loaded with 100 μM DCFH-DA in DMEM for 30 min. Each well was loaded with PBS. One half of the plate was irradiated at a single dose of 3 Gy. Since the ROS oxidize intracellular DCFH to the fluorescent DCF dye, the DCF fluorescence intensity is taken as being directly

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