BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
12 (2016) 1931–1940

Original Article

nanomedjournal.com

Studies of silicon nanoparticles uptake and biodegradation in cancer cells by Raman spectroscopy

Elen Tolstik, PhD[a,b,*], Liubov Andreevna Osminkina, PhD[c,d], Christian Matthäus, PhD[a,b], Markus Burkhardt, BS[a], Konstantine Eduardovich Tsurikov, BS[c], Uliana Alexandrovna Natashina, BS[c], Victor Yurievich Timoshenko, PhD[c,d], Rainer Heintzmann, PhD[a,b], Juergen Popp, PhD[a,b], Vladimir Sivakov, PhD[a]

[a]*Leibniz Institute of Photonic Technology, Jena, Germany*
[b]*Institute of Physical Chemistry, Abbe Center of Photonics, Friedrich-Schiller-University, Jena Germany*
[c]*Lomonosov Moscow State University, Physics Department, Moscow, Russia*
[d]*National Research Nuclear University MEPhI, Moscow, Russia*

Received 16 February 2016; accepted 6 April 2016

Abstract

In-vitro Raman micro-spectroscopy was used for diagnostics of the processes of uptake and biodegradation of porous silicon nanoparticles (SiNPs) in breast cancer cells (MCF-7 cell line). Two types of nanoparticles, with and without photoluminescence in the visible spectral range, were investigated. The spatial distribution of photoluminescent SiNPs within the cells obtained by Raman imaging was verified by high-resolution structured-illumination optical microscopy. Nearly complete biodegradation of SiNPs inside the living cells was observed after 13 days of the incubation. The results reveal new prospects of multi-modal visualization of SiNPs inside cancer cells for theranostic applications.

Key words: Silicon nanoparticles; Raman spectroscopy; HR-SIM microscopy; Biodegradation; Cancer theranostic

The importance of cancer diagnosis at a very early stage and the need for more personalized medical treatment require an improvement of already existing diagnostic procedures and treatment modalities. The ability to detect and to localize malignant cells *in-vivo*, as well as to medicate and to eliminate the tumour cells as early as possible, is the key to a carefully targeted therapy.[1] The application of nano-sized particles for both detection and treatment of tumours is one of the most important areas in current biomedical research. During the growth of the tumour it is possible to observe the accumulation of luminescent semiconductor nanocrystals, i.e. quantum dots (QDs), or metal-coated nanoparticles.[2–4] These nanoparticles can be loaded with anti-cancer chemotherapeutics for targeted drug release at the affected regions.[5] However, the widespread biological application of conventional QDs based on semiconductor compounds is hampered by problems related to their poor biocompatibility and lack of biodegradability which lead to inhibition of cell growth and viability. This is especially true for cadmium or lead-containing QDs.[3-5]

In comparison with QDs, which can contain toxic metals, silicon nanoparticles (SiNPs) are biocompatible,[6-9] low both in

Abbreviations: SiNPs, silicon nanoparticles; QDs, quantum dots; PL, photoluminescence; PL-SiNPs, photoluminescent SiNPs; NL-SiNPs, non-luminescent SiNPs; HR-SIM, high-resolution structured illumination microscopy; SEM, scanning electron microscopy; FE-SEM, field-emission scanning electron microscopy; TEM, transmission electron microscopy; MAWCE, metal-assisted wet-chemical etching

In the article "Studies of Silicon Nanoparticles Uptake and Biodegradation in Cancer Cells by Raman Spectroscopy" no conflicts of interest are presented.

This work was supported by the German Federal Ministry of Education and Research under Grant No. 01DJ13010 and Baltic Sea Research Network "NanoPhoto" (Grant No. 01DS14017), by the scientific project No. 13 N12166 and "QuantiSERS" and "Jenaer Biochip Initiative 2.0" of "InnoProfile Transfer – Unternehmen Region". The photoluminescence study was supported by the Russian Science Foundation (Grant No. №14-50-00029). L.A.O. greatly acknowledges the financial support of the joint program of MSU-DAAD "Vladimir Vernadsky".

*Corresponding author at: Leibniz Institute of Photonic Technology, Jena, Germany.

E-mail addresses: Elen.tolstik@ipht-jena.de, Elenatolstik@gmail.com (E. Tolstik).

http://dx.doi.org/10.1016/j.nano.2016.04.004
1549-9634/

cytotoxicity[10] and genotoxicity.[11] Furthermore, SiNPs were found to be biodegradable, which is extremely important for their complete elimination from living cells and tissues.[12] In addition, useful properties for Brachy- and photodynamic therapies have been demonstrated for silicon nanostructures.[6,11-13] *In-vitro* and *in-vivo* ultrasound- or radio-frequency radiation-induced hyperthermia using SiNPs-based sensitizers seems promising for mild cancer therapy.[14,15] Also porous SiNPs can be used as dissolved nano-containers for drug delivery.[16] Along with the observation of photoluminescence (PL) of Si nanocrystals[17] the possibility of drug delivery by SiNPs coined the rapid development of their application in biomedicine.[6,18,19] The potential of SiNPs to degrade in physiological fluids in combination with the solubility of orthosilicic acid, which is the main degradation product and occurs as the natural form of silicon in living organisms, has resulted in extensive research on SiNPs and their application in the field of cancer theranostics.[20] The complete release of SiNPs from the body within several weeks without showing any toxicity has been demonstrated *in-vivo*.[21] Nonetheless, the SiNPs degradation rate depends on the particle size, porosity, surface coverage, pH value and ambience and can vary from a few days up to several months.[12] SiNPs biodegradation is associated usually with the disappearance of their PL property. Alternatively it can be assessed by histological methods or by the analysis of silicon content in the tissues by inductively coupled plasma optical emission spectrometry.[12,15,20,21] However, these methods of analysis are not very precise, fast or/and straightforward. Moreover, the PL quenching of SiNPs cannot directly be related to their biodegradation.[22] Therefore, the development of new fast methods to monitor the biodegradation of SiNPs is urgently required.

The uptake and the localization of SiNPs with efficient PL can be studied by fluorescence-based detection techniques.[20,23-25] A high-resolution structured illumination microscopy (HR-SIM) is an optical fluorescence imaging method, which allows to record highly resolved multi-colour fluorescence images of living systems.[26-29] By using HR-SIM the images can be recorded at a lateral resolution of 100 nm and an axial resolution down to 150 nm depending on the excitation wavelength. This results in a resolution twice as high compared to conventional light microscopy, which was originally applied in previous studies of SiNPs.[23,30] Thus, by applying HR-SIM together with conventional staining techniques it is possible to visualize the main cellular organelles simultaneously with SiNPs.

Raman micro-spectroscopy is a powerful tool for label-free detection of the biochemical distribution of different compounds and elements in a sample with subcellular resolution.[31-33] It is especially useful to detect possible chemical changes and modifications of the SiNPs under the applied experimental conditions. In this context Raman microscopy can be used to study the intra-cellular uptake of nanostructures and to monitor their intercellular distribution over time, especially in the case of non-luminescent nanoparticles where standard fluorescent techniques are not applicable.[34-38] Previous studies of silicon-based materials by means of Raman spectroscopy were mostly performed to study crystallinity and sizes of SiNPs.[39-43]

Here, we report a novel approach for *in-vitro* examination of the intracellular behaviour of SiNPs, their localization and biodegradation inside the cancer cells by Raman micro-spectroscopy. For SiNPs with bright visible PL Raman microscopy measurements are confirmed by HR-SIM.

Methods

Nanoparticles formation

Heavily boron-doped (doping level of 10^{20} cm^{-3}; specific resistivity of 0.005 Ω*cm) 4-inch crystalline silicon (c-Si) wafers with crystallographic orientation of (100) were used. Photoluminescent SiNPs (PL-SiNPs) were formed by using metal-assisted wet-chemical etching (MAWCE) to fabricate photoluminescent Si nanowires (PL-SiNWs) followed by their fragmentation by using in an ultrasound bath (37 kHz, 90 W). The MAWCE method is based on a two-step process, as previously reported.[44] First, silver (Ag) nanoparticles were deposited on silicon wafer surfaces by immersing the wafers for 30 s in an aqueous solution of 0.02 M $AgNO_3$ and 5 M hydrofluoric acid (HF) in a 1:1 volume ratio (solution I). For the second step, silicon wafers covered with Ag nanoparticles were immersed in a second etching solution of 5 M HF and 30% H_2O_2 in a 10:1 volume ratio (solution II) in a Teflon vessel for 60 min at room temperature. Then the samples were rinsed several times in deionized water and additionally immersed in concentrated (65%) nitric acid (HNO_3) for 15 min to remove the residual Ag nanoparticles from the sample surfaces. Finally, SiNW arrays were rinsed several times in de-ionized water and dried at room temperature.

Non-luminescent SiNPs (NL-SiNPs) were formed from electrochemically prepared non-luminescent mesoporous silicon (NL-PSi) in HF(48%):C_2H_5OH solution (1:1) at the current density of 60 mA/cm^2 and etching time of 60 min. The obtained films were separated from c-Si substrates by applying a pulse of etching current with the current density of 600 mA/cm^2 and then the films were rinsed several times in de-ionized water. The prepared free standing films were milled in distilled deionized water by using a planetary mill FRITSCH "Pulverisette 7 premium line" for 20 min. Afterwards the SiNPs suspension was centrifuged for 3 min at 2000 rpm rotating speed and then filtered through a 0.22 μm filtration membrane (Millipore).

Structural analysis

Structural analysis of the samples was carried out by scanning electron microscopy (SEM, Tescan Lyra), field-emission scanning electron microscopy (FE-SEM, Carl Zeiss ULTRA 55) and transmission electron microscopy (TEM, LEO912 AB OMEGA). The cross-sectional SEM images were done for the samples cleaved according to the corresponding in-plane crystallographic directions of the c-Si substrates. The samples of SiNPs for TEM studies were prepared by deposition of a drop of the aqueous suspension of SiNPs on the standard TEM-grids followed by their drying in air for 10 min. The dynamic light scattering (DLS) measurements were performed with a Malvern Zetasizer Nano ZS instrument to determine the size distribution of nanoparticles in aqueous suspensions. PL spectra of SiNPs were measured under excitation with a cw Ar-ion laser at 364 nm (power of 10 mW, spot diameter of 1 mm). The PL signal was