Stabilization of porous silicon nanoparticles by PEGalization in water

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Abstract. Mesoporous silicon (mPSi) nanoparticles (NPs) are stabilized by polyethylene glycol (PEG) chains during mechanical grinding in a ball mill that is used to form mPSi-PEG-NPs. The structure, composition, and properties of the obtained samples are studied by means of the dynamic light scattering, scanning electron microscopy and Fourier-transform infrared spectroscopy. The proposed PEGalization procedure is an effective way of regulating the dissolution of mPSi-NPs in water and it is promising for potential application of mPSi-NPs in drug delivery.

1. Introduction
Mesoporous silicon (mPSi) obtained by electrochemical etching of crystalline silicon (c-Si) is a promising material for biomedical applications because of its biodegradability, low toxicity, and ability to act as an agent for various therapeutic effects [1]. Nanoparticles (NPs) of mPSi are promising for delivery and gradual release of payload, they have been successfully tested in vivo as nanocontainers of anticancer drugs, such as, for example, doxorubicin [2-4].

Nevertheless, that kind of NPs are either hydrophobic (as-prepared) or in case of surface oxidation, can rapidly dissolve into water with the formation of silicic acids. The goal of our work was the creation of mPSi-NPs conjugates with PEG to reduce the dissolution rate of silicon in an aqueous medium.

In the case of mPSi, the NPs are 100-200 nm aggregates of small (2-5 nm) crystallites [5]. It was found that silicon NPs can gradually dissolve in water and aqueous solutions [6,7]. Although the dissolution rate of bulk c-Si is determined by the pH of the solution and the orientation of the c-Si surface [8] and varies from 1 nm/day to 1 μm/day [9], the dissolution rate of silicon NPs mainly depends on their size, morphology and surface coverage [7, 10]. The stability of mPSi-NPs colloids is one of the challenges for their biomedical applications. The surface coating allows to prevent the rapid dissolution of NPs and stabilize their properties.

The surface conjugation can be performed using electrostatic forces, more strong covalent bonds, as it releases the charge over days, weeks, or months [2, 11]. Another way of physical absorption on the surface is often realized in the form of a polymer coating [12]. For example, in [13] it was found that coating mPSi-NPs with dextran can significantly improve their efficiency and stability.

PEG is the most commonly used surface protection molecule, which is an inert hydrophilic polymer invisible to an immunological response. PEG is an inexpensive, well-studied polymer for many pharmaceutical and biotechnical applications, namely PEGalization [14-19]. PEG is rapidly cleared in
vivo without structural changes, clearance is molecular weight dependent. In our case, PEG-400 Da is excreted in the urine [15].

2. Experimental part

The method for preparing aqueous suspensions of mPSi-NPs included the formation mPSi films by electrochemical etching of hole-type conduction c-Si wafers with surface orientation (100) and a resistivity of 20 mΩ·cm. Electrochemical etching of c-Si plates was performed into the mixture of 48% fluoric acid and ethanol (HF:EtOH, 1:1 vol.), for 60 minutes with a current density of 60 mA/cm². Ethanol has been used as a surfactant that promotes the penetration of hydrofluoric acid into the pores due to the hydrophobic nature of silicon, as well as to remove hydrogen bubbles that are released during the anodizing process. Etching was carried out in a polytetrafluoroethylene cell with a platinum counter electrode at room temperature [13]. Films were separated from the substrate using a short electric current pulse of increased density (500-600 mA/cm²), washed in deionized water, and dried at room temperature. Next, the mPSi films were ground in an agate mortar to obtain powders of micrometer-sized particles, and in deionized water or 30% aqueous solution of PEG milled into a FRITSCH "Pulverisette 7" planetary ball mill using balls with a diameter of 5 mm from zirconium oxide for 40 minutes at speeds from 500 to 800 rpm [9]. As a result, suspensions of mPSi-NPs were formed, which were further centrifuged to separate non-conjugated PEG (30 min, 20817 rcf). The centrifuged samples were resuspended, placed in dialysis bags with pore sizes of 6-8 kDa, and kept in distilled water (5l) at room temperature with constant stirring. Aliquots of suspensions were analyzed after 3, 6 and 24 hours of dialysis. The stability of mPSi-NPs colloid in aqueous medium was monitored by dynamic light scattering (Zetasizer Nano ZS), scanning electron microscopy (Tescan maia 3, at an accelerated voltage of 7 kV), FTIR spectroscopy (BRUKER IFS-66v/S) with a spectral resolution of 4 cm⁻¹.

3. Results and discussion

3.1. Dynamic light scattering

To verify that a PEG layer can be attached to the surface of mPSi-NPs by physical adsorption [20, 21], we analyzed aqueous solutions of mPSi-NPs by dynamic light scattering (figure 1).

We observe a decrease in the hydrodynamic diameter of NPs and a transition of the zeta potential to a more negative region in samples coated with PEG. Size change can be attributed to weaker
agglomeration in presence of surfactant. A decrease in zeta potential also indicates a decrease in the aggregation of mPSi-NPs due to the passivation of their surface with hydrophilic neutral PEG particles.

3.2. Scanning electron microscopy
Based on the results of scanning microscopy, we can observe the porous structure of the mPSi and mPSi-PEG samples and determine the average size of the NPs (figure 2).

![Figure 2. SEM images: (a) mPSi-NPs, (b) mPSi-NPs after 24h dialysis, (c) mPSi-PEG-NPs, (d) mPSi-PEG-NPs after 24h dialysis.](image)

The SEM images clearly show the spherical shape of the particles and their agglomeration. The average particle size in mPSi-NPs is 100 nm.

3.3. FTIR spectroscopy
The method of FTIR spectroscopy was also applied to study the structure and composition of functionalized silicon NPs. Samples were mixed with KBr to make tablets and tested for the presence of PEG in mPSi-PEG-NPs, tested for PEG gradual release and silicon retention.

Figure 3 shows FTIR transmission spectra of mPSi-NPs after 6 hours of dialysis, PEG, mPSi-PEG-NPs and mPSi-PEG-NPs after 6 hours of dialysis. The spectrum of a 30% aqueous PEG solution was recorded in a ZnSe prism.

The method is based on the analysis of the intensity of the characteristic absorption band of PEG molecules corresponding to the stretching vibrations of the C-H bond located at a frequency of 1456 cm\(^{-1}\). This band is quite descriptive and does not overlap with functional groups corresponding to mPSi-NPs.
Figure 3. FTIR transmission spectra of mPSi-NPs, mPSi-PEG-NPs and PEG 400 Da in water (30%); 30 scans, 4 cm$^{-1}$ spectral resolution.

Black curve demonstrates that the inner surface of mPSi-NPs is covered with hydrogen, as evidenced by absorption peaks at 600-800 cm$^{-1}$ associated with surface Si-H bonds. Intense absorption bands were found indicating the formation of an oxide phase (467 and 553 cm$^{-1}$ Si=O). Stretching vibrations of the O$_x$Si-H$_x$ fraction (806, 883, 2252 cm$^{-1}$) are also observed. The appearance of bands corresponding to the Si-O-Si (1095 cm$^{-1}$) and O$_x$Si-H$_x$ groups is the result of the oxidation of the samples in the air. Thus, the FTIR spectrum of mPSi-NPs demonstrates a mixed oxide-hydride composition of the pore surface coating, which provides a combination of hydrophilic properties and effective loading of NPs with drugs, including those with a low degree of solubility in water.

The FTIR spectrum of PEG is represented by sharp peaks (green curve), but after interaction with mPSi-NPs, their broadening and shift occur, it can be assumed that PEG molecules become more rigid, because adhere to the surface of the mPSi-NPs. Red and blue curves show good binding of mPSi-NPs and PEG. The characteristic PEG bands were found. The intense band at 1095 cm$^{-1}$ refers to the stretching vibrations of the C-O-C group, but it is superimposed on the silicon band (Si-O-Si). The absorption bands in the region of 1470-1300 cm$^{-1}$ are due to bending vibrations of -CH$_2$ groups. The absorption bands in the region of 2950-2850 cm$^{-1}$ correspond to stretching vibrations of C-H bonds.

The PEG content in mPSi-PEG-NPs was determined from the intensity of the peak at 1456 cm$^{-1}$. During grinding and centrifugation, about 50% of PEG was bound to mPSi-NPs. Dialysis for 6 hours reduces the PEG content by 30%. We also observe the retention of Si-H and O$_x$Si-H$_x$ bonds after the interaction of mPSi-NPs with PEG, which indicates the protective effect of PEG.
Thus, according to the FTIR spectra, we can say that PEG is a preservative for mPSi-NPs not only at the initial stage but also remains on the surface after a while.

4. Conclusions
The advantages of the proposed method are the mild reaction conditions (in terms of temperature, pressure, experimental equipment and reagents), the absence of the contamination of NPs during coating, and the short duration of the procedure since PEGalization is performed simultaneously with the grinding of NPs. A high-density surface coating is obtained that successfully stabilizes the NPs against dissolution in aqueous media.

The ability to inhibit the dissolution of mPSi-NPs by PEGalization has been confirmed by DLS and FTIR spectroscopy. This is promising for the use of NPs as nanocontainers for drug delivery. Silicon NPs have a well-defined structure with multiple compartments that can be used to accommodate various payloads. Undoubtedly, the use of PEGs and similar stabilizers on NPs surfaces will continue. Strategies for combining the signaling or therapeutic benefits of NPs with efficient delivery in vivo remain one of the main challenges in biomedicine.

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