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Improved stability and biocompatibility of nanostructured silicon drug carrier for intravenous administration

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ABSTRACT

Nanotechnology has attracted considerable interest in the field of biomedicine, where various nanoparticles (NPs) have been introduced as efficient drug carrier systems. Mesoporous silicon (PSi) is one of the most promising materials in this field due to its low toxicity, good biodegradability, high surface area, tunable pore size and controllable surface functionality. However, recognition by the reticuloendothelial system and particle agglomeration hinder the use of PSi for intravenous applications. The present paper describes a dual-PEGylation method, where two PEG molecules with different sizes (0.5 and 2 kDa) were grafted simultaneously in a single process onto thermally oxidized PSi NPs to form a high-density PEG coating with both brush-like and mushroom-like conformation. The material was characterized in detail and the effects of the dual-PEGylation on cell viability, protein adsorption and macrophage uptakes were evaluated. The results show that dual-PEGylation improves the colloidal stability of the NPs in salt solutions, prolongs their half-lives, and minimizes both protein adsorption and macrophage uptake. Therefore, these new dual-PEGylated PSi NPs are potential candidates for intravenous applications.

1. Introduction

Intravenously injectable nanoparticles (NPs) have important therapeutic applications in site-specific drug delivery and medical imaging. The naked NPs, however, quickly agglomerate and get captured and removed from the bloodstream by the reticuloendothelial system (RES) within a few minutes after being introduced into the cardiovascular system [1]. Clearly, such short circulation time of NPs limits their successful application in nanomedicine.

Functionalization of NPs with polyethylene glycol (PEG) is widely used to sterically stabilize NPs for medical applications. The PEG coating can prevent agglomeration [2], reduce protein adsorption, prevent phagocytosis by immunodefensive cells such as macrophages [3,4] and increase circulation time [1,5]. Therefore, many studies have investigated the effect of PEG molecular size and surface density in particular. It is generally accepted that longer PEG chains are better for shielding the NPs [6], but some studies have reported that grafting density is at least as important as the length of the chains [4,7]. However, these two factors, size and density, both affect the PEG layer conformation on the particle surface: a low density of PEG or long PEG molecules generally form mushroom-like layers and a high density or short PEG molecules tend to form brush-like layers [6,8]. The functionalities of these conformations are different but both alternatives have been shown to decrease protein adsorption, which in theory means also decreased uptake by RES [6,8,9].

Mesoporous silicon (PSi) NPs have attracted considerable attention in biomedical applications due to their good biocompatibility, high surface area and large pore volume, which enables effective drug loading [10–13]. In addition, the degradation product of PSi (orthosilicic acid) is nontoxic and has even been reported to be essential for optimal bone and collagen growth [14,15]. These properties have been the impetus for the development of PSi for drug delivery [12,16–18], imaging [19,20] and therapeutic applications [21]. However, up to now, the development of injectable PSi NPs has not been well studied.

In the present work, the aim was to find an optimal PEGylation method for PSi NPs. Efficient PEGylation of the PSi NPs is a challenge due to their porous structure with pore openings on the particle surface, which reduces dramatically the number of the available binding sites for PEG molecules on the external surface.

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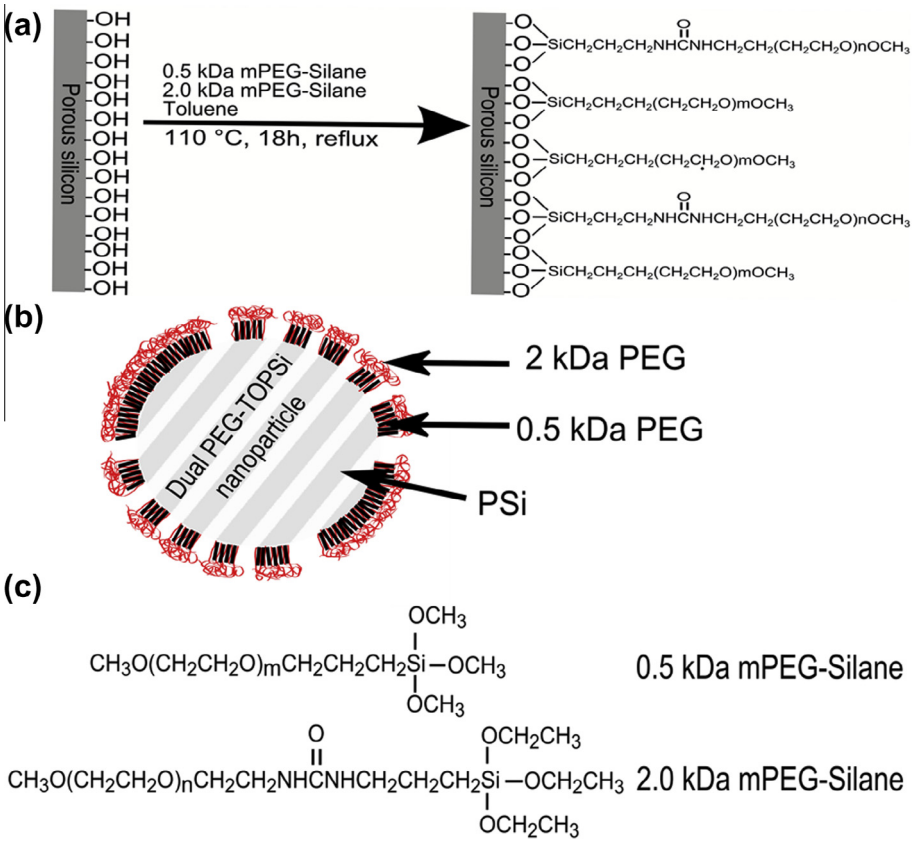

Fig. 1. (a) Scheme for the production of dual PEG-TOPSi. (b) Dual PEG-TOPSi NP with 0.5 kDa PEG forming a brush-like layer (black) and 2 kDa PEG forming a mushroom-like layer (red) at the top of the 0.5 kDa PEG layer. (c) Chemical structures of 0.5 kDa and 2 kDa PEG molecules. In 0.5 kDa PEG m = 9–12 and in 2 kDa PEG n = 44–45.

Here, we demonstrate the effect of dual-PEGylation on thermally oxidized PSi (TOPSi) by using two kinds of low-molecular-sized PEG molecules (0.5 and 2 kDa). Up to now, there have been a large number of studies related to PEGylation of various NPs [2–5], PSi microparticles [22] and even double PEGylation on the same particle [23]. However, as far as we are aware, this is the first report on the simultaneous PEGylation of the PSi NPs with two different molecular sized PEGs. We hypothesized that by simultaneous dual-PEGylation (0.5 and 2 kDa PEG) (Fig. 1a) in a single process, we would be able to create a high-density PEG coating with both brush-like and mushroom-like conformations on the particle surface (Fig. 1b). Furthermore, we will demonstrate that the developed dual PEG-TOPSi NPs display improved colloidal stabilities, prolonged half-lives and increased resistance to protein adsorption and decreased uptake by macrophages in vitro as compared to the uncoated TOPSi NPs.

2. Material and methods

2.1. Preparation of PSi

Single-crystal p^+-type Si wafers (100) (Siegert Wafer GmbH) with resistivity values of 0.01–0.02 Ω cm were anodized in 1:1 (v/v) 38% hydrofluoric acid (Merck Millipore) and ⩾99,5% AA ethanol (Altia Oyj) solution with the pulse sequences described elsewhere [24]. After etching, the PSi films were dried at 65 °C for 1 h. Some of the PSi films were stored for further use with the rest being ball-milled (Planetary Micro Mill, Pulverisette 7, Fritsch GmbH) to produce micro- and nanoparticles, which were separated by sieving and centrifugation, respectively, to obtain different size fractions of PSi particles.

2.2. Thermal and chemical oxidation of PSi NPs

PSi NPs were oxidized at 300 °C for 2 h in ambient air to produce TOPSi. TOPSi was then chemically oxidized to increase the surface density of hydroxyl groups. First, TOPSi was oxidized in H_2O_2:NH_4OH:H_2O (1:1:5 v/v) solution at 85 °C for 5 min and subsequently in H_2O_2:HCl:H_2O (1:1:6 v/v) solution at 85 °C for 15 min. The material obtained was denoted as TOPSi-OH.

2.3. PEGylation of TOPSi-OH NPs

100 μl of 90% 0.5 kDa methoxy-PEG-silane (ABCR GmbH & Co.), 50 mg of 2 kDa methoxy-PEG-silane (Laysan Bio Inc.) or their mixture (100 μl of 0.5 kDa and 50 mg of 2 kDa PEG) was dissolved in 4 ml of anhydrous toluene (Sigma–Aldrich), after which 10 mg of TOPSi-OH NPs was added to the solution. The dispersion was sonicated for 3 min and bubbled with nitrogen (N_2) gas for 20 min to deoxygenate the dispersion. The dispersion was heated overnight (~18 h) at 110 °C under reflux. At the end of the process, the reaction solvent was evaporated and the PEGylated PSi NPs were dispersed in ethanol. The PEGylated PSi NPs were rinsed with ethanol twice and 5–10 min sonication was applied during each rinsing to remove any physically adsorbed PEG. The obtained samples were stored in ethanol for further use. The PEGylated PSi samples were denoted as 0.5 kDa PEG-TOPSi, 2 kDa PEG-TOPSi and dual PEG-TOPSi depending on the PEG molecules being used. For the convenience with N_2 ad/desorption and Fourier transform infrared spectroscopy (FTIR) analyses, PEGylated microparticles and film samples were also prepared by using the same method.