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Engineering multi-stage nanovectors for controlled degradation and tunable release kinetics

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ABSTRACT

Nanovectors hold substantial promise in abating the off-target effects of therapeutics by providing a means to selectively accumulate payloads at the target lesion, resulting in an increase in the therapeutic index. A sophisticated understanding of the factors that govern the degradation and release dynamics of these nanovectors is imperative to achieve these ambitious goals. In this work, we elucidate the relationship that exists between variations in pore size and the impact on the degradation, loading, and release of multistage nanovectors. Larger pored vectors displayed faster degradation and higher loading of nanoparticles, while exhibiting the slowest release rate. The degradation of these particles was characterized to occur in a multi-step progression where they initially decreased in size leaving the porous core isolated, while the pores gradually increased in size. Empirical loading and release studies of nanoparticles along with diffusion modeling revealed that this prolonged release was modulated by the penetration within the porous core of the vectors regulated by their pore size.

1. Introduction

Porous silicon (pSi) is a biomaterial that is non-cytotoxic [1], degradable [2] and photoluminescent [3]. The ability to control its fabrication, surface, loading and release of imaging and therapeutic moieties transformed pSi into an extremely powerful and versatile material for biomedical applications [4,5]. As a result, pSi finds applications ranging from tissue engineering [6,7], to optoelectronics [8,9], to biomedical devices [10], and brachytherapy [11].

Recently, significant research efforts have been devoted to pSi platforms for the delivery of drugs [12–14] and proteins [15,16]. The use of mesoporous (i.e., pores ranging from 2 to 50 nm) silicon allows for the targeting and delivery of payloads (drugs, biologics, nanoparticles, etc.) to diseased sites, while providing controlled release over the embedded agents and effectively resulting in their confinement, protection, and entrapment along the journey [17]. Nanoparticles have emerged with the potential to target and deliver immense payloads to the site of action, maximizing efficacy while limiting adverse side effects [18,19]. However, due to limitations regarding their shape, size, surface charge, and inadvertent environmental activation, they present themselves imperfectly leading to their sequestration by biological barriers [20].

Thus by using advanced modeling of the microvasculature, blood flow dynamics, mechanisms of endothelial cells endocytosis and mononuclear phagocyte system (MPS) sequestration [21–25], our group engineered multi-stage nanovectors (MSV) designed specifically to overcome biological barriers and deliver therapeutic and diagnostic agents to the target site [26–29]. This approach decouples the multitude of tasks typically required by nanoparticles and distributes them onto multiple stages. The first stage is responsible for the storage and protection of the nanoparticles (i.e., second stage) upon systemic administration. This stage is based on nanoporous silicon particles of defined size and shape with tunable pore size (5–150 nm) and porosities (30–90%) [30].

Previous research into the degradation of pSi has shown that their byproducts are released in the form of monomeric orthosilicic acid, a highly abundant trace element in organisms vital for normal bone homeostasis [31]. Furthermore, research efforts demonstrated that slight increases in orthosilicic acid in the bloodstream were well-maintained and excreted in an efficient and timely manner [32]. Additional endeavors employing inductively coupled

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plasma–atomic emission spectroscopy (ICP–AES), showed that the dissolution kinetics of pSi films could be tuned through the control of pH and temperatures [31]. These results yielded interest in the development of injectable pSi nanovectors that degrade in a controlled spatial and temporal manner. The complete degradation of these materials can generally span from minutes to hours to days and is significantly shorter than polymeric particles (weeks–months) [19].

Motivated by these studies, we aimed to understand the effect of modifications in the engineering of MSV pores has on their degradation and release profiles. Here, we investigated MSV with pore sizes ranging from 10 to 50 nm and monitored their degradation using ICP–AES, scanning electron microscopy (SEM), and flow cytometry to quantify distinct degradation rates attributed to each pore size. The loading and release of model nanoparticles, quantum dots (QD), was followed using flow cytometry and release data was interpreted using a continuum diffusion model to elucidate the contribution of pore size to release.

2. Materials and methods

2.1. Multi-stage nanovector particle fabrication

MSV were microfabricated according to our published protocols [30]. We formed arrays of 2 μm diameter, 300 nm deep cylindrical trenches into SiN masked, 0.005 W cm, p-type silicon wafer by UV photolithography and reactive ion etch. We selectively porosified the trenches by electrochemical etch of the patterned substrate in HF ethanoic solution, first applying a porosification current profile and then increasing the current to form a highly porous layer at the particle/substrate interface to allow particle release. The solution composition and porosification current density depends on the target pore size and porosity for every specific particle and is detailed in our previous publication [30]. Higher current or higher ethanol to HF ratio leads to higher porosity and larger pores. Nonporous MSV were fabricated by forming an array of 2 μm disks in a 330 nm thick layer of polycrystalline Si (PolySi) grown over 800 nm of LPCVD oxide. MSV were detached from the substrate by lift-off through buffered oxide etch of the LTO sacrificial layer. All particles were thoroughly rinsed in DI water multiple times to ensure removal of processing reagents. This procedure is depicted in Fig. 1A, along with a solid works representation of MSV (Fig. 1B) and representative SEM images of the MSV shape/size (Fig. 1C) and pores (Fig. 1D). The particles were then stored in isopropanol in controlled environmental conditions.

2.2. Porous structure characterization

The pore size distribution and porosity of the particles were characterized by N_2 adsorption/desorption isotherms according to the Barret–Joyner–Halenda and the Brunauer–Emmett–Teller models respectively. A collection of oxidized MSV from 10 independent fabrication processes were mixed, centrifuged to form a pellet, the supernatant was removed and the pellet was transferred to a sample cell and allowed to dry at 80 °C overnight in a vacuum oven. The sample was degassed at 200 °C for 12 h and the isotherms were measured at 77 K in a Quantachrom Autosorb-3B.

2.3. Surface modification of porous silicon microparticles

Surface modification of MSV was achieved using established protocols [26]. Briefly, MSV were oxidized using a piranha etch treatment (1 volume H_2O_2 and 2 volumes of H_2SO_4) for 2 h followed by extensive washings in water. MSV were modified 3-aminopropyl triethoxysilane (APTES; Sigma Aldrich, St. Louis, MO) using a 2% (v/v) solution in IPA for 2 h at 35 °C with mixing.

2.4. Experimental procedure for degradation in simulated physiological conditions

Phosphate Buffered Saline (PBS; Invitrogen; Carlsbad, California), without $CaCl_2$ and $MgCl_2$ at pH 7.2, was used to investigate the degradation of MSV. 1×10^8 MSV were equally split into three low-binding micro-centrifuge tubes (VMR; West Chester, PA) and diluted to a concentration of 1.515×10^7 particles per mL with 0.025% Triton X-100 (Sigma–Aldrich) in PBS. Samples were rotated in triplicate on a LabQuake® tube rotator (Thermo Fischer Scientific; Waltham, MA) at 10 rpm for 72 h at 37 °C.

2.5. Inductively coupled plasma–atomic emission spectroscopy (ICP–AES)

At pre-determined times, 100 μL aliquots were removed, placed in nylon centrifugal 0.45 μm filter tubes (VWR), and centrifuged for 10 min at 4200 rpm to remove any particle debris from the sample. The flow through was collected and stored at 4 °C for further analysis. Samples were diluted 1:100 in water containing 1 ppm of yttrium and measured using a Varian Vista Pro Simultaneous Axial Inductively Coupled Plasma–Atomic Emission Spectrometer (Varian; Palo Alto, CA) housed at Rice University's Geochemistry Laboratory, as previously described [27].

2.6. Scanning electron microscopy

Samples were washed thrice in deionized water to remove salt, placed on aluminum mounts (Ted Pella; Redding, CA), and left in a vacuum desiccator to dry overnight. Samples were analyzed in a Zeiss Neon 40 microscope equipped with an in-lens detector at an acceleration voltage between 2 and 5 keV at a working distance of approximately 4 mm.

2.7. Flow cytometry

Aliquots from each specified time point were spun down at 4200 rpm for 10 min and the supernatant was discarded. The pellet was re-suspended in 150 μL of DI water and changes in side and forward scatter were measured using a BD FACSCalibur™ system (BD Biosciences; San Jose, CA) analyzed with CellQuest. The instrument's detectors (FSC & SSC) were calibrated and adjusted for particle recognition as previously described [26], in order to accurately evaluate intact and non-degraded particles. Bivariate counter plots and three-dimensional plots were generated, that displayed SSC versus FSC to evaluate the changes in shape and size of the particles over time. The overall shapes and distributions of particles were observed and the geometric means of the FSC and SSC were recorded.

2.8. Loading and release of quantum dots

SP, MP, LP, and XLP MSV were loaded with QD following established protocols [26,30]. Briefly, MSV were exposed to 525-carboxyl QD (Invitrogen) in 200 mM Tris–HCl (Sigma–Aldrich) for 15 min under rotation. MSV were centrifuged and washed in water to remove unloaded QD and a small aliquot was collected for initial analysis. QD-loaded MSV were suspended in PBS containing 0.025% Triton X-100 and placed on a tube rotator (10 rpm) incubated at 37 °C. At pre-determined times, aliquots were removed and centrifuged at 3500 × g, supernatants were discarded, and MSV were stored at 4 °C until analysis could be preformed. Fluorescent signal associated with MSV was acquired using a BD FACS Fortessa (BD Biosciences) equipped with a 488 nm excitation source and a forward scatter photomultiplier tube housed within The Methodist Hospital Research Institute's Flow Cytometry Core.

2.9. Diffusion modeling

A continuum diffusion model was created using a discretized continuum Finite Element (FE) method [33,34]. In brief, the fundamental relation in the continuum description of diffusion is Fick's law:

$$\mathbf{J} = -D\nabla c \tag{1}$$

where $\mathbf{J}$ is the mass flux and c is the concentration gradient. The governing mass balance equation can be written as

$$-\frac{\partial c}{\partial t} + \frac{\partial}{\partial x_i}\left(D\frac{\partial c}{\partial x_i}\right) + q = 0 \tag{2}$$

where q is a source term, and summation is implied on the repeated index $i = 1,2,3$. This equation is further transformed into a balance equation of a single finite element by using a Galerkin procedure. Since the diffusion coefficient D is a function of concentration c, an incremental-iterative scheme is employed within the implicit solution algorithm (governing equations are satisfied at the end of the time step) that suppresses error propagation. This FE model is incorporated into the FE software PAK [35] used for linear and nonlinear analysis of solids, fluids, field and coupled problems, and in bioengineering [33,34].

Pore models were constructed as cylinders with 700 nm length and 15, 26 and 51 nm diameters, having one end closed. Based on geometrical considerations, the maximum concentration of 10 nm QD was calculated to be 3.2 mM if pores are filled 100%. First, 51 nm XLP pores were assumed to be filled 100%. Then diffusion coefficient D was predicted by matching experimental release profile of QD release, where D was found 8×10^{-6} μm^2/s. Next, using derived D, MP and LP experimental release profiles were matched by adjusting pore filling. In our approach, D serves as a transport coefficient that integrates restrained QD diffusion inside nanoscale-confined spaces [36–38] and silica matrix degradation effects on release.

2.10. Statistical analysis

All the data are the result of samples measured in triplicates. Statistics were calculated with Prism GraphPad software. Linear regression analysis was performed and significance was calculated by testing whether slopes. Nonlinear regression analysis was performed using a one-phase association fit and constraints of Y_0 at 0.0 and plateau equal to 100. The rate constants were then compared to each other using an extra sum of squares F test. Loading results were tested for significance using a one-way ANOVA followed by a Tukey post-test to compare all pairs of columns.