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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.

Keywords

Degradation; Drug delivery; Nanoparticle; Porosity; Porous silicon; Nanovector

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].

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Appendix A. Supplementary material: Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biomaterials.2013.07.049>.

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 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 $^{\circ}\text{C}$ overnight in a vacuum oven. The sample was degassed at 200 $^{\circ}\text{C}$ 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 $^{\circ}\text{C}$ 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 $\times$ -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 $^{\circ}\text{C}$.

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 $^{\circ}\text{C}$ 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 ×–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 performed. 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:

$$\mathbf{J} = -D\nabla c \quad (1)$$

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

$$\frac{\partial c}{\partial t} + \frac{\partial}{\partial X_i} \left(D \frac{\partial c}{\partial X_i} \right) + q = 0 \quad (2)$$

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 $8 \times 10^{-6} \mu\text{m}^2/\text{s}$. 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.

3. Results and discussion

3.1. MSV characteristics

Fig. 1A displayed the procedures by which MSV were fabricated resulting in uniform populations of particles (Fig. 1C) that can be imparted with varying pore sizes (Fig. 1D). In this study, five MSV types were investigated: nonporous (NP); small pores (SP); medium pores (MP), large pores (LP) and extra-large pores (XLP). NP were non-porous and as previously reported [30], SP had a 10 nm pore diameter and 46.3% porosity, MP 15 nm pores and 51.1% porosity, LP 26 nm pores and 66.1% porosity, and XLP 51.3 nm pores and 82% porosity (Fig. 1E). The volume dispersed as measured by coulter counter and the mass of each MSV was compared to all MSV investigated (Fig. 1F). As expected, decreased volumes per MSV were observed as pore size increased, as did the mass of the individual MSV ranging from 3 to 9 pg/particle for XLP to SP respectively. NP does not conform to this rule as they were fabricated using silicon of a different density and were smaller than SP-XLP MSV. Furthermore, zeta potential measurements (Fig. 1G) revealed that the surface for both oxidized and APTES modified MSV was equivalent.

3.2. Degradation of MSV

SEM and flow cytometry were used to characterize the qualitative and quantitative dynamics that occur to the overall shape, size, and pores of MSV during degradation. Upon inspection of Fig. 2, we observed a progression in the manner and time-scale of degradation that highlighted a correlation between pore size and MSV degradation rate. SEM images demonstrated that MSV initially had consistent shapes consisting of a central region with vertically aligned pores of uniform size, surrounded by a porous ring with angled pores constituting the corona. This corona is less prominent on XLP and not present in NP due to the different fabrication strategy. As time progressed, NP preserved their overall size and shape displaying minimal signs of degradation over 72 h. On the other hand, MSV experienced a significant effect on their size and shape during degradation due to their porous nature. As a matter of fact, as the porosity increased (SP to XLP) we observed a shift in their overall size with time. From these observations, certain windows could be delineated where MSV were no longer present or had completely degraded: SP, after 48 h; MP, after 24 h; LP, after 18 h; and XLP, after 12 h.

Flow cytometry analysis was performed to confirm and quantitate the shape and size changes of MSV over time. Fig. 2 shows bivariate contour plots and 3D plots at each time, for the different MSV analyzed. Contour plots were used to examine the size (FSC) and shape (SSC) uniformity of MSV. While 3D plots were useful in determining the size and shape distribution with respect to the number of events/counts (z -axis). Contour plots illustrated that MSV began with compact and uniform distributions displaying consistent size and shape throughout the events acquired prior to the start of degradation. However, given sufficient time the size and shape distribution progressively became decentralized, displaying wide variations and gradually drifting toward the origin (lower left) of plots (indicative of dramatic changes in their structure). The progression of this distribution

followed a similar porosity-dependent trend where those with low porosity (SP) retained structure stability for longer periods of time compared with higher porosity (LP, XLP) MSV. SP predominately began accumulating at the origin at 48 h, MP between 18 and 24 h, LP at 18 h, and XLP at 12 h. Comparably the 3D plots originated with a centralized peak (black arrow-heads) and with time, a shift toward the origin was similarly seen (indicated by red arrows). This shift indicated that a larger concentration of MSV was undergoing degradation with increasing events acquired with smaller sizes and shapes. In addition, equivalent patterns were observed after plotting the geometric means of FSC and SSC (Supplementary Figure S1A&B) over time. The data confirmed that MSV degraded in distinct windows such that the higher pore sized particles were completely degraded (i.e., reached zero) at different times.

Inspection of magnified SEM images revealed a distinctive pattern common to all MSV undergoing degradation (Fig. 3A). As time increased, the corona (or porous ring) is observed as the first major feature to experience the effects of degradation. Consequently, MSV experienced a significant decrease in size as the corona eroded away leaving the highly porous central core occupied predominantly with vertically aligned pores. At the same time, the pores contained within the core were also subjected to degradation. However compared with the size of MSV, the pores on the backside and front-side of MSV became larger with time thus increasing the effective porosity of MSV (Fig. 3B). When comparing the pores on the front-side of different pore sized MSV (SP & XLP), we observed the same phenomenon but over distinct time frames.

ICP–AES was used to measure the dissolution of silicon that accumulated in solution during MSV degradation. Fig. 3C plotted the rates observed in different MSV of varying pore sizes by plotting time versus the percentage of total silicon detected in solution and fitting the values with a one-phase association exponential equation with Y_0 and plateau set at 0 and 100, respectively. We observed highly significant ($p < 0.0001$) and distinct rate constants for each MSV analyzed. In addition, using simple linear regression analysis to test if the slopes were significantly different revealed that the slopes governing the rate of degradation of MSV were very significant throughout the entire data set and extremely significant within the first 24 h with 0.12% and 0.012% (respectively) chance of randomly choosing data with slopes this different. The exponential fitting of the points provided specific rate constants for each MSV such that we could extrapolate values where the particles have degraded 50% and 95% (Supplementary Table 1). As expected, the rate constants for each MSV increased from NP to XLP ranging from 0.0034 to 0.1711 h⁻¹. Fig. 3D plotted the pore size (P , nm) versus the time (t , h) needed to achieve 50% and 95% degradation revealing a negative correlation between this pairing. This type of analysis permits one to engineer MSV such that they degrade at a given percentage within a specified time frame (i.e., degradation index) based on tailoring its pore size. A linear fit gave us equations to determine the time in order to achieve 50% (3) and 95% (4) degradation:

$$t_{50\%} = -0.239 \times P + 15.86 \quad (3)$$

$$t_{95\%} = -1.031 \times P + 68.53 \quad (4)$$

3.3. Loading of QD into MSV

The retention of 10–15 nm QD was used to understand the effect porosity has on the loading and release kinetics of nanoparticle payloads within MSV. Opposing electrostatic charges between QD and MSV, specifically using carboxyl and amino terminal groups on their

surface respectively, was used to enhance loading, as previously demonstrated [26]. The retention, distribution, and depth of penetration of QD within MSV porous matrix were investigated using scanning transmission electron microscopy on embedded ultrathin sections of loaded particles (Fig. 4A and B). High magnification of images in part A for MP MSV (Fig. 4B) exhibited a large extent of size exclusion toward QD showing high concentrations of nanoparticles at the surface (red arrows, Fig. 4B) with minimal penetration. On the other hand, MSV with larger pores demonstrated substantial QD penetration within their porous matrix (red arrows, Fig. 4B) with XLP MSV displaying uniform distribution throughout the entire pore. Fluorescent quantification of QD loading using flow cytometry confirmed that MSV exhibit an extremely significant size-dependent correlation between the pore size of MSV and the diameter of QD (Fig. 4C). As the pore size increased from 10 nm (SP) to >50 nm (XLP), the median fluorescence associated with the MSV progressively escalated from 12,000 to 65,000 indicating increased loading in MSV with larger pores.

3.4. Release of QD from MSV

Following the loading, the release dynamics of QD from MSV were investigated from MP, LP, and XLP (SP was not followed as negligible QD penetration was observed) using flow cytometry. The percentage released at various times of each MSV was fitted with a one-phase association exponential equation using constraints of Y_0 equal to 0.0 and the plateau at 100 (Fig. 5A). We observed that MSV with larger pore sizes, and containing higher concentrations of QD, released at a slower rate (i.e., smaller rate constant) compared to those with smaller pore sizes. For example, at 2 h MSV released 75%, 63%, and 38% of the QD payload for MP, LP, and XLP respectively. While at 24 h, MP and LP had released 97% and nearly its entire payload while XLP only had released 83% of its initial payload within the same time period. These values then corresponded to distinct, highly significant rate constants that decreased as the pore size of MSV increased, ranging from 0.66 to 0.18 h^{-1} for MP to XLP respectively. Similar to the analysis for degradation, Fig. 5E plotted the pore size (P , nm) versus the time (t , h) estimated to achieve 50% and 95% release of embed payloads (Supplementary Table 2). However, in this case, a positive correlation was achieved for this pairing and enables future investigators to tune their release rate by adjusting the pore size of MSV. A linear fit through these points, gave us equations to determine the time in order to achieve 50% (5) and 95% (6) release:

$$t_{50\%} = 0.079 \times P - 0.234 \quad (5)$$

$$t_{95\%} = 0.334 \times P - 1.010 \quad (6)$$

3.5. Mechanism of MSV degradation

Surveying the degradation results (Figs. 2 and 3), we observed distinct time frames for each MSV investigated that displayed a consistent evaluation among the various techniques used (ICP–AES, flow cytometry and SEM). For example, NP showed minimal degradation and their shape remained conserved throughout the duration of the experiment, SP showed complete degradation within 48–60 h, MP within 36 h, and LP and XLP completely degraded within 24 and 18 h respectively.

Taking this into consideration, we proposed a mechanism (Fig. 6A) that describes how MSV progressively degrade. Inspection of SEM images revealed that MSV degraded such that the highly porous corona was the first main feature to experience significant degradation and began eroding away, allowing for increased surface area and decreasing the amount of bulk

silicon. While the corona of MSV degraded at a much quicker rate, the central highly porous region was isolated where the pores demonstrated an overall increase in their diameters. This increase was induced by the degradation of the pore wall resulting in decreased wall thickness and larger pore diameters ultimately resulting in unstable MSV whose pores collapsed due to insufficient structural integrity. Hence, the overall degradation of MSV occurred such that the outer envelop (corona) experienced faster degradation attributed to increased exposure of this region to the environment, compared to the core, resulting in smaller sizes over time. Upon eroding the corona, the pores contained within the core experienced a drastic enlargement due to the pore wall degradation process. The presence of larger pores accelerates this process as it results in premature core instability triggering their eventual collapse within a much faster time frame than MSV with smaller pores.

The degradation of MSV can be likened to the two main mechanisms that govern that of polymer-based material, specifically surface and bulk degradation [39]. Surface degradation was characterized as occurring only at the surface resulting in smaller sized particulates, leaving the core of the material intact during degradation. On the other hand bulk degradation results in the uniform erosion throughout the material yielding fragments as byproducts [40,41]. Hence, together, these two mechanisms can be used to describe the mechanism of degradation that is observed on the overall shape and pores of MSV, respectively. The initial erosion of the MSV corona left a circular region (or core) composed of pores intact a feature distinctive of surface degradation. While the pores exhibited a uniform increase in diameter resulting in pore fragment-like structures, consistent with bulk degradation.

3.6. Interplay between pore size and QD release

Tuning the pore size of MSV functioned as an effective method to regulate the loading and release of nanoparticles. QD loading in MSV was confirmed using high-resolution electron microscopy and flow cytometry demonstrating QD infiltrating pores of MSV large enough to accommodate their size (10–15 nm) and highly significant increased loading in MSV with larger pores. We postulated that this occurred due to the higher penetration of QD within the porous matrix of MSV with larger pore sizes. Monitoring the gradual release over time of QD from MSV permitted the investigation of this assumption. A diffusion model was developed to calculate the mass release of QD for MP, LP and XLP using a recently developed numerical technique [33] under the assumption of varying penetration of QD attributed to potential obstruction by pore confinement. Hence on these observations, we assumed QD-induced stabilization of MSV and XLP were fully loaded with QD throughout the 700 nm pore length (Fig. 6B). The first assumption was justified due to the prolonged release not consistent with degradation kinetics that exhibited more than 80% silicon dissolution within the first 10 h. The second assumption relied on the large XLP and QD pore size to diameter ratio, where QD penetration is least obstructed by pore confinement. While the pores of XLP may be loaded 100%, the model analysis showed that experimental release may be matched only if LP and MP are filled to a penetration of 300 nm (40%) and 130 nm (18%), respectively (Fig. 6C). This finding concurs with our previous experimental and theoretical work, where we have shown that nanoparticle penetration into nanochannels was controlled by varying the channel height [42].

Although common convention predicts that the degradation rate controls the release of embedded payloads, it fails to account for the transport of QD diffusion inside nanoscale-confined space and the effect of payload on the degradation of the silicon matrix. Previous reports have demonstrated that nanochannels can experience significant pH shifts due to properties of within the loading milieu [43,44]. In our case, the acidic nature of the carboxyl groups on QD can reduce the local pH within the pores resulting in slower dissolution of MSV, as observed in other pSi materials [31]. Hence in our experiments the impact of MSV

degradation on the payload release was minimal and largely dependent on the payload penetration within the porous matrix imparted by the pore size of MSV.

In summary, tuning the pore size of MSV revealed variations in their overall degradation and loading/release dynamics. Engineered MSV with larger pores exhibited accelerated degradation kinetics, higher loading concentrations of nanoparticles, and prolonged release of payloads attributed to deeper pore penetration. Thus tailoring the pore size of MSV has substantial repercussions on several of their functions, including degradation and release.

4. Conclusion

The effective and timely delivery of drugs is the ultimate goal of any delivery system. Adjusting the pore size of MSV prompted tunable degradation kinetics and unique release profiles corresponding to the degree of payload penetration, a correlation that could potentially give rise to tailored drug delivery vectors. These types of nanovectors allow for the engineering of particles whose pore size can control the release profile of a drug or nanoparticles nested within the pores. Thus, by tuning the pore size of MSV one can control the rate of degradation and potentially optimize the release of nanovectors to tailor the pharmacokinetics of the agent within.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. MSV fabrication and characterization. A) Schematic of MSV fabrication showing order of procedures from photolithography and electrochemical etch to release of MSV (black arrow) under sonication. B) Solid works model of MSV used to approximate mass. C) Low magnification SEM micrograph of MSV illustrating uniform size and shape, scale bar = 1 μm . D) High magnification SEM micrograph of MSV with different pore sizes, starting from top left and proceeding clock-wise: SP, MP, LP, XLP; scale bar = 50 nm. E–F) Bar graphs comparing pore size (G) and volume (F) on the left axis and porosity (G) and mass (F) on the right axis for the MSV investigated. F) Bar graph comparing the surface charges of MSV after oxidation (stripped) and APTES (solid).

Fig. 2.

MSV degrade in distinct times after pore size adjustment. SEM micrographs of MSV at various times emphasizing the impact pore size have on the overall changes in shape and size of MSV, scale bar = 1 μm . Contour and 3D plots of FSC versus SSC (z-axis is counts) acquired from flow cytometry confirm the observed changes in MSV size and shape over time. Black arrows indicate the peak of intact, or non-degraded, MSV while red arrows are used to specify where the degradation by-products accumulate. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3.

Qualitative and quantitative validation of degradation impact on MSV. A) SEM micrographs depict the degradation experienced by MSV over time on both their frontside and backside. Images were split to show the effect on the particle's shape and size (left, scale bar = 1 μm) and on its pores (right, scale bar = 100 nm). B) High magnification SEM images of MSV pores comparing SP & XLP at different points during degradation, scale bar = 100 nm. C) ICP–AES quantification of the concentration of silicon deposited in solution for each MSV. Values were then fitted with an exponential equation resulting in distinct degradation rates. D) Values extrapolated from the exponential fit to determine 50% and 95% degradation were plotted as pore size versus time. A linear straight fit was then applied to give an equation relating the two variables at each percentage of degradation.

Fig. 4. Loading of QD into MSV of varying pore sizes. A) Low magnification of scanning transmission electron microscopy images of MSV loaded with QD. B) High magnification images of the inset within A (red box), confirming the accumulation of QD (red arrows) at the surface (MP) or penetration within the porous matrix (LP, XLP). C) QD loading quantification within the different MSV tested. Each MSV demonstrated a highly significantly different ability to load QD ($***p < 0.001$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

Release of QD from MSV. A) The release of QD was investigated and plotted with an exponential fit producing distinct release rates for each MSV. B) Values extrapolated from the exponential fit to determine 50% and 95% release were plotted as pore size versus time. A linear straight fit was then applied to give an equation relating the two variables at each percentage.

Fig. 6. Mechanism of MSV degradation and modeling release based on payload penetration. A) Schematic illustrating the proposed degradation mechanism of MSV. B) A diagram depicting the parameters and assumptions used to develop the diffusion model. The diagram shows that QD (green spheres) penetrate deeper in XLP and that the concentration of QD decreases as they approach the pore opening. C) The resulting data from the model is plotted against the actual values corresponding to their pore size and pore penetration of 18%, 40% and 100% for MP (15 nm), LP (26 nm), and XLP (51 nm) respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)