ABSTRACT SUMMARY
The influence of porosity and pore size on the degradation, loading, and release dynamics of multistage nanovectors (MSV) was studied. Adjusting the pore size from 10 to 50 nm resulted in distinct degradation rates, loading concentrations, and release kinetics such that larger pores yielded faster degradation but could contain more nanoparticles and extend their release kinetics compared to smaller pores. Diffusion models and electron microscopy images confirmed that these prolonged release rates were attributed to deeper payload penetration within MSV. Hence, a desired release and degradation rate can be achieved by tuning the pore size of MSV.

INTRODUCTION
Nanoparticles emerged as potential carriers capable of concentrating poorly soluble drugs and accumulate at the tumor site relying on the enhanced permeability and retention effect. However, depending on their size, shape, charge, and surface properties they present themselves imperfectly to the multiplicity of biological barriers encountered upon systemic administration resulting in minimal clinical success [1,2]. It is unreasonable to request a single nanoparticle to be bestowed with all the necessary tools needed to evade these barriers while retaining selectivity for the tumor site.

We used advanced mathematical modeling and engineering to develop a multi-stage delivery system designed to sequentially overcome biological barriers to safely deliver therapeutic and imaging agents to the target site [3,4]. This delivery strategy decouples the tasks required to a delivery system onto multiple stages. The first-stage is composed of porous silicon, a well-known biomaterial for its biocompatibility, degradability, photoluminescence, versatile fabrication parameters, and tunable pore sizes [5]. Embedded within the first stage there are second-stage nanoparticles that can retain drugs, biologics, or contrast agents (third-stage). We have previously demonstrated the loading of a variety of nanoparticles [6] and extended release in vivo resulting in prolonged gene silencing [7] and tumor reduction [8]. In this study, we further investigated the effect of pore size on the degradation, loading, and release dynamics of multistage nanovectors (MSV) and its potential implications towards controlled drug delivery.

EXPERIMENTAL METHODS
3 µm hemispherical MSV were fabricated as previously described with different porosities [9], oxidized using a piranha treatment, and modified with amino proplytriethoxysilane (APTES) to obtain a positive charge. The following MSV pore sizes were used: 10 nm, small pores (SP); 15 nm, medium pores (MP); 25 nm, large pores (LP); 50 nm, extra-large pores (XLP). For APTES modification, MSV were incubated with a 3-5% (v/v) APTES solution in IPA and reacted for two hours at 35°C with mixing.

Degradation of MSV was characterized using inductively coupled plasma–atomic emission spectroscopy (ICP-AES), scanning electron microscopy (SEM), flow cytometry, and size distribution analysis. 1x10⁶ APTES MSV were suspended in phosphate buffered saline and rotated at 15 rpm at 37°C for 72 hours. Samples for ICP-AES were measured using a Varian Vista Pro housed at Rice University’s Geochemistry Laboratory. Samples were diluted in water, containing an internal standard to account for instrument drift, and measured for silicon content. Samples were imaged using a Field Emission SEM. Flow cytometry was acquired using a BD FACS Calibur previously calibrated for MSV [4]. Size distribution analysis was obtained using a Beckman Coulter Counter.

The loading and release studies were performed using 15 nm 525-carboxylated-quantum dots (QD) into APTES MSV. 3x10⁶ MSV were loaded in Tris-buffer containing 2 µM QD and measured using flow cytometry using the FITC filter settings. Release was performed at 37°C, rotating at 15 rpm for 48 hours in PBS. At pre-determined times, aliquots were removed and read at flow cytometry.

RESULTS AND DISCUSSION
The degradation rates of MSV with different pore sizes exhibited a distinct rate with a linear correlation to its pore size (i.e., larger pores, faster degradation rate).

![Figure 1. MSV degradation rate. The amount of silicon deposited in solution was determined using ICP and graphed vs time. MSV showed distinct degradation rates dependent on their pore size.](image)
ICP-AES (Figure 1) demonstrated unique linear degradation rates within the first 24 hours for SP (10 nm), MP (15 nm), LP (25 nm), and XLP (50 nm). Linear regression analysis had a significantly different rate from each other. SEM images illustrated that each MSV degraded in a specific manner. The first main feature to be lost is the highly porous ring surrounding the central core of MSV (Figure 2A). This degradation allowed for an increased surface area and decreased the total amount of silicon remaining in MSV. This resulted in MSV of smaller sizes containing the porous central core. SEM images of the pores initially exhibited slow increases with time, but once the porous ring had degraded the pores experienced rapid increases until the MSV could no longer support itself resulting in the collapse of the pores and MSV (Figure 2A,B). Flow cytometry and size analysis confirmed that the overall size and shape of MSV decreased in a time-dependent manner consistent with pore size, validating the ICP-AES data. Flow cytometry showed MSV’s size and shape became less uniform and clustered, beginning to deteriorate at different time frames regulated by their porosity. Size analysis illustrated dramatic shifts in their size, indicating overall changes in diameter that occurred, as expected, to depend on the pore size of the MSV. Flow cytometry of MSV degradation. A. MSV and pore degradation as time increases, porous ring around MSV degrades rapidly followed by increases in pore sizes. B. XLP experienced quicker pore enlargement compared to SP.

The loading of QD into MSV displayed a pore-size dependency such that larger pores were capable of retaining more QD. The release of QD from MSV revealed that larger pores enabled prolonged release. Release occurred rapidly with 75%, 65%, and 40% being released within the first two hours from MP, LP, and XLP respectively (Figure 3). At 24 hours, both MP and LP had released nearly 100% while only 80% had been released from XLP within the same time frame. A diffusion model was developed to investigate the mass release of QD from MSV. Under the assumption that QD could fully penetrate XLP, the model analysis was able to match experimental release rates only if LP and MP were filled to a penetration of 300 nm (~40%) and 130 nm (18%), respectively. Hence, the prolonged release rates in larger pore MSV is attributed to the deeper penetration experienced in these pores.

![Figure 2](image2.png)

**Figure 2.** SEM of MSV degradation. A. MSV and pore degradation as time increases, porous ring around MSV degrades rapidly followed by increases in pore sizes. B. XLP experienced quicker pore enlargement compared to SP.

![Figure 3](image3.png)

**Figure 3.** QD release from MSV. The release of QD from MSV was quantified using flow cytometry. XLP and LP exhibited prolonged release compared to MP.

**CONCLUSION**

The timely and efficient delivery of agents is the ultimate goal for any delivery system. Here, we explore the effect that pore size has on the degradation, loading, and release of a novel multi-stage delivery system. We demonstrated that MSV degrade faster as the pore size is increased. The release of MSV exhibited prolonged release for larger pores based on deeper penetration of nanoparticles. This study provides guidance on how to optimally tailor MSV to obtain a desired degradation or release rate.

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