Controlling Silica-induced Oxidative Stress to Facilitate Therapeutic Applications of Engineered Silica

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Statement of Purpose: Ordered mesoporous silica materials have emerged as promising bio-materials for tissue engineering and drug delivery due to their high surface areas and easy pore size control.[1] There exist innumerable studies in literature that have demonstrated controlled retention and release of pharmaceuticals from silica systems. However, these ideas are not practical as silica generates Reactive Oxygen Species in biological systems.[2] One way to address this setback of otherwise medically potential silica materials is by using antioxidant enzymes. We hypothesize that loading silica particles with an anti-oxidant enzyme would overcome the oxidative stress that induces silica toxicity. In order to begin addressing this hypothesis, in the present work, we study the loading and protection of the antioxidant enzyme, catalase loaded into and onto selected engineered mesoporous silica materials.

Methods: *Nanoparticle Synthesis and Characterization.* We synthesized four types of mesoporous silica materials by precipitation of tetraethoxysilane in ethanol / water /ammonia solution - (a) nonporous spherical silica (NPSP)[3] without a template (b) spherical silica with radially oriented pores (SP-R)[3] using cetyltrimethylammoniumbromide to template the pores (c) and hollow spherical silica particles with pores oriented either parallel to the hollow core (HSSP-P)[4] or expanded, interconnected bimodal pores (HSSP-I1 and HSSP-I2) using latex microspheres and cetylpyridiniumchloride as templates. The synthesized materials were characterized for particle size distribution using Scanning Electron Microscopy. The surface area and pore size measurements were performed using N_2 adsorption-desorption.

Enzyme Loading and Analysis. 1.4 mg/ml of catalase and 1 mg/ml of each nanoparticle were suspended in PBS (pH=7.4) buffer solution. After incubating for 1 hour at room temperature, the enzyme loaded particles were centrifuged and used for loading and activity analyses. Each individual particle solution was prepared in triplicates. Amount of enzyme adsorbed on to the particles was determined by measuring ^{125}I labeled catalase content in solution pre and post-configurations using a Γ-counter. The activity of catalase was determined using UV-Vis spectrophotometer from 0.1 mg/ml loaded nanoparticles in PBS added to 4.5 mM H_2O_2. The degradation of H_2O_2 was monitored by measuring absorbance with time at 242 nm. For protection studies, enzyme loaded particles were resuspended in PBS solution with 0.2 wt% solution of pronase and incubated for an hour before activity measurements.

Results: The particles sizes were found to be 0.2, 0.15-0.85 and 0.6 nm for NPSP, SP-R and HSSP particles respectively. BJH pore diameters obtained from N_2 sorption are 2.9 and 3.1 nm for SP-R and HSSP-P particles. HSSP-I1 exhibited bimodal pore sizes of 3.8 and 5.4 nm while HSSP-I2 had 4.2 and 7.5 nm pores. Figures 1a and 1b describe the loading and activity of catalase on silica nanoparticles determined by mass loading and activity analyses before and after an hour of pronase incubation. The advantage of silica particles was clearly seen, as the best performing material possessed ~50wt% and ~28wt% catalase pre and post pronase digestion, respectively. This is in stark contrast to polymer particles synthesized by our group which were still therapeutically effective in vivo, but contained only 2wt% and 0.5wt% catalase pre and post pronase, respectively.[5] In comparison to NPSP, SP-R exhibits higher loading and activity. This indicates that mesoporosity helps stabilize catalase on silica surface. In comparison to HSSP-P, HSSP-I particles exhibit higher loading and activity due to acccessible hollow cores. Larger pores may be accessible to pronase in addition to catalase leading to poorer protection in HSSP-I2.

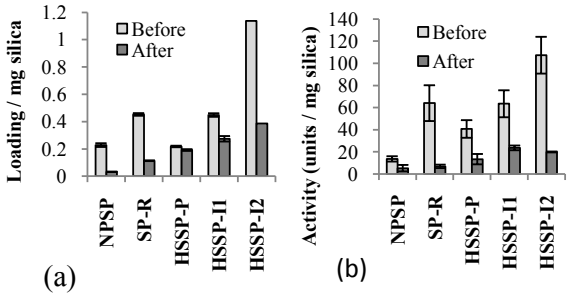

Figure 1. (a) Amount and (b) activity of catalase loaded on silica nanoparticles before and after an hour of proteolysis.

Conclusions: All the mesoporous silica particles displayed the potential for effective catalase loading and protection when compared to non-porous silica. Particles with hollow cores displayed higher loading, activity and protection against pronase when compared to SP-R and NPSP. HSSP-I2 exhibited highest loading but poor protection. HSSP-P offered greatest protection despite having inaccessible pores. This indicates that pore size tuning may be necessary to obtain protection without compromising loading.

References:

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