Published in final edited form as:

Microporous Mesoporous Mater. 2013 July 15; 175: 157–160. doi:10.1016/j.micromeso.2013.03.022.

Non-destructively shattered mesoporous silica for protein drug delivery

Chenghong Lei*, Baowei Chen, Xiaolin Li, Wen Qi, and Jun Liu*

Pacific Northwest National Laboratory, P. O. Box 999, Richland, WA 99352, USA

Abstract

Mesoporous silicas have been extensively used for entrapping small chemical molecules and biomacromolecules for drug delivery. We hypothesize that the loading density of biomacromolecules such as proteins in mesoporous silicas could be limited due to disordering in the pore structure and long diffusion time in the pore channels. We shattered mesoporous silicas non-destructively resulting in improved intramesoporous structures and reduced particle sizes in aqueous solutions by a powerful sonication, where the mesoporous structures were still well maintained. The sonication-shattered mesoporous silica can increase the protein loading density to nearly 2.7 times as high as that of the non-shattered one, demonstrating that significantly more mesopore space of the silica could be accessible by the protein molecules, which may result in more sustained protein drug delivery.

Keywords

Mesoporous silica; Non-destructive shattering; Intramesoporous structure; Protein drug delivery

Introduction

Mesoporous silica can be synthesized with pore sizes from 2–50 nm and particle size from a few hundreds of nanometers to tens of micrometers. The surface area can be up to 1000 m² g⁻¹ with the ordered pore surface accounting for >95% of the total surface area; There are roughly 5 × 10¹⁸ silanol groups per m² on the local pore surface that can be easily functionalized specifically for different applications [1–3]. With rigid, open-pore structures and controllable pore sizes, mesoporous silica can facilitate delivery of small chemical molecules such as ibuprofen as well as biomacromolecules such as proteins [4–18]. Mesoporous silica can also protect the biomolecules from the bacterial decomposition because the pore size is sufficiently small to eliminate any invading bacteria (at least 2 μm in size), making mesoporous silica suitable for hosting many biomolecules. Although the wet chemistry can allow mesoporous silicas to be prepared with small pore sizes (<5 nm) and particles sizes of ~100–300 nm [15, 19–21], mesoporous silicas with large pore sizes are needed to obtain large loading density for many biomacromolecules such as proteins. However, the final step to prepare mesoporous silicas with large pore sizes usually needs calcination at more than 550 °C [1–3]. Such synthetic processes produce agglomerated

© 2013 Elsevier Inc. All rights reserved.

*To whom correspondence should be addressed. chenghong.lei@pnnl.gov; jun.liu@pnnl.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

particles as large as 10–30 μm . We hypothesize that the protein loading density could be limited because some of the pore volume may be inaccessible due to some disordered structures and the long diffusion pathways in the large particles [1, 22]. We further hypothesize that it is possible to mechanically break up mesoporous silica particles and increase the protein loading density due to better accessibility to the pore volume by improving the intramesoporous structures and reducing the particle sizes provided the shattered mesoporous silica can still maintain its mesoporous structure. However, because of the rigidity of as-made mesoporous silica (AMS), it cannot be shattered by a mechanic grinding without the destruction of the mesoporous structure. In this work, we used a powerful sonicator which is normally used to lyse biological cells, and reduced the particle sizes of mesoporous silicas by 3 to 4 times in an aqueous solution and significantly improved the parameters of intramesoporous structure. Remarkably, the sonication-shattered mesoporous silica (SSMS) with the reduced particle sizes still maintained its mesoporous structure and can increase the protein loading density up to 2.7 times as high as that of the corresponding AMS.

Materials and Methods

The commercially as-made mesoporous silica (mesocellular foam-type) was obtained from Aldrich (product number 560979). As-made mesoporous silicas (AMS, mesocellular foam-type) were prepared according to procedures modified from our earlier work [6, 7]. In a typical synthesis, 4.0 g of Pluronic P-123 (MW = 5,800) was dissolved in 2 M HCl solution (120 mL) at 35–40°C. Then 6.0 g of mesitylene (49.9 mmol) and 8.5 g (40.8 mmol) of tetraethylorthosilicate (TEOS) were added to the milky solution and stirred for 6 h at the same temperature. The mixture was transferred into a Teflon-lined autoclave container and heated up to 150°C for 24 h. The white precipitate was then collected by filtration, dried in air, and finally calcined at 550°C for 6 hours.

20 mg of AMSs were suspended in water in 50 mL of falco tube. The AMS suspensions were shattered using Branson Sonifier 250 with a sonicator tip 102C (CE). The sonicator was set at the output level 10 and the sonications were carried out for 5×1 min sonication with 1 min interval time between sonications on an ice bath. Final concentration of SSMSs were 0.5 mg/mL.

4 mL of the AMS or SSMS suspensions (0.5 mg/mL) were filtered with Millipore filter (10,000MWCO) to concentrate the suspension to 2 mg/mL. Then, 0.27 mL of rat IgG (2.956 mg/mL) was added to 1 mL of the concentrated AMS or SSMS suspension. The incubation was carried out at 21 °C shaking at 1200 min^{-1} on an Eppendorf Thermomixer 5436 for 36 h. Next, the mesoporous silica-protein composite was separated by centrifugation at 13 K rpm for 10 min and the supernatant was removed (the elution number: 0). Protein release experiments were carried out at room temperature (21 ± 1 °C) using PBS (pH 7.4, 10 mM sodium phosphate, 0.14 M NaCl) as the release buffer. For each elution, 0.2 mL of the release buffer was added following 5 min shaking and 5 min centrifuge at 13 k rpm. For each subsequent elution with the same elution buffer, the mesoporous silica-protein composite was repeatedly separated by centrifugation and the amount of the released protein in the supernatant was measured by UV at 280 nm. Protein loading densities decreased along the series of elutions.

High resolution TEM was carried out on a Jeol JEM 2010 Microscope with a specified point-to-point resolution of 0.194 nm. The operating voltage on the microscope was 200 kV. Nitrogen adsorption-desorption isotherms for surface-area and pore analysis were measured with a Quantachrome Autosorb Automated Gas Sorption Systems. Particle sizes of

mesoporous silicas were measured using Horiba Laser Scattering Particle Size Distribution Analyzer LA-950.

Results and discussion

In this work (*Materials and Methods*), two mesostructured silicas were tested. One is AMS prepared in this lab (AMS-L), the other one is the commercially available AMS (AMS-C), while the corresponding sonication-shattered ones were called SSMS-L and SSMS-C, respectively. Figure 1 shows transmission electron microscopy (TEM) images of AMSs and SSMSs. Both AMSs and the corresponding SSMSs are made of disordered pores with mesocellular foam porous structure. However, the intramesoporous structural parameters and the particle sizes of mesoporous silicas were changed after the sonication.

Table 1 shows the surface area, pore volume, adsorption pore size and desorption pore size of the AMSs and the corresponding SSMSs via N₂ isotherm sorption measurements. It is worthwhile to note that the pore sizes were estimated using the Barrett-Joyner-Halenda (BJH) method [23]. Although, for the mesocellular foam-type silica, it has been reported that it is more accurate to estimate the pore size using Broekhoff-de Boer-Frenkel-Halsey-Hill (BdB-FHH) method [24], in our pore size range, the pore sizes obtained from the BdB-FHH method are only slightly shifted in the same trend comparing to the BJH method. The adsorption pore size corresponds to the wide cage diameter while the desorption pore size is related to the narrow pore entrance size [25–28]. The results from the N₂ sorption measurements show that, comparing to AMS-C, the Brunauer-Emmett-Teller (BET) surface area and the BJH adsorption pore size of SSMS-C were significantly increased due to the sonication (Table 1), but its desorption pore size (~12 nm) had no change. Comparing to AMS-L, the surface area and adsorption pore size of SSMS-L had no significant change, but its desorption pore size was significantly increased by 52% from 11.13 nm to 16.90 nm due to the sonication (Table 1). Figure 2 shows the particle size distribution of AMSs and SSMSs. AMS-C has a mean particle size of 9.53 μm, while SSMS-C has a mean particle size of 2.57 μm. AMS-L has a mean particle size of 21.77 μm, while SSMS-L has average bead size of 7.01 μm. These results demonstrated clearly that the AMS particles were shattered by the sonication, their particle sizes could be reduced by 3–4 times under the present sonication conditions without the destruction of mesoporous structures (Figure 1). We believe that, under the same powerful sonication, these changing differences are ascribable to the difference of AMS-C from AMS-L in views of the intramesoporous structural disorderness and the depth of pore channels, and the initial particle sizes of AMSs might be thermodynamically important in determination of the changes of the shattered particle sizes, the surface areas, the adsorption/desorption pore sizes.

Next, we tested the protein loading density with AMSs and SSMSs using rat immunoglobulin G (IgG, monoclonal antibody, M.W. 150 kDa). Individual AMS or SSMS was incubated in the antibody solution respectively, where the antibody would be spontaneously entrapped in. We defined the protein amount (mg) of an antibody entrapped with 1 mg of AMS or SSMS as the protein-loading density (P_{LD}). We exploited the IgG loading density in AMSs and SSMSs and studied the IgG release from AMSs and SSMSs in a physiological buffer (*Materials and Methods*) (Figure 3). The resulting AMS- or SSMS-IgG composites were transferred to fresh buffers and eluted multiple times to determine the release kinetics of IgG from the particles. The protein contents of the supernatants in between each cycle of shaking-elution-centrifugation were measured. At the “0 elution” data point, P_{LD} of IgG on both AMS-C and AMS-L was 0.14 mg/mg, while that on SSMS-C and SSMS-L was 0.23 mg/mg and 0.38 mg/mg, respectively (Figure 3). Comparing to the AMSs, P_{LD} of IgG was increased 64% on SSMS-L and 171% on SSMS-L, respectively. We believe that the improved intramesoporous structure and the reduced particle size (Table 1

and Figure 2) of SSMSs allowed more protein access to their internal pore volumes. Our previous work has confirmed that for the neutral protein IgG with a Y-like molecular shape, mesoporous silica with larger desorption pore size allowed larger IgG loading, while the surface area of the material has less effect on IgG loading [29]. Therefore, we believe that more significant increase of P_{LD} of IgG with SSMS-L than SSMS-C was resulted from the larger desorption size of SSMS-L than SSMS-C (Table 1), where the surface area and adsorption pore size of SSMS-L had no significant change comparing to AMS-L. However, the increase of P_{LD} of IgG with SSMS-C were mostly ascribed to the significant increase of the surface area and the adsorption pore size of SSMS-C due to the shattered smaller size, rather than its desorption size which had no change comparing to AMS-C. Nevertheless, the fact that SSMS-L displayed the largest increase in P_{LD} indicated that the desorption pore size affected the most for P_{LD} of IgG since the particle sizes of SSMS-L after shattering was actually much larger than that of SSMS-C.

Because of non-covalent interactions, that is, the electrostatic, H-bond, hydrophilic and hydrophobic interactions of the amino acid residues of IgG molecules with various AMSs and SSMSs, when IgG-AMS or IgG-SSMS composites are dispersed in a fresh buffer solution, a new thermodynamic balance must be reached and thus IgG would be released from AMSs and SSMSs. The subsequent release of IgG from AMSs and SSMSs was carried out in pH 7.4, 10 mM sodium phosphate, 0.14 M NaCl (PBS) (Figure 3). As expected, the spontaneously entrapped IgG were gradually released from AMSs and SSMSs along the series of elutions with the release buffer (Figure 3), where P_{LD} of IgG on SSMSs were still much higher than that on AMSs after last elution, demonstrating the potential advantages of SSMSs over AMSs for more sustained protein drug delivery under physiological or *in vivo* conditions.

In summary, we improved the intramesoporous structures and reduced the particle sizes of mesoporous silicas by 3 to 4 times in aqueous solutions by a non-destructive shattering via powerful sonication, where the mesoporous structures were still well maintained. The sonication-shattered mesoporous silica can result in the protein loading density increased up to 2.7 times as high as that of the non-shattered one, demonstrating that significantly more mesoporous space of the silicas could become accessible for protein loading after the non-destructive shattering which may result in a more sustained protein drug delivery.

Acknowledgments

This research is supported by the NIH National Institute of General Medical Sciences (grant number R01GM080987), the U.S. Department of Energy (DOE) Office of Basic Energy Sciences (Award KC020105-FWP12152), and the Transformational Materials Science Initiative of Pacific Northwest National Laboratory (PNNL). PNNL is a multiprogram national laboratory operated for DOE by Battelle under Contract DE-AC05-76RL01830. Wen Qi thanks the partial financial support from the China Scholarship Council.

References

1. Wan Y, Zhao DY. On the controllable soft-templating approach to mesoporous silicates. *Chemical Reviews*. 2007; 107:2821–2860. [PubMed: 17580976]
2. Zhao DY, Feng JL, Huo QS, Melosh N, Fredrickson GH, Chmelka BF, Stucky GD. Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 angstrom pores. *Science*. 1998; 279:548–552. [PubMed: 9438845]
3. Feng X, Fryxell GE, Wang L-Q, Kim AY, Liu J, Kemner KM. Functionalized monolayers on ordered mesoporous supports. *Science*. 1997; 276:923–926.
4. Han YJ, Watson JT, Stucky GD, Butler A. Catalytic activity of mesoporous silicate-immobilized chloroperoxidases. *Journal of Molecular Catalysis B-Enzymatic*. 2002; 17:1–8.

5. Han YJ, Stucky GD, Butler A. Mesoporous silicate sequestration and release of proteins. *J. Am. Chem. Soc.* 1999; 121:9897–9898.
6. Yiu HHP, Wright PA, Botting NP. Enzyme immobilization using SBA-15 mesoporous molecular sieves with functionalised surfaces. *Journal of Molecular Catalysis B-Enzymatic.* 2001; 15:81–92.
7. Yiu HHP, Wright PA, Botting NP. Enzyme immobilisation using siliceous mesoporous molecular sieves. *Microporous and Mesoporous Materials.* 2001; 44–45:763–768.
8. Takahashi H, Li B, Sasaki T, Miyazaki C, Kajino T, Inagaki S. Catalytic activity in organic solvents and stability of immobilized enzymes depend on the pore size and surface characteristics of mesoporous silica. *Chem. Mater.* 2000; 12:3301–3305.
9. Gomes DEB, Lins RD, Pascutti PG, Lei CH, Soares TA. The Role of Nonbonded Interactions in the Conformational Dynamics of Organophosphorous Hydrolase Adsorbed onto Functionalized Mesoporous Silica Surfaces. *Journal of Physical Chemistry B.* 2010; 114:531–540.
10. Lei C, Soares TA, Shin Y, Liu J, Ackerman EJ. Enzyme Specific Activity in Functionalized Nanoporous Supports. *Nanotechnology.* 2008; 19:125102. [PubMed: 21817721]
11. Lin YS, Haynes CL. Impacts of Mesoporous Silica Nanoparticle Size, Pore Ordering, and Pore Integrity on Hemolytic Activity. *Journal of the American Chemical Society.* 2010; 132:4834–4842. [PubMed: 20230032]
12. Rosenholm JM, Peuhu E, Eriksson JE, Sahlgren C, Linden M. Targeted Intracellular Delivery of Hydrophobic Agents using Mesoporous Hybrid Silica Nanoparticles as Carrier Systems. *Nano Letters.* 2009; 9:3308–3311. [PubMed: 19736973]
13. Rosenholm JM, Meinander A, Peulhu E, Niemi R, Eriksson JE, Sahlgren C, Linden M. Targeting of Porous Hybrid Silica Nanoparticles to Cancer Cells. *ACS Nano.* 2009; 3:197–206. [PubMed: 19206267]
14. Trewyn BG, Nieweg JA, Zhao Y, Lin VSY. Biocompatible mesoporous silica nanoparticles with different morphologies for animal cell membrane, penetration. *Chemical Engineering Journal.* 2008; 137:23–29.
15. Torney F, Trewyn BG, Lin VSY, Wang K. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nature Nanotechnology.* 2007; 2:295–300.
16. Hartmann M, Jung D. Biocatalysis with enzymes immobilized on mesoporous hosts: the status quo and future trends. *Journal of Materials Chemistry.* 2010; 20
17. Russo PA, Ribeiro Carrott MML, Mourao PAM, Carrott PJM. Tailoring the surface chemistry of mesocellular foams for protein adsorption. *Colloids and Surfaces a-Physicochemical and Engineering Aspects.* 2011; 386
18. Cauda V, Fiorilli S, Onida B, Verne E, Brovarone CV, Viterbo D, Croce G, Milanese M, Garrone E. SBA-15 ordered mesoporous silica inside a bioactive glass-ceramic scaffold for local drug delivery. *Journal of Materials Science-Materials in Medicine.* 2008; 19:3303–3310. [PubMed: 18496735]
19. Trewyn BG, Giri S, Slowing, Lin VSY. Mesoporous silica nanoparticle based controlled release, drug delivery, and biosensor systems. *Chemical Communications.* 2007:3236–3245. [PubMed: 17668088]
20. Slowing, Trewyn BG, Giri S, Lin VSY. Mesoporous silica nanoparticles for drug delivery and biosensing applications. *Advanced Functional Materials.* 2007; 17:1225–1236.
21. Slowing, Trewyn BG, Lin VSY. Mesoporous silica nanoparticles for intracellular delivery of membrane-impermeable proteins. *Journal of the American Chemical Society.* 2007; 129:8845–8849. [PubMed: 17589996]
22. Liu J, Shin Y, Nie ZM, Chang JH, Wang L-Q, Fryxell GE, Samuels WD, Exarhos GJ. Molecular assembly in ordered mesoporosity: A new class of highly functional nanoscale materials. *J. Phys. Chem. A.* 2000; 104:8328–8339.
23. Flood, EA.; Linsen, BG.; van den Heuvel, A. *The Solid Gas Interface.* New York, USA: Marcel Dekker; 1967.
24. Lukens WW, Schmidt-Winkel P, Zhao DY, Feng JL, Stucky GD. Evaluating pore sizes in mesoporous materials: A simplified standard adsorption method and a simplified Broekhoff-de Boer method. *Langmuir.* 1999; 15:5403–5409.

25. Kim SS, Karkamkar A, Pinnavaia TJ, Kruk M, Jaroniec M. Synthesis and characterization of ordered, very large pore MSU-H silicas assembled from water-soluble silicates. *Journal of Physical Chemistry B*. 2001; 105:7663–7670.
26. Kim TW, Ryoo R, Kruk M, Gierszal KP, Jaroniec M, Kamiya S, Terasaki O. Tailoring the pore structure of SBA-16 silica molecular sieve through the use of copolymer blends and control of synthesis temperature and time. *Journal of Physical Chemistry B*. 2004; 108:11480–11489.
27. Kruk M, Jaroniec M. Argon adsorption at 77 K as a useful tool for the elucidation of pore connectivity in ordered materials with large cage-like mesopores. *Chemistry of Materials*. 2003; 15:2942–2949.
28. Matos JR, Kruk M, Mercuri LP, Jaroniec M, Zhao L, Kamiyama T, Terasaki O, Pinnavaia TJ, Liu Y. Ordered mesoporous silica with large cage-like pores: Structural identification and pore connectivity design by controlling the synthesis temperature and time. *Journal of the American Chemical Society*. 2003; 125:821–829. [PubMed: 12526683]
29. Qi W, Li X, Chen B, Yao P, Lei C, Liu J. Intramesoporous silica structure differentiating protein loading density. *Materials Letters*. 2012; 75:102–106. [PubMed: 22745517]

- Mesoporous silica was shattered by sonication resulting in improved intramesoporous structure;
- Sonication-shattered mesoporous silica still kept its mesoporous structure;
- Sonication-shattered mesoporous silica allowed much higher protein loading density;
- The resulting higher protein loading density may allow a more sustained protein drug release.

Figure 1. TEM images of as-made and sonication-shattered mesoporous silicas. (A) AMS-C; (B) SSMS-C; (C) AMS-L; (D) SSMS-L.

Figure 2.
Particle size distribution of as-made and sonication-shattered mesoporous silicas. (A) AMS-C and SSMS-C; (B) AMS-L and SSMS-L.

Figure 3.
Rat IgG loading density in as-made and sonication-shattered mesoporous silicas and the gradual release of IgG from the mesoporous silicas in pH 7.4, PBS buffer.

Table 1

BET data of AMSs and SSMSs

Sample ID	Surface area (m ² /g)	Volume (total, cc/g)	Adsorption pore size (nm)	Desorption pore size (nm)
AMS-C	407.9	1.84	16.63	12.14
SSMS-C	485.2	2.03	28.25	12.12
AMS-L	371.4	2.45	28.40	11.13
SSMS-L	357.3	2.30	28.46	16.90