Published in final edited form as:

J Am Chem Soc. 2012 August 29; 134(34): 13997–14003. doi:10.1021/ja3036114.

Iron(III)-doped, silica nanoshells: a biodegradable form of silica

Kristina K. Pohaku Mitchell^a, Alexander Liberman^b, Andrew C. Kummel^a, and William C. Trogler^{a,*}

^aUniversity of California San Diego, Department of Chemistry and Biochemistry, 9500 Gilman Drive, La Jolla, CA 92093

^bUniversity of California San Diego, Department of Materials Science and Engineering, 9500 Gilman Drive, La Jolla, CA 92093

Abstract

Silica nanoparticles are being investigated for a number of medical applications; however, its use *in vivo* has been questioned because of the potential for bioaccumulation. To obviate this problem, silica nanoshells were tested for enhanced biodegradability by doping iron(III) into the nanoshells. Exposure of the doped silica to small molecule chelators and mammalian serum was explored to test whether the removal of iron(III) from the silica nanoshell structure would facilitate its degradation. Iron chelators such as EDTA, desferrioxamine, and deferiprone were found to cause the nanoshells to degrade on the removal of iron(III) within several days at 80 °C. When the iron(III)-doped, silica nanoshells were submerged in fetal bovine serum (FBS) and human serum (HS) at physiological temperature, they also degrade via removal of the iron by serum proteins, such as transferrin, over a period of several weeks.

Keywords

nanoshells; biodegradable; silica; iron(III)

INTRODUCTION

Silica nanoparticles are being investigated for a variety of applications such as catalysis, ultrasound contrast imaging agents, photonic band gap materials, adsorptive materials, and drug delivery.^{1–8} There are several characteristics that are desirable for materials to be used in drug delivery: a long shelf life, facile commercial synthetic scale up, and clearance from the body. Calcined silica is a potential nanoporous material for biomedical applications because it is a robust, chemically inert material of high surface area whose surface chemical properties are easily modified. However, the stability of silica makes it problematic for *in vivo* medical applications because of the potential of inert nanoparticles to bioaccumulate.⁹ Mesoporous silica nanoparticles have been found to accumulate in the liver, kidneys, bladder, and spleen with a small fraction of nanoparticles also found in the lungs.^{10–15} The location of accumulation depends however, on the size, shape, and surface properties of the nanoparticles.^{11–15} Industrial uses of nanosilica also suffer from the occupational hazard that

*Corresponding Author: William C. Trogler, Professor, Dept. Chemistry & Biochemistry, University of California San Diego, 9500 Gilman Drive, #0358, La Jolla, CA 92093, wtrogler@ucsd.edu.

Supporting Information. The supporting information contains: ICP-OES methods for sample preparation and quantification of iron in nanoshells; methods for preparation of sample for magnetic susceptibility measurements; Curie-Weiss plot of mass susceptibility vs. temperature; DLS and zeta potential measurements of 200nm iron(III)-doped, hollow silica nanoshells; and tabulated EDS data of degradation of plain silica and iron(III)-doped, silica nanoshells; control images of iron(III)-doped, silica nanoshells in water; TEM images of 200 nm iron(III)-doped, silica nanoshells. This material is available free of charge via the Internet at <http://pubs.acs.org>.

it poses, since inhalation may lead to silicosis.^{16–18} Therefore, methods that increase the biodegradable nature of silica could have a wide impact in mitigating potential hazards with its use.

The rate at which silica degrades depends on a number of factors including composition, preparation, calcination temperature, surface area, and pore structure.^{13, 19–22} Many studies have looked at using various forms of silica based materials (xerogels, sols, bioactive glasses, etc.) as biocompatible, biodegradable materials.^{20, 21, 23–28} Of the materials studied, compounds that had been calcined, or sintered, at high temperatures reduced the amount of silica released or dissolved from the bulk material.^{13, 19–22, 29, 30} Calcination causes the dehydration of silica and imparts it with a dense network structure. These changes to the network structure cause calcined silica to have a slow rate of degradation, thereby increasing the potential for bioaccumulation.³⁰ There are reports of attempts to overcome this obstacle by altering the composition of silica nanoparticles through the incorporation of cations to increase the rate at which silica degrades, for example. The incorporation of Ca^{2+} into calcined silica has been shown to increase the silica degradation rate compared to plain silica.³⁰ However, the mechanism of silica degradation was not clear. It was difficult to determine if the changes observed were due to the enhanced removal of silica or just the dissolution of the incorporated calcium.

This work explores the incorporation of ferric iron (Fe^{3+}) into a silica nanoshell structure. Iron, despite its large abundance, is a limiting nutrient for most forms of life due to the limited solubility of its common form, $\text{Fe}(\text{OH})_3$, at neutral pH. As a result, nature has developed a variety of methods for the solubilization and sequestration of iron using iron chelating proteins.³¹ Transferrin is an iron transport protein found in the blood serum of vertebrates.³² Human transferrin binds iron as Fe^{3+} with a high affinity as evidenced by its dissociation constant (K_d) of 10^{-22} M at pH 7.0.³¹ This strong binding affinity raised the possibility that the incorporation of Fe^{3+} into the silica structure would make silica biodegradable. Furthermore, there are clinical drugs, such as desferrioxamine and deferiprone, which are approved for chelation therapy to treat individuals with iron overload diseases such as hemochromatosis and thalassemia. Such drugs could conceivably be administered as a secondary treatment, if necessary, to assist the removal of iron(III)-doped, silica nanoparticles.

This work explores iron(III) doping into hollow silica nanoshells prepared by the sol-gel method and polymer templating approach shown in Scheme 1.³³ Degradation of Fe-doped, hollow silica nanoshells was studied in the presence of strong iron chelates, as well as in the presence of the natural chelating ligands found in mammalian serum.

EXPERIMENTAL

Materials

Tetramethoxy orthosilicate; 3-hydroxy-1,2-dimethylpyridin-4(1H)-one 98% (Deferiprone), desferrioxamine mesylate salt 95% (desferrioxamine), ethylenediamine-tetraacetic acid disodium salt (EDTA), anhydrous ethanol, and absolute ethanol were obtained from Sigma Aldrich. The 200nm amino polystyrene templates were obtained from PolySciences. Iron(III) ethoxide was purchased from Gelest. Heat inactivated human serum was obtained from Lonza. Fetal bovine serum was obtained from Mediatech, Inc. All reagents were used as obtained without further purification. SEM images were acquired using a FEI/Phillips XL30 FEG ESEM with an accelerating voltage of 10kV. Energy-dispersive X-ray Spectroscopy (EDS) was performed on the same instrument using the Oxford EDX attachment and INCA software. UV-visible spectroscopy was performed using a Perkin

Elmer Lambda 35 model spectrophotometer. Dynamic light scattering and zeta potential measurements were obtained using a Malvern Zetasizer Nano ZS.

Synthesis of iron(III)-doped, silica nanoshells

A 20 mg/mL solution of iron(III) ethoxide was prepared by dissolving 20 mg of iron(III) ethoxide in 1 mL of anhydrous ethanol under an inert atmosphere. This translucent, dark brown solution was saved until needed for synthesis of the iron(III) doped hollow silica nanoparticles. The particles were synthesized as follows: 1 mL of absolute ethanol and 50 μ L of 200 nm amino polystyrene beads were added to a 1.5 mL microcentrifuge tube. The mixture was vortexed to suspend the polystyrene in the ethanol. As the polystyrene beads and ethanol were mixing, 3.2 μ L of tetramethoxyorthosilicate (TMOS) was mixed with 10 μ L of a 20 mg/mL solution of iron(III) ethoxide. When the TMOS and iron(III) ethoxide appeared to be thoroughly mixed, the translucent brown solution was added to the polystyrene bead suspension. The mixture was vortex stirred for 5 h. The nanoparticles, which appear yellow in color, were centrifuged at 7000 rpm for 5 min, the supernatant removed, and the pellet resuspended in water. This wash process was performed three times. After the last wash, the supernatant was removed and the pellet was dried under ambient conditions. The dry pellet was removed from the microcentrifuge tube, placed in a crucible, and calcined at 550 $^{\circ}$ C in a standard muffle furnace for 18 h. An orange-brown powder was obtained from the crucible after calcination.

Nanoshell degradation using UV-vis spectroscopy

0.1 M aqueous solutions were prepared for disodium EDTA, desferoxamine mesylate salt, and 1,2-dimethyl-3-hydroxy-4(1H)-pyridon (deferiprone) in water. A 1 mg portion of 200 nm iron(III)-doped, hollow silica nanoparticles was added to a 1.5 mL microcentrifuge tube to which 1 mL of the chelate solution was added. The samples were vortexed to suspend the particles and placed in an 80 $^{\circ}$ C water bath. From each 100 μ L aliquots were removed, and replaced with fresh chelate solution. The aliquots were diluted with 900 μ L of water and the UV-visible spectra were obtained. Aliquots were sampled and measured every hour for a total of 8 h. After the last measurement was taken at 8 h, the suspension was left in the water bath for 16 h (a total of 24 h). Every 24 h, the samples were centrifuged and the supernatant was removed and replaced with 1 mL of fresh chelate solution. The UV-vis spectrum was obtained for the supernatant, diluted as necessary, and the process was repeated every 24 h until there were no recoverable solids after approximately 7–10 days.

Nanoshell degradation in mammalian serum

A 1 mg portion of 200 nm iron(III)-doped, hollow silica nanoparticles was placed in a 1.5 mL microcentrifuge tube. 1 mL of either fetal bovine serum or human serum was added and the samples were vortexed to suspend the particles. The samples were placed in a 37.5 $^{\circ}$ C constant temperature water bath. The samples were vortexed every 24 h with the serum being replaced with fresh serum every third and seventh day. A pellet was isolated by centrifugation every seven days, washed twice with water, and calcined. The calcined pellets were imaged using SEM.

RESULTS AND DISCUSSION

Synthesis and Characterization of Fe-doped, Silica Nanoshells

The synthesis of Fe-doped, hollow silica nanoparticles was adapted from the method developed by Yang et al.^{33, 34} As seen in Scheme 2, a polyamine was not required in the synthesis as long as amine-functionalized polystyrene (PS) beads are used as the template. Iron(III) ethoxide was dissolved in anhydrous ethanol and the resulting solution was premixed with tetramethylorthosilicate (TMOS) prior to its addition to the PS bead

suspension. Water from the PS template suspension was the source of trace water for the sol-gel hydrolysis reaction to occur. The reagents are combined with the template beads and allowed to react for 5 h. The pellet is isolated by centrifugation, washed with deionized water, dried under vacuum, and then calcined. The removal of the PS core by heating to 550 °C results in the isolation of a fine powder with an orange-brown color.

Scanning electron microscopy (SEM) was performed to determine the structure, seen in Figure 1. The SEM images reveal that the particles obtained are hollow with a continuous wall with no visible holes, cracks, or other deformations. The images also reveal that the resulting nanoshells are fairly uniform in size having an average diameter of approximately 180 nm. Dynamic light scattering (DLS) was explored to determine the average size of the particles suspended in water. After sonication, the 200 nm Fe-doped, silica nanoshells exhibit an average diameter of 1180 nm, with a PDI of 0.54 by DLS and have a zeta potential of -48.6 mV. The DLS distribution peak, however, indicated that the mean diameters of the nanoshells measured were 318 nm. The size discrepancy observed between the DLS fitting parameters and the SEM images suggested problems due to the iron-doped, silica nanoshells absorbing the laser (633 nm) used in DLS thereby distorting the size measurements. In order to better characterize the dispersed nanoshells in solution, wet SEM capsules, from QuantoMix, were used to directly image the particles dispersed in water (Figure 2). The nanoshells imaged using the wet SEM capsules appear to be predominantly single, 180 nm particles. The images also show particles that appear to be smaller in size. The apparent size range is due to the increased distance between the particles and the capsule membrane. The SEM images in Figure 1 on a flat substrate show that the individual nanoshells are uniform in size.

The iron content of the Fe-doped, silica nanoshells was determined using energy-dispersive X-ray spectroscopy (EDS) and inductively coupled plasma-optical emission spectroscopy (ICP-OES). EDS measurements were obtained for 20 different frames and three different samples during SEM imaging. The mole percent (mol %) values were then averaged. The expected mol % for iron, from the fraction added during synthesis, is 4.7%. EDS of the Fe(III)-doped nanoshells shows a mol % of $5.7 \pm 1.8\%$.

ICP-OES was used as a bulk analysis method to quantify the amount of iron doped into the silica nanoshells. A 1 mg portion of Fe(III)-doped, silica nanoshells were suspended in 10 mL of 2% nitric acid. The ICP-optical emission spectrum was measured at five wavelengths (see Experimental section) and the resulting concentrations were averaged. The dilution factor was corrected by multiplying the averaged value by 100. The 1 mg of particles suspended in 1 mL of solution should produce an iron concentration of 0.83 mM (4.7 mol %). After correcting for the dilution of the particles, the concentration of iron in the nanoshells was determined to be 0.72 ± 0.04 mM ($\sim 4.1 \pm 0.2$ mol %). It can be concluded that approximately 88% of the added iron(III) ethoxide used in the synthesis reaction is incorporated into the nanoshell. This is also the maximum amount of iron that can be incorporated into the nanoshell without adversely affecting its strength, as attempts to introduce higher amounts of iron(III) ethoxide in the synthesis led to the formation of colloidal matter and broken nanoshells.

The EPR spectrum at 77 K showed no appreciable signal, suggesting that the iron present in the nanoshells is antiferromagnetically coupled.^{35, 36} Bulk magnetic susceptibility measurements were performed. Mass susceptibility measurements, χ_g , were plotted as χ_g^{-1} vs. the absolute temperature (a Curie-Weiss plot, see Supplemental Information). The data closely follows Curie behavior, as evidenced by the linearity of the data to low temperatures. The Weiss temperature was determined to be -10.5 K. The negative Weiss temperature suggests that the iron(III) in the nanoshells is weakly antiferromagnetically coupled to other

iron(III) centers within the nanoshell or between individual particles.^{37, 38} This observation agrees with the EPR results. Antiferromagnetic behavior of iron(III) doped into silica has also been observed in silica films and attributed to the presence of small iron nanoparticles.³⁹ This suggests a heterogeneous structure where small clusters of hydrated iron(III) oxide and silica gel are fused together on the template surface during nanoshell formation.^{38, 39}

Small Molecule Chelation and Degradation of Iron(III)-doped, SiO₂ Nanoshells

Small molecule chelation was initially used to determine whether nanoshell degradation could be promoted by iron removal. Deferiprone, EDTA, and desferrioxamine were used because of their known high affinity for iron(III), as well as their use as metal chelation therapy agents. EDTA strongly binds iron(III) with a formation constant of $\sim 10^{25}$. Its high binding affinity and use in a variety iron(III) chelating applications made it a suitable compound for this study. Desferrioxamine and deferiprone were investigated because of their therapeutic use to treat iron overload in patients. Desferrioxamine mesylate (Desferal) is FDA approved to treat individuals with iron overload disorders, such as haemochromatosis or thalassemia. It chelates iron(III) with a binding constant of 10^{30} . Desferrioxamine (desferal) is usually administered intravenously due to its low absorption in the gastrointestinal tract. Deferiprone (Ferriprox®) is used in Europe as an alternative, orally administered, iron overload treatment and has a iron(III) binding constant of 10^{20} .

Desferrioxamine and deferiprone both form orange colored solutions after binding iron(III). This allows colorimetric quantification of iron removal from the nanoshells. Panel (a) in Figure 3 shows the supernatant from the nanoshell degradation using deferiprone as the chelating agent. The procedure for nanoshell degradation is provided in detail in the Experimental section. As seen in Figure 3, the color intensity of the supernatant decreases over time thus indicating that less iron is being removed with successive extractions. It should be noted that the nanoshell pellet isolated from the supernatant also becomes paler as the iron is removed. The solid pellet also decreases in size and is more difficult to isolate, by centrifugation, over time as the nanoshells dissolve. Iron(III)-doped, SiO₂ nanoshells were submerged in MilliQ water and DPBS, without CaCl₂, and incubated at 80 °C as a control. No change was observed in the color of the supernatant or in the morphology of the nanoshells after 7 days indicating that a chelating agent is required for the removal of iron from the nanoshells. Adding deferiprone to the supernatant failed to produce any colorimetric response for iron(III), visually or spectrophotometrically.

These results establish that small molecule chelation can be used to remove iron(III) from the nanoshells and cause their breakdown into soluble species. The total amount of iron removed, Figure 3 panel (B), by the three chelating agents are approximately equal to the amount of iron incorporated into the nanoshells as determined by EDS and ICP-OES.

The results from Figure 3b show that 57%, 39%, and 42% of the iron doped into the nanoshells is removed after 24 h exposure to EDTA, desferrioxamine, and deferiprone, respectively. Desferrioxamine has the largest formation constant yet removes the smallest amount of iron in the first 24 h period relative to EDTA and deferiprone. Compared to EDTA and deferiprone, desferrioxamine is the largest molecular weight chelator and must fold appropriately in order to bind the iron in an octahedral complex. As a result, the process of removing iron appears to be kinetically hindered during the first 24 h. EDTA is the next largest of the chelators but is able to readily form the octahedral complex due to its compact structure. The minimal required reorganization of EDTA, along with its high formation constant, lead to the increased removal rate of iron from the nanoshells within the first 24 h. Deferiprone is the smallest of the chelators used, but also has the lowest formation constant of the three. It has ready steric access to the iron in the nanoshells; however, because of its

lower formation constant, it does not remove as much iron as EDTA within the first 24 h period. There are obvious differences in the rate of iron removal in the first 24 h period, yet approximately 84% of the iron(III) doped into the nanoshells is removed after exposure to the iron chelates for three days, irregardless of the type of chelator.

It can therefore be concluded that the chelating agents are removing all the iron in the nanoshells. The decreased yield of recoverable nanoshells as the iron is removed suggests that the chelated iron comes from a structural site, thereby leading to the collapse and dispersal of the nanoshell into smaller, soluble fragments.

The collapse of the nanoshell structure was examined using deferiprone to chelate the iron. The SEM images, seen in Figure 4, show thinning of the nanoshell wall as iron is removed, collapse of the structure, and the presence of colloidal silica as iron is removed in stages until no colloidal silica is recoverable by centrifugation. Thinning of the nanoshell wall is observed during the first 3 days of exposure of the iron(III)-doped nanoshells to the chelating solutions. No holes are observed in the TEM images of the parent nanoshells (see Supplemental Information) indicating that the pores in the silica are less than 1 nm in diameter. Given the small dimensions of the pores, the chelate may slowly diffuse within the pores thus, the iron may be removed preferentially from the outer surface of the nanoshell. As iron is removed from the outer surface the walls of the nanoshells become thinner until the structure begins to collapse (Figure 4c). After the nanoshell ruptures, the chelates should have better access to both surfaces of the nanoshell leading to enhanced iron removal and the production of colloidal silica (the haze seen in Figure 4d).

These results show that doping ferric iron into silica during the sol-gel synthesis results in a material that even after calcination can be degraded by removal of iron(III). The degradation seen using chelates such as desferrioxamine, an FDA approved drug, and deferiprone suggest a potential method for removing iron(III)-doped, silica from living organisms. The next test was to determine whether iron-sequestering proteins present in mammalian serum, such as transferrin, would be able to remove the iron(III) from the nanoshells and promote biodegradation.

Degradation of iron(III)-doped, SiO₂ nanoshells in mammalian serum

Small molecule chelation experiments show that iron removal resulted in the collapse of the iron(III)-doped, silica nanoshell structure with eventual dissolution to non-centrifugable fragments. To determine whether the biological ligands for iron(III) present in serum could affect a similar degradation process under conditions relevant to *in vivo* dosing, the biodegradability of the iron(III)-doped, silica nanoshells was tested by immersing them in serum. Serum, the liquid portion of blood with the cells and clotting factors removed, contains the iron(III) chelating protein transferrin. Its high binding constant, 10^{20} , is similar to that of the small molecule chelators used in the preliminary study. Therefore, it seemed likely that the binding of iron(III) by transferrin should also lead to the collapse and dissolution of the nanoshell structure.

Figure 5 displays SEM images of 200 nm silica nanoshells, which serve as a control, and iron(III)-doped, silica nanoshells that have been immersed in fetal bovine serum (FBS) and human serum (HS) for various time periods. The samples were incubated at 37 °C (physiological temperature) and images were taken of the pellet that was isolated at different time points. As seen in panels (a) and (c), the undoped silica nanoshells remain intact in both FBS and HS over the course of 24 d and are easy to isolate via centrifugation. It should be noted that the last images of the plain silica nanoshells are shown at day 24 for comparison to iron(III)-doped, silica nanoshells. However, the plain silica nanoshells remain intact after 24 days, confirming the inertness of calcined silica. The slight haze that appears as time

progresses are due to small amounts of serum salts, such as NaCl and CaCl₂, which were confirmed using EDS.

When comparing the 200 nm iron(III)-doped, silica nanoshells, seen in panels (b) and (d) of Figure 5, to the control undoped silica nanoshells it is apparent that the results differ markedly. Although there are many imageable, intact nanoshells at 10 d, seven days later it becomes difficult to find any nanoshells and only irregular solid fragments are seen. The EDS analysis, seen in Tables S1 and S2 (found in the Supplemental information), showed that these solids contain large fractions of calcium and phosphorous, which can be attributed to remineralization of the iron-doped silica with the calcium phosphate present in serum after breakdown and dissolution of the iron(III)-doped silica nanoshells.

The silicon content measured using EDS (see Tables S1 and S2 in the Supporting Information) also decreases as the amount of calcium and phosphorous increase. The iron(III)-doped, silica nanoshells dispersed in FBS could only be isolated, via centrifugation, up to 21 d. The pellet that was obtained from FBS at 21 d was less than 1% of the initial amount of nanoshells, of which the entire recovered sample was imaged. Of the sample that was imaged, it was not possible to find any nanoshells. Only fragments primarily composed of calcium and phosphorous were in the recovered solid. The same trend is seen for the iron(III)-doped, silica nanoshells that had been immersed in HS; however, the last pellet that could be isolated was at 24 d rather than after 21 d. Again, less than 1% of the original sample weight was recoverable by centrifugation after 24 d, and again the isolable solid particles were high in calcium and phosphorus, which is indicative of extensive remineralization of the silica.

There was not a similar significant increase in the amount of measured calcium and phosphorous in the undoped silica, control samples. The increased amount of calcium and phosphorous seen in the iron(III)-doped, silica nanoshells is attributed to the degradation of the nanoshells into smaller, soluble fragments and subsequent remineralization with the soluble calcium(II) and phosphate ions present in serum, yielding tiny amounts of a mixed calcium phosphate silicate.^{20, 22, 25, 27, 40} The iron(III)-doped, silica nanoshells have to degrade for at least 17 d at physiological conditions before degradation is observed by SEM. After 21–24 d, the trace solid that was recovered and imaged by SEM images consists chiefly of calcium phosphate and very low levels of silica are detected. It has been observed previously that the formation of calcium phosphate depends on its ability to crystallize at a silica surface.²² These results strongly suggest that the iron in the nanoshell structure can be removed by iron sequestering proteins found in serum leading to the collapse of the structure and the formation of molecular or extremely small silica clusters that cannot be recovered by centrifugation.

CONCLUSION

It has been demonstrated that iron(III) can be doped into a silica nanoshell during sol-gel synthesis. Incorporation of iron(III) into the silica matrix was shown to make the nanoshells degradable in the presence of iron chelating agents. The biodegradability of the nanoparticles under conditions relevant to *in vivo* use was demonstrated through their solubilization in fetal bovine serum and human serum after approximately 20–25 d at physiological temperature. This approach provides a general method to make porous, calcined silica biodegradable and may enable broad application of silica nanoparticles in biomedical technologies.^{41,42}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project described was supported by Award Number P42ES010337 from the National Institute of Environmental Health Sciences and NIH Grant U54 CA 119335. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Environmental Health Sciences or the National Institutes of Health. We acknowledge the use of the UCSD Cryo-Electron Microscopy Facility which is supported by NIH grants to Dr. Timothy S. Baker and a gift from the Agouron Institute to UCSD. We also thank the Scripps Institution of Oceanography Analytical Facility (ICP-OES); Prof. Michael Tauber and Dr. Hannah Shafaat at the University of California San Diego (UCSD) for performing the EPR measurements; and Prof. David Hendrickson, Dr. Christopher Beedle, and Dr. Katie Heroux for collecting and aiding in the analysis of the DC magnetic susceptibility data. Alex Liberman is supported by an R25 CA153915 training grant as well as R01CA095298 from the National Cancer Institute. Kristina K. Pohaku Mitchell thanks the UCSD Socrates Program for an NSF GK-12 STEM fellowship.

ABBREVIATIONS

DPBS	Dulbecco's Phosphate Buffered Saline
EDS	energy dispersive X-ray spectroscopy
FBS	fetal bovine serum
HS	human serum
ICP-OES	inductively coupled plasma-optical emission spectroscopy
SEM	scanning electron microscopy

References

1. Arnal PM, Comotti M, Schueth F. *Angew Chem, Int Ed.* 2006; 45(48):8224–8227.
2. Jin P, Chen Q, Hao L, Tian R, Zhang L, Wang L. *J Phys Chem B.* 2004; 108(20):6311–6314. [PubMed: 18950116]
3. Martinez HP, Kono Y, Blair SL, Sandoval S, Wang-Rodriguez J, Mattrey RF, Kummel AC, Trogler WC. *Med Chem Comm.* 2010; 1:266–270.
4. Slowing II, Trewyn BG, Giri S, Lin VSY. *Adv Funct Mater.* 2007; 17(8):1225–1236.
5. Slowing II, Trewyn BG, Lin VSY. *J Am Chem Soc.* 2007; 129(28):8845–8849. [PubMed: 17589996]
6. Wang H, Yang ZY, Lu YF. *J Appl Phys.* 2007; 101(3):033129/1–033129/5.
7. Xu X, Asher SA. *J Am Chem Soc.* 2004; 126(25):7940–7945. [PubMed: 15212543]
8. Zhu Y, Shi J, Shen W, Dong X, Feng J, Ruan M, Li Y. *Angew Chem, Int Ed.* 2005; 44(32):5083–5087.
9. Alexis F, Pridgen E, Molnar LK, Farokhzad OC. *Mol Pharmaceutics.* 2008; 5(4):505–515.
10. He Q, Shi J. *J Mater Chem.* 2011; 21:5845–5855.
11. He X, Nie H, Wang K, Tan W, Wu X, Zhang P. *Anal Chem (Washington, DC, U S).* 2008; 80(24):9597–9603.
12. Huang X, Li L, Liu T, Hao N, Liu H, Chen D, Tang F. *ACS Nano.* 2011; 5(7):5390–9. [PubMed: 21634407]
13. Barbe C, Bartlett J, Kong L, Finnie K, Lin HQ, Larkin M, Calleja S, Bush A, Calleja G. *Adv Mater (Weinheim, Ger).* 2004; 16:1959–1966.
14. Lee CH, Cheng SH, Wang YJ, Chen YC, Chen NT, Souris J, Chen CT, Mou CY, Yang CS, Lo LW. *Adv Funct Mater.* 2009; 19(2):215–222.
15. Lu J, Liong M, Li Z, Zink JJ, Tamanoi F. *Small.* 2010; 6:1794–1805. [PubMed: 20623530]

16. Chen Y, Chen J, Dong J, Jin Y. *Toxicol Ind Health*. 2004; 20:21–7. [PubMed: 15807405]
17. Han B, Guo J, Abrahaley T, Qin L, Wang L, Zheng Y, Li B, Liu D, Yao H, Yang J, Li C, Xi Z, Yang X. *PLoS One*. 2011; 6:e17236. [PubMed: 21359146]
18. Napierska D, Thomassen LCJ, Lison D, Martens JA, Hoet PH. *Part Fibre Toxicol*. 2010; 7:39. [PubMed: 21126379]
19. Andersson J, Rosenholm J, Areva S, Linden M. *Chem Mater*. 2004; 16(21):4160–4167.
20. Korteso P, Ahola M, Karlsson S, Kangasniemi I, Kiesvaara J, Yli-Urpo A. *J Biomed Mater Res*. 1999; 44:162–167. [PubMed: 10397917]
21. Korteso P, Ahola M, Karlsson S, Kangasniemi I, Yli-Urpo A, Kiesvaara J. *Biomaterials*. 2000; 21:193–8. [PubMed: 10632401]
22. Li P, Ohtsuki C, Kokubo T, Nakanishi K, Soga N, Nakamura T, Yamamuro T. *J Am Ceram Soc*. 1992; 75(8):2094–7.
23. Lee EJ, Teng SH, Jang TS, Wang P, Yook SW, Kim HE, Koh YH. *Acta Biomater*. 2010; 6(9): 3557–3565. [PubMed: 20304111]
24. Radin S, El-Bassyouni G, Vresilovic EJ, Schepers E, Ducheyne P. *Biomaterials*. 2005; 26:1043–52. [PubMed: 15369693]
25. Radin S, Falaize S, Lee MH, Ducheyne P. *Biomaterials*. 2002; 23:3113–3122. [PubMed: 12102182]
26. Seleem MN, Munusamy P, Ranjan A, Alqublan H, Pickrell G, Sriranganathan N. *Antimicrob Agents Chemother*. 2009; 53(10):4270–4274. [PubMed: 19667284]
27. Vaekiparta M, Jokinen M, Vaahtio M, Vallittu PK, Yli-Urpo A. *Key Eng Mater*. 2004; 254–256:553–556.
28. Xue JM, Tan CH, Lukito D. *J Biomed Mater Res, Part B*. 2006; 78B(2):417–422.
29. Jokinen M, Peltola T, Veittola S, Rahiala H, Rosenholm JB. *J Eur Ceram Soc*. 2000; 20(11):1739–1748.
30. Li X, Zhang L, Dong X, Liang J, Shi J. *Microporous Mesoporous Mater*. 2007; 102(1–3):151–158.
31. Bertini, I.; Gray, HB.; Stiefel, EI.; Valentine, JS. *Biological Inorganic Chemistry: Structure and Reactivity*. University Science Books; Sausalito, CA: 2007. p. 739
32. Crichton RR, Charlotiaux-Wauters M. *Eur J Biochem*. 1987; 164(3):485–506. [PubMed: 3032619]
33. Yang J, Lind JU, Trogler WC. *Chem Mater*. 2008; 20(9):2875–2877.
34. Trogler, WC.; Esener, SC.; Messmer, D.; Lind, JU.; Pohaku, K.; Yang, J. *Hollow silica nanospheres for drug delivery and gene transfer PCT Int. Appl. WO 2009023697*. 2009. p. 76
35. Tanaka K, Kamiya K, Matsuoka M, Yoko T. *J Non-Cryst Solids*. 1987; 94(3):365–373.
36. Yanez-Limon JM, Perez-Robles JF, Gonzalez-Hernandez J, Vorobiev YV, Romano JA, Gandra FCG, Da SEC. *J Sol-Gel Sci Technol*. 2000; 18(3):207–217.
37. Carlin, RL. *Magnetochemistry*. Springer-Verlag; Berlin Heidelberg: 1986.
38. Heinrichs B, Rebbouh L, Geus JW, Lambert S, Abbenhuis HCL, Grandjean F, Long GJ, Pirard JP, van Santen RA. *J Non-Cryst Solids*. 2008; 354(2–9):665–672.
39. Babonas GJ, Reza A, Simkiene I, Sabataityte J, Baran M, Szymczak R, Karlsson UO, Suchodolskis A. *Appl Surf Sci*. 2006; 252(15):5391–5394.
40. Jokinen M, Peltola T, Veittola S, Simola J, Yli-Urpo A. *Key Eng Mater*. 2002; 218–220:283–286. (Bioceramics-14).
41. Martinez HP, Kono Y, Blair S, Mattrey R, Kummel AC, Trogler WC. *Med Chem Commun*. 2012; 1:266–270.
42. Liberman A, Martinez HP, Ta CN, Barback CV, Mattrey RF, Kono Y, Blair SL, Trogler WC, Kummel AC, Wu Z. *Biomaterials*. 2012; 33:5124–5129. [PubMed: 22498299]

Figure 1. SEM images of 200 nm Fe-doped, SiO₂ calcined nanoshells

Scanning electron microscopy (SEM) images of iron(III)-doped, silica nanoshells. The PS template was removed during the calcination process. The scale bar in panel a is 1 μm and 200 nm (0.2 μm) in panel b.

Figure 2. Wet SEM images of calcined, 200 nm Fe-doped, SiO₂ nanoshells
Nanoshells were suspended in water and placed in a QuantoMix wet SEM capsule for imaging. The scale bar in panel a is 1 μm and 5 μm in panel b.

Figure 3. Small molecule chelation of Fe(III) in Fe-doped, SiO₂ nanoshells

1 mg of 200 nm iron(III)-doped, SiO₂ nanoshells was submerged in 1 mL of 0.1 M aqueous chelating solution (EDTA, desferrioxamine, or deferiprone). The supernatant was removed and analyzed by UV-visible spectroscopy every 24 h. The photographic image on the top (a) shows the color intensity of the aliquots of Fe-deferiprone complex extracted over 7 d. The graph (b) shows the cumulative concentration of Fe(III) removed from the nanoshells on chelation by EDTA (blue curve), desferrioxamine (red curve), and deferiprone (yellow curve).

Figure 4. Degradation of 200 nm Fe-doped, silica nanoshells using deferiprone
Collapse of the nanoshell structure, imaged by SEM, as iron is removed, via deferiprone chelation, over a) 0 days, b) 1 day, c) 3 days, d) 7 days. The haze observed in panel d was determined by EDS to be primarily colloidal silica.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Scheme 1.
Synthesis of SiO_2 Nanoshells

Scheme 2.
Synthesis of Fe-doped, Silica Nanoshells