Surface Modification of Silica Core−Shell Nanocapsules: Biomedical Implications

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Received October 28, 2005; Revised Manuscript Received December 30, 2005

In this article we present the synthesis of oil core silica shell nanocapsules with different shell thicknesses. The surface of the nanocapsules was modified with polyethyleoxide (PEO) and succinic anhydride. Two biomedical tests were then used to study the biocompatibility properties of these nanocapsules with different surface treatments, hemolysis and thromboelastography (TEG). PEO surface modification greatly reduced the damaging interactions of nanocapsules with red blood cells (RBCs) and platelets and attenuated particle size effects. It was found that the blood toxicity of charged particles increased with the acid strength on the surface. Experiments toward the assessment of detoxification of these nanocapsules in model drug overdose concentrations are currently underway.

Introduction

The scientific crossover between nanotechnology and medicine has attracted great attention, due to advances the former has provided for improvement of health care.[1] Among many nanoscience breakthroughs in recent years the potential of reducing toxic drug concentration in cases of overdose is particularly interesting to us. The importance of this approach comes from the fact that there are neither specific pharmacological antidotes nor immuno-toxicotherapeutic agents to overcome the effects of many drugs, at overdose concentrations, among them tricyclic antidepressants.[2] Recently, two approaches to detoxification therapy using nanoparticles[3] and nanoemulsions[4] showed superior performance compared to their "macro" counterparts. The rationale is that smaller entities will have larger surface-to-volume ratio, thus more effectively partitioning toxic drugs from an aqueous environment to the hydrophobic core or oil/water interface.

Our previous work highlights another approach to the drug detoxification problem, using nanocapsules (ncs), prepared by using microemulsion droplets as templates and subsequently polymerizing a silica shell around the template. Previous publications have presented the synthesis, size control, and uptake efficiency features of ncs.[5] The main focus in this work is the evaluation of biocompatibility via assessing blood toxicity through study of ncs surface characteristics. It is well-known that the surface characteristics of nano entities play a key role in processes such as cell adhesion,[6] biorecognition, and biosensing.[7] In this study we will compare oil core silica shell ncs having a nonmodified (i.e., silica) surface, nonionic surface using poly(ethyleneoxide), PEO, and a charged surface, using succinic anhydride modifiers. Furthermore, two simple tests will be used to asses the biocompatibility of these different ncs, hemolysis and thromboelastography (TEG).

Synthesis and Modification of ncs. An oil-in-water microemulsion was synthesized as previously reported.[5a] The silica shell thickness was controlled by the concentration of the sol−gel agent tetramethoxysilane (TMOS).[5b] Three different types of ncs were analyzed, "thin" (52 ± 7 nm), "medium" (90 ± 10 nm), and "thick" (125 ± 15 nm) shell ncs, depending on the thickness of the formed shell. The statistical analysis confirmed the difference in diameter to be $P < 0.05$ (P is the significance test) for different ncs. These ncs samples of different shell thicknesses were subjected to reaction with [2-methoxy (polyethyleneoxy) propyl] trimethoxysilane ("PEO−TMS") and 3-(triethoxysilyl) propyl-succinic anhydride to obtain the noncharged and charged ncs surfaces, respectively. The size of the ncs was determined by quasi elastic light scattering (QELS) and transmission electron microscopy (TEM). The surface characterization was achieved by IR, zeta potential, and TEM. The TEM micrographs of ncs without PEO, along with zeta potential curves for the ncs of different surfaces are shown in Figure 1. The contrast for TEM was achieved by the addition of unsaturated oil and subsequent staining with OsO_4. Nanocapsules with a silica surface have a visible dark circle in the center, i.e., an oil core, surrounded by a lighter, unstained silica shell, while PEO-modified ncs appear darker as a whole due to staining of the ethylene oxide groups on the surface. All tested samples were thoroughly dialyzed to remove the excess of Tween-80 (major surfactant used in the synthesis) micelles and soft microemulsion droplets of Tween-80 and ethyl butyrate, as these species were very damaging to the integrity of red blood cells (RBC) (see the Supporting Information, p 10) and greatly reduced the platelet activity (see the Supporting Information, p 14) in TEG experiments.

Hemolysis. It is well-known that silica causes a lung disease called *Silicosis*,[8] and quartz has been classified as a carcinogen by the International Agency for Research on Cancer (IARC) in 1997.[9] However, there are reports on amorphous silica-based materials, such as Bioglass,[10] showing good biocompatible properties in contact with other tissues. Most of the silica studies related to biocompatibility are cytotoxicity studies with respect to pulmonary diseases.[11] The reports focused on the interaction of silica in the blood, more specifically to red blood cells (RBCs), are rather scarce in the literature. A study of Ayers and Hunt[12] shows that chitosan−silica aerogels have good

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10.1021/bm050820+ CCC: $33.50
Published on Web 02/18/2006

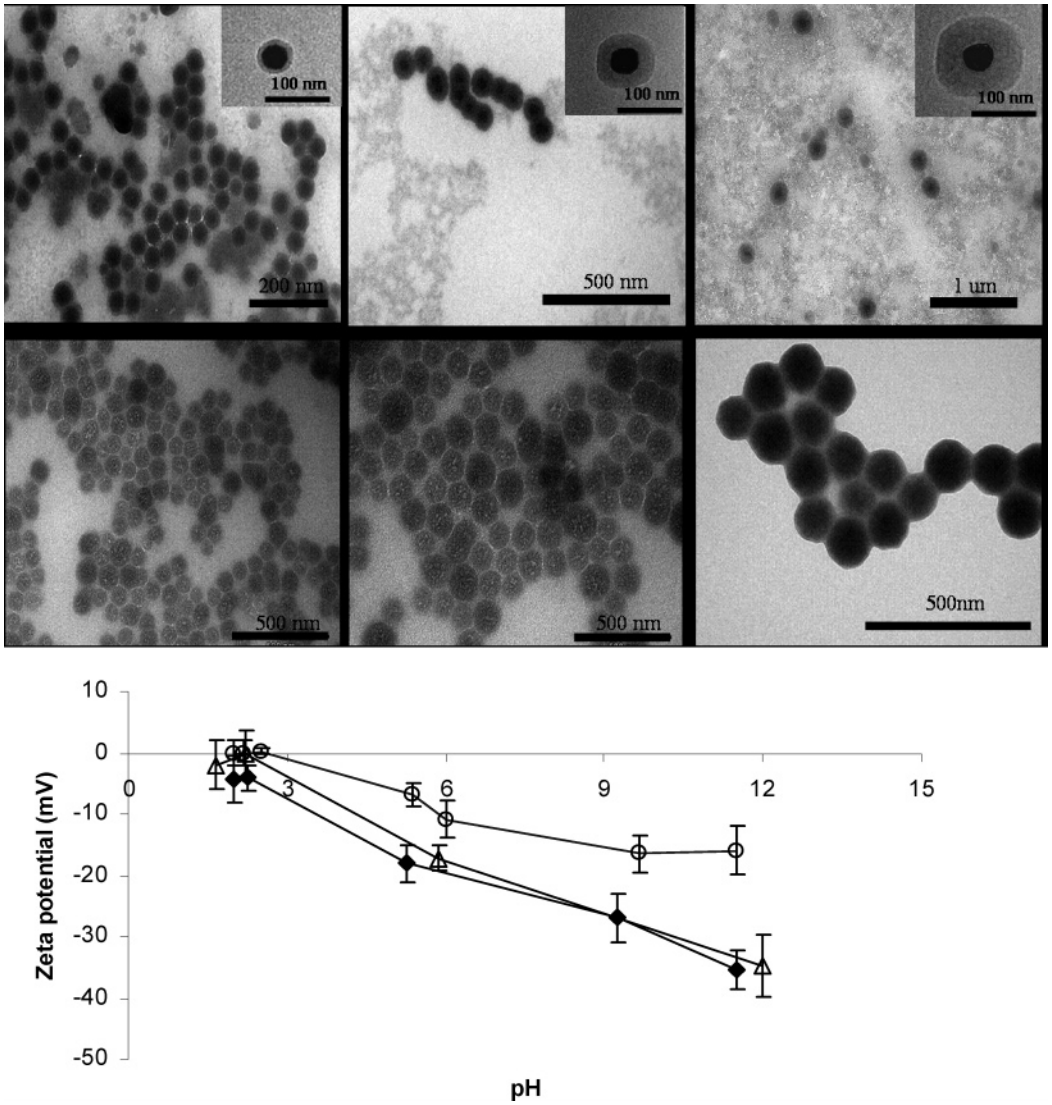

Figure 1. (a) TEM pictures of unmodified ncs (top row) and PEO-modified ncs (bottom row), (from left) thin, medium, and thick shell. (b) Zeta potential curves for ncs with different surfaces: (◆) silica surface, (○) PEO surface, and (△) PEO and succinic acid surface.

cytotoxic profile but are toxic with respect to RBCs. One hypothesis is that silica is causing the hemolysis of RBCs,[13] through the interaction of the negatively charged surface of the siliceous materials at physiological pH with the quaternary amines of the phospholipids at the cell membrane surface. This implies that the extent of hemolysis is a function of surface area, i.e., contact area between cells and particles. The hemolytic activity for ncs of distinct surfaces at three different thicknesses is represented in Figure 2. All of the samples tested showed negligible hemolytic activity upon 20 min of exposure to RBCs with the exception of thick ncs with carboxylic acid functionality on the surface ("0 h" point in the Figure 2a−c). Furthermore, hemolytic activity increased with prolonged exposure for every sample regardless of surface properties (i.e., from 0 to 24 h, from 24 to 48 h, etc.). Analysis of samples without PEO showed that there is a statistically significant decrease in polymer-modified ncs hemolysis of RBCs. This result is not surprising as it was shown that PEO greatly reduces cytotoxicity of quantum dots (QDs) in cell viability studies.[14] Moreover, if the extent of hemolysis of ncs without PEO is compared for ncs with different thicknesses for 24 h of exposure, it is a function of the overall surface area for silica ncs, while the contact area is not a significant factor for PEO-modifiedncs (Figure 2d). It is obvious that hydrophilic, chemically inert polymer at the surface of ncs slows down the hemolytic process, although it still occurs. We believe that this result implies that the surface coverage is not complete, allowing low concentrations of unreacted Si−OH to eventually bind to the RBCs surface. It is important to note that the hemolytic activity of ncs was at the same level even after ncs were modified under extreme conditions (high concentration of PEO−TMS at pH = 11 for several days). A deeper insight into the influence of the surface on the RBCs hemolysis is gained by testing ncs with succinic acid and polymer moiety together (PEO/succinic anhydride at 80/20 w/w) and acid alone. The former showed activity statistically similar to that of nonmodified ncs, while ncs with acid functionality alone were the most damaging of all tested ncs. According to these data the surface acidity greatly influences the RBC hemolysis, since succinic acid is a much stronger acid than silicic acid (pK_a = 4.2 and 5.8 for succinic and pK_a = 9.8 and 13.8 for silicic acid).

Thromboelastography (TEG). The second biomedical test, TEG, was used in this study to assess the evolution of blood clotting parameters in the presence of ncs. The two important TEG parameters are represented in Figure 3 for all three types of ncs: maximum amplitude (MA) and *k*-time. The reduction of MA is an indication of thrombocytopenia (platelet dysfunction), and prolongation of *k*-time is characteristic for haemo-

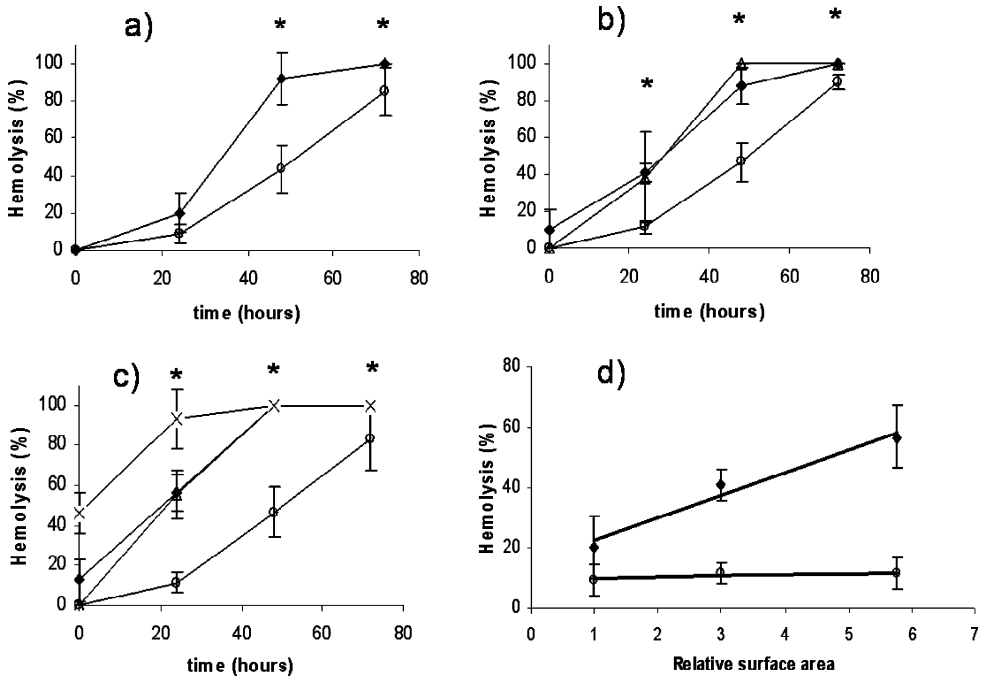

Figure 2. Hemolysis of RBCs in the presence of ncs having different surfaces at 0.1 wt % oil content: (a) thin shell (PEO and silica ncs shown), (b) medium shell (PEO, silica, and PEO/succinic acid shown), (c) thick shell (PEO, silica, PEO/succinic acid, and succinic acid alone shown) (in a−c the lines are a guide to an eye), and (d) hemolysis of RBCs after 24 h for ncs with silica and PEO surfaces (in d the lines represent statistical trends). (◆) Silica surface, (○) PEO surface, (△) PEO and succinic acid surface, and (×) succinic acid surface. (∗ $P < 0.05$ for silica and PEO-modified surface ncs.)

philia.[15] MA represents the mechanical strength of the formed clot, and all types of ncs decrease the MA in a concentration-dependent fashion ($P < 0.001$). The other parameter, *k*-time, is a measure of the kinetics of clot formation, and similarly, the ncs act to increase the *k*-time in a concentration-dependent manner ($P < 0.001$). Under the conditions used here, the ncs at 1 wt % are too bioreactive regardless of surface treatment, while at 0.01 wt % they were too diluted to have any significant impact on the coagulation process. The statistical difference for both MA and *k*-time is observed between ncs of different surfaces (without PEO) at the concentration of 0.1 wt % for medium and thick ncs. At intermediate concentration, the *k*-time for PEO-modified ncs is essentially the same, regardless of surface area, while the *k*-time for unmodified ncs is prolonged dramatically with the increase of surface area (from thin to thick ncs). It is well-known that PEO, in many aspects, is a universal biocompatibilization polymer.[16] The most striking feature is that PEO reduces protein adsorption[17] and the prolongation of circulation in the bloodstream. It seems that the former feature of PEO is not dominant with respect to blood coagulation processes in this case, that is, *r*-time, which is a reflection of initiation of coagulation in the protein cascade process, is constant for all of the samples regardless of concentration and surface chemistry (see the Supporting Information, p 14). On the other hand, MA and *k*-time reflect the platelet activity and are strongly affected by the nature of the ncs surface. Non-modified ncs dramatically reduce the platelet activity, which is contrary to what is commonly observed with artificial surfaces, such as titanium oxide,[18] which actually promote platelet adhesion and lead to hypercoagulation. Nonionic surfactants are commonly noted as platelet activity reducers.[19] However, all free surfactant used in the synthesis of the ncs was removed by multiple dialysis, filtration, and sedimentation (for large ncs), to the point where no surfactant was traceable by UV−vis in the supernatant to a detection limit below the critical micelle concentration (cmc). Moreover, the surfactant concentration at the interior oil interface was the same for modified and nonmodified ncs regardless of size (i.e., the size of the oil templates was the same for all three sizes of ncs). This fact leads to a conclusion that reduction of platelet activity is likely caused by surface properties of the ncs, rather than surfactant effects.

Conclusions

In this article we showed the importance of surface properties of nanocapsules on blood compatibility. We believe that the key feature in reducing the hemolytic activity of silica core−shell ncs is decreasing the surface charge, therefore preventing the electrostatic interaction between particles and positively charged lipids on the cell surface. Similarly, hemolysis is more profound when an acid stronger than silicic acid is present at the particle surface. Similar arguments can be made for the influence of nanocapsules on the blood coagulation process, where a nonionic, nonreactive surface minimizes the effect of the ncs. The most probable cause of this behavior is the preservation of platelet activity, caused by the presence of poly(ethylene oxide). Furthermore, polymer mitigates the surface area effects (i.e., "particle size"), since all tested ncs modified with PEO had similar biomedical properties regardless of particle size, which can be important for further drug detoxification studies. Experiments leading toward the assessment of toxic drug removal efficiency in media of increasing complexity (i.e., from normal saline to whole blood) of ncs having different surfaces along with detoxification in vivo and in vitro are currently underway.

Acknowledgment. The authors thank Karen L. Kelley from ICBR at the University of Florida. The authors also thank Dr.

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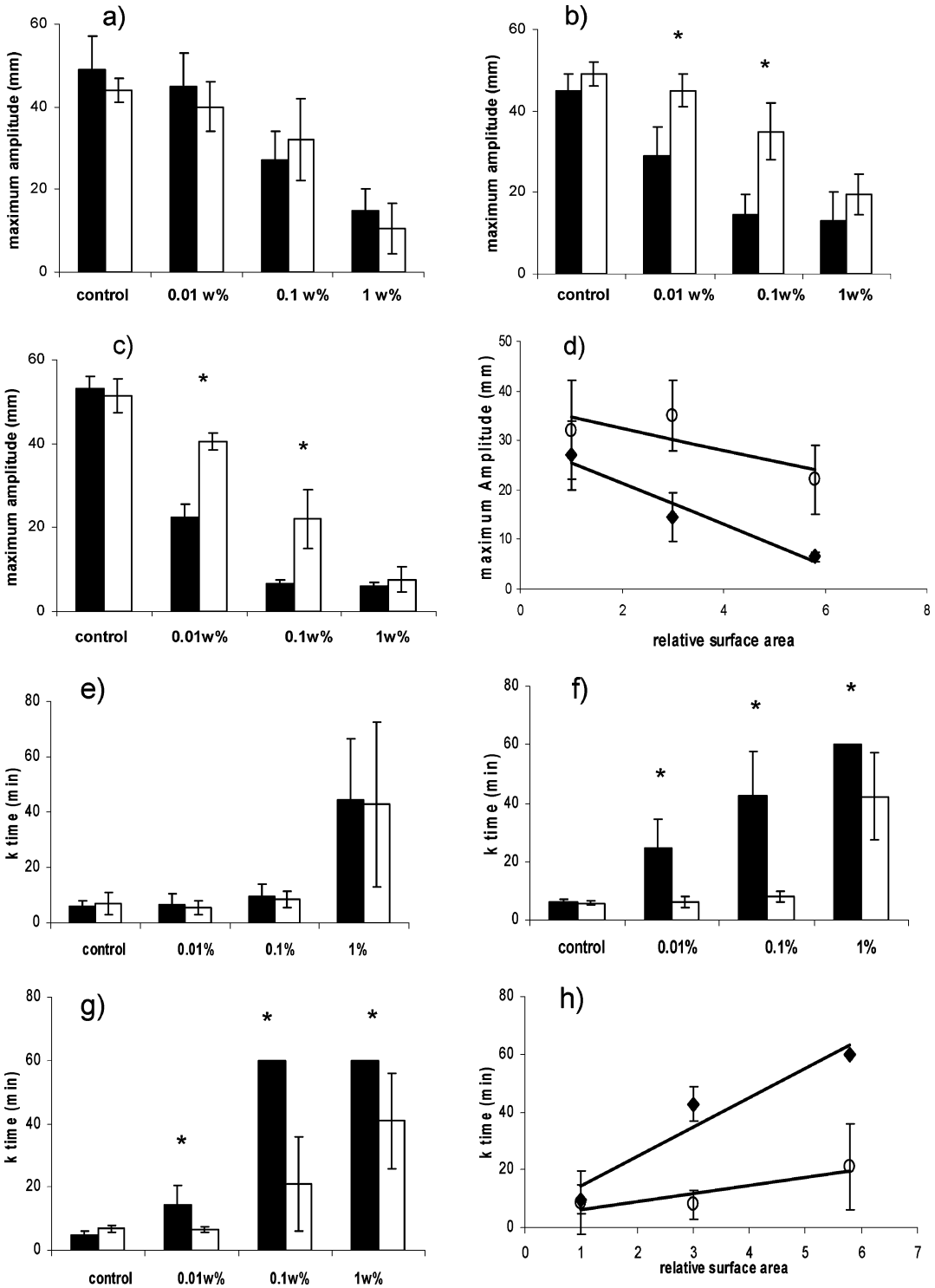

Figure 3. MA for ncs with silica and PEO-modified surfaces (a) thin shell, (b) medium shell, (c) thick shell, and (d) comparison of MA for ncs of different sizes at 0.1 wt %, silica surface and PEO-modified (the lines represent statistical trends). The *k*-time for ncs with silica and PEO-modified surfaces (e) thin shell, (f) medium shell, (g) thick shell, and (h) comparison of *k*-times for silica and PEO-modified ncs at 0.1 wt % based on graphs 3e−g (the lines represent statistical trends). (∗ $P < 0.05$ for silica and PEO-modified surfaces; plots a−c and e−g (open bar) PEO-modified surface, (solid bar) silica surface; graphs d and h (○) PEO-modified surface, ◆ silica surface.)

Brij Moudgil and Dr. Dinesh Shah for their helpful comments and suggestions. Financial support was provided by the Particle Engineering Research Center (PERC) for Particle Science and Technology at the University of Florida and the National Science Foundation (NSF) (Grant Nos. EEC-94-02989 and NSF-CPE 80005851). Additional funding was provided by DOE-BES (Grant No. DE-FG02-96ER45589) and the NSF SGER programs.

Supporting Information Available. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Tolles, W. M.; Rath, B. B. *Curr. Sci.* **2003**, *85*, 1746.
(2) (a) Yu-Ling, M.; Henry, J. A. *Toxicology* **2001**, *169*, 133. (b) Brucculeri, M.; Kaplan, J.; Lande, L. *Pharmacotherapy* **2005**, *25*, 119. (c) Gabel, M.; Hinkelbein, J. *Anaesthesist* **2004**, *53*, 53.

(3) Morey, T. E.; Varshney, M.; Flint, J. A.; Rajasekaran, S.; Shah, D. O.; Dennis, D. O. *Nano Lett.* **2004**, *4*, 757.
(4) Varshney, M.; Morey, T. E.; Shah, D. O.; Flint, J. A.; Moudgil, B. M.; Seubert, C. N.; Dennis, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 5108.
(5) (a) Jovanovic, A. V.; Underhill, R. S.; Bucholz, T. L.; Duran, R. S. *Chem. Mater.* **2005**, *17*, 3375. (b) Underhill, R. S.; Jovanovic, A. V.; Carino, S. R.; Varshney, M.; Shah, D. O.; Dennis, D. O.; Morey, T. E.; Duran, R. S. *Chem. Mater.* **2002**, *14*, 4919.
(6) (a) Parak, W. J.; Gerion, D.; Pellegrino, T.; Zanchet, D.; Micheel, C.; Williams, S. C.; Boudreau, R.; Le Gros, M. A.; Larabell, C. L.; Alivisatos, P. *Nanotechnology* **2003**, *14*, R15. (b) Zhang, Y.; Li, J.; Shen, Y.; Wang, M.; Li, J. *J. Phys. Chem. B* **2004**, *108*, 15343. (c) Hiddessen, A. L.; Rodgers, S. D.; Weitz, D. A.; Hammer, D. A. *Langmuir* **2000**, *16*, 9744. (d) Zhu, J.; Yudasaka, M.; Zhang, M. F.; Kasuya, D.; Lijima, S. *Nano Lett.* **2003**, *3*, 1239.
(7) (a) Chan, W. C. W.; Maxwell, D. J.; Gao, X.; Bailey, R. E.; Han, M.; Nie, S. *Curr. Opin. Biotechnol.* **2002**, *13*, 40. (b) Xiao, Y.; Pavlov, V.; Levine, S.; Niazov, T.; Markovitch, G.; Willner, I. *Angew. Chem.* **2004**, *43*, 4519. (c) Weizmann, Y.; Patolsky, F.; Lioubashevski, O.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 1073. (d) El-Sayed, I. H.; Huang, X. H.; El-Sayed, M. A. *Nano Lett.* **2005**, *5*, 829.
(8) Mossman, B. T.; Churg, A. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 1666.
(9) International Agency for Research on Cancer (IARC). *Silica, Some Silicates Coal Dust and Para-aramid Fibrils*; Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 68; IARC Press: Geneva, 1997.
(10) (a) Hench, L. L. *J. Am. Ceram. Soc.* **1991**, *81*, 1497. (b) Hench, L. L.; Paschall, H. H. *J. Biomed. Mater. Res.* **1974**, *5*, 49.
(11) (a) Fenoglio, I.; Croce, A.; Di Renzo, F.; Tiozzo, R.; Fubini, B. *Chem. Res. Toxicol.* **2000**, *13*, 489. (b) Johnston, C. J.; Driscoll, K. E.; Finkelstein, J. N.; Baggs, R.; O'Reilly, M. A.; Carter, J.; Gelein, R.; Oberdörster, G. *Toxicol. Sci.* **2000**, *56*, 405. (c) Fubini, B.; Zanetti, G.; Altilia, S.; Tiozzo, R.; Lison, D.; Saffioti, U. *Chem. Res. Toxicol.* **1999**, *12*, 737. (d) Kim, Y. H.; Kim, K. S.; Kwak, N. J.; Lee, K. H.; Kweon, S. A.; Lim, Y. *J. Biosci.* **2003**, *28*, 77. (e) Schins, R. P. F.; Duffin, R.; Hohr, D.; Knaapen, A. M.; Shi, T.; Weisaupt, C.; Stone, V.; Donaldson, K.; Borm, P. J. A. *Chem. Res. Toxicol.* **2002**, *15*, 1166.
(12) Ayers, M. R.; Hunt, A. J. *J. Non-Cryst. Solids* **2001**, *285*, 123.
(13) Depasse, J.; Warlus, J. *J. Colloid Interface Sci.* **1976**, *56*, 618.
(14) (a) Selvan, S. T.; Tan, T. T.; Ying, J. Y. *Adv. Mater.* **2005**, *17*, 1620. (b) Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Javier, A. M.; Gaub, H. E.; Stolze, S.; Fertig, N.; Parak, W. J. *Nano Lett.* **2005**, *5*, 331.
(15) For a review see: (a) Mallet, S. V.; Cox, D. J. A. *Br. J. Anaesth.* **1992**, *67*, 307. (b) Kurata, M.; Horii, I. *J. Toxicol. Sci.* **2004**, *29*, 13.
(16) Xu, H.; Yan, F.; Monson, E. E.; Kopelman, R. *J. Biomed. Mater. Res.* **2003**, *66A*, 870.
(17) Mosqueira, V. C. F.; Legrand, P.; Gulik, A.; Bourdon, O.; Gref, R.; Labarre, D.; Barratt, G. *Biomaterials* **2001**, *22*, 2967.
(18) Kenausis, G. L.; Voros, J.; Elbert, D. L.; Huang, N.; Hofer, R.; Ruiz-Taylor, L.; Textor, M.; Hubbell, J. A.; Spencer, N. D. *J. Phys. Chem. B* **2000**, *104*, 3298.
(19) Morey, T. E.; Varshney, M.; Flint, J. A.; Seubert, C. N.; Smith, W. B.; Bioraker, D. G.; Shah, D. O.; Dennis, D. O. *J. Nanopart. Res.* **2004**, *6*, 159.

BM050820+