J|A|C|S COMMUNICATIONS

Published on Web 07/16/2010

Noninvasive Remote-Controlled Release of Drug Molecules in Vitro Using Magnetic Actuation of Mechanized Nanoparticles

Courtney R. Thomas,[†,‡] Daniel P. Ferris,[†,‡] Jae-Hyun Lee,[§] Eunjoo Choi,[§] Mi Hyeon Cho,[§] Eun Sook Kim,[∥] J. Fraser Stoddart,[⊥] Jeon-Soo Shin,[∥] Jinwoo Cheon,[*,§] and Jeffrey I. Zink[*,†,‡]

Department of Chemistry and Biochemistry and California NanoSystems Institute, University of California, Los Angeles, California 90095-1569, Department of Chemistry, Yonsei University, 262 Seongsanno Seodaemun-Gu, 120-752 Seoul, Korea, Department of Microbiology, College of Medicine, Yonsei University, 120-749 Seoul, Korea, and Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113

Received March 30, 2010; E-mail: jcheon@yonsei.ac.kr; zink@chem.ucla.edu

Abstract: Mesoporous silica nanoparticles are useful nanomaterials that have demonstrated the ability to contain and release cargos with mediation by gatekeepers. Magnetic nanocrystals have the ability to exhibit hyperthermic effects when placed in an oscillating magnetic field. In a system combining these two materials and a thermally sensitive gatekeeper, a unique drug delivery system can be produced. A novel material that incorporates zinc-doped iron oxide nanocrystals within a mesoporous silica framework that has been surface-modified with pseudorotaxanes is described. Upon application of an AC magnetic field, the nanocrystals generate local internal heating, causing the molecular machines to disassemble and allowing the cargos (drugs) to be released. When breast cancer cells (MDA-MB-231) were treated with doxorubicin-loaded particles and exposed to an AC field, cell death occurred. This material promises to be a noninvasive, externally controlled drug delivery system with cancer-killing properties.

Mesoporous silica nanoparticles (MSNs) have attracted widespread research interest as functional materials.[1−7] They are endocytosed by cells,[1] are nontoxic,[2] and can be used to deliver drugs.[3] Recently, an amazing array of methods for controlling pores to trap and release cargos have been developed. These range from coatings on particles to intricate nanovalves that control the pore openings using light,[4] pH,[5] or redox[6] activation. For therapeutic applications, an external and noninvasive method of actuation is preferable for control of therapeutic effects. Light control has been demonstrated, but its practical applicability is limited because of shallow tissue penetration for photodynamic therapies. Nanovalves based on changes in pH are self-opening but cannot be controlled by an external stimulus. This lack of an effective, external control for in vivo applications can be overcome by a new class of materials driven by a magnetic core.

Magnetic nanocrystals (NCs) are of importance in biomedical applications, as they can be used for both therapeutics and imaging. The usefulness of magnetic materials for inducing hyperthermic effects when placed in an oscillating magnetic field[7] and for T_2-weighted magnetic resonance imaging (T2MRI) contrast[8] make magnetic NCs theranostic. Among those developed, zinc-doped iron oxide nanocrystals (ZnNCs)[9] improve upon existing materials by offering a 4-fold increase in hyperthermic effects and a roughly 10-fold increase in MRI contrast relative to undoped iron oxide NCs.

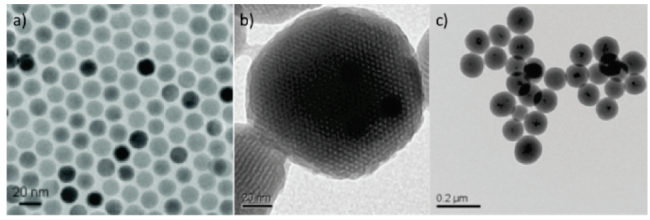


Figure 1. Electron micrographs of (a) zinc-doped iron oxide nanocrystals (ZnNCs) and (b, c) ZnNCs encapsulated within mesoporous silica.

We have combined the advantages of mechanized silica (MSNs with nanovalves) with those of zinc-doped iron oxide (Figure 1) to create a new generation of drug delivery systems responsive to heat activation. To this effect, a nanovalve was chosen that is non-self-opening in biological systems, exhibits thermal stability at room temperature, and can be operated under heating.

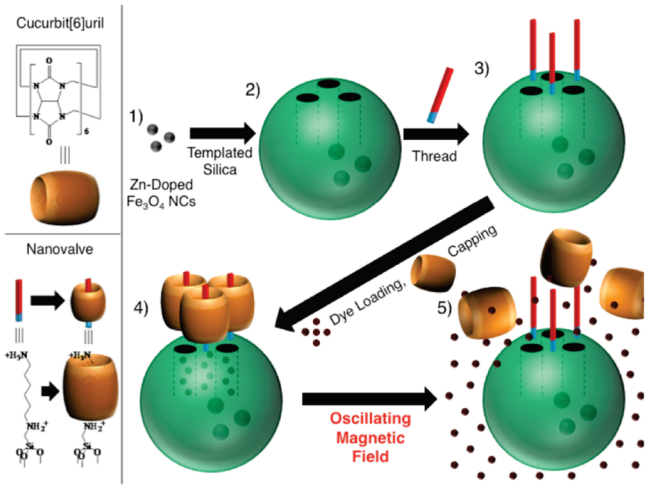
When this nanovalve is attached to the surface of a mesoporous particle, an increase in temperature causes the valve to open, allowing materials contained within to diffuse out. If the nanoparticles contain ZnNCs, then application of an oscillating magnetic field induces local heating, which should result in the same drug-release effect. This novel approach to drug delivery allows cargo to be contained within the nanoparticle at body temperature but results in controlled release of the therapeutic agent to induce apoptosis upon local heating generated by the ZnNCs.

In this study, we discuss four experiments performed on this magnetically activated release system (MARS): (1) macroscopic heating of the solution to induce guest release; (2) magnetic heating via application of an oscillating magnetic field as an external control; (3) localized magnetic heating without increasing the solution temperature in a thermostatted medium; and (4) remote-controlled actuation of the nanovalves to demonstrate controlled drug delivery in cancer cells.

Magnetic-core silica nanoparticles (MCSNs, Scheme 1) were synthesized by modifying a standard MCM-41-type synthesis.[2c] To contain the ZnNCs within the silica cores, the ZnNCs were first stabilized in a surfactant solution. The silica precursor tetraethyl orthosilicate (TEOS) was added to a solution containing the cetyltrimethylammonium bromide (CTAB)-stabilized ZnNCs with sodium hydroxide. The base catalyzed the hydrolysis of the silica precursor to form the mesostructured nanoparticles around the ZnNCs. Particle characterization confirmed the size and pore diameter, and inclusion of ZnNCs was confirmed by transmission

[†] Department of Chemistry and Biochemistry, University of California, Los Angeles.
[‡] California NanoSystems Institute, University of California, Los Angeles.
[§] Department of Chemistry, Yonsei University.
[∥] Department of Microbiology, Yonsei University.
[⊥] Northwestern University.

Scheme 1. Sketch of Nanoparticles, Machines, and Assembly[a]



[a] ZnNCs (1) are synthetically positioned at the core of the mesoporous silica nanoparticles (2). The base of the molecular machine is then attached to the nanoparticle surface (3). Drug is loaded into the particle and capped (4) to complete the system. Release can be realized using remote heating via the introduction of an oscillating magnetic field (5). The particles and machines are not drawn to scale.

electron microscopy (Figure 1 and Table S1 and Figure S1 in the Supporting Information). To assemble the nanovalve for facilitation of magnetic actuation, a molecular machine was assembled on the particle. *N*-(6-*N*-Aminohexyl)aminomethyltriethoxysilane was first condensed on the particle surface. Cargo loading was accomplished by soaking the nanoparticles in a saturated solution of rhodamine B or doxorubicin to fill the mesoporous structure by diffusion, resulting in a 4 wt % loading. Containment of cargo in the pores was achieved by adding cucurbit[6]uril, which electrostatically binds the molecular thread on the silica nanoparticle surface to the interior of the 1 nm cyclic cucurbit[6]uril cavity.[5b,10] After this step, the MARS nanoparticles were washed thoroughly with water to remove excess dye adsorbed on the silica surface.

A nanovalve was selected for the MARS that remained closed at physiological temperature and opened when heated. The valves were attached to the surfaces of MSNs without magnetic cores, and external heat was applied (Figure S3). At room temperature, the valves remained closed, and as the applied temperature was increased, dye was released.

The complete MARS was tested to determine whether magnetically induced heating would open the nanovalves, causing the release of the contained fluorescent molecules. To perform this study, MARS particles at room temperature were placed into an oscillating magnetic field, and dye release was observed as a function of time. Although the source of heat was changed from an external source to the internal heating caused by magnetic actuation, dye release was still observed (Figure 2a).

A sample of MCSNs was placed into an oscillating magnetic field to measure their effect on solution temperature. A sample at a concentration of 10 mg/mL was placed inside a water-cooled copper coil producing an alternating current (AC) magnetic field having a frequency of 500 kHz and a current amplitude of 37.4 kAm^{-1} (Taeyang Instrument Company, Korea). The temperature of the 1 mL of water above the particles was monitored and found to increase to a maximum temperature of 52 °C (Figure S2). This effect was also observed for the ZnNCs in solution.[9]

For therapeutic applications, it is important to know whether the opening of the nanovalve is a result of internal heating of the nanoparticle or an increase in the ambient temperature. To determine

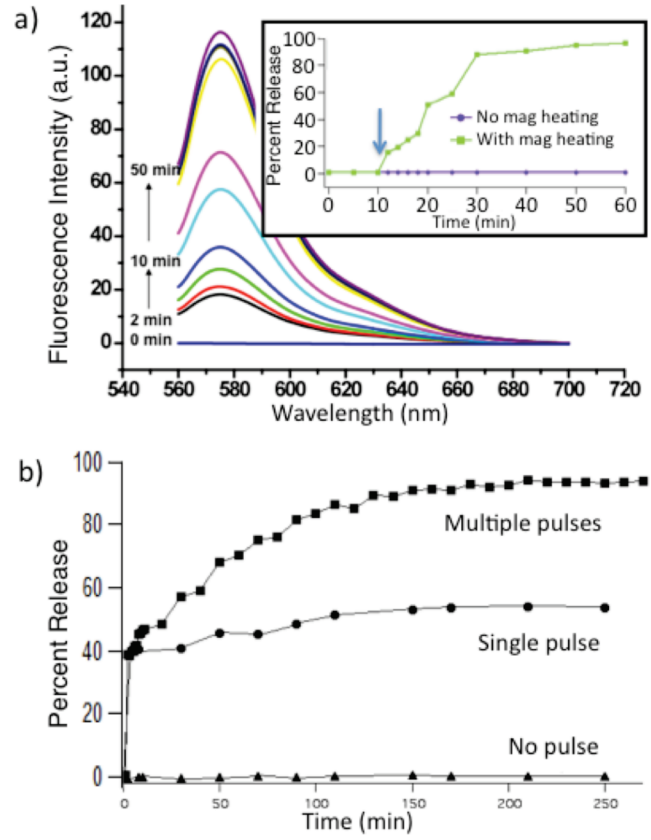


Figure 2. Cargo release using magnetic actuation. In (a), the MARS nanoparticles were continuously exposed to the magnetic field. The inset shows the data as a release profile. In (b), a sample was kept at 0 °C and exposed to pulses of the magnetic field. A single AC magnetic field exposure (●) exhibited ~40% cargo release after an initial 1 min pulse. Multiple pulses performed at 1, 3, 5, 7, and 9 min and then every 20 min for 270 min (■) enabled more dye release until all of the dye diffused out. A baseline (▲) was obtained by monitoring the fluorescence with no pulses. The low temperature of the surrounding solution (0 °C) was maintained in order to observe the effects only from the magnetic field and not from heating of the surrounding solution.

whether internal heating alone causes the valve to open, a sample of the MARS was kept at 0 °C and placed into the oscillating magnetic field. The MARS was then activated by applying 1 min pulses of the AC field, and dye release was monitored using small aliquots of the particle solution placed in a fluorometer. A single pulse caused 40% of the rhodamine B dye to be released with a dramatic increase in solution fluorescence (● in Figure 2b), which we attribute to rapid internal particle heating and valve opening. A second sample, pulsed intermittently, not only showed the same initial release of cargo but also continued dye release upon each additional pulse (■ in Figure 2b). It is clear that under these conditions, the local internal heating is important for dye release but macroscopic heating of the bulk solution is not necessary for valve actuation.

These materials are useful for in vitro drug delivery, as demonstrated by the release of anticancer drugs in the breast cancer cell line MDA-MB-231 (Figure 3a). The MARS nanoparticles were taken up by the cells, and minimal drug release was observed because the surface-attached valves were closed (Figure 3a, images 1 and 2). In the presence of the oscillating field, the local heating caused by the magnetic ZnNCs facilitated the release of doxorubicin from the silica pores, inducing apoptosis in the breast cancer cells (Figure 3a, images 5 and 6). In the images taken after a 5 min exposure to the magnetic field, a dramatic increase in intensity from

the doxorubicin (red color) was seen as a result of drug delivery into the cells.

The effect of the MARS on the cells was examined without drug loading under the same conditions as those with drug-loaded particles. When a sample not containing doxorubicin was endocytosed into the cells and exposed to the oscillating magnetic field, 16% cell killing was observed, while 37% cell killing resulted from exposure to the magnetic field when doxorubicin was contained in the mesopores (Figure 3b). Thus, both hyperthermia and drug delivery contributed to cell death.

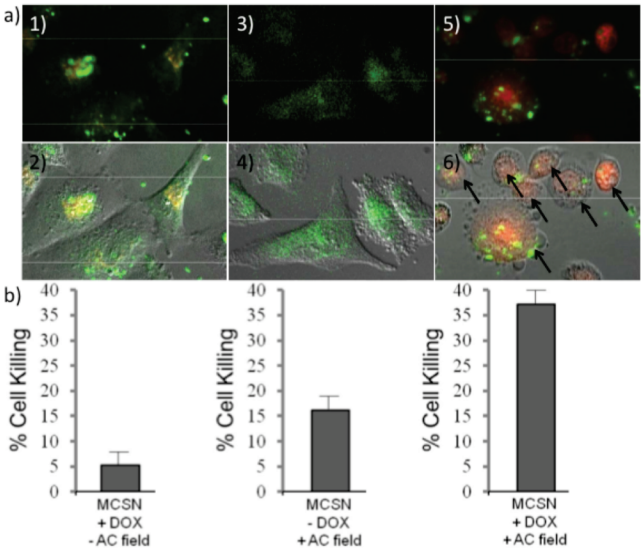


Figure 3. Results of MDA-MB-231 exposure to the MARS. Panel (a) shows fluorescent microscope images (1, 3, and 5) and fluorescent images with differential interference contrast (2, 4, and 6). Color scheme: green, fluorescently labeled MARS; red, doxorubicin (DOX); yellow, merged green and red. MARS nanoparticles containing DOX were taken up into the cells, but before the AC field was applied, no drug release (images 1 and 2) and negligible cell death [∼5%; panel (b), left bar] occurred. Images 3 and 4 show the effects of the magnetic field on MARS nanoparticles without DOX in the pores. Heating from the particles accounted for 16% of the cell killing [panel (b), middle bar]. Images 5 and 6 demonstrate DOX release after a 5 min AC field exposure, which caused 37% of the cell death [panel b, right bar]. The arrows in image 6 indicate the location of apoptotic cells.

In summary, we have demonstrated that novel magnetic-core silica nanoparticles are effective in actuating nanovalves and releasing anticancer drugs upon exposure to an oscillating magnetic field. Additionally, we have shown the feasibility of using this system as a drug delivery system in cancer cells. Optimization to balance the hyperthermic and apoptotic effects by varying the length of the magnetic actuation is under investigation.

Acknowledgment. The authors thank Monty Liong and Sarah Angelos for discussions and help in testing the theoretical and experimental framework for this study. This work was supported in part by the UC Toxic Substances Training and Research Program, the National Science Foundation (NSF CHE 0809384), the Nanomedical National Core Research Center (R15-2004-024-00000-0), the Creative Research Initiative (2010-0018286), the National Research Laboratory (M10600000255), the World Class University Program, a KOSEF grant funded by the Korean Government (2009-00810010), and Second Stage BK21 Project for Chemistry and Medical Sciences of Yonsei University.

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

References

(1) (a) Slowing, I.; Trewyn, B. G.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2006**, *128*, 14792–14793. (b) Lu, J.; Liong, M.; Zink, J. I.; Tamanoi, F. *Small* **2007**, *3*, 1341–1346. (c) Slowing, I.; Trewyn, B. G.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2007**, *129*, 8845–8849. (d) Vallet-Regi, M.; Balas, F.; Arcos, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548–7558. (e) Liong, M.; Lu, J.; Kovochich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. *ACS Nano* **2008**, *2*, 889–896. (f) Rosenholm, J. M.; Meinander, A.; Peuhu, E.; Niemi, R.; Eriksson, J. E.; Sahlgren, C.; Lind, M. *ACS Nano* **2009**, *3*, 197–206.

(2) (a) Lin, Y.-S.; Tsai, C.-P.; Huang, H.-Y.; Kuo, C.-T.; Hung, Y.; Huang, D.-M.; Chen, Y.-C.; Mou, C.-Y. *Chem. Mater.* **2005**, *17*, 4570–4573. (b) Lin, Y.-S.; Wu, S.-H.; Hung, Y.; Chou, Y.-H.; Chang, C.; Lin, M.-L.; Tsai, C.-P.; Mou, C.-Y. *Chem. Mater.* **2006**, *18*, 5170–5172. (c) Liong, M.; Lu, J.; Kovochich, M.; Xia, T.; Ruehm, S. G.; Nel, A. E.; Tamanoi, F.; Zink, J. I. *ACS Nano* **2008**, *2*, 889–896.

(3) (a) Lai, C.-Y.; Trewyn, B. G.; Jeftinija, D. M.; Jeftinija, K.; Xu, S.; Jeftinija, S.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2003**, *125*, 4451–4459. (b) Giri, S.; Trewyn, B. G.; Stellmaker, M. P.; Lin, V. S.-Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 5038–5044. (c) Aznar, E.; Marcos, M. D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Amoros, P.; Guillem, C. *J. Am. Chem. Soc.* **2009**, *131*, 6833–6843. (d) Bernardos, A.; Aznar, E.; Marcos, M. D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Barat, J. M.; Amoros, P. *Angew. Chem., Int. Ed.* **2009**, *48*, 5884–5887. (e) Schlossbauer, A.; Kecht, J.; Bein, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 3092–3095. (f) Coti, K. K.; Belowich, M. E.; Liong, M.; Ambrogio, M. W.; Lau, Y. A.; Khatib, H. A.; Zink, J. I.; Khashab, N. M.; Stoddart, J. F. *Nanoscale* **2009**, *1*, 16–39. (g) Zhao, Y.; Trewyn, B. G.; Slowing, I. I.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2009**, *131*, 8398–8400.

(4) (a) Zhu, Y.; Fujiwara, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2241–2244. (b) Lu, J.; Choi, E.; Tamanoi, F.; Zink, J. I. *Small* **2008**, *4*, 421–426. (c) Mal, N. K.; Fujiwara, M.; Tanaka, Y. *Nature* **2003**, *421*, 350–353. (d) Ferris, D. P.; Zhao, Y.-L.; Khashab, N. M.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2009**, *131*, 1686–1688. (e) Park, C.; Lee, K.; Kim, C. *Angew. Chem., Int. Ed.* **2009**, *48*, 1275–1278. (f) Vivero-Escoto, J. L.; Slowing, I. I.; Wu, C.-W.; Lin, V. S.-Y. *J. Am. Chem. Soc.* **2009**, *131*, 3462–3463. (g) Nguyen, T. D.; Leung, K. C.-F.; Liong, M.; Liu, Y.; Stoddart, J. F.; Zink, J. I. *Adv. Funct. Mater.* **2007**, *17*, 2101–2110.

(5) (a) Park, C.; Oh, K.; Lee, S. C.; Kim, C. *Angew. Chem., Int. Ed.* **2007**, *46*, 1455–1457. (b) Angelos, S.; Khashab, N. M.; Yang, Y.-W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. *J. Am. Chem. Soc.* **2009**, *131*, 12912–12914. (c) Leung, K. C.-F.; Nguyen, T. D.; Stoddart, J. F.; Zink, J. I. *Chem. Mater.* **2006**, *18*, 5919–5928.

(6) (a) Nguyen, T. D.; Tseng, H.-R.; Celestre, P. C.; Flood, A. H.; Liu, Y.; Stoddart, J. F.; Zink, J. I. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10029–10034. (b) Liu, R.; Zhao, X.; Wu, T.; Feng, P. *J. Am. Chem. Soc.* **2008**, *130*, 14418–14419. (c) Saha, S.; Johansson, E.; Flood, A. H.; Tseng, H.-R.; Zink, J. I.; Stoddart, J. F. *Chem.—Eur. J.* **2005**, *11*, 6846–6858.

(7) (a) Fortin, J.-P.; Wilhelm, C.; Servais, J.; Menager, C.; Bacri, J.-C.; Gazeau, F. *J. Am. Chem. Soc.* **2007**, *129*, 2628–2635. (b) Derfus, A. M.; Maltzahn, G.; Harris, T. J.; Duza, T.; Vecchio, K. S.; Ruoslahti, E.; Bhatia, S. N. *Adv. Mater.* **2007**, *19*, 3932–3936. (c) Hu, S.-H.; Chen, S.-Y.; Liu, D.-M.; Hsaio, C.-S. *Adv. Mater.* **2008**, *20*, 2690–2695.

(8) (a) Weissleder, R.; Moores, A.; Mahmood, U.; Bhorade, R.; Benveniste, H.; Chiocca, E. A.; Basilion, J. P. *Nat. Med.* **2000**, *6*, 351–354. (b) Jun, Y.-W.; Lee, J.-H.; Cheon, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 5122–5135. (c) Laurent, S.; Forge, D.; Port, M.; Roch, A.; Robic, C.; Elst, L. V.; Muller, R. N. *Chem. Rev.* **2008**, *108*, 2064–2110. (d) Lee, J.-H.; Huh, Y.-M.; Jun, Y.-W.; Seo, J.-W.; Jang, J.-T.; Song, H.-T.; Kim, S.; Cho, E.-J.; Yoon, H.-G.; Suh, J.-S.; Cheon, J. *Nat. Med.* **2007**, *13*, 95–99.

(9) Jang, J.-T.; Nah, H.; Lee, J.-H.; Moon, S. H.; Kim, M. G.; Cheon, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 1234–1238.

(10) (a) Mock, W. L. *Top. Curr. Chem.* **1995**, *175*, 1–24. (b) Kim, K. *Chem. Soc. Rev.* **2002**, *31*, 96–107. (c) Fusaro, L.; Locci, E.; Lai, A.; Luhmer, M. *J. Phys. Chem. B* **2008**, *112*, 15014–15020. (d) Masson, E.; Lu, X.; Ling, X.; Patchell, D. L. *Org. Lett.* **2009**, *11*, 3798–3801.

JA1022267