ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

Composite of Functional Mesoporous Silica and DNA: An Enzyme-Responsive Controlled Release Drug Carrier System

Guilong Zhang,[∥,†,‡] Minglei Yang,[∥,†] Dongqing Cai,[†] Kang Zheng,[¶] Xin Zhang,[§] Lifang Wu,*[,†] and Zhengyan Wu*[,†]

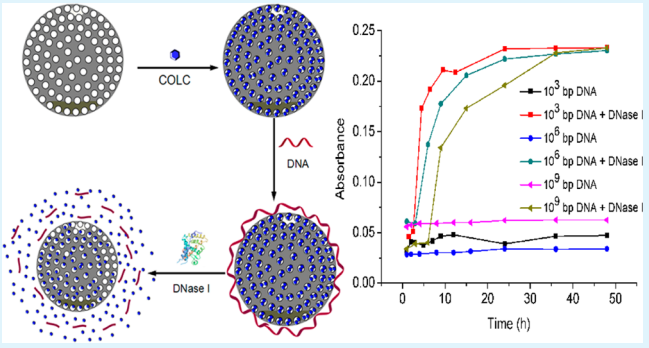
[†]Key Laboratory of Ion Beam Bioengineering, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, China

[‡]University of Science and Technology of China, Hefei 230026, China

[§]School of Life Sciences, Anhui Agricultural University, Hefei 230036, China

[¶]Key Laboratory of Materials Physics, Institute of Solid State Physics, Chinese Academy of Sciences, Hefei 230031, China

ABSTRACT: An efficient enzyme-responsive controlled release carrier system was successfully fabricated using single-stranded DNA encapsulated functional mesoporous silica nanoparticles. Mesoporous silica nanoparticles were initially fabricated through hydrolysis of tetraethyl orthosilicate (TEOS) in cetyltrimethylammonium chloride (CTAC) solution, and the surface of nanoparticles could be encapsulated with single-stranded DNA. This nanomaterial has excellent bioactivity and its hydrolysate cannot cause damage to normal cell, thus the biocompatibility of the nanomaterial is improved. In addition, this nanomaterial showed an excellent drug release performance when loaded with drugs, which would be helpful to increase the treatment efficiency and decrease side effects of drugs.



KEYWORDS: *mesoporous silica, DNA, enzyme-responsive, controlled release*

INTRODUCTION

Recently, controlled release drug carrier systems have attracted wide interest because they can maintain the valid drug concentrations and improve the therapeutic efficacy.[1−5] Because of nontoxicity, high biocompatibility, chemically stable porous nanostructure, and outstanding drug delivery performance,[6−10] mesoporous silica nanoparticles (MSNs) have been used as the most promising carrier materials for a controlled release system (CRS). Some CRSs based on MSNs have been described,[11−15] and controlled release performances of these systems have also received more and more attention.

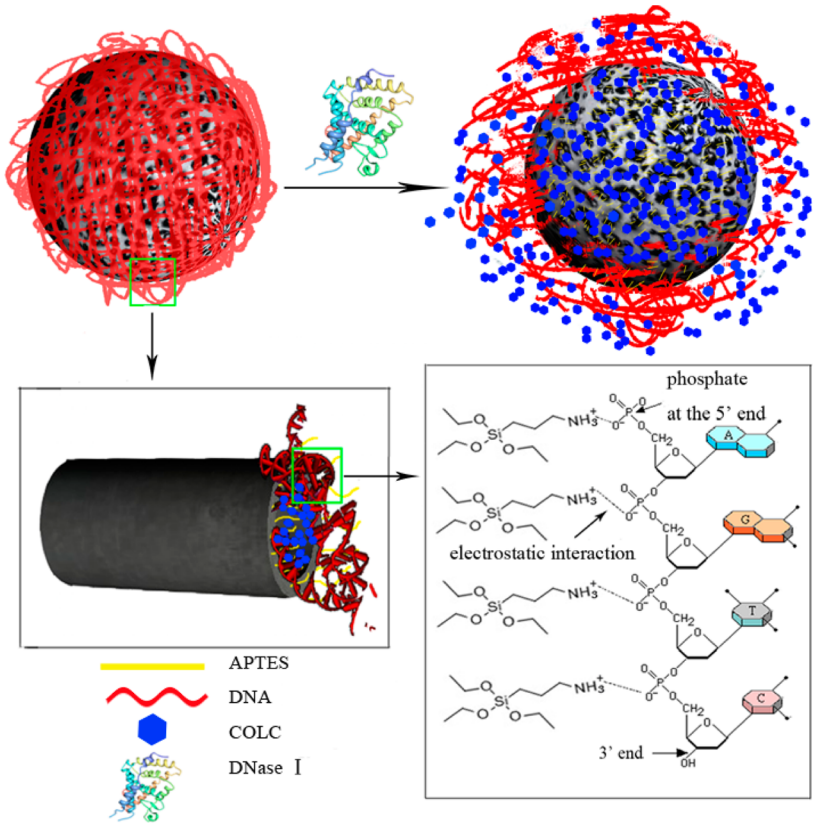
A number of CRSs with excellent controlled release performances have been developed successfully as carriers of nanomedicines, and the prevailing ones are usually fabricated using organic substances as the coating,[16] and their organic coating materials under enzyme catalysis could be decomposed into small molecules in vivo. It is well-known that lysosomes possess a variety of enzymes to resist the intrusion of exogenous substances into normal cell through enzyme catalysis.[17,18] When organic substance-coated nanomedicine is injected into living body, the nanomedicine can be delivered to lysosomes through endocytosis, and then, the organic substances will be degraded through the enzyme catalysis; thus, the drugs in CRS are released. Currently, some organic-based delivery systems (PLA, PEG, liposome, etc.) have been approved through FDA. However, some organic CRSs such as polymer still have potential risks because the decomposed small organic molecules are usually harmful to living body. Recently, some CRSs have been fabricated using DNA modified nanoparticle.[19−24] However, the majority of these DNA need to be modified with organic substance, which would result in the same problem. To avoid the problem, it is necessary to develop a new coating material. In this work, we chose DNA of edible plants, a natural and safe biomolecule, as the coating of nanomedicine, which makes CRS possess higher biosafety and biocompatibility compared with organic substances.

Here, we described an enzyme-responsive CRS using (3-Aminopropyl)triethoxysilane (APTES) modified mesoporous silica nanoparticles (AMSNs) encapsulated with the DNA from natural edible plants as the carrier. Currently, some reports on gene therapy indicated that DNA could possess excellent safety.[25,26] DNA of edible plants especially has excellent bioactivity, and its hydrolysate cannot cause damage to normal cells; thus, the biocompatibility of nanomedicine can be improved. In this work, it is the first time that enzyme-responsive CRS was fabricated through the nanoparticle

Received: December 17, 2013
Accepted: May 12, 2014
Published: May 12, 2014

   dx.doi.org/10.1021/am502154w | *ACS Appl. Mater. Interfaces* 2014, 6, 8042−8047

Scheme 1. Schematic Representation of Drug Release Process of Drug-Loaded AMSN Encapsulated with DNA



modified with single-stranded DNA of edible plants. The modification of APTES makes MSNs aminated and the surface of nanopaticles positively charged because of the amino protonation in simulated body fluid (SBF), which is beneficial for making negatively charged DNA encapsulate AMSNs through electrostatic interactions. As depicted in Scheme 1, once mesopores in AMSNs were loaded with drugs, the curly DNA molecules on the surface of nanoparticles could encapsulate the nanoparticles, acting as gates of the mesopores to keep the drugs enclosed. When DNase I enzyme was added into SBF, the DNA was hydrolyzed to plenty of tiny fragments, and obviously, the gates started to open and drugs could be released from the mesopores. Moreover, in comparison with the reported CRSs, this system may possess higher biocompatibility and lower side effect.

EXPERIMENTAL SECTION

Materials. All chemical reagents were used as received without further purification. Triethanolamine (TEA), cetyltrimethylammonium chloride (CTAC), and (3-aminopropyl)triethoxysilane (APTES) were purchased from Sinopharm Co. (Shanghai, China). Colchicine (COLC) and tetraethyl orthosilicate (TEOS) were received from Aladdin Co. (Shanghai, China). These chemical reagents were all of analytical grade. In addition, RNAprep Pure Plant kit was obtained from Tiangen Co. (Beijing China); RNaseA and LA Taq DNA polymerase were purchased from Takara Co. (Japan). Nanoclay hydrophilic bentonite was received from Sigma-Aldrich Co. (U.S.A.). Potassium acetate and GelRed were purchased from Sangon Biotech Co. (Shanghai, China). M-MLV Reverse Transcriptase was obtained from Promega Co. (Madison, U.S.A.). These reagents were all of bioreagent grade.

Synthesis of MSN. MSN was synthesized using a soft template method.[27] Briefly, CTAC (2.5 g) was added into ethanol solution (10 mL ethanol in 76 mL deionized water) under magnetic stirring at 60 °C. After 30 min, TEA (2 mL) was added into the resulted solution and stirred continuously for 2 h. Then, TEOS (1 mL) was added into the mixture solution dropwise and stirred continuously for 12 h, and the product was centrifuged under 15 000 rpm for 20 min. Afterward, the soft template was removed through reflux twice in acetone for 24 h at 60 °C.

Amine Modified MSNs. MSNs were added into the mixture of toluene and APTES to aminate the silica surface. The detailed procedures are as follows: MSNs (50 mg) was dispersed into 20 mL toluene under magnetic stirring, and then, 50 μL of APTES was added into the resulted solution under nitrogen and stirred for 24 h. Finally, the aminated nanoparticles were centrifuged and washed with ethanol and deionized water for three to five times.

DNA Extraction. To extract the genome DNA, leaves of tomato (*Solanum lycopersicum*) and wheat (*Triticum aestivum*) (approximately 5 g) were ground into powder by mortar in liquid nitrogen, respectively. Then, the powder was gently dispersed in extraction buffer containing 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.5 M NaCl, 10% (W/W) CTAB, 0.5% (W/W) bentonite, 2% (W/W) PVP-10, and 0.2% (W/W) 2-mercaptoethnol at 65 °C for 30 min, and then 34% (V/V) potassium acetate (5M, pH 4.8) was added to extraction buffer in ice bath for 20 min. Afterward, the following steps of chloroform washing, DNA precipitation and washing, and RNaseA treatment were carried out as previously described.[28]

To obtain 2000 bp DNA fragments, the total RNA was extracted from the flowers of *Pistacia chinensis* using RNAiso plus. The cDNAs obtained from RNA using the M-MLV Reverse Transcriptase were used for polymerase chain reaction (PCR) as a template, and then, the PCR reaction (25 μL reaction volume containing template DNA, dNTPs, $MgCl_2$, Taq DNA polymerase, primer, and PCR buffer) was carried out. The primer was designed to amplify 12-ox-phytodienoic acid reductase gene, which has the length of approximately 1200 base pairs. Amplification was performed in a thermocycler. After programmed for an initial denaturation at 94 °C for 8 min, 94 °C for 30 s, and 55 °C for 30 s, 72 °C for 1 min for 35 cycles and a final

dx.doi.org/10.1021/am502154w | ACS Appl. Mater. Interfaces 2014, 6, 8042−8047

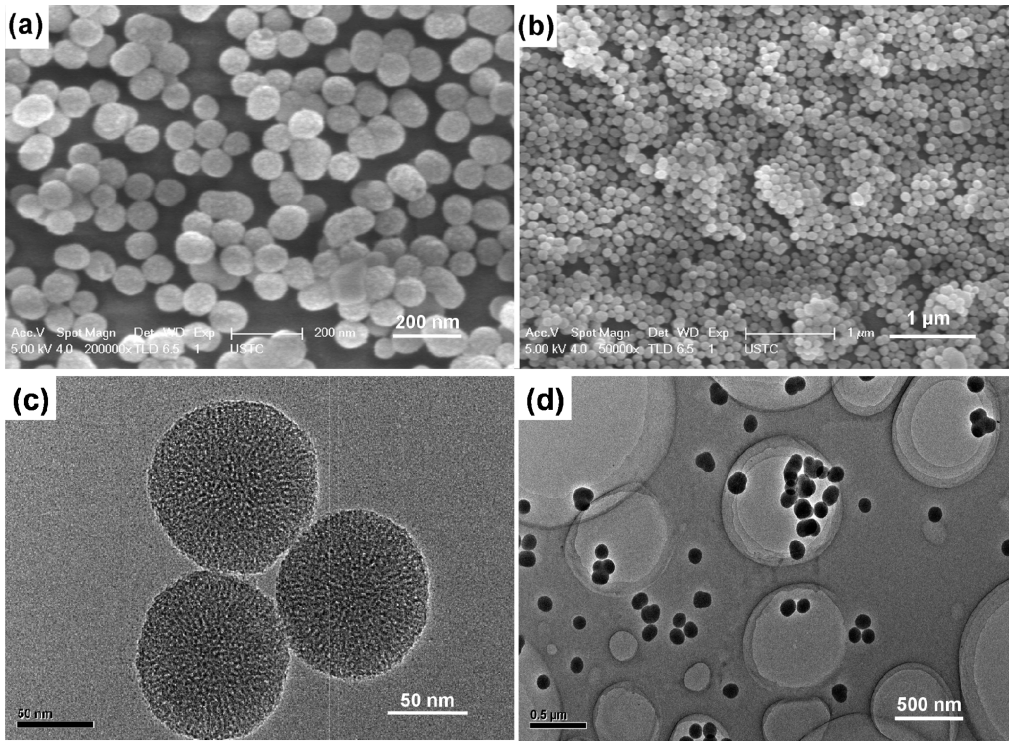


Figure 1. SEM (a, b) and TEM (c, d) images of mesoporous silica nanoparticles.

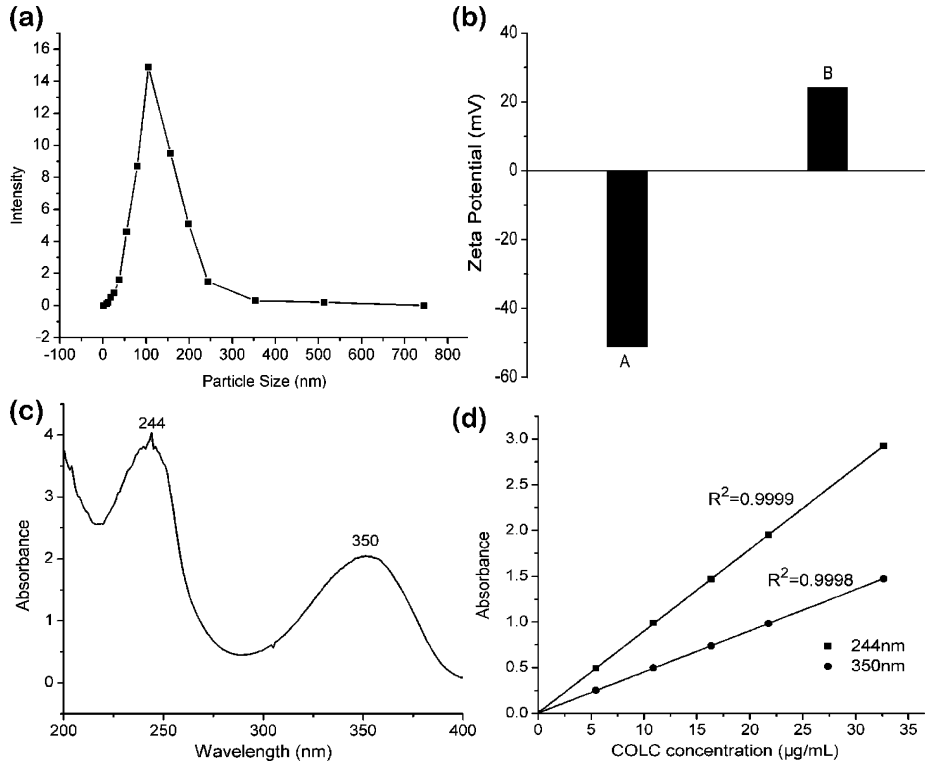


Figure 2. (a) Particle size distribution of MSN, (b) zeta potential of (A) MSN and (B) AMSN, (c) UV−vis absorption wavelength, and (d) calibration curve of COLC.

auto extension step, the amplification products were analyzed by agarose (1.5%) and stained with GelRed.

Storage and Release of Drug. (a) AMSNs (10 mg) were dispersed in 5 mL of COLC solution (10 mg/mL) by shaking at ambient temperature for 24 h, making COLC loaded in pores of AMSNs. Then, 500 μL of DNA (1.5 mg/mL) was added into the mixture solution and heated at 95 °C for 1 h to obtain flexible single strands. After that, urea (50 mg) was added into the resulted solution to prevent DNA single strands from forming rigid double strands. The resulted system was shaken at an ice bath for 24 h to obtain DNA-encapsulated AMSNs. (b) 10 mg of DNA-encapsulated AMSNs were dispersed in 6 mL SBF by shaking for 24 h at 36.5 °C. The COLC

dx.doi.org/10.1021/am502154w | ACS Appl. Mater. Interfaces 2014, 6, 8042−8047

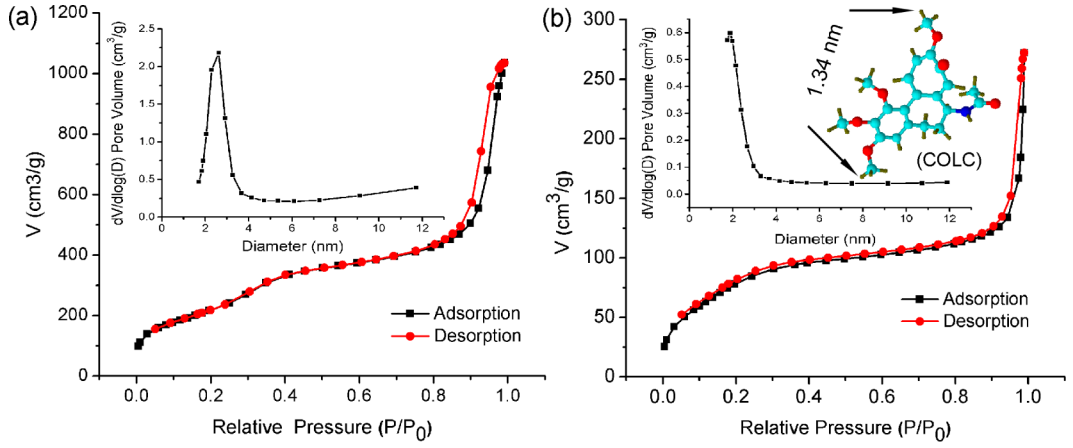


Figure 3. N_2 adsorption−desorption isotherms of (a) MSN and (b) AMSN (inset of a, pore size distribution of MSN; inset of b, pore size distribution of AMSN, and structure and size of COLC molecule).

concentration in the supernatant after centrifuging was determined using a UV−vis spectrophotometer (UV 2550, Shimadzu Co., Japan) at wavelength of 244 or 350 nm to get the drug release performance.

Characterization. The morphology and microstructure were observed on a scanning electron microscope (Sirion 200, FEI Co., U.S.A.) and an H-800 transmission electron microscope (Hitachi Co., Japan). The particle size distribution was determined by a dynamic light scattering (DLS) detector (Zetasizer 300, Malvern Co., U.K.). The pore size distribution was measured using a porosimetry analyzer (Tristar II, 3020M, Micromeritics Co., U.S.A.). The structure and interaction were analyzed using a Fourier transform infrared spectrometer (Bruker Co., Germany).

RESULTS AND DISCUSSION

MSN was synthesized by adding a mixture of TEOS and TEA to homogeneous CTAC solutions at 60 °C, and the size of MSN depends on the adding amount of TEA. Scanning electron microscope (SEM) images showed that MSNs had homogeneous size of approximately 100 nm (Figure 1a and b). In addition, the particle size distribution of MSNs is shown in Figure 2a, and the same result was obtained (approximately 100 nm). Transmission electron microscope (TEM) images showed that MSN in the solution possessed obvious mesopores and high dispersibility (Figure 1c and d). To make MSN positively charged, APTES was used to aminate MSN, which could increase the zeta potential of nanoparticles because of the protonation of amino group in simulated body fluid (SBF). It was shown that AMSN had more positive charges than MSN (Figure 2b).

The nitrogen adsorption−desorption isotherm curves of MSNs and AMSNs and their corresponding pore size distribution curves are shown in Figure 3. It was found that the amounts of nitrogen absorption at 0.8−1.0 of P/P_0 increased sharply, which is probably because of the pores among the aggregated particles (Figure 3a). In addition, the isotherm curves of MSNs at 0.15−0.6 of P/P_0 exhibited that MSN possessed ordered mesoporous structure and high porosity, and the pore size distribution curve showed that pore sizes narrowly distributed over 2−4 nm (dominantly 2.9 nm) (inset of Figure 3a). However, the isotherm curve of AMSNs indicated lower porosity and smaller pore size of AMSN compared with MSN (Figure 3b), because the amino groups on the surface of AMSN decrease the porosity and pore size. COLC molecule size was estimated through Chemdraw software to be around 1.34 nm (inset of Figure 3b). Therefore, although the pore size of AMSN was decreased to 2.0 nm, it was also suitable for loading drug molecules.

Colchicine (COLC), a drug used in cancer treatment, was selected as a model drug to be loaded in AMSN, and DNA molecules could act as the "guard" to control the release of drugs from the pores of AMSN. Drug-loading capacity of AMSN was determined using a UV−vis spectra. The absorption wavelength and corresponding calibration curve of COLC are shown in Figure 2c and d. It was shown that AMSN possessed high drug storage capacity (242 μg/mg) through UV−vis analysis, which is helpful to provide continuous treatment to diseased tissue. The rigid duplex DNA was thermally treated at 95 °C and then treated with urea to obtain the stable flexible single strands, which is beneficial for encapsulating the AMSN. As is shown in Figure 4a, molecular weight of DNA could be verified through agarose gel

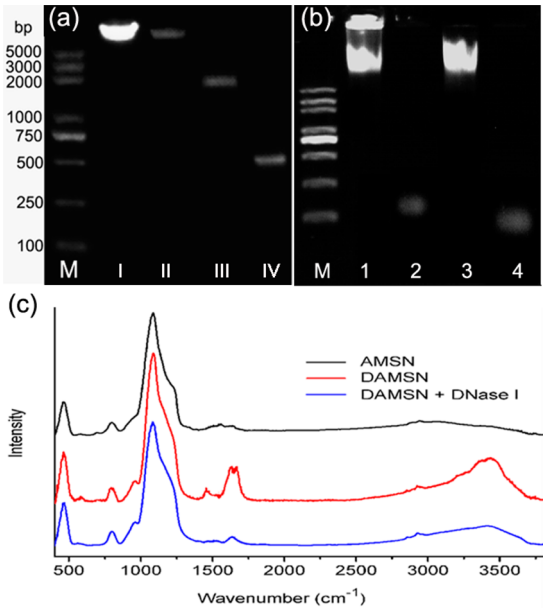


Figure 4. (a) DNA electrophoresis images (Marker (M), 10^9 bp DNA (I), 10^6 bp DNA (II), 2×10^3 bp DNA (III), 2×10^3 bp DNA after melting at 95 °C (IV)); (b) DNA electrophoresis images (M, marker; samples 1 and 3, 10^9 and 10^6 bp DNA; samples 2 and 4, 10^9 and 10^6 bp DNA after DNase I degradation); (c) FTIR spectra of AMSN, DAMSN, and DAMSN after enzyme degradation.

dx.doi.org/10.1021/am502154w | ACS Appl. Mater. Interfaces 2014, 6, 8042−8047

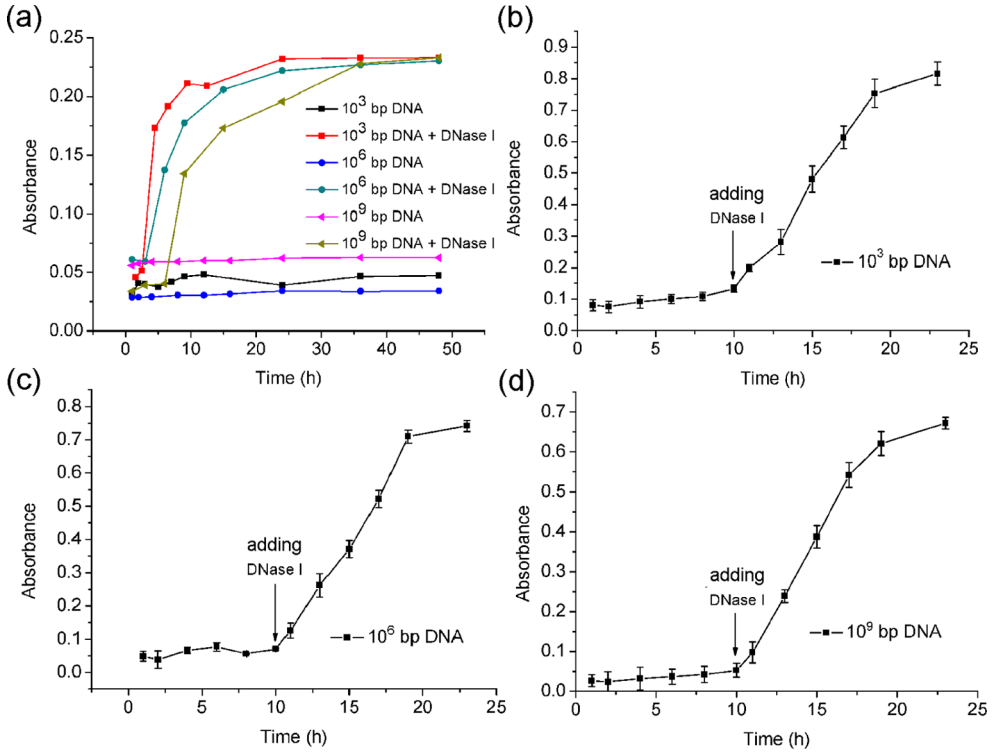


Figure 5. Release behavior of COLC in AMSN encapsulated with DNA: (a) with and without addition of DNase I; (b–d) before and after adding DNase I for 10^3 bp (b), 10^6 bp (c) and 10^9 bp (d) DNA.

electrophoresis (AGE). Compared with sample III, sample IV showed smaller molecular weight and possessed flexible chains, which indicated that duplex DNA was successfully divided into single strands. In order to obtain the interactions among AMSN, AMSN encapsulated with DNA (DAMSN), and DAMSN after enzyme degradation, FTIR analysis was performed (Figure 4c). For AMSN, the peak at 1089 cm^{-1} assigns to Si—O stretching vibration. For DAMSN, the peak at 3430 cm^{-1} ascribes to the NH stretching vibration of DNA, indicating the binding of nucleic acid bases onto the surface of AMSN. The peaks at 1667 and 1634 cm^{-1} are assigned to the C═O stretching vibration of nucleotide. This splitting of the C═O stretching vibration peak is assigned to polynucleotide double helix structure because of the antiparallel orientation of each helix.[29] In addition, the peaks at 1456 and 1472 cm^{-1} reflect the purine and pyrimidine (DNA bases) ring modes.[30] These results indicated that DNA successfully encapsulated AMSN. The FTIR spectrum of DAMSN after enzyme degradation displayed that some DNA characteristic peaks became weakened or even disappeared, indicating that the DNA coating on AMSN has been hydrolyzed by DNase I enzyme.

The hydrolysis of DNA coating was also investigated by agarose gel electrophoresis (Figure 4b). Large electrophoresis spot could be seen clearly for both sample 1 and 3, because DNA with high molecular weight was unable to penetrate the agarose gel. Nevertheless, some DNA fragments could be found in SBF when DNase I was added into the solution (sample 2 and 4 in Figure 4b). These results implied that the DNA gates of AMSN could be opened effectively through the hydrolization effect of DNase I.

To investigate enzyme-responsive release performance of COLC, COLC-loaded DAMSNs (CDAMSNs) were placed in DNase I SBF at 36.5 °C. Figure 5a showed that few drugs were released when AMSNs were encapsulated with DNA single strands, which indicated that the gates of the mesopores were almost closed because of the strong electrostatic interactions between the positive groups (NH_3^+) on the surface of AMSN and the negative groups of single-stranded DNA. However, after about 2 h since the addition of DNase I, the COLC molecules began to be released in varying degrees, because the gates started to open resulting from the hydrolysis of DNA strands. Additionally, the COLC release rate decreased with the increase of the molecular weight of DNA, which is because DNA with higher molecular weight was harder to hydrolyze, as shown in Figure 4b (10^6 bp DNA was hydrolyzed faster compared with 10^9 bp DNA). Interestingly, the release rate of COLC from CDAMSN with DNA of 10^9 bp increased steadily and gradually, suggesting that this drug delivery system might be long-acting and continuous, which is helpful to maintain the effective concentration of drugs in diseased tissue, enhance treatment efficiency, and decrease drug toxicity for normal cells.

This enzyme-responsive nanomedicine was investigated further, and the release behaviors of COLC in DAMSN before and after addition of DNase I are shown in Figure 5b–d. The absorbance intensity of COLC before addition of DNase I was monitored for 10 h, and the flat baselines showed that drug leakages from nanomedicine were weak and could be negligible. After DNase I was added into release system solution, the absorbance intensity of COLC increased rapidly, which indicates that the hydrolysis of DNA through DNase I could open the gates of MSN. The similar release behaviors appeared in all three DAMSNs (coated with 10^3, 10^6, or 10^9 bp DNA). These results demonstrated that CDAMSN was efficient for the storage of drugs and could meanwhile release drugs after addition of DNase I.

dx.doi.org/10.1021/am502154w | ACS Appl. Mater. Interfaces 2014, 6, 8042–8047

CONCLUSION

In summary, an efficient enzyme-responsive controlled release carrier system was successfully fabricated using a single-stranded DNA encapsulated AMSN. Based on the electrostatic interactions between flexible single-stranded DNA and AMSN, the single-stranded DNA coating could act as gates for storage of drugs. These gates could be opened to release drugs through the enzyme hydrolysis effect of DNase I, and the drug release performance could be controlled by the molecular weight of DNA; therefore, this drug delivery system might make a long-acting and continuous effect of drugs, which would be helpful to increase the treatment efficiency and decrease the side effects of drugs.

AUTHOR INFORMATION

Corresponding Authors

*Tel.: +86-551-65595012. Fax: +86-551-65591413. Email: lfwu@ipp.ac.cn.

*Tel.: +86-551-65591413. Fax: +86-551-65591413. Email: zywu@ipp.ac.cn.

Author Contributions

‖G.Z. and M.Y. are cofirst authors.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge financial support from the National Natural Science Foundation of China (No. 10975154, No. 11075178) and Hefei Center of Physical Science and Technology of China (No. 2012FXCX006).

REFERENCES

(1) Gullotti, E.; Yeo, Y. Extracellularly Activated Nanocarriers: A New Paradigm of Tumor Targeted Drug Delivery. *Mol. Pharmaceutics* **2009**, *6*, 1041.

(2) Angelos, S.; Khashab, N. M.; Yang, Y. W.; Trabolsi, A.; Khatib, H. A.; Stoddart, J. F.; Zink, J. I. pH Clock-Operated Mechanized Nanoparticles. *J. Am. Chem. Soc.* **2009**, *131*, 12912.

(3) Kyriakides, T. R.; Cheung, C. Y.; Murthy, N.; Bornstein, P.; Stayton, P. S.; Hoffman, A. S. Anti-tumor Effect of Intraperitoneal Administration of Cisplatin-Loaded Microspheres to Human Tumor Xenografted Nude Mice. *J. Controlled Release* **2002**, *78*, 295.

(4) Song, S. W.; Hidajat, K.; Kawi, S. pH-Controllable Drug Release Using Hydrogel Encapsulated Mesoporous Silica. *Chem. Commun.* **2007**, *42*, 4396.

(5) Lu, J.; Choi, E.; Tamanoi, F.; Zink, J. I. Light-Activated Nanoimpeller-Controlled Drug Release in Cancer Cells. *Small* **2008**, *4*, 421.

(6) Hsiao, J. K.; Tsai, C. P.; Chung, T. H.; Hung, Y.; Yao, M.; Liu, H. M.; Mou, C. Y.; Yang, C. S.; Chen, Y. C.; Huang, D. M. Mesoporous Silica Nanoparticles as a Delivery System of Gadolinium for Effective Human Stem Cell Tracking. *Small* **2008**, *4*, 1445.

(7) Vallet-Reg, M.; Balas, F.; Arcos, D. Mesoporous Materials for Drug Delivery. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548.

(8) Yang, P. P.; Quan, Z. W.; Hou, Z. Y.; Li, C. X.; Kang, X. J.; Cheng, Z. Y.; Lin, J. A Magnetic, Luminescent, and Mesoporous Core−Shell Structured Composite Material as Drug Carrier. *Biomaterials* **2009**, *30*, 4786.

(9) Zhu, C. L.; Song, X. Y.; Zhou, W. H.; Yang, H. H.; Wen, Y. H.; Wang, X. R. An Efficient Cell-Targeting and Intracellular Controlled-Release Drug Delivery System Based on MSN-PEM-Aptamer Conjugates. *J. Mater. Chem.* **2009**, *19*, 7765.

(10) Lee, C. H.; Cheng, S. H.; Huang, I. P.; Souris, J. S.; Yang, C. S.; Mou, C. Y.; Lo, L. W. Intracellular pH-Responsive Mesoporous Silica Nanoparticles for the Controlled Release of Anticancer Chemotherapeutics. *Angew. Chem., Int. Ed.* **2010**, *49*, 8214.

(11) Langer, R. Polymer-Controlled Drug Delivery Systems. *Acc. Chem. Res.* **1993**, *26*, 537.

(12) Yokoyama, M.; Okano, T. Targetable Drug Carriers: Present Status and a Future Perspective. *Adv. Drug Delivery Rev.* **1996**, *21*, 77.

(13) Guo, X.; Szoka, F. Chemical Approaches to Triggerable Lipid Vesicles for Drug and Gene Delivery. *Acc. Chem. Res.* **2003**, *36*, 335.

(14) Liu, J. N.; Bu, W. B.; Pan, L. M.; Shi, J. L. NIR-Triggered Anticancer Drug Delivery by Upconverting Nanoparticles with Integrated Azobenzene-Modified Mesoporous Silica. *Angew. Chem., Int. Ed.* **2013**, *52*, 4375.

(15) Pikabea, A.; Ramos, J.; Forcada, J. In Situ Monitoring of DNA-Aptavalve Gating Function on Mesoporous Silica Nanoparticles. *Part. Part. Syst. Charact.* **2014**, *31*, 161.

(16) Vemula, P. K.; Cruikshankb, G. A.; Karp, J. M.; John, G. Self-Assembled Prodrugs: An Enzymatically Triggered Drug-Delivery Platform. *Biomaterials* **2009**, *30*, 383.

(17) Torchilin, V. P. Recent Approaches to Intracellular Delivery of Drugs and DNA and Organelle Targeting. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 343.

(18) Kroemer, G.; Jaattela, M. Lysosomes and Autophagy in Cell Death Control. *Nat. Rev. Cancer* **2005**, *5*, 886.

(19) Climent, E.; Martínez-Máñez, R.; Sancenón, F.; Marcos, M. D.; Soto, J.; Maquieira, A.; Amorós, P. Controlled Delivery Using Oligonucleotide-Capped Mesoporous Silica Nanoparticles. *Angew. Chem., Int. Ed.* **2010**, *49*, 7281.

(20) Ma, X.; Nguyen, K. T.; Borah, P.; Ang, C. Y.; Zhao, Y. Functional Silica Nanoparticles for Redox-Triggered Drug/ssDNA Co-delivery. *Adv. Healthcare Mater.* **2012**, *1*, 690.

(21) Li, H.; Ma, T. Y.; Kong, D. M.; Yuan, Z. Y. Mesoporous Phosphonate−TiO_2 Nanoparticles for Simultaneous Bioresponsive Sensing and Controlled Drug Release. *Analyst* **2013**, *138*, 1084.

(22) Chen, C.; Pu, F.; Huang, Z.; Liu, Z.; Ren, J.; Qu, X. Stimuli-Responsive Controlled-Release System Using Quadruplex DNA-Capped Silica Nanocontainers. *Nucleic Acids Res.* **2011**, *39*, 1638.

(23) He, D.; He, X.; Wang, K.; Chen, M.; Cao, J.; Zhao, Y. Reversible Stimuli-Responsive Controlled Release Using Mesoporous Silica Nanoparticles Functionalized with a Smart DNA Molecule-Gated Switch. *J. Mater. Chem.* **2012**, *22*, 14715.

(24) Chen, L.; Wen, Y.; Su, B.; Di, J.; Song, Y.; Jiang, L. Programmable DNA Switch for Bioresponsive Controlled Release. *J. Mater. Chem.* **2011**, *21*, 13811.

(25) Wang, H.; Jia, T. H.; Zacharias, N.; Gong, W.; Du, H. X.; Wooley, P. H.; Yang, S. Y. Combination Gene Therapy Targeting on Interleukin-1β and RANKL for Wear Debris-Induced Aseptic Loosening. *Gene Ther.* **2013**, *20*, 128.

(26) Ginn, S. L.; Alexander, I. E.; Edelstein, M. L.; Abedi, M. R.; Wixon, J. Gene Therapy Clinical Trials Worldwide to 2012—An Update. *J. Gene Med.* **2013**, *15*, 65.

(27) Möller, K.; Kobler, J.; Bein, T. Colloidal Suspensions of Nanometer-Sized Mesoporous Silica. *Adv. Funct. Mater.* **2007**, *17*, 605.

(28) Bokszczanin, K.; Przybyla, A. A. New Simple and Efficient Method of DNA Isolation from Pear Leaves Rich in Polyphenolic Compounds. *Int. J. Hortic. Sci.* **2006**, *12*, 21.

(29) Arora, K.; Chaubey, A.; Singhal, R.; Singh, R. P.; Pandey, M. K.; Samanta, S. B.; Malhotra, B. D.; Chand, S. Application of Electrochemically Prepared Polypyrrole−Polyvinyl Sulphonate Films to DNA Biosensor. *Biosens. Bioelectron.* **2006**, *21*, 1777.

(30) Tien, C. L.; Lafortune, R.; Shareck, F.; Lacroix, M. DNA Analysis of a Radiotolerant Bacterium Pantoea Agglomerans by FT-IR Spectroscopy. *Talanta* **2007**, *71*, 1969.