Electronic Supplementary Information (ESI)

Preparation of Multicompartment Silica-Gelatin Nanoparticles with Self-Decomposability as Drug Containers for Cancer Therapy *in Vitro*

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1. Methods:

1.1. Labeling the MSGN with dyes

To label the MSGN with FITC and TRITC, an amount of 0.2 g MSGN with different size was dispersed in 10 mL water. Then, an anhydrous DMSO of FITC and TRITC (100 μL, 1 mg mL^{-1}) were added into the above solution. After adjusting the pH value above 9 with NaOH (1 M), the mixture solution was gently shaken over night at 8 ^{o}C in the dark. Finally, the MSGN was separated and washed three times with PBS by centrifugation.[1]

1.2. Loading the MSGN with DOX

10 mg MSGN (480±80 nm, mean value±SD, n=100) was incubated in DOX aqueous solution (DOX concentration was 1.0 mg mL^{-1}, the total volume was 1.5 mL) for 24 h with gentle shaking, then the MSGN was centrifuged and washed with PBS for several times until no DOX could be detected by UV-vis in the supernatant. The supernatant was collected carefully and assessed by UV-vis spectrometer to determine the absorption at peak of 495 nm (characteristic absorption peak of DOX). Finally the amount of DOX loaded by MPS could be estimated by standard curve of DOX**.**

1.3. The DOX release from MSGN in response to pH change

An aliquot of the MSGN-DOX suspension (2 mL, 5 mg mL^{-1}) was removed from the stock solution. The sample suspension was then centrifuged and washed by PBS for twice more. After the final washing process, the MSGN-DOX was resuspended in 2 mL desired pH buffer solution at 37 °C. PBS buffer was used for the release at pH 7.4, while acetate buffer solution

was used for the release at pH 5.0. At predetermined intervals, 0.5 mL supernatant was removed, and assessed by UV-vis spectrometer. After that, 0.5 mL fresh buffer solution was added to the sample suspension. This process was repeated until no further release was observed.

1.4. The DOX release from MSGN in aqueous solution with different concentration of NaCl

An aliquot of the MSGN-DOX suspension (5 mL, 2 mg mL^{-1}) was removed from the stock solution. The sample suspension was then centrifuged and washed by Milli-Q water for twice more. After the final washing process, the MSGN-DOX was resuspended in 5 mL aqueous solution with different NaCl concentration (0, 0.01, 0.05 M) at room temperature. At predetermined intervals, 1 mL supernatant was removed, and assessed by UV-vis spectrometer. After that, 1 mL NaCl aqueous solution was added to the sample suspension.

1.5. Cell culture

MCF-7 cells were cultured at 37 °C in a DMEM medium (Gibco BRL, USA) complemented with FBS (10%), L-glutamine (2 mM), penicillin (100 U mL^{-1}) and streptomycin (25 mg mL^{-1}) in a humidified atmosphere with 5% CO_2. For the following experiments, cells were detached from culture flasks using PBS containing EDTA (0.02%) and trypsin (0.05%) and seeded to 96-well plates at a density of 10^5 cells $well^{-1}$.

1.6. Staining the cells for CLSM observation

Cellular internalization behavior of the MSGN was studied using MCF-7 cells. For CLSM observation, the MSGN was labeled with FITC or TRITC, and the cell nuclei and membrane were stained with Hoechst 33342 and Alexa 488 WGA or FM-4-64, (0.025 mg mL^{-1}, 10 μL) respectively. After being co-incubated with the dyes for 20 min, the cells were washed by PBS for three times, and supplemented with fresh cell culture medium.

1.7. Biocompatibility of MSGN (480±80 nm, mean value±SD, n=100)

Biocompatibility assessments were conducted on MCF-7 cells in 96-well plates grown to ~70-80% confluency. Cells were incubated in triplicate with specified concentrations of the MSGN (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mg mL^{-1}) for 24 h. After that, cells were washed for three times by PBS and then assessed for viability using the CCK-8 assay

1.8. Cytotoxicity assay of MSGN-DOX *in vitro*

Cells were allowed to adhere to 96-well plates for 12 h, and then were incubated with different concentration of MSGN-DOX (0.02, 0.04, 0.08 mg mL^{-1}, the DOX loading amount was 0.105 mg per 1 mg MSGN, the size of MSGN was 480±80 nm, mean value±SD, n=100). Cells incubated with free DOX were taken as a comparison (the amount of free DOX was the same with those loaded by MSGN). After 24 h co-incubation, cells were then assessed for viability using the CCK-8 assay.

1.9. Self-decomposition of MSGN

MSGN (0.1 mg mL^{-1}) was dispersed in 1 mL PBS or acetate buffer solution at 25 or 37 ^{o}C with gentle shaking. At predetermined intervals, a series of aliquots (5 μL) of solution were removed at different time points and dropped on copper grid for TEM observation.

1.10. Getting the statistical size of MSGN

JEM-1011 and JEM-2011 equipment (JEOL, Tokyo, Japan) were used to take TEM images of MSGN, then Image J 1.45 (Download from the website: http://imagej.en.softonic.com/) was employed to get the statistical size of MSNG from 100 samples.

Characterization and Instrumentation

UV−visible spectra (UV−vis) were recorded with a HITACHI U-3010 UV−visible spectrophotometer. Confocal laser scanning (CLSM) micrographs were taken with an Olympus FV1000 confocal system, which has a 60× oil-immersion objective and a numerical aperture of 1.4. The transmission electron microscopy (TEM) images were acquired by using a JEM-1011 and JEM-2011(JEOL, Tokyo, Japan). The scanning electron microscopy (SEM) images and energy-dispersive X-ray spectrum (EDX) patterns were obtained with an S-4800

instrument with 10 kV accelerating (HITACHI, Tokyo, Japan). ζ- potential was documented by a dynamic light scattering technique (Zetasizer Nano, Malvern).

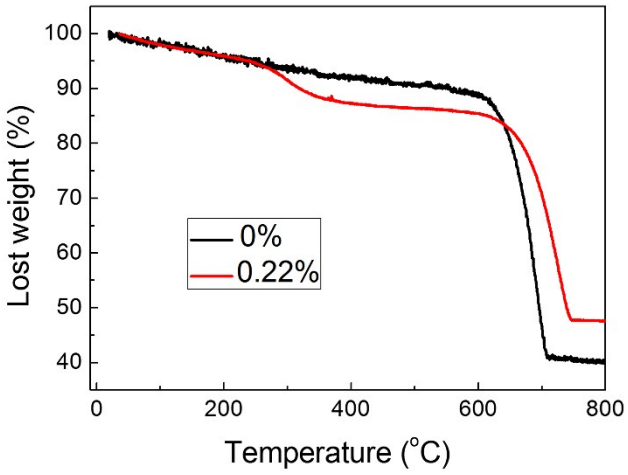

Fig. S1. TGA analysis of $CaCO_3$ particles (230±30 nm, mean value±SD, n=100). The content of gelatin in the reaction medium was 0% and 0.22% respectively.

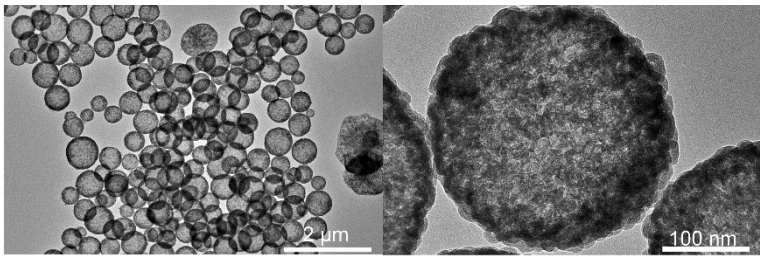

Fig. S2. TEM images of MSN (around 500 nm) templated by $CaCO_3$ particles without gelatin.

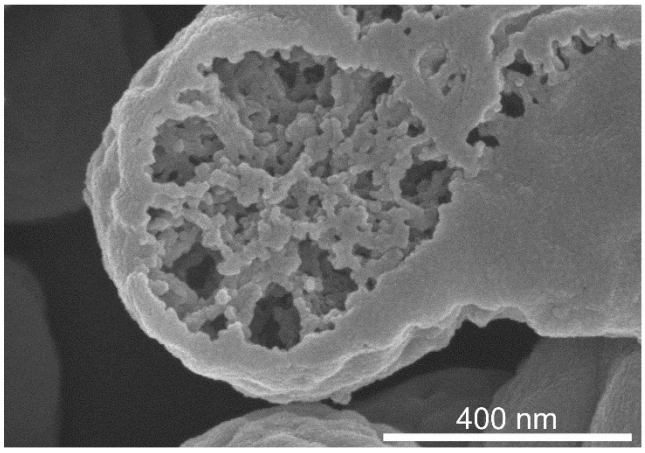

Fig. S3. SEM image of cross section profile of a single MSGN.

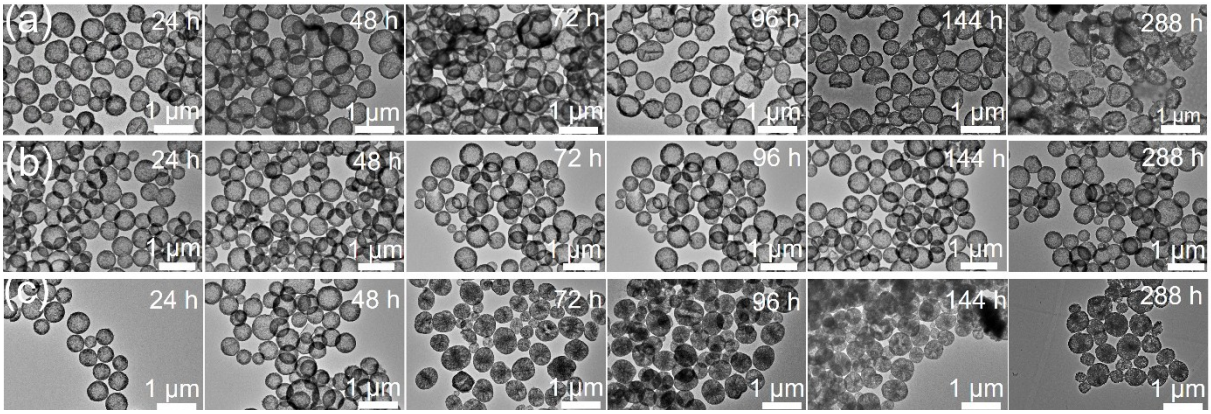

Fig. S4. Typical TEM images of MSGN with size about 480±80 nm (mean value±SD, n=100) after being incubated in PBS or acetate buffer solution (0.1 mg mL^{-1}) at different conditions, (a) pH 7.4, 25 °C; (b) pH 5.0, 37 °C; (c) MSN incubated in pH 7.4 buffer solution, 37 °C as a control.

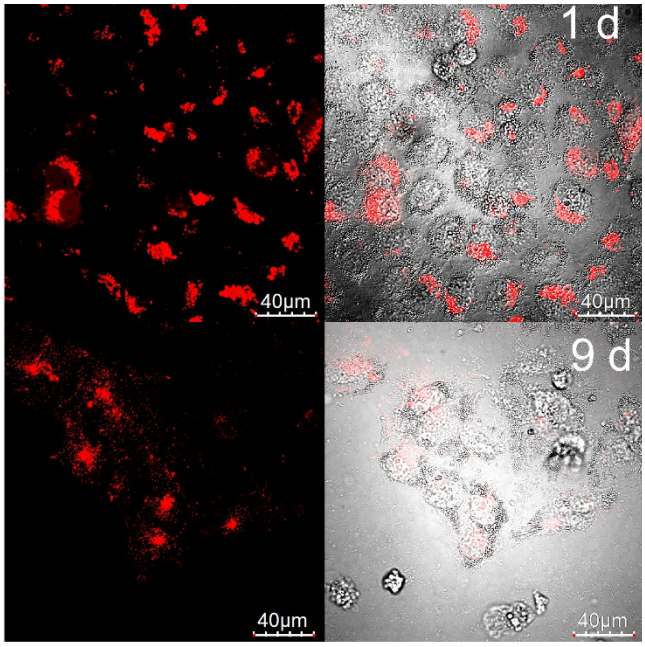

Fig. S5. CLSM images of MSGN (250±50 nm (mean value±SD, n=100), lableled by TRITC) incubated with MCF-7 cells for 1 and 9 days, respectively.

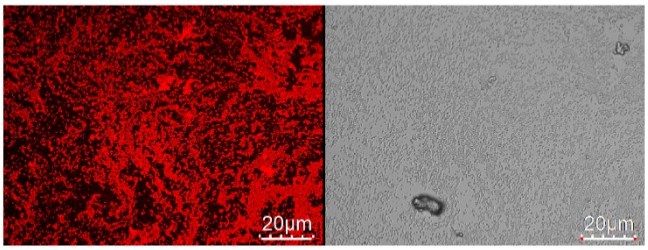

Fig. S6. CLSM images of MSGN (480±80 nm, mean value±SD, n=100) after being loaded with DOX (excited by 559 nm laser).

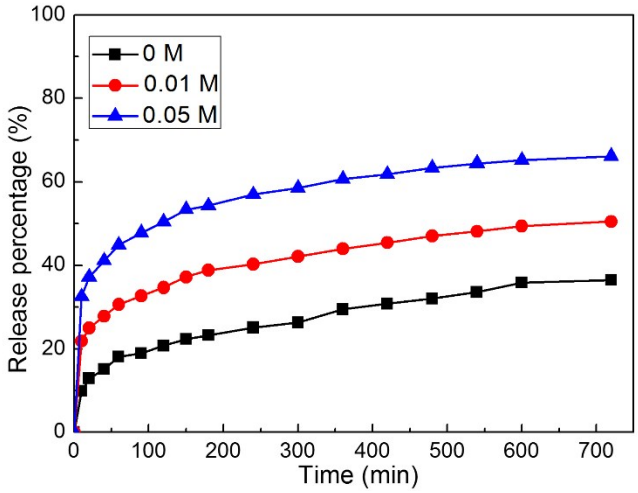

Fig. S7. The release profile of encapsulated DOX from MSGN in aqueous solution with different concentration of NaCl.

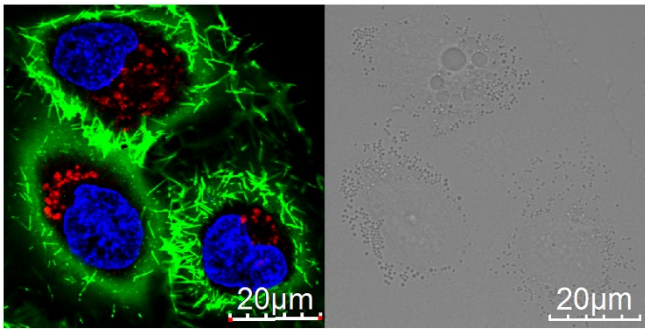

Fig. S8. CLSM image of MCF-7 cells incubated with MSGN-DOX for 24 h (fluorescence from DOX excited by 559 nm laser). The nuclei and membrane of MCF-7 were stained by Hoechst 33342 (blue one) and Alexa 488 (green one), respectively.