

## Supporting Information

### Facile One-Pot Preparation of Calcite Mesoporous Carrier for Sustained and Targeted Drug Release for Cancer Cells

*Yuming Guo,\* Jie Zhang, Lili Jiang, Lin Yang,\* Xiaoman Shi, Qilong Fang, Kui Wang and Kai Jiang*

College of Chemistry and Chemical Engineering, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, Henan Normal University, Xinxiang 453007, P.R. China

## 1 Materials and Methods

### 1.1 Materials

Calcium chloride ( $\text{CaCl}_2$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), and chondroitin sulfate were purchased from China National Pharmaceutical Group Corp.. All chemicals used in this study were of analytical grade and were used as received without further purification. Double distilled water (DD water) was used in all of the experiments.

### 1.2 Preparation of $\text{CaCO}_3$

The preparation of the CS-CaMRs was preformed as follows. Briefly, the aqueous solution of CS (25 mg/mL, 20 mL) and  $\text{CaCl}_2$  (33.3 mg/mL, 15 mL) were mixed together under vigorous stirring. The pH of the system was adjusted to 7.00 by the dropwise addition of the 1 M KOH and 1 M HCl under stirring and the stirring was continued for another 2 hr at 25 °C to allow the completely interaction between the calcium ions and the CS. Then, the aqueous solution of  $\text{Na}_2\text{CO}_3$  (33.3 mg/mL, 15 mL) was added dropwise into the system and stirred for 10 min. Subsequently, the reaction system was incubated for 24 hr at 25 °C. Finally, the product was collected by centrifugation for 5 min with 12,000 rpm and washed several times with DD water and absolute ethanol. The obtained product was dried under vacuum for 24 hr at 40 °C and denoted as CS-CaMRs. For comparison, the  $\text{CaCO}_3$  was also prepared with the CS concentration of 0.1%, 0.2%, 0.3%, and 0.5% under similar conditions and denoted as 0.1CS-Ca, 0.2CS-Ca, 0.3CS-Ca, and 0.5CS-Ca, respectively. In addition, the preparation of

the  $\text{CaCO}_3$  was also performed with the  $[\text{Ca}^{2+}]$  of 30 mM and 50 mM under nearly identical conditions as the typical experiment.

### 1.3 Characterization

The size and morphology of the as-prepared products were characterized by scanning electron microscopy (SEM, JSM-6390LV, JEOL). High-resolution transmission electron microscopy (HR-TEM) investigations, accompanied by selected-area electron diffraction (SAED), were conducted on a JEOL JEM-2100 transmission electron microscope with the acceleration voltage of 200 kV. The phases of the as-prepared products were determined by powder X-ray diffraction (XRD) using a D8ADVANCE X-ray diffractometer (Bruker axs Com., Germany) with graphite monochromatized  $\text{Cu K}\alpha$  radiation ( $\lambda = 0.15406 \text{ nm}$ ). A scanning rate of  $0.05 \text{ deg/s}$  was applied to record the pattern in the  $2\theta$  range of  $20\text{--}70^\circ$ . The Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Bio-Rad FTS-40 Fourier transform infrared spectrometer in the wavenumber range of  $4000\text{--}400 \text{ cm}^{-1}$ . The thermogravimetry-differential scanning calorimetry (TG-DSC) analysis was performed in the temperature range  $25\text{--}900^\circ\text{C}$  with a linear heating rate of  $10^\circ\text{C/min}$  on an NETZSCH STA 449C instrument. The size distribution analysis of the samples was performed using the log normal function from 100 rods in an arbitrarily chosen area results in a narrow size distribution. The specific surface area and pore size of the CS-CaMRs were analyzed through the Brunauer–Emmett–Teller (BET) determination at liquid nitrogen temperature using  $\text{N}_2$  as an adsorbent (Gemini 2380, Micromeritics, USA). The samples were dried at  $100^\circ\text{C}$  for 4 h and then degassed for 2 h under vacuum prior to the analysis of the surface areas.

### 1.4 DOX·HCl loading and the incorporation efficiency

6 mg of CS-CaMRs were added into DOX·HCl aqueous solution (0.6 mg in 6 mL of double distilled water) and shaken for 24 h at  $30^\circ\text{C}$  in an orbital shaker to load the DOX·HCl. After the loading, the sample was centrifuged, rinsed with DD water for 5 times, dried under vacuum, and denoted as CS-CaMRs/DOX·HCl. For the determination of the DOX·HCl loading content, the CS-CaMRs/DOX·HCl was treated with hydrochloride aqueous solution ( $\text{pH} = 1.0$ ) to recover the DOX·HCl completely. The absorbance of the solution was determined by UV–Vis absorption spectroscopy at 500 nm. DOX·HCl incorporation efficiency was expressed both as DOX·HCl loading content (% w/w) and entrapment (%);

represented by Equation 1 and 2, respectively. The reported values are the mean values of triplicate determinations.

$$\text{Loading content (\% w/w)} = \frac{\text{mass of DOX} \cdot \text{HCl in CSCaMRs} / \text{DOX} \cdot \text{HCl} \times 100}{\text{mass of CSCaMRs}} \quad (1)$$

$$\text{Entrapment (\% w/w)} = \frac{\text{mass of DOX} \cdot \text{HCl in CSCaMRs} / \text{DOX} \cdot \text{HCl} \times 100}{\text{mass of DOX} \cdot \text{HCl used in formulation}} \quad (2)$$

## 1.5 Cell culture

HeLa human cervical carcinoma cells (ATCC No. CCL-2) and V79-4 Chinese hamster lung cells (ATCC No. CCL-93) were cultured in Eagle's minimum essential medium supplemented with heat-inactivated FBS (10%), Penicillin (100 units/mL), Streptomycin (100 µg/mL), amphotericin B (fungizone, 0.25 µg/mL) and sodium bicarbonate (2.2 mg/mL) in a humidified incubator at fully humidified atmosphere at 37°C, 5% CO<sub>2</sub> and 95% room air.

## 1.6 Cytotoxic effects of CS, pure DOX, and CS-CaMRs/DOX on HeLa and V79-4 Cells

One hundred microliters of culture media containing cells, HeLa or V79-4, with initial cell density of  $2.5 \times 10^4$  cells/mL were seeded separately in the wells of sterile 96-well flat bottom culture microplates and acclimated for 24 hr. It was then mixed with one hundred microliters of CS, pure DOX, and CS-CaMRs/DOX at concentrations of 0.0244, 0.0488, 0.0977, 0.1953, 0.3906, and 0.7813 µg/mL each for 120 hr of incubation in a humidified incubator at fully humidified atmosphere at 37°C, 5% CO<sub>2</sub> and 95% room air. The treatment of the cells with culture medium rather than sample was prepared as the control. The cytotoxic effects of the CS, pure DOX, and CS-CaMRs/DOX on HeLa and V79-4 cells were determined by MTT colorimetric assay. Briefly, after treated with samples, freshly prepared MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 20 µL, 5 mg/mL) in PBS was added to each well of the control and the treated cells and incubated at 37°C, under 5% CO<sub>2</sub> for 5 hr. After the incubation, the medium was carefully removed and 150 microliters of DMSO were added to each well to dissolve the dark blue crystals completely. The absorbance of the solution in each well at the wavelength 570 nm was measured by a microplate reader. The extent of cell proliferation was reflected by the average value of absorbance while the cytotoxic effects of the CS, pure DOX, and CS-CaMRs/DOX were calculated by Equation 4. The data were reported as mean ± standard deviation (SD) based on the measurements of the triplicate samples.

## 1.7 Determination of the [Ca<sup>2+</sup>]

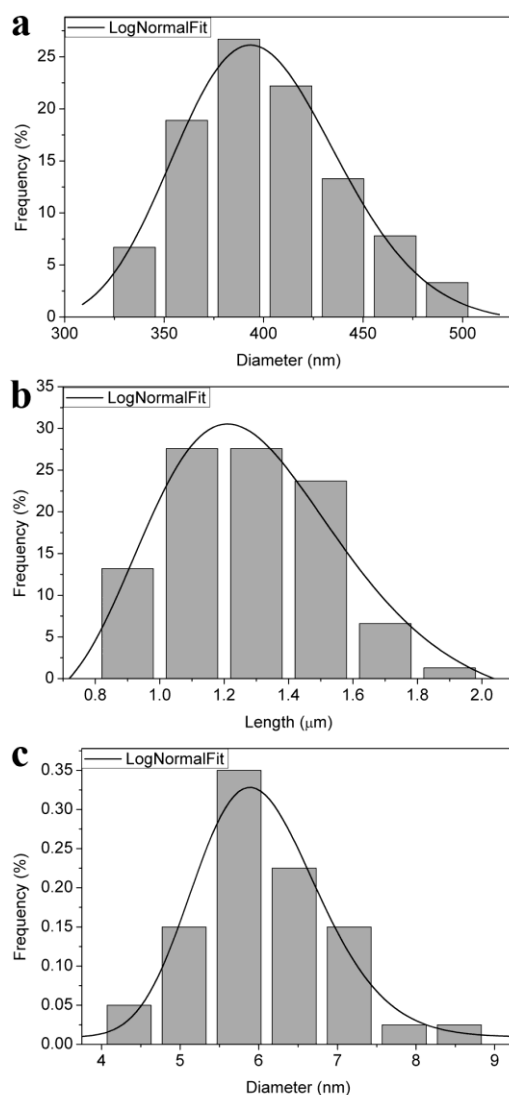
For the determination of the  $[\text{Ca}^{2+}]$ , the CS-CaMRs were incubated in EMEM medium and the cultivation system of HeLa cells under standard cell culture conditions, respectively. After incubated for 5 days, the mixtures were centrifuged for 10 min with 12,000 rpm to remove any solid samples completely. Subsequently, the supernatants were diluted with 2%  $\text{HNO}_3$  and analyzed using the inductively coupled plasma-mass spectrometer (ELAN DRC-e, Perkin–Elmer Sciex, Canada) to determine the  $[\text{Ca}^{2+}]$  in the supernatants. For comparison, the  $[\text{Ca}^{2+}]$  in the pure EMEM medium was also determined.

### **1.8 Transmission electron microscopy of cells**

HeLa cells were seeded on culture flasks and treated with CS-CaMRs for 5 days. After treatment, cells were collected, fixed with glutaraldehyde (2.5%) and washed three times with PBS. Subsequently, post-fixation with osmium tetroxide (1%) was performed followed by washed with PBS, dehydration with ascending series of alcohol before embedding in Spurr. Ultrathin sections with 70 nm were cut by Leica Reichert ultracut and doubly stained with uranyl acetate and lead citrate. Images were acquired using transmission electron microscope.

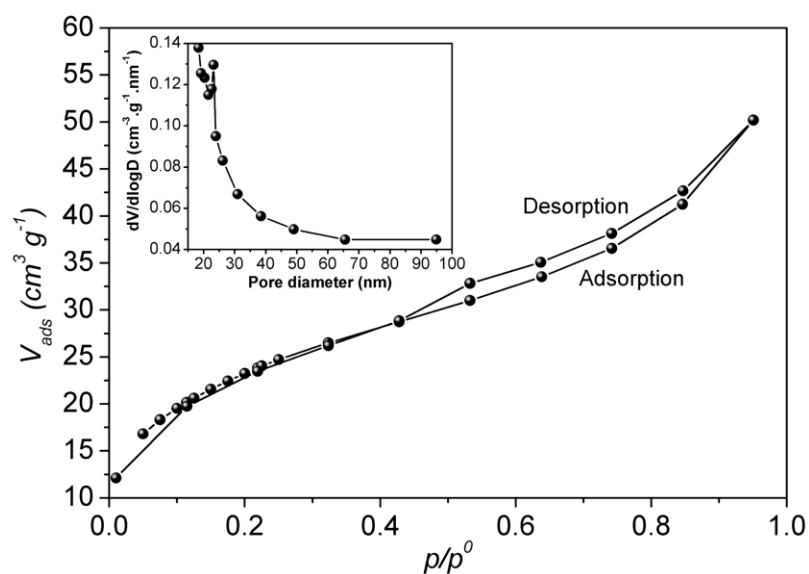
## 2 Results

### 2.1 Size distribution analysis of the diameter, length of the CS-CaMRs and the diameter of the small nanoparticles in the CS-CaMRs



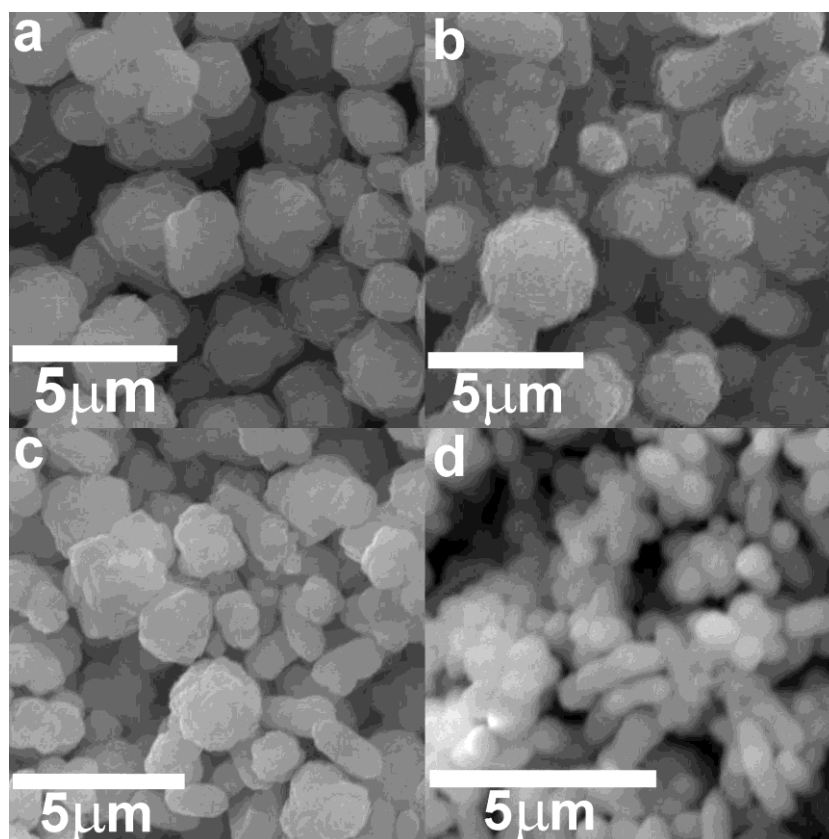
**Figure S1.** (a) Diameter distribution histogram of the CS-CaMRs. (b) Length distribution histogram of the CS-CaMRs. The size distribution analysis through a lognormal distribution function from 100 rods in an arbitrarily chosen area results in a narrow size distribution. (c) Size distribution histogram of the small nanoparticle in the CS-CaMRs. The size distribution analysis through a lognormal distribution function from 100 nanoparticles in an arbitrarily chosen area results in a narrow size distribution.

## 2.2 Multipoint Brunauer-Emmett-Teller (BET) analysis of the CS-CaMRs



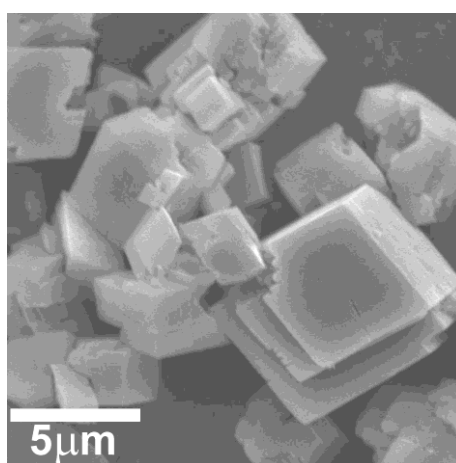
**Figure S2.** Nitrogen adsorption/desorption isotherm of the CS-CaMRs (i.e. the volume of nitrogen adsorbed,  $V_{ads}$ , versus relative pressure,  $p/p^0$ ). Inset: Pore size distribution curve calculated from desorption branch of the nitrogen isotherm by the BJH method.

### 2.3 SEM observation of the products in the presence of different concentrations of CS



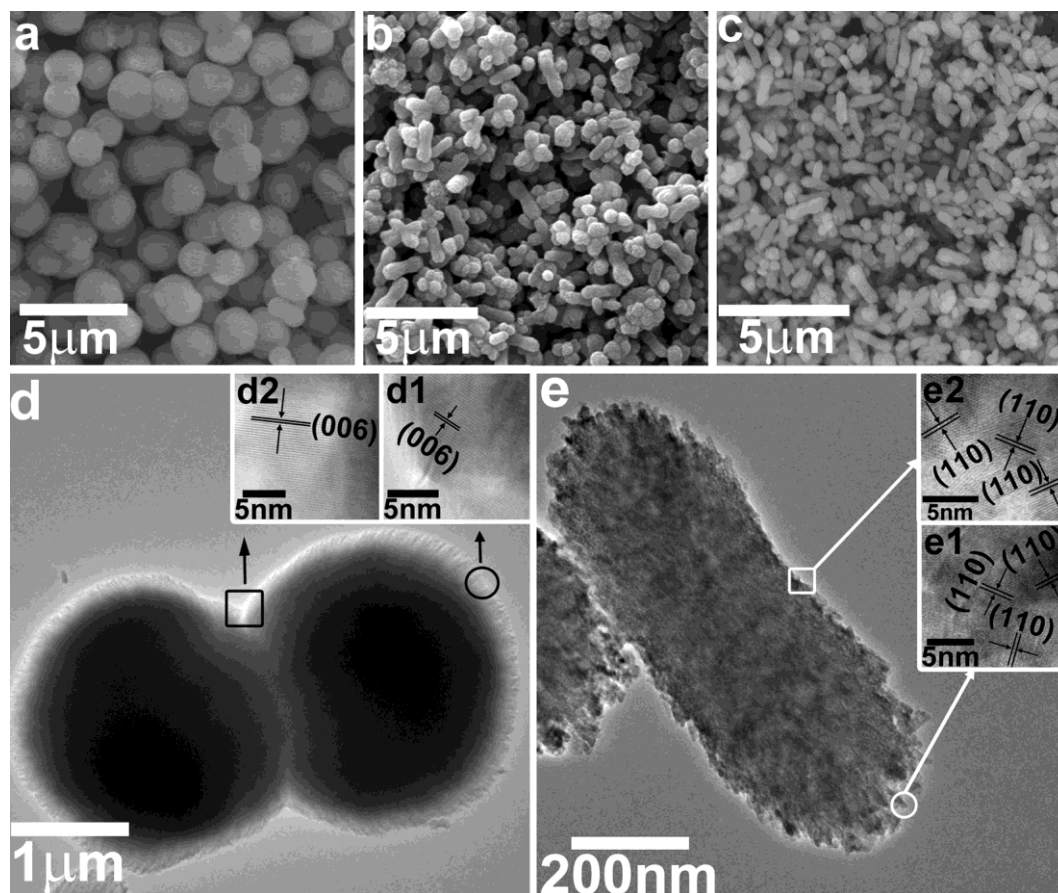
**Figure S3** SEM images of the products obtained in the presence of different concentrations of CS. (a) 0.1%, (b) 0.2%, (c) 0.3%, and (d) 0.5% CS. The products were prepared under the similar conditions to the typical experiment.

### 2.4 SEM observation of the control product in the absence of CS



**Figure S4.** SEM images of the control products obtained in the absence of CS under the similar conditions to the typical experiment.

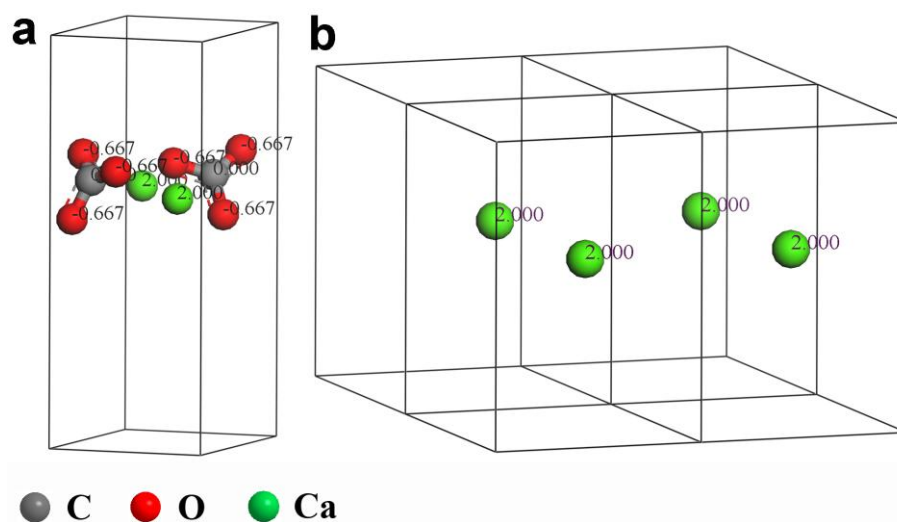
## 2.5 SEM observation of the products obtained at different reaction intervals



**Figure S5.** SEM images of the products obtained at different reaction intervals. (a) 10 min, (b) 3 h, and (c) 24 h. (d) TEM image of the product obtained at 10 min. d1-d2: magnified images of the regions circled by the black ring and square. (e) TEM image of the product obtained at 24 h. e1-e2: magnified images of the regions circled by the white ring and square.

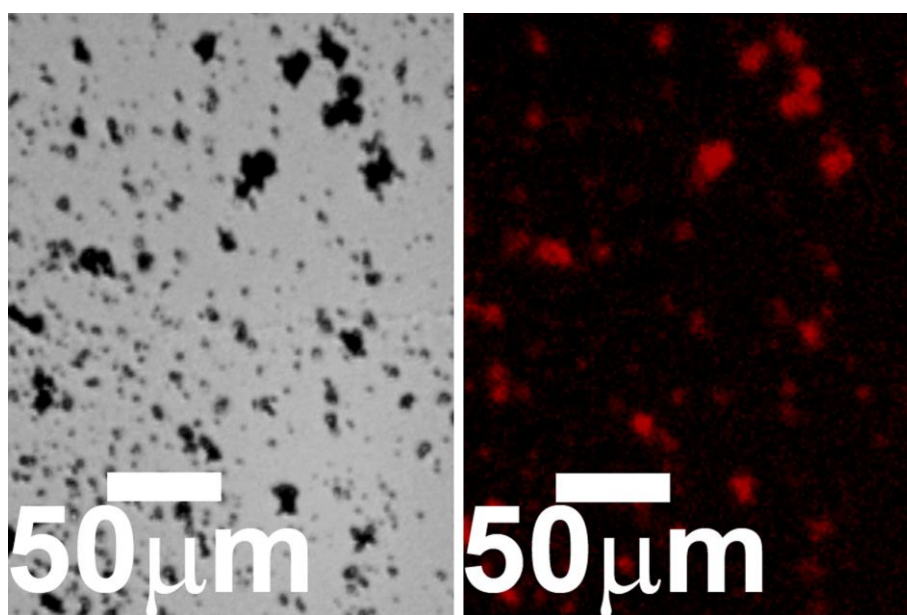


## 2.6 The theoretical model of (110) and (006) faces of calcite



**Figure S6.** The theoretical model of (a) (110) face and (b) 2 × 2 (006) faces of calcite built by “Materials Studio”. The model is obtained through the cleavage of the optimized crystal cell of the calcite and the subsequent vacuum slab. The numbers attached onto the balls show the charges of different elements in the models.

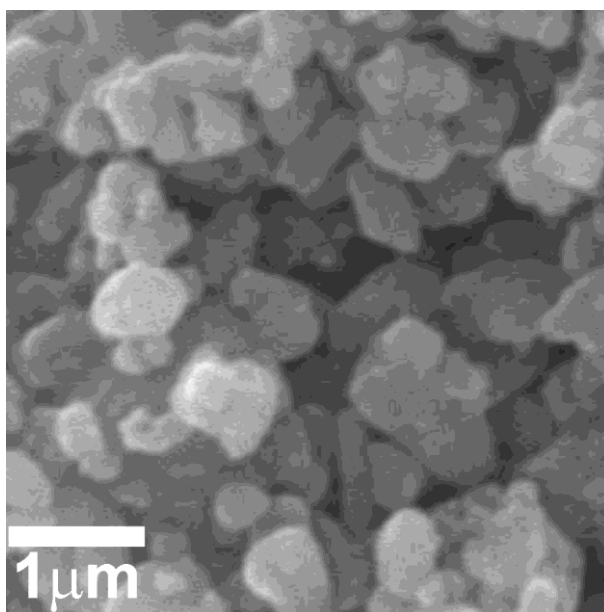
## 2.7 Light and fluorescence microscopy observations of the CS-CaMRs/DOX·HCl



**Figure S7.** Light (left) and fluorescence (right) micrographs of the CS-CaMRs/DOX·HCl. The red autofluorescence of DOX·HCl is used to monitor its loading into CS-CaMRs.

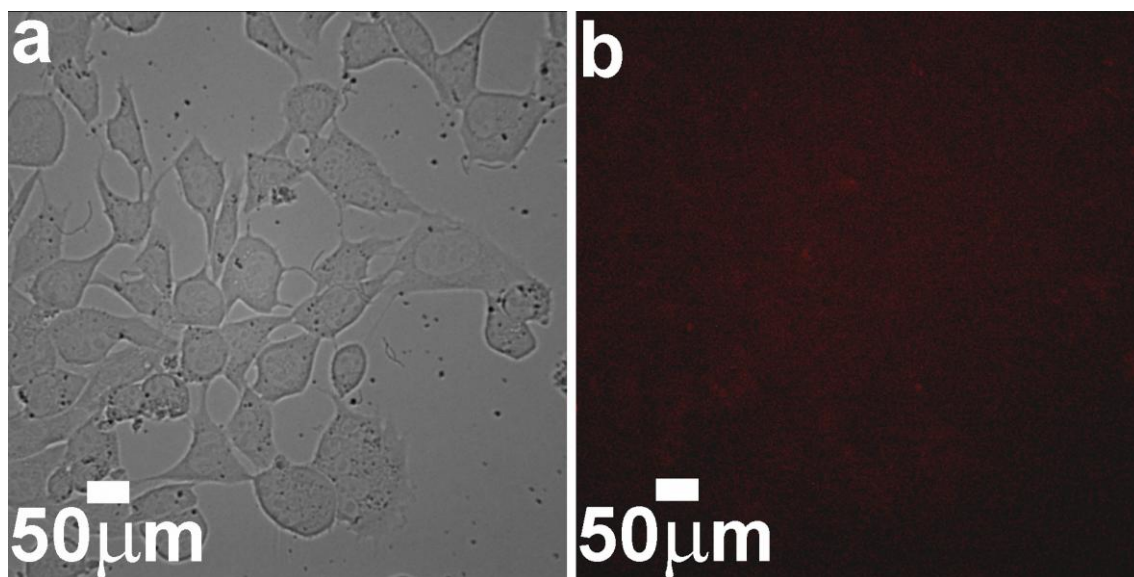
## 2.8 Stability of the CS-CaMRs in the weak acidic environment

From inductively coupled plasma-mass spectrometry (ICP-MS) analysis, after incubated under different conditions, the  $[\text{Ca}^{2+}]$  under weak acidic condition (231.2 mg/L) was much higher than the neutral condition (82.3 mg/L). In addition, after incubated under weak acidic condition, the rod-like sample changed to nanoparticles (Fig. S8, ESI†). These results indicated that the calcite in the CS-CaMRs could be decomposed gradually under the weak acidic condition. Therefore the pH-sensitive sustained release feature of the carrier can be attributed to the gradual dissolution and decomposition of the calcite.



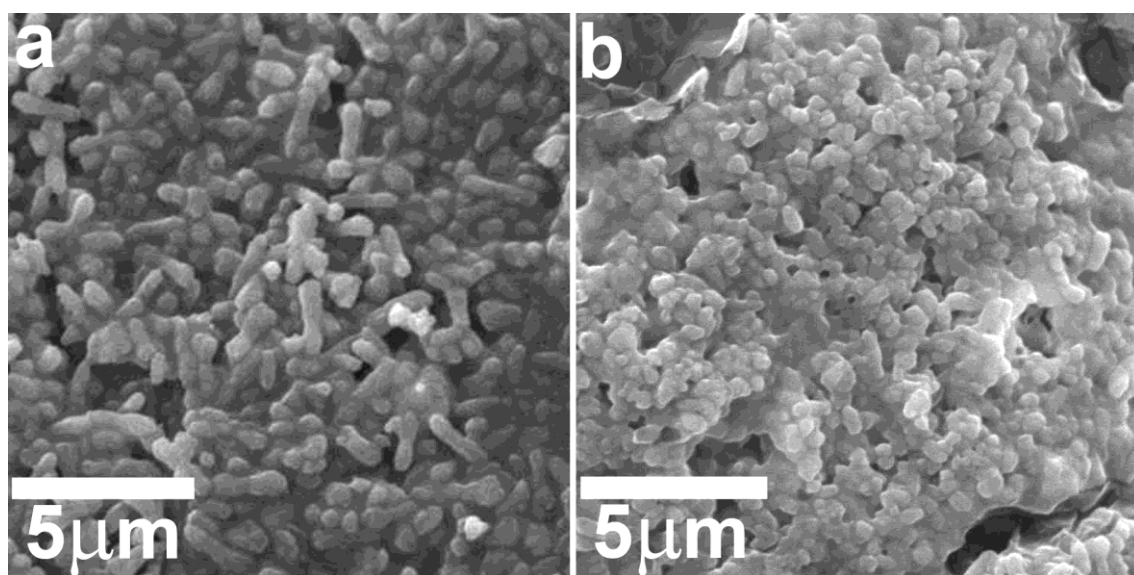
**Figure S8.** SEM image of the CS-CaMRs after incubated under weak acidic environment for 5 days.

## 2.9 Light and fluorescence microscopy observations of the V79-4 cells after treated with CS-CaMRs/DOX·HCl for 12 h



**Figure S9.** (a) Light micrograph and (b) fluorescence micrograph of the V79-4 cells after treated with CS-CaMRs/DOX·HCl for 12 h. The red fluorescence of the DOX·HCl in the CS-CaMRs/DOX·HCl is used to visualize the location of the CS-CaMRs/DOX·HCl.

## 2.10 The stability of the CS-CaMRs in the control EMEM medium and the cultivation system of the HeLa cells



**Figure S10.** SEM images of the CS-CaMRs after incubated under different conditions for 5 days. (a) EMEM medium. (b) Cultivation system of the HeLa cells.