Supporting Information for

pH-responsive polysaccharides microcapsules through covalent bonding assembly

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Experimental Section

Materials

Sodium alginate (ALG, Low Viscosity Grade, viscosity of 2% solution 250 cps at 25 ^{o}C), Chitosan (CHI, medium molecular weight) and fluorescent isothiocyanate-dextran (FITC-dextran, Mw~20 KDa) were purchased from Sigma-Aldrich. Sodium metaperiodate($NaIO_4$) and Heparin sodium were purchased from Sinopharm Chemical Reagent Co., Ltd. Starch soluble was purchased from Beijing AoBoXing Universeen Bio-tech Co., Ltd. Sodium chloride (NaCl), ethylene glycol, ethanol, Na_2EDTA were obtained from Beijing Chemical Corporation. Pancreatin powder was obtained from Yakanglinuo bioengineering Co. (Jinan, China). All chemicals were used without further purification. The water used in all experiments was prepared in a three-stage Millipore Milli-Q plus 185-purification system and had a resistivity higher than 18.2 MΩ.

Periodate oxidation of sodium alginate, heparin sodium and starch soluble

5 g sodium alginate was dissolved in 200 mL distilled water, then 5.4 g sodium periodate was added and magnetic stirred in dark at room temperature for 24 h to obtain the product. Then, 20 mL ethylene glycol was added to the above solution under dark with continuous magnetic stirring and kept for 15 min to neutralized the solution. After reaction, the mixture was dialysed against distilled water (2 L) for 48 h with several changes of water. The obtained dialyzate was further purified by three times centrifugations and washing with water, and then dried at room temperature under

vacuum.

Periodate oxidation of heparin sodium or starch soluble was synthesized via the same method above mentioned.

Characterization of microcapsules

The transmission electron microscopy (TEM) micrograph was acquired by using a Philips CM200-FEG instrument operated at 120 kV. Fourier transform infrared (FTIR) spectra were recorded by using a Tensor 27 instrument (Bruker, Germany). Atom force microscopy (AFM) images were taken by means of a Digital Instrument Nanoscopy IIIa instrument in the tapping mode. Confocal laser scanning microscopy (CLSM) micrographs were taken with an Olympus FV500 confocal system (Carl Zeiss) equipped with 60× oil-immersion objective and a numerical aperture of 1.4. Individual aliquots of 20 μL were placed on a glass slide and a cover glass was coated before observation. The microcapsules were excited at 488 nm and two fluorescent images were obtained at 510-540 nm (green) and 570-600 nm (red).

Cell culture

HeLa cells were cultured at 37 ^{o}C in a DMEM medium (Gibco BRL, USA) supplied 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 24-well plates or a 35mm glass-bottom Petri dish.

Microcapsules' permeability test

To investigate the permeability in different pH buffers at room temperature, the microcapsules from $MnCO_3$ were immersed in a series of pH buffers, respectively. FITC-dextran (Mw~20 KDa) was added in, and the samples were investigated by CLSM 15 min later.

Microcapsules' biocompatibility test

Cells were incubated with microcapsules for 24 h and washed three times with PBS. Then the cell membrane was labeled with FM 4-64 for 30 min, followed by washing. The cells were examined by CLSM and Z-sectioning was performed for identification of capsules intracellular location.

Microcapsules' stability test

The microcapsules were immersed in 0.1 M HCl solution and 0.1 M NaOH solution for at least 24 h, respectively, and then were investigated by CLSM.

Microcapsules' biodegradability test

To prove the biodegradability of the microcapsules, the microcapsules were incubated in the pancreatin solution and stirred at room temperature overnight. The microcapsules suspension was washed with deionized water carefully and then investigated by CLSM and AFM.

In vitro cytotoxicty assay

Cells were allowed to adhere to 24-well plates for 24 h, and then were incubated with the microcapsules for 24 h. After incubation, 40 μL sterile filtered MTT in PBS was added to each well and incubated with the cells for 4 h at 37 ^{o}C. Then dimethyl sulfoxide (DMSO) was added to dissolve the purple formazan crystals, followed by measuring the absorbance at 600 nm with PerkinElmer 1420 Multilable Counter.

Figure S1. CLSM images of $(CHI/ADA)_5/CHI$ microcapsules (A) after incubation in 0.1 M HCl solution and (B) in 0.1 M NaOH solution for 24 h.

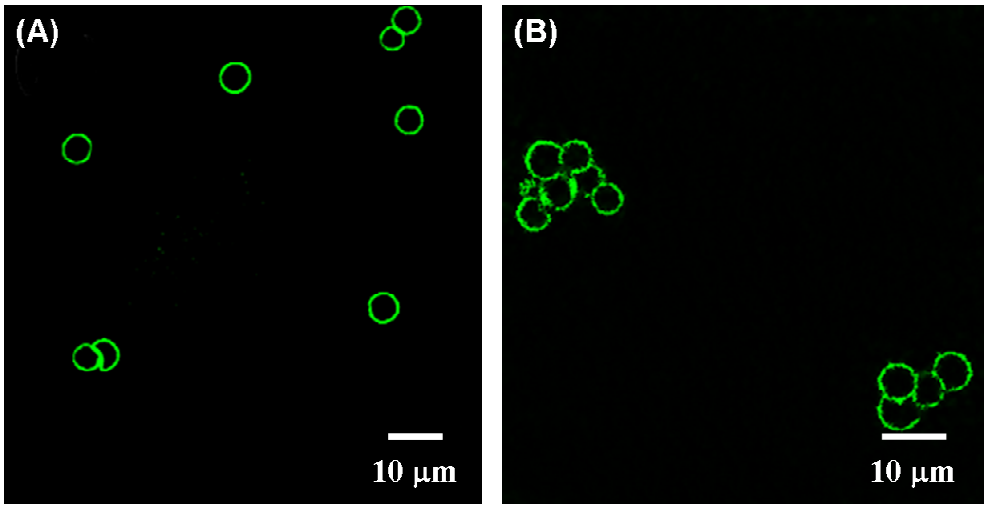


Figure S2. In vitro cytotoxicity of microcapsules on HeLa cells: (A) a negative control, the cells were incubated under normal conditions without microcapsules; (B) cells were incubated with hollow $(CHI/ADA)_5/CHI$ microcapsules. Each error bar represents the mean of at least three measurements（±S.D.).

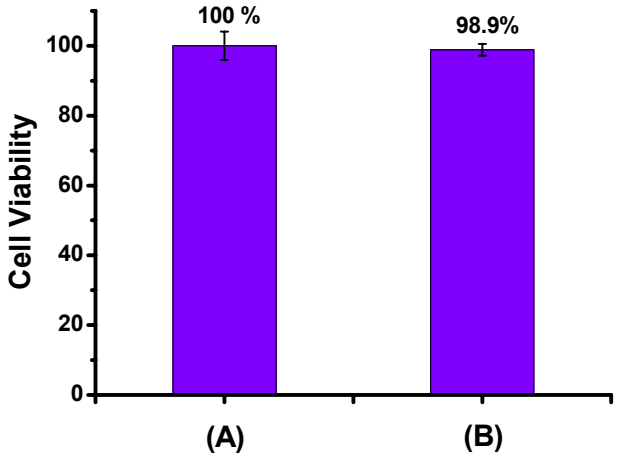
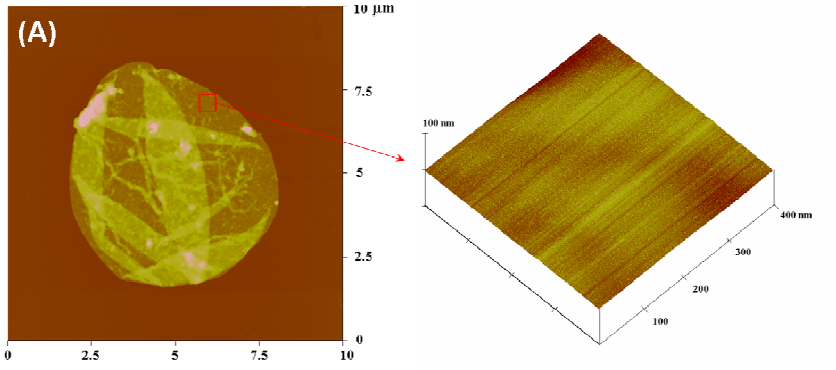


Figure S3. AFM images of $(CHI/ADA)_5/CHI$ microcapsules (A) before and (B) after degradation by pancreatin.



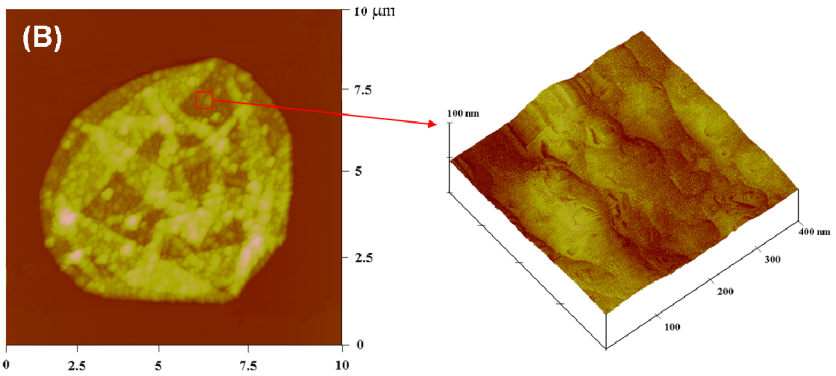


Figure S4. CLSM images of $(CHI/ADA)_5/CHI$ microcapsules (A) before and (B) after degradation by pancreatin. The corresponding fluorescence intensity profiles were shown below.

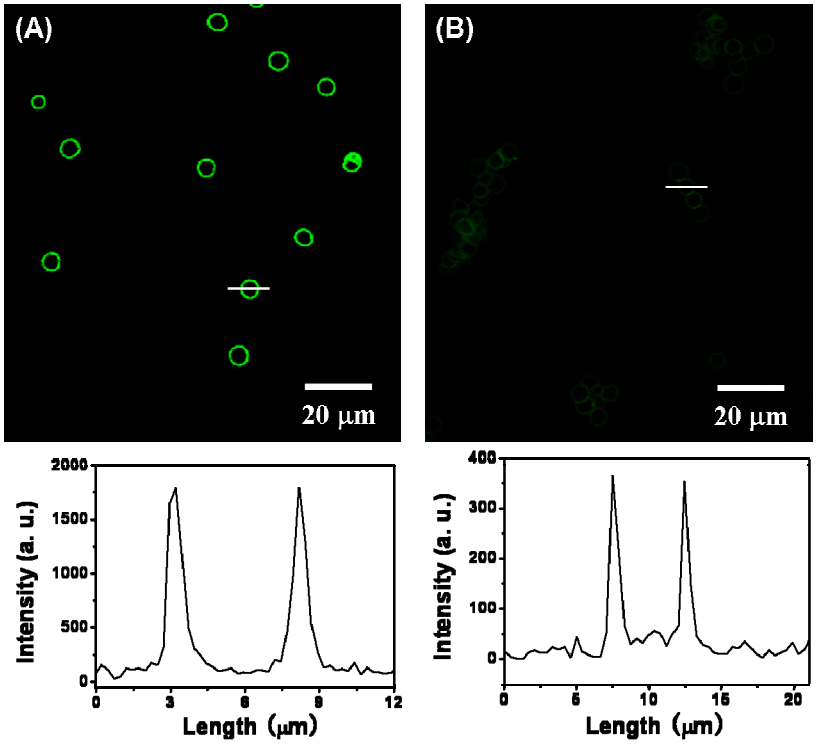


Figure S5. CLSM images of (A) $(CHI/DHP)_5/CHI$ and (B) $(CHI/DAS)_5/CHI$ microcapsules, the scale bars represent 30 μm, respectively.