Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

A Highly Expandable and Tough Polyacrylamide-Alginate Microcapsule

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Experimental Section

Materials

Acrylamide (AAm), N,N-methylene bis-acrylamide (MBA), tween-85 (HLB=11) and span-80 (HLB=4.3) were purchased from Aladdin (Shanghai, China). Potassium peroxydisulfate (KPS), sodium alginate from brown algae (medium viscosity)was bought from Sigma-Aldrich, ethylene diamine tetraacetic acid disodium (EDTA), potassium carbonate (K_2CO_3), n-heptane and calcium chloride ($CaCl_2$) were bought from Sinopharm chemical Regent Beijing Co., Ltd (Shanghai, China). Dox (Doxorubicin Hydrochloride) was purchased from Sangon Biotech (Shanghai, China). Deionized water was prepared by secondary distillation technique using double distilled water device (YMNL, Nanjing, China). All chemicals were analytically pure reagent.

Fabrication of $CaCO_3$ particles

To prepare the $CaCO_3$ particles, 25 ml $CaCl_2$ solution (0.33 M) was rapidly added into a mixture of 25 ml K_2CO_3 solution (0.33M) and 10 ml ethanol under vigorous agitation[26]. Then the mixture was continuously stirred for five minutes. The precipitated $CaCO_3$ particles were separated using a Buchner funnel (G6) and then washed with water and ethanol for three times respectively. The $CaCO_3$ particles were preserved after drying in a vacuum. Then morphologies of $CaCO_3$ microspheres were measured with scanning electron microscope (SEM). The size distribution of $CaCO_3$ microspheres was tested by laser particle analyzer (Microtrac

S3500).

Fabrication of high strength capsules

The oil phase were got by mixing 2.94 g Span80, 0.06 g Tween85, 93 mg MBA and 30ml n-heptane in a round-bottom flask with stirring of 1000 r/min under slowly blowing of nitrogen for 30 min. At the same time, 200 mg alginate was decomposed in 8.5 ml distilled water, followed by the addition of $CaCO_3$ microspheres at 5% the weight of alginate. The mixture was sonicated for 5 min and incubated for 30min to get a stable suspension. Then 1.4g AAm was added into the above suspension to form water phase. Then the aqueous phase was added into the oil phase and the mixture was stirred at 1000 r/min for 40min to get a stable emulsion. After that, 54μl KPS solution with the concentration of 10 mg/ml was added to the reaction system to start the polymerization of Am and the stirring speed was turned to 300 r/min. After 5 hours, 15 mg Hydroquinone was added to end the polymerization of Am. This reaction condition was got after many times of try and uniform PAAm/alginate microgel without core in it was got during this process (Supplementary Figure 6). Then 2 ml acetic acid was added to the reaction to decompose the $CaCO_3$ core of the capsules and Ca^{2+} released during this process crosslinked alginate in the capsules. In addition, CO_2 produced by the reaction of calcium and acetic acid could blow the capsules to make the capsule about fifty bigger than its original size. Reaction solution was moved out 3 ml /time after a period of time to observe the size change after acid was added into the emulsion. The control reaction was token by adding 10 ml 0.02 M EDTA to the emulsion after ending the

polymerization of Am. The whole reaction was taken under 45 °C.

The capsules produced before and after the decomposing of the $CaCO_3$ core were separated from the emulsion by adding acetone to the system under gentle stirring. Then the capsules were washed using acetone and ethanol by centrifugation for three times respectively. Capsules were suspended in water for further study.

Release profile of microcapsules

Dox was used as the model molecule to test the release profile of microcapsules. Dox was loaded in microcapsule by postloading method as in our previous study[26,27]. That is, 50mg dry microcapsules was added to 500 μl of 8.8mg/ml Dox aqueous solution and cultured in 37°C for 12 h. The amazing phenomenon is that model molecule solution was absolutely absorbed by microcapsules after culture. Then the model molecule loaded microcapsules were washed with deionized water for 5 times. To test the stimulate sensitivity of microcapsule, the molecule loaded microcapsule was immersed in a 2ml tube with 1.5ml release media of acid solution (0.1 M, pH 1.2) , PBS buffer (pH 7.4) respectively. Then the tube was cultured in a Thermostatic shaker incubator (Kexi Instrument, Jintan, China) at 37 °C with 100rpm. 50 μl supernate was taken out of the tube to test the concentration of model molecule after a certain time.

UV-Vis (Agilent Technologies, Cary 60) was used to test the UV-Vis absorption of the released solution and the absorption value of 485nm was take to calculate the amount of model molecules released with time. Each controlled release experiment was repeated for at least three times, the amount of model molecules released was the

average value of different experiments.

Fourier transform infrared (FTIR).

The structures of alginate, PAAm and the shell of alginate and AAm capsule were analysed using an FTIR Spectrometer (Nicolet 6700). Capsules were freeze dried before FTIR measurement. Capsules were grinded with potassium bromide and then sheeted for measurement.

Scanning electron microscopy (SEM) measurement.

The morphology of $CaCO_3$ particles, microgels and capsules were observed by SEM (Hitachi S-3400- II). The suspension of $CaCO_3$ particles and capsules were dropped on the fresh mica and then slowly dried in the super clean bench for 6 hours before scanning.

Optical microscope measurement

The size changes of microgels after adding acetic acid to the emulsion were measured by optical microscope (Olympus BX53). Capsule diameters were caculated using image J software. The data for each experimental condition were caculated from at least 10 capsules. The internal structures of microgels and capsules were also investigated using the same microscope. The expansion times of the capsule was caculated using the average diameter after expansion and average diameter of initial capsule. As the capsule was in ball shape, the expansion times of volum was calculated using the formula: $V=4/3\pi r^3$ (r was half of the diameter).

Microgels and capsules were dyed by adding10 μL acetone solution of Nile red with a

concentration of 0.038 mM into 40 μL aqueous dispersion liquid of microgels or gel capsules. After blowing slowly in Clean Bench for 12h, acetone was volatilized and microgels or capsules were dyed by Nile red.

The cross section observation

The cross section of microgel was measured by TEM (Hitachi, H-7650C, Japan) and the microgels were processed as follows. Microgels were fixed at 4 °C in 4 % glutaraldehyde (in PBS, pH 7.4) overnight. This was followed by another rinsing in PBS and subsequent gradual dehydration in acetone series with increasing concentrations (30, 50, 75, 90, and 100%). Finally, the microgels were embedded in epon araldite. Ultrathin sections (Leica, EM UC7, Germany) of 60–80 nm were stained with uranyl acetate and lead citrate for 2–3 min at room temperature[41].

The cross section images of capsules were observed using SEM. And the cross sections were got as follows. First, capsules were suspended in water and then moved into freezing room of fridge. Next, the frozen capsule suspension was random sliced with a knife in 4 °C and frozen solid turn into aqueous suspension during this process. Then 10μL capsule suspension was dropt on a fresh mica and then slowly dried in the super clean bench for 6 hours before scanning.

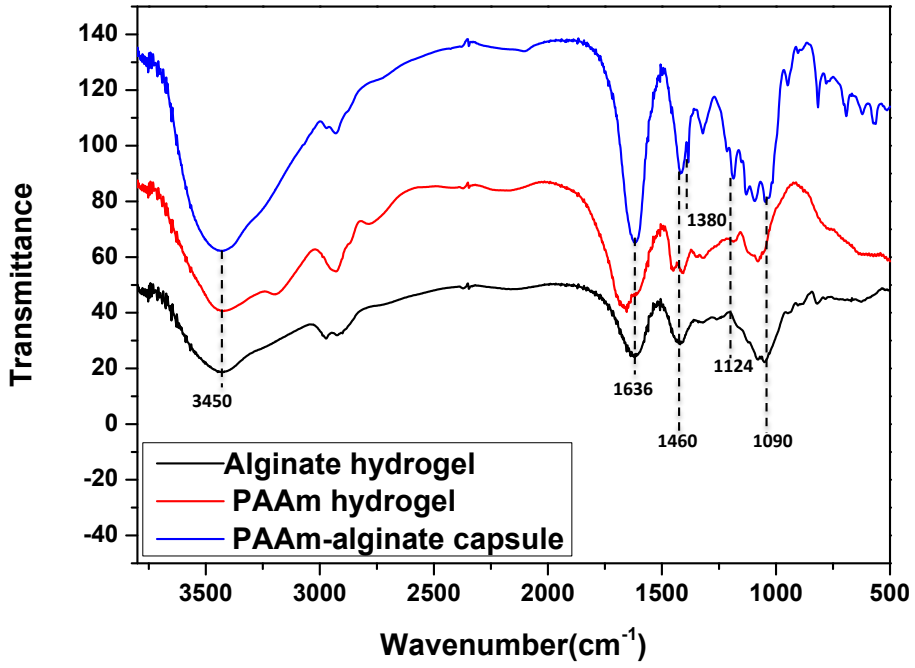

Figure S1. FTIR of PAAm-alginate capsules. A new peak at 1383 cm^{-1} for C-N stretching of secondary amide was found in the spectrum of the alginate-PAAm capsule (blue curve) compared with that of PAAm (red curve) and alginate (black cure). Furthermore, the intensity of primary amide peaks (1636 and 1460 cm^{-1}) and NH_2 in-plane rocking peak (1124 cm^{-1}) increased, as well as the intensities of O-H stretching peak (3450 cm^{-1}) and symmetric C-O stretching (1090 cm^{-1}).

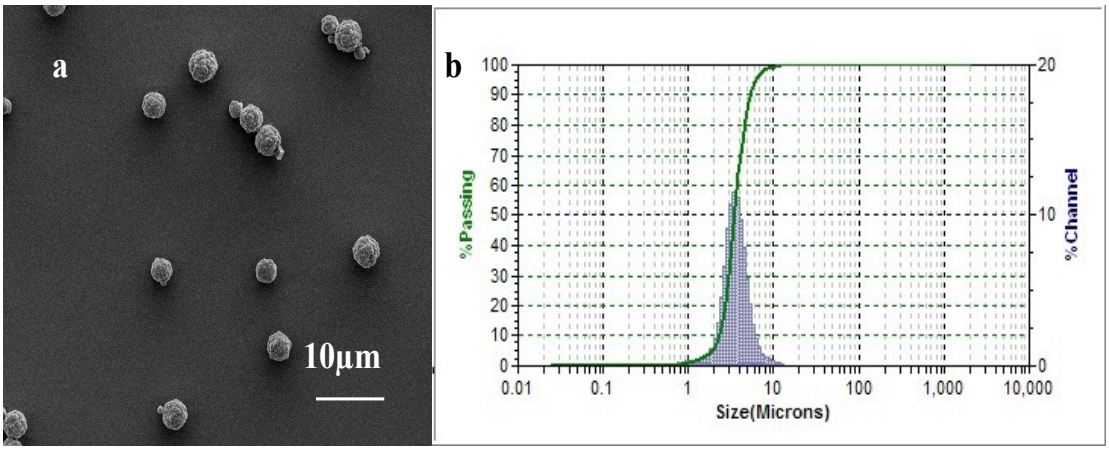

Figure S2. (a) SEM image of $CaCO_3$ microspheres. (b) Size distribution of $CaCO_3$ microspheres determined by laser particle analyzer (Microtrac S3500).

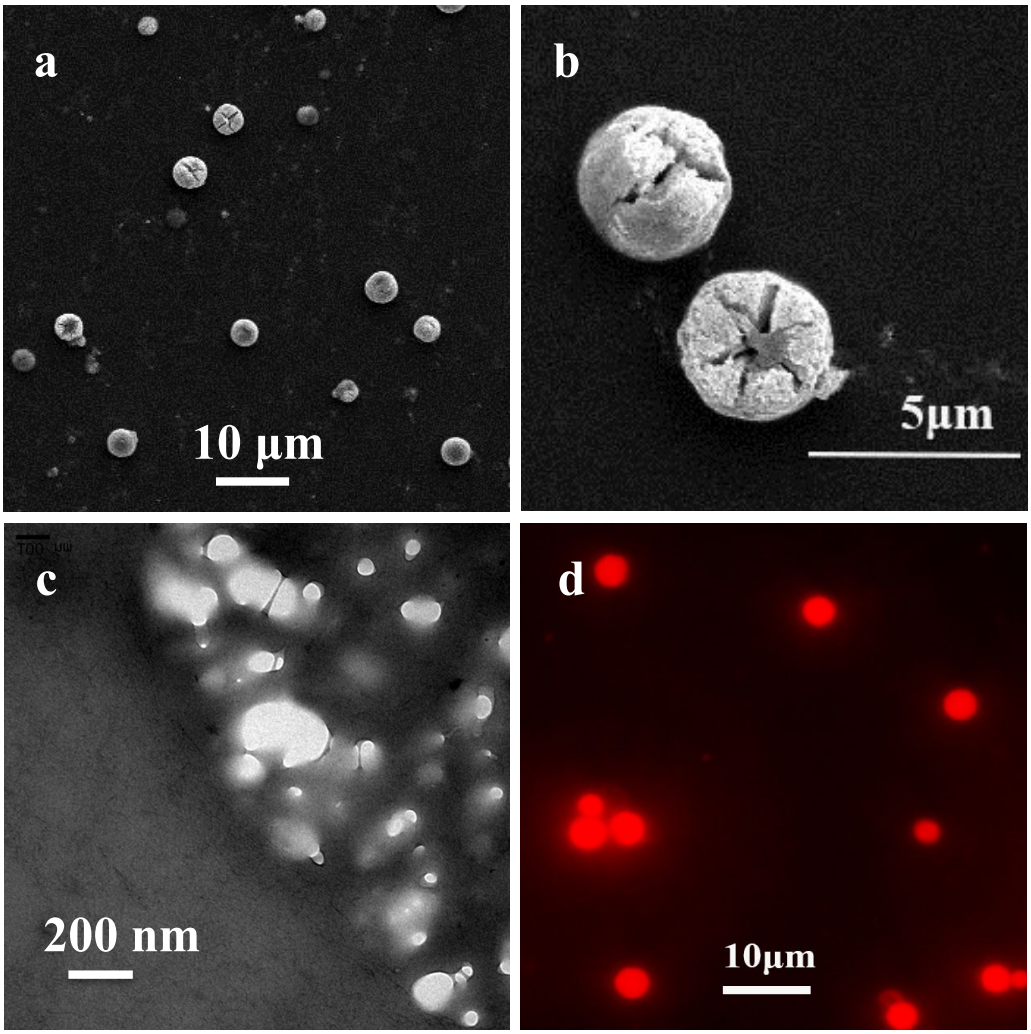

Figure S3. Morphologies of the prepared microgels. (a) Microgels are almost in the same size and some microgels have smoothly membranes but some others have fractured hydrogel membranes. (b) Microgels were ruptured after a period of exposure in electron beam with high energy. (c) Detailed TEM image of microgel cross section. Loose structure of $CaCO_3$ core (white) and compact and thin hydrogel membrane (black) can be seen clearly in this image. (d) Microgels dyed with Nile. Both the core and hydrogel membrane can be dyed by Nile and this confirm the loose $CaCO_3$ core can be used as drug vehicle as well.

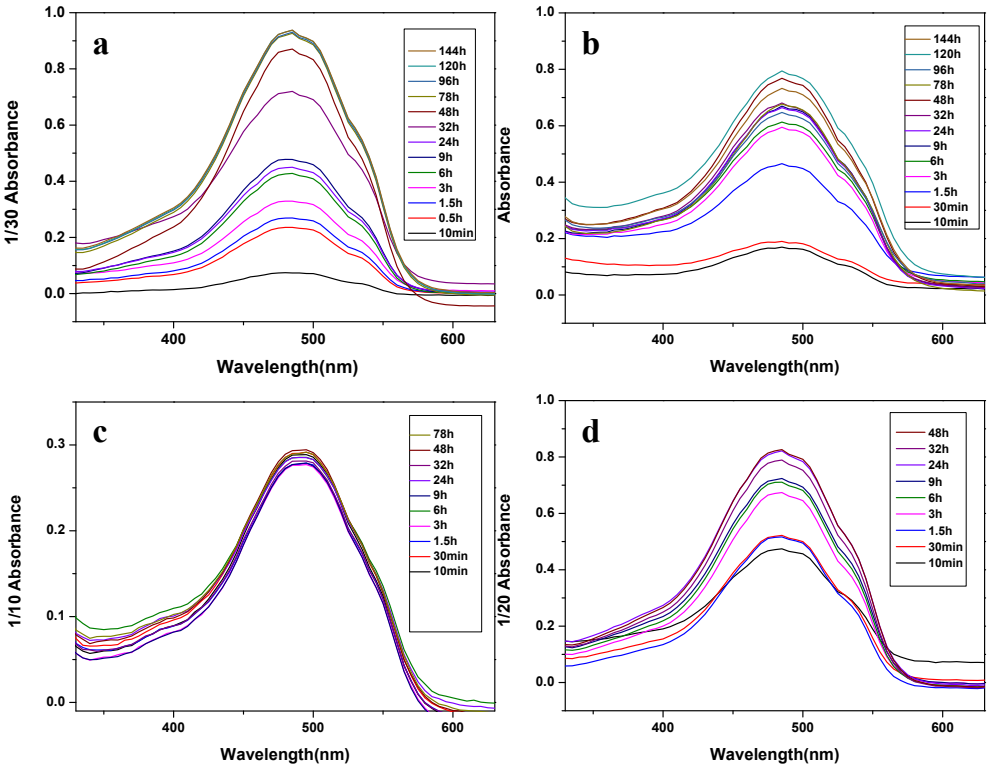

Figure S4. UV-Vis absorption spectra data in different condition. (a) Adding acid solution (pH=1.2) (released model molecule solution was diluted 30 times before UV measurements). (b) Adding EDTA (released dox solution was diluted 10 times before UV measurements). (c) After microcapsules were treated by EDTA for 78h as showing in Figure 4b, adding acid (pH=1.2) to the capsules again (released dox solution was diluted 20 times before UV measurements). (d)Adding PBS buffer as control.

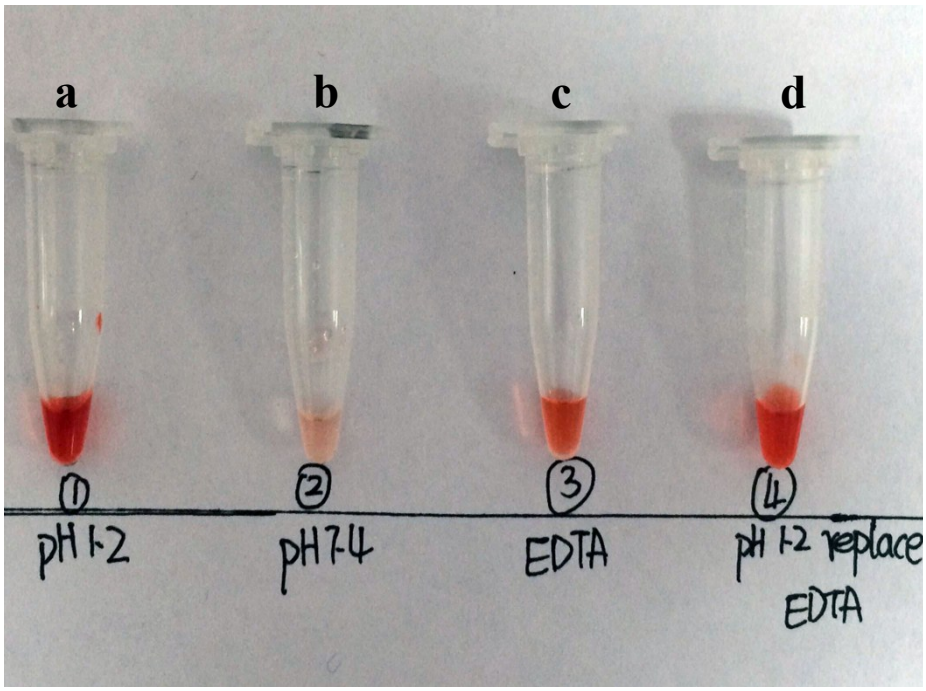

Figure S5. Images of the final released Dox solution at four different conditions. The total amount of released Dox can be determined simply by their color. (a) The supernatant solution was darkest red after adding HCl at pH=1.2 to microcapsule suspension, which means most amount of Dox was released. (b) The supernatant solution was lightest red after adding PBS buffer at pH=7.4, which means few Dox was released (control experiments). (c) The supernatant solution was light red after adding EDTA. (d) The supernatant solution was dark red after adding HCl to microcapsule suspension that was first treated by EDTA as shown in Figure S5c.

Figure S6. The Schematic drawing of the possible mechanism for molecule release of the capsules in acid and EDTA solution.