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Supplementary Information for

Electrochemically stimulated drug release from dual stimuli responsive chitin hydrogel

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1. Materials

BSA was purchased from Sigma. Ferrous ammonium sulfate, phenanthroline, hydroxylamine hydrochloride were purchased from Shanghai Reagent Co., Ltd (China). Dulbecco's Modified Eagle's Medium (DMEM), penicillin–streptomycin, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Invitrogen Corp. All other reagents were of analytical grade and were used without further purification.

2. The amount of Fe^{3+} in the hydrogel

To determine the amount of Fe^{3+} in the hydrogel, phenanthroline spectrophotometric method was used[1, 2]. Briefly, the electrodeposited hydrogel on the platinum foil was dissolved in 10 mL 0.1 M Na_2SO_4 containing sodium erythorbate (10 mM). Then, 10 mL of sample solution, 5.0 mL of HAc/NaAc (pH=4.6), 2.5 mL of hydroxylamine hydrochloride (1%), 5.0 mL of phenanthroline (0.1%) were added into a 50 mL volumetric flask. Ultrapure water was added to the scale. The volumetric flask was shaken to mixing the solution. The final solution was put still for 10min before test. The Fe^{3+} concentration in the solution was recorded on a UNICO UV-2000 Spectrophotometer at 510 nm. The solution without Fe^{3+} was used as control. Ferrous ammonium sulfate was used for calibrating the Fe^{3+} analysis.

3. The amount of BSA in the hydrogel

The amount of BSA loaded in the acrylamide-modified chitin hydrogel electrodeposited on titanium plate or on platinum foil was measured by spectrophotometric method. Briefly, the acrylamide-modified chitin hydrogel was dissolved in 10 mL 100 mM Na_2SO_4, pH 7.0 (adjusted by 1%NaOH) solution at room temperature. In the case of Fe^{3+} crosslinked hydrogel electrodeposited on platinum foil, the hydrogel was dissolved in 10 mL 0.1 M Na_2SO_4 containing sodium erythorbate (10 mM). The BSA concentrations in

the solutions were recorded on a UNICO UV-2000 Spectrophotometer at 280nm. A blank acrylamide-modified chitin gel dissolved in 10 mL 100 mM Na_2SO_4 was used as a control. Protein standard was used for calibrating the BSA analysis.

4. SEM of the hydrogel

Scanning electron microscopy (SEM) observation was carried out on a TESCAN VEGA 3 LMU microscope. The hydrogels were frozen in liquid nitrogen, freeze-dried, coated with gold and analyzed.

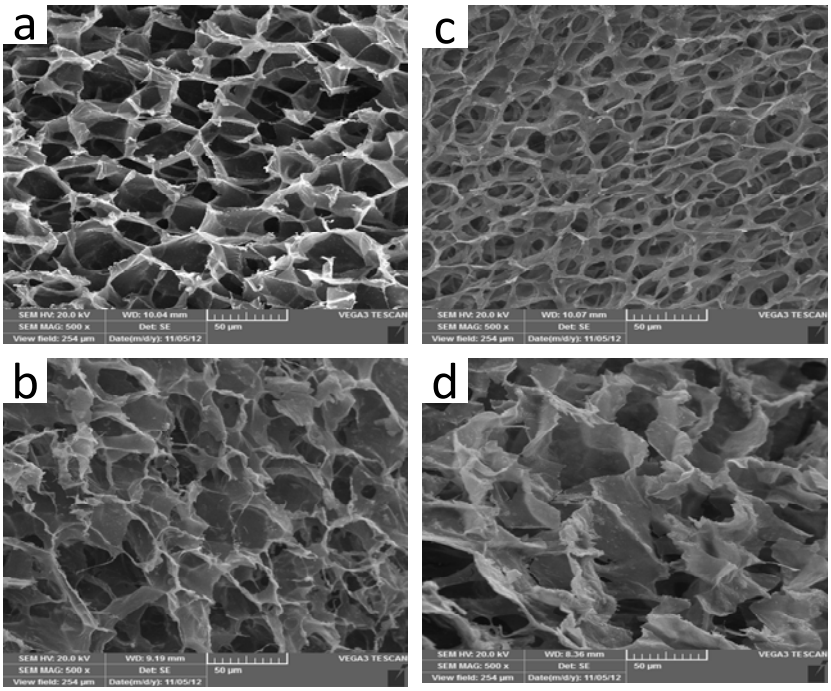

Fig.S1 SEM images of iron(Ⅲ)-crosslinked acrylamide-modified chitin hydrogel after freeze-drying with a magnification of 500× , surface (a) and cross-section (b), SEM images of the acrylamide-modified chitin hydrogel after freeze-drying with a magnification of 500× , surface (c) and cross-section (d)

5. Cytotoxicity assay

COS7 cells were used to evaluate the cytotoxicities of AMC4, Fe^{3+} crosslinked hydrogel and pH sensitive hydrogel by MTT assay. Briefly, the cells were seeded in a 96-well plate at a density of 6000 cells per well and incubated in 100 mL DMEM containing 10% FBS for 24 h. Then, sample solutions were added to the media. In the case of Fe^{3+} crosslinked hydrogel and pH sensitive hydrogel, the released solutions were chosen to evaluate the cytotoxicities. The concentrations of the samples were 23.41, 35.12, 52.67, 79.01, 118.52 mg L^{-1}. After 48 h, 20 μL MTT (5 mg mL^{-1}) solution was added to each well and further cultured for 4 h. After that, the medium was removed and replaced with 150 mL DMSO. The absorbance was measured at a

wavelength of 570 nm by a microplate reader (Bio-Rad,Model550, USA) to determine the cell viability. The relative cell viability (%) was calculated by ($OD570_{sample}/OD570_{control}$) × 100, where $OD570_{control}$ was obtained in the absence of samples and $OD570_{sample}$ was obtained after co-incubation with the samples[3, 4].

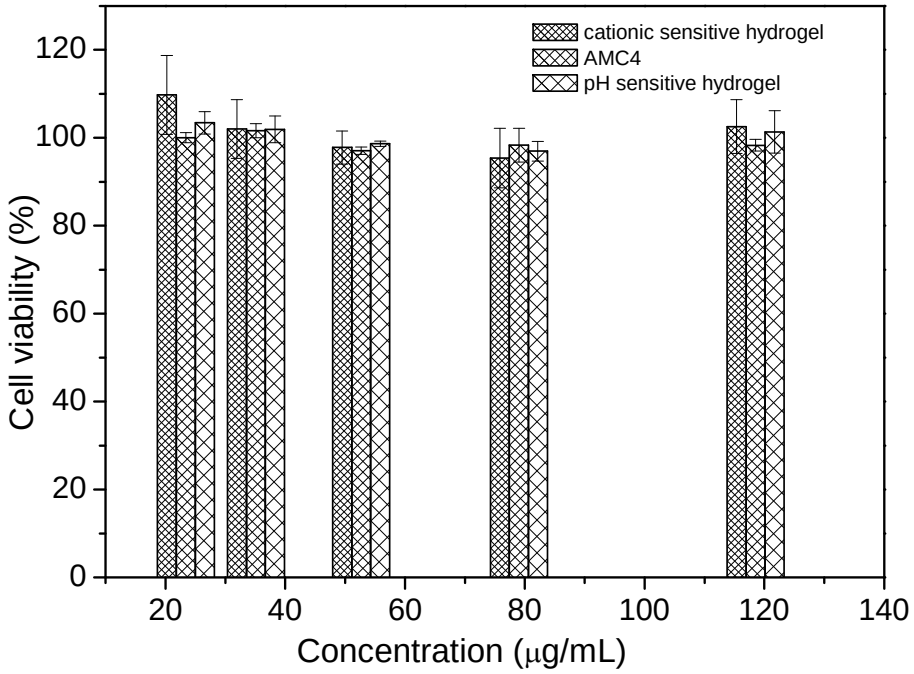

Fig.S2 Cell viabilities of released solutions from Fe^{3+} crosslinked hydrogel and pH sensitive hydrogel and the AMC4 using COS7 cells.

References

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