Supporting Information for:

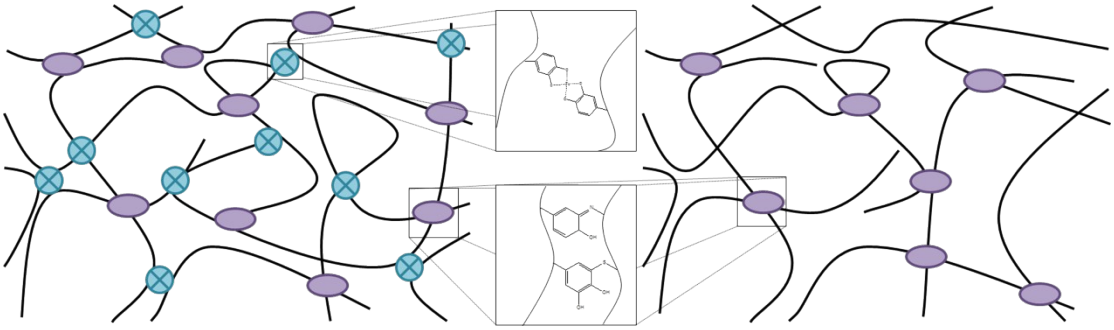
Fe^{3+}-induced bioinspired chitosan hydrogels for the sustained and controlled release of doxorubicin

Jinmao Zhang,[a] Xinyi Tao,[a] Jianwen Liu,[b] Dongzhi Wei[a] and Yuhong Ren*[a]

[a]State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, China. E-mail: yhren@ecust.edu.cn; Fax: +86 21 6425 0068; Tel: +86 21 6425 2163

[b]School of Pharmacy of East China University of Science and Technology, Shanghai 200237, China

Scheme S1



Scheme S1 The mechanism of crosslinking in the Fe^{3+}-induced CCS-NACCS hydrogel (left) and IO_4^--induced CCS-NACCS hydrogel (right).

Fig. S1

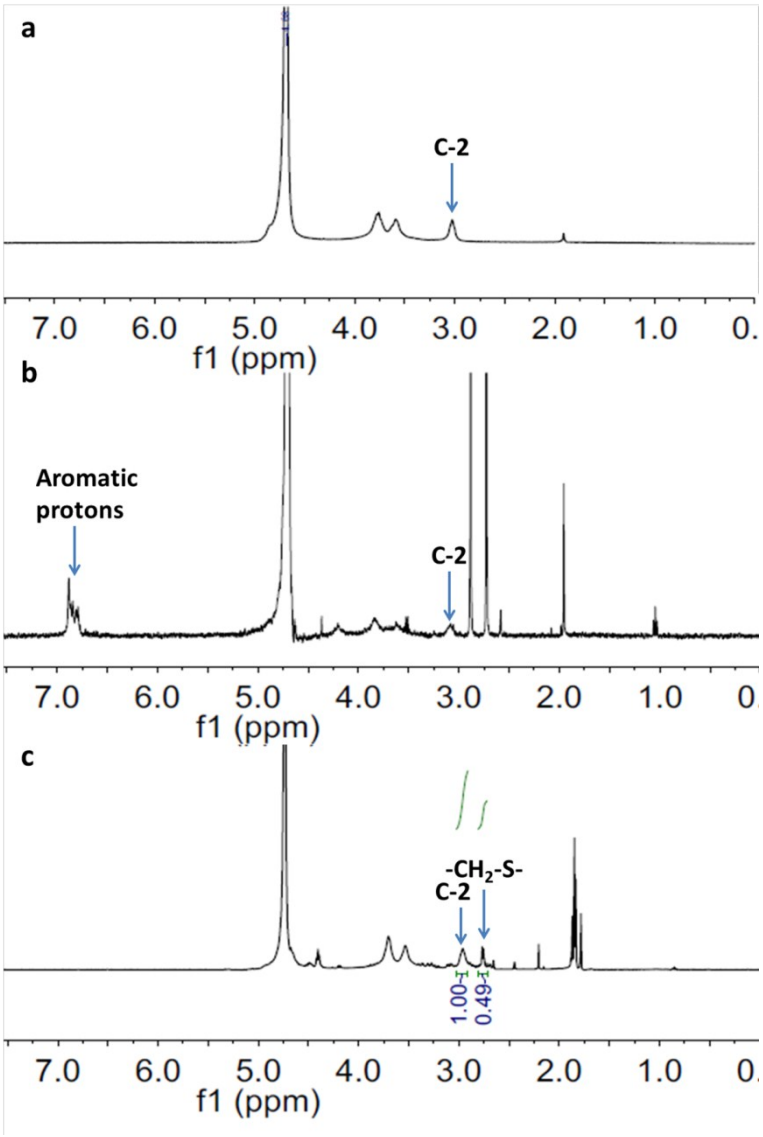


Fig. S1 ^{1}H NMR spectra of CS (a), CCS (b) and NACCS (c). Solvent: D_2O containing 1% DCl .

Fig. S2

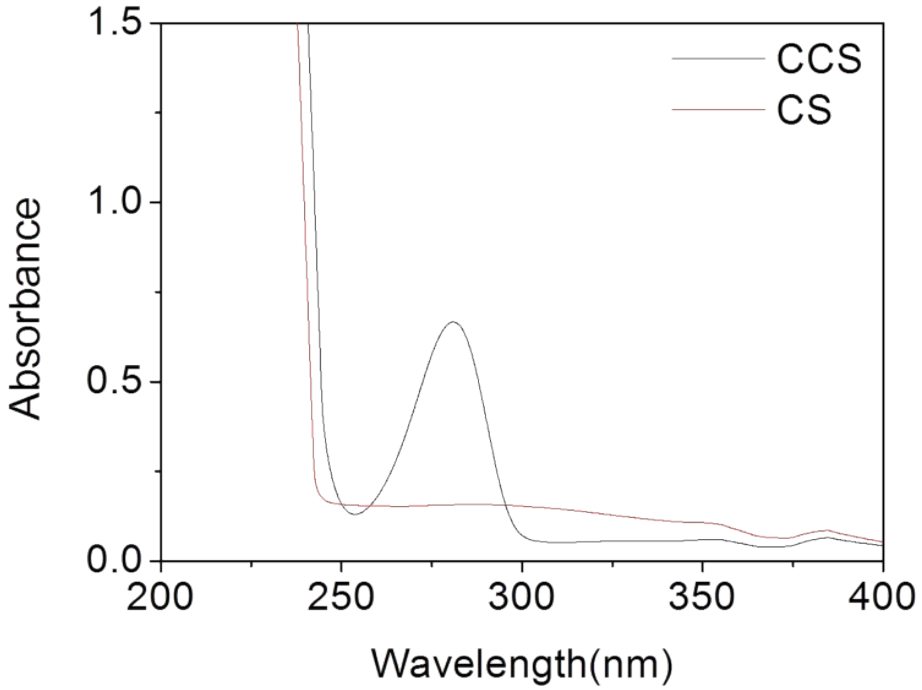


Fig. S2 UV-vis spectra of CS and CCS.

Fig. S3

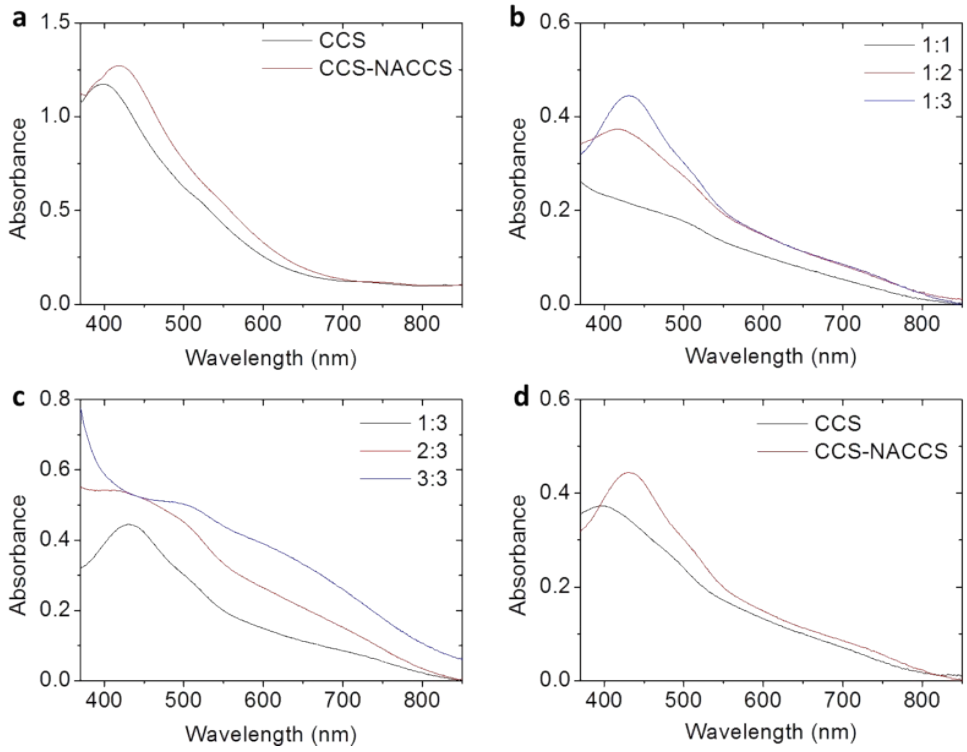


Fig. S3 UV-vis spectra of $NaIO_4$-CCS-NACCS and $NaIO_4$-CCS (a), $FeCl_3$ -CCS-NACCS at different molar ratio of Fe^{3+} and catechol (b, c), $FeCl_3$-CCS-NACCS and $FeCl_3$-CCS (d) in a 1% acetic acid solution.

UV-vis spectroscopy

Fe^{3+}-induced covalent and coordinate interactions were monitored on a UV-vis spectrophotometer (U-5100, Hitachi, Tokyo, Japan) using a quartz cuvette with a path length of 1 cm according to previous reports.[1, 2] All samples were initially blanked against a 1% acetic acid solution (v/v). Solutions were obtained by mixing 50 μL of the appropriate CCS solution (catechol concentration: 49.5 mM, 33.0 mM, 16.5 mM), 15 μL of the appropriate NACCS solution (thiol concentration: 55.0 mM, 36.7 mM, 18.3 mM), and 5 μL of the corresponding $FeCl_3$ or $NaIO_4$ solution (165 mM, 330 mM, 495 mM) in 2 mL of a 1% acetic acid solution. The solutions were thoroughly mixed before testing.

References:

1 Z. Guo, K. Ni, D. Wei and Y. Ren, *RSC Adv.*, 2015, **5**, 37377-37384

2 N. Holten-Andersen, M. J. Harrington, H. Birkedal, B. P. Lee, P. B. Messersmith, K. Y. C. Lee and J. H. Waite, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 2651-2655.

Fig. S4

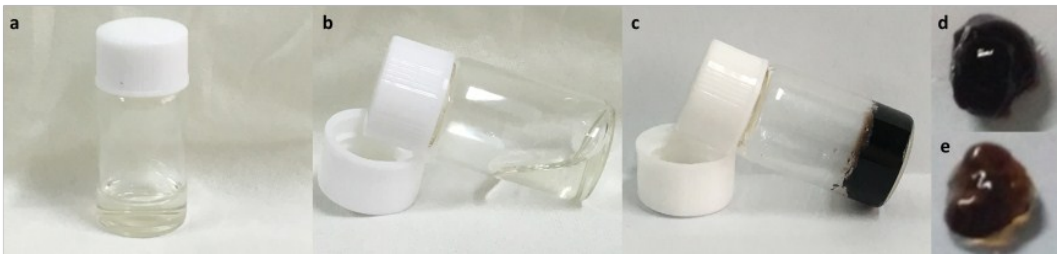


Fig. S4 Photos of Fe^{3+}-induced hydrogel before (a, b) and after gelation (c); photos of the Fe^{3+}-induced hydrogel (d) and IO_4^--induced hydrogel (e).

Fig. S5

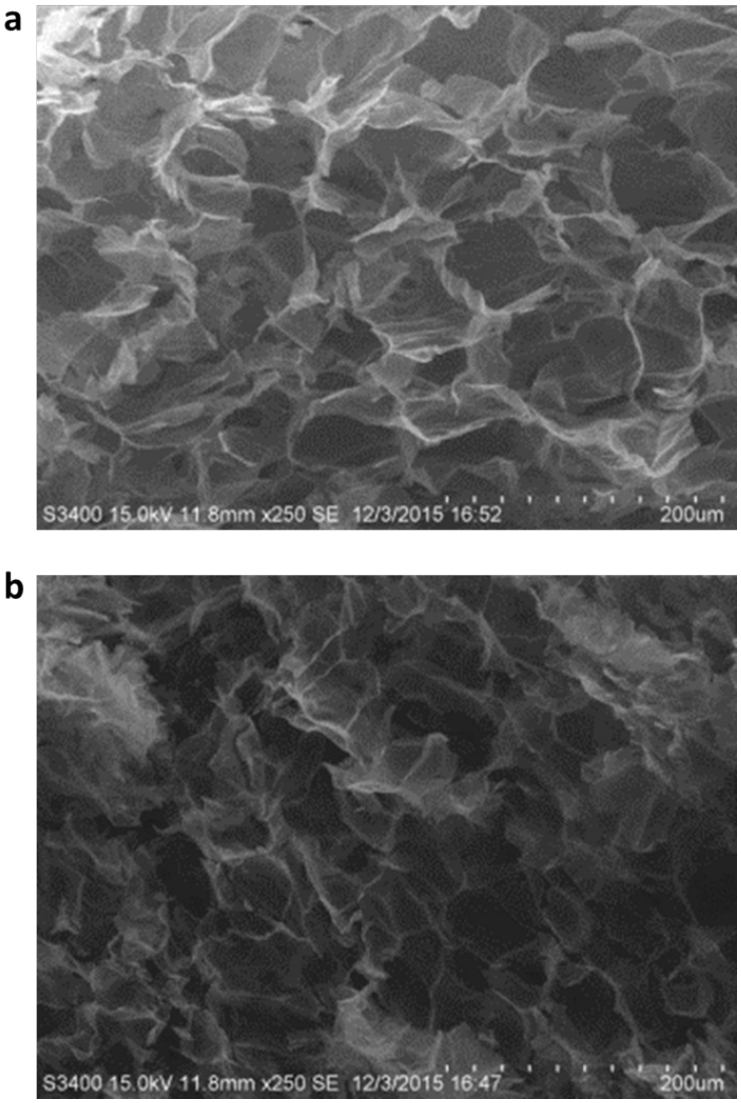


Fig. S5 SEM images of the Fe^{3+}-induced CCS hydrogel (a) and CCS-NACCS hydrogel (b).

Fig. S6

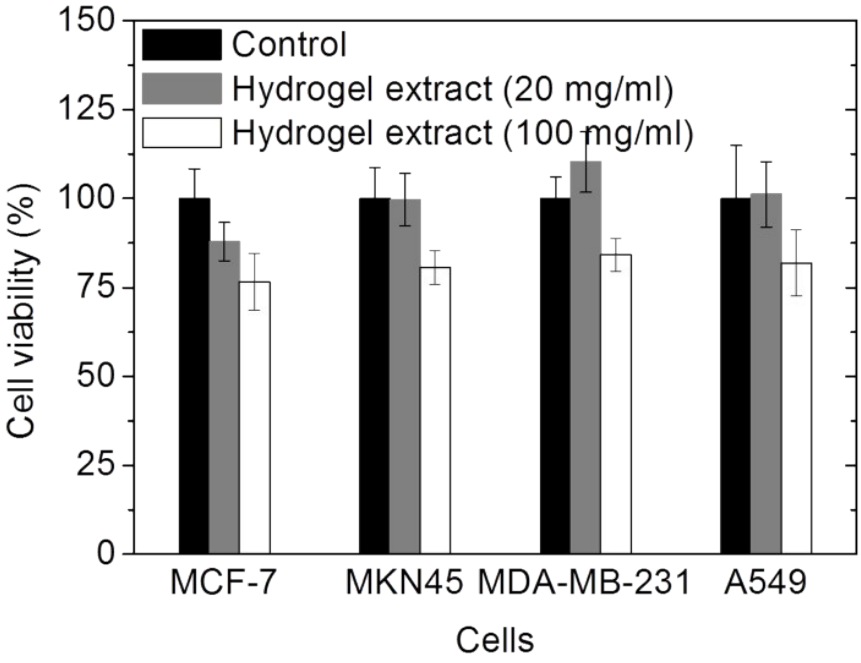


Fig. S6 Cytotoxicity test of an Fe^{3+}-induced CCS-NACCS hydrogel for different cell lines.

Fig. S7

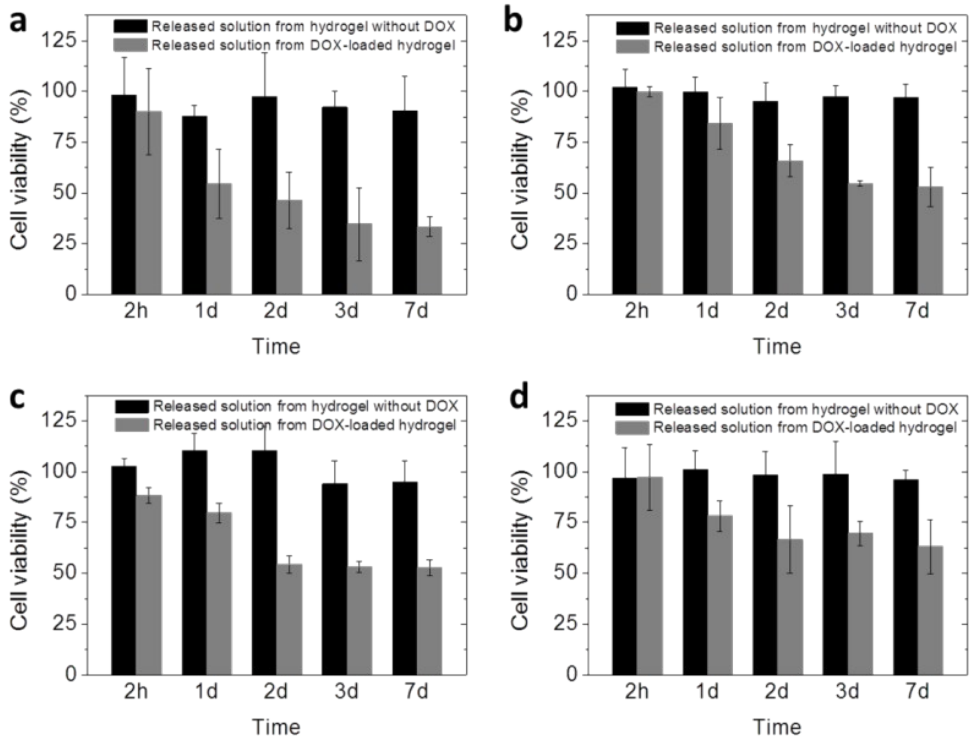


Fig. S7 The viability of MCF-7 cells (a), MKN45 cells (b), MDA-MB-231 cells (c) and A549 cells (d) incubated with released solutions from DOX-loaded CCS-NACCS hydrogels and CCS-NACCS hydrogels without DOX at various release time points based on the MTT assay.

Table S1 Synthesis of CCS with different DSs.

Conjugate	The molar ratio of CS and 3,4-dihydroxy benzaldehyde	DS(%)	Yield(%)
CCS-1	1:0.6	40.7 ± 0.40	81.6
CCS-2	1:0.8	62.4 ± 0.26	71.7
CCS-3	1:3.0	77.7 ± 0.47	72.1

Table S2 Summary of the drug loading efficiency (DLE), drug loading capacity (DLC), diffusional exponent (n), and correlation coefficient (R^2).

pH	DLE（%）	DLC（mg DOX/g hydrogel）	Diffusional exponent, n	R^2
7.4	99.5 ± 0.26	0.998 ± 0.0062	0.1326	0.8301
6.8	98.8± 0.15	0.991 ± 0.0046	0.1492	0.8837
5.0	99.3± 0.17	0.996 ± 0.0072	0.2426	0.9876

Table S3 The half maximal inhibitory concentration (IC_{50}) of free DOX and the DOX-loaded hydrogel for different cell lines.

Cell lines	IC_{50} of free DOX (μg/mL)	IC_{50} of the DOX-loaded hydrogel (mg/mL)
NCI-H460	4.8 ± 0.31	11.5 ± 0.38 (5.1 ± 0.17 μg DOX/mL)
MCF-7	6.2 ± 0.35	16.6 ± 0.98 (7.4 ± 0.44 μg DOX/mL)
MKN45	6.3 ± 0.25	19.8 ± 0.85 (8.8 ± 0.38 μg DOX/mL)
MDA-MB-231	8.4 ± 0.40	18.7 ± 0.67 (8.3 ± 0.30 μg DOX/mL)
A549	12.5 ± 0.21	30.7 ± 0.87 (13.7 ± 0.39 μg DOX/mL)
Hela	20.2 ± 1.06	
SKOV-3	37.1 ± 1.25	