Supporting Information

Visualization of *in situ* hydrogels by MRI *in vivo*

Jia Liu[a], Ke Wang[b], Jie Luan[a], Zhi Wen[b], Lei Wang[a], Zhilan Liu[a,*], Guangyao Wu[b] and Renxi Zhuo[a]

[a] Key Laboratory of Biomedical Polymers of the Ministry of Education & College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

[b] Medical Imaging Division, Zhongnan Hospital of Wuhan University, Wuhan 430072, P. R. China

*** Corresponding author: Zhilan Liu**

Tel.: +86-27-68755200; fax: +86-27-68754509.

E-mail address: liuzl@whu.edu.cn (Zhilan Liu)

1 Synthesis and characterization of diarylaldehyde PEG (PEG-DA)

The PEG-DA was synthesized by esterification of 4-formylbenzoic acid with PEG under DCC and DMAP according to the literature[1]. Briefly, PEG2000 (2.00 g, 1.0 mmol) was dried in vacuum oven at 50°C overnight and then dissolved in 50 mL dried THF with 4-formylbenzoic acid (0.66 g, 4.40 mmol) and DMAP (0.05 g, 0.4 mmol). DCC was dissolved in 15 mL dry THF and then added into the former solution dropwise. The mixed solution was magnetically stirred for 20 h at room temperature. The white sediment was filtered, and the filtrate was concentrated to 5 mL by rotary evaporation. The residual was precipitated in 200 mL diethyl ether, yielding the white waxy solid. The product was refined by reprecipitating in diethyl ether for two times (yield 85%). ^{1}H NMR and IR spectrums were showed in Fig. S1. H_δ (300 MHz, $CDCl_3$, ppm) = 10.11 (s, 2H, C*H*O), 8.22 (d, 4H, Ar-*H*), 7.96 (d, 4H, Ar-*H*), 4.51 (m, 4H, COOCH_2), 3.86-3.83 (m, 4H, COOCH_2CH_2), 3.69-3.58 (m, 172176H, OCH_2CH_2O). IR (KBr): ν (cm^{-1}) = 3490, 2882, 1976, 1715, 1466, 1345, 1280, 1104, 961, 842.

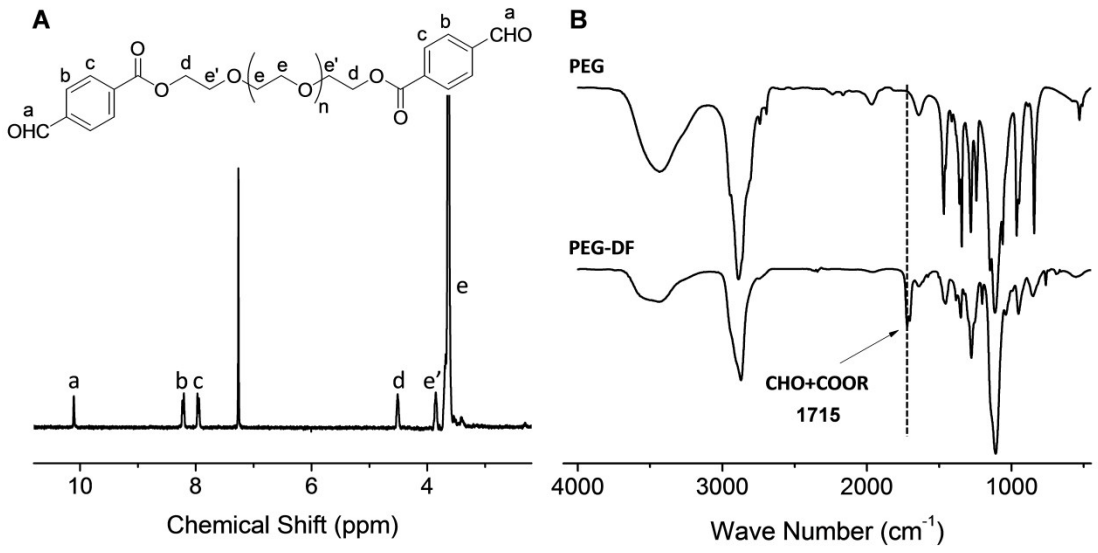

Fig. S1. ^{1}H NMR spectrum of PEG-DA in $CDCl_3$ (A) and FT-IR spectrums of PEG and PEG-DA (B)

2 Synthesis and Characterization of Ch-DTPA

Ch-DTPA was prepared according to previous work[2]. Chitosan (1.00 g, 6.21 mmol $–NH_2$) was dissolved in 70 mL 50 mM diluted aqueous HCl, and heated to 65°C for 3 h until it dissolved completely. Then the solution was cooled to room temperature. TEMED was added to adjust the pH to 4.7. DTPA (0.4886 g, 1.2 mmol) was dissolved in proper amount of deionized water using TEMED to adjust the pH to 4.7. NHS and EDC (both 1.2 equivalent to DTPA) were dissolved in 50 mM aqueous HCl and added into DTPA aqueous dropwise in ice bath. The DTPA/EDC/NHS mixture solution was finally added to the chitosan solution and reacted for 30 h at 70°C, followed by another 12 h at ambient temperature. The resulting solution was purified by dialysis through cellulose tube (Mw cutoff: 8k~14k g/mol) against pure water for 3 days. The product was then lyophilized and kept in powder form before use. The product (yield 70%) was characterized by FT-IR spectrum.

Fig. S2 shows FT-IR spectra of DTPA, Chitosan, Ch-DTPA 20% and chitosan physically mixed with 20% DTPA. Chitosan was found to exhibit characteristic absorptions at 1652 and 1589 cm^{-1}, which are attributed to residual amide groups due to incomplete deacetylation. The characteristic peaks of saccharide structure appeared at 1160, 1080 and 1035 cm^{-1} are attributed to C-O-C anti-symmetric stretching and skeletal vibrations involving the C-O stretching. The spectrum is similar to that of the former works[3]. The product Ch-DTPA has a very similar curve shape compared to chitosan, but the peaks intensity at 1654 and 1589 cm^{-1} was extremely extended and became to a much stronger peak at 1653 cm^{-1}. This result demonstrated that much more -CONH- linkages exist in Ch-DTPA than in chitosan. Furthermore, in zoomed picture, there is a new shoulder peak at 1530 cm^{-1} which does not appear in the physical mixture of chitosan and

DTPA with a proportion of 20% (w/w). This shoulder peak could be attributed to amide II band reported in former literatures[4, 5], which further confirmed that DTPA was covalently bonded to chitosan with amide bond rather than physical mixture.

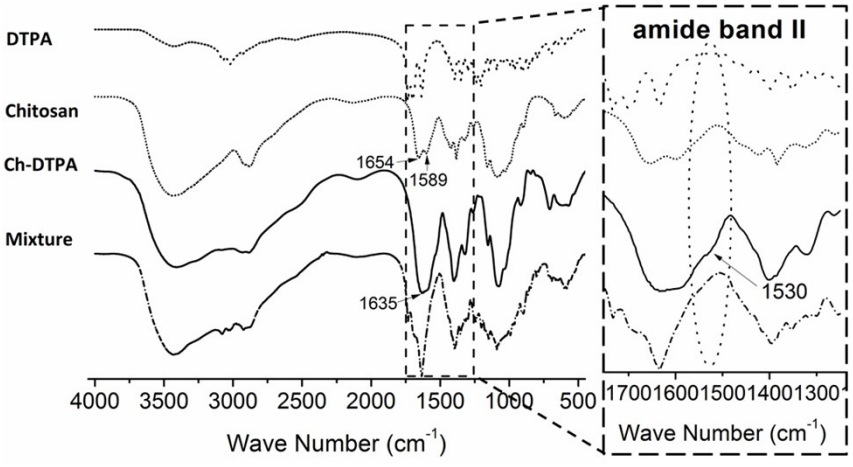

Fig. S2. FT-IR spectra of DTPA, Chitosan, Ch-DTPA 20% and chitosan/DTPA 20% mixture.

3 Qualitative and quantitative analysis of chelating capability (CC)

Xylenol orage (XO) is a commonly used indicator for metal titrations, and its color changes dramatically when meet free metal ions. As show in Fig. S3A, trace Gd^{3+} ions make the color of XO solution changed from yellow to purple red. However, when the solution is composed of Ch-DTPA and Gd^{3+} at the same time, the color of the XO solution stays light yellow because the macromolecular chelator Ch-DTPA could chelate Gd^{3+} completely leaving no free Gd^{3+} in the solution. As the control group, chitosan itself doesn't have the ability to chelate Gd^{3+}, so there are plenty of free metal ions to change the color of XO into purple red.

In addition, the UV absorbance spectrum in Fig. S3B also verifies the actual association between Gd^{3+} and DTPA bonded to Chitosan. Comparing XO/Gd^{3+} (purple line) to XO (black

line), free Gd^{3+} in solution could make the maximum absorption wavelength λ_{max} move from 433 nm to 570 nm. The absorbance curve of Chitosan/ Gd^{3+}/OX (green line) is very similar to that of XO/ Gd^{3+} (purple line), which demonstrates that plenty of free Gd^{3+} exist in the solution. However, the absorbance curve of Ch-DTPA/ Gd^{3+}/XO (red line) is much closer to that of XO (black line), both of which have strong absorbance at 570 nm but no signal at 433 nm. This fact implies that Ch-DTPA could chelate Gd^{3+} effectively under such conditions and leave no free Gd^{3+} in the solution.

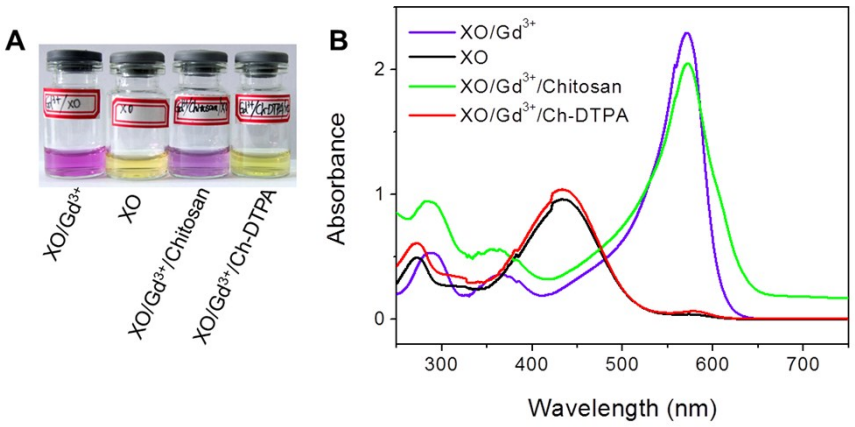

Fig. S3. (A) Photos (from left to right) assigned to XO in medium with $GdCl_3$; XO in medium without $GdCl_3$; XO in medium with the mixture of $GdCl_3$ and chitosan; XO in medium with the mixture of $GdCl_3$ and Ch-DTPA, respectively. (B) The UV-visible absorption spectra of corresponding four samples.

In order to quantitatively determine the complexing capacity (CC) of Ch-DTPA to Gd^{3+}, two assessment methods, complexometric titration and ICP-AES spectroscopy analyses, were performed.

Firstly, the XO assay based on the former research[6] with slight modification was performed. Briefly, quantitative Ch-DTPA (about 5.0~10.0 mg) was dissolved in 50 mL acetic buffer (50 mM,

pH 5.8), two drops of XO indicator (dissolve 3 mg XO in 250 mL acetic buffer and stored in 4 °C refrigerator before use) was added and the solution turned to light yellow color. The mixture is then titrated with 0.1 M $GdCl_3$ solution ($GdCl_3 \cdot 6H_2O$ dissolved in acetic buffer) until the color turned to violet and could not fade in 30 s. The volume of Gd^{3+} titrant was recorded to calculate the chelating capacity according to following equation.

$$Capacity\ of\ chelate = \frac{c \times V_m \times M_{Gd}}{m_{Ch-DTPA}} mg/g$$

Where $m_{Ch\text{-}DTPA}$ represents the mass of Ch-DTPA; c represents the concentration of $GdCl_3$ titrant; V_m is the volume of Gd^{3+} titrant; M_{Gd} is the molar mass of Gd^{3+} equal to 157.25 g/mol.

Secondly, inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Thermo, Waltham, MA, USA) was also applied to determine the Gd^{3+} chelating capability of Ch-DTPA. Briefly, Ch-DTPA was dissolved in acetic buffer (50 mM, pH 5.8), excessive 10 times of $GdCl_3$ solution was added. The mixed solution was stirred for 2 h and then poured into a cellulose tube (Mw cutoff: 8k~14k g/mol) and dialysed against deionized water for 3 days, the dialysis medium was changed every 3 h until no Gd^{3+} could be detected. Finally, the solution in dialysis tube was freeze-dried. The product powder Ch-DTPA/Gd^{3+} was dissolved in aqueous dilute HCl to the concentration about 1 mg/mL and the samples were propelled through the tube into the ICP-AES for determination. The standard solution was prepared by $GdCl_3 \cdot 6H_2O$ dissolve in dilute HCl aqueous. Absorption was measured at the wavelength of 335 nm. The linear range was found between 0 and 100 ppm. The chelating capacity was calculated according to following equation.

$$Capacity\ of\ chelate = \frac{c}{c_{Ch-DTPA}} mg/g$$

Where c is the concentration of Gd^{3+} which recorded by ICP-AES in ppm unit; $c_{Ch\text{-}DTPA}$ is the

concentration of polymer in mg/mL unit.

The CCs of two samples which feed ratio of DTPA was 20% and 40% were listed in Table S1. The results suggest that the ICP-AES spectroscopy analyses have lower detection limit than the method of titration. Even though trace amount of Gd^{3+} was detected in Chitosan by the ICP-AES, the content of Gd^{3+} in Chitosan is much lower than that in Ch-DTPA. The plenty of $-NH_2$ on Chitosan main chain probably absorbed very little of Gd^{3+}. Besides, the CC values of products are lower than that in feed. There should be two reasons. Firstly, the esterification does not react completely leading to the substitute degree of DTPA not as high as that in feed. Secondly, the stereo-hindrance effect probably reduces the complexing potency of DTPA moiety, which means some of DTPA immobilized on chitosan main chain could not chelate the Gd^{3+} actually.

Table S1. Complexing Capacity of Ch-DTPA to Gd(III)

Sample	Feed ratio of DTPA/-NH_2 (mol%)	Theoretic CC to Gd^{3+} (mg/g)	CC to Gd^{3+} (mg/g)[a]	CC to Gd^{3+} (mg/g)[b]
Chitosan	-	0	0	0.67±0.33
Ch-DTPA1	20	134	78.63±2.28	70.15±0.09
Ch-DTPA2	40	202	121.08±2.00	88.98±1.95

[a] determined by titration

[b] determined by ICP-AES spectrum anlysis

Reference

1. L. Martin-Gomis, J. Ortiz, F. Fernandez-Lazaro, A. Sastre-Santos, B. Elliott and L. Echegoyen, *Tetrahedron*, 2006, **62**, 2102-2109.
2. C. E. Smith, A. Shkumatov, S. G. Withers, B. X. Yang, J. F. Glockner, S. Misra, E. J. Roy, C.

H. Wong, S. C. Zimmerman and H. Kong, *Acs Nano*, 2013, **7**, 9599-9610.

3. T. W. Chung, J. Yang, T. Akaike, K. Y. Cho, J. W. Nah, S. I. Kim and C. S. Cho, *Biomaterials*, 2002, **23**, 2827-2834.
4. M. Huang, Z. X. L. Huang, M. Bilgen and C. Berkland, *Nanomed-Nanotechnol*, 2008, **4**, 30-40.
5. V. Darras, M. Nelea, F. M. Winnik and M. D. Buschmann, *Carbohydrate Polymers*, 2010, **80**, 1137-1146.
6. A. Barge, G. Cravotto, E. Gianolio and F. Fedeli, *Contrast Media Mol I*, 2006, **1**, 184-188.