Stimuli-Induced Gel-Sol Transition of Multi-Sensitive Supramolecular β-Cyclodextrin Grafted Alginate/Ferrocene Modified Pluronic Hydrogel

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Supplementary Information

CONTENTS:

1. Materials and instruments

Alginate was purchased from Tianjin Yuanhang Chemicals Co., Ltd., China. Pluronic F127 (Mn = 13450), was purchased from Aldrich Chemical Co. and dried in a vacuum at 50 °C for 6 h before use. β-CD was purchased from Tianjin Bodi Chemical Co. Ltd. Glucose oxidase (GOD, 250 U/mg) was purchased from Toyobo, Japan. Carboxyferrocene (Fc-COOH), morpholinoethanesulfonic acid (MES), dicyclohexylcarbodiimide (DCC), 4-(dimethylamino) pyridine (DMAP), N-hydroxysuccinimide (NHS), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide hydrochloride (EDCI), and other reagents were local commercial products and used as received.

^{1}H NMRs were measured on an Avance Bruker-600 spectrometer. The chemical shifts of ^{1}H NMR are expressed in parts per million downfield relative to the internal tetramethylsilane (δ = 0 ppm). Rheological studies were carried on a rotating rheometer (Bohlin advanced Rheometer, Malvern Instruments, UK) equipped with a temperature controller.

2. Synthesis of β-CD-conjugated alginate (Alg-β-CD)

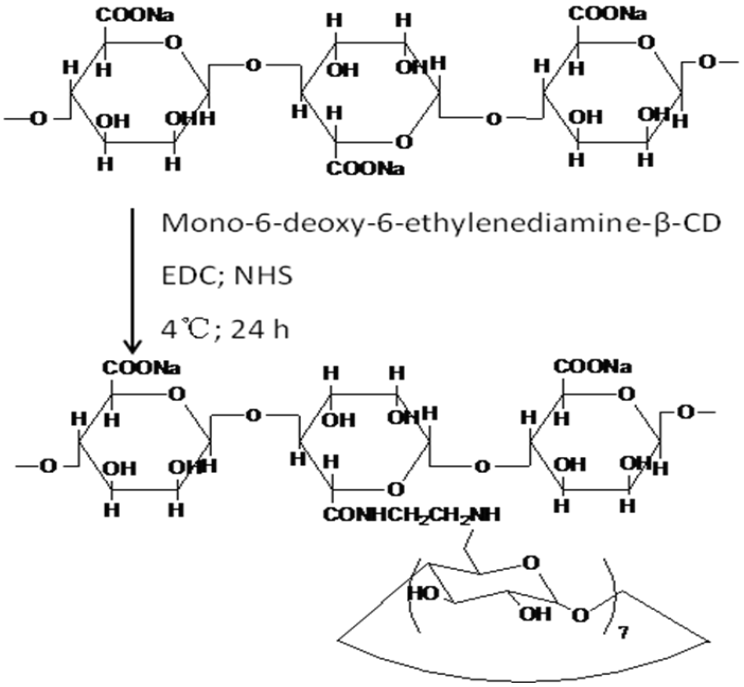
Alg-β-CD was synthesized in two steps with slight modification (Scheme S1). Firstly the mono-6-(*p*-tolylsulfonyl)-β-cyclodextrin (6-CD-OTs) and mono-6-deoxy-6-ethylenediamine-β-CD (6-CD-EDA) were synthesized according to the method reported in the literature. [1,2] β-CD was grafted onto the sodium alginate backbone via amido link condensation reaction. A sodium alginate solution [0.6% (w/v)] was prepared in MES buffer solution (0.1 M) and NaCl (0.5 M), and the pH was adjusted to 6.0. A sample of NHS (69 mg) and of EDC (230 mg, molar ration of EDC: NHS: COO^- = 1: 0.5: 1) were added to alginate solution (40 mL) to activate the carboxylic acid groups on the polymer backbone. The solution was agitated for 30 min to obtain a homogeneous solution followed by the addition of 10 mL 6-CD-EDA solution [9.4 % (w/v)]. The reaction was carried out at 4 ℃ for 24 h. The resulting mixture was dialyzed against pure water for 3 days and then dealt with lyophilization. The degree of β-CD substitution (DS) to alginate was calculated according to equations (1) and (2):

$$\frac{I_1}{I_2} = \frac{7X}{28X + 4n} \quad (1) \qquad DS = \frac{X}{n} \times 100\% \quad (2)$$

where I_1 is the digital integration of anomeric protons of β-CD, I_2 is the digital integration of the tertiary hydrogels on the cycle of alginate and β-CD, X is the mole number of the grafted β-CD, n

is the average number of monosaccharides in one alginate chain. The final DS of Alg-β-CD used was 12.4 % by this calculated method.

^{1}H NMR (600 MHz, D_2O, δ): 5.01 (s, anomeric protons of β-CD), 3.53-3.97 (m, H-$_{2\sim5}$) of alginate and H-$_{2'\sim5'}$) of β-CD, 3.30 (d, H-$_6$ of β-CD), 2.84 (t, H-$_{8'}$), 2.82 (t, H-$_{7'}$). The spectrum of Alg-β-CD was shown in Figure S1.



Scheme S1. The synthesis and chemical structure of Alg-β-CD.

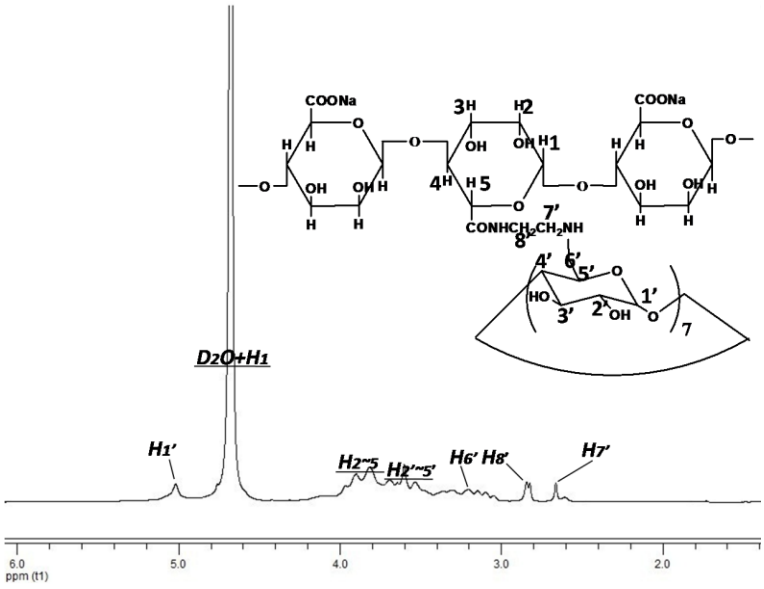
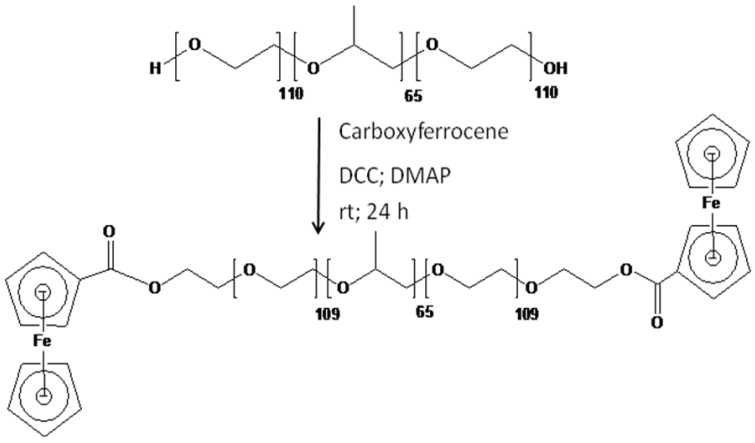


Figure S1. ^{1}H NMR spectrum of Alg-β-CD in D_2O.

3. Synthesis of ferrocene-terminated pluronic F127 (F127-Fc)

F127-Fc was synthesized with one pot method (Scheme S2)[2]. DMAP (0.75 mmol) and DCC

(1.5 mmol) were added successively to a solution of Pluronic F127 ($(PEO)_{110}(PPO)_{65}(PPO)_{110}$, M_W = 13450, 0.5 mmol) and carboxyferrocene (1.5 mmol) in dry dichloromethane (CH_2Cl_2, 40 mL). The mixture was stirred at room temperature for 24 h. After removing dicyclohexylurea (DCU) by filtration, the filtrate was concentrated in a vaccum at room temperature. The dry resultant was washed with Et_2O for three times. Subsequently, the product was dissolved in a moderate amount of water, and the solution was filtrated through a filter membrane (0.45 μm) in order to detach other impurities. Finally, the light yellow product was obtained after lyophilization. ^{1}H NMR (600 MHz, D_2O, δ): 4.78 (d, H-$_1$), 4.42 (t, H-$_2$), 4.36 (t, H-$_d$), 4.17 (s, H-$_3$), 3.54-3.68 (m, H-$_{c+c'+c''}$), 3.21-3.38 (m, H-$_{b+b'}$), 1.11 (s, H-$_a$). The spectrum of F127-Fc was shown in Figure S2. The final DS of F127-Fc used was 24.3% which was calculated though the integral ratio between H-$_1$ and H-$_a$.



Scheme S2. The synthesis and chemical structure of F127-Fc.

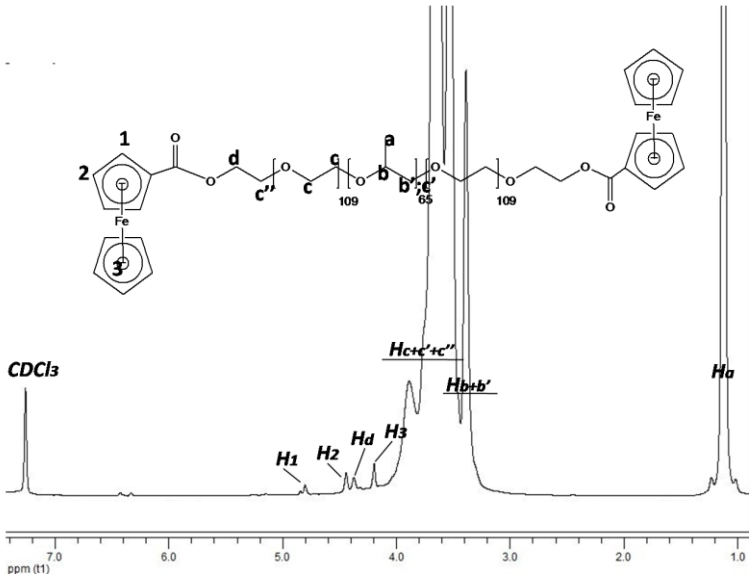


Figure S2. ^{1}H NMR spectrum of F127-Fc in $CDCl_3$.

4. Fabrication of Alg-β-CD/F127-Fc hydrogels.

Alg-β-CD/F127-Fc hydrogels were prepared by mixing different amount of Alg-β-CD and F127-Fc in pH 7.4 phosphate-buffered saline (PBS) at 4 ℃. The final concentration of Alg-β-CD was fixed at 3 wt% with varying concentrations of F127-Fc. After the solution was mixed homogeneously for 24 h as usual, it was stored at 0-4 ℃ before use which can make sure the host-guest interactions between β-CD and ferrocene group were kept. And the inclusion interaction of complex hydrogel was also investigated by 2D NMR NOSEY in D_2O.

5. Sol-gel-sol transition phase diagram.

The sol-gel-sol transition phase diagram dependent on temperature for Alg-β-CD/F127-Fc hydrogel, pluronic F127-Fc, pluronic F127 were determined in pH 7.4 PBS solution using a test tube inverting method and temperature increment of 1℃. Each sample with a given concertration was added to the PBS solution for 24 h at 0-4 ℃ and heated gradually. The state was determined by inverting the vial when no fluidity was visually observed for 1 min. The sol-gel transition phase diagram obtained by this method is known to have a precision of ±2 ℃. All the measurements were triplicated.

6. Rheological measurement.

Rheological studies were carried on a rotating rheometer (Bohlin advanced Rheometer, Malvern Instruments, UK) equipped with a temperature controller. The viscosity was recorded with increasing temperature by 2.0 ℃/min using a gap size 200 μm. The frequency of 1 Hz and constant stress of 75 Pa were used for the temperature sweep measurement. The testing samples were 3% Alg-β-CD, 22 % F127-Fc and 3 % Alg-β-CD/22 % F127-Fc complex.

7. Glucose- and redox- sensitivity determination.

To demonstrate the glucose-senstivity of Alg-β-CD/F127-Fc hydrogel, our approach was to immobilize GOD (5 mg/mL) into the cross-linking matrix and kept the prepared samples at 0-4 ℃ before use. The glucose-sensitivity determination was carried by adding saturated glucose solution into the surface of complex hydrogel, and since glucose solution diffuse into the inner of complex hydrogels through micropore gradually, the feedback-responsive state could be observed and photographed. The final concentrations of Alg-β-CD, F127-Fc of the testing samples were fixed at 3 %, 22 %. Moreover, the response of hydrogel to sodiumhypochlorite solution was also

investigated, the operations and final concentrations of Alg-β-CD, F127-Fc were same as before.

8. Evaluation of biocompatibility

In order to demonstrate the biocompatibility of Alg-β-CD/127-Fc hydrogel system, the agar diffusion test was taken into consideration which was a classic method of evaluating toxicity of materials. [3] The agar diffusion test was carried out according to international standard ISO 10993-5 at a quality controlled testing laboratory. L929 cell line was chosen because it had a significant history of use in assays of this type. There were three test specimens in each of the test groups, and the test was run twice with the positive and negative controls on the dish. After incubation, the cytotoxicity of the test samples was determined by measuring the inhibition zone index (Zi, nominal scale 0-5) around the test specimens and by assessing microscopically the cell lysis index (Li, nominal scale 0-5) under a light microscope. The concentrations of Alg-β-CD, F127-Fc for testing were fixed at 3 %, 22 %. Before testing, the sample was sterilized at 40℃ using oxacyclopropane, and vacuumized at least one day in order to detach the residual oxacyclopropane.

9. Reference.

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