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Supporting Information

Synthesis and Characterization of enzyme-degradable zwitterionic dextran hydrogel

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1. Materials and Methods

1.1 Materials

Dextran (70K), *N*,*N*-Dimethylglycine ethyl ester, sodium hydroxide, epichlorohydrin, glycidyl methacrylate, cellulose dialysis membrane (14K cut-off), phosphate buffered saline (PBS) and fluorescein diacetate were purchased from Sigma-Aldrich (St. Louis, MO). MMP (H-CKSGGPQGIWGQGSKC-OH) peptide was purchased from GenScript (Piscataway, NJ). RGD (H-CGRGDS-OH) peptide was purchased from NEO Bioscience (Woburn, MA). Tris-(carboxyethyl) phosphine hydrochloride (TCEP) was purchased from CHEM-IMPEX INT'L INC (Wood Dale, IL). NIH-3T3 fibroblast and Bovine aorta endothelial cells (BAECs) were purchased from American Type Culture Collection (Rockville, MD). Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Invitrogen (Grand Island, NY). Water used in all experiments was purified using a Millipore Milli-Q Direct 8 Ultrapure Water system (Billerica, MA).

1.2 Synthesis of CB-Dex and preparation of peptide cross-linked hydrogels

Zwitterionic dextran 70K with different degrees of carboxybetaine substitution was synthesized via a facile one pot reaction[1] as we reported before and then CB-Dex was further reacted with glycidyl methacrylate to introduce methacrylate (MA) to produce CB-Dex-MA.[2] MMP peptide with a cysteine on both terminuses was used as a cross-linker. Before the hydrogel formation, TCEP was used to reduce the partially oxidized cysteine on MMP to thiol group. Hydrogel was formed via Michael Type reaction of thiol groups on MMP peptides to methacrylate groups on CB-Dex-MA at a molar ratio of thiol to methacrylate of 1:1. CB-Dex-MA was dissolved in PBS pH 7.4 and mixed with MMP peptide in PBS solution to the desired concentration (CB-Dex-MA). The pH value of the solution was firstly adjusted to around 7.8, then quickly vortexed, transferred into a mold and placed in an incubator at 37°C for 30 min. The formed hydrogel was equilibrated in PBS for 3 days and then was punched as a dish with diameter of 8 mm and thickness of 2 mm for further characterization. For RGD-CB-Dex-MA hydrogel preparation, the CB-Dex-MA was conjugated with RGD at a molar ratio of thiol to methacrylate of 0.15 to 1 first at 37

°C for 60 min and then hydrogel was formed following the same procedure as previous. CB-Dex-MA hydrogels made by free radical-induced cross-linking with the same weight percentage were used as control and formed with the previous procedure as we reported[1].

1.3 Swelling ratio experiments and rheological studies of hydrogels

The MMP cross-linked CB-Dex-MA hydrogels were immersed in water with water change every day and the hydrogels were weighted to determine the hydrated mass (W_h) after incubation for 72 hours. The dry mass was obtained by weighting the hydrogel after freeze-dry for 48 hours. The swelling ratio was defined as $Q = \frac{W_h - W_d}{W_d} * 100\%$. The equilibrated hydrogel was punched as a dish with diameter of 8mm and thickness of 2mm for rheological characterization. The hydrogels were loaded in 8mm parallel plate geometry on a TA instruments ARES-G2 rheometer with a strain of 10% and an angular frequency from 100 to 1 rad/s. The storage and loss modulus was monitored during the measurement process. Dextran hydrogel without CB substitution was used as the control.

1.4 Cytotoxicity assay for CB-Dextran

The cytotoxicity of linear CB-Dex (100 mg/mL, 10 mg/mL, 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL and 0.001 mg/mL) were evaluated against NIH-3T3 fibroblast cells in a 96 wells plate using Vybrant MTT Cell Proliferation Assay (Life Technology, Carlsbad, CA). The NIH-3T3 fibroblast cells were seeded on a 96 wells plate with concentration of 1x 10^5/ml NIH-3T3 cells and incubated for 24 hours in culture medium ((Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% Pen-Strep). Then the old medium was replaced with 100 μL of CB-Dex solution in DMEM at different concentrations and the cells were incubated at 37 °C for 4 hours or 24 hours. The medium was then replaced with 100 μL of the fresh medium and 10 μL of MTT stock solution (5 mg/mL in PBS), and the cells were incubated for another 4 hours. 150 μL of DMSO was then used to dissolve purple crystal after removing the medium and the absorbance of DMSO supernatant was measured at 540nm using a Tecan

Infinite 200 microplate reader (Switzerland). Dextran without CB substitution solution was used as the control, fresh DMEM medium without dextran or CB-Dex was used as a negative control and the cell viability was expressed as a percentage of the control. Each sample hass 6 replicates. Data were analyzed using single-factor analysis of variance (ANOVA) and the comparison between CB-Dex and Dextran was performed using student's t test. A p values less than 0.05 was considered statistically significant.

1.5 Enzyme degradation

To study the enzymatic degradability, the hydrogel samples were firstly equilibrated in PBS, pH 7.4 with 0.2 mg/mL sodium azide and 1mM $CaCl_2$ at 37°C for 72 hours. The MMP cross-linked CB-Dex-MA hydrogel was immersed in 0.1 mg/mL and 0.01 mg/mL of collagenase solution in PBS (pH 7.4, 0.2 mg/mL sodium azide and 1 mM $CaCl_2$). At each time point, the hydrogel sample was weighted separately. Two controls were used: (1) CB-Dex-MA hydrogels without collagenase and (2) CB-Dex-MA hydrogels made by free radical-induced cross-linking with the same amount of collagenase (0.1mg/mL). The enzyme degradation behavior was investigated by monitoring the change of normalized hydrogel weight (NW_h) in the buffer and NW_h was defined as:

$$NW_h = \frac{W_t}{W_0}$$

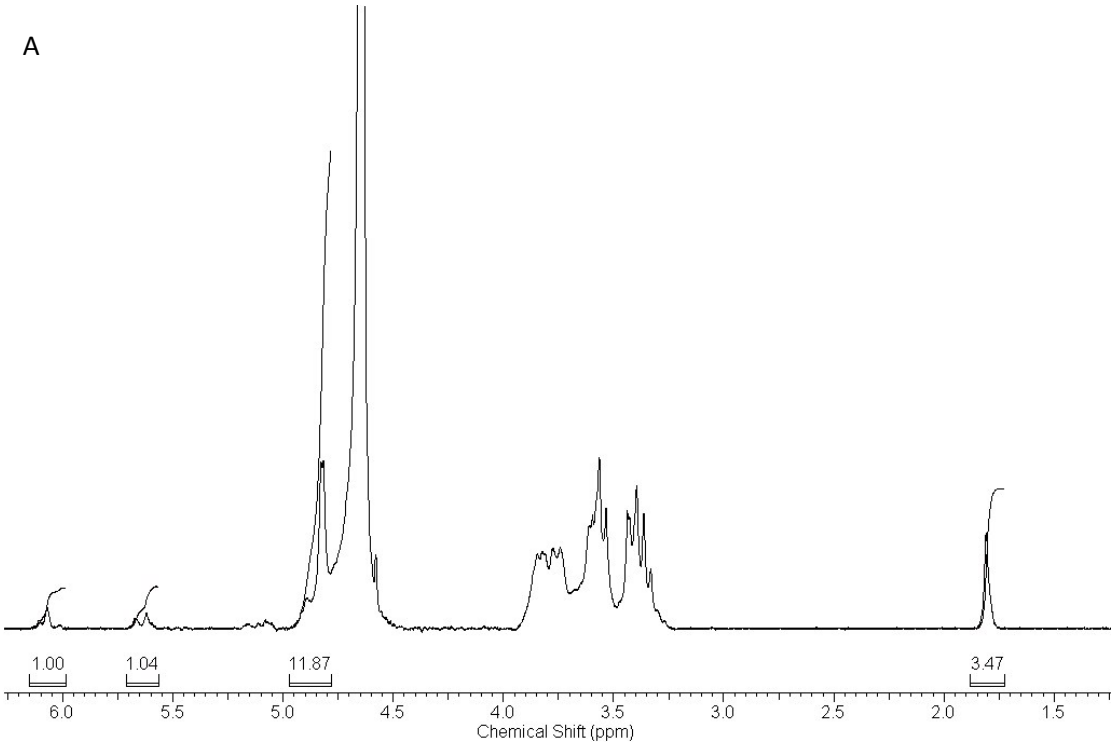
Where W_t is the hydrated weight at time t and W_0 is the initial hydrated weight of the hydrogel.

1.6 Cell Adhesion study

Three hydrogel surfaces were characterized with cell adhesion study against BAECs: MMP cross-linked Dextran-MA hydrogel, MMP cross-linked CB-Dex-MA hydrogel and MMP cross-linked CB-Dex-MA modified with RGD hydrogel. The hydrogel samples were equilibrated in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% Pen-Strep first for 72 hours first, then the BAECs was seeded on each hydrogel surface with the concentration of $1x10^5$/mL and the cells were incubated with 5% CO_2 at 37°C for 48 hours. Tissue culture polystyrene plate (TCPS) was used as a positive control. After removing the

medium, the fluorescein diacetate was used to stain the cells and then surface cell coverage and cell morphology was visualized with an Olympus IX81 fluorescent microscopy (Olympus, Japan) with 10x objective lens through FITC filter. The cell density of each surface was obtained by counting the number of cell on certain area of surface.

2. Figures



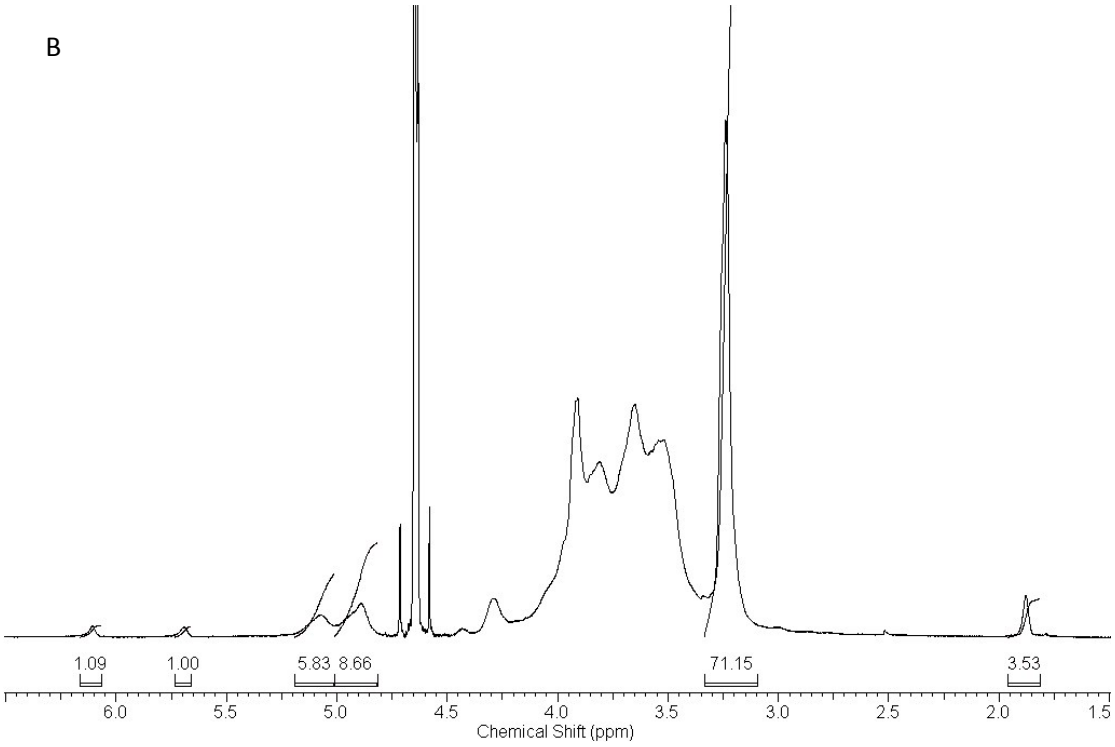


Figure S1. 300MHz ^{1}H NMR spectrum of (A) Dextran-MA; (B) CB-Dex-MA in D_2O.

Reference

1. B. Cao, L. Li, H. Wu, Q. Tang, B. Sun, H. Dong, J. Zhe and G. Cheng, *Chem. Commun.*, 2014, **50**, 3234-3237.
2. W. van Dijk-Wolthuis, J. Kettenes-Van Den Bosch, A. Van der Kerk-Van Hoof and W. Hennink, *Macromolecules*, 1997, **30**, 3411-3413.