Supporting Information

Mimicking The Mechanical Properties of Cartilage using Ionic- and Hydrogen-Bond Cross-linked Hydrogels with High Equilibrium Water Content above 70%

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The detail procedures of tensile, cyclic tensile and compression tests of hydrogel

The mechanical properties of hydrogels were tested by using an Instron 3343 testing machine with a 1 KN load cell (Instron Corporation, MA) at room temperature. Hydrogel samples with standard dumbbell-shapes of 20 mm in length and 4 mm in width (GB/T-528-2008) were subjected to tensile and cyclic tensile test. The thickness of hydrogel samples was about 2 mm. For tensile testing, the extension speed was set as 100 mm/min. For cyclic testile test, the cyclic extension speed was set as 50 mm/min and a layer of silicone oil was coated onto the surface of the hydrogel samples in order to prevent water evaporation from the hydrogels. The elastic moduli of hydrogel samples were calculated from tensile strain ranging 5% to 15%. The elongation at break and fracture strength were directly given by the program. For compression test, the hydrogels were cut into cylinders with diameter of 5 mm and height of about 2 mm. The compression rate was set as 5 mm/min. The compression moduli of hydrogels were calculated at compression strain from 60% to 80% of the compression curve.

Materials and Methods for Cytotoxicity test of hydrogels

Cell Culture

The Mouse fibroblasts L929 cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai) and cultured in Eagle's Minimum Essential Medium (EMEM, CR-11700, Cienry, China) with 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin in a 5% CO_2 humidified atmosphere at 37 °C. Cells were seeded at a density of 1.5×10^4 or 4×10^5 cells/well in 96-well and 6-well plates, respectively, to conduct subsequent experiments.

Preparation of hydrogel extract

Hydrogel extracts were prepared in accordance with national standards in GB/T 16886.12-2005/ISO 10993-12:2002. Briefly, the hydrogel was cut into small pieces about 5 mm × 25 mm for extraction. The extraction ratio is that 0.1 g hydrogels were added into 1 mL sterile EMEM medium, and then placed in a constant temperature shaker at 37 ^oC for 24 h (rotation speed: 100 rpm). After

the extraction, the hydrogel extracts were filtrated with a 0.22 μm sterile Millipore Express PES Membrane Filter (GPWP02500, Merck Millipore, Germany) and added with 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin, then stored at 4 °C under sterile conditions for further uses. The osmotic pressure and pH value of the extracts were measured by Osmometer and pH meter respectively.

Cell Morphology Observation

L929 cells were seeded in 6-well plates (3516, Corning, USA) at a density of 4×10^5 cells/well and incubated at 37 °C under 5% CO_2 for 12 h. Then cells were treated with medium extract (negative control, the EMEM medium placed in shaker at 37 °C for 24 h), three kinds of hydrogel extracts and 4.5% Phenol medium extract (positive control) for 24 h. Olympus IX53 was used to observe cell morphological changes. The medium extract was used as negative control, and 4.5% phenol medium extract was used as the positive control.

Cell Cytotoxicity Assay

The viability of L929 cells was determined using Cell Counting Kit-8 (CCK-8, C0039, Beyotime, China) according to the manufacturer's instructions. Briefly, Cells were seeded at a density of 1.5×10^4 cells/well in 96-well plates (3599, Corning, USA) and incubated at 37 °C under 5% CO_2 for 12 h. The cells were then treated with hydrogel extracts or control extracts for 24 h. Subsequently, 10 μL CCK-8 was added to each well and the cells were incubated for 1 h before detection with multi-function detection system (SynergyMx M5, Bio-Tek, USA). The absorption was measured at 450 nm and the absorption at 630 nm was set as the reference wave.

Apoptosis Measurement

Apoptosis of L929 cells induced by hydrogel extracts was analyzed using an Annexin V-FITC/PI apoptosis kit (AP101-100, Multi Sciences, China). Cells were seeded in 6-well plates at the density of 4×10^5 cells/well, incubated for 12 h and then treated with hydrogel extracts or control extracts for 24 h. Following exposure, both floating and attached cells were collected, washed twice with PBS, and resuspended in 1× binding buffer. Then, 500 μL of the cell suspension was incubated with 5 μL

Annexin V-FITC and 10 μL propidiumiodide (PI) for 10 min at room temperature in dark. The level of apoptosis was determined by flow cytometry (BD Fortessa, BD Biosciences, USA).

Cell Cycle Analysis

Cell cycle assay of L929 cells was analyzed using a cell cycle staining kit (CCS012, Multi Sciences, China). In brief, Cells were seeded in 6-well plates at the density of 4×10^5 cells/well, incubated for 12 h and then treated with hydrogel extracts or control extracts for 24 h. Following exposure, both floating and attached cells were collected, washed twice with PBS, and resuspended in 1 mL PBS. Then, the cells were slowly added to 3 mL anhydrous ethanol (-20°C), stirred at a high speed and fixed overnight. The cells were washed with PBS and subsequently stained with 500 μL propidiumiodide (PI) at room temperature for 30 min. Cell cycle distribution was determined by flow cytometry (ACEA NovoCyteTM, ACEA Biosciences, USA). A total of 1×10^4 cells were analysed for each sample.

Statistical analysis.

All data were shown as Mean ± SD and each experiment was repeated at least three times. Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test for comparison between groups, with $P < 0.05$ considered to be statistically significant. Prism 6 software was used for all statistical analyses.

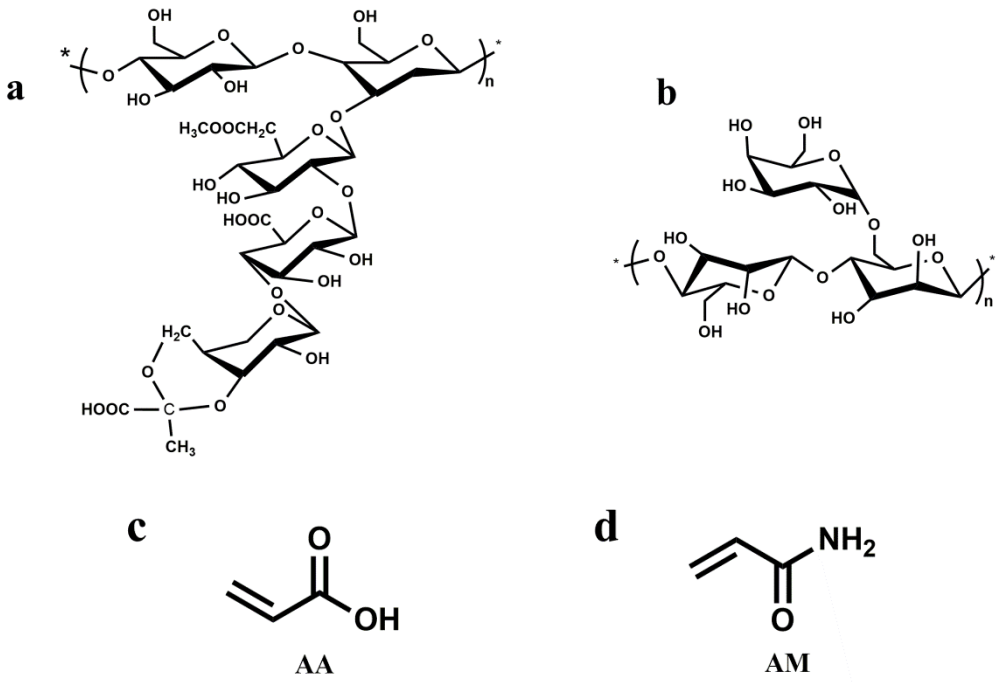

Figure S1. The chemical structures of (a) XG, (b) GG, (c) AA and (d) AM.

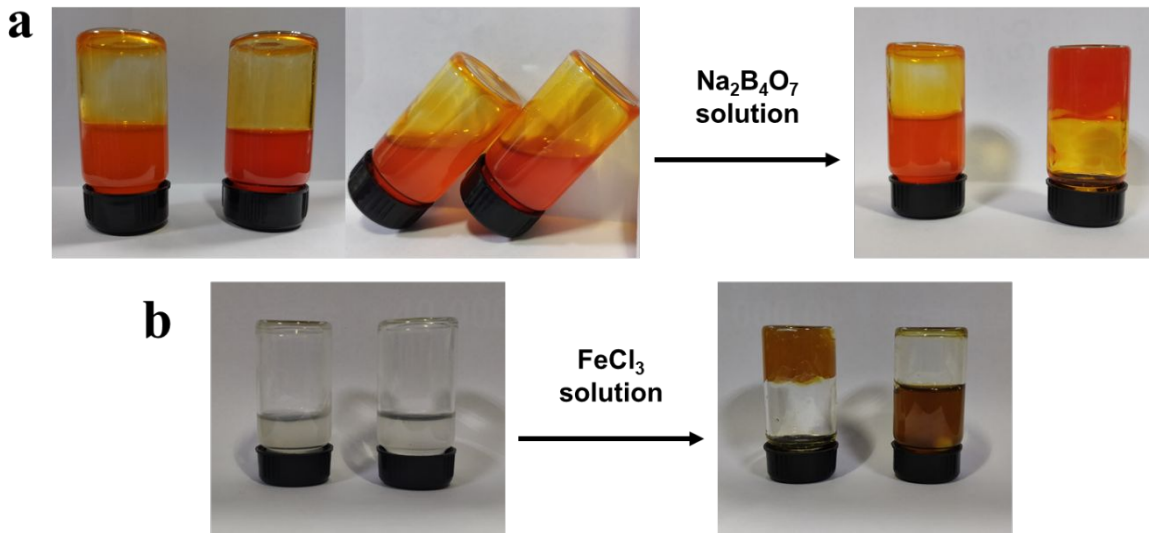

Figure S2. (a) Gelation behavior of XG (left) and GG (left) aqueous solutions with concentration of 1 wt% dyed with methyl orange after adding 100mg/L $Na_2B_4O_7$ aqueous solutions. (b) Gelation behavior of XG (left) and GG (left) aqueous solutions with concentration of 1 wt% after adding 0.3M $FeCl_3$ aqueous solutions.

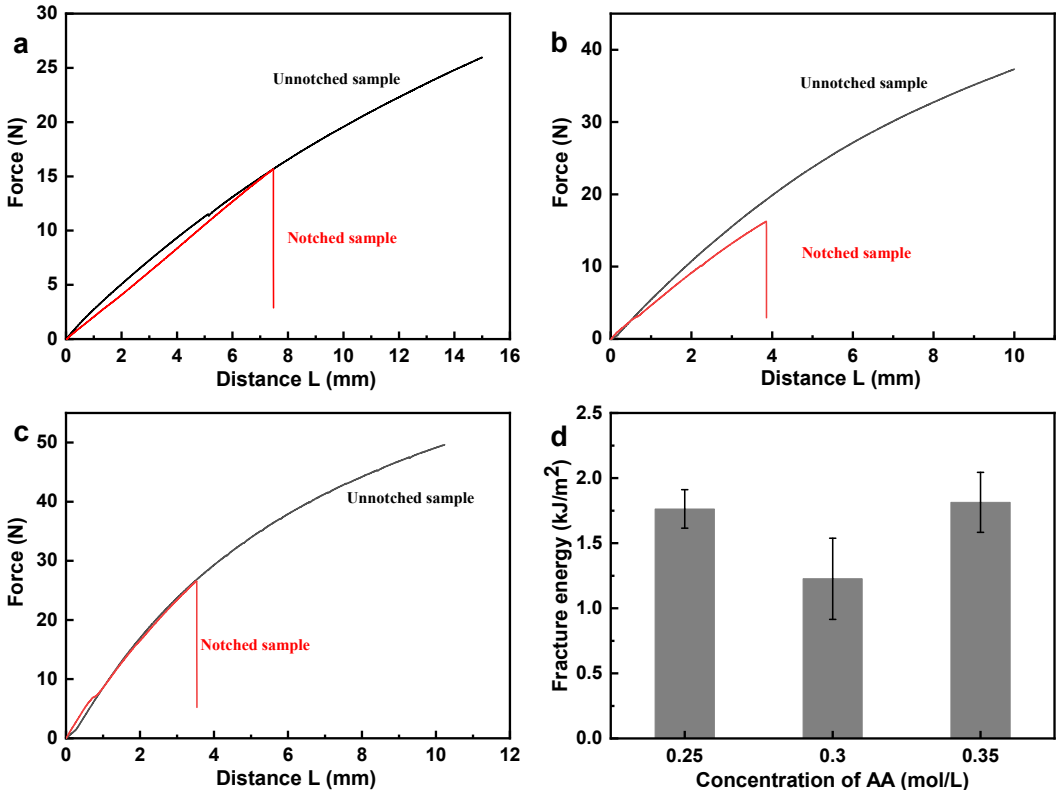

Figure S3. The pure shear curves of notched and unnotched samples of (a) P($AA_{0.25}$-*co*-$AM_{2.0}$)/XG_1-GG_1/Fe^{3+}/B hydrogel, (b) P($AA_{0.3}$-*co*-$AM_{2.0}$)/XG_1-GG_1/Fe^{3+}/B hydrogel and (c) P($AA_{0.35}$-*co*-$AM_{2.0}$)/XG_1-GG_1/Fe^{3+}/B hydrogel. (d) The corresponding toughness of the three hydrogels.

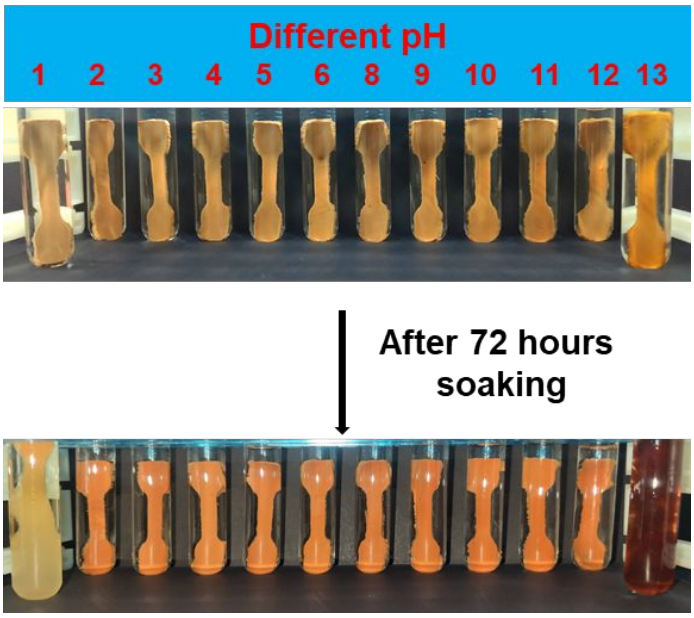

Figure S4. Pictures **of** dumbbell shaped P($AA_{0.35}$-*co*-$AM_{2.0}$)/$XG_{1.0}$-$GG_{1.0}$/Fe^{3+}/B hydrogels immersed into aqueous solutions with various pH values ranging from 1 to 13 for 72 h. Up: just immersing in the solution. Bottom: after immersing in the solution for 72 h.