Supplementary Information

A self-healing hydrogel formation strategy via exploiting endothermic interactions between polysaccharides

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Experimental Methods

1 Materials

Alginate (ALG, 100 mPa s^{-1}, G/M ratio = 34/66, Qingdao Mingyue Bio-tech Co., Ltd), Chitosan (CHI, Mw = 45 000, deacetylation degree ≥ 95%, Aladdin Industrial Inc.), 2-hydroxypropyltrimethyl ammonium chloride chitosan (HACC, Mw = 78 000, substitution degree = 85%, Zhejiang Aoxing Bio-tech Co., Ltd) and Carboxymethyl chitosan (CMC, 22 mPa s^{-1} , substitution degree = 96.5%, Qingdao Honghai Bio-tech Co., Ltd) were used after dissolution and filtration. All other chemicals were of analytical grade and commercially available.

2 Methods

2.1 Preparation of Hydrogel

The solutions of ALG (2 wt%) or CMC (2 wt%) were mixed with solution containing CHI (4 wt%)or HACC (4 wt%) at molar ratios of charge neutrality, respectively. The mixture was mixed uniformly by vortex and eventually homogeneous hydrogels were obtained after precipitation 12h (or centrifugation).

2.2 Macroscopic Self-Healing Experiments

Two pieces of hydrogel disks (15 mm in diameter) stained by fuchsin basic and methylene blue, were cut into equal 4 pieces, respectively. Then the total 8 pieces of alternate colors were alternate combined into two blended integral hydrogel disks, and were kept at 25 °C.

2.3 Rheological Measurements

(1) The storage moduli (G') of hydrogel disks (30 mm in diameter) were tested by rheometer fitted with parallel dentate antiskid plates. Under a fixed strain level, 1.0%, the angular frequency was swept from 0.01 rad s^{-1} to 100 rad s^{-1}. (2) The hydrogel disk (30 mm in diameter) was measured under strain amplitude sweep (10%–1500%) at a fixed angular frequency (10 rad s^{-1}). (3) The alternate step strain sweep of hydrogel disk (30 mm in diameter) was measured at a fixed angular frequency (10 rad s^{-1}). Amplitude oscillatory strains were switched from small strain with 100 s for every strain interval. (4) Similar experiments were carried out for a fixed large strain to subsequent small strain with loading

period changed from 100 to 300 s for each strain level.

2.4 Isothermal Titration Calorimetry.

The interactions between ALG and HACC was probed by isothermal titration calorimetry (ITC), using a MicroCal ITC-200 instrument (Malvern Instruments Ltd.). In each measurement, thirty-eight 1 μL injections of 0.5 wt % HACC (16.8 mM monomer units) were added to a sample cell filled with 0.05 wt % ALG (2.5 mM monomer units) solution. The calorimeter measured the heat supplied to the sample cell to keep the cell at a constant temperature. The binding heat was then obtained from this thermogram by subtracting the heat of dilution, which was negligibly small. In the case of ALG-CHI binding, thirty-eight 1 μL injections of 0.5 wt % CHI (29.5 mM monomer units) were added to a sample cell filled with 0.07 wt % ALG (5.1 mM monomer units) solution.

2.5 Characteristics of ALG-HACC hydrogels

ATR- FTIR Spectra:

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Equinox 55, Germany) was used to analyze the functional groups of ALG powder, HACC powder and dried ALG-HACC gel.

SEM:

The sample was sputtered to form conductive coating prior to SEM analysis. The dried conductive coating samples were scanned at high voltage of 20 kV by SEM (JSM-6360LV, JEOL, Japan).

Swelling tests:

The dried ALG-HACC hydrogels were immersed in solutions containing different concentration of NaCl. The weight of hydrogels were measured in 24h.

Cell compatibility:

Mouse fibroblast cells L929 were purchased from the Riken Cell Bank. The cells were maintained at 37 ^{o}C in a humidified atmosphere of 5% CO_2 in RPMI-1640 medium supplemented with containing 10% fetal bovine serum (FBS), 1% antibiotics (penicillin/streptomycin). Cells were harvested with 0.25% trypsin solution (Invitrogen) and resuspended in the medium to achieve a final concentration of 6×10^4 cells mL-1. After loading the ALG-HACC hydrogel into a six-well plate, cells were seeded onto the corresponding hydrogel gently and incubated for another 24 h (2×10^4 cells cm^2)**.** Qualitative analysis of live and dead cells was determined by live/dead viability assay according to the specification provided by the manufacturer. Hydrogels and cells were incubated with dual-color dyes (2 μm ED-1 and 1μm calcein AM, Sigma, USA) at 37^{o}C for 1 h. Then, the samples were scanned with Confocal laser scanning microscopy (CLSM, Leica TCS-SP2, Germany). CLSM was equipped with an inverted microscope (Leica, DMIRE2, Germany) and laser sources blue (Ar 488 nm/5 mW).

Supplementary results

1. The adhensive characters of ALG-HACC hydrogels

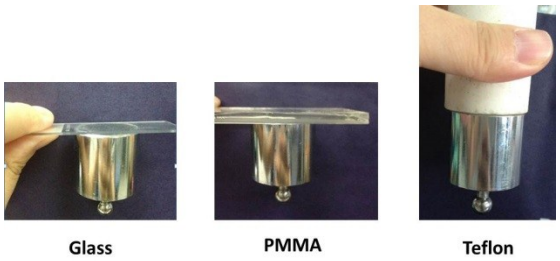

Fig 1. Demonstration of adhensive characters on the glass, PMMA and Teflon by putting wet hydrogels between the substance and weight (200g).

2. Swelling tests

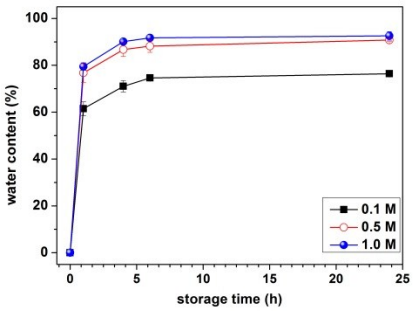

Fig 2. The water content of ALG-HACC hydrogels in different concentration of NaCl solutions.

3. SEM

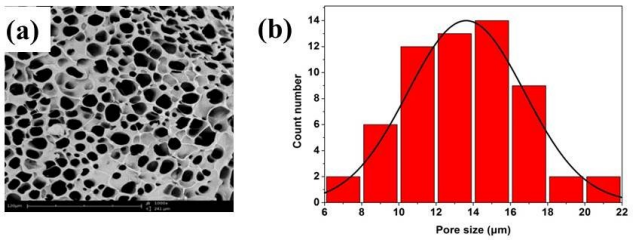

Fig 3. a) SEM images of interior microstructures in ALG-HACC hydrogels. The scale bar provided is 120 μm. b) The distribution of pore size in hydrogels.

4. FTIR

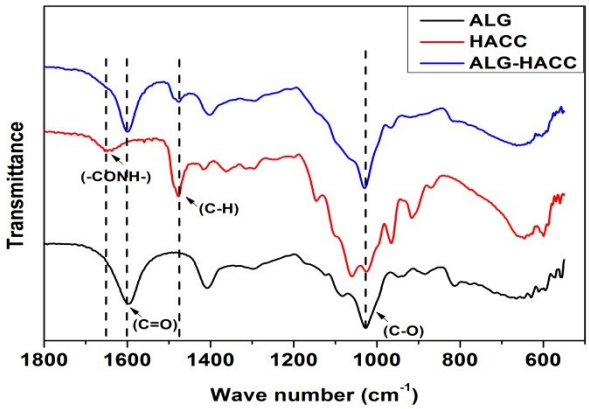

Fig 4. ATR-FTIR spectra of the ALG, HACC and ALG-HACC hydrogel.

5. ITC

Table 1. Enthalpy of Complexation ΔH of different polyelectrolyte complexes

Sample	ΔH (kJ/mol)	Molar ratio, N
ALG-CHI	-35.3	0.6
ALG-HACC	38.5	1.0
ALG-HACC(Ac)	-176.4	0.7
ALG-HACC(HAc)	-229.3	1.0
CMC-HACC	32.6	0.8

Table 2. Enthalpy of Complexation ΔH of ALG and HACC in 0, 30 and 150 mM NaCl solution

NaCl (mM)	ΔH (kJ/mol)	Molar ratio, N
0	38.5	1.0
30	30.1	0.9
150	14.5	0.8