Supporting Information

From kPa to MPa: An environmental-friendly way to prepare a polysaccharide hydrogel with tunable mechanical properties

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Experimental Section

1.1 Materials

Gellan gum (GG) was purchased from Xieli Biological Chemical Reagent Co. Ltd. (Sichuan, China). Calcium chloride and phosphate buffered saline (PBS, Gibco) were purchased from Baoxin Biotechnology Co. Ltd. (Chengdu, China).

1.2 Fabrication of hydrogels

Briefly, GG (1 g or 2 g) was dissolved in deionized water (100 mL, 1% w/v, 2% w/v) at 90 ^{o}C for 30 min, as described elsewhere [S1], under which complete and homogeneous dispersion of the material was obtained. Afterwards, each 15 mL polymer solution is divided and was progressively decreased to 60 ^{o}C. When it reached 60 ^{o}C, 1 mL $CaCl_2$ solution (0.4% w/v, 0.8% w/v) was added and kept stirring for another 20-30 min. Gellan gum cylindrical hydrogels was fabricated by casting the solution into a 24-well plate and allowing it to rest at room temperature for 2-5 min and form a solid gel. In addition, extra high concentration $CaCl_2$ solution was added on the top of the hydrogels overnight. The cylindrical hydrogel was then taken out from the well plate to do further tests.

1.3 Mechanical properties

To assess the effects of the concentrations of both polymers and Ca cations on the mechanical properties of the developed hydrogels, compression tests were performed on a HZ-1004B tester (Lixian instrument Co. Ltd, China). Freshly prepared GG hydrogels (14 mm in thickness, 9 mm in diameter, n = 3) were compressed in the direction normal to the circular face of the cylindrical samples at a rate of 0.2 mm per

minute until failure of the hydrogel. The Young's modulus was defined as the slope of the linear region of the stress- strain curve in the 30-40 % of the strain range (Group A,C,D and E). For group B, it is in the 5-15 % of the strain range since it cracks at about 37 % strain. Ultimate stress and ultimate strain values were taken as the point where failure of the hydrogel occurred.

1.4 Dynamic thermomechanical analysis

The mechanical performance concerning the viscoelastic properties of GG hydrogels was assessed by dynamic mechanical analysis (DMA). The viscoelastic measurements were performed using DMA with the compressive mode. DMA analysis was carried out in all GG hydrogels with a cylindrical shape of around 8 mm in diameter and 9 mm in thickness at 37 ℃. DMA spectra were acquired during a frequency scan between 0.1 and 10 Hz. The experiments were performed under constant strain amplitude. A small preload was applied to each sample to ensure that the entire surface was under compression. For all hydrogels being tested, the distance between plates was kept the same. DMA analysis was performed in triplicate (n = 3) for each condition.

1.5 Morphology characterization

Scanning electron microscopy (SEM , 2013A532 Quanta 250) was employed to examine the morphology of the GG hydrogels with an emphasis on the porous characteristics. Prior to the observation, the hydrogels were frozen in liquid nitrogen and immediately lyophilized for 48 h. Then the lyophilized hydrogels were cut to observe their cross-sections and sprayed with gold for 40 seconds before testing.[S2]

1.6 Translucency assessment

The hydrogels' optical translucency was qualitatively and quantitatively evaluated. The qualitative evaluation was performed optically and microscopically against white paper sheet with the text “SCU” to evaluate the light distortion occurred through the hydrogel. The quantification was determined by visible light spectrophotometry at 495 nm wavelength, and ultrapure water in 96-well plate was used as a control. [S3]Five measurements per condition were performed in triplicate (n = 3).

1.7 Swelling and degradation studies

Swelling and degradation studies were carried out in all GG hydrogels by immersing them in 5 mL of phosphate buffered saline solution (PBS, Gibco) with 0.025% w/v NaN3, followed by incubating at 37 ℃ under mild shaking up to 28 days. At day 1, 2, 4, 7, 21 and 28 days, the hydrogels (n = 3) were removed from the solutions and gel surfaces were quickly blotted on a filter paper and the swelling ratio and degradation of all GG hydrogels were calculated. Their wet weight was measured （Ws） and compared to the initial wet weight (Wi) and the weight of freeze-dried hydrogel (Wd) after swelling or degradation at each time point. The swelling ratio (%) and remaining weight (%) was defined according to the following two equations.

$$\text{Swelling ratio (\%)}=\frac{W_s-W_d}{W_d}\times 100$$

$$\text{Remaining weight (\%)}=\frac{W_s}{W_i}\times 100$$

2. The optical images of the mechanical tests

The initial height of the hydrogels is both around 10 mm. When applying a certain compressive forces, the height decreases. However, the group A, the softer one, is

broken and an obvious defect can be seen in Figure S1. a,b. On the contrary, hydrogels of group E shows an ability to resist the force and maintain its integrity.

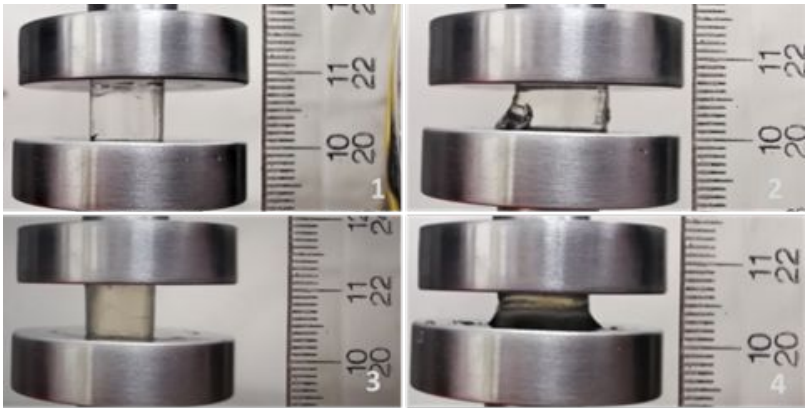

Figure S1. Optical images of the hydrogels of group A (a, b) and group E (c,d) before and after applying forces.

3. The water contents of the five groups.

The water content is calculated by the initial wet weight (Ws) and freeze-dried weight (Wd). [S4]

$$\text{Water content (\%)}=\frac{W_s - W_d}{W_d} \times 100$$

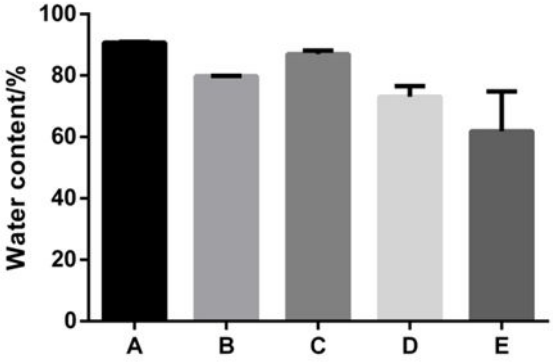

Figure S2. Water contents of the five groups.

4. The ultimate strains of the five groups.

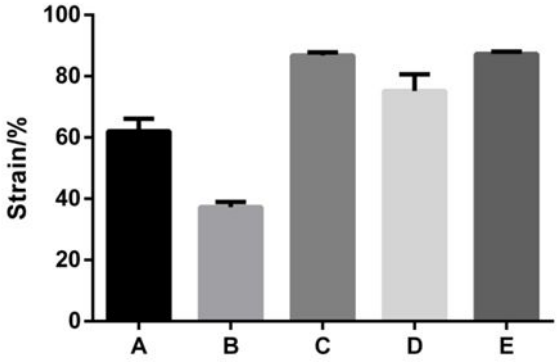

Figure S3. The ultimate strains of the five groups.

Reference

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(S2) Chunyan Cui, Tengling Wu, Fei Gao, Chuanchuan Fan, Ziyang Xu, Hongbo Wang,Bo Liu, and Wenguang Liu., An Autolytic High Strength Instant Adhesive Hydrogel for Emergency Self-Rescue. *Adv. Funct. Mater.* **2018**, 1804925

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