Synergistically complexation of phenol functionalized polymer induced in-situ microfiber formation for 3D printing of marine-based hydrogel

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Materials abbreviation list:

Ch-Ph: chitosan conjugated propionic acid (refer to the gel precursor)

Alg-Ty: Alginate conjugated tyramine (refer to the gel precursor)

PHEC: Phenolated polyelectrolyte complex (mixture of Ch-Ph and Alg-Ty, refer to gel precursor)

PEC: Polyelectrolyte complex

Ch: Chitosan hydrogel using Ch-Ph gel precursor

Alg: Alginate hydrogel using Alg-Ty gel precursor

DCCA: Double crosslinked chitosan-alginate hydrogel using PHEC gel precursor

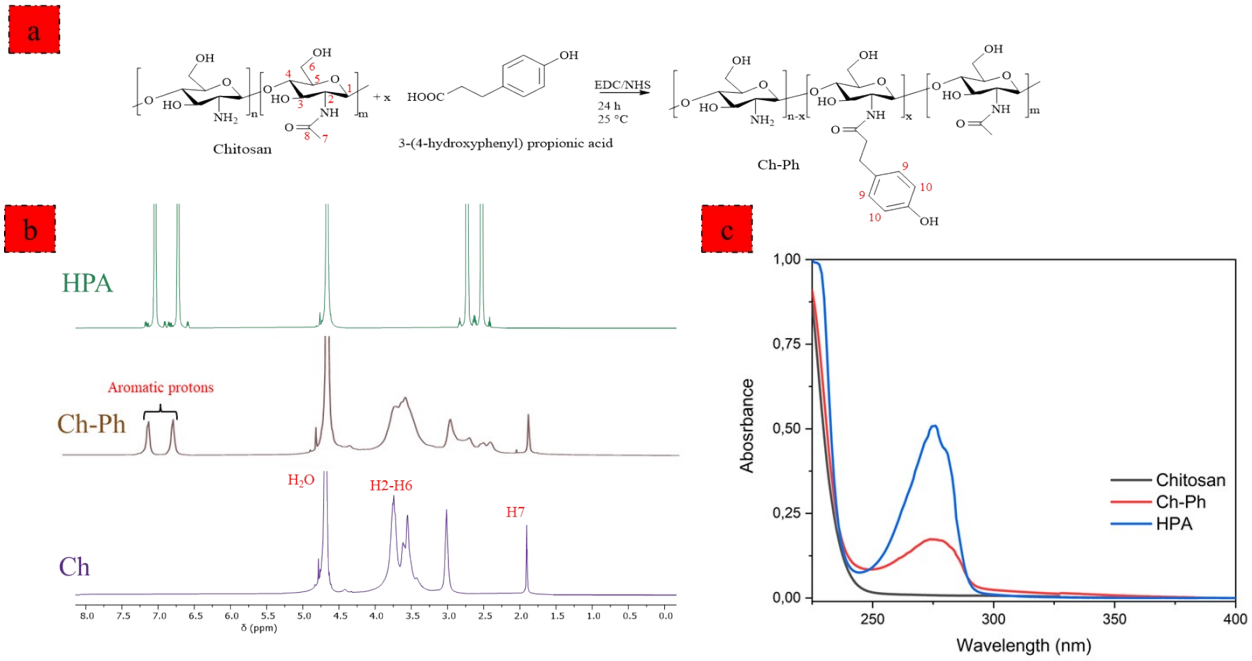

Figure S1. a) Synthetic mechanism of Ch-Ph preparation b) ^{1}H NMR in D_2O and c) UV-vis spectra of the Ch-Ph conjugate, chitosan and HPA,

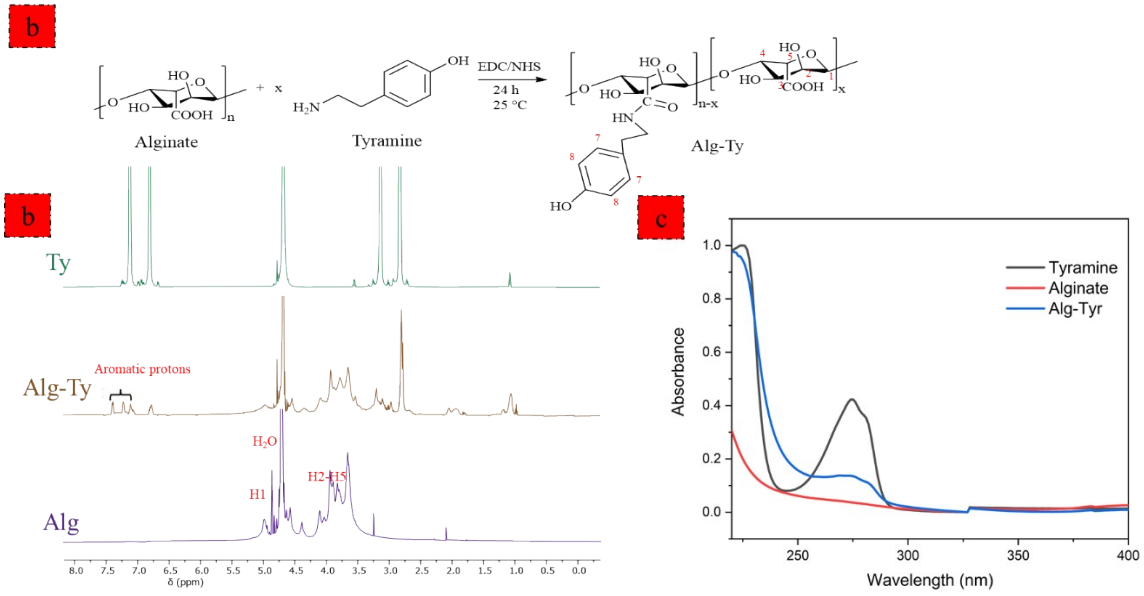

Figure S2. Synthetic scheme of the Alg-Ty, e) ^{1}H NMR in D_2O, and f) UV-Vis spectra of tyramine, Alg-Ty, and alginate.

Table S1. Gelation time of hydrogels based on chitosan, alginate, and their mixture.

Sample name	Concentration (wt%)		Gel time (sec)
	Chitosan-Phenol	Alginate-Tyramine	
$Ch_{0.5}$	0.5	0	52 ± 4
Ch_{1}	1	0	28 ± 3
$Ch_{1.5}$	1.5	0	14 ± 1
$Alg_{0.5}$	0	0.5	422 ± 9
Alg_{1}	0	1	354 ± 5
$Alg_{1.5}$	0	1.5	189 ± 7
$Ch_{0.5}$- $Alg_{0.5}$	0.5	0.5	68 ± 4
$Ch_{0.5}$- Alg_{1}	0.5	1	57 ± 5
$Ch_{0.5}$- $Alg_{1.5}$	0.5	1.5	47 ± 3
Ch_{1}- $Alg_{0.5}$	1	0.5	54 ± 5
Ch_{1}- Alg_{1}	1	1	42 ± 4
Ch_{1}- $Alg_{1.5}$	1	1.5	36 ± 3
$Ch_{1.5}$- $Alg_{0.5}$	1.5	0.5	34 ± 4
$Ch_{1.5}$- Alg_{1}	1.5	1	28 ± 4
$Ch_{1.5}$- $Alg_{1.5}$	1.5	1.5	23 ± 2

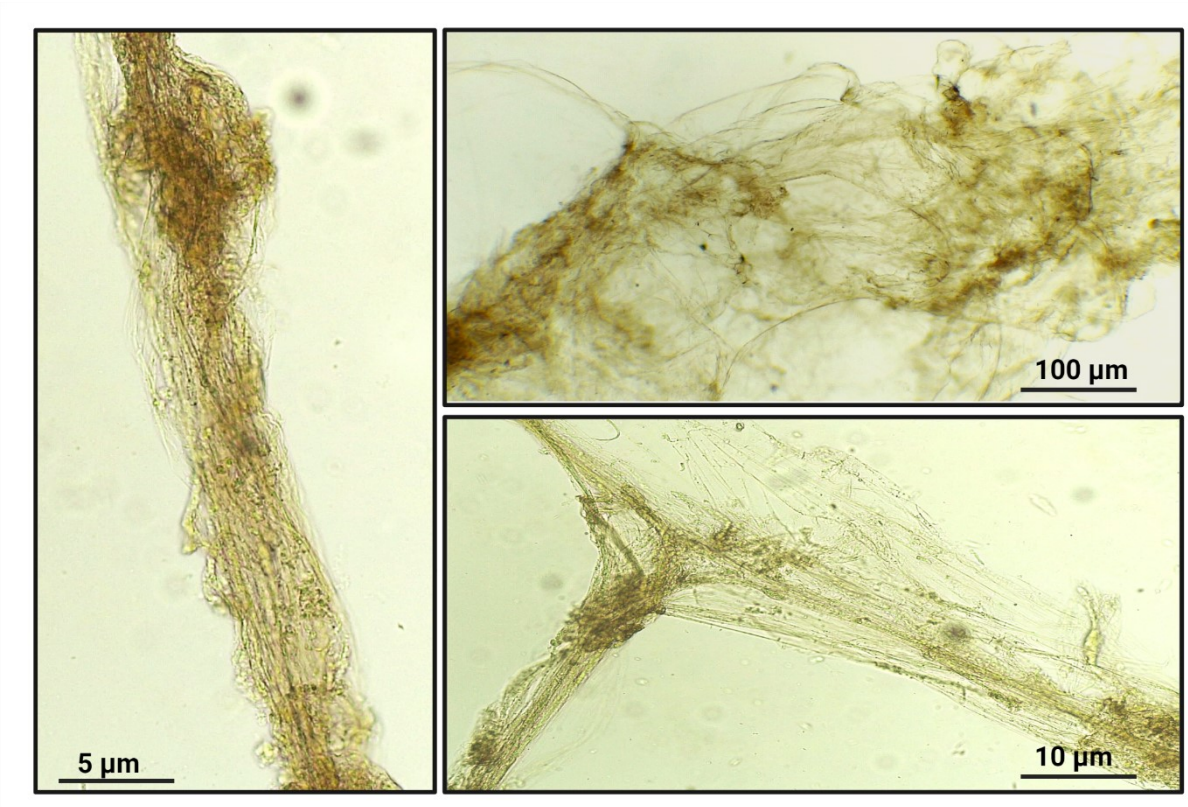

Figure S3. Optical microscopy images of in situ phenol functionalized microfibers

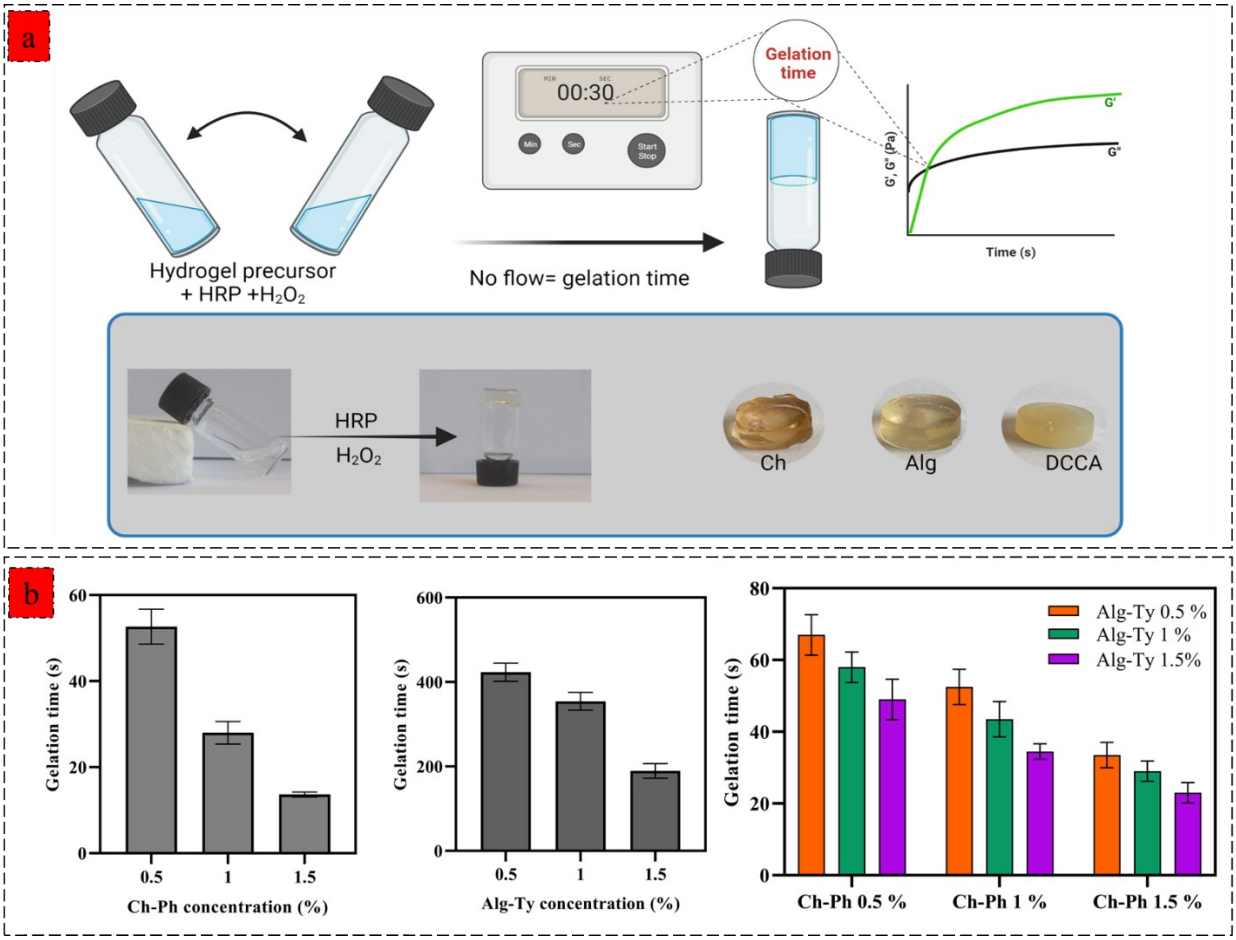

Figure S4. a) Schematic representation of the vial tilting method for gelation time record and photograph of Ch, Alg and DCCA hydrogels. b) Gelation time of Ch hydrogels at different concentrations (0.5, 1, 1.5 wt%) , d) Gelation time of Alg hydrogels at different concentrations (0.5, 1, 1.5 wt%). e) Gelation time of dual crosslinked (DCCA) hydrogels at different concentrations of Ch-Ph (0.5, 1, 1.5 wt%) and Alg-Ty (0.5, 1, 1.5 wt%). Results are expressed as gel time and are the mean ± SD of three independent experiments.

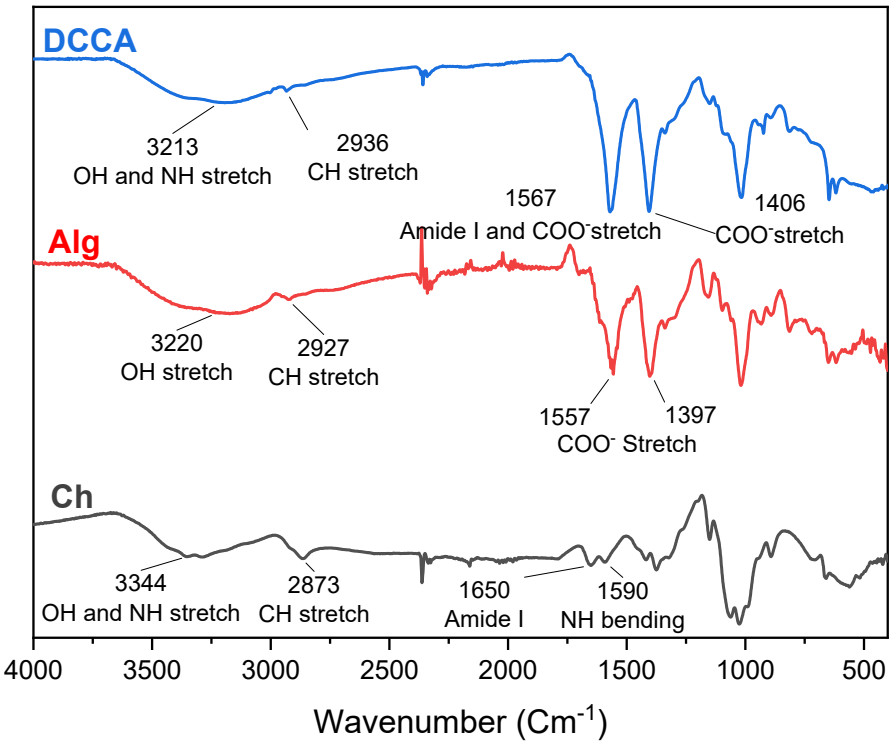

Figure S5. FT-IR spectra of Ch, Alg, and DCCA hydrogel.

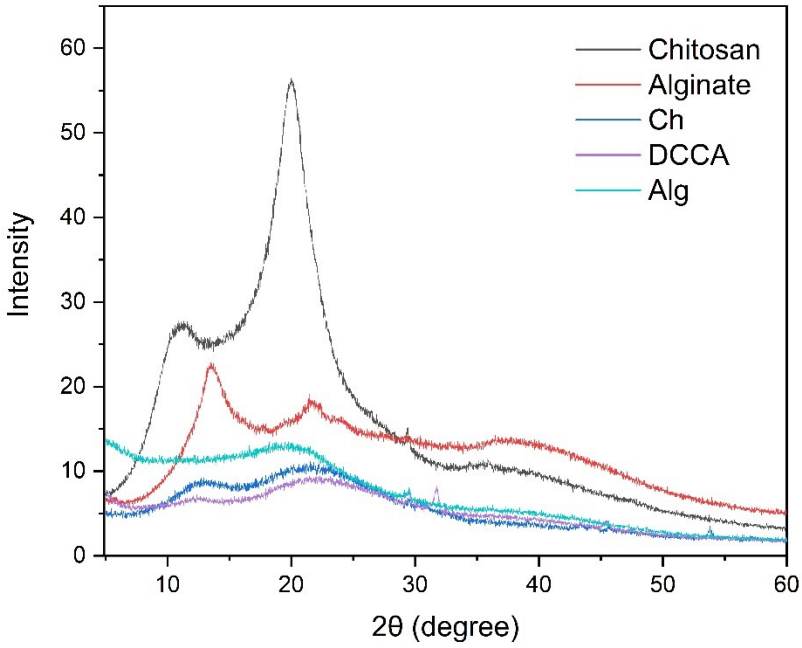

Figure S6. X-ray diffraction analysis of chitosan powder, alginate powder, freeze-dried Ch, Alg, and DCCA hydrogels.

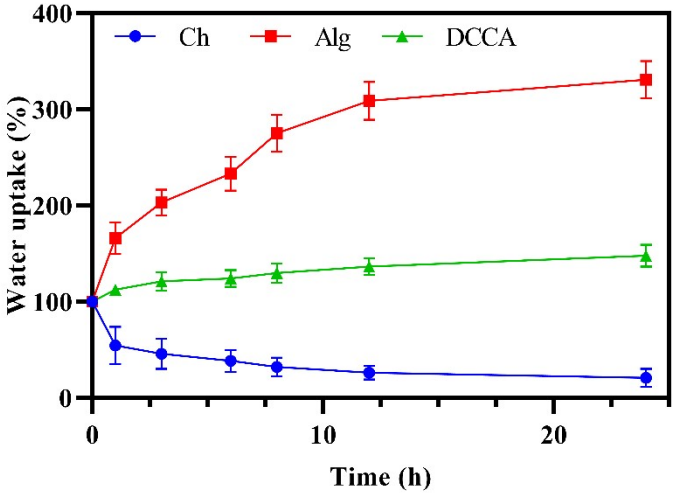

Figure S7. The swelling ratio of Ch, Alg and DCCA hydrogels over a 24h period in PBS at 37 °C

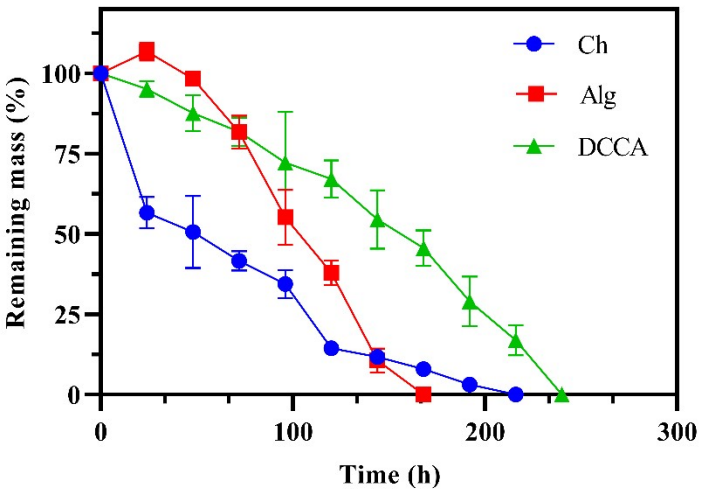

Figure S8. The degradation rate of Ch, Alg and DCCA hydrogels in lysozyme solution (1 mg/mL) at 37 °C.

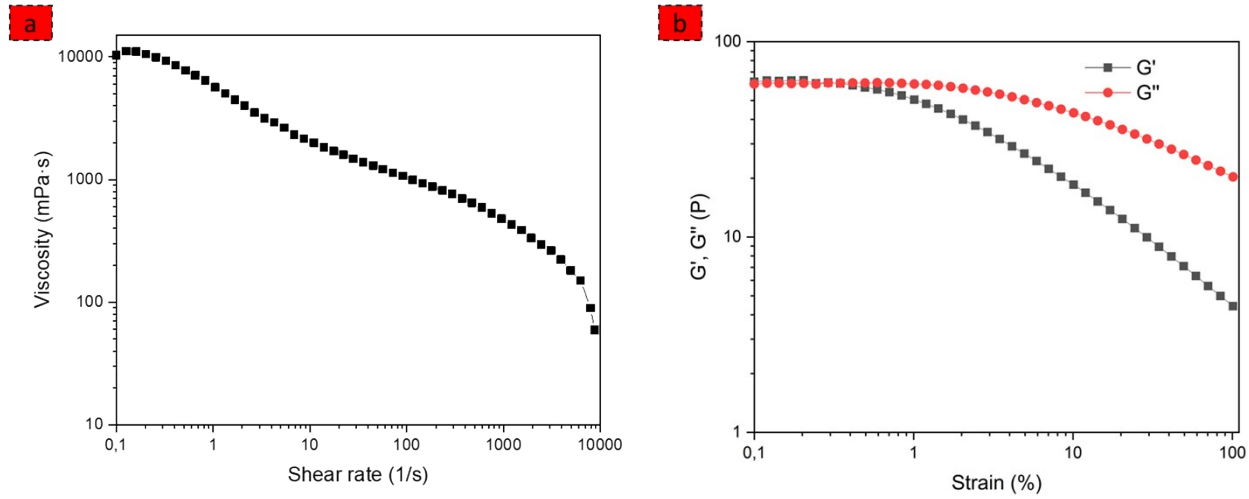

Figure S9. Dynamic viscosity of PHEC ink (2% polymer concentration) over the range of shear rates (0.1-1000 1/s at 37 °C). Storage modulus (G') and loss modulus (G")–strain dependence of Ch, Alg and DCCA ink (2% polymer concentration) at a constant frequency of 1 Hz at 37 °C.

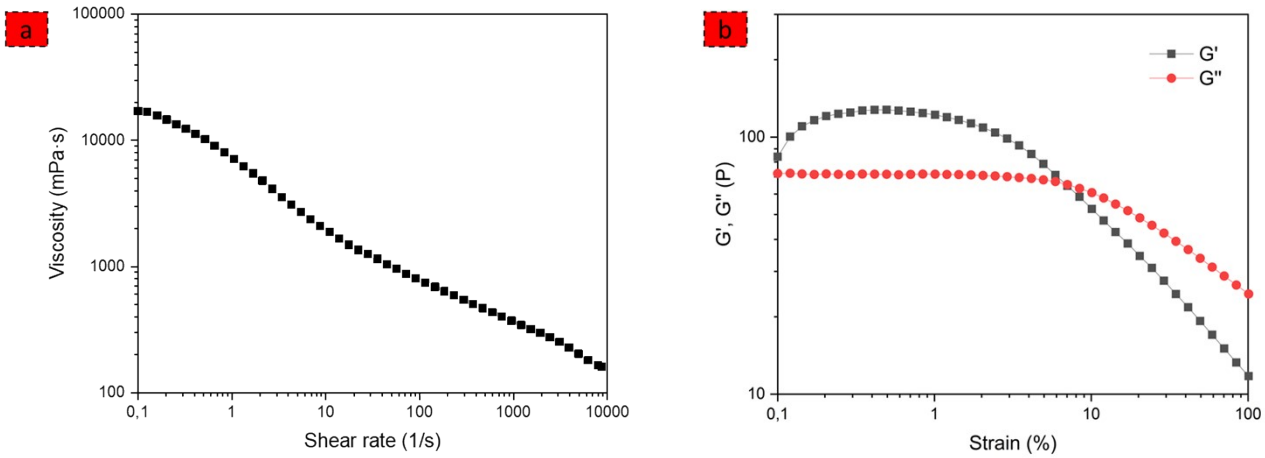

Figure S10. Dynamic viscosity of PHEC ink (2.5 % polymer concentration) over the range of shear rates (0.1-1000 1/s at 37 °C). Storage modulus (G') and loss modulus (G")–strain dependence of Ch, Alg and DCCA ink (2.5 % polymer concentration) at a constant frequency of 1 Hz at 37 °C.

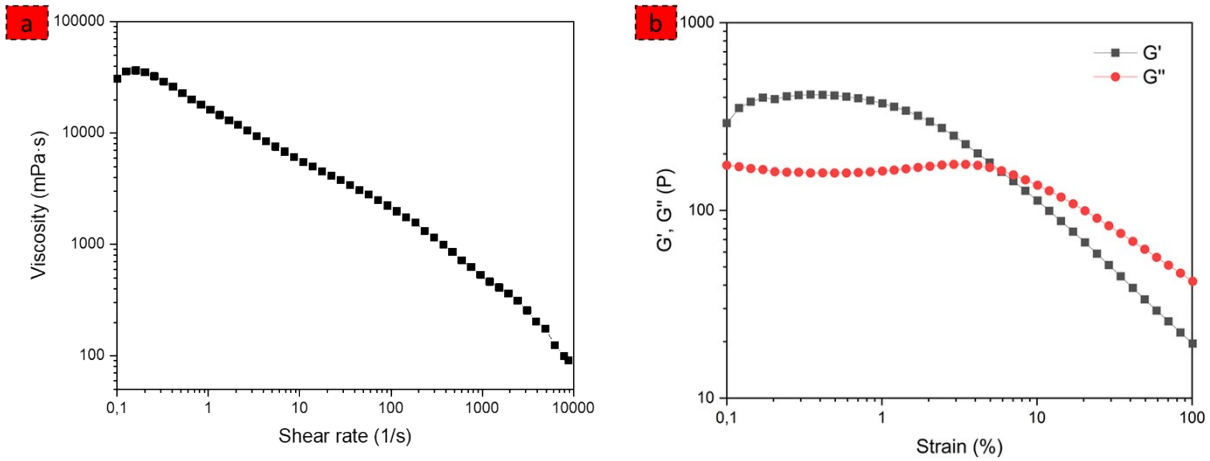

Figure S11. Dynamic viscosity of PHEC ink (3 % polymer concentration) over the range of shear rates (0.1-1000 1/s at 37 °C). Storage modulus (G') and loss modulus (G")–strain dependence of Ch, Alg and DCCA ink (3 % polymer concentration) at a constant frequency of 1 Hz at 37 °C.

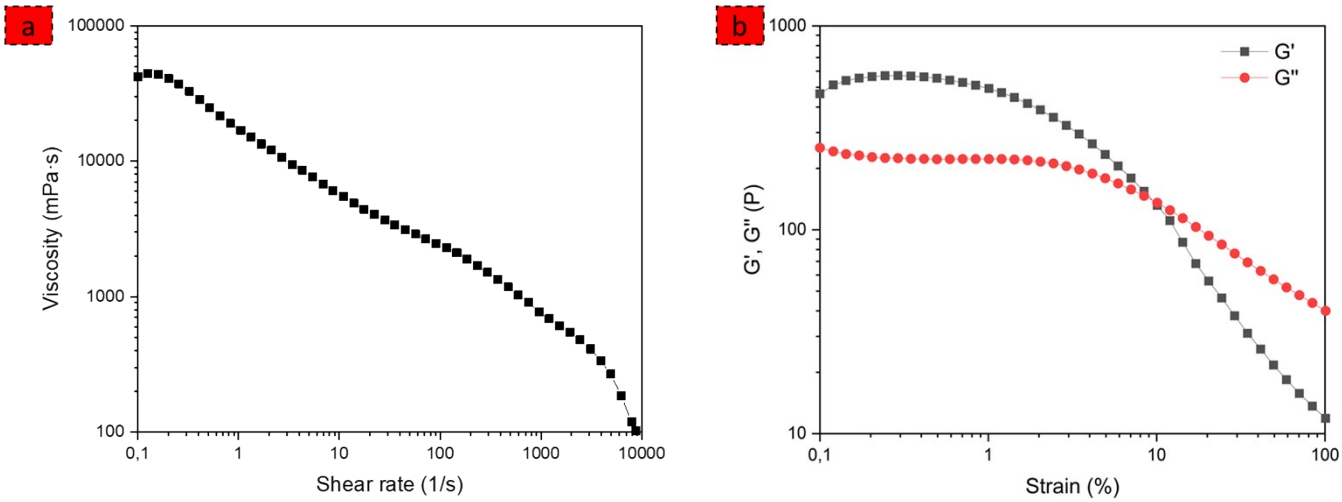

Figure S12. Dynamic viscosity of PHEC ink (3.5 % polymer concentration) over the range of shear rates (0.1-1000 1/s at 37 °C). Storage modulus (G') and loss modulus (G")–strain dependence of Ch, Alg and DCCA ink (3.5 % polymer concentration) at a constant frequency of 1 Hz at 37 °C.

Supplementary Notes 1: **Swelling ratio of hydrogels**

The swelling ratio of hydrogels was evaluated using the method previously described [1]. First, 400 µL of hydrogels was formed and incubated in a PBS solution (pH=7.4) at 37°C. The hydrogels were weighed over time. Briefly, the hydrogels were removed from the solution, and the excess water was removed with filter paper before weighting. The swelling ratio was then determined with the following equation:

$$\text{Swelling ratio } (\%) = \frac{Wt}{Wi}.100\% \quad (1)$$

where W_t and W_i are respectively the wet weight measured at each interval of time and the initial weight.

Supplementary Notes 2: **Degradation rate of hydrogels**

The hydrogel mass change in lysozyme solution was monitored as described in previous studies [2]. Briefly, 400 µL hydrogels were weighted (W_i) and then immersed in a PBS solution containing 1mg/mL of lysozyme (pH 7.4) and incubated at 37°C. The lysozyme solution was renewed every three days during the experiment. At a certain time-point, the hydrogels were removed from the solution and weighed (W_t). The remaining weight ratio is calculated using equation (2).

$$\text{Remaining weight } (\%) = \frac{Wt}{Wi}.100\% \quad (2)$$

Supplementary Notes 3: **Antioxidant activity**

DPPH[1] radical scavenging assay was performed to investigate the antioxidant activity of hydrogels according to a previously reported method [3]. 300µL of hydrogels were prepared in a 48-well plate. Then, 1 mL of ethanol containing 100µL of DDPH solution (0.5 mM) was added and incubated for 60 min in the dark. 300µL of deionized water (DIW) was used for the control group. The absorbance at 517 nm of the reaction mixture was measured, and the scavenging effect of hydrogels on DPPH radicals was calculated using equation (3).

$$Radical\ scavenging\ activity\ (\%) = [1 - (As/Ac)] \times 100 \quad (3)$$

Where A_s and A_C are the absorbances of samples, and the control (DIW), respectively.

Supplementary Notes 4: **Antibacterial activity**

The antibacterial activity of the hydrogels against gram-negative bacteria (*E.Coli*) and gram-positive bacteria (*S.aureus*) were investigated using the growth inhibition assay [4] and disk diffusion test [5]. Briefly, 100 µL/well hydrogels were formed in a 48 wells plate, and 1 mL of bacterial suspension (10^5 CFU/mL) inoculated into Muller–Hinton (M–H) was added to the wells and incubated for 24 h at 37 °C. 200 µL of bacterial suspensions were transferred to 96-well plates, and the absorbance was measured using a microplate reader (Epoch plate reader, BioTek®, USA).

For the disk diffusion test, 100µL of the bacterial suspension (10^6 CFU/mL) was uniformly spread on an agar plate. The hydrogels prepared in cylindrical shape were deposited on the top of the agar plate and incubated for 24 h at 37 °C. The inhibition zone around each sample was recorded as the antibacterial effect of the hydrogels

Supplementary Notes 5: **3D cell encapsulation**

For 3D cell encapsulation, sterile gel precursors and a 3T3-L1 cell suspension were mixed with HRP to obtain a homogenous solution with a final cell density of 5×10^5 cells/mL. Then, 50 µL of cell encapsulated gel precursors containing HRP were mixed with 50 µL gel precursors containing H_2O_2

[1] 2,2-diphenyl-1-picryl-hydrazyl-hydrate

(1mM) in a Millicell EZ SLIDE 8-well glass (Merck, Kenilworth, NJ, USA) and the hydrogels were incubated at 37 °C for 30 min to form a stable hydrogel. The cell encapsulated hydrogels were cultured with 1 mL DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10 % fetal calf serum (FCS), 200U/mL penicillin and 200U/mL streptomycin. The hydrogels were incubated at 37 °C for 1 or 3 days, and cell viability at days 1, and 3 was determined using Hoechst/ethidium homodimer I (EH1) staining. Briefly, cell nuclei were stained using 10 μM Hoechst (H33342 Sigma, MA, USA) and EH1 (E1903 Sigma MA, USA) and incubated for 20 min. Then, the cells were washed two times with PBS. The cell nuclei distribution and morphology were analyzed using a fluorescent microscope (Zoe fluorescent cell imager, Biorad, Hercules, CA).

References

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