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An injectable, self-healing phenol-functionalized chitosan hydrogel with visible light-crosslinking possibility for 3D printing

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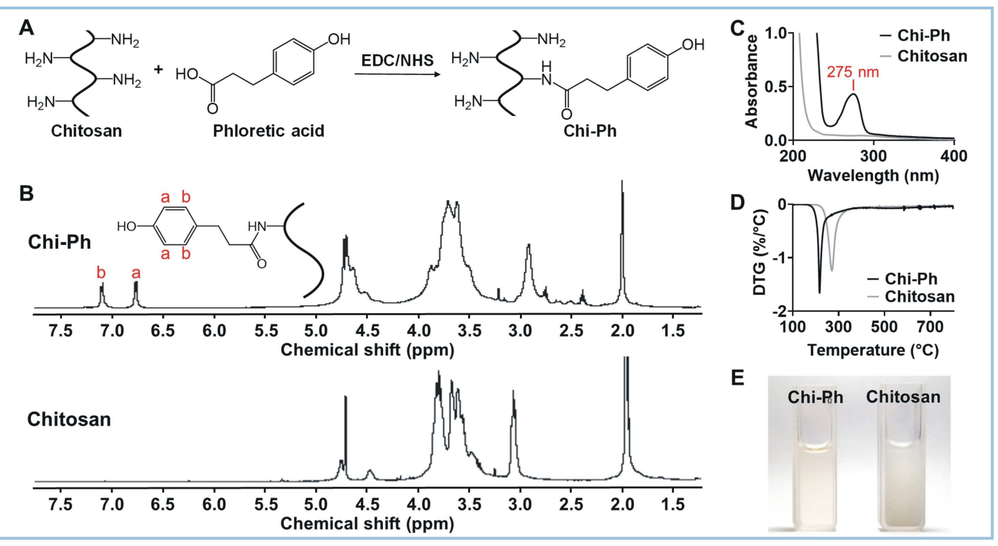
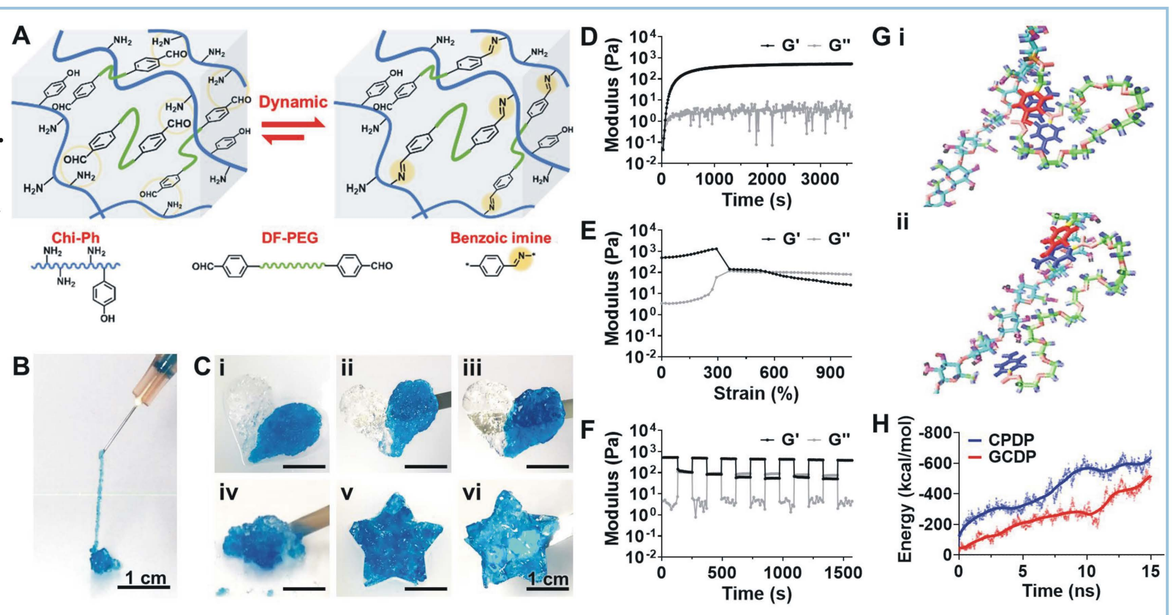
Introduction A chitosan-based self-healing hydrogel is developed for injectable hydrogel and printable ink using phenol-modified chitosan and dibenzaldehyde-terminated telechelic poly(ethylene glycol). Phenol functionalization of chitosan can introduce unique interaction that allows the hydrogel to possess fast gelling rate, good self-healing ability, and long-range critical gel behavior, as well as the possibility to be reinforced by secondary visible light-crosslinking. The cell-laden hydrogel is successful printed into a 3D construct. Moreover, the hydrogel is developed for modular 3D printing, i.e. hydrogel modules (LEGO-like building blocks) are individually printed and assembled into an integrated construct with secondary crosslinking. The versatile phenol-functionalized chitosan self-healing hydrogel will open up numerous potential applications, particularly in 3D bioprinting and modular 3D bioprinting.

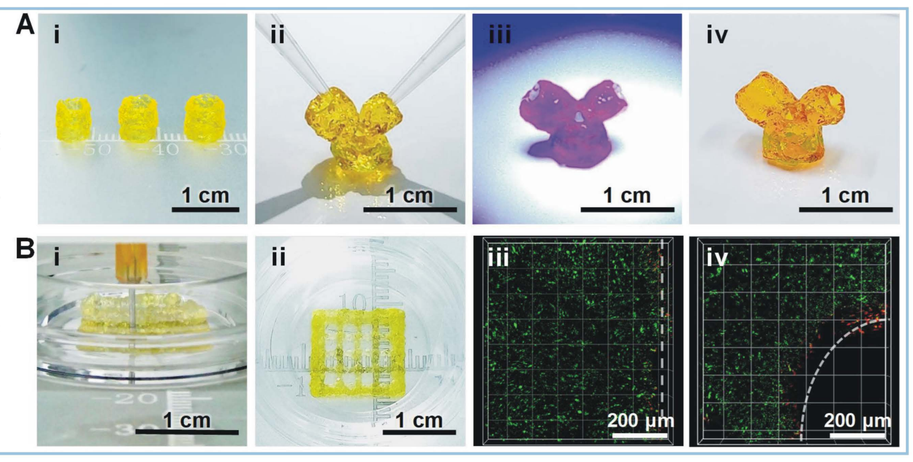
Synthesis of phenol-modified chitosan (A) Phenol-modified chitosan (Chi-Ph) was synethesized by conjugation of phloretic acid to chitosan using EDC/NHS chemistry. (B) ^{1}H NMR spectrum of Chi-Ph. The peaks at 6.8 and 7.1 ppm confirmed the successful conjugation of phenol groups to chitosan. (C) UV-Vis spectra for Chi-Ph solution (1 mg/mL in water) and for chitosan solution (1 mg/mL in 0.005 N HCl). A new peak at 275 nm supported the functionalization of chitosan by phenol groups. (D) DTG curves of Chi-Ph and chitosan. Chi-Ph was pyrolyzed at a lower temperature, suggesting that phenol groups decreased the inter- and intra-molecular interactions of the original chitosan chains. (E) Photograph of TBS buffer (pH 7.4) containing Chi-Ph or chitosan at 20 mg/mL. Absorbances for Chi-Ph and chitosan in TBS were 0.11 and 2.60 at 600 nm, respectively, indicating that Chi-Ph but not chitosan was soluble in aqueous medium at neutral pH.



Preparation of self-healing CPDP hydrogel (A) Scheme for preparation of CPDP hydrogel by dynamic benzoic imine crosslinking between Chi-Ph and DF-PEG. (B) Injection of CPDP hydrogel through a 26-gauge needle for filament formation. (C) Images of self-healing. (D) The storage modulus (G') and loss modulus (G'') of CPDP hydrogel against gelling time. (E) The strain-sweep experiment of CPDP hydrogel in the range of 1% to 1000% dynamic strain amplitudes. (F) Continuous rheological measurements for CPDP hydrogel damage and healing at alternate 1% and 500% dynamic strains. At 500% strain, the hydrogel was close to a critical gel (tanδ very close to 1). (G) Atomistic simulation of Chi-Ph and DF-PEG during the gelling process, revealing the formation of stacked geometry between a phenol group on Chi-Ph and (i) two benzaldehyde groups or (ii) one benzaldehyde group on DF-PEG. Phenol groups: red, benzaldehyde groups: blue. (H) The difference in the interaction energy between the CPDP hydrogel and the GCDP hydrogel system (from glycol chitosan and DF-PEG, control group).



3D printing of CPDP hydrogel (A) Assembling of modular 3D printed hydrogel constructs: (i) photo-crosslinkable CPDP hydrogel was printed into the tube-like components; (ii) three tube-like components were assembled as a Y-like construct, and (iii) subsequently irradiated with blue light for 60 s; and (iv) the Y-like construct maintained the shape. (B) 3D bioprinting of hMSC-laden photo-crosslinkable CPDP hydrogel. (i) The extrusion-based bioprinting onto a dish surface. (ii) The top view of the construct with lattice structure. (iii, iv) 3D confocal microscopic images showing the live/dead staining of hMSCs in the filament (iii) and intersecting filaments (iv) of the construct after 4 h. The approximate filament borders were marked by white dashed lines. Live cells: green, dead cells: red.



Conclusion A versatile CPDP hydrogel was developed as the injectable self-healing hydrogel and the visible light-crosslinkable bioink. Compared to the known chitosan-based self-healing hydrogels, the new CPDP hydrogel showed faster gelling rate, higher modulus, and long-term stability. The hydrogel behaved as a critical gel in a wide range of strains, which may account for the printability and stackability. Moreover, the individually printed constructs could be assembled into an integrated construct due to the adhesive and self-healing nature of the hydrogel. The CPDP hydrogel shows fast gelling, self-healing, and injectable properties that can find extra applications in 3D bioprinting and modular 3D bioprinting.