Supplementary Information

Pickering Emulgels Reinforced with Host-Guest Supramolecular Inclusion Complexes for High Fidelity Direct Ink Writing

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Experimental Methods

Materials: Microcrystalline cellulose (MCC) and polyethylene glycol (PEG) 35000 were received from Sigma Aldrich. Glycolic acid (98%) and ammonium formate (97%) were obtained from Alfa Aesar. α-cyclodextrin (α-CD) (≥98%) was obtained from Carl Roth. Sunflower oil was purchased from a local market. All chemicals were used as received without further purification.

Preparation of cellulose nanocrystals (CNCs): CNCs were extracted using deep eutectic solvent (DES) according to our previous work with a slight modification. The DES was synthesized by mixing ammonium formate with glycolic acid at a molar ratio of 2/1. The mixture was heated at 80 °C until a transparent solution was obtained. After that, 3 g of MCC was added into the as-prepared 30 g of DES. Then, the reaction was carried out in an autoclave at 140 °C for 1 h. After reaction, the solid residue was collected by centrifugation and purified by washing with deionized water until the pH of the suspension was kept constant. Thereafter, the obtained suspension was subjected to ultrasonication for 1 h by using a titanium tip sonicator (Branson Sonifier 450D) at 30% amplitude with alternating on/off cycles (2/1 s, respectively). The resulting suspension was then centrifuged at 5000 rpm for 15 min to remove the larger aggregate. After that, the collected supernatant CNCs suspension was purified by dialyzing against ultrapure water. Finally, the obtained CNCs suspension was stored at 4 °C for further use.

Preparation of Pickering emulsion stabilized solely CNCs and α-CD-based host-guest supermolecular hydrogel reinforced Pickering emulsion gels: Oil-in-water Pickering emulsions with different internal phase fractions were prepared by using CNCs as stabilizers. Briefly, a desired volume of sunflower oil (1.28, 2, 3 and 4.5 ml) was added into 1 ml of CNCs suspension (8 mg/ml). The mixture was then emulsified at 24000 rpm for 1 min using a high-speed homogenizer (IKA T25 basic). The resultant Pickering emulsion gels with the internal phase fraction of 56 vol%, 67 vol%, 75 vol% and 82 vol% were named as emugel-56, emulgel-67, emulgel-75 and emulgel-82, respectively. The inks for DIW were fabricated based on the as-prepared emugels. The obtained emulgel was firstly mixed with 1 ml of α-CD (0.386 g/ml) aqueous solution at 24000 rpm for 1 min followed by thorough mixing with 1 ml of PEG aqueous solution (0.532 g/ml) at 13500 rpm for another 1 min. The obtained emulgels with a continuous phase containing α-CD-based host-guest supermolecular hydrogel were named as HG-emulgel-X, where x represents the final internal phase fraction. In this work, the HG-emulgels derived from emugel-56, emulgel-67, emulgel-75 and emulgel-82 by homogenizing with 1 ml of α-CD and 1 ml of PEG aqueous solution have an internal phase fraction of 30%, 40%, 50% and 60%, respectively, which were therefore named as HG-emulgel-30, HG-emulgel-40, HG-emulgel-50, and HG-emulgel-60, respectively. The freshly prepared HG-emulgels were stored at 4 °C for further use.

Direct Ink Writing of Pickering emulgels: A BIOX Bioprinter (CELLINK, Sweden) equipped with a pneumatic printhead was used to 3D print the structures. The samples were transferred to a 3 ml clear pneumatic syringe and were extruded by sterile high-precision conical bioprinting nozzles 20-25 G. (diameter 250 μm, 410 μm, and 630 μm). Plastic Petri dish was utilized as solid support for 3D printed samples (60 mm and 100 mm diameter). The presented 3D models were designed by Open Source Thinkercad and the CELLINK 3D model archive.

Light microscopy: The emulsion droplets were observed using a digital microscope (Leica DVM6) at room temperature. The emulsion sample was placed onto the glass slide and then scratched into a thin layer in order to get a clear visualization.

Scanning electron microscopy (SEM): SEM images were recorded at an accelerating voltage of 3.00 kV on a Zeiss Gemini 1550 microscope. In order to prevent charging, the freeze-dried samples were sputter-coated with a thin layer of gold before observation.

Rheology: The rheological measurements were carried out at 20 °C using an MCR 301 rheometer (Anton Paar, Germany) with a parallel-plate geometry (PP25) at a fixed gap height of 1 mm. The steady shear flow measurements were performed in the shear rate ranging from 0.01-500 S^{-1}. The linear viscoelastic region (LVR) was determined by strain amplitude sweeps in the range of 0.01-1000% at a fixed frequency of 10 rad/s. After that, the frequency sweeps were performed at a fixed strain of 0.1% (within the LVR) for a frequency range of 0.01-100 rad/s.

Shrinkage: The extent of shrinkage in the samples was monitored by the geometrical changes after printing and freeze-drying conditions. The printed sample volumes before (V_w) and after (V_d) drying were measured, and the following equation was used to calculate the shrinkage.

$$\text{Shrinkage (\%)} = \frac{V_w - V_d}{V_w} \times 100 \tag{1}$$

Supplementary Figures

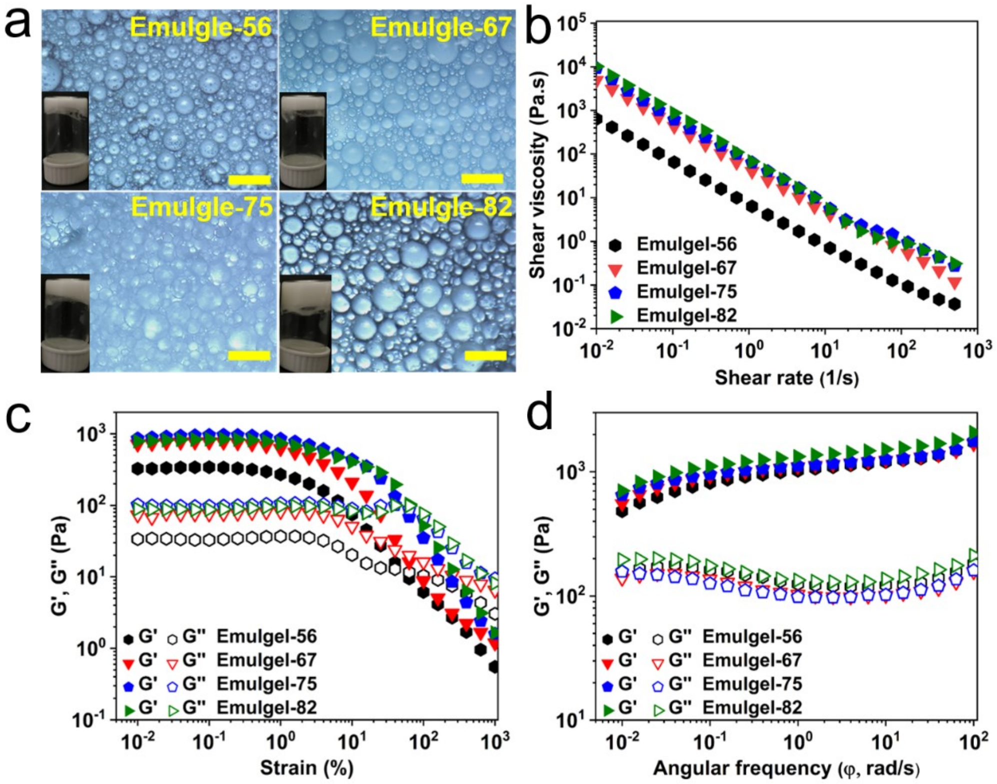

Fig. S1 a) Optical microscope images of emulgels stabilized solely by CNCs with different oil fractions. Inset: photograph of the corresponding emulgel. b) Steady-state flow curves of the emulgels. c) Strain amplitude sweep and d) frequency sweep curves for the emulgels stabilized solely by CNCs with different oil fractions.

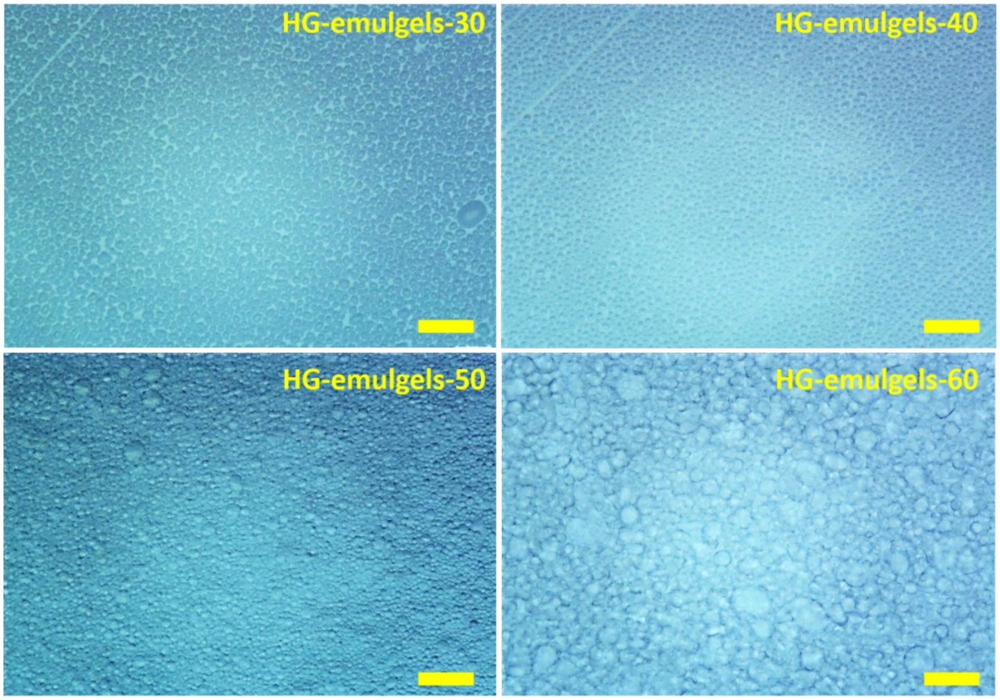

Fig. S2 Optical microscopy images of HG-emulgels-30, HG-emulgels-40, HG-emulgels-50 and HG-emulgels-60. Scale bar: 20 μm

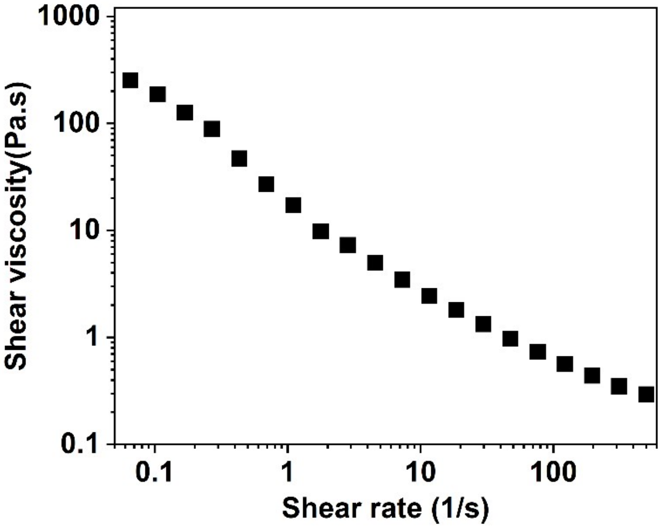

Fig. S3 Steady-state curves for supramolecular hydrogel synthesized with 1 ml of α-CD and 1 ml of PEG aqueous solution.

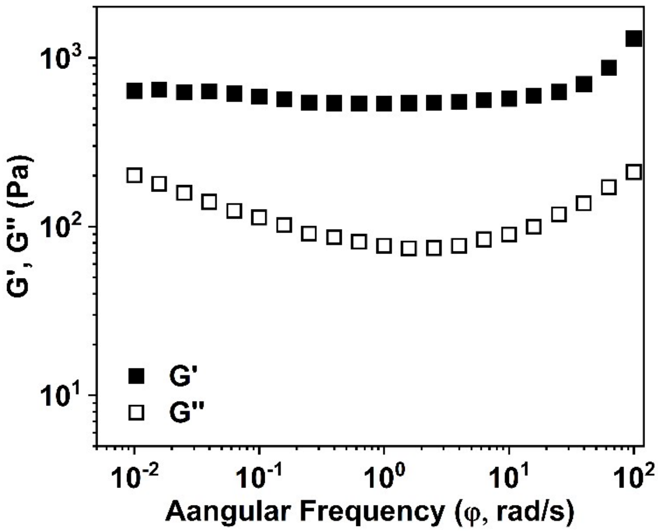

Fig. S4 Frequency sweep curves for supramolecular hydrogel synthesized with 1 ml of α-CD and 1 ml of PEG aqueous solution.

Table S1. The printing parameters including the nozzle diameter, print speed, print pressure, and layer thickness.

Sample	Nozzle diameter, µm	Print speed, mm/s	Print pressure, kPa	Layer thickness
HGE-30	250 (25 G)	-	-	Layer thickness is equal to the nozzle diameter, with the exception of the first layer to be 66% of the nozzle diameter.
	410 (22 G)	18	18-19	
	630 (20 G)	16-18	16	
HGE-40	250	18	-	
	410	18	15-18	
	630	16-18	15	
HGE-50	250	18	19 - 20	
	410	18	14 - 17	
	630	16-18	13 - 15	
HGE-60	250	-	-	
	410	18	20-23	
	630	16-18	18	

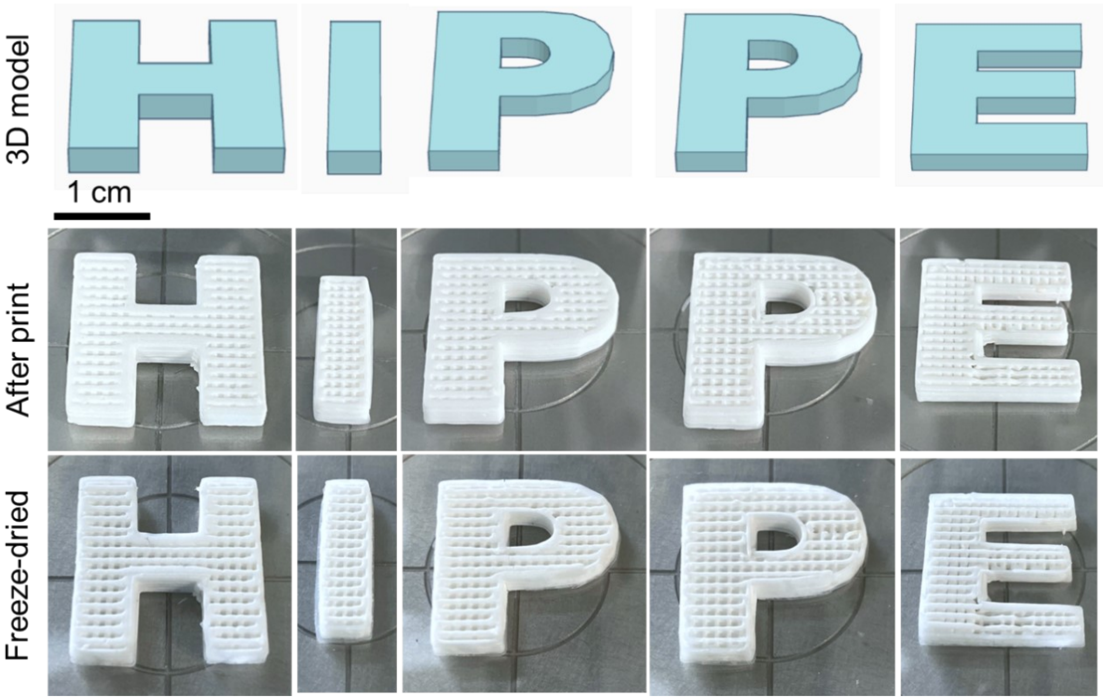

Fig. S5 3D printed letters demonstrating the print quality in wet condition and shrinkage of the structure after freeze-drying. The scale bar is 1 cm.

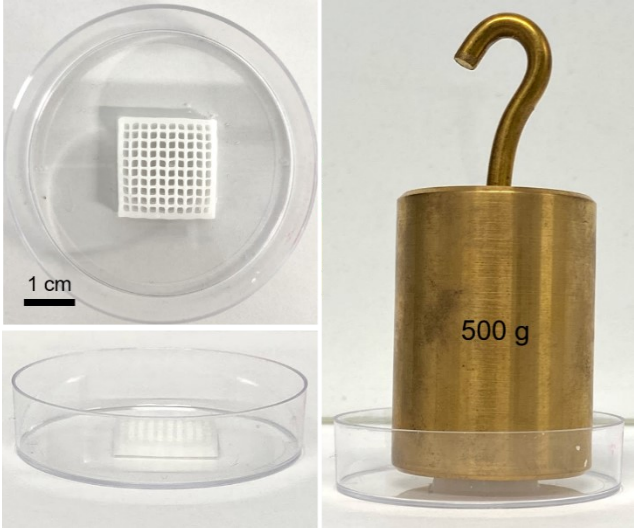

Fig. S6 Photograph of the dried cubic grid printed with HGEs-50 supporting a mass loading of 500 g.