Supplementary Information for

One-step Synthesis of composite hydrogel capsules to support liver organoids generation from hiPSCs

Yaqing Wang†, Haitao Liu †, Min Zhang, Hui Wang, Wenwen Chen, and Jianhua Qin*

*Corresponding author. Email: jhqin@dicp.ac.cn

†These authors contribute equally

Table S1 ART-FTIR peaks and their assignments for Na-alginate and chitosan.

Wavenumber (cm^{-1})	Na-alginate	Chitosan
3700-3000	O-H stretch	
3340		O-H and N-H stretch
3000-2850	C-H stretch	
2923		C-H stretch
1630		amide I
1610	Antisymmetric COO- stretch	
1590		N-H bending from amine and amide II
1420		$-CH_2$ bending
1415	Symmetric COO- stretch	
1385		$-CH_3$ symmetrical deformation

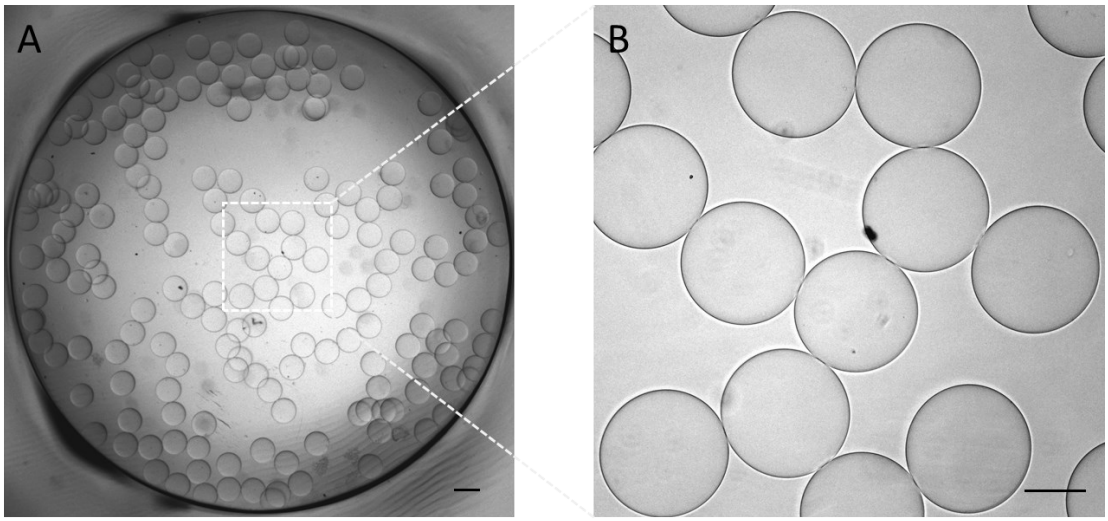

Fig. S1 High-throughput production of capsules in the oil-free droplet microfluidic system. (A) Massive capsules collected in 384-well plate. Scale bar: 200μm. (B) Enlarge image of (A). Scale bar: 100μm. These capsules are fabricated under such conditions: rates of core, middle and shell flow: 0.2, 2, and 4 μl/min; valve switch cycle: 0.4 s; concentrations of NaA /CS: 1% (w/w).

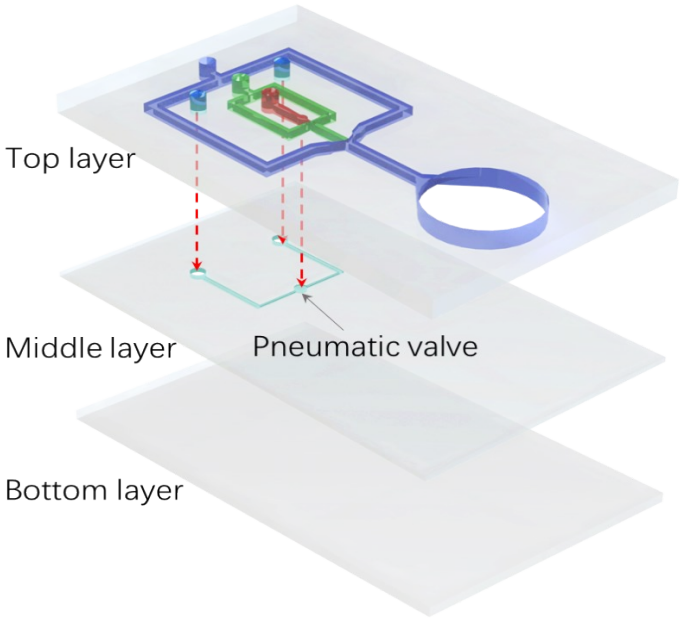

Fig. S2 The layered components of the microfluidic chip for HHCs generation. The top layer is used for manipulating the core, middle and shell flows to form droplet templates and capsules. The middle layer is used for controlling the compressed air to drive the pneumatic valve. The bottom layer is served as seals and upholder.

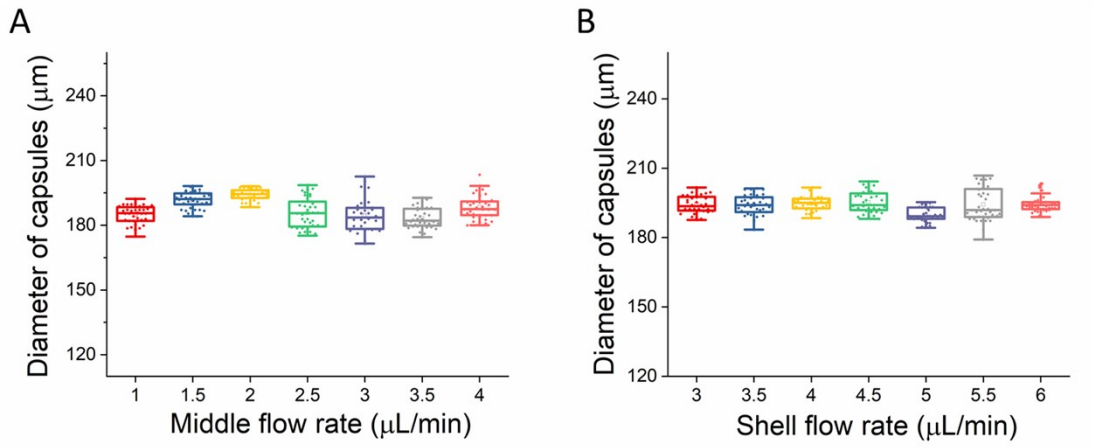

Fig. S3 Effects of middle and shell flow rates on diameter and polydispersity of the generated capsules. (A) The plot of capsule diameter under different middle flow rates (1 to 4 μl/min). (B) The plot of capsule diameter under different shell flow rates (3 to 6 μl/min). Quantitative analysis of the diameter of capsules were performed on at least 30 capsules.

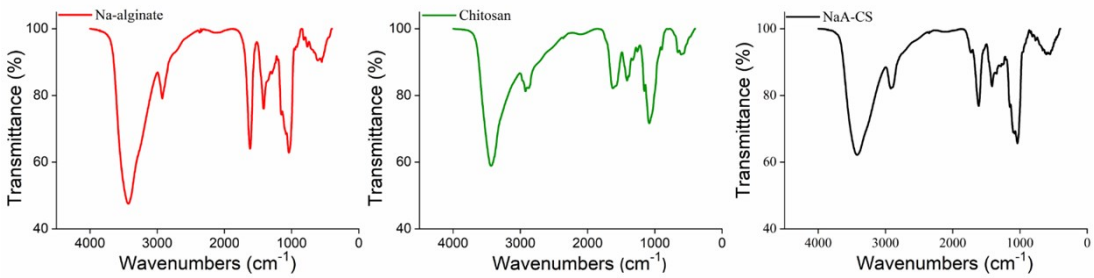

Fig. S4 ATR-FTIR spectra of Na-alginate, chitosan, and NaA-CS capsules. These capsules were produced without fibrinogen/thrombin.

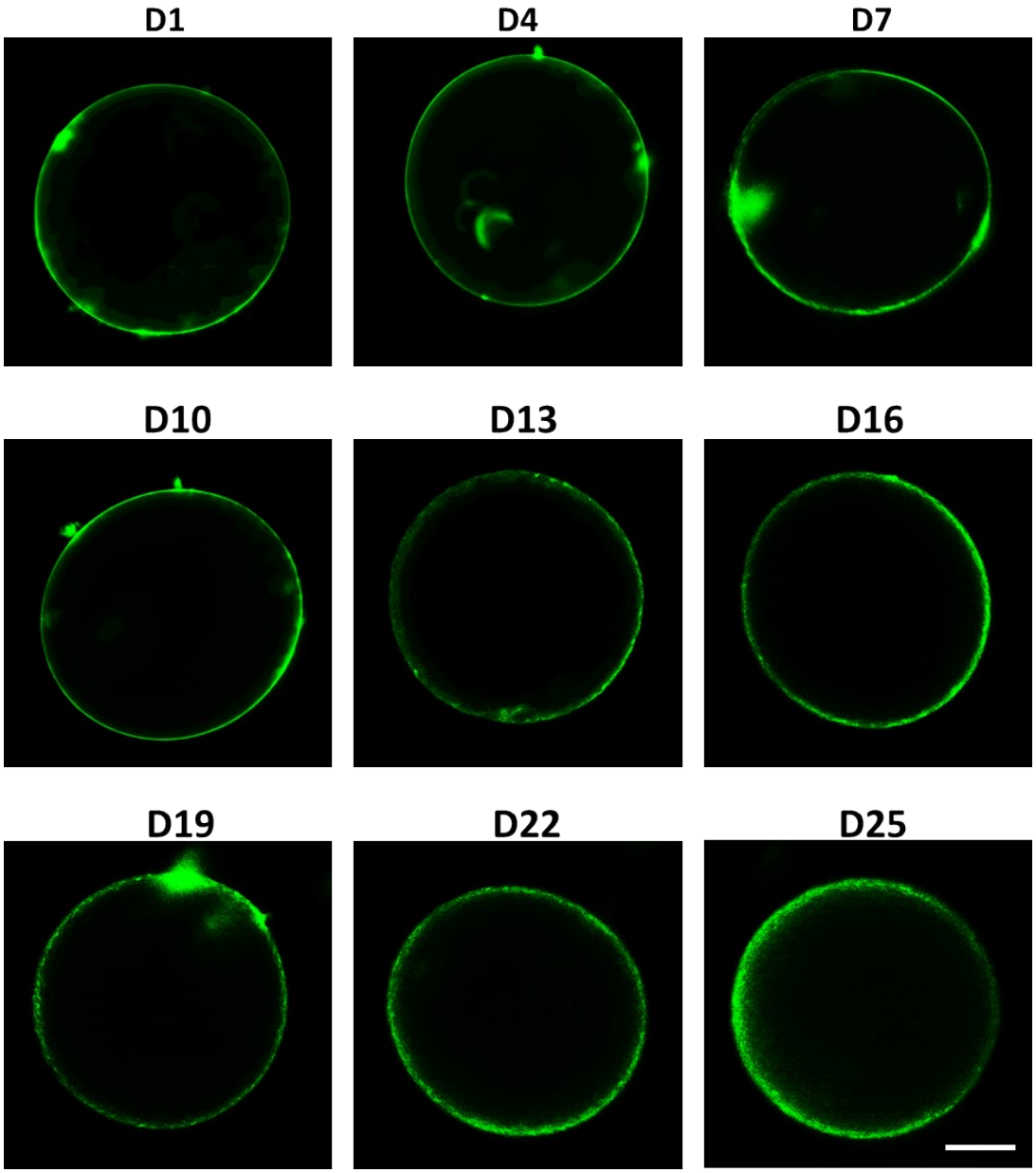

Fig. S5 The integrity of hybrid hydrogel capsules under cell culture conditions. These capsules are fabricated with FITC-chitosan, which are immersed in DMEM medium supplemented with 1% streptomycin and penicillin, 10% fetal bovine serum in a humidified atmosphere composed of 5% CO2 at 37 oC for 25 days. Scale bars: 50μm. These capsules are fabricated under the given conditions (rates of core, middle and shell flow: 0.15, 2, and 4 μl/min; valve switch cycle: 0.4 s; concentrations of NaA /FTIC-CS: 1% (w/w); concentration of fibrinogen: 5 mg/ml; concentration of thrombin: 2.5 U/ml).

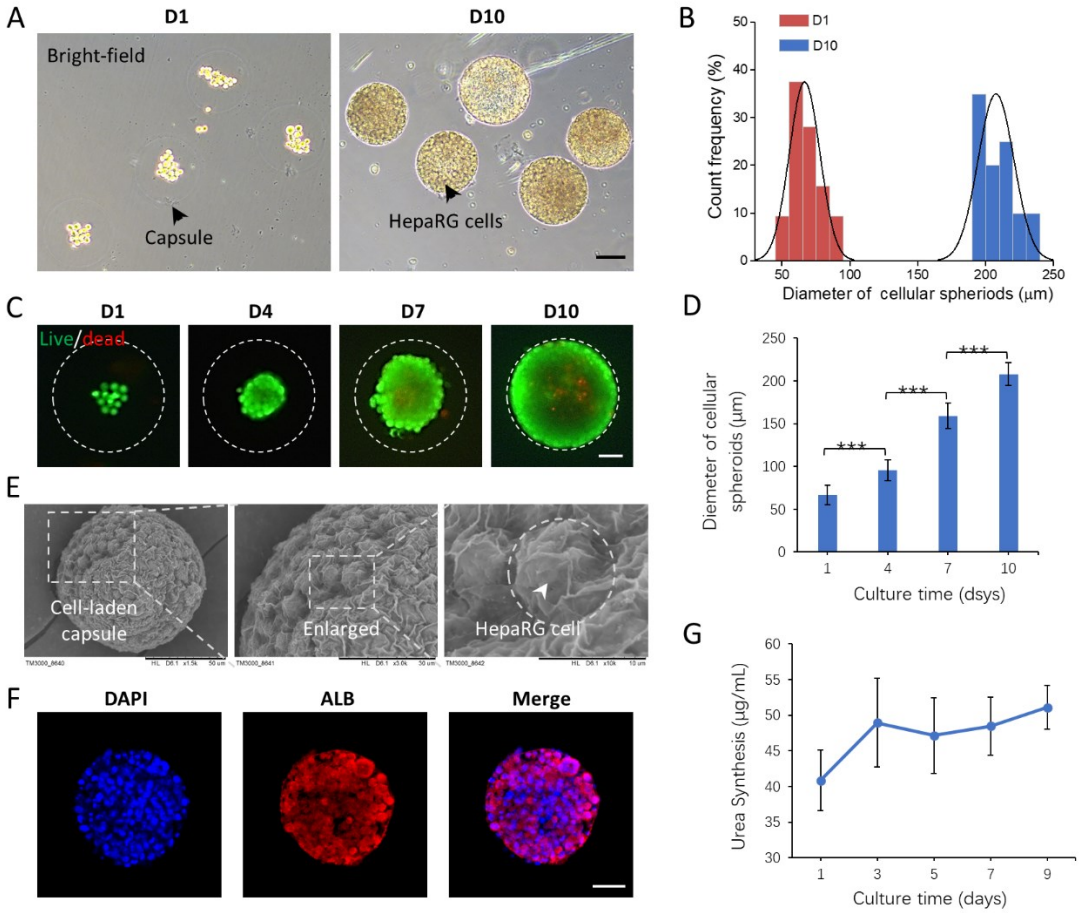

Fig. S6 Encapsulation and 3D culture of HepaRG cells in HHCs. (A) Bright-field images of cell-laden capsules after encapsulation of 1 and 10 days. Scale bar: 100 μm. (B) Size distribution was assessed by the diameter of cellular spheroids in capsules on days 1 and 10. (C) Cell viability of cellular spheroids in the capsules. The green and red fluorescence represent live and dead cells, respectively. Scale bar: 50 μm. (D) Diameter profiles of cellular spheroids in capsules on days 1, 4, 7 and 10. Student's t-tests were performed, ***$p < 0.001$. (E) SEM images of freeze-dried capsules. (F) Immunofluorescence staining for albumin in HepaRG cell spheroids in capsules at day 10. Blue fluorescence represents nucleus and red fluorescence represents secreted albumin. (G) Urea synthesis in cellular spheroids encapsulated in defined capsules on days 1, 3, 5, 7 and 9. Quantitative analysis of the diameter of cellular spheroids were performed on at least 20 spheroids. Data are shown as mean ± SD.

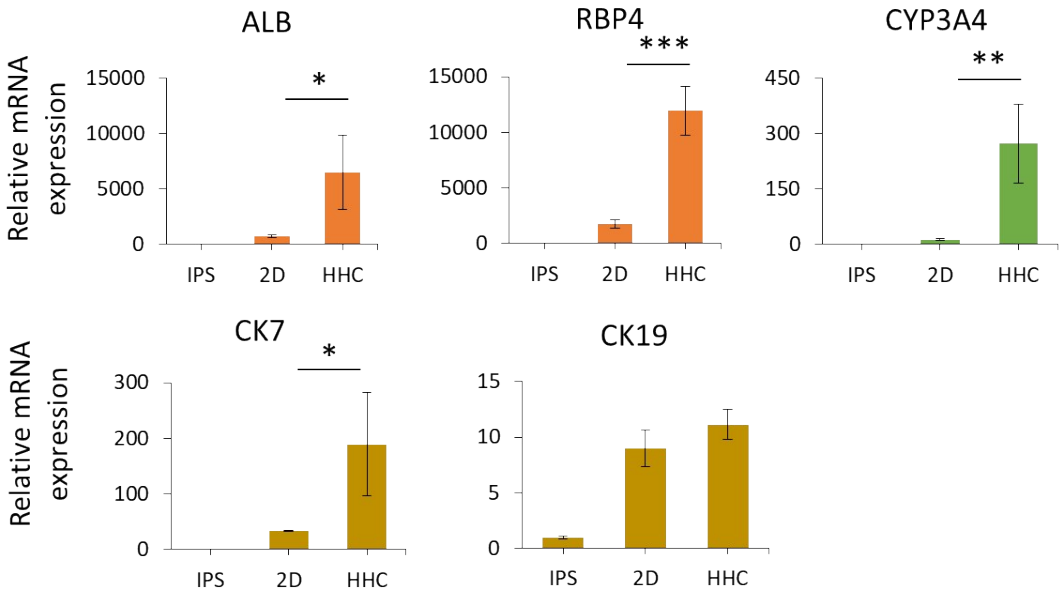

Fig. S7 The expressions of hepatocyte-associated genes (AFP, ALB, RBP4 and CYP3A4) were examined in hiPSCs, 2D liver cells and encapsulated liver organoids in CHCs using real-time PCR, respectively. The expression values were normalized to GAPDH. Three independent experiments were performed. Data are shown as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.