Supporting Information

Semi-Interpenetrating Polymer Network of Hyaluronan and Chitosan Self-Healing Hydrogels for Central Nervous System Repair

Yi Liu[1], Yi-Hua Hsu[2], Abel Po-Hao Huang[1,2], and Shan-hui Hsu[1,3,*]

[1] Institute of Polymer Science and Engineering, National Taiwan University, Taipei, Taiwan

[2] Department of Surgery, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan

[3] Institute of Cellular and System Medicine, National Health Research Institutes, Miaoli, Taiwan

Supplemental data: total 17

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Movies: 7 (Movie S1 – S7)

[*] Shan-hui Hsu (Corresponding author)

Institute of Polymer Science and Engineering, National Taiwan University, No. 1, Sec. 4 Roosevelt Road, Taipei 10617, Taiwan, R.O.C.; E-mail: shhsu@ntu.edu.tw

Table S1. The primer sequences used for real-time RT-PCR analysis in the in vitro experiment.

Gene	Primer	
	Forward	Reverse
GAPDH	GGCTACAGCAACAGGGTGGT	CGAGTTGGGATAGGGCCTCT
Nestin	ACTGTGGAATCACCAGGAGG	ATTCCACCTCTCCCAGAGAC
Tubb3	CAGGGCCAAGACAAGCAGCA	GGAGCCCTAATGAGCTGGTGA
MAP2	TTCTCCACTGTGGCTGTTTG	GAGCCTGTTTGTAGACTGGAAGA
GFAP	CTGAACCCTCTGAGCAAATG	GAATCAAACACAGAGCCTGC
CNPase	ACCCTGAGCTGGCAAGAGTA	GGTAGGAGCATACATCCCAG

Table S2. The primer sequences used for real-time RT-PCR analysis in the in vivo experiment.

Gene	Primer	
	Forward	Reverse
CCL2	GGTCTCTGTCACGCTTCTG	TTCTCCAGCCGACTCATTG
TLR2	GGATCTTGATGGCTGTGATAGG	CTTTGTGTTTGCTGTGAGTCC
IL-1β	CCTCAAGGGGAAGAATCTAT	GAGGTGCTGATGTACCAGTT
Arg1	ATATCTGCCAAGGACATCGTG	AGGTCTCTTCCATCACTTTGC
Caspase3	AATTCAAGGGACGGGTCATG	GCTTGTGCGCGTACAGTTTC

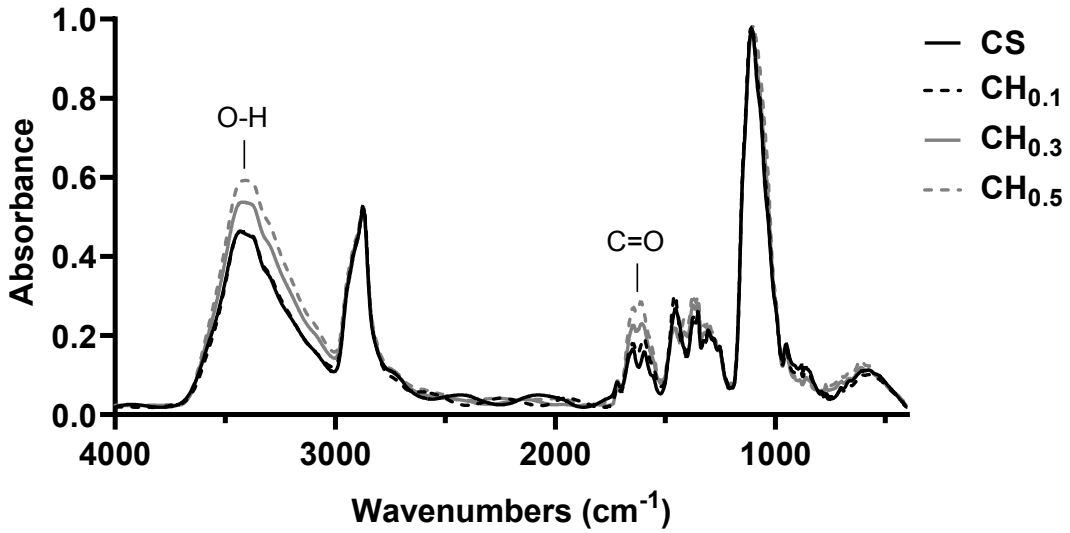


Figure S1. FTIR spectra of the hydrogels.

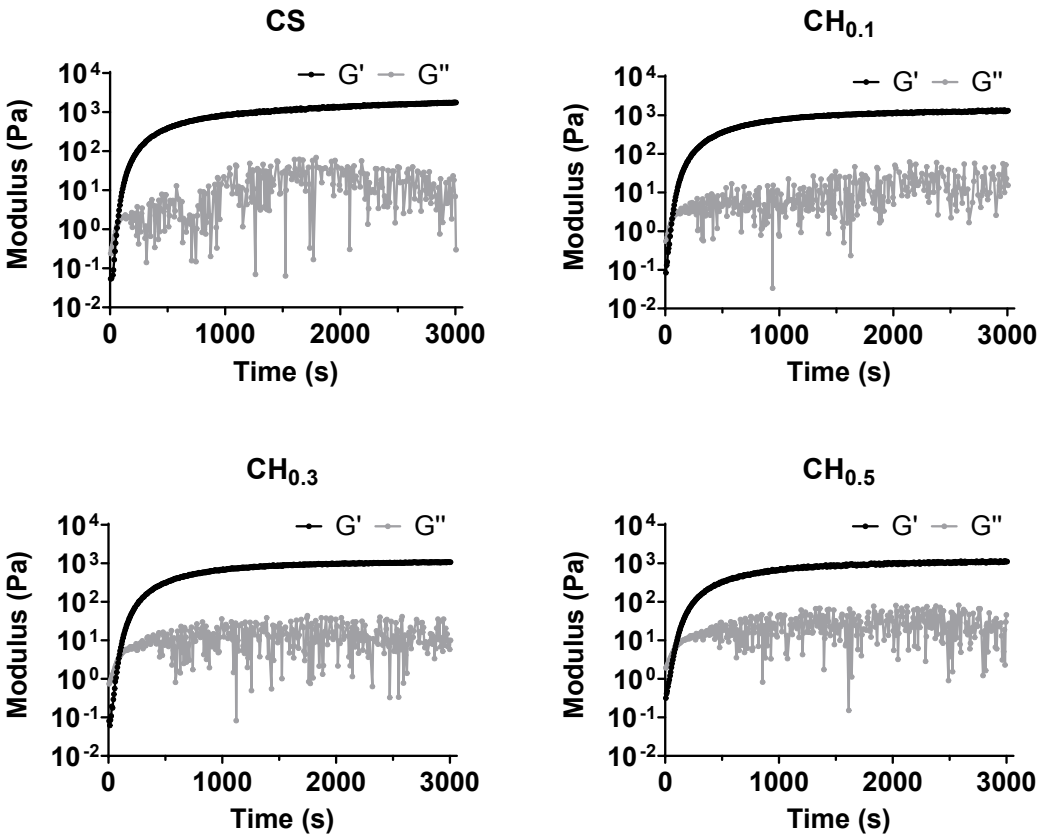


Figure S2. Time-sweep experiments for a longer duration of 3000 s showing the time-dependent change of storage moduli (G') and loss moduli (G'') of the hydrogels at 1 Hz frequency and 1% dynamic strain. G' was quite stable after 1000 s, while G'' kept oscillating.

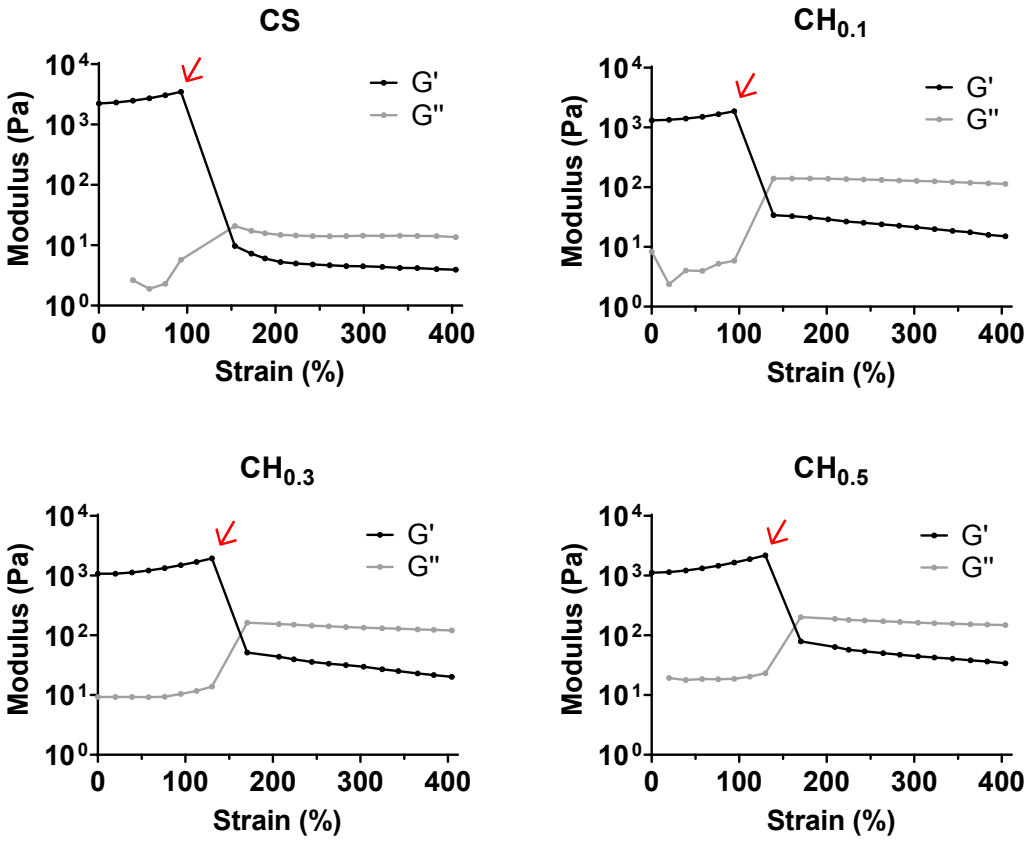


Figure S3. The strain-sweep experiments for the hydrogels in the range of 0.1% to 400% dynamic strain amplitudes at 1 Hz frequency. Strain hardening (G' increase with the increased strain, indicated by arrows) was observed before the structural damage (gel-to-sol transition) occurred.

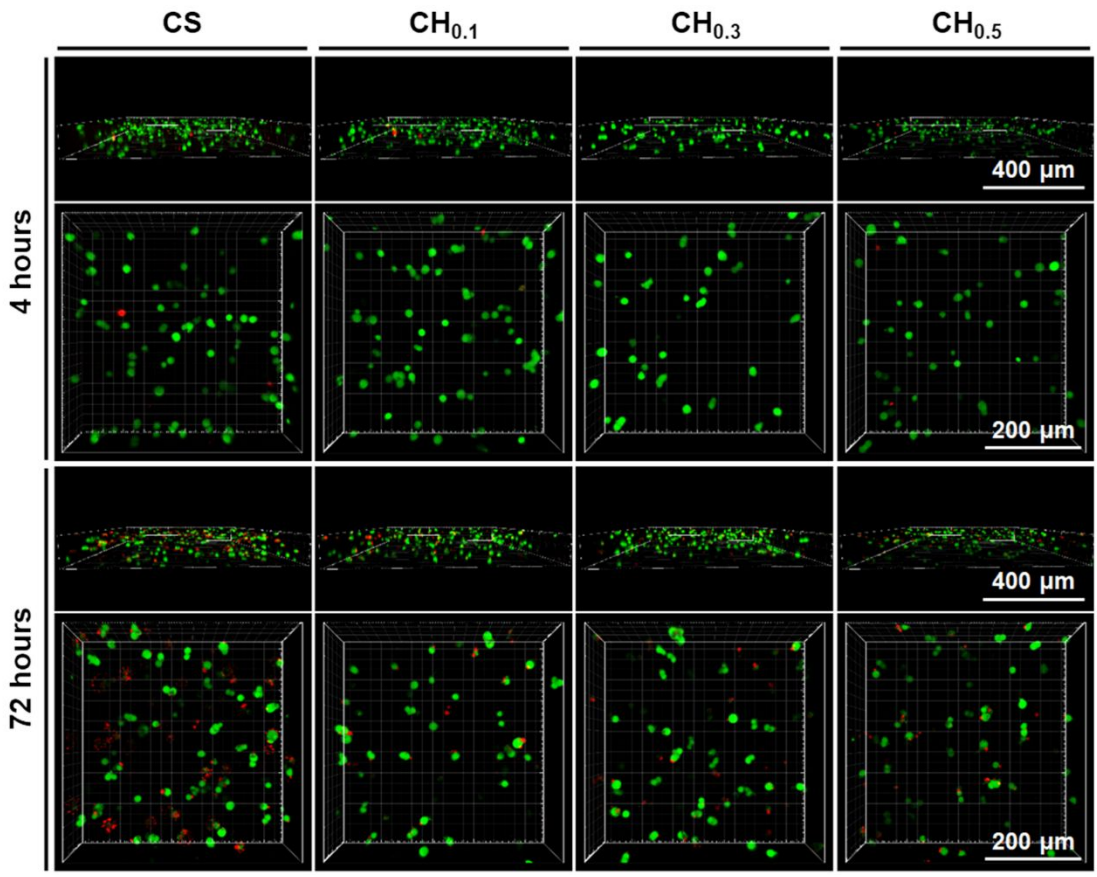


Figure S4. The side views and the enlarged top views of 3D confocal microscopic images for the live/dead staining of NSCs encapsulated in CS or CH hydrogel. The apoptosis bodies of NSCs were clearly observed after 72 hours of culture in CS hydrogel. Live cells: green, dead cells: red.

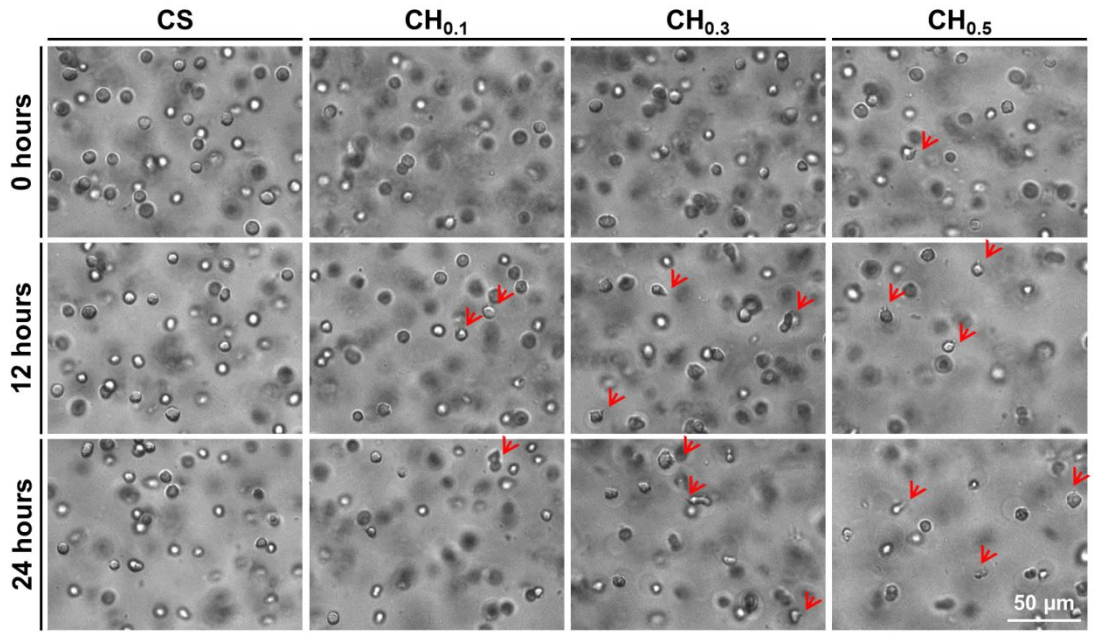


Figure S5. Morphology of NSCs encapsulated in CS or CH hydrogels. Bright-field microphotographs of NSCs obtained from live-cell time-lapse imaging videos after 0, 12, and 24 hours of encapsulation in the hydrogels. Filopodia or lamellipodia are indicated by arrows (↑).

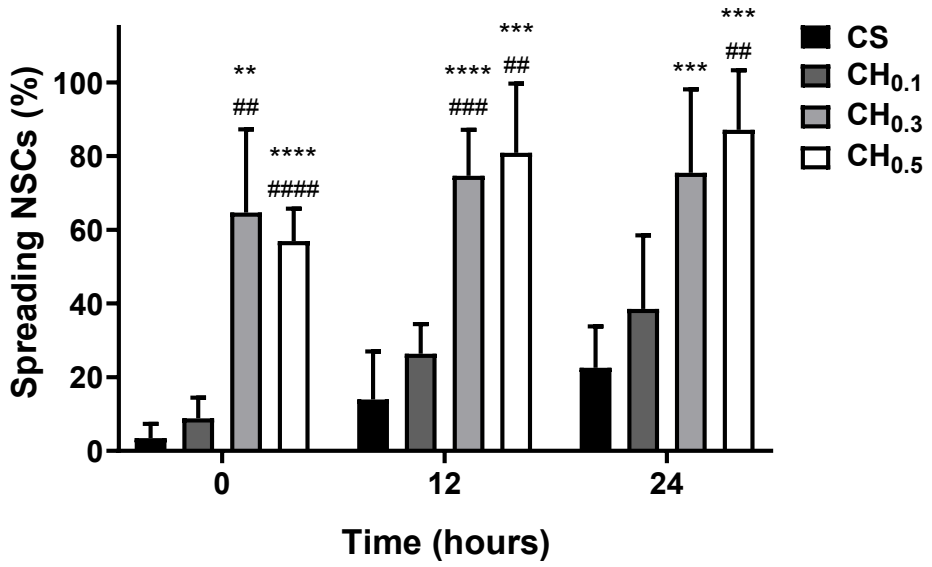


Figure S6. The percentage of spreading NSCs (non-circular cells) in the total population of NSCs encapsulated in CS and CH hydrogels. These data were quantified from the live-cell images at the specified time points. Two-way ANOVA was applied for comparison. **, ***, and **** represent $p < 0.01$, $p < 0.001$, and $p < 0.0001$ compared to the CS hydrogel group, and ##, ###, and #### represent $p < 0.01$, $p < 0.001$, and $p < 0.0001$ compared to the $CH_{0.1}$ hydrogel group (n = 6).

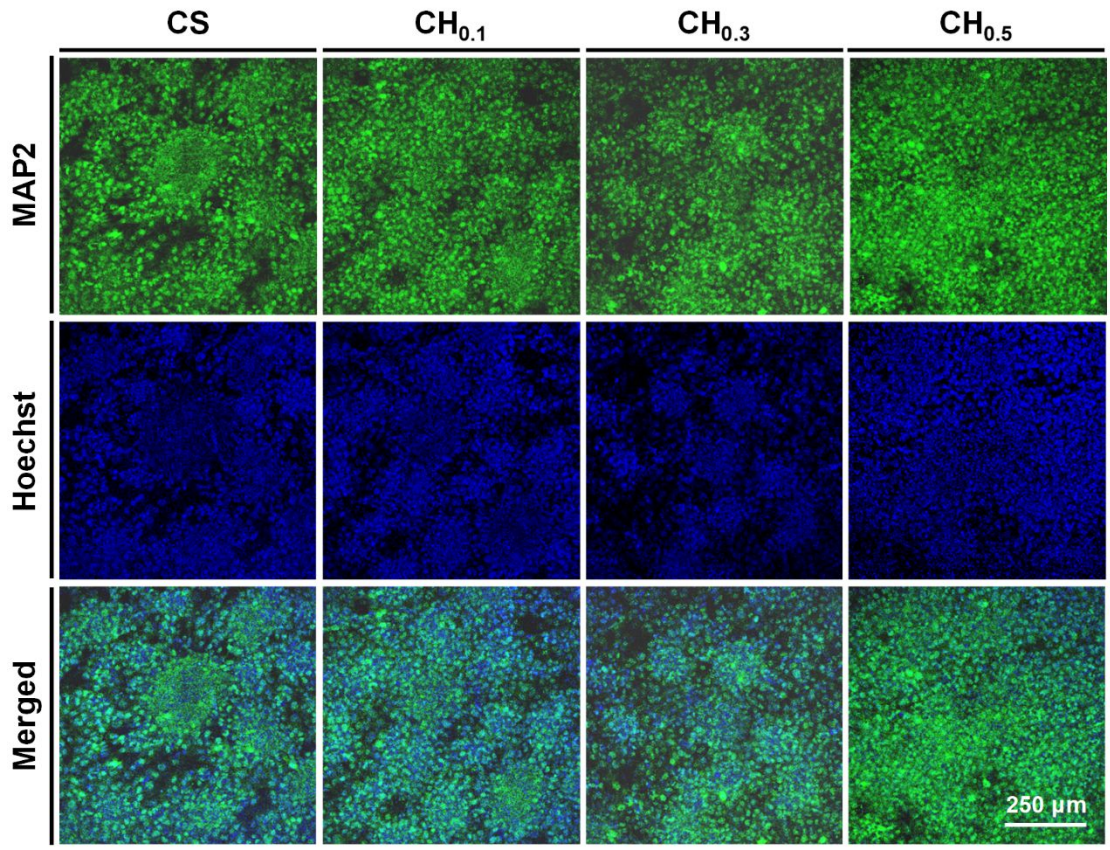


Figure S7. The expression of MAP2 in NSCs analyzed by immunofluorescence staining after 7 days of encapsulation in CS and CH hydrogels. The expression of MAP2 was upregulated slightly as the HA content of the hydrogels increased. MAP2: green, Hoechst: blue.

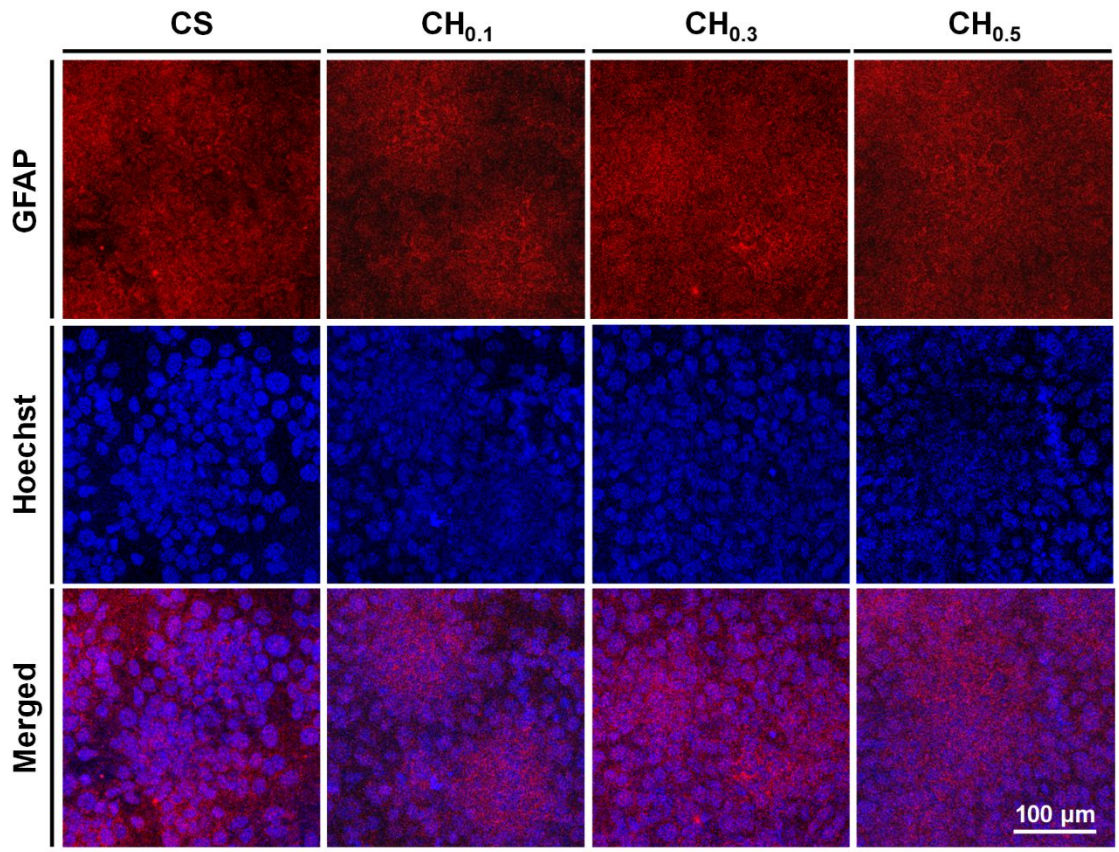
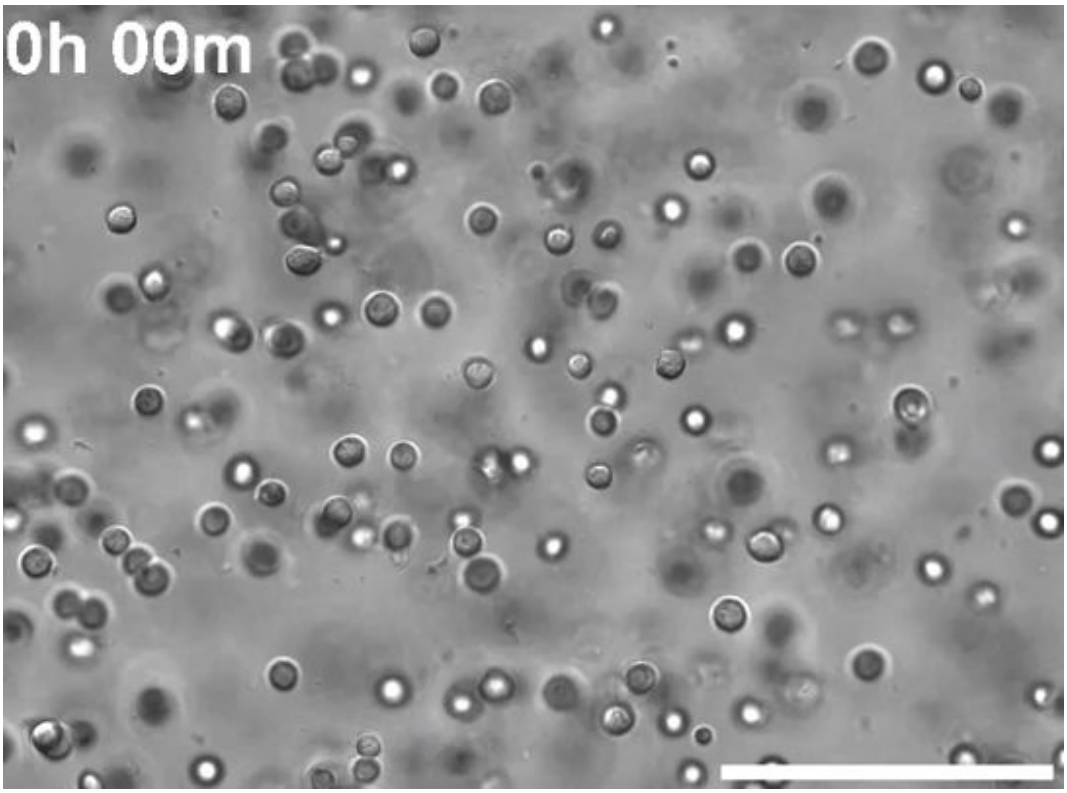
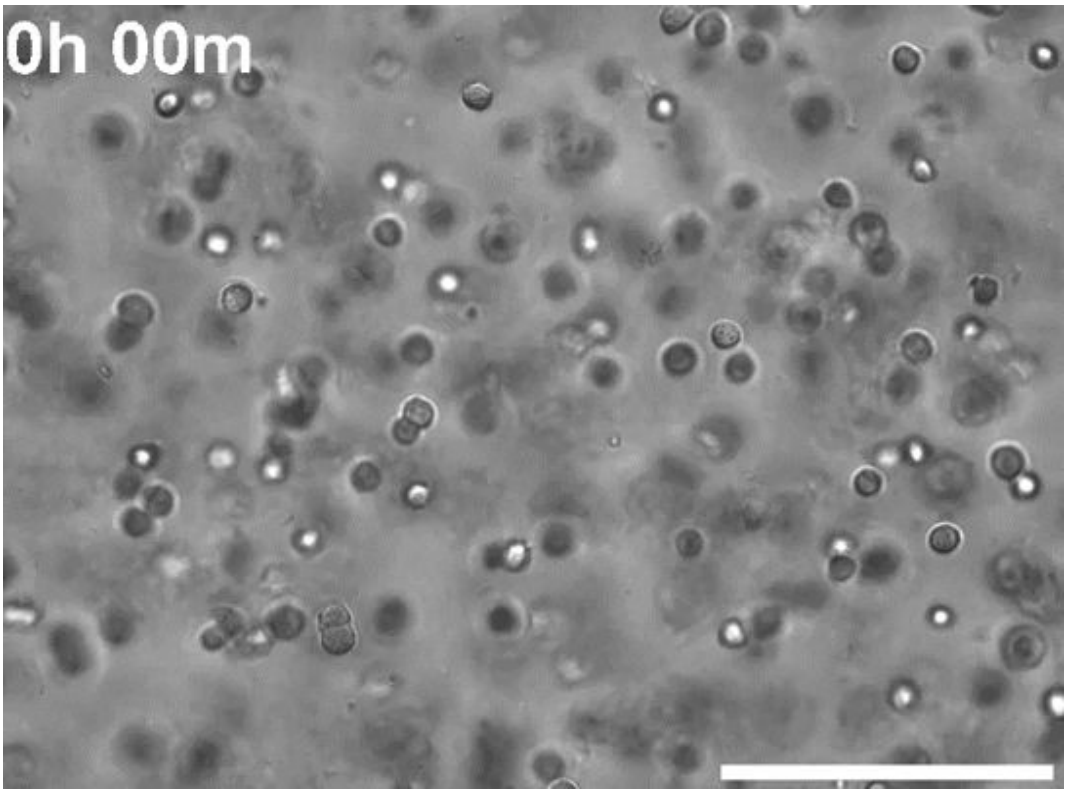


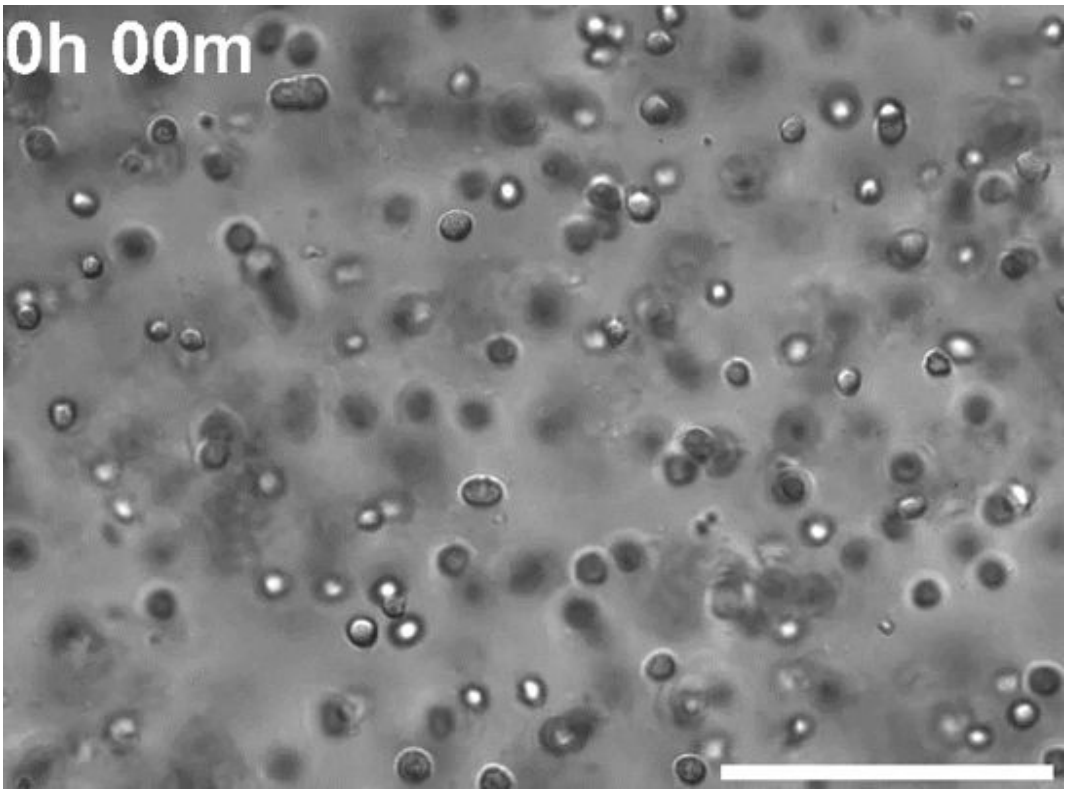
Figure S8. The expression of GFAP in NSCs analyzed by immunofluorescence staining after 7 days of encapsulation in CS and CH hydrogels. The expression of GFAP revealed no significant difference among the hydrogels. GFAP: red, Hoechst: blue.



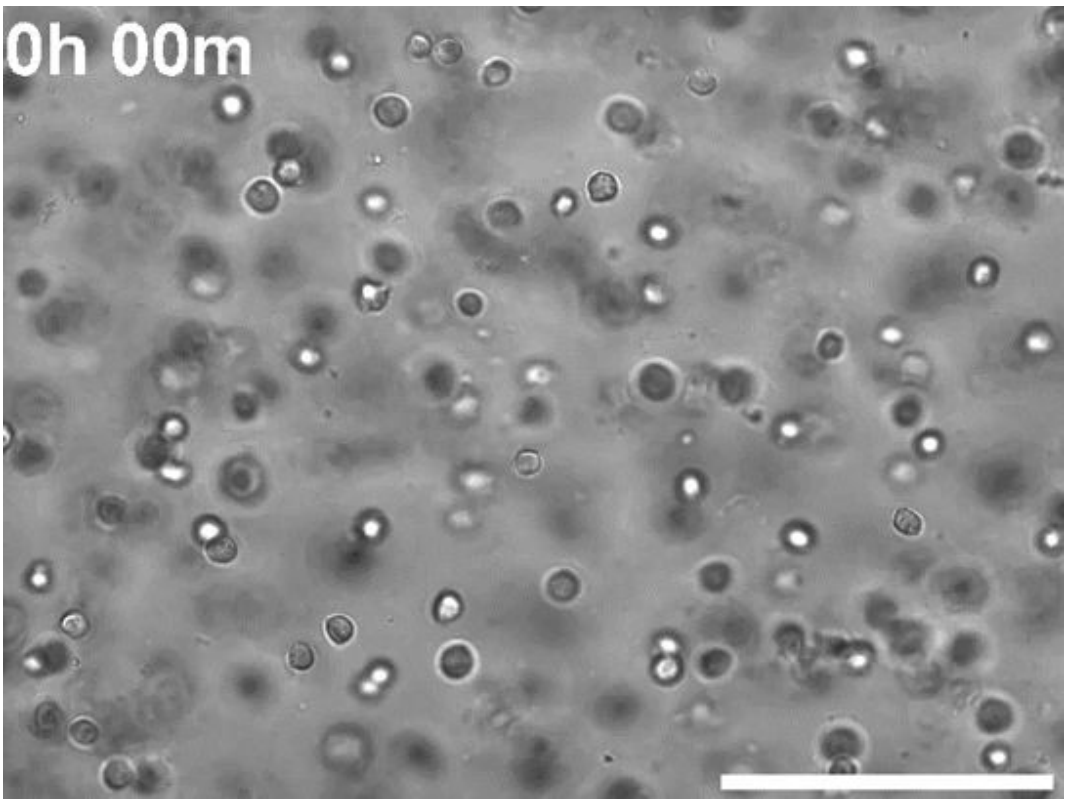
Movie S1. The live-cell time-lapse video of NSCs encapsulated in CS hydrogel during 24 hours of culture. Scale bar: 100 μm.



Movie S2. The live-cell time-lapse video of NSCs encapsulated in $CH_{0.1}$ hydrogel during 24 hours of culture. Scale bar: 100 μm.



Movie S3. The live-cell time-lapse video of NSCs encapsulated in $CH_{0.3}$ hydrogel during 24 hours of culture. Scale bar: 100 μm.



Movie S4. The live-cell time-lapse video of NSCs encapsulated in $CH_{0.5}$ hydrogel during 24 hours of culture. Scale bar: 100 μm.

Moive S5. The swimming behavior of adult zebrafish with traumatic brain injury (TBI) after the treatment of PBS.

Movie S6. The swimming behavior of adult zebrafish with traumatic brain injury (TBI) after the treatment of CS hydrogel.

Movie S7. The swimming behavior of adult zebrafish with traumatic brain injury (TBI) after the treatment of CH hydrogel.