

Electronic Supporting Information (ESI)

Dual-enzymatically cross-linked gelatin hydrogel enhances neural differentiation of human umbilical cord mesenchymal stem cells and functional recovery in experimental murine spinal cord injury

*Minghao Yao^{a, §, *}, Jinrui Li^{a, §}, Junni Zhang^{a, §}, Shanshan Ma^a, Luyu Wang^a, Feng Gao^a, Fangxia Guan^{a, b, *}*

^a School of Life Science, Zhengzhou University, 100 Science Road, Zhengzhou 450001, P. R. China

^b Institute of Neuroscience, Zhengzhou University, Zhengzhou 450000, P. R. China

E-mail: guanfangxia@126.com, yao453343550@126.com

[§] Minghao Yao, Jinrui Li, and Junni Zhang are equal contributors

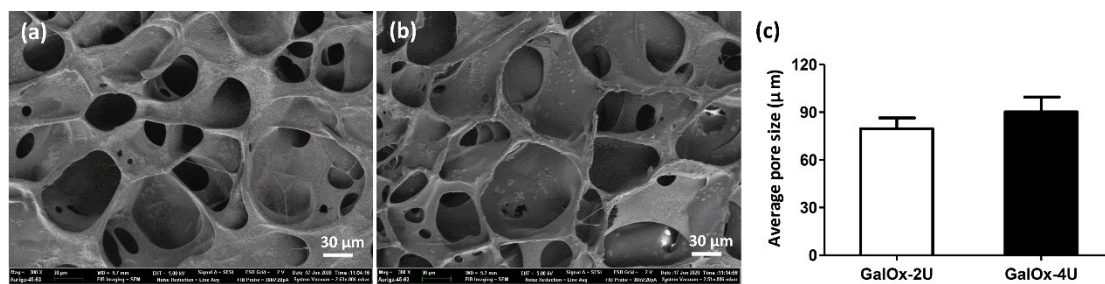


Figure S1 Internal microtopography of GH hydrogels under scanning electron microscope and average pore size.

GalOx-2U hydrogel (a), GalOx-4U hydrogel (b), average pore size of GalOx-2U and GalOx-4U hydrogels (c).

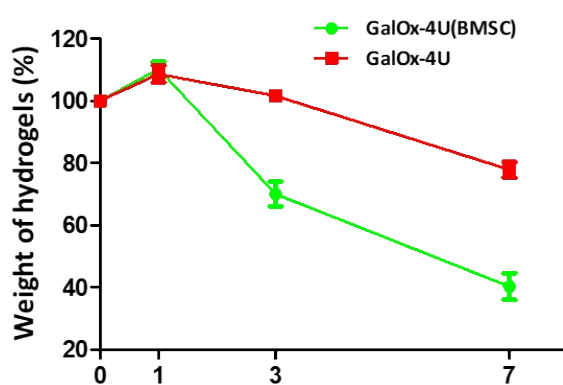


Figure S2 The degradation of hUC-MSCs-loaded GalOx-4U hydrogel and cell-free GalOx-4U hydrogel on day 1, 3,

and 7.

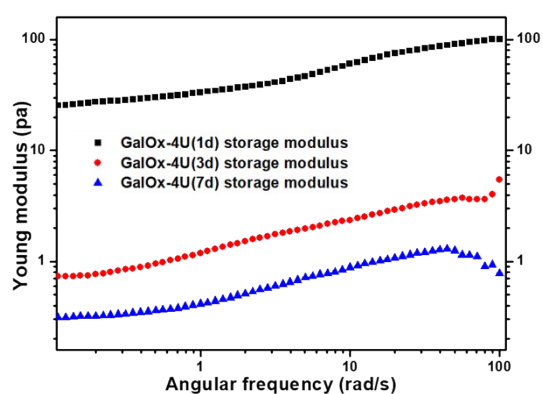


Figure S3 Storage modulus of GalOx-4U hydrogels 1, 3, and 7 days after the fabrication measured by rheometer.

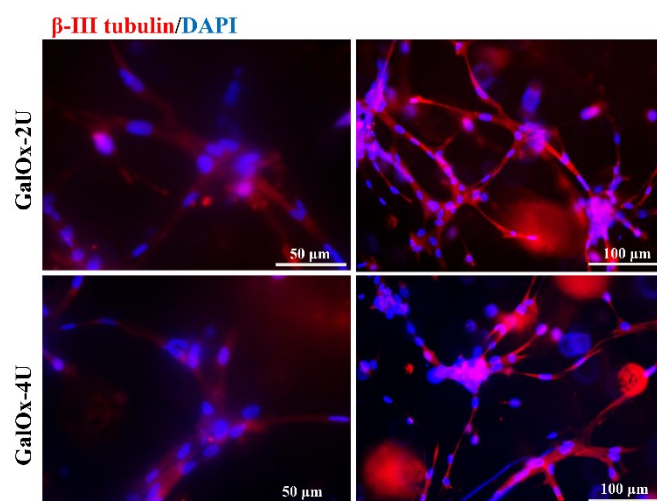


Figure S4 Images of hUC-MSCs cultivation within GalOx-2U and GalOx-4U hydrogels by β -III tubulin immunofluorescent staining on day 3 (Enlarged images).

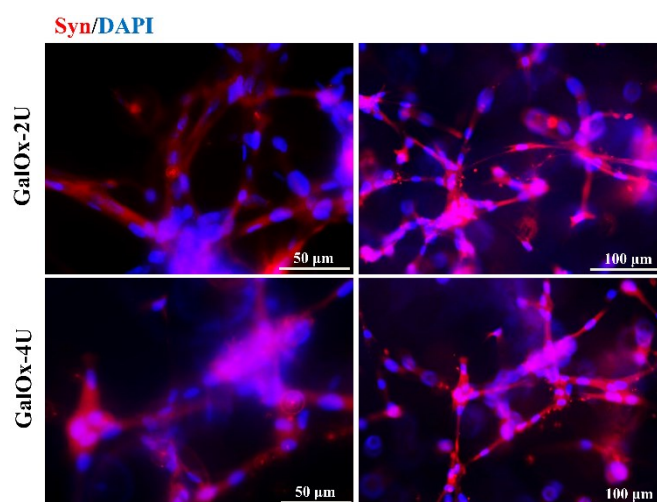


Figure S5 Images of hUC-MSCs cultivation within GalOx-2U and GalOx-4U hydrogels by synapsin-1 immunofluorescent staining on day 5 (Enlarged images).

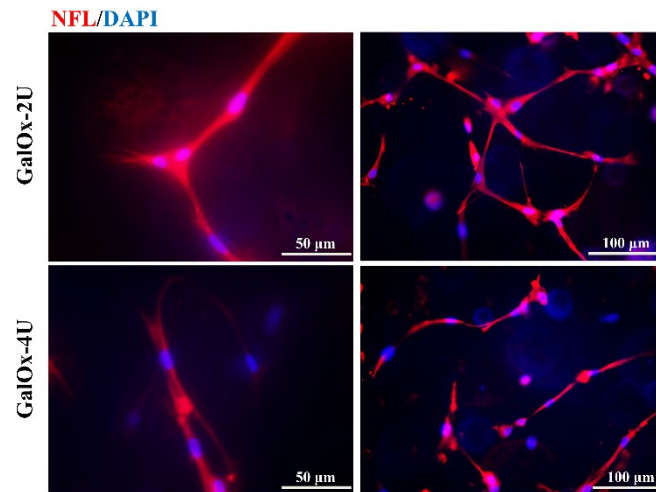


Figure S6 Images of hUC-MSCs cultivation within GalOx-2U and GalOx-4U hydrogels by NFL immunofluorescent staining on day 7 (Enlarged images).

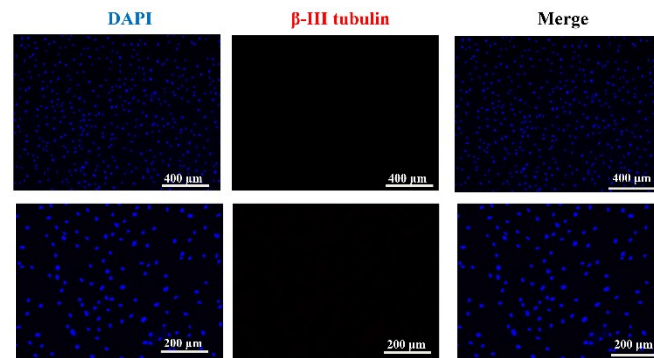


Figure S7 β-III tubulin immunostaining of hUC-MSCs after the cultivation on cell culture plate for 3 days before encapsulated in hydrogel.

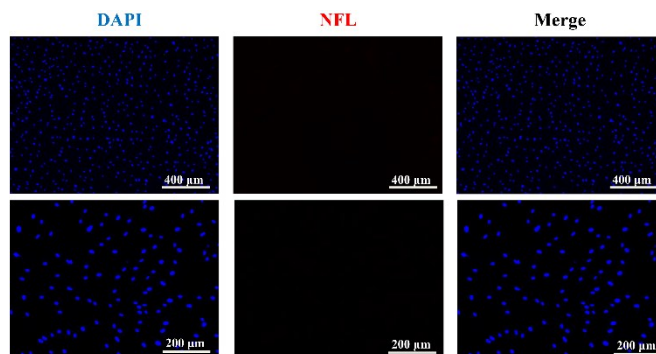


Figure S8 NFL immunostaining of hUC-MSCs after the cultivation on cell culture plate for 3 days before encapsulated in hydrogel.

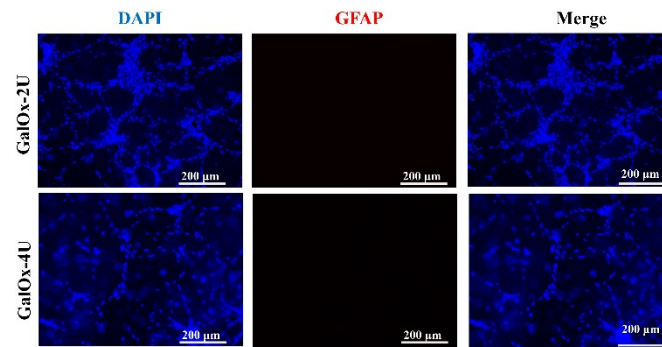


Figure S9 GFAP immunostaining of hUC-MSCs within GalOx-2U and GalOx-4U hydrogels on day 3.

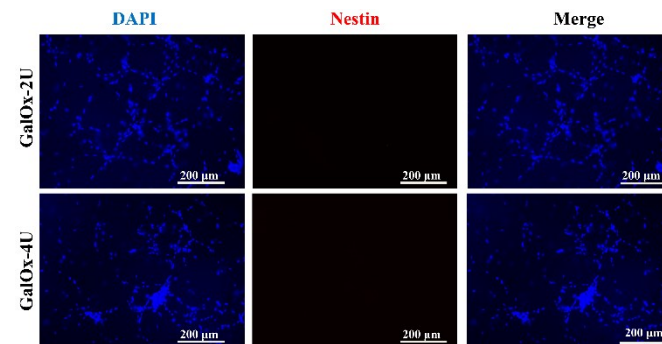


Figure S10 Nestin immunostaining of hUC-MSCs within GalOx-2U and GalOx-4U hydrogels on day 3.

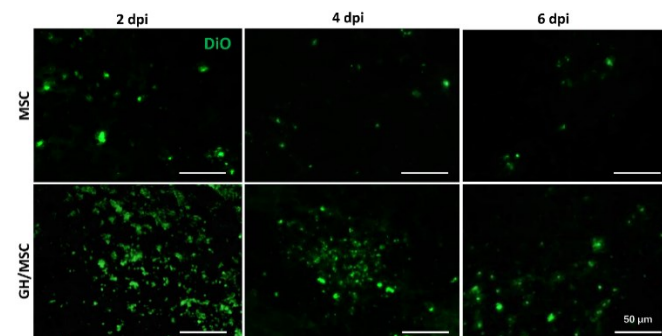


Figure S11 Images of DiO-labeled hUC-MSC in the damaged spinal cords on 2, 4, and 6 dpi of GH/MSC group and MSC group for analyzing cellular retention.

Table S1 Wet weight, dry weight, and water content of spinal cords in Normal, NS, GH, MSC, and GH/MS C groups.

| | Normal | | | NS | | | GH | | | MSC | | | GH/MS C | | |
|-------------------|------------|------|------|------------|------|------|------------|------|------|------------|------|------|------------|------|------|
| wet weight (mg) | 37.0 | 37.2 | 36.7 | 26.4 | 40.0 | 34.1 | 33.2 | 35.7 | 36.2 | 31.0 | 33.9 | 33.3 | 42.6 | 31.7 | 32.6 |
| dry weight (mg) | 10.7 | 12.0 | 11.3 | 3.9 | 5.7 | 3.5 | 7.3 | 6.6 | 6.3 | 6.2 | 7.3 | 7.8 | 11.6 | 10.9 | 9.6 |
| water content (%) | 71.1 | 67.7 | 69.2 | 85.2 | 85.8 | 89.7 | 78.0 | 81.5 | 82.6 | 80.0 | 78.5 | 76.6 | 72.8 | 65.6 | 70.6 |
| Mean±SD (%) | 69.3 ± 1.7 | | | 86.9 ± 2.5 | | | 80.7 ± 2.4 | | | 78.4 ± 1.7 | | | 69.7 ± 3.7 | | |