

Tunable viscoelastic collagen/polyethylene glycol composite hydrogels modulate neural and tumor cell behavior in 3D microenvironments

Supplementary File

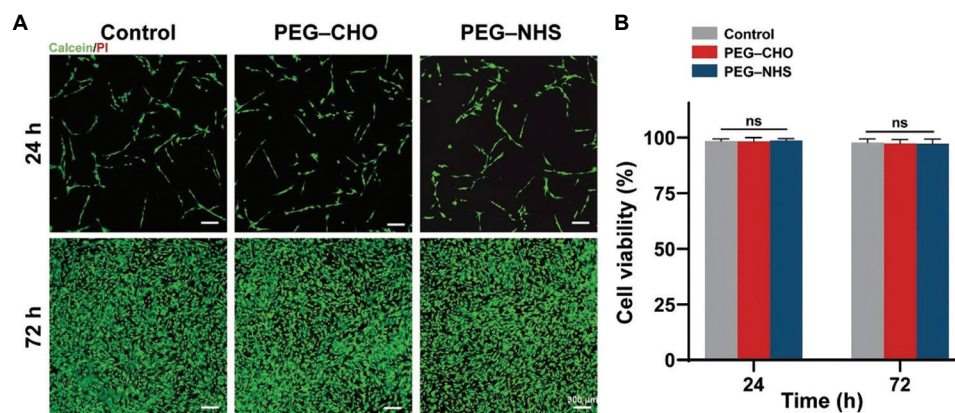


Figure S1. Representative live/dead staining images of PC12 cells after culture with normal medium, PEG-CHO, and PEG-NHS solutions (scale bar: 300 μ m; magnification: $\times 100$). "ns" indicates no statistical significance.

Abbreviations: PEG-CHO: 4-arm poly(ethylene glycol)-benzaldehyde; PEG-NHS: 4-arm poly(ethylene glycol)-succinimidyl carbonate; PI: Propidium iodide.

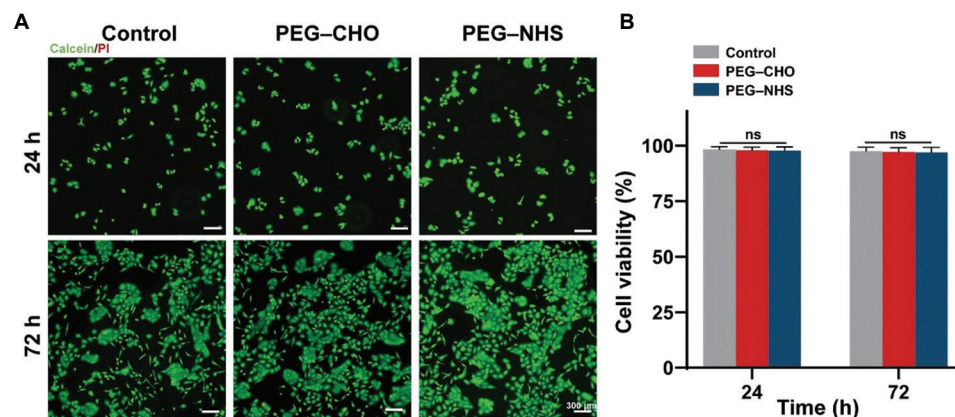


Figure S2. Representative live/dead staining images of DU145 cells after culture with normal medium, PEG-CHO, and PEG-NHS solutions (scale bar: 300 μ m; magnification: $\times 100$). "ns" indicates no statistical significance.

Abbreviations: PEG-CHO: 4-arm poly(ethylene glycol)-benzaldehyde; PEG-NHS: 4-arm poly(ethylene glycol)-succinimidyl carbonate; PI: Propidium iodide.

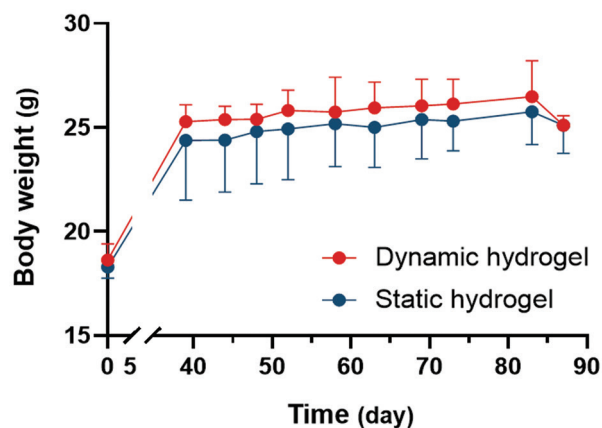


Figure S3. Body weight profiles of mice in the dynamic and static hydrogel groups ($n = 4$).

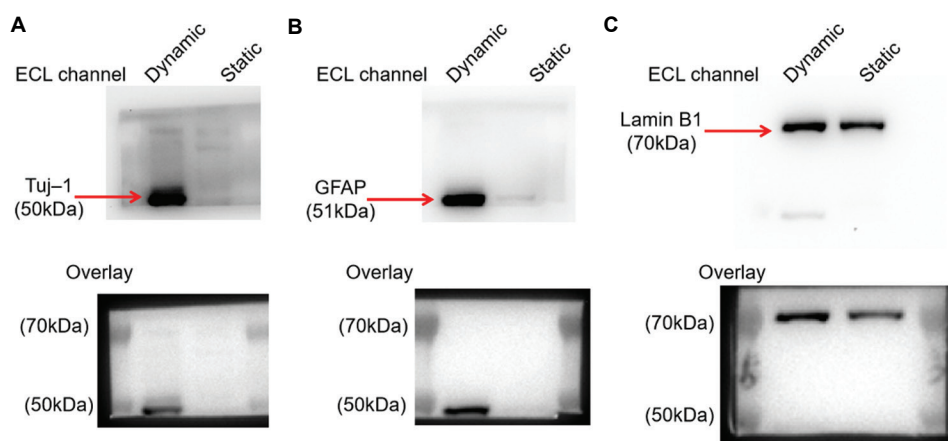


Figure R1. Original images for Western blotting for Tuj-1, GFAP, and Lamin B1 in Figure 3D. (A) Western blotting for Tuj-1 (50kDa), (B) Western blotting for GFAP (51kDa), and (C) Western blotting for Lamin B1 (70kDa).

Table S1. Composition of the neural stem cell proliferation medium (50 mL)

Component	Volume	Company name	Product number
Sodium pyruvate	500 μ L	Gibco	11360070
Penicillin-streptomycin	500 μ L	Gibco	15140122
L-glutamine	500 μ L	Gibco	25030081
Non-essential amino acids	500 μ L	Gibco	11140050
B27	1 mL	Gibco	7504044
Epidermal growth factor (0.1 mg/mL)	10 μ L	Sino Biological	10605
Basic epidermal growth factor (0.1 mg/mL)	10 μ L	Sino Biological	10014-HNAE
30% glucose	1 mL	Biosharp	BS085
Heparin (0.025 g/mL)	3.66 μ L	Sigma	H3149
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12	Fill until the final volume reaches 50 mL	Gibco	C11330500BT

Table S2. Composition of the neural stem cell adhesion medium (50 mL)

Component	Volume	Company name	Product number
Sodium pyruvate	500 µL	Gibco	11360070
Penicillin–streptomycin	500 µL	Gibco	15140122
L-glutamine	500 µL	Gibco	25030081
Non-essential amino acids	500 µL	Gibco	11140050
Fetal bovine serum	5 mL	Gibco	10270-106
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12	Fill until the final volume reaches 50 mL	Gibco	C11330500BT

Table S3. Composition of the neural stem cell differentiation medium (50 mL)

Component	Volume	Company name	Product number
Sodium pyruvate	500 µL	Gibco	11360070
Penicillin–streptomycin	500 µL	Gibco	15140122
L-glutamine	500 µL	Gibco	25030081
Non-essential amino acids	500 µL	Gibco	11140050
Fetal bovine serum	2.5 mL	Gibco	10270-106
B27	1 mL	Gibco	7504044
30% glucose	1 mL	Biosharp	BS085
Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12	Fill until the final volume reaches 50 mL	Gibco	C11330500BT

Table S4. Physicochemical properties of dynamic and static hydrogels

Parameter	Dynamic hydrogel	Static hydrogel
Cross-linking reaction type	Imine bonds	Amide bonds
Chemical bond strength	Reversible, weak	Irreversible, stable
Swelling properties	Below 10%	Below 10%
Storage modulus (G')	Approximately 100 Pa ($> G''$)	Approximately 100 Pa ($> G''$)
Stress-relaxation half-time ($\tau_{1/2}$)	Within 45 s	Approaching infinity

Abbreviation: G'' : Loss modulus.

Table S5. Neural cell behavior in dynamic and static hydrogels

Parameter	Dynamic hydrogel	Static hydrogel
Cell viability	Cytocompatible	Cytocompatible
Cell behavior	Neurite outgrowth	Rounded and compact
Level of Tuj-1 protein	High	Low
Level of GFAP protein	High	Low

Abbreviations: GFAP: Glial fibrillary acidic protein; Tuj-1: β -III tubulin.

Table S6. Cancer cell behavior in dynamic and static hydrogels

Parameter	Dynamic hydrogel	Static hydrogel
Cell viability	Cytocompatible	Cytocompatible
Cell behavior	Larger spheroids with cellular protrusions	Smaller and smooth spheroids
<i>In vivo</i> tumorigenicity	Larger tumors	Smaller tumors
<i>In vivo</i> organ damage	Low	Low