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Supporting Information

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A 3D Bioprinted in vitro Model of Neuroblastoma Recapitulates Dynamic Tumor-Endothelial Cell Interactions Contributing to Solid Tumor Aggressive Behavior

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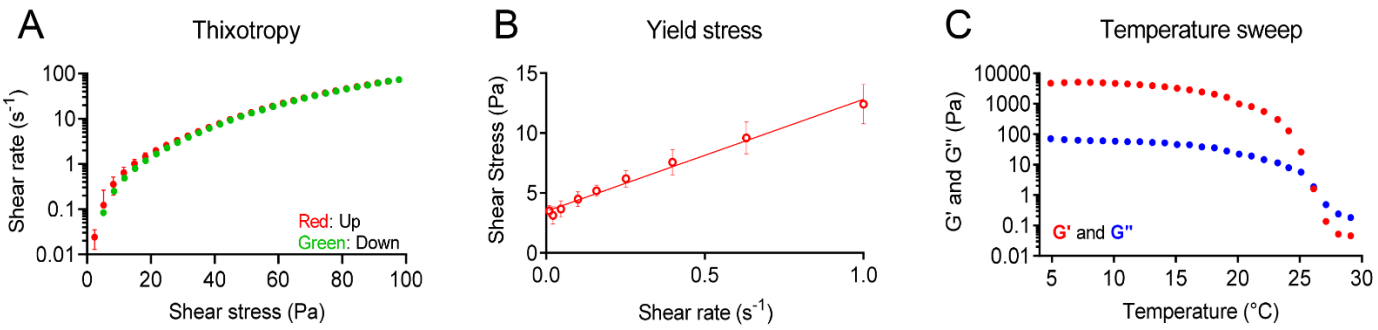


Figure S1. **Rheological analysis of Carbopol support bath and gelMA bioink**. **A**: Thixotropy results of 0.4% w/v Carbopol bath. The hysteresis loop was plotted based on an increase-decrease control on shear stress between 0 to 100 Pa. The loop demonstrates limited thixotropic time with almost overlapping up and down curves, indicating the ability of Carbopol bath to rapidly recover under shearing. **B**: Yield stress of 0.4% Carbopol bath, achieved by applying a range of small shear rates from 0.1 to 1 s^{-1}, and then interpolating the obtained shear stress at zero share rate. The yield stress is 3.48 Pa. **C**: Temperature sweep to identify the gelling point of 10% w/v gelMA. Lower temperatures increased storage (G') value, while moving to high temperatures produced a more liquid-like solution with increased loss (G'') value. The gelling/liquefying point was identified as ~26°C. These results ensured the printability of the bioink printed at room temperature in this study.

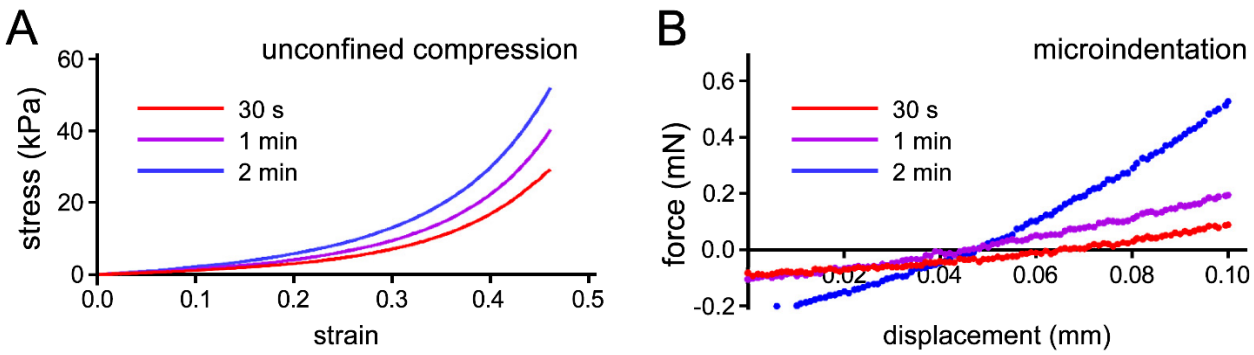


Figure S2. **Characterization of printing mechanical properties of bioprinted gelMA constructs**. **A-B**: Stress-strain curves obtained from unconfined compression test (**A**) and unloading force-displacement curves obtained from microindentation test (**B**) at different UV exposure times (30 s, 1 min, and 2 min).

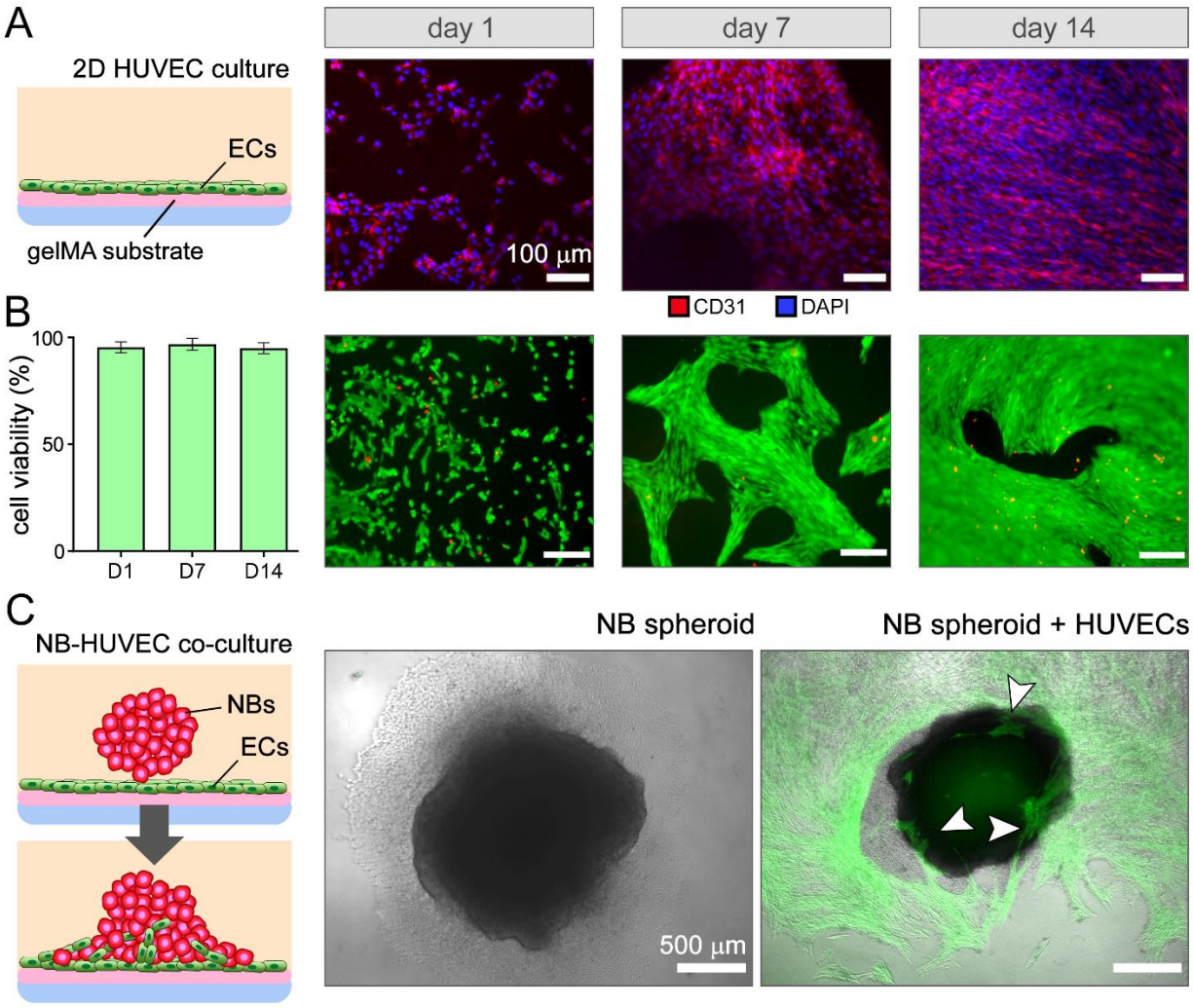
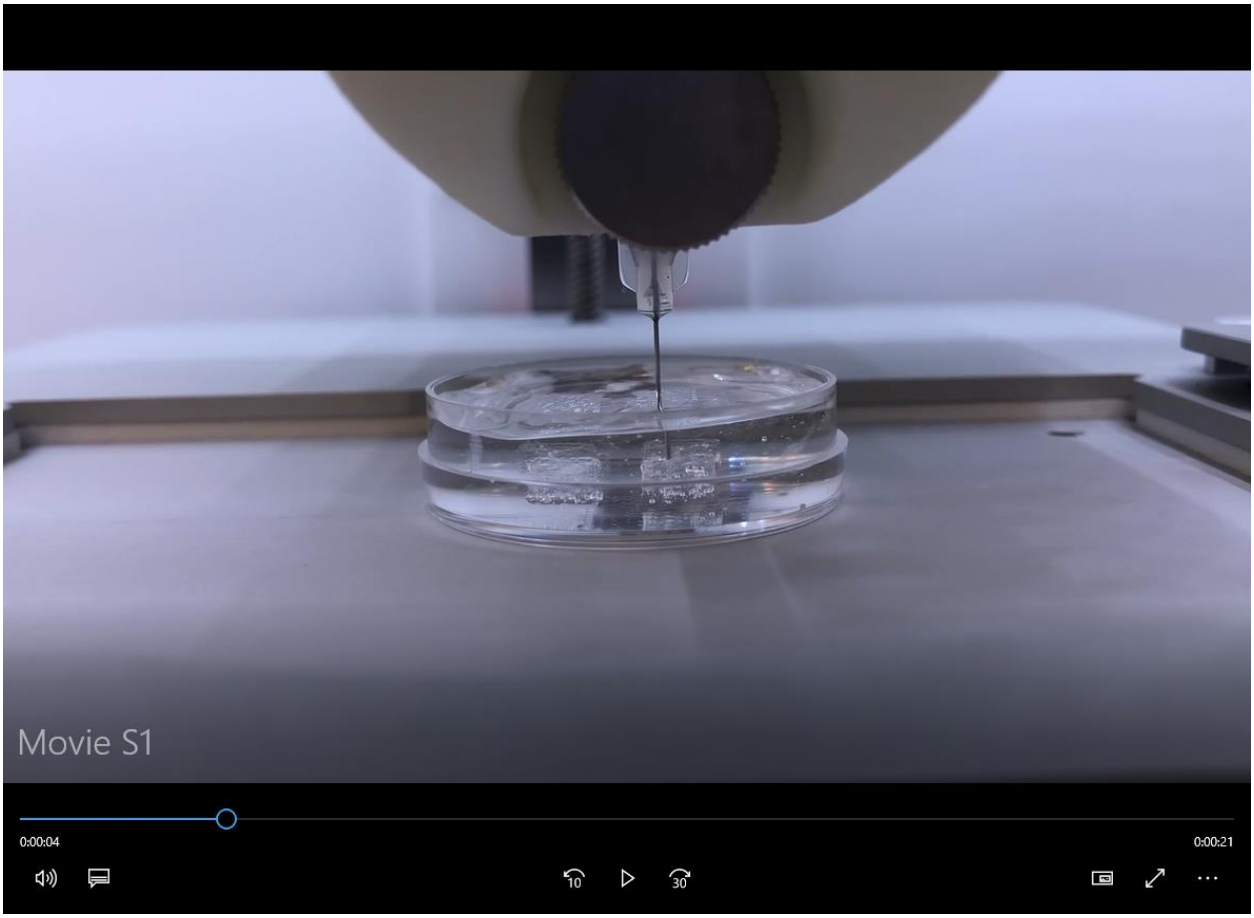


Figure S3. **Interactions between human umbilical vein endothelial cells (HUVECs), gelMA substrate, and neuroblastoma (NB) cells**. **A-B**: 2D culture of cytoplasmic-GFP HUVECs on the cast gelMA substrate. **A**: CD31 and DAPI staining of GFP HUVECs demonstrates high affinity of the cells to the gelMA bioink and their rapid growth. **B**: Live/dead assay on HUVECs at days 1, 7, and 14 of culture (n = 3). Scale bars in **A** and **B** represent 100 µm. **C**: Coculture of NB spheroid, loaded onto the endothelial layer grown on gelMA substrate (as in **A-B**). NB and HUVECs showed spreading and infiltration into each other. White arrows highlight penetration of HUVECs into the NB spheroid. Scale bars in **C** represent 500 µm.

Table S1: List of primers used in the qRT-PCR analysis of NB spheroids extracted from 3D bioprinted gelMA constructs.

Primers (human)	Forward (5'-3')	Reverse (5'-3')	Blast confirmed
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG	YES
OCT4	GGAGGAAGCTGACAACAATGAAA	GGCCTGCACGAGGGTTT	YES
SOX2	TGCGAGCGCTGCACAT	TCATGAGCGTCTTGGTTTTCC	YES
VIM	GACAATGCGTCTCTGGCACGTCTT	TCCTCCGCCTCCTGCAGGTTCTT	YES
SNAI2	CAGACCCTGGTTGCTTCAA	TGACCTGTCTGCAAATGCTC	YES



Movie S1: Representative bioprinting process used to simultaneously manufacture multiple vascular gelMA constructs.