ADVANCED HEALTHCARE MATERIALS

Supporting Information

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Embedded Bioprinting of Breast Tumor Cells and Organoids Using Low-Concentration Collagen-Based Bioinks

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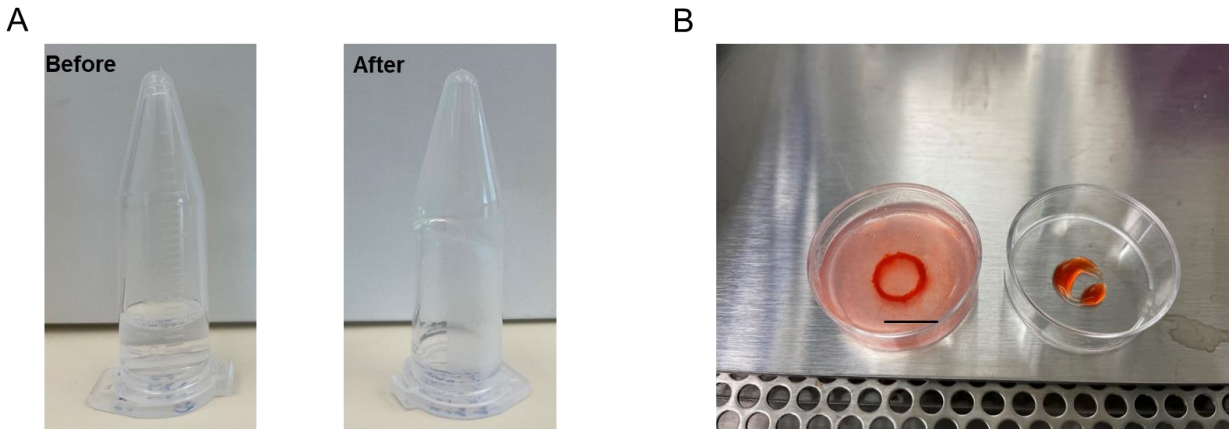

Figure S1. A) Successful formation of silk fibroin hydrogel as shown by the inverted tube assay. B) Comparison of embedded printing (left) and traditional extrusion printing (right) using the low concentration collagen I solution. Scale bar 1 cm.

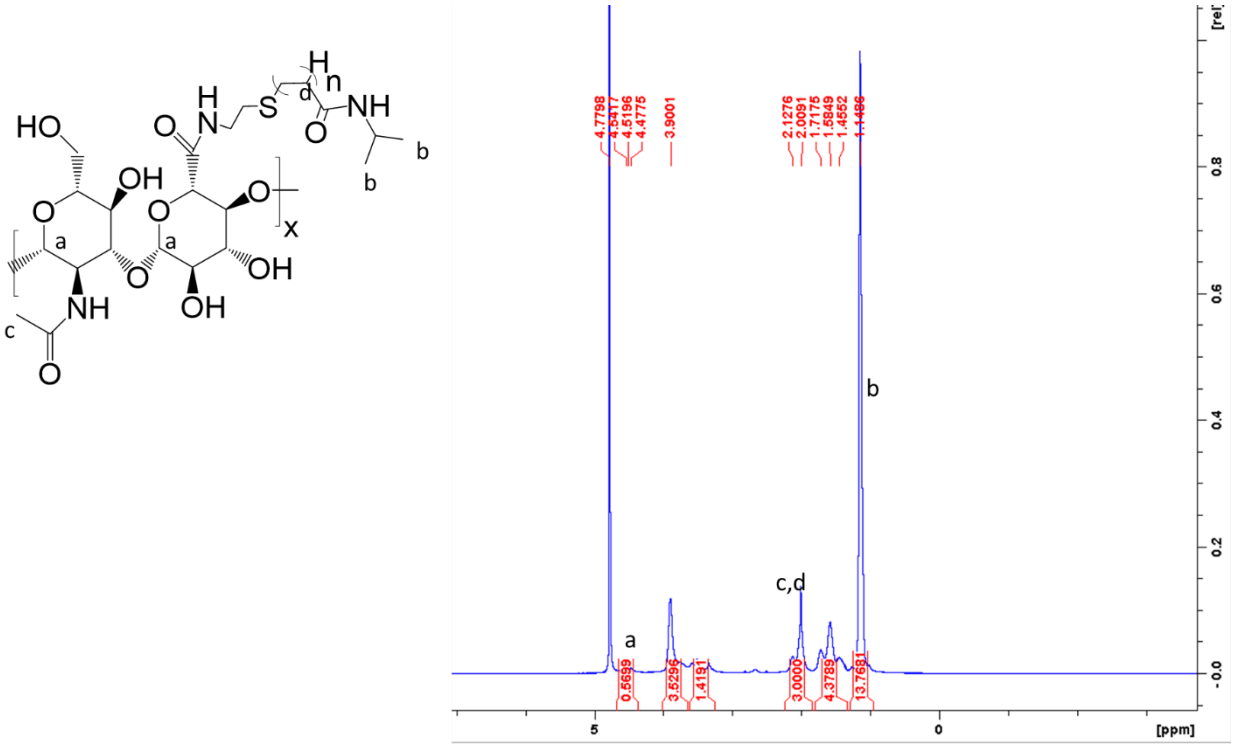

Figure S2. 1H-NMR spectrum of HA-pNIPAM and the corresponding peak annotation.

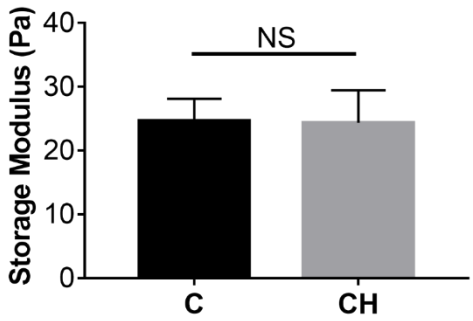

Figure S3. Comparison of storage modulus of collagen (C) and collagen-HA-pNIPAM (CH) (n=3).

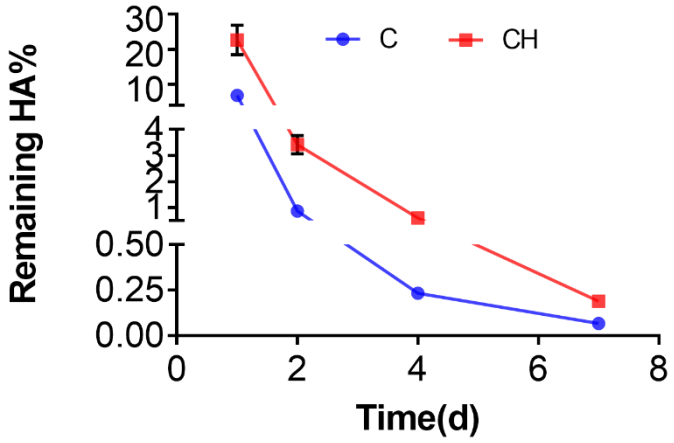

Figure S4. The remaining ratio of rhodamine labeled HA or HA-pNIPAM in the collagen gels at different time points (n=3).

Figure S5. Illustration of 3D Printing a spiral inside the silk bath using the red dye colored collagen solution (left) and printing a disk inside the silk bath using the CH solution (right, the bioink turned white at 37°C).

A

Bioink ID	Compositions (with 1*10^5/ml cells)	
Collagen (C)	Collagen 3 mg/ml	
Collagen HA-pNIPAM (CH)	Collagen 3 mg/ml	HA-pNIPAM 16 mg/ml
Collagen medium HA-pNIPAM (CH-m)	Collagen 3 mg/ml	HA-pNIPAM 8 mg/ml
Collagen low HA-pNIPAM (CH-l)	Collagen 3 mg/ml	HA-pNIPAM 4 mg/ml

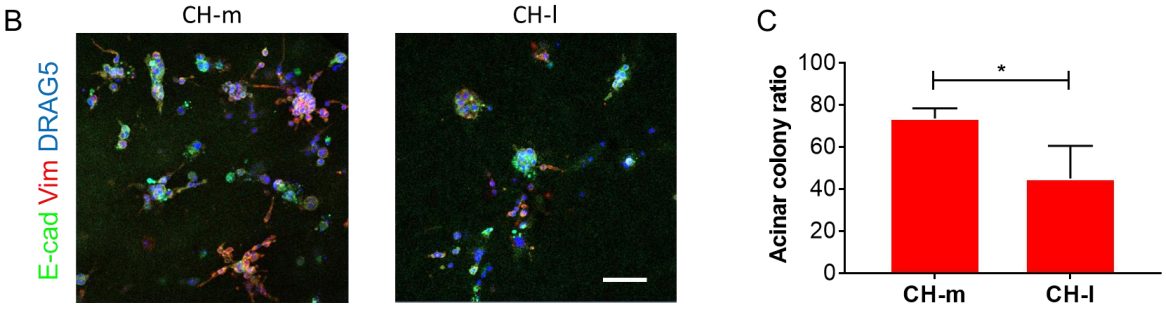

Figure S6. A) Summary table of different bioink components used for the optimization study. B&C) Immunofluorescent images of 21PT in two additional CH bioinks at day 7 and the respective acinar colony ratio in two bioinks.

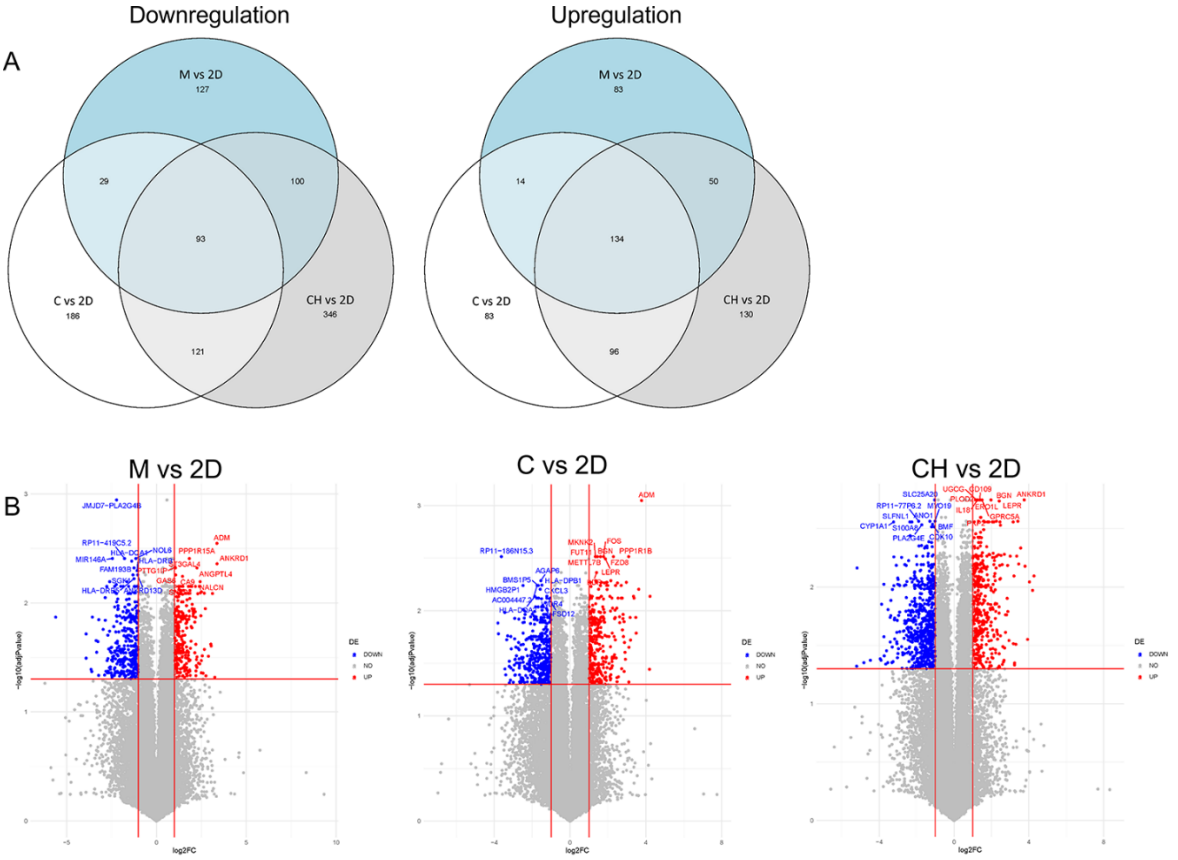

Fig S7. RNA seq analysis. A) Venn diagrams illustrating the number of overlapping DEGs among the three groups. B) Volcano plots displaying up- and down- regulated RNAs by comparing the M, C, CH groups to the 2D culture group.

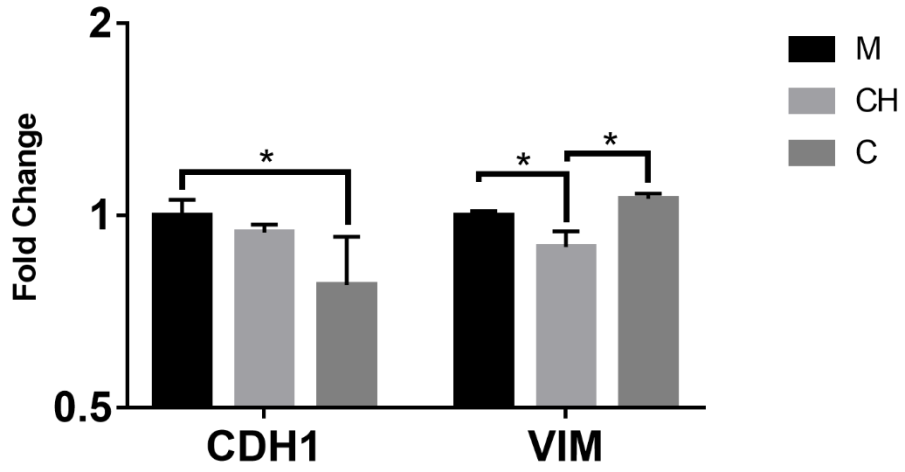

Fig S8. Comparison of gene expressions of E-cadherin (CDH1) and vimentin (VIM) based on RNA-seq analysis. Normalized to the Matrigel group (n=3, *$p<0.05$).

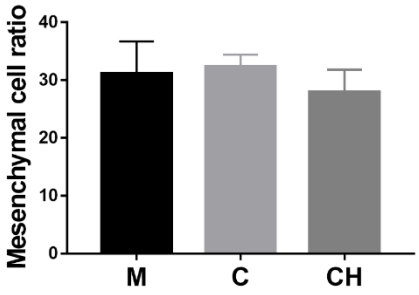

Figure S9. Quantification of the mesenchymal cell ratio of MDA-MB-231 cancer cells cultured in different bioinks at day 7 (n=3, no statistical difference was observed).

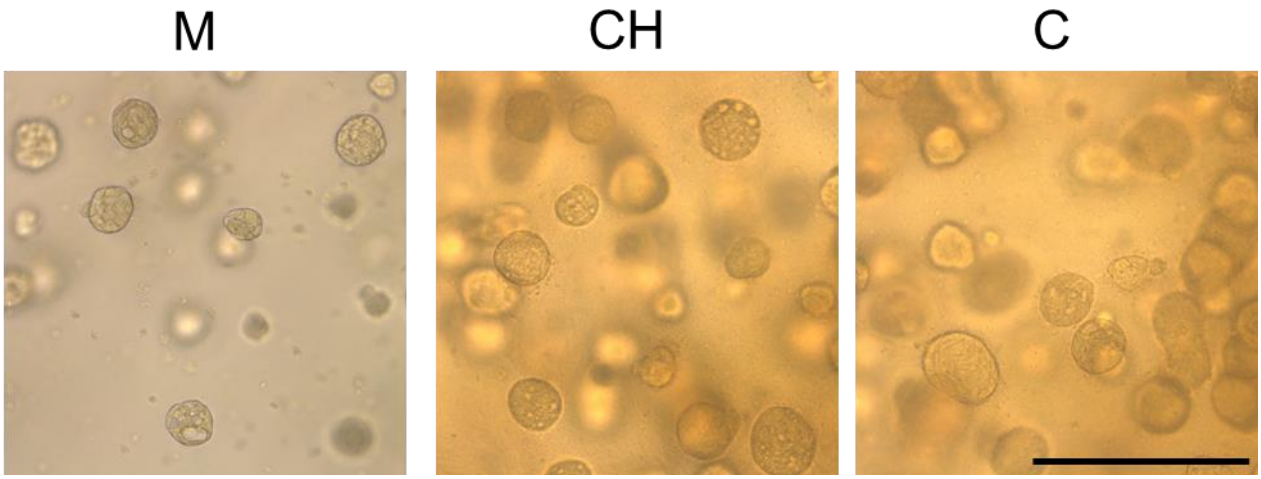

Figure S10. Morphology of 76N TERT breast epithelial cell in different bioinks at day 7. Scale bar 250 µm.

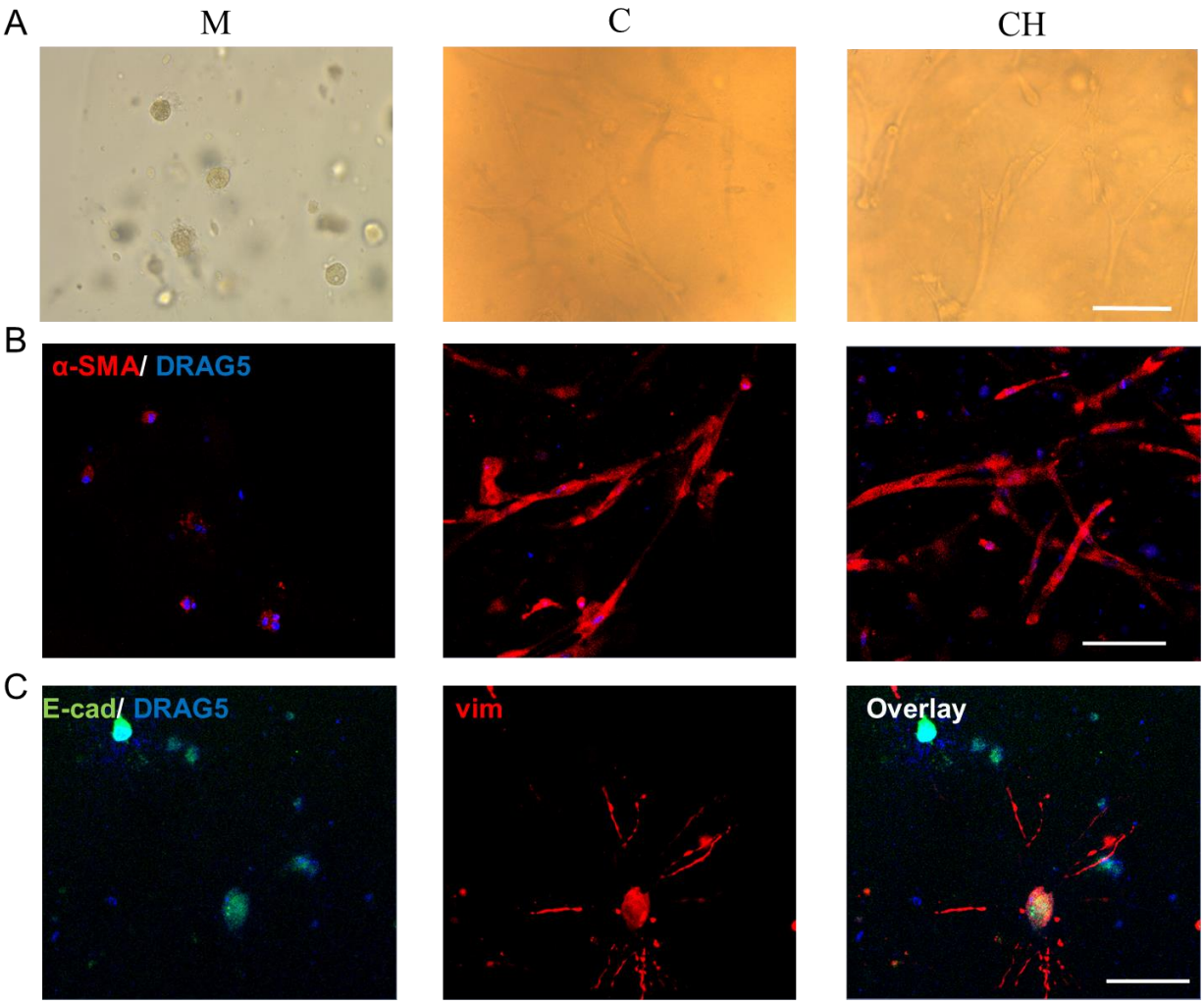

Figure S11. Behavior of cancer associated fibroblast (CAF) cells in different matrix conditions. A&B) Morphology of CAF and immunofluorescent staining of alpha-SMA protein on CAF in different bioink model on day 3. C) Immunofluorescent staining of E-cadherin and vimentin of co-cultured 21PT cells and CAF in the CH bioink on day 7. Scale bar 100 µm

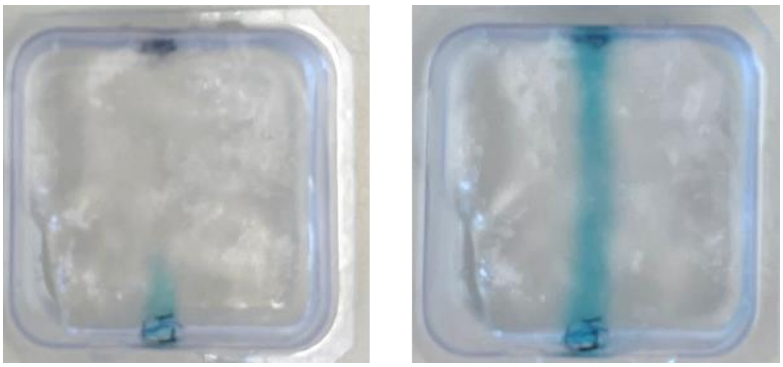

Figure S12. Demonstration of the perfusability of sacrificial F-127 ink printed channel in SF bath.