Supporting Information

Bioprinting the Tumor Microenvironment with an Upgraded Consumer Stereolithographic 3D Printer

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Figure S1: CAD files for the modifications on the printer available on GitHub.

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinting_8xIbidi_plate-holder.stl

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinter_8xPlatform_Template.stl

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinting_Support_platform_Ibidi.stl

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinter_CompressedAirInlet.stl

Figure S2: Picture of the finished bioprinter after adaptations. The changes have been marked with red arrows.

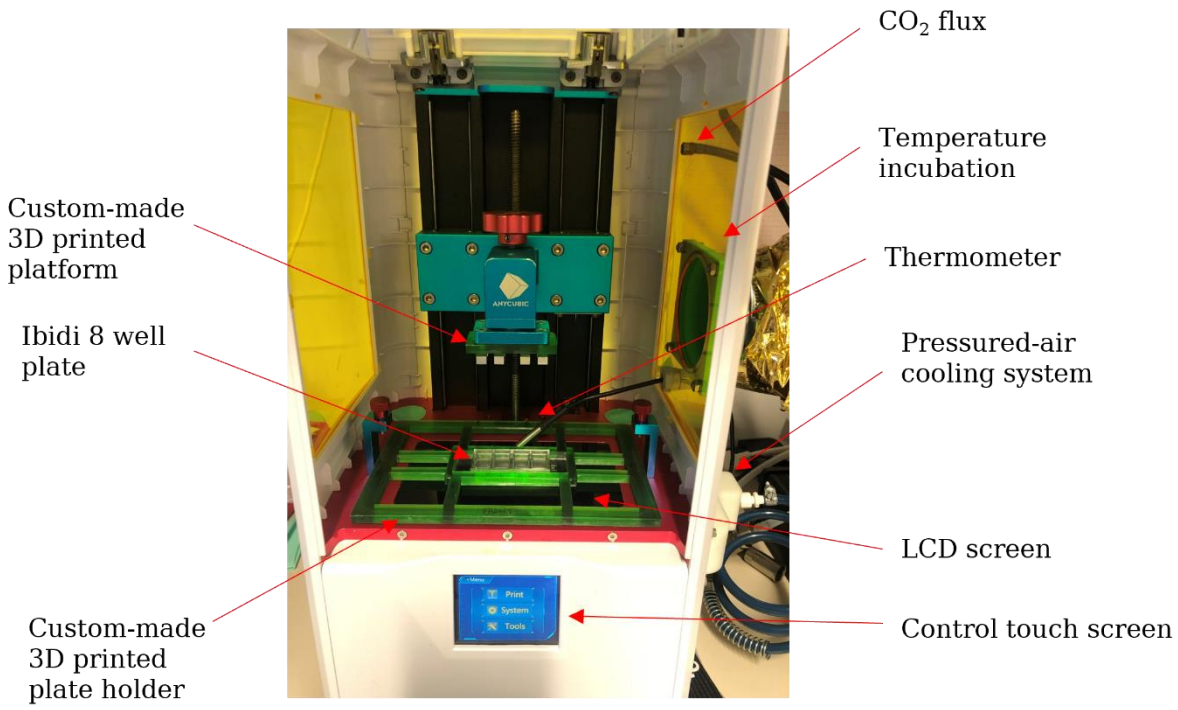

Figure S3: Complete measurements for the rheological analysis available on GitHub.

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinter_Summary_Rheology.xls

Figure S4: Cryo-FIB SEM picture of milling tests on a 3%/3% GelMA/PEGDA hydrogel sample. Squares (20 x 20 µm) were etched with increasing time to reveal deeper regions of the hydrogel and determine the optimal etching time.

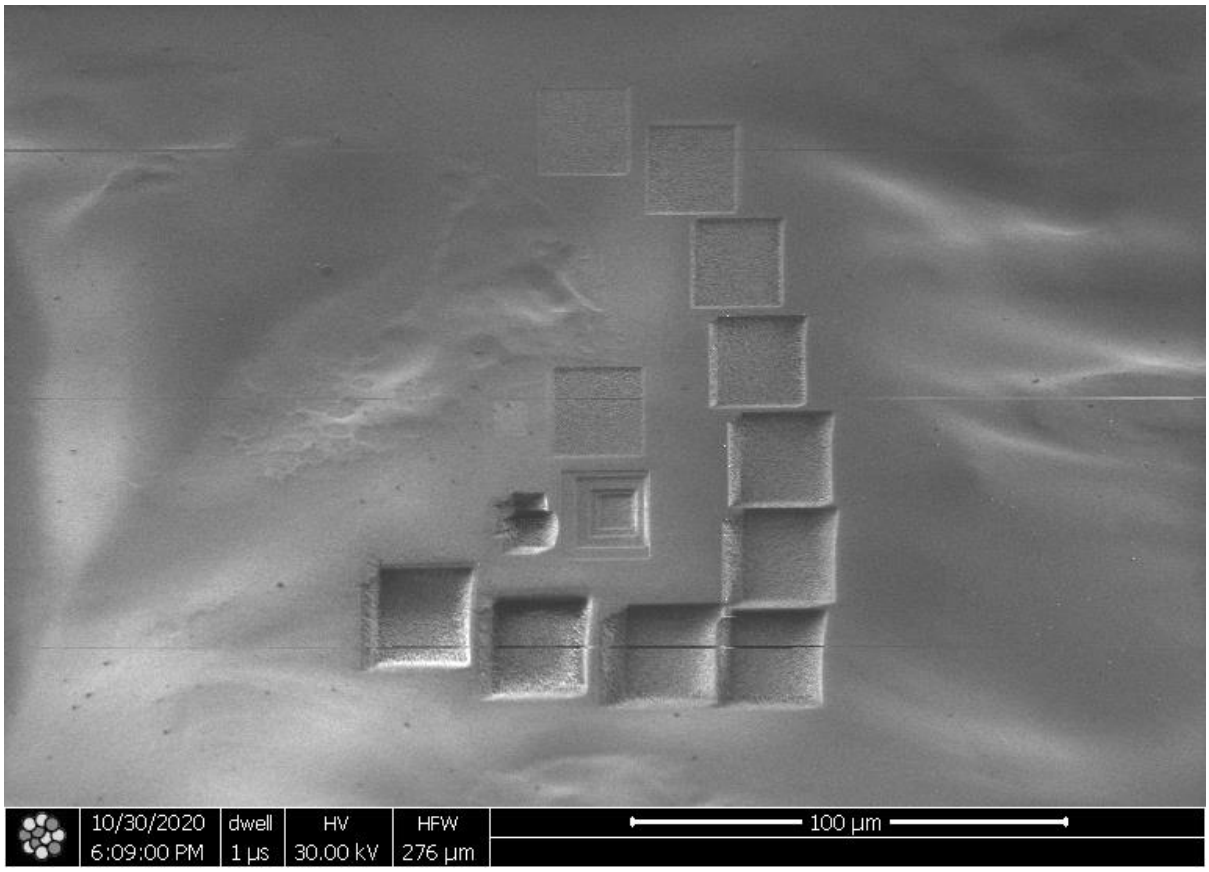

Figure S5: Imaging of CC organoids displaying a compact morphology, taken at three different timepoints during a 7-day timelapse. As a writing convention, first the GelMA concentration was written, then the PEGDA concentration (for example, 3%/1.5% referred to 3% GelMA and 1.5% PEGDA). Microscope: Zeiss Axio Observer Z1. Objective: Plan-Apochromat 5x/0.16. Camera: AxioCam MR R3. Voxel size: 1.29 x 1.29 x 60 µm³. Scale bar: 100 µm.

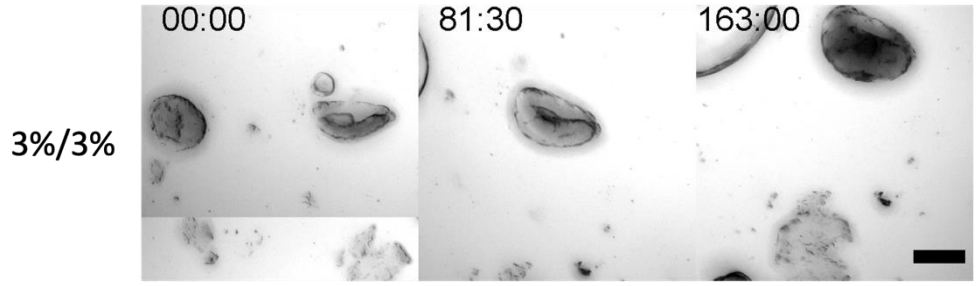

Figure S6: Cell viability of the CC organoids in the bioprinted constructs after 7 days in culture.

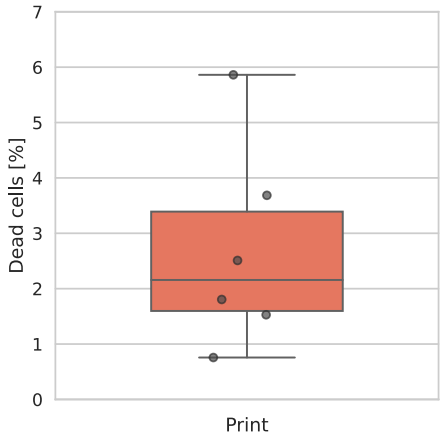

Figure S7: Complete RT-qPCR analysis available on GitHub.

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinting_RT-qPCR_results.xlsx

Figure S8: Additional tumor-related markers were investigated. The variation in the results (upregulation versus downregulation) shows the heterogeneity of the tumor. The samples were normalized to the Matrigel samples. (A) Axin 2 (*AXIN2*) is part of the WNT signaling pathway which is a pharmacological target in cholangiocarcinoma treatments (Boulter et al. 2015, J Clin Invest.). *AXIN2* was found to be upregulated. *TNFRSF19* (TNF Receptor Superfamily Member 19) is part of the TGFβ pathway to promote tumorigenesis (Deng et al. 2017, Cancer Res.). *TNFRSF19* was downregulated. (B) Cystic fibrosis transmembrane conductance regulators (*CFTR)* are an indicator of high fibrosis in diseased tissues (Kim et al. 2002, Dig Dis Sci.). All samples were downregulated but the square one. Epithelial cell adhesion molecule (*EpCAM*) is a prognosis marker in cholangiocarcinoma (Sulpice et al. 2014, J Surg Res.). EpCAM was slightly downregulated compared to Matrigel samples.

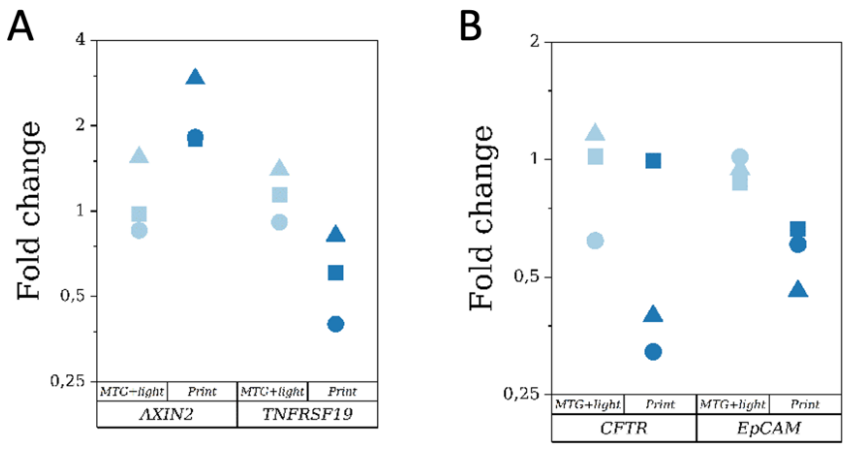

Figure S9: CAD of the platform for the Anycubic Photon S bioprinter for a 96 well-plate available on GitHub. https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/96-well-plate_Anycubic-Bioprinter.stl

Figure S10: List of antibodies and dyes.

Table 1: List of primary antibodies

Antigen	Supplier	Cat. number	Clonality	Origin	Reactivity	Dilution
Caspase 3	Cell Signaling	9661	Polyclonal	Rabbit	Human, mouse, rat, monkey	1:400
Ki67	Abcam	Ab6526	Monoclonal	Rabbit	Human, mouse	1:100
Keratin 19	St John's Laboratory	STJ24355	Polyclonal	Rabbit	Human, mouse, rat	1:100
YAP	Santa Cruz Biotechnology	sc-101199	Monoclonal	Mouse	Human, mouse, rat	1:400

Table 2: List of secondary antibodies

Antigen	Supplier	Cat. number	Origin	Fluorophore	Dilution
Rabbit	Thermo Fisher Scientific	A11008	Goat	Alexa Fluor 488	1:400
Rabbit	Thermo Fisher Scientific	A11011	Goat	Alexa Fluor 546	1:400
Mouse	Thermo Fisher Scientific	A21131	Mouse	Alexa Fluor 488	1:400

Table 3: List of dyes

Dye	Supplier	Cat. number	Dilution
Phalloidin 488	Thermo Fisher Scientific	A12379	1:100
Phalloidin 546	Thermo Fisher Scientific	A22283	1:200
Phalloidin 647	Thermo Fisher Scientific	A22287	1:100
Hoechst 33342	Thermo Fisher Scientific	H1399	1:500

Figure S11: List of primers used for this work available on GitHub.

https://github.com/LouiseBreide/BreidebandSupportingInformation/blob/main/Bioprinter_RT-qPCR_primerslist.xlsx