

**A high-throughput 3D bioprinted cancer cell migration and invasion model with
versatile and broad biological applicability**

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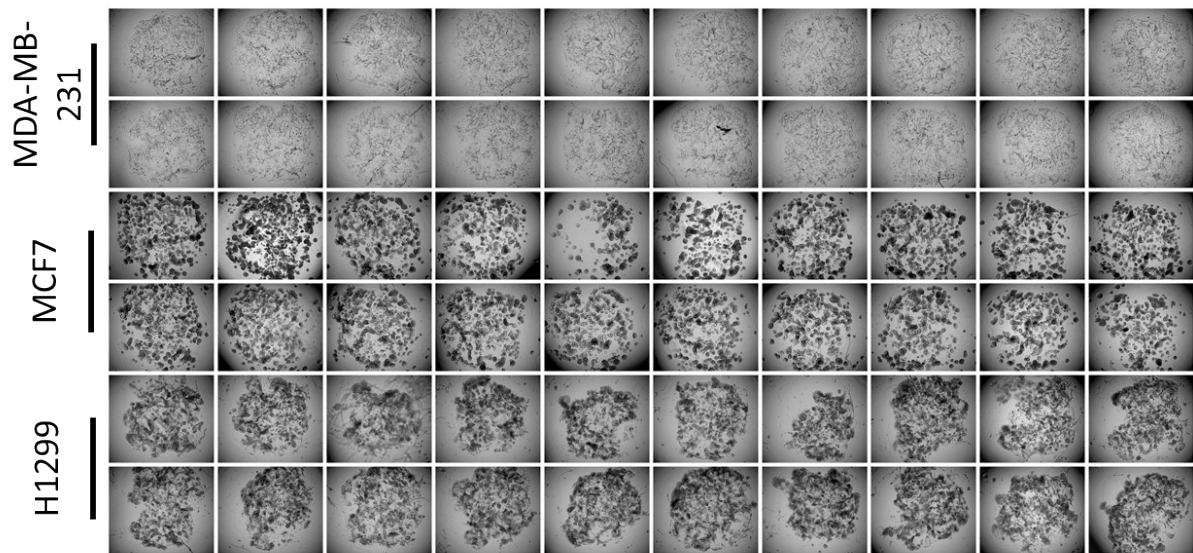
Supplementary data

Supplementary Figure 1.

Supplementary Figure 2.

Movies

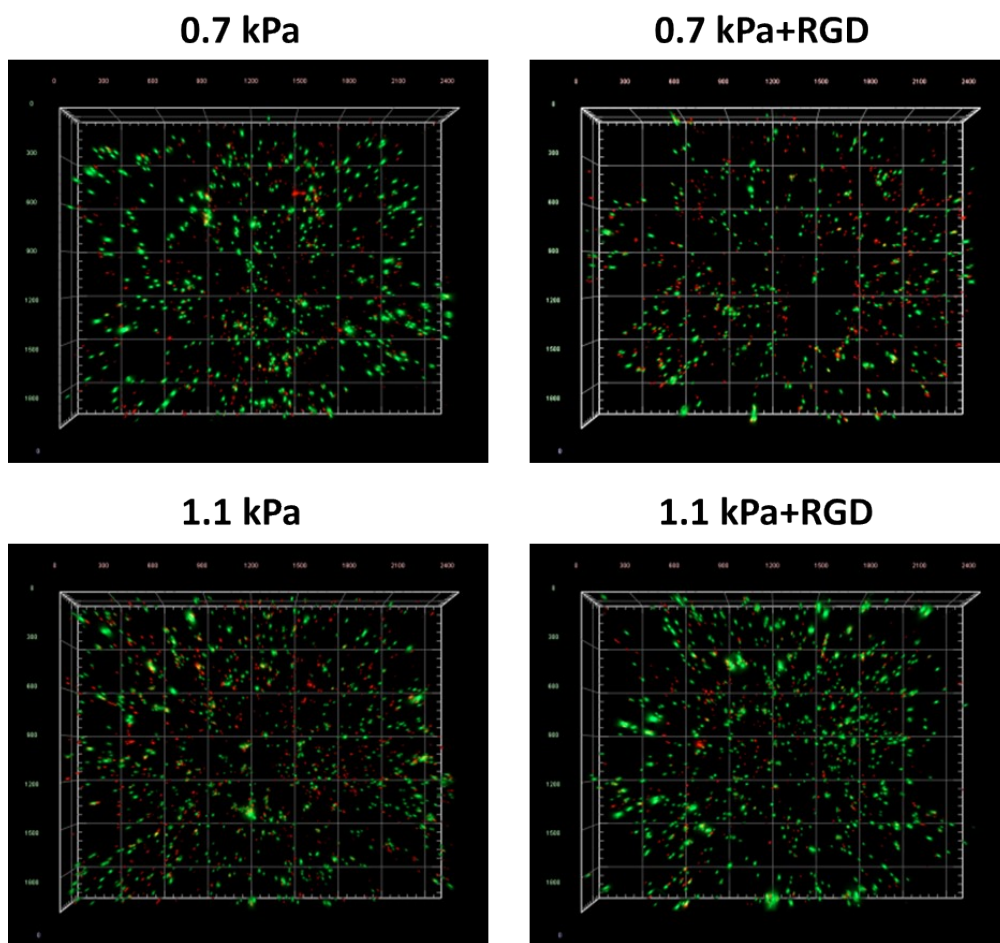
Supplementary data



Supplementary Figure 1. Generation of multiple cell models in a 96 well plate

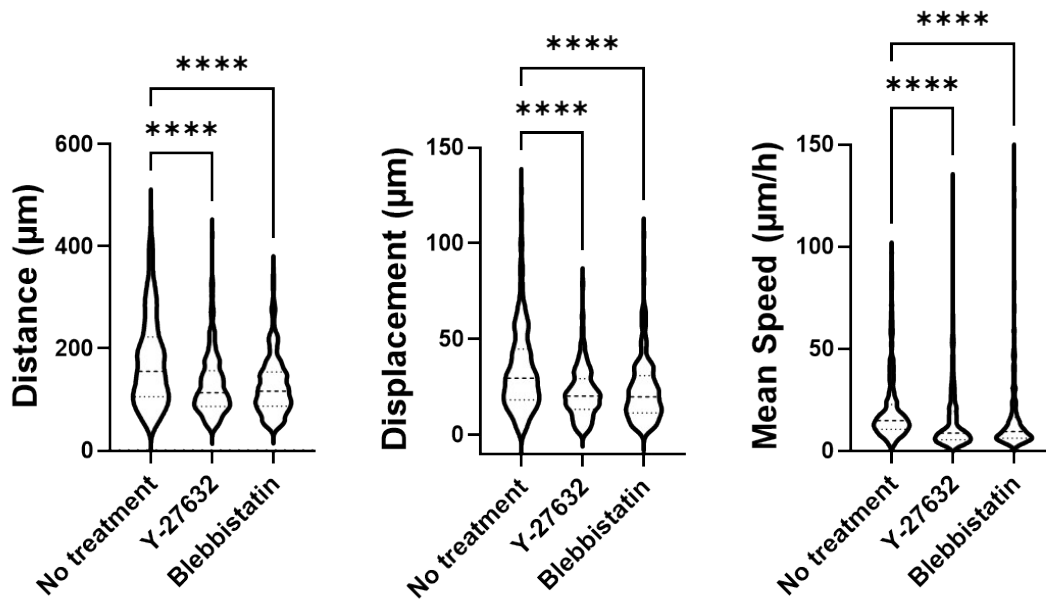
The HTP 3D bioprinting platform allows to print up to 3 different cancer models in a multi-well plate. MDA-MB-231, MCF7 and H1299 cells were bioprinted with 0.7 kPa+RGD hydrogels in the inner 60 wells of a 96 well plate. The plate was incubated at 37 °C for 7 days. Bright-field images were taken at a single plane (5X objective).

MCF7 cells at Day 0 (2 h post-printing)



Supplementary Figure 2. Cell viability of MCF7 bioprinted cells at Day 0 post-printing

MCF7 cancer cells were bioprinted with each hydrogel combination (0.7 kPa±RGD or 1.1 kPa±RGD) in 96 well plates and incubated for 2 h at 37 °C. Cells were stained with calcein-AM (green; live)/ethidium homodimer (red; dead) Live/Dead Assay and z-stack 3D images were taken at day 0 post-printing (5X objective).



MDA-MB-231 in 0.7kPa+RGD gels	No Treatment	Y-27632	Blebbistatin
Number of cells	358	300	326
Median Distance (µm)	155.5	113.9	117.0
Median Displacement (µm)	29.59	20.18	19.86
Median Mean Speed (µm/h)	14.75	8.741	9.484

Kruskal-Wallis test ****P<0.0001

Supplementary Figure 3. Chemical inhibitors of MDA-MB-231 cell migration within 0.7 kPa+RGD hydrogels

MDA-MB-231 cells were bioprinted in 0.7 kPa+RGD hydrogels. Either Y-27632 (a ROCK inhibitor) or Blebbistatin (a global myosin inhibitor) were treated in different wells in a 96 well plate. 3D cell movement of MDA-MB-231 cells in the absence or presence of the inhibitors was compared and quantitated. Kruskal–Wallis one-way analysis with a post hoc Dunn test was performed. ****P<0.0001

Movies.

[Movie 1.](#) MCF7 in Matrigel

[Movie 2.](#) MDA-MB-231 in Matrigel

[Movie 3.](#) MCF7 in a 0.7 kPa bioprinted gel

[Movie 4.](#) MDA-MB-231 in a 0.7 kPa+RGD bioprinted gel

[Movie 5.](#) MDA-MB-231 tracking in 1.1 kPa RGD_No Treatment

[Movie 6.](#) MDA-MB-231 tracking in 1.1 kPa RGD_Blebbistatin

[Movie 7.](#) MDA-MB-231 tracking in 1.1 kPa RGD_ROCK inhibitor

[Movie 8.](#) H1299 tracking in 0.7 kPa RGD_No Treatment

[Movie 9.](#) H1299 tracking in 0.7 kPa RGD_ROCK inhibitor

[Movie 10.](#) H1299 tracking in 0.7 kPa RGD_Blebbistatin