Electronic Supplementary Material (ESI) for Lab on a Chip.

Electronic Supporting Information (ESI)

Surface-tension driven open microfluidic platform for hanging droplet culture

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This Electronic Supporting Information includes:

(1) 3D model files for both the hanging well device used for cell culture and modeling as well as OmniTray insert used for holding and hydrating the device during culture.

(2) Files used for creating the computational model of fluid transfer and distribution in the device.

(3) Schematic of humidification setup for device to prevent fluid evaporation.

(4) Live/dead epifluorescence images of MDA-MB-231 cells at normoxic and hypoxic conditions imaged in the droplet.

(5) Average concentration of solute in sections of the device over 42 hours.

(6) ESI references.

(1) 3D Model files used for design and fabrication of devices and device holders (Model S1, S2, S3, and S4):
The model design for each model was created in Solidworks 2014. Models S1 and S2 are the hanging well device used in the experiment attached to the ESI as .SLDPRT and .IGS file types, respectively. Files S3 and S4 are the OmniTray inserts used for holding and humidifying the devices during cell culture attached to the ESI as .SLDPRT and .IGS file types, respectively.

(2) Files used for creating the computational model of fluid transfer and distribution in the device (File S1):
Fluid in the device was computationally modeled using MATLAB and saved as .m files. File S1 is a zipped file containing the .m files use to create the model. The file "Main_V03.m" contains the parameters used in the model including the physical parameters of the device.

(3) Schematic of humidification setup for device to prevent fluid evaporation during cell culture (Figure S1):

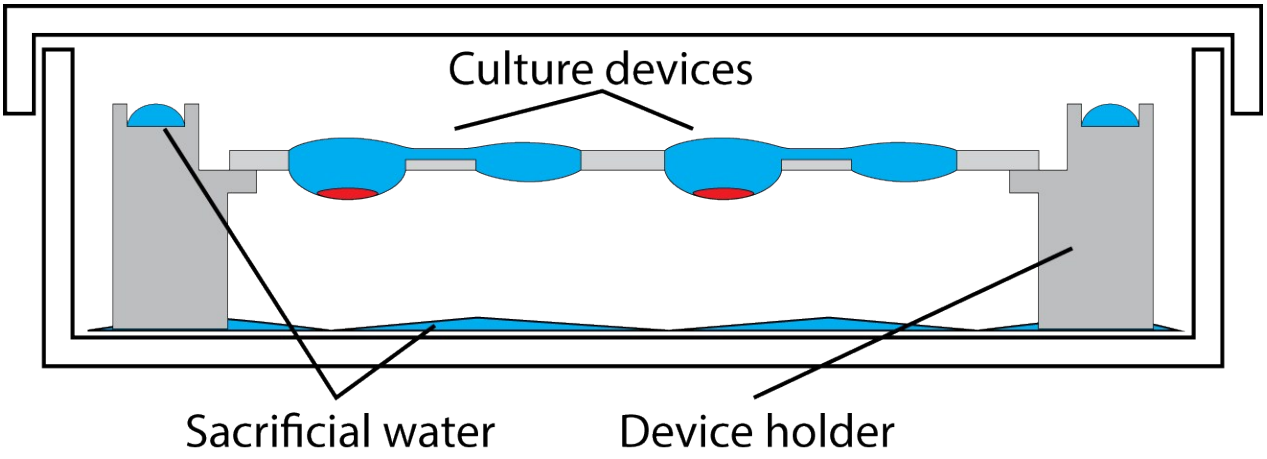

Figure S1. The device holder is placed in an OmniTray (Nunc) water is added to the bottom of the OmniTray and to the channel features of the device holder. The device is then placed into the device holder and filled with media and cells. The lid is placed on the OmniTray to ensure proper humidification of the device to prevent evaporation.

(4) Live/dead epifluorescence images of MDA-MB-231 cells at normoxic and hypoxic conditions imaged in the droplet.

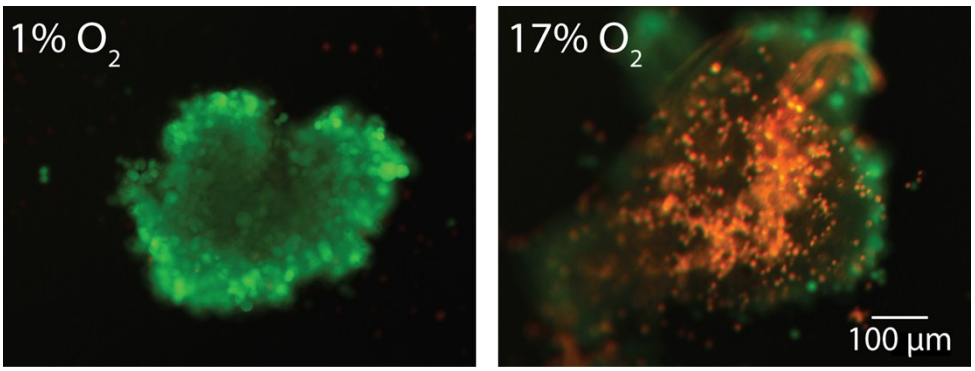

Figure S2. 10,000 MDA-MB-231 cells were seeded into the culture well of the device and were cultured at either normoxic or hypoxic conditions for 48 hours before imaging. Live cells (green) were strained with calcein AM and dead cells were strained with ethidium homodimer (red). Spheroids were extracted from the device and transferred to a well plate for imaging. The hypoxic condition formed a markedly smaller spheroid with no low-viability core, while the normoxic condition formed a spheroid with a low-viability, hypoxic core typical of what has been previously observed[1,2].

(5) Average concentration of solute in sections of the device over 42 hours.

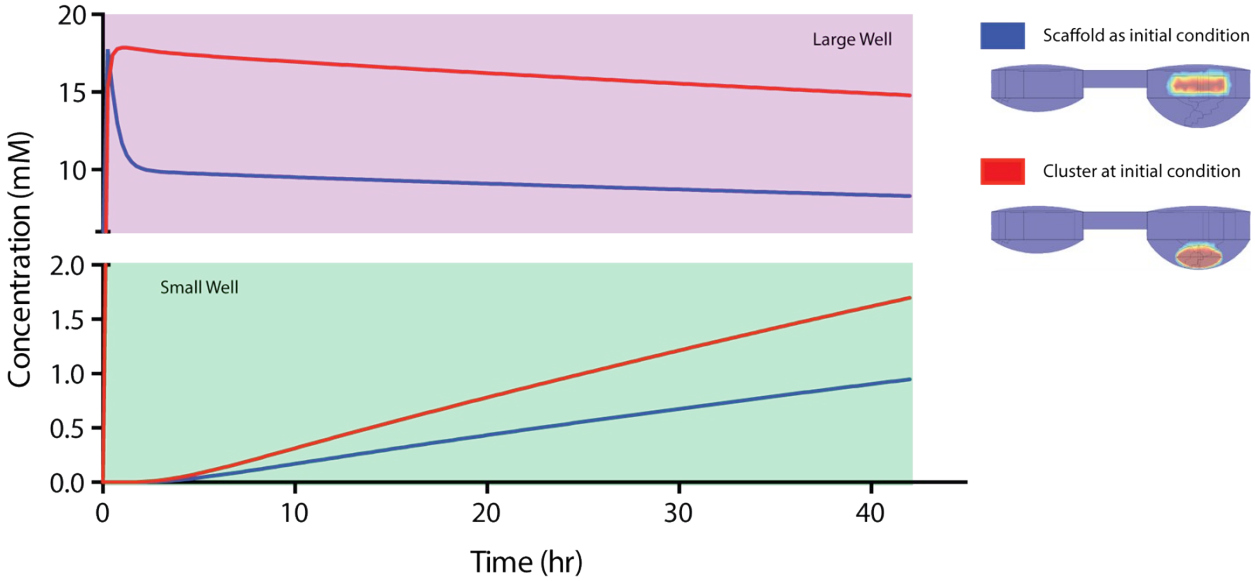

Figure S3. The average concentration in the bottom meniscus of each well following solute release in a modeled bone-like scaffold or cluster in the culture well.

(6) ESI References

1. M. T. Santini and G. Rainaldi, *Pathobiology*, 1999, **67**, 148–57.
2. D. R. Grimes, C. Kelly, K. Bloch and M. Partridge, *J. R. Soc. Interface*, 2014, **11**, 20131124.