

Supporting Information

Metabolic Response of Lung Cancer Cells to Radiation in a Paper-Based 3D Cell Culture System

Karen A. Simon¹, Bobak Mosadegh^{1,2,3}, Kyaw Thu Minn¹, Matthew R. Lockett^{1,4}, Marym R. Mohammady¹, Diane M. Boucher⁵, Amy B. Hall⁵, Shawn Hillier⁵, Taturo Udagawa⁵,
Brenda K. Eustace^{5*} and George M. Whitesides^{1,2*}

¹ Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA.

² Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford Street, Cambridge, MA 02138, USA.

³ Dalio Institute of Cardiovascular Imaging. Department of Radiology, Weill Cornell Medical College, 413 E. 69th Street Suite BRB-108, New York, NY, 10021, USA

⁴ Department of Chemistry, University of North Carolina at Chapel Hill, 125 South Road, Chapel Hill, NC 27599, USA

⁵ Vertex Pharmaceuticals Incorporated, 50 Northern Blvd., Boston, MA, 02210 USA

(*) Author to whom correspondence should be addressed:

gwhitesides@gmwgroup.harvard.edu

brenda_eustace@vrtx.com

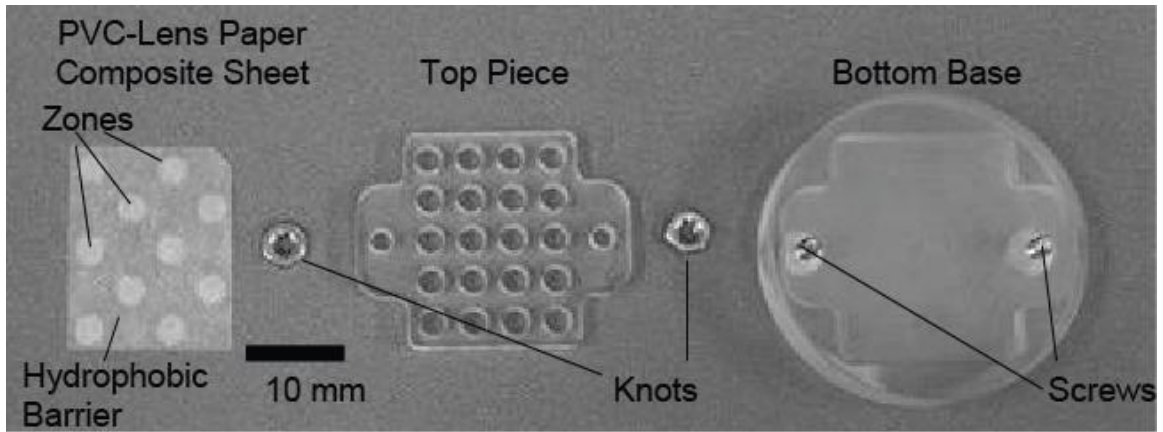


Figure S-1. Images of the PVC-lens paper composite sheet and PMMA holders for multi-layer 3D culture

Table S-1a. Number of Cells/Zone in Non-irradiated Multi-Layer Cultures^{a,b}

0 Gy	0 Gy_Stack 1	0 Gy_Stack 2	0 Gy_Stack 3	0 Gy_Mean	0 Gy_Stdev	0 Gy_Std Error	Mean > (2×Std Error) ?
L1	1.95E+05	1.76E+05	1.92E+05	1.88E+05	1.03E+04	5.95E+03	YES
L2	7.06E+04	6.38E+04	7.91E+04	7.11E+04	7.68E+03	4.44E+03	YES
L3	4.33E+04	4.42E+04	4.56E+04	4.44E+04	1.16E+03	6.67E+02	YES
L4	2.60E+04	2.37E+04	2.65E+04	2.54E+04	1.51E+03	8.72E+02	YES
L5	8.93E+03	2.22E+04	1.01E+04	1.37E+04	7.33E+03	4.23E+03	YES
L6	1.52E+03	1.99E+04	3.20E+03	8.21E+03	1.02E+04	5.88E+03	NO

Table S-1b. Number of Cells/Zone in Irradiated Multi-Layer Cultures^{a,b}

8 Gy	8 Gy_Stack 1	8 Gy_Stack 2	8 Gy_Stack 3	8 Gy_Mean	8 Gy_Stdev	8 Gy_Std Error	Mean > (2×Std Error) ?
L1	1.01E+05	1.06E+05	1.02E+05	1.03E+05	2.72E+03	1.57E+03	YES
L2	2.88E+04	4.62E+04	3.15E+04	3.55E+04	9.34E+03	5.39E+03	YES
L3	2.13E+04	3.68E+04	2.62E+04	2.81E+04	7.95E+03	4.59E+03	YES
L4	2.19E+04	2.62E+04	2.29E+04	2.37E+04	2.26E+03	1.30E+03	YES
L5	1.28E+04	2.03E+04	1.33E+04	1.54E+04	4.22E+03	2.44E+03	YES
L6	1.11E+04	1.32E+04	1.21E+04	1.21E+04	1.03E+03	5.95E+02	YES

^a Analyzed 12 days after seeding (i.e., 1 day in culture as separate layers + 4 days in culture as stacks + 7 days in culture after irradiation)

^b Values obtained using a calibration curve

Table S-2a. CTG Luminescence in Multi-Layer Cultures of A549 Cells (0 Gy)^a

A549	0 Gy Stack 1	0 Gy Stack 2	0 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	7.29E+03	6.60E+03	7.17E+03	7.02E+03	3.71E+02	2.14E+02	YES
L2	2.80E+03	2.55E+03	3.10E+03	2.82E+03	2.77E+02	1.60E+02	YES
L3	1.82E+03	1.85E+03	1.90E+03	1.85E+03	4.16E+01	2.40E+01	YES
L4	1.19E+03	1.11E+03	1.21E+03	1.17E+03	5.44E+01	3.14E+01	YES
L5	5.78E+02	1.05E+03	6.20E+02	7.51E+02	2.64E+02	1.52E+02	YES
L6	3.11E+02	9.74E+02	3.71E+02	5.52E+02	3.66E+02	2.12E+02	YES

Table S-2b. CTG Luminescence in Multi-Layer Cultures of A549 (8 Gy)^a

A549	8 Gy Stack 1	8 Gy Stack 2	8 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	3.91E+03	4.08E+03	3.92E+03	3.97E+03	9.79E+01	5.65E+01	YES
L2	1.29E+03	1.92E+03	1.39E+03	1.53E+03	3.36E+02	1.94E+02	YES
L3	1.02E+03	1.58E+03	1.20E+03	1.27E+03	2.86E+02	1.65E+02	YES
L4	1.05E+03	1.20E+03	1.08E+03	1.11E+03	8.12E+01	4.69E+01	YES
L5	7.16E+02	9.88E+02	7.34E+02	8.12E+02	1.52E+02	8.77E+01	YES
L6	6.57E+02	7.31E+02	6.91E+02	6.93E+02	3.71E+01	2.14E+01	YES

Table S-2c. CTG Luminescence in Multi-Layer Cultures of A549-HGF (0 Gy)^a

A549-HGF	0 Gy Stack 1	0 Gy Stack 2	0 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	6.02E+03	6.06E+03	7.15E+03	6.41E+03	6.38E+02	3.68E+02	YES
L2	1.50E+03	1.48E+03	1.48E+03	1.48E+03	1.01E+01	5.84E+00	YES
L3	9.07E+02	9.32E+02	6.86E+02	8.42E+02	1.36E+02	7.84E+01	YES
L4	5.90E+02	5.80E+02	5.14E+02	5.61E+02	4.11E+01	2.37E+01	YES
L5	5.71E+02	5.36E+02	4.41E+02	5.16E+02	6.75E+01	3.90E+01	YES
L6	5.86E+02	5.52E+02	4.66E+02	5.35E+02	6.22E+01	3.59E+01	YES

Table S-2d. CTG Luminescence in Multi-Layer Cultures of A549-HGF (8 Gy)^a

A549-HGF	8 Gy Stack 1	8 Gy Stack 2	8 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	4.51E+03	5.60E+03	5.54E+03	5.22E+03	6.14E+02	3.54E+02	YES
L2	1.22E+03	1.64E+03	2.20E+03	1.69E+03	4.93E+02	2.85E+02	YES
L3	7.11E+02	1.05E+03	1.39E+03	1.05E+03	3.38E+02	1.95E+02	YES
L4	5.52E+02	8.48E+02	9.82E+02	7.94E+02	2.20E+02	1.27E+02	YES
L5	5.06E+02	6.48E+02	9.75E+02	7.09E+02	2.40E+02	1.39E+02	YES
L6	4.33E+02	6.57E+02	7.29E+02	6.07E+02	1.54E+02	8.91E+01	YES

Table S-2e. CTG Luminescence in Multi-Layer Cultures of A549-HGF-M (0 Gy)^a

A549-HGF-M	0 Gy Stack 1	0 Gy Stack 2	0 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	7.11E+03	7.10E+03	8.41E+03	7.54E+03	7.53E+02	4.34E+02	YES
L2	1.40E+03	1.40E+03	1.86E+03	1.55E+03	2.68E+02	1.55E+02	YES
L3	7.46E+02	5.98E+02	8.83E+02	7.42E+02	1.43E+02	8.23E+01	YES
L4	5.20E+02	5.47E+02	6.30E+02	5.66E+02	5.73E+01	3.31E+01	YES
L5	5.03E+02	3.87E+02	5.19E+02	4.70E+02	7.22E+01	4.17E+01	YES
L6	4.14E+02	4.42E+02	5.79E+02	4.78E+02	8.81E+01	5.09E+01	YES

Table S-2f. CTG Luminescence in Multi-Layer Cultures of A549-HGF-M (8 Gy)^a

A549-HGF-M	8 Gy Stack 1	8 Gy Stack 2	8 Gy Stack 3	Mean	Stdev	Std Error	Mean > (2×Std Error) ?
L1	7.06E+03	7.29E+03	8.42E+03	7.59E+03	7.27E+02	4.20E+02	YES
L2	2.12E+03	1.43E+03	1.86E+03	1.80E+03	3.49E+02	2.02E+02	YES
L3	1.06E+03	6.93E+02	9.12E+02	8.87E+02	1.83E+02	1.05E+02	YES
L4	7.01E+02	6.04E+02	6.26E+02	6.44E+02	5.11E+01	2.95E+01	YES
L5	6.47E+02	5.40E+02	5.74E+02	5.87E+02	5.49E+01	3.17E+01	YES
L6	5.27E+02	5.31E+02	7.51E+02	6.03E+02	1.28E+02	7.41E+01	YES

^a Analyzed 12 days after seeding (i.e., 1 day in culture as separate layers + 4 days in culture as stacks + 7 days in culture after irradiation)

Table S-3. Antibodies Used for Immunoblotting

Antibody	Reactivity	Molecular Weight (kDa)	Sensitivity	Isotype	Manufacturer
Hydroxy-HIF-1 α (Pro 564) Rabbit mAb	Human	120	Monoclonal	Rabbit	Cell Signalling Technologies
GAPDH Rabbit mAb	Human, Mouse, Rat, Monkey, Bovine	37	Monoclonal	Rabbit	Cell Signalling Technologies
Carbonic Anhydrase 9	Human	58	Polyclonal	Goat	R&D Systems
IR Dye® 680 Donkey anti-Rabbit IgG (H+L)	Rabbit	unknown	Polyclonal	Donkey	LICOR
IR Dye® 800 Donkey anti-Goat IgG (H+L)	Goat	unknown	Polyclonal	Donkey	LICOR