Electronic Supplementary Material (ESI) for Lab on a Chip. This journal is © The Royal Society of Chemistry 2016

Title:

Microfluidics 3D gel-island chip for single cell isolation and lineage-dependent drug responses study

Authors and affiliations:

Zhixiong Zhang^{a^}, Yu-Chih Chen^{a, c^}, Yu-Heng Cheng^a, Yi Luan^a, and Euisik Yoon*^{a, b} ^aDepartment of Electrical Engineering and Computer Science, University of Michigan, 1301 Beal Avenue, Ann Arbor, MI 48109-2122; ^bDepartment of Biomedical Engineering, University of Michigan, 2200 Bonisteel, Blvd. Ann Arbor,

^oDepartment of Biomedical Engineering, University of Michigan, 2200 Bonisteel, Blvd. Ann Arbor, MI 48109-2099, USA

^cUniversity of Michigan Comprehensive Cancer Center, 1500 E. Medical Center Drive, Ann Arbor, MI 48109, USA

^Authors made equal contributions *Corresponding author

Zhixiong Zhang 1301 Beal Avenue, Ann Arbor, MI 48109-2122, USA Tel: 734-353-0400; E-mail: zhangzx@umich.edu.

Euisik Yoon 1301 Beal Avenue, Ann Arbor, MI 48109-2122, USA Tel: 734-615-4469; E-mail: esyoon@umich.edu.

Supplementary Figures





Figure S1. Identification of T47D Notch+ and Notch- Cells. (a) An example view under microscope. Cell area was automatically detected by Nikon Research Basics software (marked as red). And mean fluorescent intensity of certain cells were measured. (b) Distribution of cell numbers between relative fluorescent intensity ranges. Cells with relative fluorescent intensity less than 400 a.u. are recognized as Notch- cells, while those larger than 400 a.u. are recognized as Notch+ cells. (Scale bar: 100 μ m)



Figure S2. Drug susceptibility test of T47D Notch+ breast cancer cells in 96-well microtiter plate. T47D cells were cultured in 3D collagen gels for 2 days, followed by drug treatment (Doxorubicin, Cisplatin) for 3 days. Cells were stained with Live/Dead dye before taking microscopy images. Cell culture media or drug was exchanged every day: (a) Cell viability after Doxorubicin (red) or Cisplatin (blue) treatment at different concentration. (b) Culture media control. (c) Doxorubicin $0.1\mu M$. (c) Doxorubicin $0.1\mu M$. (d) Doxorubicin $0.3\mu M$. (e) Doxorubicin $0.5\mu M$. (f) Doxorubicin $1\mu M$. (g) Cisplatin 1uM. (h) Cisplatin 10uM. (i) Cisplatin 50uM. (j) Cisplatin 250uM. (Scale bar: $100 \mu m$)



Figure S3. Comparison of fluorescent signal intensity between GFP (Green Fluorescent Protein) and Live dye: (a) T47D Notch+ (GFP) image without live dye staining under 12ms fluorescent light source exposure. (b) T47D Notch+ (GFP) image without live dye staining under 1s fluorescent light source exposure. (c) T47D Notch+ (GFP) image after live dye staining under 12ms fluorescent light source exposure. (d) Relative fluorescent intensity comparison between with and without live dye staining under 12ms fluorescent light source exposure (N=10). *** refers to P < 0.001. (Scale bar: 100 µm)



Figure S4. Diffusion test using dextran-conjugated fluorescein to verify conformal mass transfer inside culture chambers: (a) measurement of relative fluorescent intensity at six different region inside culture chambers, (b) Relative fluorescent intensity changes in six different regions inside culture chambers (N=5). (Scale bar: 50 µm)



Figure S5. MDA-MB-231 and T47D breast cancer cells cultured in conventional petri-dish for 7 days: (a) MDA-MB-231. (b) MDA-MB-231. (c) T47D bulk. (d) T47D bulk. (Scale bar: 100 µm)



Figure S6. Distribution of Notch reporter gene fluorescent intensity ratio between 48h (day2) and 6h (day0) after cell loading under 2 culture conditions: 3D collagen gels (black, N=209), and 2D culture media (red, N=116). * refers to P < 0.05, *** refers to P < 0.001.



Figure S7. Examples of cell status its drug response (the green fluorescence in day0 and day2 images is from GFP, the green/red fluorescence in day5 is from Live/Dead dye). (Scale bar: $50 \mu m$)

3D_Day0	3D_Day2	Fluorescent	2D_Day0	2D_Day2	Fluorescent
		Intensity Ratio			Intensity Ratio
547.82	712.34	1. 300318	102.13	99.85	0.977676
1700.86	1845.7	1.085157	228.94	160.39	0. 700577
682.66	1367.01	2.002476	73.21	67.87	0.927059
128	315.54	2.465156	965.38	1741.72	1.804181
202.19	796.97	3. 941689	1160.69	1743.58	1. 502193
357.16	667.55	1.86905	961.17	1418.66	1. 475972
83.63	354.98	4.244649	862.82	895.82	1.038247
486.17	1584.84	3. 259847	609.12	234.62	0.385179
296	284	0.959459	611.85	475.24	0. 776726
663.57	1001.07	1.508613	449.71	375.46	0.834894
333.56	450.33	1.350072	368.23	442.92	1. 202835
751.32	1190.53	1.584584	1086.01	843.97	0. 777129
281.84	245.57	0.87131	342.56	501.56	1.464152
509.38	1195.57	2.347108	990.18	600.11	0. 606062
487.82	394.86	0.809438	1671.77	2081.21	1.244914
230.69	399.94	1.733669	873.41	2016.82	2. 309133
1368.99	1516.16	1.107503	893.9	669.51	0.748976
654.36	1519.32	2. 321841	744.06	1025.32	1.378007
87.83	448.26	5. 103723	1108.2	1625.37	1.466676
134.6	116.35	0.864413	1794.37	2257.45	1.258074
916.51	1635	1. 783941	1467.95	696.15	0. 474233
542.65	947.04	1.745213	710.97	654.89	0.921122
686.61	1779.89	2. 592287	411.41	288.53	0.70132
1063.19	1516.09	1. 425982	182.04	118.72	0.652164
893.18	1077.75	1.206644	933.97	1089.53	1.166558
72.87	160.73	2.205709	889.91	1240.51	1. 393972
95.28	435.51	4. 570844	895.74	1075.31	1. 200471
1118.7	1353.3	1.209708	102.58	683.58	6. 663872
100.54	605.74	6.024866	202.58	134.44	0. 663639
525.49	149.84	0. 285143	481.68	1536.47	3. 189815
731.81	1941.24	2.652656	282.4	263.17	0. 931905
878.75	1665.64	1.895465	505	622.07	1.231822
580.75	811.02	1.396505	535.13	595.26	1.112365
2293.97	2329.82	1.015628	562.09	590.5	1.050544
634.97	904.52	1. 424508	116.27	214.3	1.843124
295.36	475.82	1.610983	710.19	749.69	1.055619
609.8	1414.15	2. 319039	960.04	1150.16	1. 198033

Table S1.Raw data of Notch reporter gene fluorescent intensity ratio between 48h (day2) and 6h(day0) after cell loading under 3D or2D culture conditions.

43.81	91.93	2.098379	546.96	521.37	0.953214
653.22	1426.16	2. 183277	229.42	580. 52	2. 530381
1184.27	2057.25	1.737146	704.89	1662.25	2.358169
1182.54	2059.47	1.741565	1392.09	2137.64	1.535562
440.08	657.26	1. 493501	1341.6	2010. 89	1.498874
680.45	1421.39	2.088897	790.72	415.29	0. 525205
236.04	408.69	1.731444	665.1	827.18	1.243693
403.38	492.84	1.221776	562.92	525.6	0. 933703
100.43	298	2.967241	1156.24	895.85	0. 774796
372.5	721.13	1.935919	772.69	831.64	1.076292
200. 38	292.83	1. 461373	830.46	1766.39	2. 127002
1620.45	1526.25	0.941868	252.72	277.06	1.096312
370.44	746.77	2.0159	896.74	537.79	0. 599717
745.92	1494.17	2.003124	1713.54	1845.59	1.077063
468.58	1132.16	2. 416151	778.29	478.85	0.615259
674.85	1119.38	1.658709	77.29	346.15	4. 478587
56.63	22.97	0. 405615	986.6	389.73	0. 395023
442.48	729.08	1.647713	566.14	1439.09	2. 541933
462.18	472.3	1.021896	692.88	505.03	0. 728885
124.68	354.57	2.84384	330.54	841.23	2. 545017
185.84	303.8	1.63474	434.25	352.95	0.812781
728.86	1165.63	1.599251	879.31	1175.79	1. 337173
221.65	237.61	1.072005	425.46	1648.5	3. 87463
443.27	1228.32	2.771042	49.52	190.11	3. 839055
205.03	714.04	3. 482612	567.03	427.9	0.754634
872.91	973.11	1.114788	952.65	1822.56	1.913148
818.62	1458.52	1. 781681	981.59	2140.99	2. 181145
427.7	951.99	2.225836	263.27	307.73	1.168876
692.02	901.81	1.303156	629.05	511.99	0.81391
188.95	665.31	3. 52109	280.06	300.88	1.074341
325.61	451.65	1. 387089	746.34	1192.5	1. 597797
364.77	600.94	1.647449	1073.51	1933.12	1.800747
731.16	1192.78	1.631353	625.24	1084.08	1.733862
1322.84	1224.38	0. 925569	562.05	554.28	0.986176
557.78	1365.91	2. 448833	469.08	679.79	1. 449198
371.42	796.66	2. 144903	1935.84	2159.04	1.115299
93.57	298.63	3. 191514	1098.23	1316.84	1. 199057
304.6	895.64	2. 940381	249.74	129.8	0. 519741
1258.34	1862.86	1. 480411	854.7	936.51	1.095718
873.2	1385.36	1.586532	626.61	1235.7	1.97204
711.19	1127.44	1. 585287	539.49	333.14	0. 617509
990.41	1201.63	1.213265	1398.27	1634.42	1.168887
1134.9	1321.57	1.164481	1845.31	2025.3	1.097539

183.35	861.75	4. 700027	690.19	1461.71	2. 117837
906.23	1694.7	1.870055	762.79	964.49	1. 264424
296.77	376.4	1.268322	105.12	514.35	4. 892979
603.35	881.99	1.461821	856.04	119.45	0. 139538
896.08	1465.63	1.635602	717.63	1190.74	1.659267
960.29	1164.23	1.212373	665.93	607.85	0.912784
253.92	460.65	1.814154	1210.32	2126.72	1.757155
260.82	593.58	2.275822	386.18	790.56	2.047128
588.81	614.06	1.042883	241.43	294.47	1.219691
314.85	975.89	3. 099539	907.48	893.47	0.984562
547.48	412.68	0. 753781	315.58	594.5	1.883833
666.2	848.97	1.274347	649.14	445.45	0.686216
361.97	645.45	1. 783159	969.45	2176.41	2.244995
517.53	265.96	0. 513903	709.49	986.4	1. 390294
252.8	451.92	1. 787658	1035.11	308.13	0. 297679
563.58	1928.98	3. 422726	830.48	1677.46	2.019868
227.37	417.05	1.834235	119.07	111.52	0. 936592
1108.12	1802.86	1.626954	680.14	547.57	0.805084
601.23	1316.09	2. 188996	964.26	758.34	0.786448
617.47	1463.09	2.369492	291.92	231.3	0. 79234
199.82	272.38	1.363127	271.59	506.49	1.864907
843.62	1468.21	1.740369	1483.17	1155	0. 778737
294.09	495.83	1.68598	1158.21	851.93	0. 735557
505.12	538.52	1.066123	64.8	275.33	4. 24892
888.87	1819.97	2.04751	1988.43	2330.34	1. 17195
513.59	885.54	1.724216	50.79	200.8	3. 953534
501.28	828.02	1.651811	740.43	584.51	0. 78942
487.36	693.83	1. 42365	31.88	34.5	1.082183
370.83	509.61	1. 374242	639.98	1789.48	2. 79615
722.8	1850.43	2. 560086	921.65	2108.87	2.288146
166.87	769.62	4. 612093	345.02	438.39	1. 270622
349.02	575.51	1.648931	627.28	1034.14	1.64861
676.5	1363.62	2.015698	1286.42	1102.13	0.856742
1085.46	2203.1	2.029646	759.27	662.19	0.87214
504.75	774.97	1.535354	573.66	901.05	1. 570704
238.15	546.68	2. 295528	92.87	127.6	1. 373964
1031.13	1207.66	1. 171201			
1048.91	1171.03	1.116426			
411.84	621.45	1.50896			
619.63	1526.91	2.464229			
823.04	1139.88	1. 384963			
374.44	496.64	1. 326354			
520.64	584.31	1.122292			

344.54	481	1.396064
863.78	1010.49	1.169846
900. 47	1671.57	1.856331
385.12	780.1	2.025602
643.2	2509.48	3. 901555
445.96	1166.73	2.616221
767.12	1106.51	1.442421
167.89	364.53	2. 171243
251.08	565.98	2.254182
1053.34	1050.42	0.997228
250.17	681.06	2. 722389
397.33	2467.48	6. 210153
582.63	449.44	0.771399
255.45	1089.23	4. 263966
408.28	895.56	2. 193495
615.81	1275.29	2.070915
176.6	137.81	0. 780351
233. 2	434.89	1.86488
378.53	570.48	1. 507093
582.97	619.4	1.06249
1264.67	1762.86	1. 393929
747.83	1777.97	2. 377506
375.82	525.27	1. 397664
96.71	185.86	1.921828
82.84	754.63	9. 109488
780.89	1056.9	1.353456
454.69	452.45	0.995074
361.85	732.15	2.023352
1045.85	1083.23	1.035741
675.15	690.83	1.023224
115.38	700.31	6.069596
614.1	855.63	1. 393307
158.95	831.36	5. 230324
1375.64	1634.79	1. 188385
882.57	733.61	0.83122
875.36	1123.82	1.283838
83. 7	240.54	2.873835
318.65	492.77	1.54643
217.79	315.08	1. 446715
1028.57	1221.86	1. 187921
351.1	844.46	2. 405184
721.72	757.83	1.050033
779.89	1265.31	1.622421

375.74	476.37	1. 267818
983.93	2128, 45	2. 163213
382.22	896.17	2. 344644
583, 64	951.68	1. 630594
315 74	431 96	1.368088
1046 86	2231 21	2 131336
1166 77	1465 86	1 25634
182 84	386 21	2 112284
389 55	706 69	1 814119
41 82	87.34	2 088474
738 6	781 11	1 057555
246 59	371 33	1.50586
1295 15	1422 43	1.098274
1233.13 1337 04	160/ /3	1.050274
58 79	165 05	2 810707
160.06	049 25	2.061144
1151 02	940.20 2107 75	2.001144
1101.00 010 E	200 02	2.099973
213.5	209.02	0.979010
172.07	325.8	1. 893415
506.33	694.09	1. 370825
938.67	1142.8	1. 217467
316.33	467.61	1. 478235
309.4	265.46	0.857983
1018.61	2160.31	2. 120841
2511.02	2324.93	0. 925891
437.77	767.3	1.752747
174.53	332.23	1. 90357
951.4	895.14	0. 940866
367.65	491.51	1. 336897
653.42	845.81	1. 294435
1117.15	1258.74	1. 126742
331.33	415.03	1.252618
610.28	619.28	1.014747
921.32	1152.16	1.250554
383.77	821.21	2.139849
353.88	579.12	1. 636487
558.88	768.1	1. 374356
330. 45	440.32	1. 332486
255.42	557.34	2. 182053
820.08	892.02	1.087723
46.93	204.73	4. 362455
762	948. 73	1. 245052
113.08	212. 18	1.876371