Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2013

A High-Throughput Photodynamic Therapy Screening Platform with On-Chip Control of Multiple Microenvironmental Factors

Xia Lou [a], Gwangseong Kim[b], Hyung Ki Yoon[b], Yong-Eun Koo Lee[b], Raoul Kopelman[b], and Euisik Yoon*[a]

[a] *Center for Wireless Integrated Microsystems, Department of EECS, University of Michigan, 1301 Beal Ave, Ann Arbor, MI, 48109, USA*
[b] *Department of Chemistry, University of Michigan, 930 N University Ave, Ann Arbor, MI, 48109, USA*

*Corresponding author at: 1301 Beal Avenue, Ann Arbor, MI 48109-2122. Phone: (734) 615-4469; Fax: (734)763-9324; Email: esyoon@umich.edu

Supporting Information

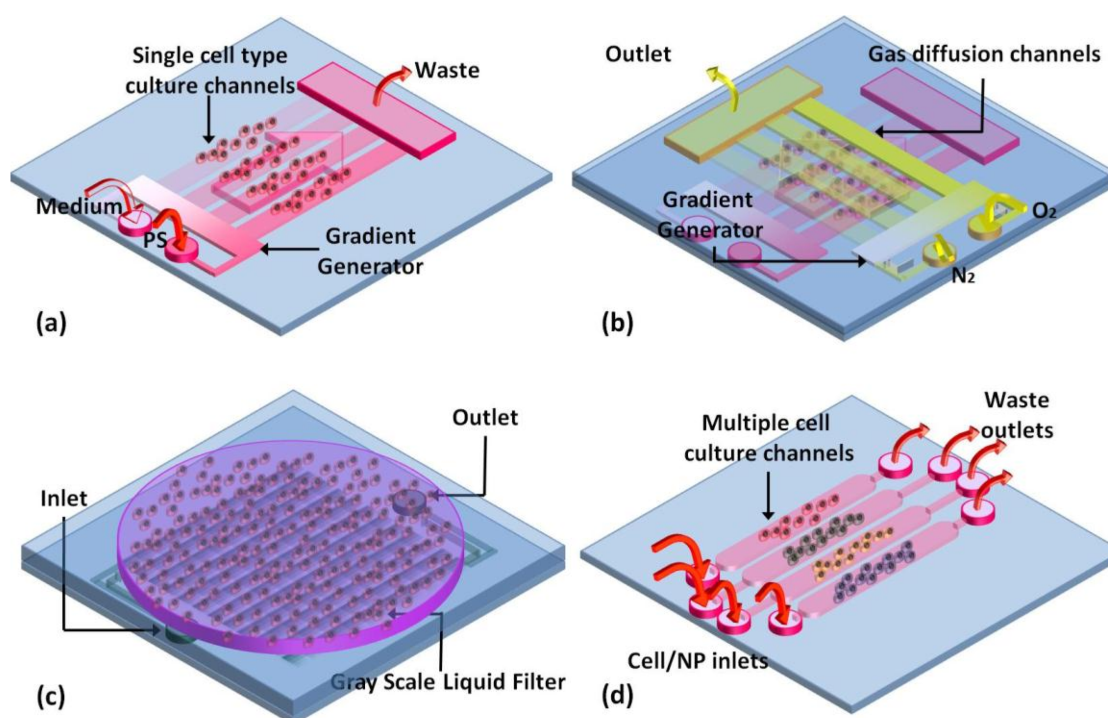


Fig. S1: Schematic diagram of various versions of prototype chips used for characterization of each individual factor: (a) Single layer chip used for testing the cell response of photosensitizer concentrations. (b) Double layer chip used for testing the cell response of oxygen concentrations or the combination of oxygen and photosensitizer concentrations. (c) Double layer chip used for testing the cell response of various fluence levels. (d) Single layer chip scheme used for parallel PDT efficacy tests of different cell lines or photosensitizers.

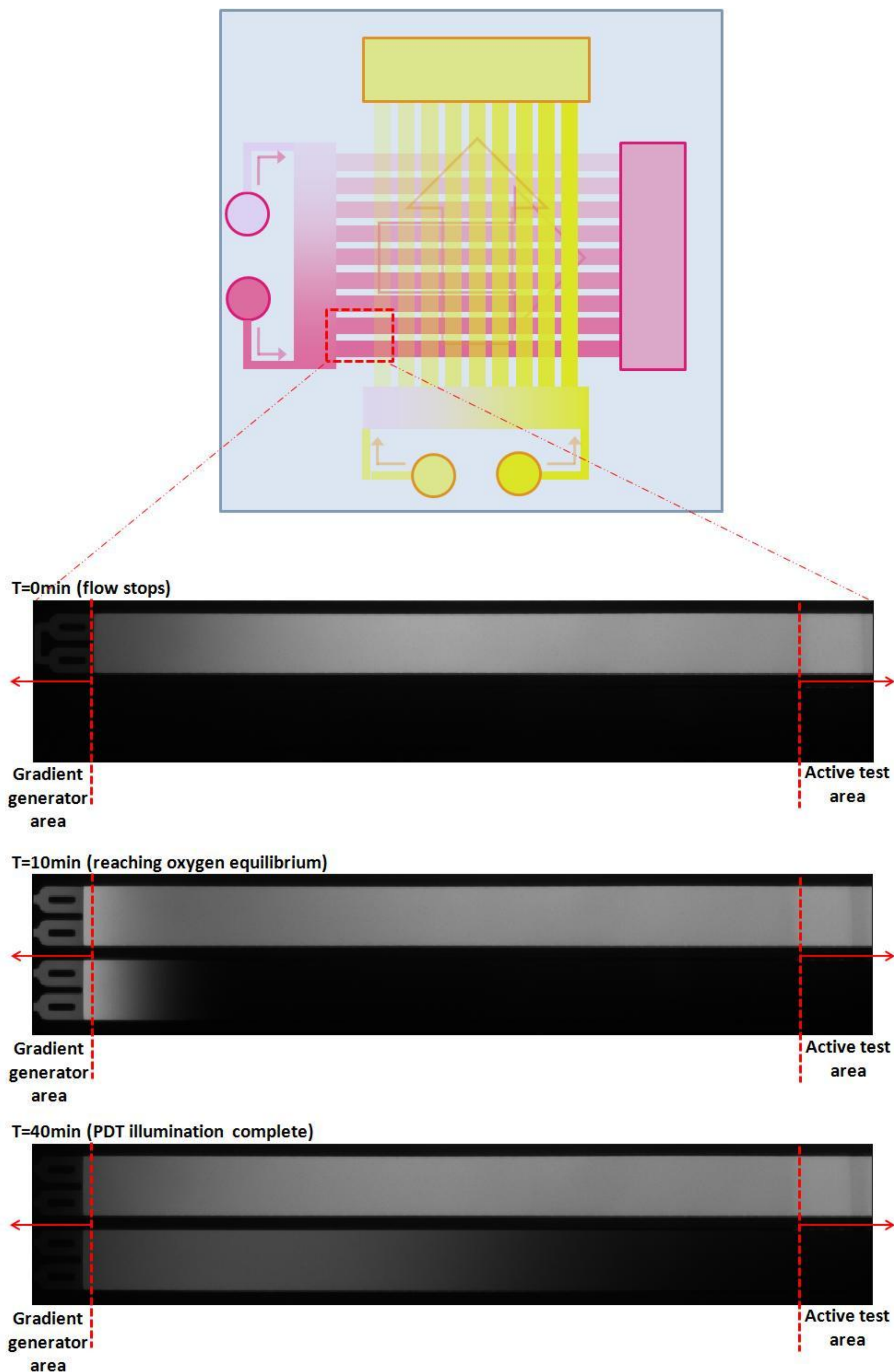


Fig. S2: Time lapse fluorescent imaging for observing diffusion of photosensitizers along the cell culture channels after stopping the gradient generation flow. The low FITC concentration channel (lower) is gradually compromised by the high FITC concentration solution (upper), however the concentration in the active PDT test area remains intact during the whole test period.

Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2013

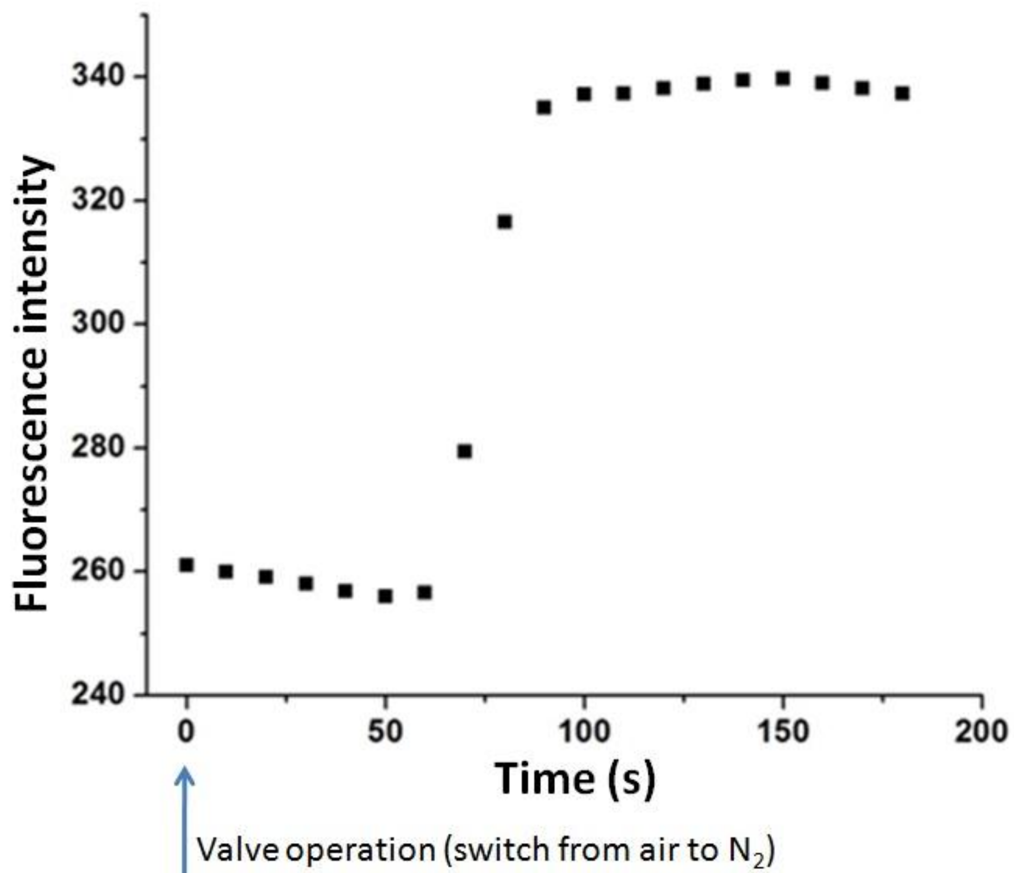
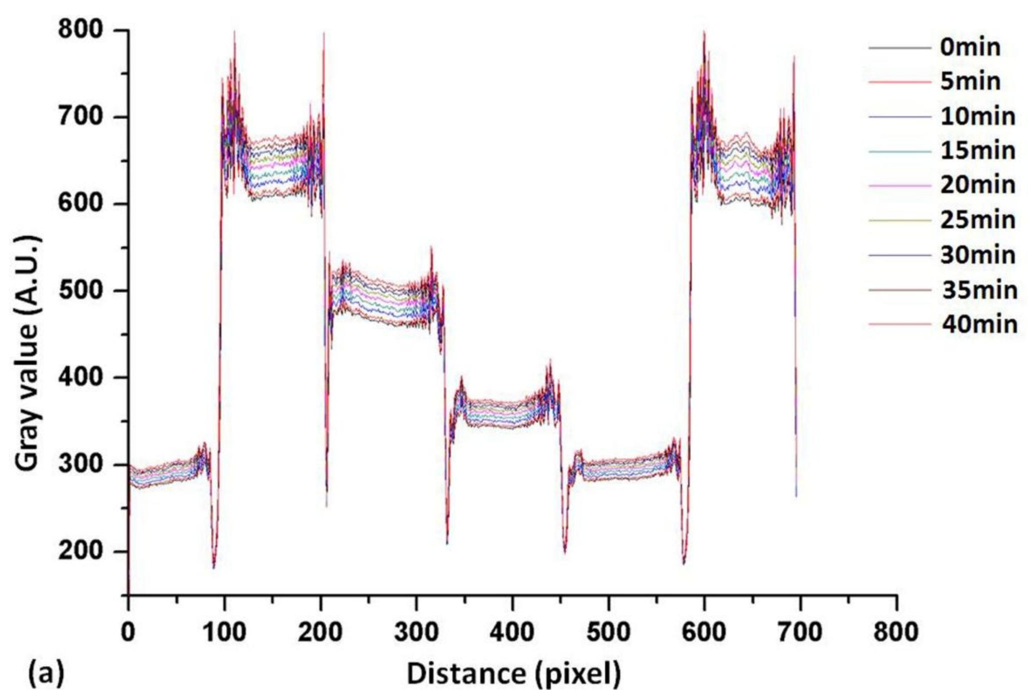


Fig. S3: Time lapse fluorescent imaging of RTDP fluorescence intensity changes over time after switching the gas concentration from the air to nitrogen. The response time is less than 50 seconds.



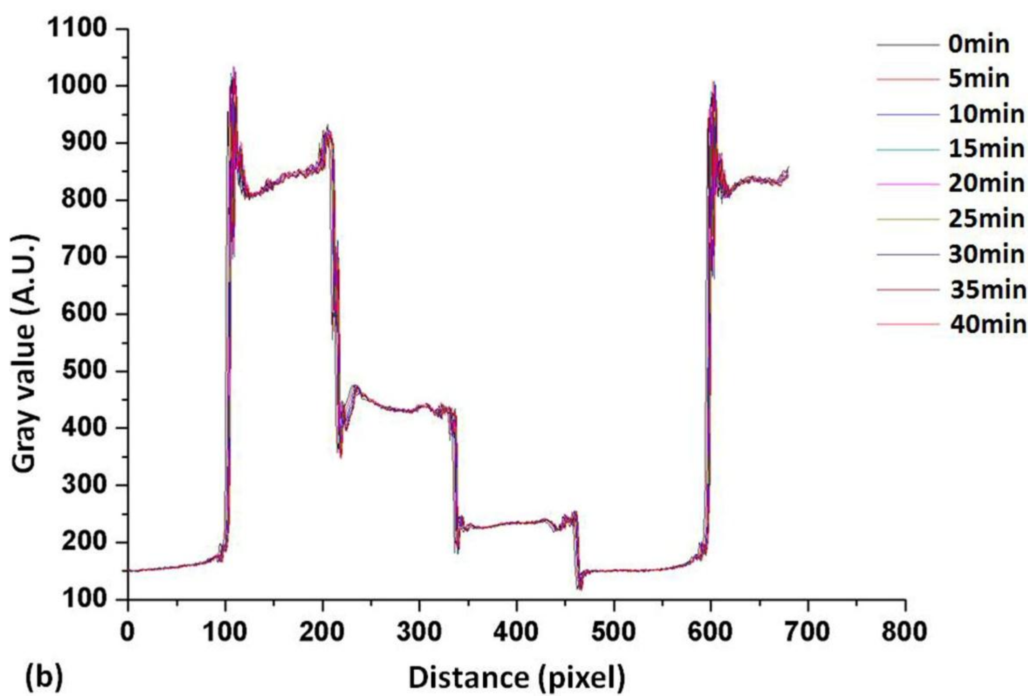


Fig. S4: Time lapse recording of illumination intensity changes across the liquid filter channels under (a) static filling of methylene blue solution and (b) continuous supply of methylene blue solution at 5 μl/min. Gray values are measured using ImageJ software.

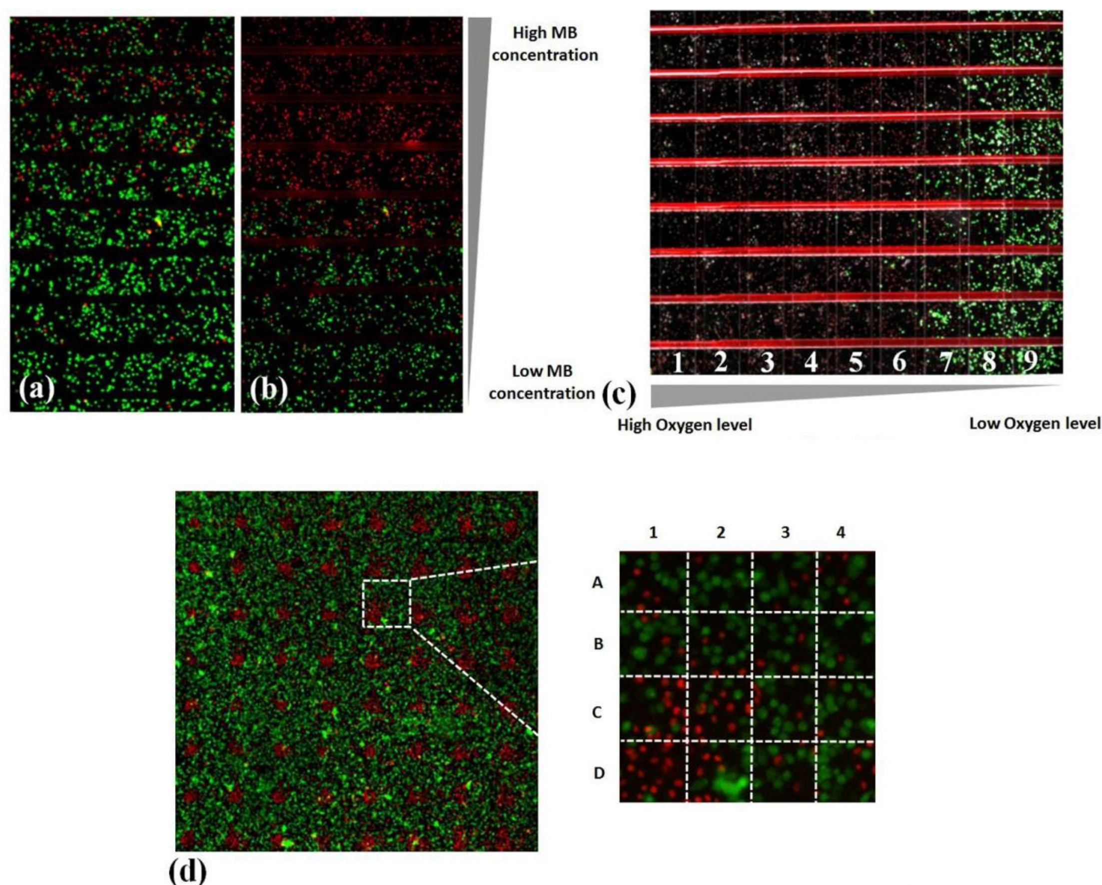


Fig. S5: PDT efficacy response to individual therapeutic factors. (a-b) C6 cell viability mapping (green: live cells, red: dead cells) for different photosensitizer (methylene blue) concentrations from the highest (top channel) to the lowest (bottom channel) for two illumination durations of (a) 10 min and (b) 20 min, respectively. (c) C6 cell viability mapping for oxygen concentration gradient from the highest (channel 1) to the lowest (channel 9). (d) C6 cell viability mapping for different illumination levels of the integrated liquid filter with an array of repeated gray patterns. The enlarged picture shows a unit cell of 4 x 4 grid patterns, with the highest illumination (1D) and the lowest (4A).

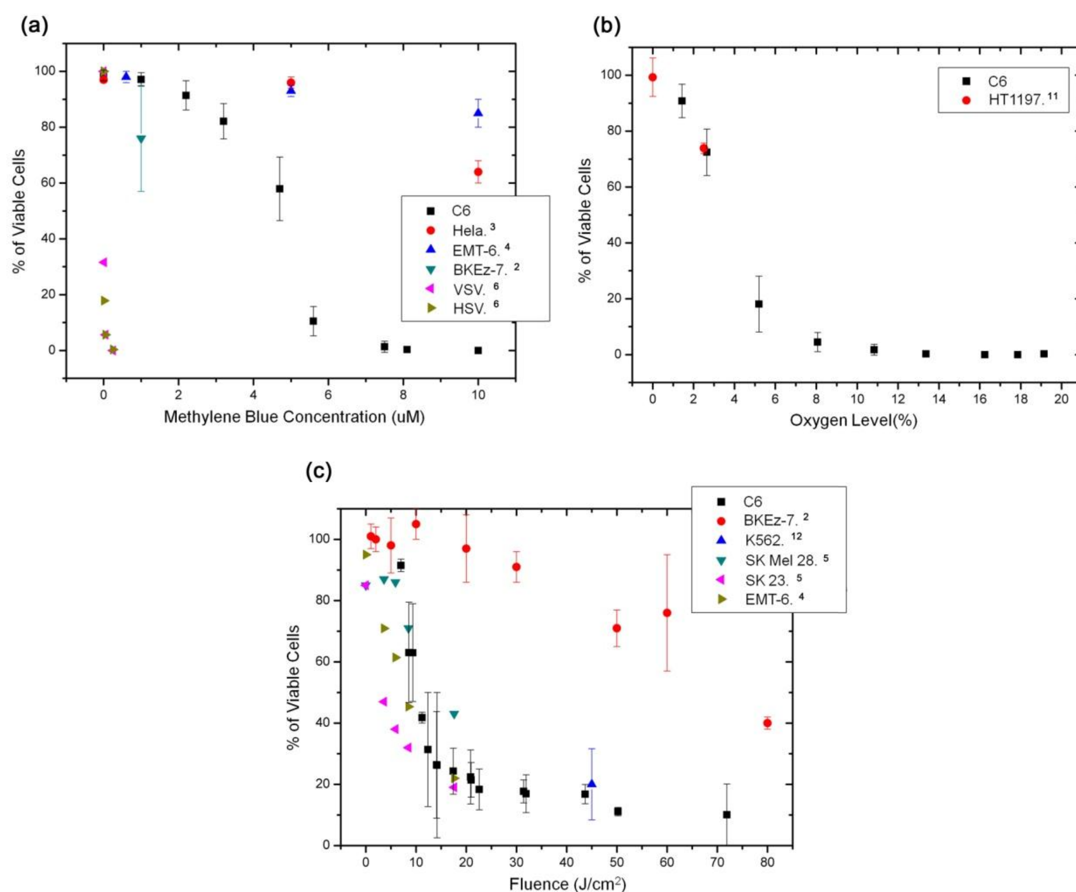


Fig. S6: Viability of C6 cells under different PDT conditions of individual varying therapeutic factors. (a) C6 cells are exposed to various concentrations of methylene blue with the same oxygen level of 21% and the same fluence of 57.5 J/cm^2. Previously-reported results of MB phototoxicity using a similar fluence (BKEz-7, Ruck *et al.*[2]), a lower fluence (10 J/cm^2, Hela, Lu *et al.*[3]; 7.2 J/cm^2, EMT-6, Wainwright *et al.*[4]), and a higher fluence (around 279 J/cm^2, VSV, HSV, Lambrecht *et al.*[6]) are plotted together for comparison. (b) C6 cells are exposed to various oxygen levels with the same methylene blue concentration of 10 μM and the same illumination dose of 71.9 J/cm^2. Similar high cell survival results under hypoxia conditions (HT1197, Wyld *et al.*[11]) using another photosensitizer (ALA) are included for comparison. (c) C6 cells are exposed to various levels of illumination doses with the same methylene blue concentration of 10 μM and the same oxygen level of 21%. Previously-reported results of MB phototoxicity using a similar MB concentration (K562, Trindad *et al.*[12]; SK Mel 28, Rice *et al.*[5]; EMT-6, Wainwright *et al.*[4]), a lower MB concentration (1 μM, BKEz-7, Ruck *et al.*[2]), and a higher MB concentration (32 μM, SK 23, Rice *et al.*[5]) are plotted together for comparison.

Electronic Supplementary Material (ESI) for Lab on a Chip
This journal is © The Royal Society of Chemistry 2013

SI Text

Methylene blue dark toxicity and phototoxicity

Methylene blue (MB) is a high-efficiency photosensitizer and can be used for inactivation of viruses, bacteria and tumor cells by photodynamic actions. It has been shown to be safe in humans and is now clinically used for PDT on some types of cancers, virus and bacterial infections in some countries.[1] Methylene blue's phototoxicity has been investigated on different types of mammalian cancer cells, non-cancer cells, bacterial cells, and virus cells.[2-14] However, its specific efficacy on C6 cells has been lacking. In our tests, dark toxicity of MB was examined first before we performed full PDT screening. We confirmed that a concentration up to 10 μM and an incubation time up to 1 hour gave no or only marginal dark toxicity for C6 cells (at least 95% viable cells) in all tests.

In Fig. S6, we summarized our MB efficacy test results on C6 cells and compared with the other previous works. First, the effect of MB concentration on cell viability is shown in Fig. S6 (a). Here the C6 cells were exposed to a fixed fluence of 57.5 J/cm^2 under atmospheric conditions. As the MB concentration increases, a concurrent decrease in cell viability was observed, with a high viability at below 1 μM while less than 5% of the cells survived above 7.5 μM. Ruck *et al.* investigated the phototoxicity on BKEz-7 endothelial cells under a similar fluence (60 J/cm^2) and found a comparable viability, around 76%, with 1 μM MB.[2] Lu *et al.* reported a lower phototoxicity on Hela cells, and also a slower viability drop as the MB concentration increases from 0 to 20 μM.[3] One possible reason is that a much lower fixed fluence (10 J/cm^2) was used during the test. Similar behaviors were also reported for EMT-6 cells, SK-23 murine melanoma cells, and SK-Mel 28 human melanoma cells.[4,5] On the other hand, Lambrecht *et al.* demonstrated with VSV and HSV cells that under a higher fluence (estimated around 279 J/cm^2), a higher MB phototoxicity was observed, and also a much faster drop in viability as the concentration increases.[6]

The effect of the ambient oxygen level on the C6 cells viability is shown in Fig. S6 (b). Cells were cultured with 10 μM MB and exposed to a fixed fluence of 71.9 J/cm^2. As the oxygen level increases, a concurrent decrease in cell viability was observed, from a high level (~ 90%) under hypoxia conditions to a low level (below 5%) with a higher oxygen supply. Unfortunately, no specific report has been found for the MB's phototoxicity dependence on oxygen level; this could be, in part, because controlling the oxygen level is challenging for conventional biomedical tests. However, the influence of hypoxia conditions on PDT treatments has been well recognized and some results, using other photosensitizers, have been reported.[7-11] Wyld *et al.* tested aminolaevulinic acid (ALA) phototoxicity on HT 1197 cells under both hypoxic and normoxic conditions.[11] It was reported that there was an obvious viability improvement under low oxygen levels (0%, 2.5%, 5%) but no significant difference was found at higher levels (7.5%, 10%). From another perspective, Chapman *et al.* also demonstrated that, under hypoxia conditions a much longer illumination time was required for Photofrin II to kill the same amount of EMT-6 cells.[8]

The effect of fluence on the C6 cells' viability is shown in Fig. 8 (c). Cells were cultured with 10 μM MB and illuminated with a series of fluences (up to 71.9 J/cm^2) under atmospheric conditions. As the fluence increases, a concurrent decrease in cell viability was observed, with a high viability (> 90%) at 71.9 J/cm^2, but less than 10% viability at above 50 J/cm^2. Using the same MB concentration (10 μM), Wainwright *et al.* investigated the phototoxicity changes on EMT-6

This journal is © The Royal Society of Chemistry 2013

cells with fluence varying from 0 to 17.6 J/cm^2, and a comparable viability change was demonstrated (from 95% to 22%).[4] Similar results were also reported on K562, SK-Mel 28, LUCENA-1, and Hela cells.[3,5,12] Using a lower MB concentration (1μM), Ruck *et al.* reported a higher viability of BKEz-7 endothelial cells and a more gradual viability drop as the fluence increases.[2] Using higher MB concentrations, lower viabilities and faster viability drops were also observed on SK 23, H. pylori 26695, RIF-1 murine fibrosarcoma, and Hela cells. [3, 5,13,14]

SI References

1. J. P. Tardivo, A. Del Giglio, C. S. de Oliveira, D. S. Gabrielli, H. C. Junqueira, D. B. Tada, D. Severino, R. de Fátima Turchiello and M. S. Baptista, Photodiagnosis and Photodynamic Therapy, 2005, **2**, 175-191.
2. A. Ruck, K. Heckelsmiller, N. Akgun, G. Beck, K. Kunzi-Rapp, E. Schick and R. Steiner, Photochem Photobiol, 1997, 66, 837-841.
3. Y. Lu, R. Jiao, X. Chen, J. Zhong, J. Ji and P. Shen, J Cell Biochem, 2008, 105, 1451-1460.
4. M. Wainwright, D. A. Phoenix, L. Rice, S. M. Burrow and J. Waring, J Photochem Photobiol B, 1997, 40, 233-239.
5. L. Rice, M. Wainwright and D. A. Phoenix, Journal of Chemotherapy, 2000, 12, 94-104.
6. B. Lambrecht, H. Mohr, J. Knüver-Hopf and H. Schmitt, Vox sanguinis, 1991, 60, 207-213.
7. S. L. Gibson and R. Hilf, Photochem Photobiol, 1985, 42, 367-373.
8. J. D. Chapman, C. C. Stobbe, M. R. Arnfield, R. Santus, J. Lee and M. S. McPhee, Radiat Res, 1991, 126, 73-79.
9. K. P. Nielsen, A. Juzeniene, P. Juzenas, K. Stamnes, J. J. Stamnes and J. Moan, Photochem Photobiol, 2005, 81, 1190-1194.
10. J. Moan and S. Sommer, Cancer Res, 1985, 45, 1608-1610.
11. L. Wyld, M. W. Reed and N. J. Brown, Br J Cancer, 1998, 77, 1621-1627.
12. G. S. Trindade, S. L. Farias, V. M. Rumjanek and M. A. Capella, Cancer Lett, 2000, 151, 161-167.
13. S. S. Choi, H. K. Lee and H. S. Chae, J Photochem Photobiol B, 2010, 101, 206-209.
14. K. J. Mellish, R. D. Cox, D. I. Vernon, J. Griffiths and S. B. Brown, Photochem Photobiol, 2002, 75, 392-397.