HIGH-THROUGHPUT OF PHOTODYNAMIC THERAPY (PDT) SCREENING FROM MULTIPLE PARAMETER ASSAYS OF 1,000 DIFFERENT CONDITIONS IN A SINGLE CHIP

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ABSTRACT

We present a microfluidic chip for high-throughput PDT screening with the capability of individual controls on light exposure dose, pericellular oxygen level and photosensitizer concentration. Both oxygen and photosensitizer gradients are produced by microfluidic gradient-generating networks and imposed on target cells. Various photo-exposures are provided by orthogonally stacking the two layers of gray scale liquid filters. All three parameters are independently controlled in the microfluidic channels monolithically integrated in a single chip. Using this platform, we successfully demonstrated the PDT efficacy on cancer cells for different exposure doses, oxygen levels and photosensitizer concentrations.

KEYWORDS: PDT, microfluidic, High-throughput, Cancer, Liquid filter, Gradient-generator

INTRODUCTION

Photodynamic therapy (PDT) has emerged as a promising treatment for cancer since 1980s, especially with advancement of new-generation photosensitizers and various photosensitizer delivery strategies [1]. However, experimental technologies for PDT lag behind to meet the need of huge numbers of screening tests for numerous photosensitizers developed recently in terms of specificity for target cancer cells, activating light delivery and optimization of PDT protocols [1, 2, 3]. While microfluidics technology has emerged as a powerful tool for high-throughput chemodrug screening and cellular assay, its application in PDT research has been rarely reported [4]. In this paper, we report a microfluidic chip that can simultaneously provide the parameter control of all three crucial factors in PDT: light exposure dose, oxygen level and photosensitizer concentration. For feasibility test, we used C6 glioma cells. After loading and culturing C6 cells in monolayer inside the chip, we tested their viability response under different PDT conditions.

DEVICE FEATURES

As shown in Figure 1, the proposed chip is composed of three layers: gas layer (generating nine oxygen level gradients), fluidic layer (providing nine photosensitizer concentrations), and filter layer (providing sixteen gray levels). All layers are made of PDMS stacking on top of each other. There is a 100μm-thick glass layer between the fluidic and filter layers, providing a rigid support as well as facilitating cell attachment and growth. While the photosensitizer concentration gradient is generated within the fluidic layer and directly flowing through the cells, the oxygen gradient is generated in the gas layer but perfusing through the 70μm-thick PDMS membrane between the two layers [5]. The gas and fluidic layers are stacked in a way that their channels run perpendicular to each other, thus giving the total 9x9=81 combination of different oxygen levels and photosensitizer concentrations. For each given combination of two parameters, the liquid filter unit stacked below provides 16 different exposure doses under single uniform light exposure. This allows the cells to be exposed to more than 1,000 different PDT conditions (9x9x16=1,296 conditions) in a single chip simultaneously.

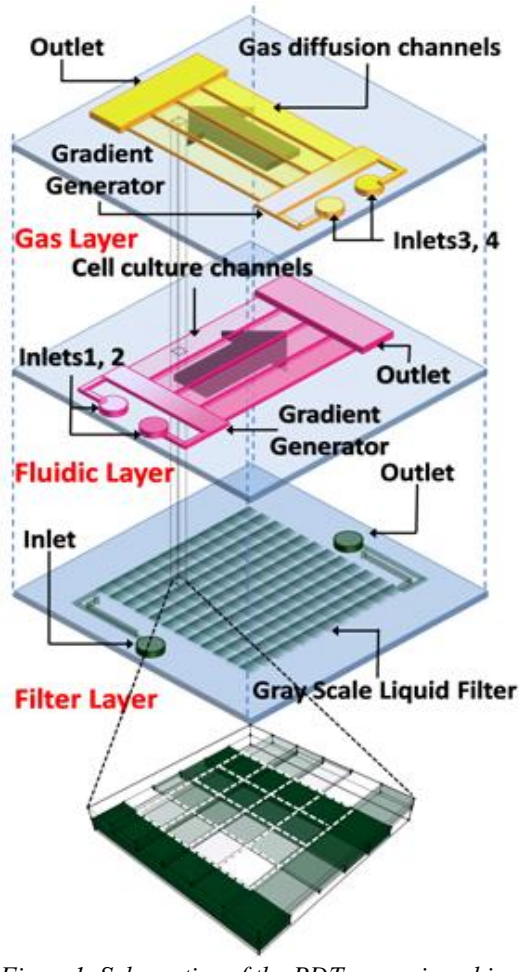

Figure1. Schematics of the PDT screening chip.

EXPERIMENTS AND RESULTS

Figure 2(a) shows the fabricated chip filled with different food dyes in each layer (yellow for gas layer, red for fluidic layer and green for filter layer; channel width of 320μm and filter pattern unit size of 80μm x 80μm). Liquid filter is filled with a photosensitive dye, Methyleneblue (MB), which has peak absorptions at 668 and 609 nm; thus generating grayscale transparencies when exposed to red light, as shown in Figure 2(b). Photosensitizer and oxygen gradients were generated by using microfluidic gradient-generating networks (Figure 2(c)). Oxygen levels in the fluidic layer were monitored using an oxygen sensitive dye, rutheniumtris(2,2'-dipyridyl) dichloride hexahydrate (RTDP), showing a gradient from high to low fluorescence intensity (low to high oxygen levels) in Figure 2(d).

Methyleneblue (MB), which has been used for a variety of applications as photosensitizer, and rat C6 glioma tumor cells are adopted for PDT screening experiments. A red LED with a peak output at 660nm is used as an exposure light source. Before cell loading, the filter layer is filled with MB and the fluidic layer is filled with culture media (DMEM+5%FBS+ 0.5%penicillin). C6 glioma cells in suspension media is prepared with a concentration of ~2.0x10^{6}cells/ml. Cells are loaded in the outlet reservoir by pippeting and introduced into the fluidic channel area by applying a negative press at the inlets. After loading, both inlets and outlet are covered with culture media and cells are incubated for 24h to reach a monolayer in the chip before PDT test. To generate a photosensitizer concentration gradient, two different culture media (DMEM, 10μM MB in DMEM) are introduced through the separated fluidic layer inlets using a syringe pump at a flow rate of 10μl/min, respectively. To generate an oxygen level gradient, two gas layer inlets are respectively connected to a compressed air source and a nitrogen source, respectively, at the same pressure of 5psi. To generate different gray scales in the liquid filter, the filter layer is filled with two different concentrations of MB (50mg/ml and 12.5mg/ml). After light exposure, cells are cultured for 20 minutes, and then stained with Calcein AM (10μM, live, green) and Ethidium homodimer-1 (10μM, dead, red) for viability test.

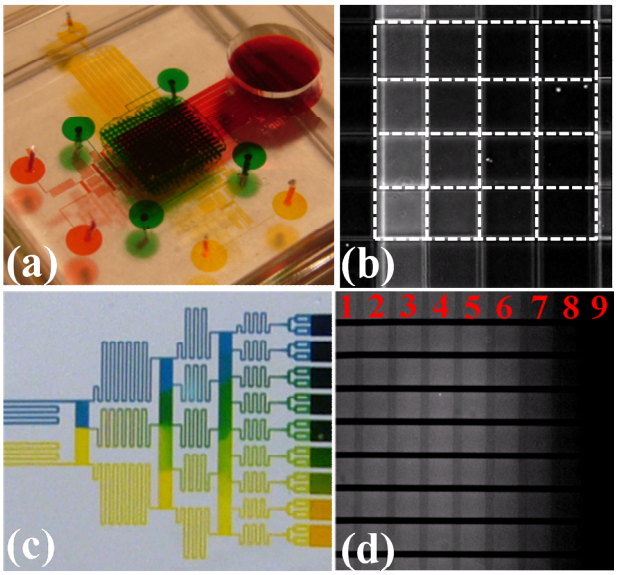

Figure2. (a) Fabricated PDT screening chip. (b)Grayscale pattern generated by the liquid filter showing transparency gradients. (c) Chemical gradient generated by a mixing network (displayed by food dye color). (d) Oxygen level gradient generated by the imposed gas layer on the top (displayed with RTDP fluorescence).

PDT test was carried out under single parameter changes first (exposure dose, oxygen level or photosensitizer concentration) and then followed by their combinations. We observed distinctive differences in viability for all three parameters: light exposure doses, Methyleneblue concentrations and oxygen levels. Figure 3 shows the C6 cells viability after different exposure dose treatments. Cells are exposed for an exposure time of 25min under the same MS concentration (50mg/ml) and oxygen level (compressed air). As we can see, the higher exposure dose (most transparent area) leads to lower cell viability while the lower exposure dose leads to high viability. Dashed grids in the enlarged part correspond to the 4x4 gray scale filter patterns. This can be confirmed in Figure 4 that the higher exposure dose (longer exposure time) results in more cell deaths. While having other parameters remaining the same, the longer exposure time (20min, Figure 4b) helps killing more cancer cells than shorter exposure time (10min, Figure 4a). Also cells in Figure 4 are treated with different MB concentrations in each channel, from high (10μM) to low (0μM), top to bottom under the same compressed air and uniform light exposure. For both conditions (Figure 4a and b), higher MB concentration helps increase PDT treatment efficacy. It is worth to notice that below certain MB concentrations, increasing the exposure dose would not help much on killing more cancer cells. This can be explained by that PDT is affected by the combination of all three pa-

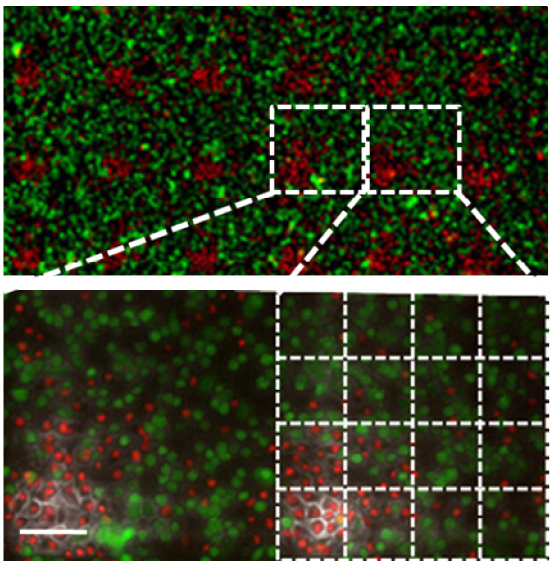

Figure3. C6 cells after live/dead (green/red) staining showing that viability changes according to transparency of the integrated liquid filter (exposure doses). (Scale bar: 80μm)

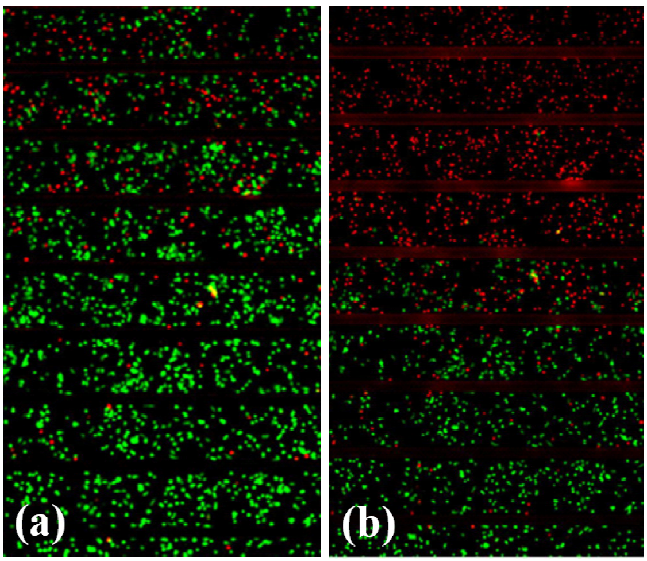

Figure4. C6 cells after live/dead staining showing that viability changes according to MB concentrations from highest (top channel) to lowest (bottom channel) with an exposure of (a) 10mins and (b) 20mins, respectively.

rameters. Under the sufficient light exposure and oxygen supply, photosensitizer concentration will become the bottleneck of its efficacy and its threshold value will show up. Same also applies to the other two factors. Figure 5 shows the result of C6 cell viability under different oxygen levels after PDT treatment. C6 cells here are exposed to an oxygen level gradient from highest (channel 1) to lowest (channel 9) with the same MB concentration (10μM) and exposure time (25min). While showing PDT treatment efficacy is greatly enhanced when the oxygen level increases, we can observe a similar threshold change in cell viability similar to the one in Figure 4 as discussed earlier in this paragraph. From this assay, we could easily screen the threshold (minimum) level of respective parameters required for effective PDT; thus provide an easy way of photosensitizer characterization at high-throughput. Figure 6 shows the C6 cell viability under the combination of different oxygen levels and MB concentrations. C6 cells are exposed to an oxygen level gradient from highest (channel 1) to lowest (channel 9), and an MB concentration gradient from highest (10μM) to lowest (0μM) with the same exposure time (15min). The cell viability map here shows an increase of cell viability from left-top corner to right-bottom corner, confirming that both sufficient oxygen and photosensitizer supply can help enhance PDT efficacy.

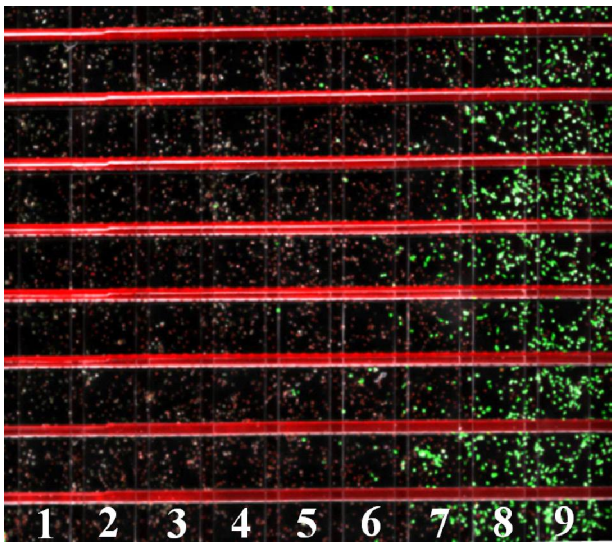

Figure5. C6 cells after live/dead staining showing that viability changes according to oxygen level gradient from highest (channel 1) to lowest (channel 9).

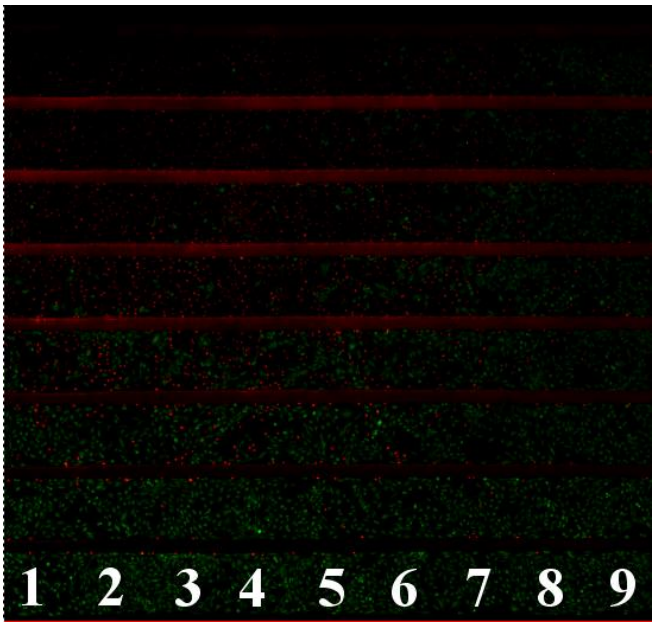

Figure6. C6 cells after live/dead staining showing that viability changes according to both oxygen level gradient from highest (channel 1) to lowest (channel 9) and MB concentration from highest (top channel) to lowest (bottom channel).

CONCLUSION

We implemented a microfluidic chip for high-throughput PDT screening and photosensitizer characterization. This chip was realized by staking multiple layers of PDMS in a compact single platform and allows the simultaneous control of three crucial parameters in PDT assays including photosensitizer concentration, oxygen level and light exposure dose. We successfully demonstrated the viability assays on C6 glioma tumor cells after PDT treatment under a variety of different conditions of parameters more than 1,000 combinations at single experiment. We could easily screen the threshold level of respective parameters required for efficacy of PDT at high-throughput and characterize different photosensitizers upon different cancers.

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REFERENCES

[1] D. Dolmans, D. Fukumura, R. Jain, "Photodynamic therapy for cancer", Nature Reviews Cancer, **3**, 380 (2003).
[2] M. Butler, P. Itotia, J. Sullivan, "A High-Throughput Biophotonics Instrument to Screen for Novel Ocular Photosensitizing Therapeutic Agents", Investigative Ophthalmology & Visual Science, **5**, 2705 (2010).
[3] I. Meerovich, V. Jerdeva, G. Meerovich, V. Derkacheva, A. Savitsky, "High-throughput screening system for the study of phototoxicity of photosensitizers invitro", Proceedings of SPIE, **4952**, 203, (2003)
[4] Z. Wang, et al, "High-density microfluidic arrays for cell cytotoxicity analysis", Lab Chip, 7, 740 (2007)
[5] M. Adler, M. Polinkovsky, E. Gutierrez, A. Groisman, "Generation of Oxygen Gradients with Arbitrary Shapes in a Microfluidic Device", Lab Chip, **10**, 388 (2010).

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