A DROPLET-BASED MICROFLUIDIC SYSTEM FOR HIGH-THROUGHPUT SCREENING OF PHOTOSENSITISERS AGAINST MICROBIAL ORGANISMS

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ABSTRACT

Herein, we present a modular droplet-based microfluidic approach for performing high throughput cytotoxicity screening of photosensitizers against microbial organisms. Multiple novel fluidic operation modalities such as large-scale chamber based light irradiation, reinjection and low voltage driven electrocoalescence are introduced. Also, photosensitiser drug cytotoxicity on *E.coli* cells are evaluated in microfluidic device using fluorescent viability assay indicator and compared with conventional colony forming unit counting cytotoxicity assay.

KEYWORDS: PDT, photodynamic, microfluidic, droplet, drug screening, lab on a chip

INTRODUCTION

Photodynamic therapy represents an efficacious alternative method for the treatment of localized microbial infections with several favourable features such as broad spectrum of action, efficient inactivation of multidrug-resistant bacteria, and low mutagenic potential. However, the cytotoxicity screening of the photosensitizers is a slow and manual process as it requires an extensive culture comparison requiring 18 hours incubation period [1]. Segmented flow microfluidics has recently emerged as a promising platform for performing high throughput cytotoxicity screening. These systems are suitable for performing high throughput experimentation as they have rapid droplet generation frequency ranging from 10Hz to kHz range, fast analysis time, reduced reagent interaction with channel walls, and integration of various functional components [2]. Previously, we introduced a microfluidic flow system for activity screening of photodynamic therapy agents [3] and other continuous flow based microfluidic methods have also been introduced demonstrating various benefits of using microfluidic devices for high throughput photosensitiser screening [4,5]. Herein, we present a modular droplet-based microfluidic approach for performing high-throughput cytotoxicity screening of photosensitizers against microbial organisms. This new method introduces multiple fluidic operations within one high throughput screening platform such as large-scale chamber based light irradiation, reinjection and low voltage driven electrocoalescence.

DEVICE DESIGN

Figure 1 shows schematics of the integrated microfluidic platform allowing for the encapsulation of cells and photosensitizers in nanolitre sized compartments, simultaneous irradiation of tens of thousands of these droplets, and viability scoring of the exposed samples. The cytotoxicity screening workflow consists of three key steps as shown in Figure 1. As shown in Figure 1 (a), microdroplets containing cells and photosensitizer are generated and aliquoted into large thermoplastic microfluidic chambers (2mm x 4 mm x 200 μm) for light exposure shown in Figure 1 (b). The collected droplets are incubated in dark, exposed to light and reinjected into another device for further processing by simply pushing droplets with oil. By using these microfluidic chambers for drug treatment, it is possible to incubate and expose light on droplets for more than a few hours. Microfluidic chamber devices are fabricated using hard thermoset polyester material with large Young's modulus to avoid expansion of the device material. Thus, droplets can be loaded into the device using high flow rates (>40 μl/min) and reinjected into another device without significant expansion, e.g. Figure 1 (c). After light exposure within the chamber structure, droplets are reinjected, merged with viability assay droplets and incubated for 30 minutes for cell viability scoring. Parallel chromium-gold electrodes (Figure 1 (d)) with 50 μm widths and 30 μm spacings, coated with a 3 μm thick spin-coated PDMS layer to prevent inter-droplet contamination, are employed to merge droplets. Using this system, droplet electrocoalescence was achieved using a 10V AC electric field whilst maintaining a one-to-one droplet fusion ratio.

EXPERIMENTAL

E.coli Top10 strain suspensions were used to evaluate Toluidine Blue O (TBO) cytotoxicity. Cells were cultured overnight and resuspended in phosphate buffered saline at an optical density of 0.25. SYTO9 and Propidium Iodide (PI) were used for all viability scoring experiments. SYTO9 is a green fluorescent dye that is cell membrane permeable while PI is a red fluorescent dye which is membrane impermeable. To evaluate the viability assay performance, cells were treated with -20°C methanol for 10 minutes and mixed with live cell samples to prepare cell stock solutions containing various proportions of dead and live cells. Cells were then mixed with 10μM SYTO9 and 4μM Propidium Iodide and injected into microfluidic device for analysis. A biocompatible fluorinated surfactant dissolved in an FC40 oil phase ensured stable

creation of droplets, as previously described by Han and coworkers [6].

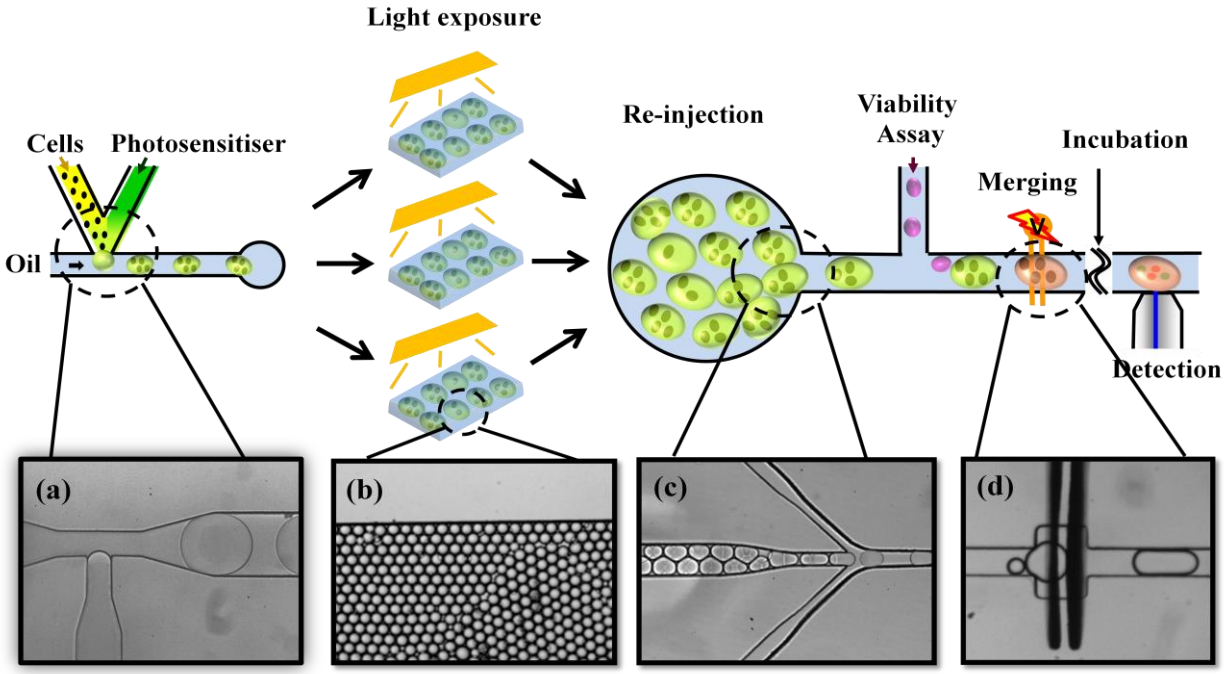

Figure 1. Schematic diagram of the microfluidic-based screening workflow consisting of four key steps. (a) Droplet Generation. (b) Microfluidic chamber for light exposure. (c) Reinjection of droplets from the chamber. (d) Electrocoalescence of droplets for cytotoxicity detection.

RESULTS AND DISCUSSION

In order to screen cytotoxicity of photosensitisers against microbial organisms, it is vital to demonstrate robust assay performance. The viability assay (comprising of SYTO-9 and propidium iodide) was successfully characterized in both well plate and droplet formats, allowing interrogation of both dead (green and red fluorescence) and live (green fluorescence) *escherichia coli* cells as shown in Figure 2 (a) and (b). A linear increase in the red/green fluorescence signal ratio with dead cell proportion was observed for both well plate-based and droplet-based formats.

The viability assays were then used to evaluate the efficacy of Toluidine Blue O (TBO) against *escherichia coli* cells at a fixed light exposure dose of 180J/cm^2. *E.coli* cell suspensions were treated in bulk with various concentrations of TBO and incubated for thirty minutes in the dark. The treated cell suspensions were then irradiated using bright white transmission lamp at a fixed intensity of 180 J/cm^2 over 20 minutes. Treated cell suspension samples were then mixed with the viability assay components. Cellular emission was detected using confocal fluorescence spectroscopy and compared with TBO cytotoxicity screening data originating from conventional colony forming unit measurements under identical conditions. Figure 3(a) shows the cytotoxicity screening results using conventional colony forming unit measurements where an aliquot (10µl) of treated cell suspensions are plated on a petri dish, cultured overnight and the number of growing

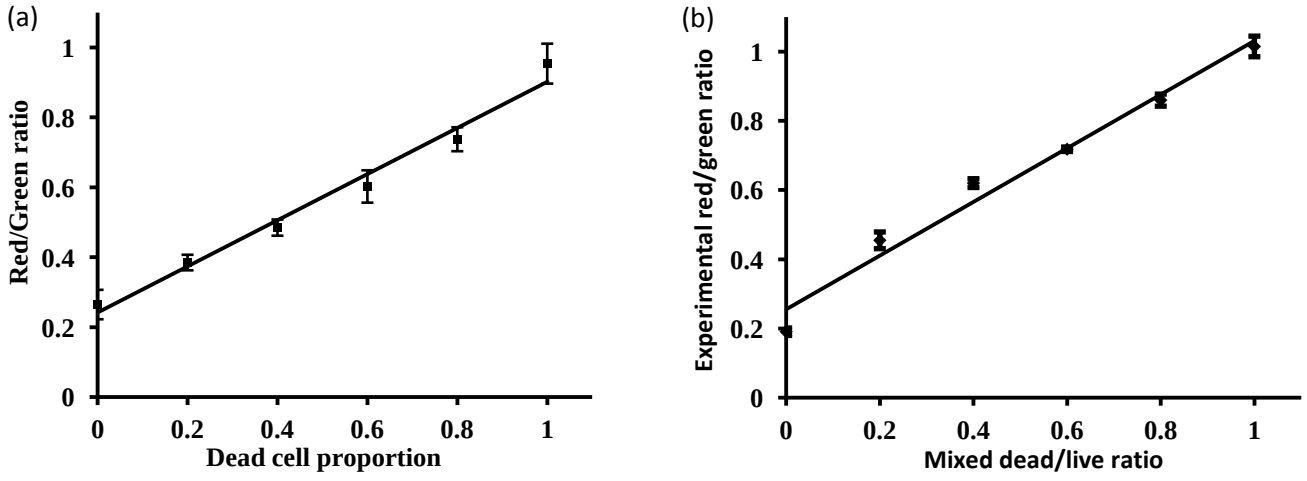

Figure 2. Viability Assay Calibration on (a) micro-wellplate and (b) droplet-based microfluidic platform.

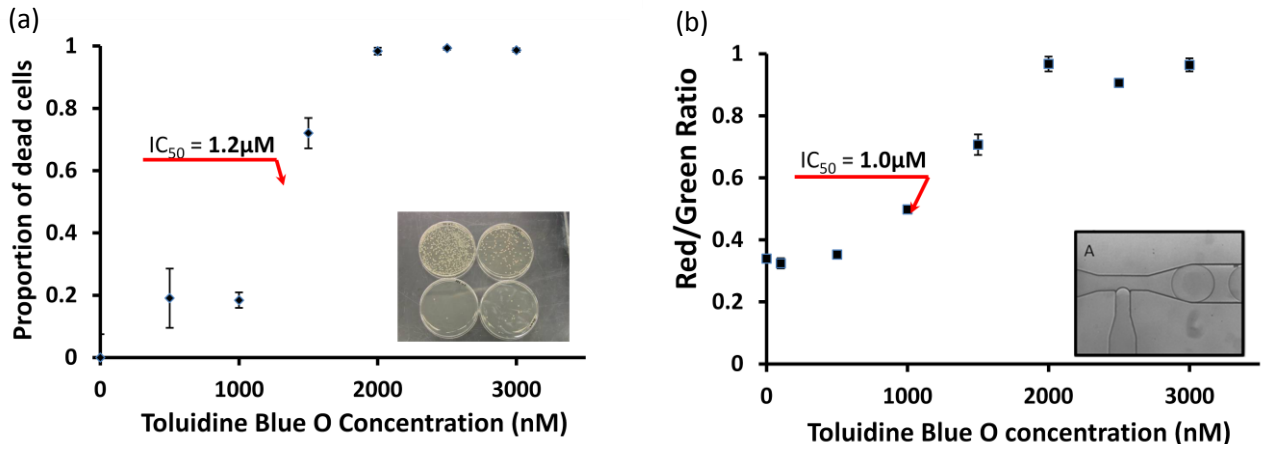

Figure 3. The effect of Toluidine Blue O concentration on e.coli cells under a fixed light dose of 180 J/cm^2. (a) TBO cytotoxicity screening using conventional colony forming unit measurements. (b) TBO cytotoxicity screening using cell based fluorescence measurements.

colonies counted for each condition. Figure 3(b) shows the microfluidic TBO cytotoxicity data under identical conditions. The variation of red/green ratio as a function of TBO concentration is sigmoidal in nature and closely corresponds to that obtained using cfu counting measurements although 30% of the cells in live cell samples were scored as dead in confocal fluorescence measurements. Such discrepancies between bulk and microfluidic measurements are due to the fact that cells suffer shear stress upon encapsulation into droplets as previously reported [7]. Regardless, the results show a strong correlation between the droplet-based microfluidic approach and conventional methods, with similar IC_{50} values of 1.0 µM and 1.2 µM, respectively.

CONCLUSION

We have developed robust droplet-based microfluidic modalities for investigating the photodynamic therapy efficiency of Toluidine Blue O on *E.coli* cells. The viability assay performance was calibrated using both well plate and microfluidic-based fluorescence detection, with a strong correlation between both methods. In addition, the viability assay was used to assess the TBO cytotoxicity on *E.coli* cells and compared to conventional CFU measurements.

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