A Microfluidic Flow System for Activity Screening of Photo-Dynamic Therapy Agents

Robert Jarvis,[1] Xize Niu, [1] Andrew J. de Mello[1] Mark Wainwright[2] and Robert C.R. Wootton [2]

[1]*Imperial College London, UK*

[2]*Liverpool John Moores University, UK*

ABSTRACT

This paper describes a new and rapid method for the continuous flow screening of photodynamic therapy (PDT) agents for activity and background toxicity. The methodology is extremely rapid and flexible and removes the need for extensive culture comparison. The methodology increases the potential screening throughput exponentially when compared with current systems. Activity of an incubated medium can be assessed in continuous flow rather than after an 18h incubation period.[1]

KEYWORDS: Photodynamic therapy, high-throughput screening, microdroplets, cell-based assays

INTRODUCTION

Photodynamic therapy agents are slowly gaining ground in clinical treatments of difficult organisms such as methicillin-resistant *Staphylococcus Aureus* but most testing is being undertaken with first generation sensitizing agents such as methylene blue and toluidine blue. One of the bottlenecks in the development of new therapeutic agents is the lack of a rapid screening system for toxicity, activity and uptake rate of each new agent. Without such a system structure activity relationships can only slowly be realised.

In our technique cell toxicity is evaluated directly rather than from later culture. The data provided by this method are difficult or impossible to reproduce in a single measurement system reliant on post-exposure culture counting and will contribute a great deal to the understanding of structure/activity relationships in this burgeoning area of therapy.

EXPERIMENTAL

Briefly, a suspension of cells, in this case *E. Coli* is incubated with a PDT agent in aqueous culture medium, then the suspension is broken into microdroplets using a fluorous medium. The droplet encapsulated cells are then passed through a channel where the droplet is split into two. One droplet is irradiated with light of a wavelength matched to the PDT agent and the other is kept dark. Two competitive fluorescent agents, STO-9 and propidium iodide, fluorescing green and red respectively, are then introduced to each droplet, and the droplet incubated. STO-9 can cross cell membranes but propidium iodide cannot, meaning that red fluorescent cells have been killed by the PDT agent and green fluorescent cells are alive. Cell fluorescence is powered by irradiation with a laser operating at 488nm and detected via PMT. A comparison of the counts of both emitted wavelengths in the light channel and dark

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channel gives data on the effectiveness of the PDT agent and also on it's background toxicity. By incorporating continuous flow incubation lipophilicity and cell uptake rates can also be measured.

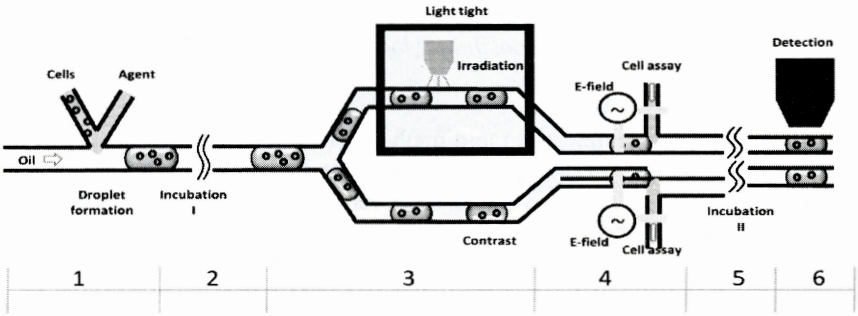

Figure 1. Schematic view of the microdroplet chip design. It consists of six parts: 1. droplet formation; 2. Incubation I to allow the photosensitizer to be absorbed into the cells; 3. Irradiation with white light; 4. Electric field induced addition of cell assays to the droplets; 5. Incubation II; 6. Detection

RESULTS AND DISCUSSION

The method was used to evaluate two parameters; the optimum incubation time for sensitiser uptake and the optimum illumination time at a set incubation time for maximum cell death.

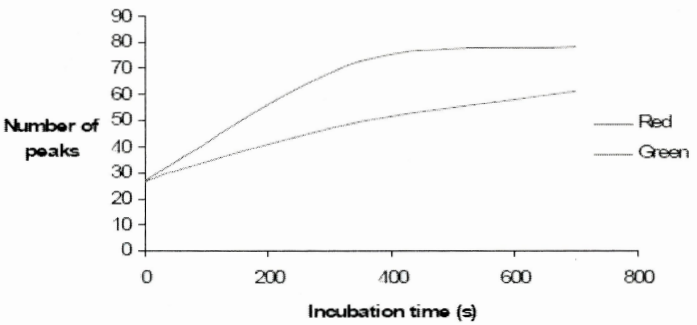

Figure 2. A graph showing the number of peaks observed per second for droplets of solutions of cells incubated for different times.

It was found that an incubation time of approximately 7 minutes (Figure 2) was required for the number of cells detected per unit time to stabilise on-chip. This compares well with previous 96- and 384-well plate studies which showed that incubation times of 5 minutes are necessary.[2] The same chip was used to determine a

suitable illumination period. Cell suspensions were prepared in bulk with TBO present at a concentration of 100nM, and illuminated using a bright white light (100mW) for different lengths of time, to give a range of illuminations from 0 Jcm^{-2} to 60 Jcm^{-2}. A suspension of cells to which no photosensitiser was added was used as a control.

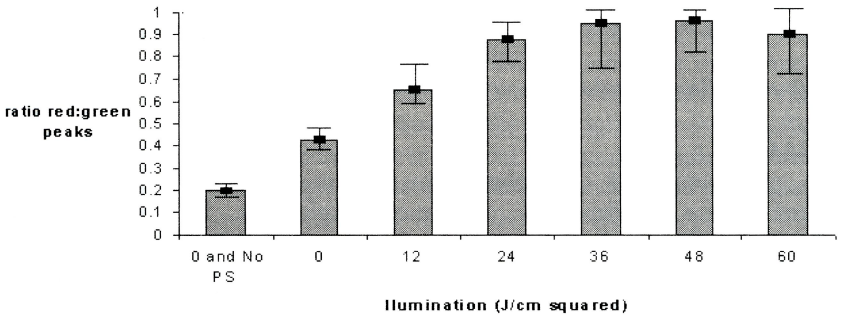

Figure 3. A graph showing the ratio of red peaks to green peaks for cell suspensions in a TBO solutions after illuminations of varying lengths. Using the cells stains outlined above, live cells show only a green peak, whilst dead cells show both green and red peaks. As such as a ratio of 0 represents a population of 100% viable cells, whilst a ratio of 1 represents a totally killed population. The data is shown as the average of 5measurements, with the error bars representing the spread of that average.

After exposure to light, the solutions were mixed with the assay compounds on-chip, droplets were formed (oil flow rate=3μl/min, cell solution flow rate=1.5μl/min, assay flow rate 1.5 μL/min, giving final concentrations of 0.50μg/ml STYO 9 and 0.30μg/ml. PI), and the fluorescence trace recorded after a 10 minute incubation. It can clearly be seen that cell numbers are greatly reduced after a 6 minute exposure (Figure 35), with cell numbers not being greatly reduced with longer exposures.

CONCLUSIONS

A robust and extremely rapid system for the high-throughput screening of photodynamic therapy agents has been demonstrated.

ACKNOWLEDGEMENTS

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