CELL-BASED SCHEDULE DEPENDENT DRUG COMBINATION SCREENING WITH A DROPLET-BASED MICROFLUIDIC SYSTEM

Guansheng Du[1,2], Jaap M.J. den Toonder[2,3], Qun Fang[1]

[1]Zhejiang University, P.R. China, [2]Eindhoven University of Technology, The Netherlands,
[3]Philips Research Laboratories, The Netherlands

ABSTRACT

We proposed an integrated and robust microfluidic platform to perform cell-based schedule dependent drug combination screening. Multiple manipulations of cells in droplets, including cell dispensing, culture, drug stimulation and viability test were performed based on a 2D microfluidic droplet array. The screening of flavopiridol (Fla) and 5-fluorouracil (5Flu) in different sequence was carried out in 342 addressable droplets, with a consumption of ~3 μg drug for each experiment.

KEYWORDS

Drug combination, high-throughput, microfluidic droplet, cell

INTRODUCTION

Drug combination therapy can increase therapeutic efficacy with low drug toxicity due to its multi-target mechanisms [1]. It has become the leading choice for some cancer and infectious diseases, such as prostate cancer and AIDS. Identification of effective drug combination in vitro requires high throughput screening, because the efficacy of drug combinations and drug interactions is dose-dependent [2]. Droplet-based microfluidic systems have been applied in high-throughput screening including protein crystallization, single cell analysis and drug screening due to its low cost and high sensitivity. However, in most droplet-based high throughput screening, it is difficult to replace the medium and drug solution in droplets, which is not optimal for schedule dependent drug combination screening [3]. To address this challenge, we have developed a flexible droplet manipulation system called DropLab [4]. In this paper, we further developed the system into cell culture in droplets and applied it to schedule dependent drug combination screening of flavopiridol and 5-fluorouracil.

In this work, multiplex cell-based and nanoliter-scale schedule dependent drug combination screening was achieved in a droplet-based microfluidic system for the first time. Multi-step droplet operation including controlled timing, dispensing, removal, mixing, transfer, as well as cell analysis which involves cell culture, drug stimulation and viability testing, was realized on a semi-open 2D droplet array. This system was applied to screen schedule dependent drug combinations of flavopiridol and 5-fluorouracil. We achieved 342 addressable and different drug combination conditions on a single PDMS chip with the size of 6 cm × 6 cm. The drug consumption for each screening test was only ~3 μg, which is a 1,000-fold reduction compared with traditional drug screening systems.

EXPERIMENT

In this system, a tapered capillary connected with a syringe pump was used for multi-step droplet operation. A PDMS chip covered with oil was used as the platform for cell analysis. Droplets with a volume of 500 nL were generated containing a number of cells. After 24-hours of incubation, the reagent in the droplet was replaced and the cells were exposed to specific drugs for single or schedule dependent screening. Finally, the drugs in the droplets were replaced by culture medium for 24-hour culture before the cell viability was determined by calcein AM and EthD-1. An array of droplets containing cells is shown in Figure 1.

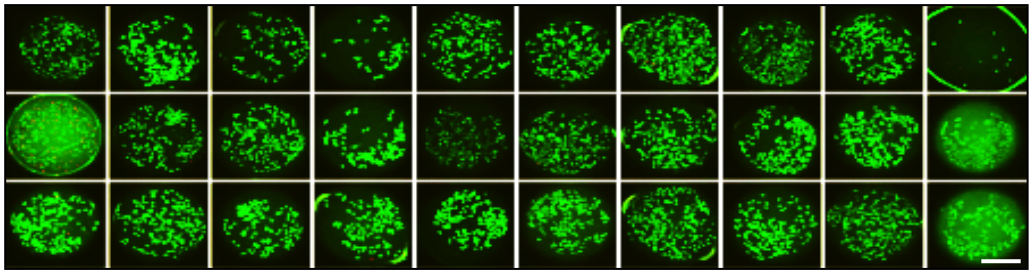

Figure 1. Array of droplets on the chip (scale bar: 5 mm)

Flavopiridol is a cyclin-dependent kinase inhibitor with preclinical activity, which has attracted wide attention due to its unique cell target. As shown in Figure 2, A549 cell line was stimulated by flavopiridol from 2 μM to 1 nM. The EC50 value was calculated to be 210.7 nM. We applied 5-fluorouracil, a widely used anticancer drug, to perform a schedule dependent drug combination screening assay, whose concentrations are shown in Table 1. As shown Figure 3, the cell viability was significantly decreased in the drug combination assay in both of these schedules compared with a single flavopiridol assay. These results indicate that the schedule drug combination of flavopiridol and 5-fluorouracil has a higher efficacy compared with single flavopiridol stimulation.

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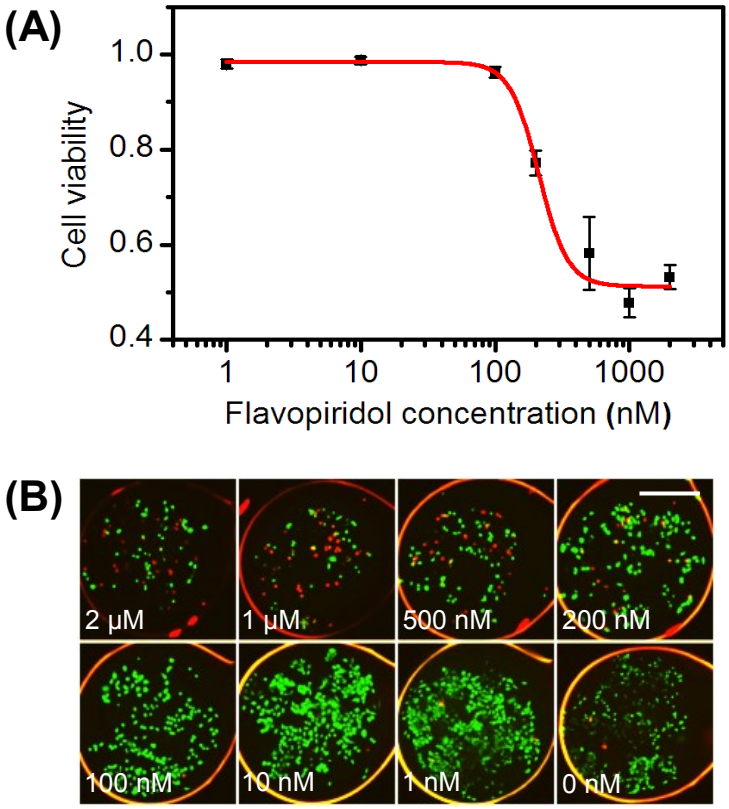
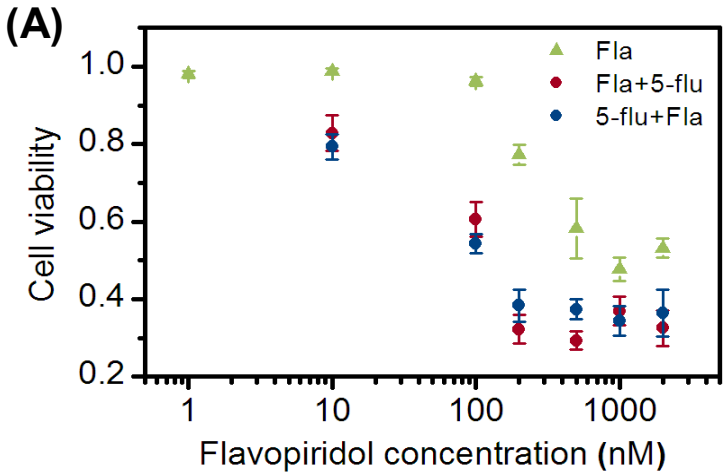


Figure 2. (A) Cell viability after exposed to flavopiridol (B) Fluorescence images of cells treated by different concentrations of flavopiridol (scale bar: 5 mm)

Group of drug combination	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Fla	2 μM	1 μM	500 nM	200 nM	100 nM	10 nM
5Flu	2 mM	1 mM	500 μM	100 μM	50 μM	10 μM

Table 1. Different concentrations of flavopiridol and 5-fluorouracil in schedule dependent drug combination screening



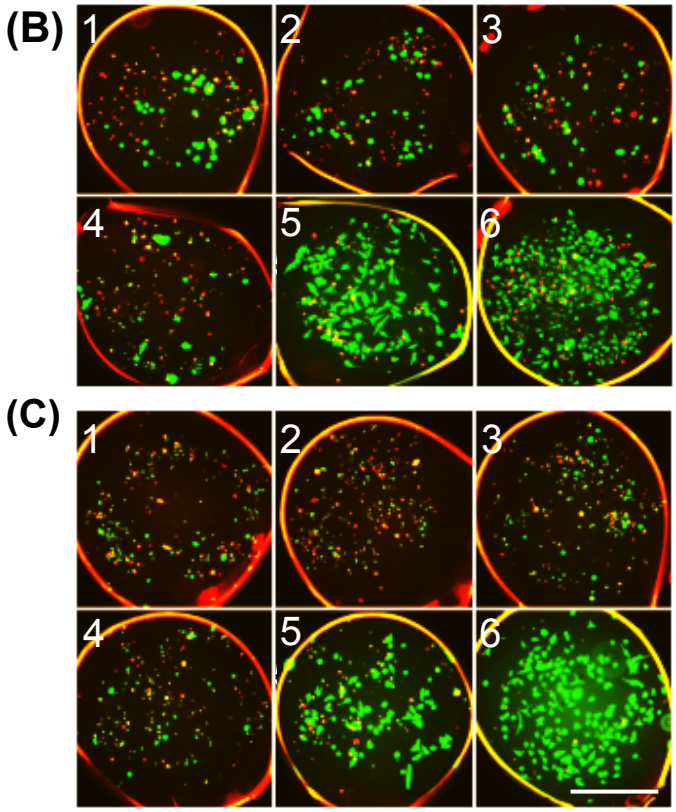


Figure 3. (A) Cell viability after assays of schedule dependent drug combinations. Fluorescence images of cells in droplets with different concentrations with different groups (the numbers) of (B) first of Fla then 5Flu, and (C) first of 5Flu then Fla (scale bar: 5 mm)

CONCLUSION

In this report, we demonstrated an effective system based on microfluidic platform for cell-based drug screening. In this system, we can integrate 342 addressable 500-nL droplets on a single PDMS with size of 6 cm × 6 cm. We applied this system to the assay of A549 exposed to flavopiridol combined with 5-fluorouracil. The combination of these two drugs can significantly decrease the cell viability compared with single flavopiridol treatment. The drug consumption of for each experiment was only ~3 μg, a nearly 1,000 fold decrease compared with traditional methods.

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CONTACT

*Q. Fang, Tel: +86-571-88206771; Email: fangqun@zju.edu.cn