CYTOTOXICITY ANALYSIS ON A CHIP

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ABSTRACT

We have developed a microfluidic reactor array enabling cytotoxicity analysis with a single experiment. This system creates a wide range of concentration gradient of an inhibitor and tests the effect of each concentration on cell viability. From a single on-chip experiment, we determined the effects of two inhibitors on NIH/3T3 fibroblast viability and obtained their IC_{50} values in a fast and efficient manner. The present system represents a common set of tools for the screening of small molecules on all kinds of cells and observing their effects on cell behavior, keeping the integrity of the qualitative and quantitative information.

KEYWORDS: cell-based assay, concentration gradient, cell culture

INTRODUCTION

To evaluate the effectiveness of drug candidates on target diseases or disorders, the half maximal inhibitory concentration (IC_{50}), the value indicating the amount of drug molecules needed to suppress the biological or biochemical functions by half, is widely used [1]. Conventionally, targeted cells are cultivated *in vitro* in flasks, petri dishes, or well-plates and exposed to a wide range concentration gradient of the drug candidates in a linear or logarithmic dilution series[2], using milliliters of reagent and thousands of cells for each single data point. To increase the number of tests executed in one experiment with less volume of reagents, a cellular microarray and microfluidic cell chambers were reported. [3, 4] Although those methodologies could easily create a controlled concentration gradient, enormous amounts of reagents and samples should be provided, which is a major limitation when applied to a limited stock of chemicals. We report an integrated multicompartment device cell-based cytotoxicity test through a log-scale concentration gradient of a molecule on adherent cells, such as NIH/3T3 fibroblasts. By integrating cell culture microchambers with our previous microfluidic system for log-scale gradient formation[5-7], we obtained cell-based IC_{50} values of hydrogen peroxide (H_2O_2) on NIH/3T3 fibroblasts, in a fast and efficient manner.

THEORY

Our microfluidic system, shown in **Figure 1**, is composed of 3 gradient formers (GFs), each composed of 4 discrete processors, flanked by 2 other processors for positive and negative controls, as shown in Figure 1. Each processor is composed of a metering section, a mixing section, and a cell culture section with top open well-shaped microchambers. The metering section allows the formation of a wide range concentration gradient of chemicals that can be introduced into the microchambers to be tested on the cells. By introducing hydrogen peroxide (H_2O_2) at different concentrations into the GFs, we can generate a concentration gradient from 16.88 mM to 0 mM in the 14 cell culture microchambers. By introducing

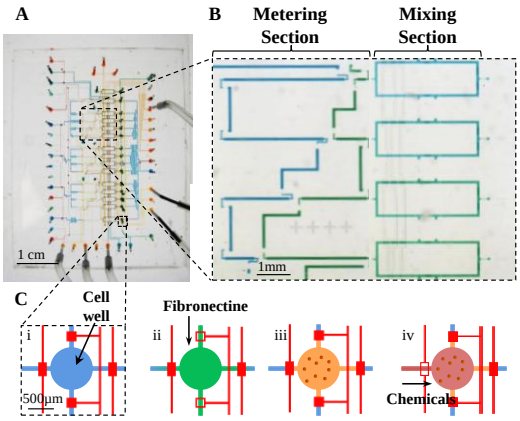

Figure 1. Pictures of the device (A) and a gradient former (B). C Step by step process for surface treatment (i and ii), cell inoculation (iii), and chemical introduction (iv)

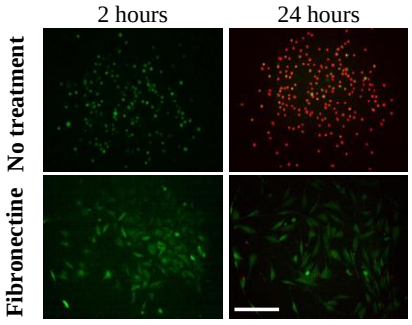

Figure 2. NIH/3T3 cell viability after 2h and 24h of culture in micro-chambers with untreated surface (No treatment) or after fibronectin coating (Fibronectin). Scale bar = 200 µm.

cells from the top of the microwells, we can seed each microchamber with a controlled amount of cells.

EXPERIMENTS

The first step to the evaluation of IC_{50} values of H_2O_2 in our system was to have a sterile environment where cells can be cultivated. We sterilized the microchambers by autoclaving the device. After drying, we treated the well-shaped microchambers for cell culture by coating the surfaces with fibronectin. NIH/3T3 cells were then introduced into the well-shaped microchambers from the open top using capillary tips. After introduction into the microwells, the cells settled to the bottom of the chamber and attached to the treated surface. After 2 hours, different concentrations of inhibitor were introduced into the microchambers and incubated with the cells. After incubation, live/dead cell assays were performed by introducing fluorescent dyes into the culture medium, calcein AM, which stain live cells green, and propidium iodide, which stain dead cells red.

RESULTS AND DISCUSSION

We demonstrated the capability to cultivate NIH/3T3 cells with our system. Two hours after introduction, cells settled in the microchamber, attached, and spread on the fibronectin-treated surface, as shown in **Figure 2**. Live/dead cell assay shows high cell viability after 24 hours. The capability to measure IC_{50} values with our device was demonstrated by performing cell based assays in our chip with different cytotoxic chemicals at different concentrations. After 24 hours of culture with the chemical (H_2O_2), cells were stained with calcein AM and propidium iodide. Pictures of the fluorescent cells were taken, as shown in **Figure 3**. We observed that the cell viability remained high (~80%) when cells were cultivated with low concentration of inhibitors (below 0.422 mM) and decreased when the concentration of inhibitors increased. When the concentration was higher than 4.22 mM, cell viability was minimal (~0%). We chartered the cytotoxicity of the different chemicals by assessing the cell viability patterns with different chemical concentrations through direct cell counting. We have plotted the dose-response curves of the cell viability in function of the different chemical concentrations as shown in **Figure 4** and determined the IC_{50} value. The values observed on chip were in accordance with the value observed off chip, which was obtained using conventional methodology, 1.5 mM and 1.6 mM, respectively.

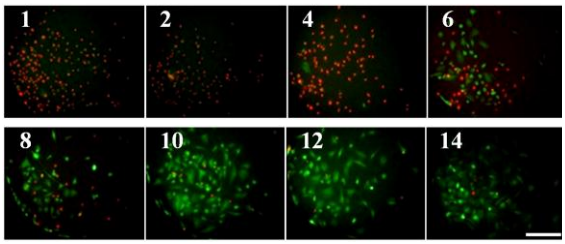

Figure 3. Dose-response of NIH/3T3 cell viability after 24h of culture with different concentrations of H_2O_2 on chip. Numbers are well's numbers. Scale bar = 200 µm.

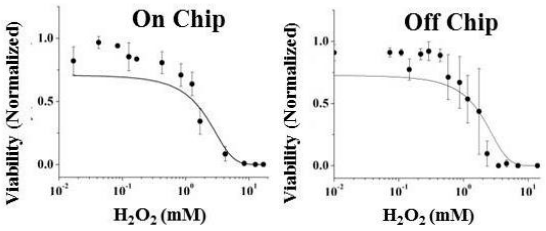

Figure 4. Dose-response curve of cell viability after 24h of culture with different concentration of H_2O_2 on chip and off chip.

CONCLUSION

Our methodology of cell screening is independent of the cell types or tested chemicals. It represents a common set of tools for the screening of small molecules on all kinds of cells and observing their effects on cell phenotype, viability, or behavior, with similar qualitative and quantitative information as conventional methods while using lower amounts of reagents and cells. For example, our method may be used in the field of drug development to quickly and accurately characterize potential new drugs on specific cancer cells, yeasts, or bacteria. Also, the ability to study the inhibition of small molecules of a cytotoxic chemical in our chip may help to majorly advance toxicology, investigating a potential inhibitor to the action of toxin (e.g. in environmental toxins, in food related toxins), or in cosmetic areas, investigating potential anti-oxidants. The present methodology could be applied in the area of regenerative medicine. By introducing stem cells into the wells and replacing inhibitors with growth factors, we could perform quick and accurate tests of different concentrations of a soluble factor and measure its impact on stem cell differentiation.

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