

DEVELOPMENT OF A MICROFLUIDIC CONCENTRATION GRADIENT GENERATOR ON A MICROWELL SLIDE FOR HIGH-THROUGHPUT CELL ANALYSIS

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ABSTRACT

The present study reports a newly developed, microfluidic concentration gradient generator that adds substantial utility to the high-throughput nature of the microwell slide that we have previously established [1, 2]. Using microfluidic dynamics, we have designed microchannels to deliver a reagent and its dilutant with precisely controlled flow volumes, generating 8 discrete steps of reagent concentration in designated microwells. As proof of concept FITC-labeled dextran and dextran-free water was used to visualize the concentration gradient. The next step will be to perform cancer drug concentration gradient experiments on an animal cell line.

KEYWORDS

Microfluidic concentration gradient, High-throughput, Cell analysis, Microwells

INTRODUCTION

To achieve reliable and precise results in high-throughput cell analysis a highly controlled environment is often required. This is often lacking in manually performed experiments or experiments done in an open chamber/well. It has been reported that microfluidic gradient devices can control the pattern and the magnitude of a continuous gradient of biomolecules [3].

Here a polydimethylsiloxane (PDMS) microfluidic 3D-structure is integrated to a microwell slide, making it an enclosed environment. The present device differs from the previous devices in that a variety of concentrations are created in a spatially discrete manner. In a dose-dependency analysis performed on the microwell platform, cells in individual microwells are subjected to a single concentration. Due to the layout of the device the cells are not affected by intercellular paracrine signaling from neighboring cells exposed to a different concentration.

EXPERIMENT

The PDMS microfluidic components consist of three layers: a microchannel layer, a mixing chamber/drain layer and a gasket layer. A schematic illustration and a photograph of the device are shown in Figure 1(A) and 1(B), respectively.

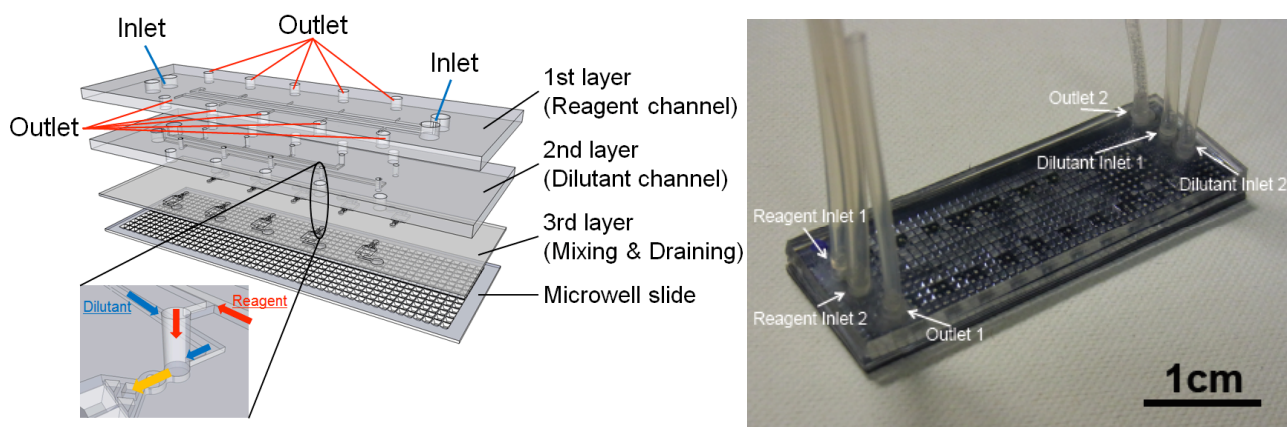


Figure 1. (A) Schematic configuration of the device. Reagents are introduced into channels on the top layer, and dilutants are introduced into channels on the second layer. (B) Photograph of the device.

Each layer was fabricated individually using photolithography and soft-lithography techniques, using a double stack of a 112 μm thick sheet of dry resist film. The components were bound together with the microwell slide on a hotplate following surface treatment with air plasma. The experimental reagent and its dilutant are introduced from separate inlets and delivered to 8 mixing chambers, shown in Figure 2.

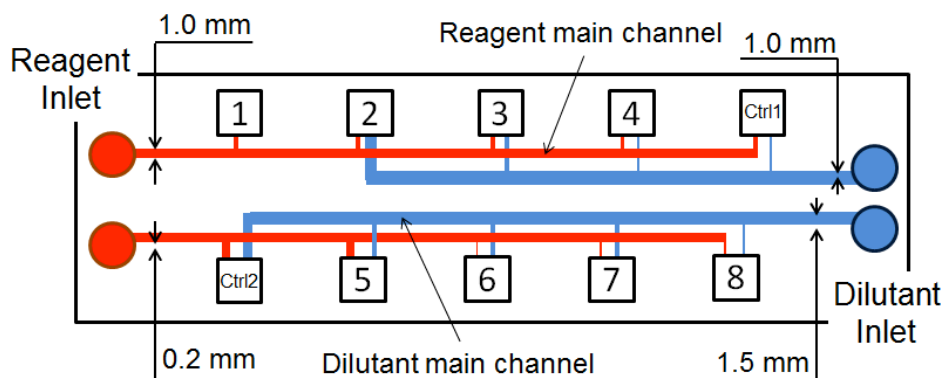


Figure 2. Specification of microchannels and numbers of mixing chambers on the device. There are 8 mixing chambers. The reagent channel, orange, in the top layer and the dilution channel, blue, in the second layer.

Each of the chambers covers four microwells of the previously described microwell slide [1, 2]. The reagent and dilutant concentration in the mixing chamber is dependent on the width and the length of the flow channels, resulting in an 8-step reagent concentration gradient, ranging from $\times 1$ (input concentration) to $\times 0.01$. To validate the device, a solution of FITC-labeled dextran in water at 0.6 mM and dextran-free water were injected using a syringe pump. Fluorescence intensity in the mixing chamber was measured using an inverted fluorescent microscope and compared to a theoretical result.

As a first cell experiment in the device, cultured bovine endothelial cells were exposed to a Saponin solution. The device gave rise to 8 Saponin concentrations and 2 controls. The result was evaluated in a fluorescent microscope after a live/dead stain assay was performed on the cells with a 5 μM Calcein AM and a 5 μM Ethidium Homodimer solution.

RESULTS AND DISCUSSION

It was confirmed that an 8-step concentration gradient of the FITC-dextran solution was created, spanning three orders of magnitude, shown in Figure 3. The experimental results correspond well with the theoretical results.

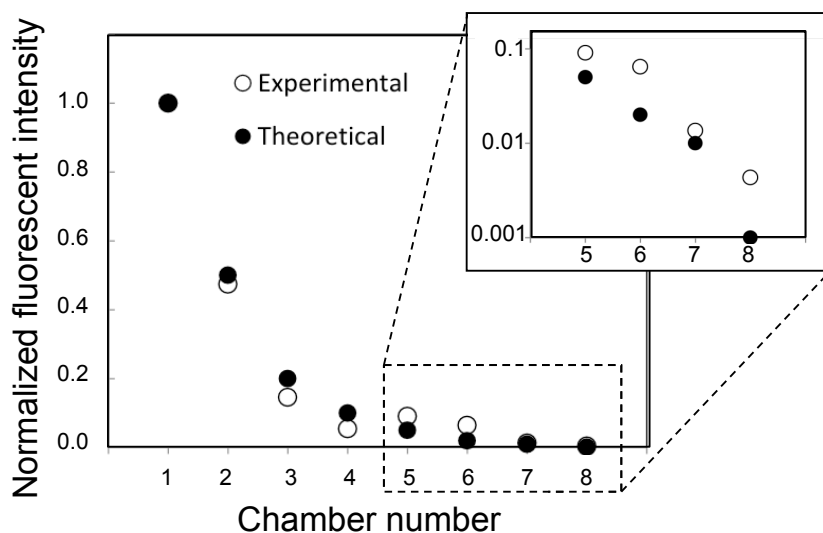


Figure 3. Comparison of concentrations in the 8 mixing chambers between the experimental result and a theoretical analysis.

Figure 4 shows fluorescent images of the 8 mixing chambers after cultured bovine endothelial cells were exposure to the Saponin solution. The amount of dead cells increased with the concentration of Saponin, as expected, while live cells decreased. The decrease was quantitatively confirmed for live cells as shown in Figure 5. The microfluidic device show great potential and can be used in e.g. drug concentration experiments when screening for an appropriate drug concentration. The device will use less reagents than traditional concentration assays and have a highly controlled environment what impedes intercellular paracrine signaling from neighboring chambers exposed to a different concentration.

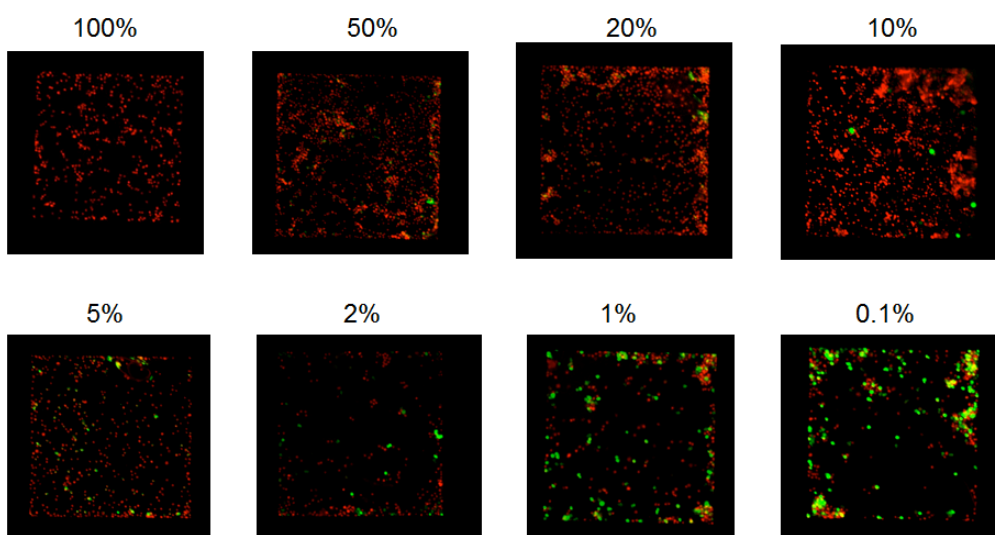


Figure 4. Fluorescent images of the 8 mixing chambers from chamber 1 (concentration of Saponin: 100%) to 8 (0.1%). Green: live cells, red: dead cells.

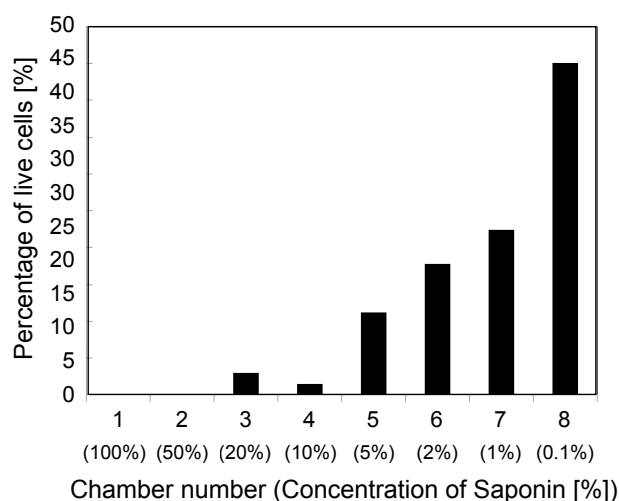


Figure 5. The percentage of live cells from chamber 1 (concentration of Saponin: 100%) to 8 (0.1%).

CONCLUSION

With this new microfluidic device we expand the capabilities of the microwell slide, enabling several parallel animal cell assays to be performed under highly controlled conditions. The results confirm the ability of the device to create 8 designated concentration steps of a reagent. Hence, the developed microfluidic components adds an important function to our microwell slide.

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