Video Article

A Microfluidic Device for Studying Multiple Distinct Strains

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Abstract

The study of cell responses to environmental changes poses many experimental challenges: cells need to be imaged under changing conditions, often in a comparative manner. Multiwell plates are routinely used to compare many different strains or cell lines, but allow limited control over the environment dynamics. Microfluidic devices, on the other hand, allow exquisite dynamic control over the surrounding conditions, but it is challenging to image and distinguish more than a few strains in them. Here we describe a method to easily and rapidly manufacture a microfluidic device capable of applying dynamically changing conditions to multiple distinct yeast strains in one channel. The device is designed and manufactured by simple means without the need for soft lithography. It is composed of a Y-shaped flow channel attached to a second layer harboring microwells. The strains are placed in separate microwells, and imaged under the exact same dynamic conditions. We demonstrate the use of the device for measuring protein localization responses to pulses of nutrient changes in different yeast strains.

Video Link

The video component of this article can be found at <http://www.jove.com/video/4257/>

Introduction

Cells are constantly reacting to a dynamically changing environment, by changing their metabolism, transcriptional profile and cellular functions. To study these phenomena, methods that can quantify such changes are needed. One type of readout for such responses is the change in localization of stress related transcription factors in response to nutritional stress.

Microfluidic devices¹ have been used to dynamically manipulate environmental conditions to cells²⁻⁴. They present several advantages for live cell imaging: the environment of the cells can be precisely controlled and dynamically changed; cells can be live-imaged at all times, including during manipulation of external conditions; and minimal reagent volumes are required. A very simple design for a microfluidic device, allowing alternation between two conditions, is a Y-shaped device². We routinely use Y-shaped microfluidic devices that allow application of dynamic changes to the cells in the channel. We use this device to follow localization changes of fluorescently tagged stress related transcription factors in yeast cells. We follow these changes under changing nutritional conditions. For example, we can provide a pulse (or series of pulses) of glucose-containing medium within a period of no-glucose medium, at a very fine temporal resolution. Often in such experiments, one is interested in comparing the responses of different cell strains (for example, different mutants, or different wild isolates). The Y-shaped channel is limited to one strain in each channel - if more than one strain is used, there is no simple way to distinguish between the strains. To overcome this, different channels can be used. If we want to apply the same dynamics conditions to multiple strains, we would like to combine the Y-channel concept with multiple channels. There are two challenges in implementing this solution: It can be hard to apply the same conditions at the same time to all channels, and there is a geometric limitation to the number of Y-channels that can be fitted in one device. We were therefore looking for a way for viewing several strains in one channel under dynamic conditions.

Here we describe a two-layered microfluidic device intended for imaging multiple strains in one channel under the same dynamic conditions. The bottom layer consists of thin PDMS with a row of holes. This layer is attached to the glass coverslip creating wells into which we will place the different strains, adhering them with Concanavalin A to the glass. The second layer is a Y-shaped microfluidic device created using the scotch tape method⁵. After placing the strains in the wells the second layer is quickly aligned and attached to the first one, creating one channel with several wells that contain the different strains. This final device allows following several strains in one channel, where all strains are subjected to the same conditions at the exact same time. The whole design and production process can be done on the benchtop, using simple means, with no soft lithography.

Protocol

1. Creating a Scotch-tape Master⁵

1. Draw or print out desired microchannel layout, to scale, on paper. In our case the design consists of two Y-shaped channels, each 3 mm wide (**Figure 2**).
2. Cover a glass-slide with layers of scotch tape. The number of layers will determine the height of the channel (about 60 μm per layer). We used 3 scotch tape layers.
3. Place the layout design on a flat surface. Align the slide over the design pattern. Carefully cut the tape on the glass slide with a scalpel according to the layout.
4. Remove the Scotch tape from all regions of the glass slide except those in the layout of the microchannel.
5. Place the slide in a heating oven at 65 °C for 2-3 min. Clean gently with ethanol.

2. Fabricating a PDMS Microfluidic Device

1. Mix the base and curing components of PDMS as recommended by the manufacturer. We use a 10:1 ratio of base to curing agent. Pour about 30 ml of PDMS mixture into a Petri dish to a height of ~0.5 cm. Degas the PDMS in vacuum if needed. Submerge the glass slide in the PDMS with the patterned Scotch tape facing up. Placing the pattern after the PDMS prevents formation of air bubbles between the slide and dish bottom. Cure for 48 hr at 65 °C, making sure the dish is horizontal using a bubble level.
2. In a new 90 mm Petri dish pour 3 ml of PDMS mix, resulting in a layer about 0.5 mm deep. (If a spin coater is available, it can be used for this stage).
3. Degas and cure as in 2.1.
4. Gently cut out the microfluidic device to the desired size with a scalpel. This layer will be termed the "flow layer".
5. Cut out a similarly sized piece of thin layer PDMS from the second Petri dish. This will be termed the "wells layer".
6. Use an appropriately sized biopsy puncher (We use 1.2 mm ID) to punch holes for the inlets and outlets in the flow layer.
7. Place the wells layer on a thick glass slide over the design layout and punch out wells, using 2 mm ID biopsy puncher, in alignment with the microchannels on the layout.
8. Clean both PDMS layers as well as a 24 mm x 60 mm glass coverslip with ethanol. Air-dry and keep clean.
9. Plasma treat both the glass coverslip and the wells layer PDMS using either standard plasma etcher (for non-reversible bonding) or a hand-held corona treater⁶ for reversible bonding. Carefully place the wells layer on top of the coverslip to cause adhesion (**Figure 3**). Gently rub out any air bubbles (if needed).

3. Cell Imaging Experiment

1. Make sure you have the desired media ready in appropriate syringes connected to flexible plastic tubes with internal diameter of 0.02" (Tygon) in the syringe pump.
2. Grow *S. cerevisiae* strains to the desired phase in liquid YPD. Vortex thoroughly and place 300 μl of each strain into a microcentrifuge tube.
3. Gently place 1 μl of Concanavalin A (sigma) 2 mg/ml into each well. Wash out excess Concanavalin A from each well twice by gently pipetting water.
4. While the Concanavalin A is drying, wash strains twice with 300 μl of SC medium lacking glucose. Washing residual glucose from the cell walls helps proper adherence to the Concanavalin A.
5. Vortex cells thoroughly. Pipette 0.75 μl or less of each cell suspension into its own well (**Figure 3**). Gently wash out residual cells with rich medium. The next two steps need to be performed quickly in order to prevent cells from drying up.
6. Plasma treat the chip and the wells thoroughly using the hand-held corona treater, being careful not to hit the wells directly. Carefully place the chip on the wells, paying close attention to the alignment between the two (this step can be done under a stereoscope). Gently press to adhere the two layers of PDMS. Slowly fill the device completely with about 50 μl rich medium, making sure no air bubbles are left inside the channel.
7. Connect the device, inserting the tubes into the appropriate inlets and start media flow. Take care that no air bubbles are introduced into the device as these can be tricky to remove; this can be accomplished by disconnecting an inlet or outlet and gently tapping on the device where the bubbles are, being careful not to break the glass coverslip.
8. Place under microscope and find appropriate imaging points in each well.
9. Keep the cells under rich medium flow for 1 hr or longer to allow recovery if needed.
10. Start the experiment: we use constant flow from each of the two syringe pumps to prevent backflow of the media, changing relative flow rates as desired. Setting pump A to 0.8 ml/hr and pump B to 0.2 ml/hr results in medium A flowing over 80% of the width of the channel (**Figure 4**). This can be dynamically changed by changing the two flow rates. The new conditions stabilize within seconds along the full length of the channel².

Representative Results

To demonstrate the separation between different strains we imaged two distinguishable yeast strains in alternate wells. Imaging the full wells shows no cell leakage between wells (**Figure 5a,b**). Both strains have the transcription factor MSN2 tagged with YFP. To test the simultaneous effect of dynamically changing conditions, we switched the flow rates in the two input channels, creating a step of no-glucose medium, which

resulted in localization of Msn2-YFP to the nucleus (**Figure 5c,d**). The response measured at all the wells occurred at the same time, showing the device can be used for application of concurrent dynamic conditions to multiple wells.

Figure 1. Device design. The device is composed of a coverslip, a thin "Wells" layer and a "Flow" layer to which tubing is connected.

Figure 2. Flow layer design. A scotch-tape mold is made for this layer. Here we used two Y-shaped channels of opposing directions.

Figure 3. Wells layer. The thin layer of PDMS with punched-out wells is adhered to a glass coverslip. Then ConA is placed in each well and allowed to dry. Cells are then loaded to the 10 wells.

A

B

Figure 4. Controlled channel coverage. By controlling the relative flow rates of the two inputs of a Y channel, a specific fraction of its width is covered with medium A vs. medium B. (a) Left channel: Flowing at 0.8ml/hr (red) and 0.2 ml/hr (clear) results in 80%/20% coverage. Right channel: Flowing both media at 0.5 ml/hr results in 50%/50% coverage. (b) Photographs of the device with 10%/90%, 50%/50% and 90%/10% flow rates.

Figure 5. Dynamic experiment with multiple yeast strains. (a,b) Whole-well images of wells containing yeast cells with (b) or without (a) HTB2-Mcherry tagging, showing no cell leakage between wells. (c,d) Response of the cells in two wells to a no-glucose step at t=0. Msn2-YFP localizes to the nucleus at the same time following the step, demonstrating simultaneous media changes in the two wells. (e) Time-tracks of nuclear Msn2-YFP levels in single cells analyzed from one of the wells in (c,d). [Click here to view larger figure.](#)

Discussion

In this paper we present a simple benchtop method for creating a microfluidic device that allows following several yeast strains simultaneously under dynamic conditions. Following several strains in one channel provides a reliable tool for comparing dynamics of single cell responses in multiple strains. One advantage of our approach is the ability to fabricate the device with simple techniques without the need for a clean room. In fact, we have also carried out the protocol skipping the plasma treatment operations (in steps 2.9 and 3.6) and got good results, so labs without any plasma equipment can still perform the protocol.

By seeding each of the different strains in its own well we avoid cell mixing during the assembly of the device, while still allowing medium flow to reach the cells during the experiment. A critical point in this protocol is not letting the cells dry out before reflowing medium over them (Steps 3.4 - 3.6). Drying out severely affects the viability of the cells, while trying to close the device with too much medium at each well results in adhesion problems between the two PDMS layers. These constraints force us to leave the wells layer in the device, rather than peel it off after the cells have settled in their spot. The wells layer, therefore, has to be deep enough to contain the initial droplet of seeded cells, but as thin as possible, so the aspect ratio of the wells allows medium flow to reach their bottom and the cells get external signals through direct flow.

The device as we describe here is limited to about 5 strains per channel. We routinely create devices with two adjacent Y channels at opposing directionalities (**Figure 2**). While this can be extended to a few more channels, or a few more wells per channel, it is still not as high throughput as some of the VLSI style devices⁷, that so far have not been applied to living cells. We note, however, that since the main purpose of this device is to subject different strains to the exact same conditions while imaging their responses, there are limitations to concurrent imaging that

stem from the number of strains imaged, regardless of the device implementation: at high magnification, strains have to be imaged sequentially, therefore the more strains imaged, the larger are the time differences of imaging between the first and last strain.

Disclosures

No conflicts of interest declared.

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