A microfluidic system based on capillary effect for high-throughput screening of yeast cells

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ABSTRACT

Microfluidic systems have been developed and used successfully for high-throughput cell-based assays. However, these systems require complicated peripherals and a computer to control the flow of liquid which limits their adoption by biologists. Here, we propose a simple-to-use high-throughput microfluidic system based on capillary effects for single cell entrapment, rapid exchange of reagents, and multiplexed assays. The system consists of (i) an autonomous capillary microfluidic system molded in PDMS, (ii) coverslips with patterned arrays of microwells sealed to the PDMS chip, (iii) a custom-built holder that can be mounted onto an inverted confocal microscope.

Keywords: High-throughput screening, microfluidic system, capillary effect, yeast

1 INTRODUCTION

Microscale science and microtechnology enable us to design, manufacture and create microfluidic systems with dimensions ranging from millimeters down to micrometers. Microfluidic systems are being increasingly used for cell biology since they provide small footprints yet offer complex functions and allow changing the fluidic environment of cells for high-throughput cell-based assays, such as high-content screening (HCS). HCS requires a special control of environmental parameters, delivery of multiple reagents, advanced microscopy, and multi-parameter readouts; using high magnification microscopy and consequently is expensive.

Microfluidic systems are promising for use in HCS because they can be used for screening multiple individual cells in a parallel, fast and flexible manner for different types of cells and conditions. For the fabrication of microfluidic devices, polydimethylsiloxane (PDMS) is widely used. It can be easily molded, it is optically transparent, and has low toxicity and high permeability to oxygen and CO_2 all of which make it suitable for cell culture purposes.

Microfluidic systems with orthogonally crossed sets of microchannels offer the opportunity for studying the interaction of large numbers of molecules with cells in a combinatorial manner. Khademhosseini *et al*. presented a soft lithographic method to fabricate multiphenotype cell arrays by capturing cells within an array of reversibly sealed microfluidic channels. In this study they designed a PDMS array of five parallel channels with independent inlets and a common outlet. Hence a combination of 25 different conditions can be tested [1]. The system is promising for HCS however it has some limitation. The channels are more than 200 µm wide, limiting imaging processes and a syringe pump was required to control the flow. The system is not compatible with high magnification imaging while experimenting. In another study Wang *et al*. reported the development of a multilayer elastomeric microfluidic array for the high-throughput cytotoxicity screening of mammalian cells [2]. This platform allowed cell-seeding as “rows” and combinatorial exposure of cells to toxins delivered along “columns”. The system is versatile, but it is complicated because it needs to be connected to a multichannel pressure controller unit requiring manifolds, valves and multiple connections.

While each of these studies presents new concepts for improved microfluidic cell culture, the overall challenge of making a practical, cost-effective platform for cellular assays has yet to be solved. Most of these systems are complicated and need specific peripheral equipment and a computer to run.

Here, we present a setup with a microfluidic capillary system (µCS) [3], a coverslip with arrays of microwells and a custom built holder that allows real time high magnification imaging using a microscope. The system can be used for patterning different “rows” of living yeast cells, and exposing them to different “columns” of chemicals. The system facilitates studying the effects of drugs on the immobilized cells in microwells because it allows high magnification imaging while exchanging reagents.

2 PROTOCOLS

µCS were fabricated by standard microfabrication procedures by replicating a master wafer with photopatterned SU-8 into PDMS. The µCS used in this study had microchannels that were 50 µm wide, 10 µm heights, and 500 µm lengths with a 50 µm gap between adjacent microchannels. The microchannels were coated with 1 mg/ml bovine serum albumin (BSA) in phosphate buffered saline (PBS) prior to use.

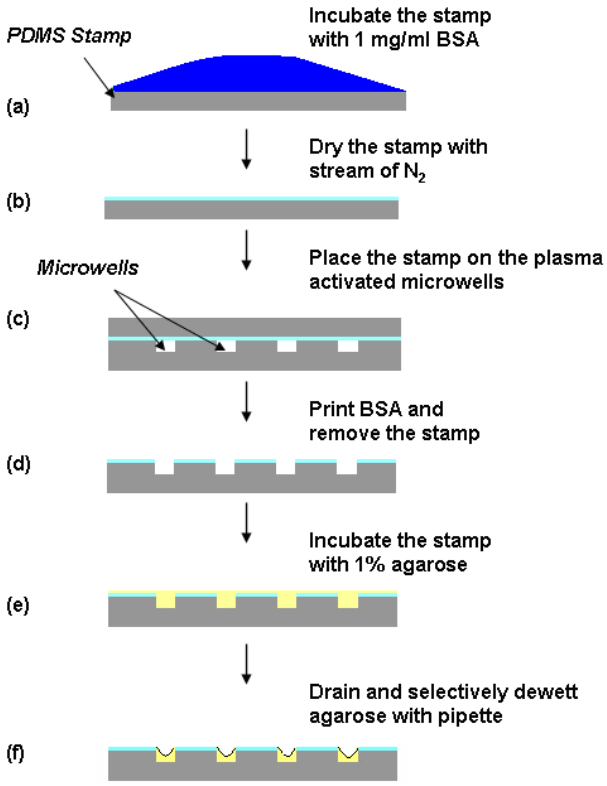

Figure1. Schematic illustration for the preparation of microwell arrays. The surface of the microwells was printed with 1mg/ml BSA to prevent adhesion of the cells on the substrate. Microwells were then selectively incubated with 1% agarose in distilled water, the solution drained followed by gelation of the agarose in the bottom of the microwell. The microwells can be stored in humid condition for several days' prior to use.

The microwells had diameters ranging from 10-50 µm and there is a 100 µm distance between each well. The microwells were formed by pressing a PDMS stamp with posts onto a coverslip coated with PDMS prepolymer which was cured in placed. Air plasma was used for 20-30 s to render microwell arrays and microchannels hydrophilic. Using microcontact printing BSA was selectively printed onto the ridges of the microwell arrays [4]. Microwells were filled with dilute agarose solution using selective dewetting. Gelation occurred after partial evaporation of the water so that the agarose formed only at the bottom of the wells (figure1).

The substrate was sealed to a µCS (with drilled inlet and outlet). Yeast cells were flowed through the microchannels and adhered selectively in the microwells. Remaining liquid was removed by applying a vacuum at the outlet for less than 10 seconds (figure 2).

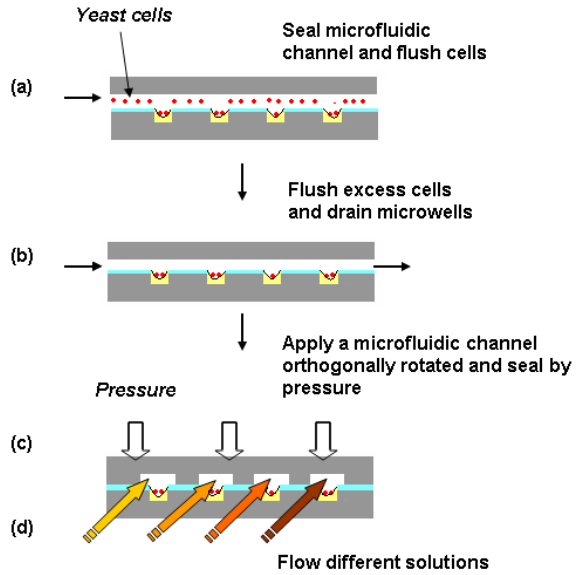

Figure 2. Schematic illustration of the selective cell deposition inside microwells and subsequent exposure to different solutions. (a) µCS was reversibly sealed on the microwells and cells were flowed through independent channels. (b) Cells lying outside of the microwells were flushed out by draining the liquid with a vacuum. Another µCS was placed orthogonally and sealed by applying light pressure. Different chemicals were delivered to different channels and flowed by capillary force.

Next the PDMS with µCS was detached, and a new chip was placed orthogonally over the microwells, so as to expose different cell "rows" to different "columns" of solutions.

3 RESULTS AND DISCUSSION

In this study we designed a microfluidic system consisting of a µCS, a coverslip with arrays of microwells and a custom built holder that allows real time high magnification imaging using a microscope.

According to the process described in figure 1, microwells selectively were filled with agarose (figure 3). Plasma treatment and hydrophilization of the microwells were critical for the selective dewetting and filling of all the microwells with very high yield. We found that BSA was necessary to prevent adsorption of agarose to the top surface during draining of the agarose solutions. If the agarose was left on the top surface, yeast cells would stick to it. BSA coating allowed complete removal of yeast from the channels as shown in figure 4. In addition complete removal of cells from chip facilitates orthogonal placement and sealing of the second µCS. Different solutions can then be flushed over the cells (figure 5).

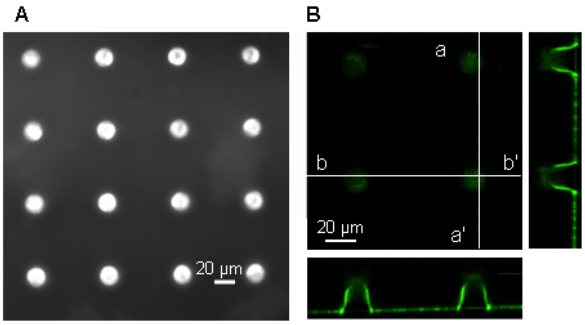

Figure 3. Selective coating of microwells with agarose. (A): microwells filled with 1% agarose solution mixed with trace amounts of fluorscein for visualization of the agarose at the bottom of the microwells. (B): confocal image of the bottom of the microwells. (aa', bb'): cross section of the microwells. Microwells with 17 µm depths which were only filled up to 1/3 with agarose.

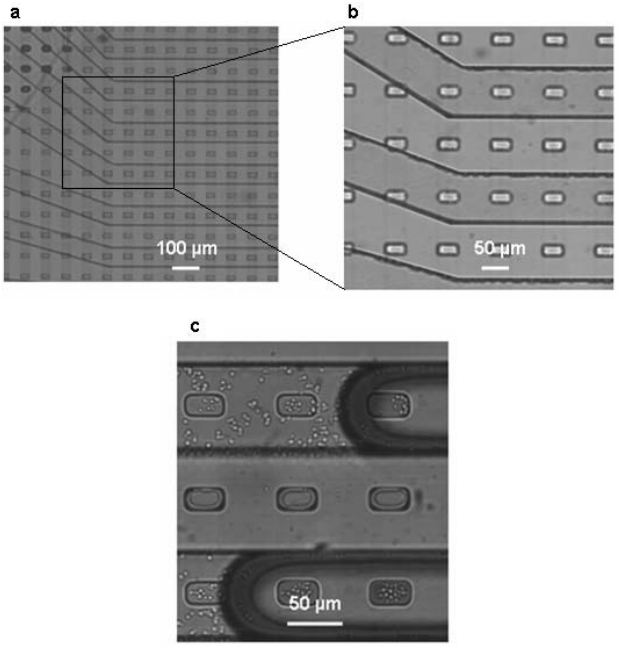

Figure 4. Microchannels sealed to microwell array for cell seeding. (a, b): arrays of microwells sealed to a capillary system. (c): microchannels are filled with cells suspension, after 10 min incubation the liquid was drained. Yeast cells seeded in the channel and were selectively removed by draining of the liquid. The yeast cells adhere to the agarose in the well but not to the BSA on the surface of the substrate.

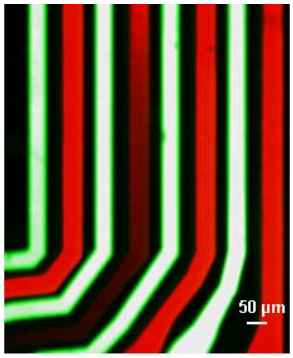

Figure 5. Delivery of different "columns" of the chemicals to different "rows" of the cells. Overview of microchannels filled with Fluorescein and Rhodamine.

4 CONCLUSION

In this study we introduced a simple-to-use high-throughput microfluidic system based on capillary effects for yeast cell entrapment, rapid exchange of reagents, and multiplexed assays. Using this system we were able to expose the cells to different solutions while simultaneously imaging them with a microscope. Our chips feature 16 µCS which by crossing can create 256 combinations if 16 different cell lines and 16 different solutions are used. Because our system is compatible with real time high magnification microscopy, it is well suited for HCS and using yeast cells.

5 ACKNOWLEDGMENT

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