Design, Fabrication, and Application of a Microfluidic Device for Investigating Physical Stress-Induced Behavior in Yeast and Microalgae

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Abstract

Purpose: The development of an efficient in vitro cell culture device to process various cells would represent a major milestone in biological science and engineering. However, the current conventional macro-scale in vitro cell culture platforms are limited in their capacity for detailed analysis and determination of cellular behavior in complex environments. This paper describes a microfluidic-based culture device that allows accurate control of parameters of physical cues such as pressure. Methods: A microfluidic device, as a model microbioreactor, was designed and fabricated to culture Saccharomyces cerevisiae and Chlamydomonas reinhardtii under various conditions of physical pressure stimulus. This device was compatible with live-cell imaging and allowed quantitative analysis of physical cue-induced behavior in yeast and microalgae. Results: A simple microfluidic-based in vitro cell culture device containing a cell culture channel and an air channel was developed to investigate physical pressure stress-induced behavior in yeasts and microalgae. The shapes of Saccharomyces cerevisiae and Chlamydomonas reinhardtii could be controlled under compressive stress. The lipid production by Chlamydomonas reinhardtii was significantly enhanced by compressive stress in the microfluidic device when compared to cells cultured without compressive stress. Conclusions: This microfluidic-based in vitro cell culture device can be used as a tool for quantitative analysis of cellular behavior under complex physical and chemical conditions.

Keywords: Lab-on-a-chip, Mechanical stress, Microalgae, Microfluidic device, Yeast

Introduction

The development of an efficient in vitro cell culture device to process various cells would be a major milestone in biological science and engineering. For example, the polystyrene cell culture dish (known as the ‘Petri dish’) has been widely used for maintaining and growing animal cells in vitro; this platform is still used as a conventional tool for biological studies such as fundamental biology and drug development for the treatment of animal or human diseases (Kim et al., 2014; Mehling et al. 2014). Plant cell culture is usually conducted using soil pots or agarose plates as an analysis platform for understanding the behavior of plant cells prior to culture and growth in traditional greenhouses (Sanati, 2014). Complete analysis and understanding of cell behavior in complex environments, however, require the design of cell culture platforms beyond the conventional Petri dish. (Charvin et al., 2008; Mehling and Tay, 2014; Sanati, 2014). Nevertheless, detailed quantitative and dynamic analysis of cell behavior in different environments is largely limited by effects of physical forces on cell behavior. Continuous tracking of...
living cells during their culture is difficult (e.g., analysis of cell behavior against time-dependent stimuli), which means that current assays of cell behavior based on conventional cell culture platforms can often be inaccurate and generate misleading results.

A 'Lab-on-a-chip device,' manipulated by micro-electro-mechanical systems (MEMS) technology, is presently recognized as an emerging strategy for the design and manipulation of in vitro cell culture platforms to solve current limitations (Charvin et al., 2008; Falconnet et al., 2011; Huh et al., 2012; Lecault et al., 2011; Mehling and Tay, 2014; Sanati, 2014; Kim et al., 2014; Vyawahare et al., 2010). Recent studies have shown the great potential of microfluidic culture platforms in providing (i) complex internal or external cell microenvironments (Kim et al., 2014; Mehling and Tay, 2014; Sanati, 2014), (ii) advantages of miniaturization for handling a small sample volume for detailed quantitative analysis of living cells (Mehling and Tay, 2014), and (iii) a platform for high-throughput analysis of cell behavior and continuous tracking of the changed behavior of cells against time-dependent external stimuli (Charvin et al., 2008; Falconnet et al., 2011; Huh et al., 2012; Lecault et al., 2011; Mehling and Tay, 2014; Sanati, 2014; Vyawahare et al., 2010). The use of microfluidic devices in current trials is an initial step, but quantitative analysis of living systems using a 'lab-on-a-chip' device would offer great opportunities for advanced biological science and engineering.

Cells are exposed to many external chemical and physical cues in their environment (Im et al., 2013). However, compared with the effects of chemical factors on cell behavior and function, the extent and importance of physical cues are still poorly understood and require further investigation. For example, yeast and algal species can adapt and grow when exposed to a variety of physical forces, including pressure (Mehling and Tay, 2014; Sanati, 2014). This suggests that physical cues may trigger behavioral and functional responses in yeasts and algae; consequently, analysis platforms for analyzing cell responses to physical cues would be helpful for optimizing cell culture conditions (Mehling and Tay, 2014; Sanati, 2014). In the present study, we used soft lithography to fabricate a simple but powerful microfluidic-based in vitro cell culture device for examining the physical stress-induced behavior of cells. We then performed a proof-of-concept study using this platform in combination with bioimaging technologies to demonstrate that external physical forces can control the shape and function of small living cells such as yeasts and microalgae.

Materials and Methods

Device design and fabrication

The device contained a double layer of polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, USA) channels bonded in a skewed position and separated by a flexible PDMS membrane; this served as the micro-valve and works by injected air pressure. The PDMS can be replicated from a master mold and plasma bonded. The master mold was fabricated following a conventional photolithography process (Figure 1(a)). A negative photoresist, SU-8 100 (Microchem, USA), was spun on a plasma cleaned 4 inch Si wafer to 100 μm thickness. The wafer was then soft-baked on a hot plate for 10 min at 65°C, immediately followed by 30 min at 95°C. A film mask (Hanalltech, KR) was then tightly contacted to the surface of this photoresist-coated wafer and exposed to 405 nm ultraviolet light (Shinu MST, Korea) at 500 mJ. After this exposure, the wafer was post-baked at 65°C for 1 min and at 95°C for 10 min. SU-8 developer (Microchem, USA) was used to remove the uncrosslinked SU-8 photoresist, leaving the patterned photoresist.

Multilayer soft lithography

We obtained a complete mold piece from the replicable mold fabricated through photolithography by using PDMS, which was chosen for its biocompatible, optically transparent, gas permeable, and elastomeric properties. PDMS was prepared following the manufacturer’s instruction: a 10:1 ratio of two parts was degassed by vacuum before pouring into the master mold (Figure 1(b)). A single channel wafer for the first layer was prepared and spin-coated (500 rpm, 60 sec) with freshly mixed uncrosslinked PDMS to make a 100 μm thick membrane over the patterns. The uncrosslinked PDMS was then cured on a hotplate for 5 min. At the same time, PDMS mixture was poured (2 mm thick) onto a wafer patterned with the same single channel representing the air-chamber. Polymerized air-channel molds were cut out and prepared. After the preparation, the air-channel was accurately aligned to the ROI part on the first layer mold and plasma bonded. The reservoir was attached and additional PDMS was poured over the patterns to adjust the total thickness to 5