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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

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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

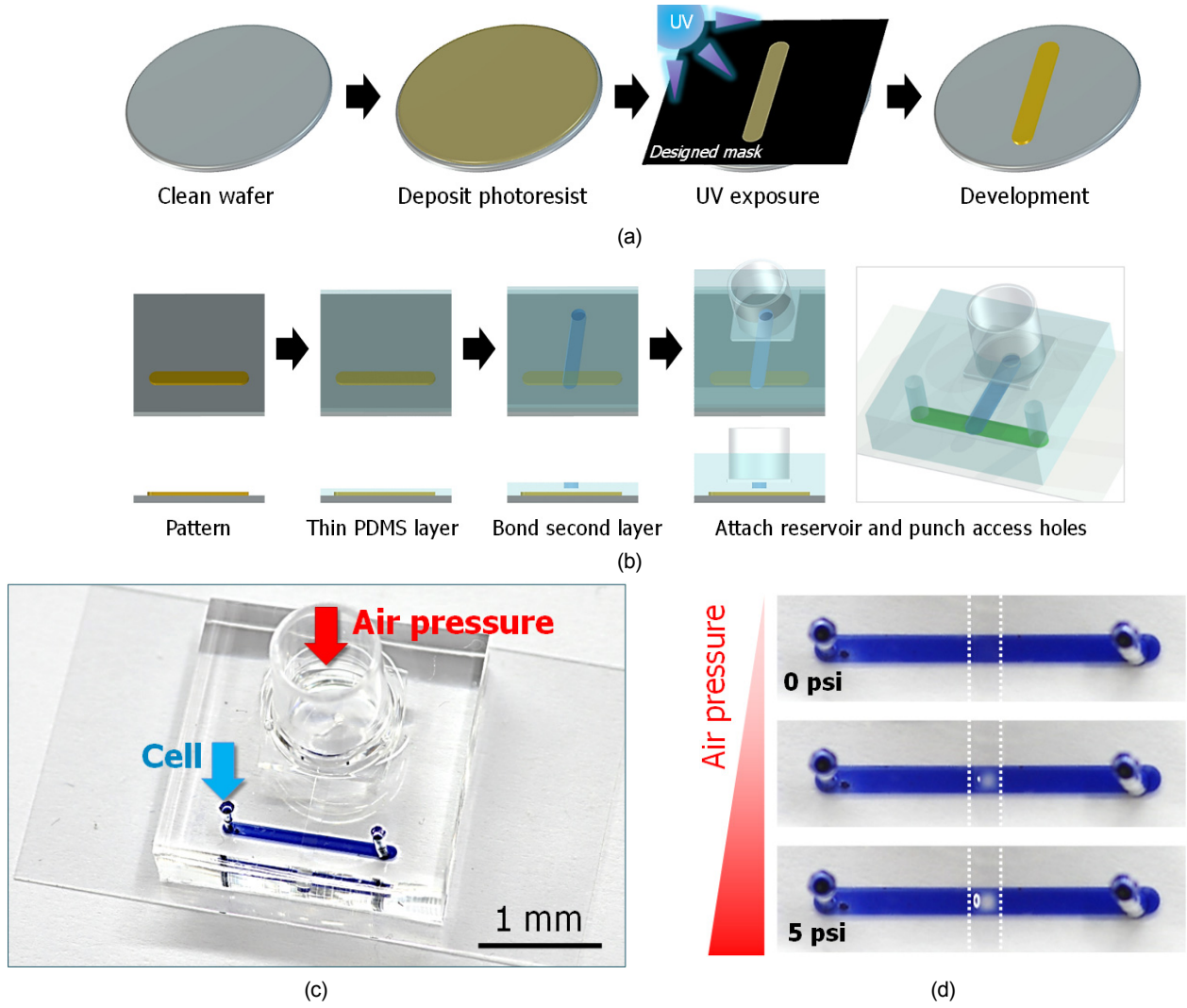

Figure 1. Design and manipulation of a microfluidic device for physical stress-induced cell culture. (a) Photolithography procedure. The positive micro-channel pattern was fabricated by the consecutive steps indicated. (b) Multilayer soft lithography: The micro-channel was covered with a layer of PDMS. The prepared upper channel layer was irreversibly bonded in a skewed position. The reservoir was attached and an additional amount of PDMS was cured to hold the layers tight. (c) Photograph of the device. Cells were injected into the lower channel through the cell inlet (indicated by blue dye). Air pressure was applied through the reservoir. (d) Device operation. The micro closure was proportional to the pressure intensity.

mm. Replica molds were cut out into appropriate pieces and access holes were punched out to form the complete piece. The final piece was plasma bonded with a cleaned glass cover slip for the desired device operation.

System installation for physical stress-induced yeast and microalgae behavior

The ONIX^{TM}FG software was used to control the pressure pump (Onix, CellASIC^{TM}, US) in the cell culture system (Figure 2(a)). Constant or pulsed type of pressure was generated at a range of 0 to 10 psi. The pressure range was controlled precisely within 0.005 psi and the time lag was controlled within an order of milliseconds. The pressure was directly delivered to the device from the pump through a 6 mm diameter tube, with the device installed firmly on a microscope stage.

Physical stress-induced yeast and microalgae culture

Saccharomyces cerevisiae in synthetic complete (SC) medium lacking leucine was pre-cultured overnight at 30°C by adding 100 μl of seed culture to 10 ml of the same medium and culturing at 30°C on an orbital shaker at 200 rpm. When the cell culture optical density (O.D.) at 595 nm was about 0.4 (early log phase), cells were introduced into the cell channel. *Chlamydomonas reinhardtii* in TAP

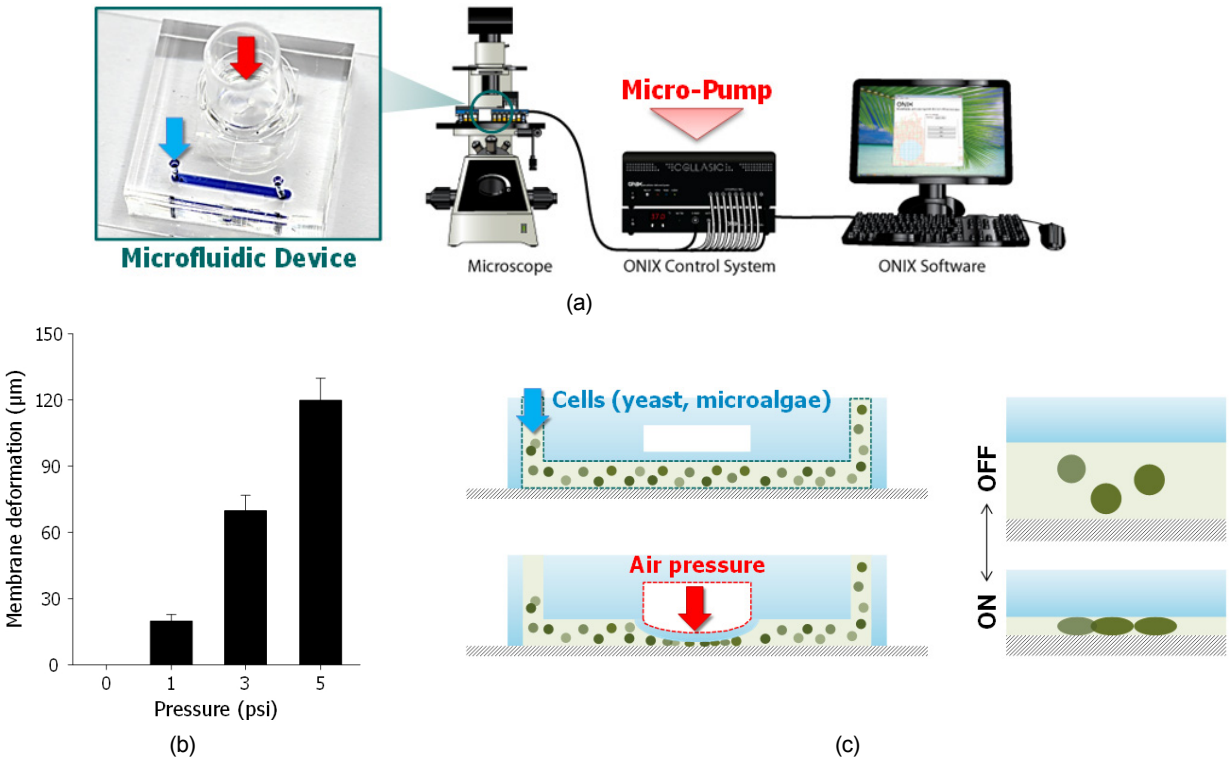

Figure 2. Experimental setup and operation scheme for physical stress-induced cell culture. (a) Micro-pump installation. The microfluidic device was fixed firmly onto the microscope stage. The reservoir was tubed to the micro-pump, which was controlled via ONIX software. (b) Membrane deformation dependence on the applied pressure. (c) Device operation scheme. Cells were introduced into the lower micro channel. When air pressure was applied, the air channel expended and the membrane contacted the bottom of the channel. The cells beneath the ROI were therefore compressed. The state of the micro-valve was controlled relative to the micro-pump activation.

(Tris-Acetate-Phosphate) medium was cultured at 23°C and 5% CO_2 on an orbital shaker at 125 rpm. When the cell culture optical density (O.D.) at 680 nm was about 1.0 (early stationary phase), cells were introduced into the cell channel.

Visualization of neutral lipids in algae with SF-44

C. reinhardtii cells in the device were washed twice with phosphate buffered saline (PBS) for 5 minutes and treated with 5 μM Seoul-Fluor 44 (SF-44) (E. Kim et al., 2012) in TAP medium for 15 minutes. After PBS washing, cells were observed using a confocal microscope in an incubation chamber set at 23°C and 5% CO_2 with an appropriate excitation and emission filter set (ex = 488 nm / em = 600 nm).

Confocal microscope imaging

Fluorescence and z-stack images of cells were obtained with a confocal microscope (Olympus FV 1000). Lasers (488 nm and 546 nm) were used to visualize green and red fluorescence, respectively.

Image post-correction analysis

Data were analyzed using Microsoft Excel 2010, images were processed with ImageJ software, and graphs were drawn with SigmaPlot 10.0. All 3D images were deconvoluted with IMARIS 7.1.0 (Bitplane, MN).

Statistical analysis

Student's t-test was used for statistical analysis using SAS software. All quantitative results were presented as mean ± standard deviation (SD).

Results and Discussion

Design and manipulation of microfluidic device for stress-induced cell culture

Platforms were manipulated by fabricating the microfluidic device containing a cell culture channel and air channel, using PDMS and soft lithography (Figure 1). The single

micro-channel patterned wafer was first prepared as a mold using photolithography (Figure 1(a)). The PDMS was then poured and cured on the patterned wafer. A second single micro-channel was bonded onto the original patterned PDMS in a skewed position (i.e., two micro-channels: cell culture channel and air channel). All the access holes of the device were punched out, and the final piece was bonded onto a cleaned glass cover slip (Figure 1(b)). As shown in Figure 1(c), the fabricated microfluidic device contained two micro-channels: one for cell culture and the other for air pressure. The air pressure channel was connected to a vacuum pump, providing 5 psi to the cells when the membrane contacted the bottom of the channel (Figure 1(d)).

Figure 2 shows a schematic of the microfluidic device-based cell culture system used for physical stress-induced living cell cultures. The microfluidic device was connected to a micro-vacuum pump to generate physical force on the cells and to a fluorescence microscope for tracking and evaluating cell behavior and function (Figure 2(a)). Deformation of the membrane in the microfluidic device to a given pressure was measured: Pressure was applied until the membrane contacted the bottom of the device (Figure 2(c)). The maximum deformation was proportional to the applied pressure, from 0 to 5 psi. When the pressure reached 5 psi, the membrane contacted the bottom of the channel, indicating pressure application to whatever was under the membrane and confirming that the microfluidic device could provide a physical force (i.e., compressive stress) to the cells during culture. We used this platform to culture yeasts or microalgae as model cells and investigated how a physical force like pressure can affect cell behavior (Figure 2(c)).

Effect of physical stress on morphology of yeast

Yeasts are eukaryotic microorganisms and are commonly used in biotechnology for bioprocessing of products such as alcoholic beverages, baked goods, ethanol, and nutritional supplements. Yeasts are susceptible to a variety of physical forces during their life cycle, but continue to be grown on solid growth media or in liquid broths in the laboratory. Environmental conditions are among the most important elements in yeast culture but the behavior of yeasts following exposure to physical pressure has not been studied because of a lack of appropriate culture conditions.

We investigated the effects of mechanical stress on yeast behavior by culturing the yeast *Saccharomyces cerevisiae*, which is used for winemaking, baking, and brewing (Figure 1). An appropriate number of yeast cells were introduced into the cell culture channel, and the cells were then cultured with or without application of mechanical stress (Figure 2). The yeast cells were highly spread out when they were compressed at a pressure of 5

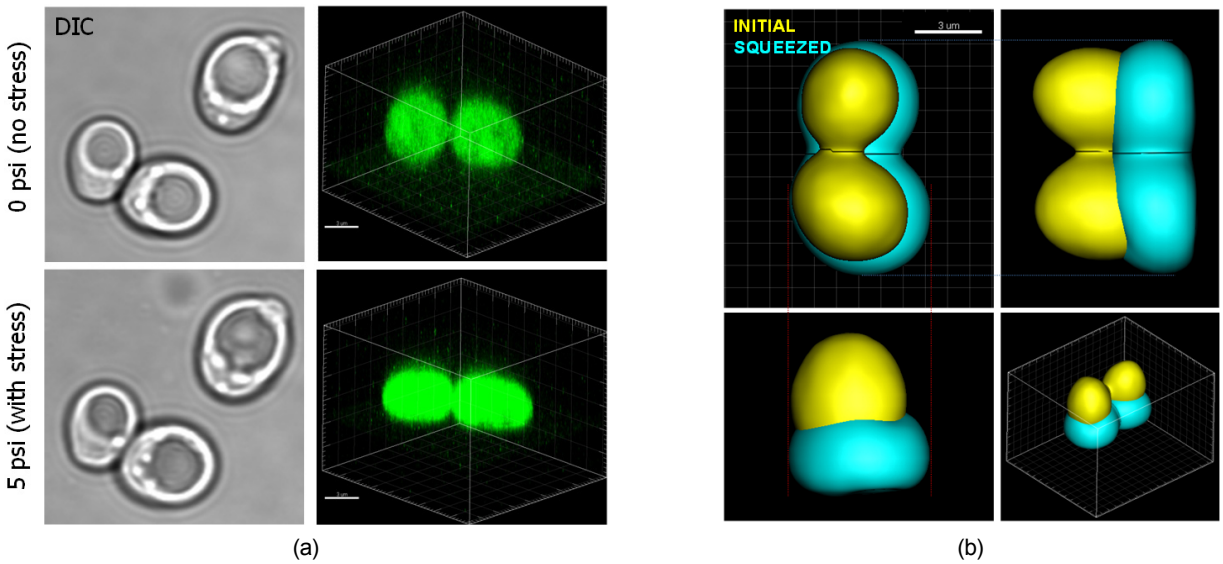

Figure 3. Effect of physical stress on morphology of *Saccharomyces cerevisiae*. (a) State of the yeast in each condition. Different interference contrast (DIC) images show the widened cross section of a yeast cell exposed to a pressure of 5 psi. Images of yeasts stained in green were captured in stacks and deconvoluted to 3D image. (b) Morphologies of the yeasts were post-corrected via IMARIS software. Yellow spheres represents the initial state of the yeasts, while the relatively flattened cyan spheres represent the yeasts in a compressed state caused by applied pressure.

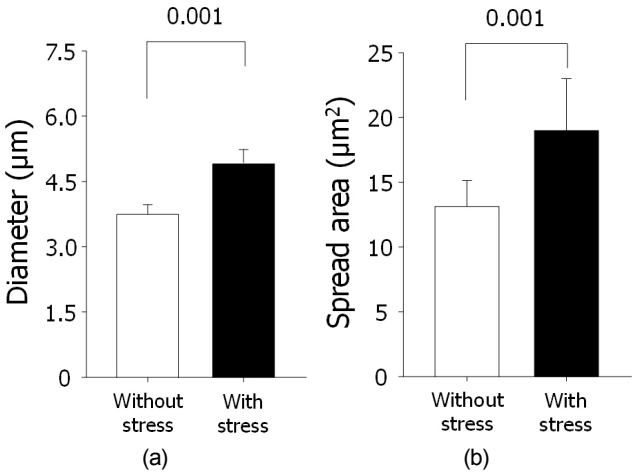

Figure 4. Quantitative analysis of the diameter and spread area of *Saccharomyces cerevisiae* cultured under compressive stress.

psi. This was also confirmed by the fluorescence microscopy image analysis of GFP-conjugated yeasts cultured under the same conditions (Figure 3(a)). We analyzed the morphologic changes in the yeasts by decovoluting image stacks into 3D images of GFP-conjugated yeasts using IMARIS software. The surfaces of the yeast cells can be monitored in a computerized view with this software (Figure 3(b)). The yellow spheres indicate the initial morphology of yeasts whereas the cyan spheres show the compressed state and indicate that the imposition of physical stress greatly affected the morphology of the yeast cells. Figure 4 shows a quantitative analysis of the diameter and spread area of the yeasts. Yeasts under mechanical stress covered approximately 50% more area compared to the yeasts without stress.

The proposed microfluidic device clearly could impose a mechanical stress on the yeasts during their culture, which allowed us to confirm that yeasts can respond to a physical stress. This response is probably a natural characteristic and capability of yeasts for adaptation to complex environments. Furthermore, manipulation of the air pressure applied by our device could control the degree of change in the morphology of the yeasts, providing us with a. platform for investigating the details of yeast responses to mechanical forces in their growth environment.

Effect of physical stress on morphology and function of microalgae

Microalgae are photoautotrophic organisms and are generally more efficient converters of solar energy compared to terrestrial plants (Eu et al., 2014; Holcomb et al., 2011; Park et al., 2013). Microalgae can produce high energy biochemicals through photosynthesis using sunlight, H_2O, and CO_2 (Eu et al., 2014). Microalgae have therefore been recently proposed as a renewable energy alternative for the production of biofuels to meet the world's growing demand for fuels (Eu et al., 2014; Holcomb et al., 2011; Park et al., 2013). However, the production of biofuel from microalgae is currently too expensive using existing processing technology, so an efficient approach is urgently needed to reduce the development costs and time if this type of biofuel production is to become viable commercially. Microfluidic-based devices may provide useful screening platforms for developing technologies for efficient production of biofuels from microalgae since these devices use only tiny amounts of sample and medium compared to current methods (i.e., culturing microalgae on large platforms).

The present proof-of-concept study explored the feasibility of the proposed microfluidic device by culturing *Chlamydomonas reinhardtii* under a mechanical force investigating how a compressive stress affects the shape and function of this microalga (Figure 2(c)). Compressive stresses were delivered to the cells through the fabricated device by applying air pressure at 5 psi to the device. The cells were compressed between the PDMS membrane and the glass bottom for 12 h. Throughout the experiment, cells were continuously incubated at 23˚C in a conventional incubator in 5% CO_2. Stressed cells and unstressed control cells were compared.

The microalgae were fully compressed (Figure 5(a)) and the compressed cells were significantly less thick and had a wider area compared to the unstressed cells (Figure 5(b)). When stained for neutral lipids with SF-44 (Figure 6(a)), the compressed cells showed higher fluorescence intensity (about 1.6 fold higher, Figure 6(b)) when compared to the uncompressed cells, indicating that the microalgae produced more lipids in response to compressive stress. Physical pressure (i.e., compressive force) can therefore apparently control the shape of microalgae and promote lipid production, which could be viewed as an efficient strategy for manipulation of microalgae in large scale platforms for production of biofuels. The present study represents a first step in evaluating the behavior of microalgae by using a microfluidic device to determine the optimum conditions for microalga processing.

Design and manipulation of *in vitro* cell culture platforms

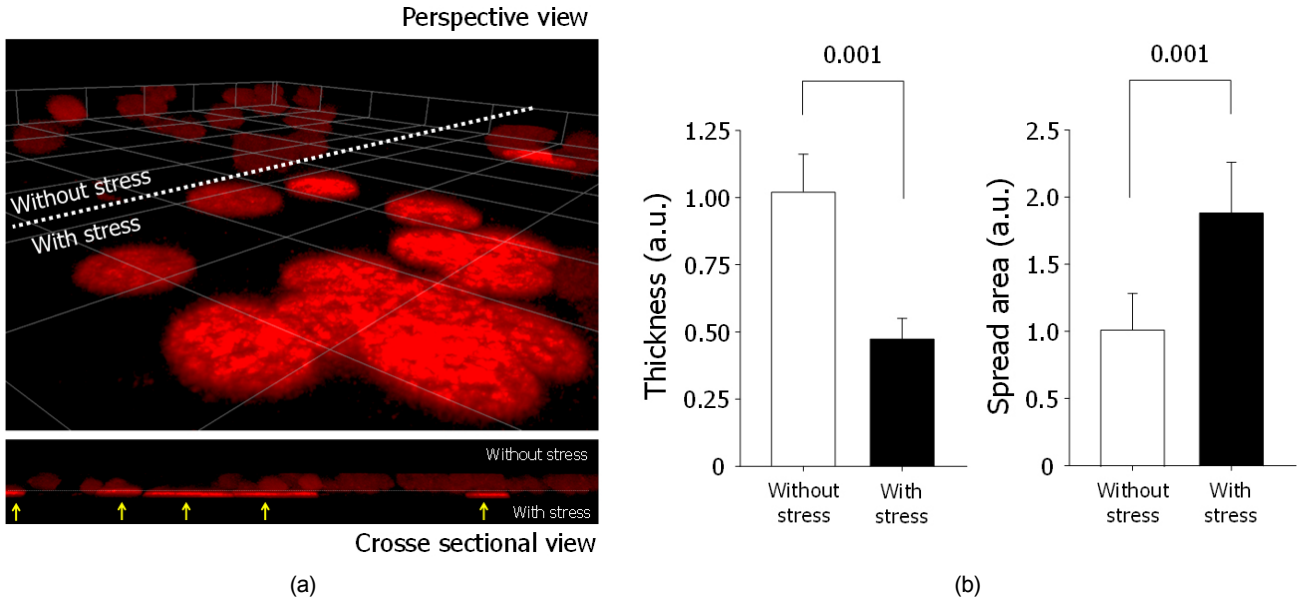

Figure 5. Effect of physical stress on morphology of *Chlamydomonas reinhardtii*. The microalgae were compressed by application of air pressure to the device. (a) 3D image of compressed microalgae beneath the device micro-valve. A perspective view clearly shows the morphological differences between the stressed and non-stressed microalgae. (b) Quantitative analysis of cell thickness and the spread area when microalgae were cultured under compressive stress.

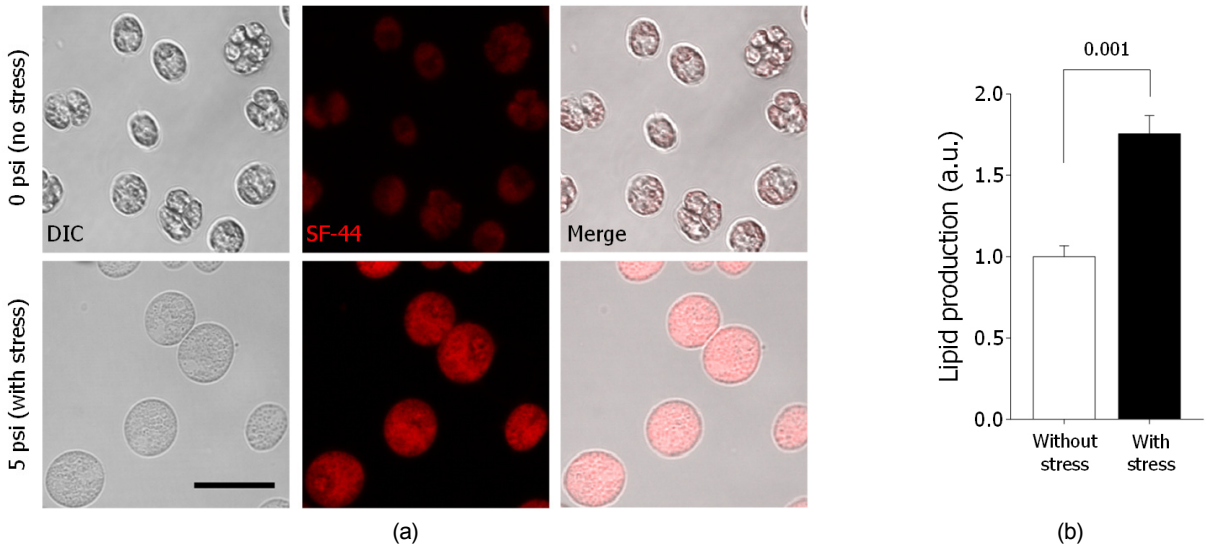

Figure 6. Effect of physical stress on function of *Chlamydomonas reinhardtii*. Neutral lipids were stained with SF-44, red. (a) Distinct morphological differences between the compressed and uncompressed states. Projection of stacked images of average intensity confirmed the increase in fluorescence intensity. (b) Enhanced lipid production by microalgae under compressive stress. Scale bar = 20 μm.

based on the concept of the 'lab-on-a-chip' can provide many advantages beyond those available with current animal or plant cell culture platforms. Experiments using current platforms are costly and time consuming, and fail to provide *in vivo*-like environments that include chemical and physical cues (Kim et al., 2014; Mehling and Tay, 2014; Sanati, 2014). In the present study, we have proposed a simple microfluidic-based *in vitro* cell culture device that allows investigation of physical stress-induced behavior of cells (Figure 1). We used our platform to culture cells under mechanical force conditions (Figure 2) and found considerable differences in the morphologies of both yeasts and microalgae (Figures 3-5). This finding is especially important because these cells are naturally exposed to a variety of stresses in their normal living conditions, so that changes in cell shape could lead to

changes in cell function (Mehling and Tay, 2014; Sanati , 2014). Therefore, external physical forces such as pressure could be expected to play key roles in cell behavior. Precise control of these conditions should allow determination of optimal conditions for a specific desired behavior. The use of our proposed microfluidic device confirmed that physical pressure stress caused definite changes in the shape and fluorescence intensity of *C. reinhardtii* cells. The fluorescence intensity data still need to be correlated with actual amounts of lipid, but we predict that the enhanced fluorescence signal in stressed cells corresponds to lipid accumulation (Figure 6). The aim of the present study was to provide preliminary results for designing and manipulating microfluidic growth conditions in an *in vitro* cell culture device. Use of this device has confirmed that we can generate physical stresses that can mimic the specific *in vivo* environment that occurs in large scale devices, thereby providing us with an efficient strategy for quantitative analysis of living systems in biological science and engineering.

Conclusions

We have modeled the physical cues generated in cell culture environments by fabricating a simple microfluidic-based *in vitro* cell culture device containing a cell culture channel and an air channel to examine physical stress-induced behavior of yeasts and microalgae. The fabricated microfluidic device was connected to a vacuum pump and fluorescence microscope as an analysis system. This system revealed that the shapes of *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii* cells were changed following imposition of a compressive stress in this microfluidic device. In particular, *Chlamydomonas reinhardtii* cells were fully compressed by the expanding PDMS membrane and the fluorescent staining signal for neutral lipids was enhanced over that seen in unstressed cells. The current study focused on the analysis of behavior of yeasts and microalgae in terms of cell shape, but the platform has potential for application to other cell systems, including animal cells, for determining physical stress-induced responses. In conclusion, the results presented in this study collectively demonstrate that this microfluidic-based *in vitro* cell culture device may be useful as a tool for quantitative analysis of living systems exposed to different physical environments.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

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References

Charvin, G., Cross, F. R and E. D. Siggia. 2008. A microfluidic device for temporally controlled gene expression and long-term fluorescent imaging in unperturbed dividing yeast cells. PLoS One 3(1):e1468.

Eu, Y., Park, H., Kim, D and J. W. Hong. 2014. A microfluidic perfusion platform for cultivation and screening study of motile microalgal cells. Biomicrofluidics 8:024113.

Falconnet, D., Niemisto, A., Taylor, R. J., Ricicova, M., Galitski, T., Shmulevich, I and C. L. Hansen. 2011. High-throughput tracking of single yeast cells in a microfluidic imaging matrix. Lab on a Chip 11:466-473.

Holcomb, R. E., Mason, L. J., Reardon, K. F., Cropek, D. M and C. S. Henry. 2011. Culturing and investigation of stress-induced lipid accumulation in micro algae using a microfluidic device. Analytical and Bioanalytical Chemistry 2011:400(1):245-253.

Huh, D., Torisawa, Y., Hamilton, G. A., Kim, H. J and D. E. Ingber. 2012. Microengineered physiological biomimicry: Organs-on-Chips. Lab on a Chip 12:2156-2164.

Im, A., Kim, J., Lim, K., Seonwoo, H., Cho, W., Choung, P and J. H. Chung. 2013. Effects of micro-electrical stimulation on regulation of behavior of electro-active stem cells. Journal of Biosystems Engineering 38(2):113-120.

Kim, E., Lee, S. H and S. B. Park. 2012. A Seoul-Fluor-based bioprobe for lipid droplets and its application in image-based high throughput screening. Chemical Communications, 48:2331-2333.

Kim, J., Kim, H. N., Lang, Y and A. Pandit. 2014. Biologically inspired micro-and nanoengineering systems for functional and complex tissues. Tissue Engineering

Part A 20:2127-2130.

Lecault, V., VanInsberghe, M., Sekulovic, S., Knapp, D., Wohrer, S., Bowden, W., Viel, F., McLaughlin, T., Jarandehei, A., Miller, M., Falconnet, D., White, A. F., Kent, D. G., Copley, M. R., Taghipour, F., Eaves, C. J., Humphries, R. K., Piret, J. M and C. L. Hansen. 2011. High-throughput analysis of single hematopoietic stem cell proliferation in microfluidic cell culture arrays. Nature Methods 8:581-586.

Mehling, M and S. Tay. 2014. Microfluidic cell culture. Current Opinion in Biotechnology 25:92-102.

Park, J. W., Na, S. C., Lee, Y., Lee, S., Park, S. B and N. L. Jeon. 2013. Measurement of lipid droplet accumulation kinetics in *Chlamydomonas reinhardtii* using Seoul-Fluor. Energies 6:5703-5716.

Sanati Nezhad A. 2014. Microfluidic platforms for plant cells studies. Lab on a Chip [Epub ahead of print].

Vyawahare, S., Griffiths, A. D and C. A. Merten. 2010. Miniaturization and parallelization of biological and chemical assays in microfluidic deceives. Chemistry and Biology 17(10):1052-1065.