MECHANICAL CHARACTERIZATION OF CYANOBACTERIA UNDER OSMOTIC STRESS

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ABSTRACT
We report the evaluation results of mechanical characterization of *Synechocystis* sp. PCC 6803. We have constructed the force measurement system using the microfluidic chip on the microscope with optical tweezers. Indentation and force sensor probes are integrated into the microfluidic chip. Using the constructed system, we evaluated the Young's modulus of two cell groups of wild type and mutant type, which was knocked out mechanosensitive channels. They are placed in two different mediums; normal and high-osmolality condition. While there was no significant difference of stiffness between two groups in normal medium, the average value and the standard deviation of Young's modulus were different in high-osmolality condition. These results indicate the basic function of mechanosensitive channels, because there was clear relationship between the stiffness and the osmotic stress. We confirmed that the measurement system will contribute to clarify unknown function of ion channels of cyanobacteria based on relationship between environmental stress and mechanical characteristics on single cell level.

INTRODUCTION
Synechocystis sp. PCC 6803 is one of unicellular cyanobacteria, and it is a kind of floating cell. It is used as a model organisms for analyzing photosynthesis [1], biofuel production [2], and its osmoadaptation mechanisms to culture environment [3]. In the adaption mechanism, mechanosensitive channels play important roles that they work as a kind of regulator to response intracellular pressure relating to osmotic condition of culture medium [4]. When cyanobacteria are exposed to the high-osmolality condition, intracellular H_2O flow out to medium condition. Thus, intracellular pressure decreases, and it cause the decrease of stress of cellular membrane. As a result, mechanosensitive channels respond to the membrane stress to keep intracellular pressure [5, 6]. Since osmolality changes cause the intracellular pressure change, the role and activity of mechanosensitive channels can be evaluated by measuring the cellular stiffness including intracellular pressure. Therefore, the measurement system of mechanical characteristics such as stiffness is highly demanded for analyzing the effects of mechanosensitive channels with alive condition. In this paper, we present mechanical characterization system consisting a microfluidic chip integrated with an indentation probe and a leaf spring type force sensor. Moreover, we show the evaluation results the Young's modulus of two types of cyanobacteria; wild type (WT) and mutant type knocked out large-conductance MS channels (ΔMscL) in two different medium condition; normal medium and high-osmolality medium.

MATERIALS AND METHODS
System configurations
Generally, it is required to deform the target cell, and to measure the reaction force for measuring the mechanical characteristics of cell such as stiffness. Particularly, we have to consider the mechanical characteristics of *Synechocystis* sp. PCC 6803 to construct the measurement system. The size of cyanobacterium is approximately only a few micrometers in diameter, and its stiffness is thought to be relatively high because of cellulose covering outside the cell. Therefore, there are two important keys for measurement the mechanical characteristics; he way to deform the cell, and the way to transport the cell.

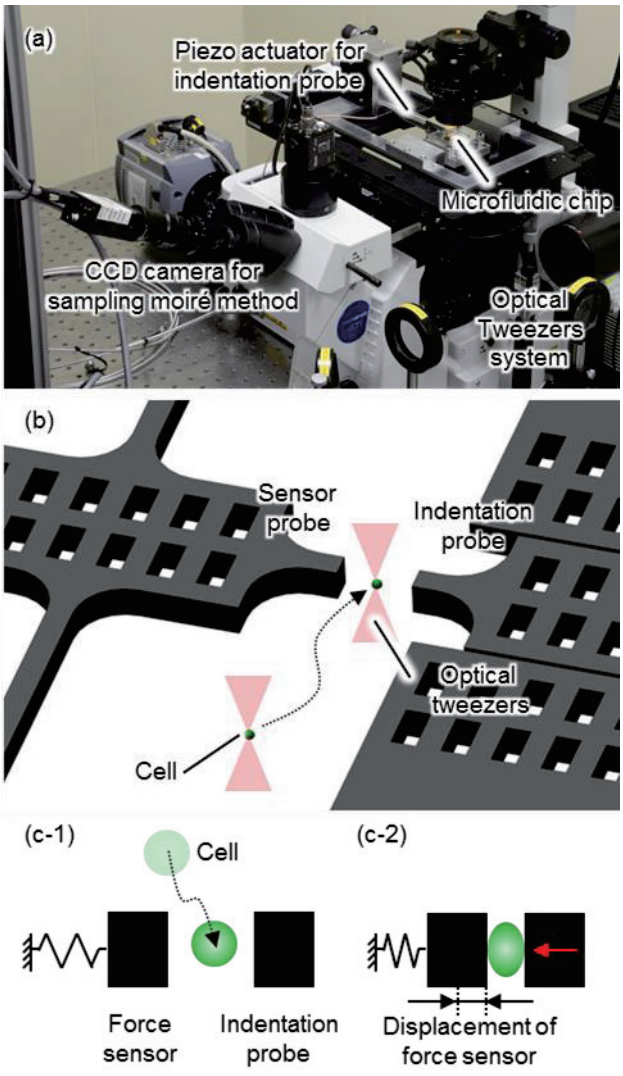

Figure 1 Mechanical characterization of *Synechocystis* sp. PCC 6803. (a) Photograph of constructed system, (b)measurement point of microfluidic chip, and (c) Mechanical characterization of after transportation.

Figure 1(a) shows the schematic image of the system for mechanical characterization of *Synechocystis* sp. PCC 6803. Unlike conventional measurement systems like AFM (atomic force microscope), the system allows us to measure a floating cell whose diameter is a few micrometers. We have previously proposed the microfluidic chip having indentation and force sensor probes for the mechanical characterization of cell [7]. In order to transport the target cyanobacteria, we have integrated the holographic optical tweezers system with the mechanical characterization system [8, 9]. The spatial light modulator is utilized to change the phase of an expanded IR laser for shaping the focus points in a microfluidic chip. We control the position of the target cell, and transport to the measurement point of microfluidic chip by using the holographic optical tweezers system. After the positioning of target cell, the indentation probe is actuated to deform the cell by using piezoelectric actuator placed outside of the microfluidic chip, as shown in Fig. 1(c). The deformation is observed through a CCD camera attached microscope. In addition, the cellular reaction force is measured from the deformation displacement of leaf spring type force sensor. From the the relationship between measured cellular reaction force and cellular deformation, we can measure the stiffness of the cell.

Fabrication process of microfluidic chip

The fabrication process of the microfluidic chip is shown in Fig. 3, and details are summarized as follows.

(a) A negative photoresist (SU-8 3010, MicroChem Corp.) is patterned on the surface of a borosilicate glass chip. The glass substrate is etched by using deep reactive-ion etching (DRIE). The etching depth is approximately 5 μm. The Cr layer is sputtered on the patterned substrate.

(b) The Cr layer is patterned by using lift-off process. In this process, only the etched part of the glass chip is covered by Cr. The Cr pattern is used for avoiding the unexpected bonding in the process of Fig. 2(d).

(c) A positive photoresist (OFPR, Tokyo Ohka Kogyo Co., Ltd.) is patterned on the device layer of the silicon-on-insulator (SOI) substrate as an etching mask, and then the device layer is etched by using DRIE.

(d) A negative photoresist (SU-8 3010, MicroChem Corp.) is patterned on the handle layer of SOI substrate after anodic bonding of the borosilicate glass and device layer.

(e) The handle layer is etched by using DRIE, and then the buried oxide layer of SOI substrate and Cr pattern of the glass substrate are removed.

The fabricated microfluidic chip is shown in Fig. 3. In this figure, two pairs of indentation and force sensor probes are integrated as the measurement point of microfluidic chip. By connecting the piezoelectric actuator through the attachment, we can actuate the indentation probe [9]. The dimensions of fabricated leaf spring of force sensor are 3000 μm, 4.65 μm and 7.5 μm in length, width and thickness, respectively. Thus, the spring constant of the force sensor is calculated as 0.038 N/m.

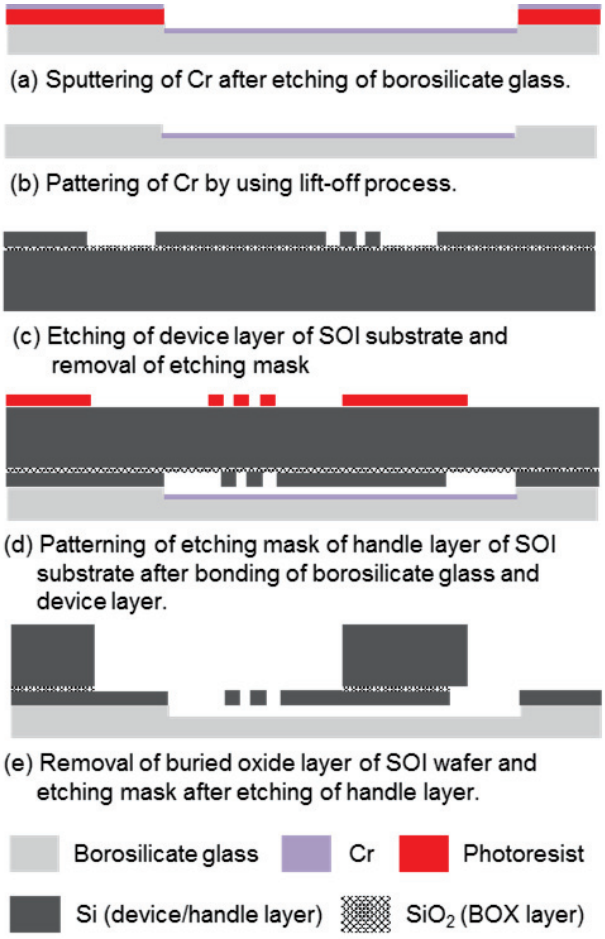

Figure 2 Fabrication process of microfluidic chip.

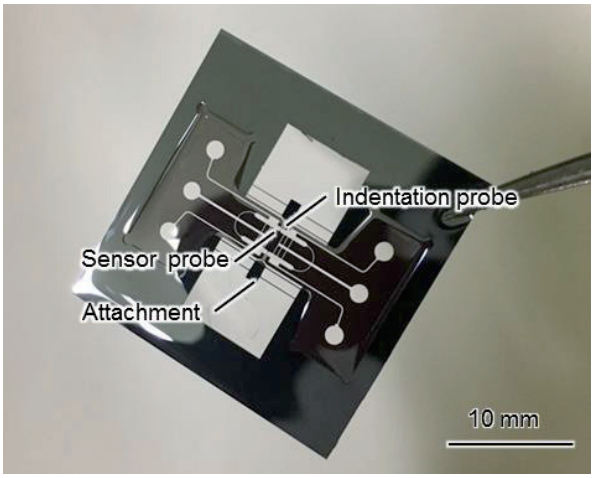

Figure 3 Photograph of fabricated microfluidic chip.

Sample preparation

Cells are cultivated with the same manner of Ref. [9]. The growth conditions of cells are summarized as follows.

(a) Synechocystis sp. PCC 6803 samples are grown at 30 °C in BG11 medium containing 20 mM TESKOH (pH 8.0).

(b) Solid medium consists of BG11 buffered at pH 8.0, 1.5 % agar or agarose, and 0.3 % sodium thiosulfate.

(c) Continuous illumination is provided by fluorescent lamps (light intensity: 50 μmol of photons $m^{-2}s^{-1}$; wavelength: 400-700 nm).

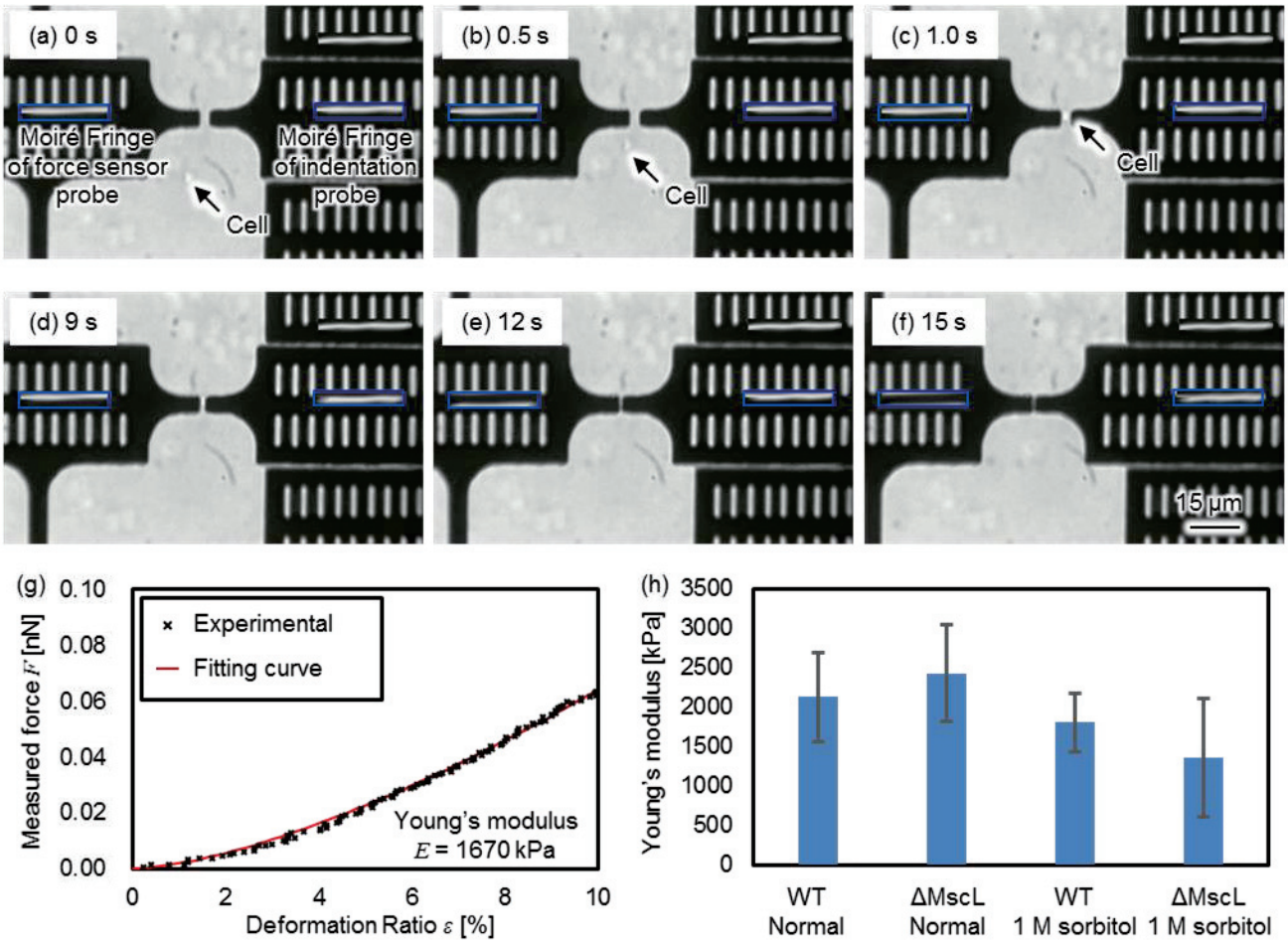

Figure 4 Experimental results. (a) through (g) A typical result of mechanical characterization. (a) through (c) transportation of target cell, (d) through (f) measurement of reaction force of deformed cell, (g) evaluation of Young's modulus of measured cell, and (h) evaluation result of WT and ΔMscL in normal and high-osmolality medium.

EXPERIMENTS

Figures 4(a) through 4(g) show a typical result of mechanical characterization of *Synechocystis* sp. PCC 6803. The target cell was transported to the measurement point of microfluidic chip by using the optical tweezers, as shown in Figs. 4(a) through 4(c). The transported cell was deformed by the indentation probe, as shown in Figs. 4(d) through 4(e). The displacement of indentation and force sensor probes were measured by the images of CCD camera. For the improvement of measurement accuracy of the position of each probe, we utilized the sampling moiré method as shown in the blue boxes in each figure [10, 11]. Considering the displacement measurement accuracy was 9 nm in this experiment, the resolution of the force sensing was 0.34 nN. Thus, we can get the deformation-reaction force curve of the cell, as shown in Fig. 4(g). From the result, we evaluated the Young's modulus of cell by using Hertzian contact model. Under the assumptions that the cell is uniform sphere and the probes are rigid, we can calculate the Young's modulus E_{cell} as Eq. (1).

$$E_{cell} = \frac{3(1-\nu^2)}{D^2\varepsilon^{3/2}}F \qquad (1)$$

where F, D, E, ν and ε are reaction force, original diameter, Young's Modulus, Poisson's ratio and deformation ratio (deformation divided by diameter) of the tested cell, respectively. In this research, the cell is considered as made of incompressible material, thus the Poisson's ratio ν is 0.5. Since the Hertzian contact model is only suitable in small deformation case, we use the data whose deformation ratio is smaller than 10%. The red curve in Fig. 4(g) shows the fitted result. Eventually, we measured the Young's modulus of cell as 1670 kPa.

We measured two groups of cells; wild type (WT) and mutant type knocked out large-conductance mechanosensitive channels (ΔMscL) in two medium conditions; normal and high-osmolality condition (1 M sorbitol). The number of measured samples is 10 for each of them. The result is shown in Fig. 4(h). The bar graph show the average value of 10 samples and each error bar show the standard deviation. From Fig. 4(h), we can see that the cellular stiffness in high-osmotic condition is smaller than that in normal medium condition. These results indicate that the mechanosensitive channels could regulate stiffness of the cell, and contribute to osmoadaptation mechanism for surviving from osmotic stress. By using the system which can measure small floating cells with high-resolution, we succeeded in the mechanical characterization of *Synechocystis* under the osmotic stress.

CONCLUSIONS

In this paper, we presented the mechanical characterization of *Synechocystis* sp. PCC 6803 under osmotic stress. By using the constructed system consisting the microfluidic chip integrated indentation and force sensor probes, we succeeded in measuring Young's modulus of a small floating cell with high resolution. The constructed system can be applied to evaluate the activity of not only mechanosensitive channels but also water channels.

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REFERENCES

[1] J. Appel, S. Phunpruch, K. Steinm U Ller, and R. U. D. Schulz, R. U. D., "The Bidirectional Hydrogenase of *Synechocystis* sp. PCC 6803 Works as an Electron Valve During Photosynthesis", *Archives of Microbiology*, vol.173-5-6, pp.333-338, 2000.

[2] N. Quintana, F. Van der Kooy, M. D. Van de Rhee, G. P. Voshol and R. Verpoorte, "Renewable Energy from Cyanobacteria: Energy Production Optimization by Metabolic Pathway Engineering", *Applied microbiology and biotechnology*, vol.91-3, pp.471-490, 2011.

[3] Y. Kanesaki, I. Suzuki, S. I. Allakhverdiev, K. Mikami and N. Murata, "Salt Stress and Hyperosmotic Stress Regulate the Expression of Different Sets of Genes in *Synechocystis* sp. PCC 6803", *Biochemical and biophysical research communications*, vol.290-1, pp.339-348, 2002.

[4] E. S. Haswell, R. Phillips and D. C. Rees, "Mechanosensitive Channels: What Can They Do and How Do They Do It? ", *Structure*, vol.19-10, pp. 1356-1369. 2011.

[5] K. Nanatani, T. Shijuku, M. Akai, Y. Yukutake, M. Yasui, S. Hamamoto, K. Onai, M. Morishita, M. Ishiura, N. Uozumi, "Characterization of the role of a mechanosensitive channel in osmotic down shock adaptation in Synechocystis sp PCC 6803" *Channels* vol. 7, Iss. 4, 2013.

[6] K. Nanatani, T. Shijuku, Y. Takano, L. Zulkifli, T. Yamazaki, A. Tominaga, S. Souma, K. Onai, M. Morishita, M. Ishiura, M. Hagemann, I. Suzuki, H. Maruyama, F. Arai, and N. Uozumi, "Comparative Analysis of kdp and ktr Mutants Reveals Distinct Roles of the Potassium Transporters in the Model Cyanobacterium Synechocystis sp. PCC 6803", *J. Bacteriol.*, vol. 197, no. 4, pp. 676-687, 2014.

[7] S. Sakuma and F. Arai, "Cellular Force Measurement Using a Nanometric-Probe-Integrated Microfluidic Chip with a Displacement Reduction Mechanism", *Journal of Robotics and Mechatronics*, vol.2-25, pp.277-284, 2012.

[8] K. Onda and F. Arai, "Multi-beam bilateral teleoperation of holographic optical tweezers", *Opt. Express*, vol. 20, no. 4, pp. 3633-3641, Feb. 2012.

[9] T. Hasegawa, S. Sakuma, K. Nanatani, N. Uozumi and F. Arai1, "Mechanical characterization system of cyanobacteria based on a robot integrated microdluidic chip", Proc. of IEEE/RSJ Int. Conf. on Intelligent Robots and Systems, pp. 6033-6038 ,2015.

[10] S. Ri, M. Fujigaki, Y. Morimoto, "Sampling moiré method for accurate small deformation distribution measurement", *Experimental Mechanics*, vol5-50, pp.501–508, 2010.

[11] H. Sugiura, S. Sakuma, M. Kaneko, and F. Arai, "On-Chip Method to Measure Mechanical Characteristics of a Single Cell by Using Moiré Fringe", *Micromachines*, vol.6-6, pp.660-673, 2015.

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