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Development of a micro cell compression stimulator for evaluating real-time cellular responses

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This paper presents a micro cell compression stimulator for evaluating real-time cellular responses to compression stimuli. The device was produced by a micro three-dimensional structure fabrication process using multiple exposures to the photoresist. The device consists of a pressure inlet port, cell inlet ports, a gasket, microchannels, cell culture chambers, and a diaphragm on the culture chamber for applying compressive pressure to cells. Compression stimuli applied to the cells can be controlled by regulating the expansion of the diaphragm via a pressure control. The device permits the observation of cellular responses to compressive pressure in real time because it is made of transparent materials and stimulates the cells without deforming the cell culture surface, when observed by optical microscopy. We demonstrated the validity of the fabrication process, evaluated the performance of the fabricated device, and compared the experimental results with the FEM structural analysis results. We found through operational testing that the diaphragm was deformed quickly by applying negative/positive pressure and that the diaphragm displacement became larger with increasing applied pressure. These results indicate that this device can be used to control the intensity and the cell stimulus profile by regulating the applied pressure. In all cases, the cellular deformation during compression stimulus was successfully observed in real time using an optical microscope. The device is expected to facilitate the control of stem cell differentiation and the clarification of cellular mechanoreceptor mechanisms and signal transduction pathways. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4717683>]

I. INTRODUCTION

The development of differentiation inducing techniques that can be applied to multipotent stem cells, such as induced pluripotent stem (iPS) cells and embryonic stem (ES) cells, is critical to regenerative medicine. Stem cell differentiation is greatly dependent on the living environment; cell differentiation is determined by timing, amplitude, and the degree of external stimuli on a cell, among other factors.¹⁻³ If the surrounding environment of these cells can be controlled artificially, then it may be possible to guide them safely and efficiently toward developing specific functionalities.

In addition, the clarification of the mechanoreception mechanism and signal transduction pathways in cells by analyzing the cellular reactions and responses to mechanical stimuli *in vivo* is an important field of study in cellular biology.⁴⁻⁶ Several studies that employed self-made experimental devices have been carried out.⁶⁻¹⁰ These experimental systems were used to control the mechanical stimuli that were applied to cells. However, they do not permit real-time observations of the cells to be made easily because the cell culture surface moves when stimuli are applied. If reactions and responses of cells to mechanical stimuli were observed in real time, then these mechanisms could be clarified.

The above problems can be overcome by fabricating microdevices using micromachining technology. Micromachin-

ing technology permits the integration of various mechanical, electrical, and chemical elements, and it has been used to create microdevices that can be used to manipulate chemical solutions, small mechanical parts, and cells among other things.¹¹⁻¹⁵ Microdevices fabricated by employing this technology can be used to artificially control stimuli that are applied to cells and can permit cellular responses to be observed in real time.

To this end, we present a micro cell compression stimulator for evaluating cellular responses to compression stimuli. We fabricated microdevices that can be used to control cell differentiation by applying dynamic extracellular stimuli based on real-time cellular responses. We examine the fabrication process, fabrication conditions, and performance of the device. We also observe the deformation behavior of cells stimulated by using the device in real time. The results indicate that the microdevice can be used not only as a technique for controlling the differentiation of stem cells in regenerative medicine but also as a basic research tool in cellular biology.

II. MATERIALS AND METHODS

A. Device design

A schematic of the micro cell mechanical stimulator is shown in Fig. 1. The device is assembled by stacking layers of polydimethylsiloxane (PDMS) that are self-adhesive (Fig. 1(a) and 1(b)). The device has cell inlet ports, a pressure inlet port, a gasket, microchannels, cell culture chambers,

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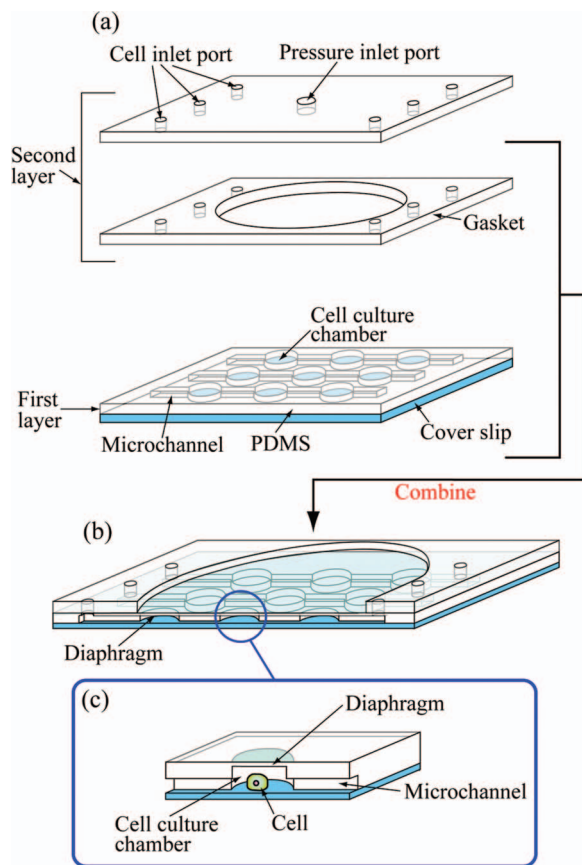


FIG. 1. Schematics of a micro cell compression stimulator. (a) Each layer constitutes the micro cell compression stimulator. (b) The structure is made by combining each layer. The layers are bonded to each other by self-adhesion. The gasket and the cell culture chambers are separated by a thin silicone film (diaphragm). (c) Enlarged view of the areas surrounding the cell culture chamber.

and a diaphragm on the cell culture chamber for applying compressive pressure to the cells (Fig. 1(c)). The compression stimuli can be controlled by regulating the expansion of the diaphragm through a pressure control. Also, the compression stimuli can be modified by changing the culture chamber because the degree of deformation of the diaphragm depends on the diameter of the chamber.

B. Principle of cell compression stimuli

Figure 2 shows the principle of how cell compression stimuli are produced by the fabricated microdevice. The compression stimuli can be controlled by regulating the expansion of the diaphragm via a pressure control. When hydraulic pressure is applied through the pressure inlet port, the PDMS diaphragm is deformed toward the cell culture chamber and the cultured cells receive the compression stimuli via the deformed diaphragm. The compression stimuli can be modified by changing the diameter of the culture chamber because the degree of deformation of the diaphragm depends on the diameter of the chamber. The device permits real-time cellular responses to the compressive pressure to be observed by optical microscopy because it is made of transparent materi-

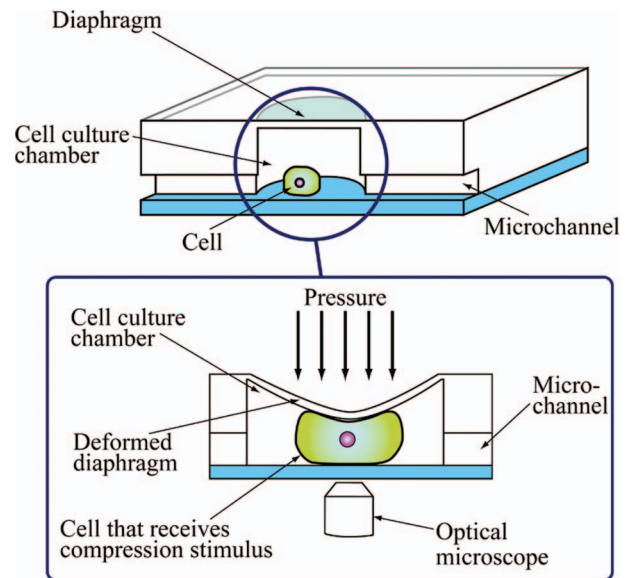


FIG. 2. Principle of creating cell compression stimuli. When pressure is applied to the diaphragm through the pressure inlet port, the diaphragm on the culture chamber expands toward the culture chamber and the cultured cells are stimulated by the diaphragm. Real-time cellular deformation and cellular responses can be observed by optical microscopy because the fabricated device is made of transparent materials.

als and stimulates the cells without deforming the cell culture surface.

C. Device fabrication

Figure 3 shows the geometry of the micro cell compression stimulator. The sizes of the microchannel, the cell culture chamber, and the cell inlet port are $200\ \mu\text{m}$, $1\ \text{mm}$, $1.2\ \text{mm}$, and $2.0\ \text{mm}$, respectively. The heights of the microchannel, the cell culture chamber, and the gasket are $50\ \mu\text{m}$, $150\ \mu\text{m}$, $160\ \mu\text{m}$, and $50\ \mu\text{m}$, respectively.

Figure 4 shows the fabrication process of the micro cell compression stimulator. The device is made of PDMS bonded to a cover slip. The cross section shown in Fig. 4 is a deformed schematic projection along line A-A' and line B-B' in Fig. 3. The device was fabricated as follows. First, a $50\ \mu\text{m}$ thick SU-8 3050 photoresist (Nippon Kayaku Co., Ltd.) layer for a microchannel mold was coated and exposed on a glass plate (Fig. 4(a) and 4(b)). Next, a $100\ \mu\text{m}$ thick SU-8 3050 photoresist layer for a mold of the cell culture chamber and cell inlet port was coated and exposed on the first SU-8 layer (Fig. 4(c)). Following this, a $10\ \mu\text{m}$ -thick SU-8 3050 photoresist layer for a diaphragm mold was coated and exposed on the second SU-8 layer (Fig. 4(d)). All of the SU-8 layers were developed subsequently and the SU-8 mold of the microdevice was completed (Fig. 4(e)). The fluoropolymer (DURASURFE DS-3304Z, HARVES Co., Ltd.) was spin-coated onto the SU-8 mold of the microdevice to form a very thin layer for mold-releasing. PDMS (Shin-Etsu Silicone KE-106, Shin-Etsu Chemical Co., Ltd.) was cast over the SU-8 mold and cured in a furnace (Fig. 4(f)). Then, the PDMS device was peeled away from the SU-8 mold and bonded to the cover slip

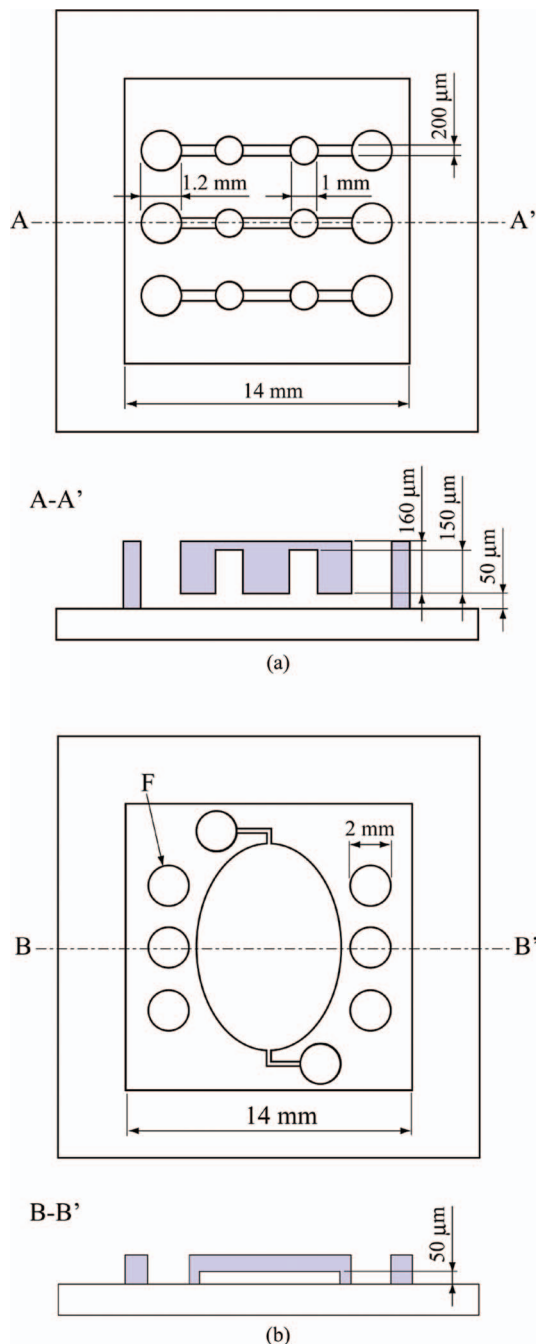


FIG. 3. Geometry of the micro cell compression stimulator. (a) Geometry of the first layer consisting of the microchannel, cell culture chambers, and cell inlet ports. (b) Geometry of the second layer consisting of the cell inlet ports, the gasket and pressure inlet ports.

(Fig. 4(g)). The second layer was fabricated in a similar process. The 50 μm thick SU-8 3050 photoresist for the pressure inlet port mold and gasket was coated, exposed, and developed on a glass plate (Fig. 4(h)–4(j)). Next, the fluoropolymer was coated and PDMS was cast over the SU-8 mold and cured in a furnace (Fig. 4(k)). Finally, the PDMS device was peeled off and bonded to the first layer (Fig. 4(l) and 4(m)). Figure 5 shows the shape of the fabricated SU-8 mold of the first layer measured using a 3D measurement laser microscope (LEXT OLS4000, Olympus Corp.). We confirmed that the fabricated

SU-8 structure had different thicknesses due to the multiple exposure process. Figure 6 is a photograph of the fabricated micro cell compression stimulator. The device was fabricated by photolithography, PDMS molding, and PDMS stacking. The mold for the PDMS structure was fabricated by micro three-dimensional structure fabrication using multiple exposures to SU-8.¹⁶

III. RESULTS AND DISCUSSION

A. Device fabrication

We evaluated our processing accuracy for characterizing the fabrication process and fabrication conditions. The width and thickness of the fabricated SU-8 mold were measured by using a 3D measurement laser microscope. The dimensional error of the fabricated SU-8 mold ranged up to approximately 1.5%. The thicknesses of the microchannel (C in Fig. 3), the cell culture chambers (D in Fig. 3), and the cell inlet ports (E and F in Fig. 3) were 51 μm , 148 μm , 161 μm , and 52 μm , and their errors were 2.0%, 1.3%, 0.6%, and 4.0%, respectively. These results indicate that the SU-8 mold was fabricated accurately by micro three-dimensional structure fabrication using multiple exposures to SU-8.

B. Micro cell compression stimulator operational testing

Operational testing of the micro cell compression stimulator was carried out using a test device (Fig. 7) to examine the fundamental characteristics of deformation of the diaphragm. The test device consisted of the diaphragm on the cell culture chamber, microchannels, cell inlet ports, and a silicone tube bonded to the cell inlet ports for pressure applying. Figure 8 shows the experimental setup for driving the diaphragm. In this experiment, hydrostatic pressure was applied through the microchannel to the diaphragm. The pressure applied from the cell inlet port was changed by adjusting the water column height, h . Figure 9 shows a photograph and 3D profile of the operational test. When negative pressure was applied, the diaphragm expanded immediately from its initial position toward the culture surface and the degree of expansion changed in accordance with the applied pressure (Fig. 9(b)). The diaphragm expanded toward the outside when we applied positive pressure (Fig. 9(c)). Figure 9(d) and 9(e) show the 3D profiles of the deformed diaphragm in the directions toward the culture surface and the outside, respectively, measured by using the 3D measurement laser microscope. When a pressure of 4 kPa was applied, the diaphragm was displaced by 120 μm . When the pressure was eliminated, the diaphragm returned to its initial shape (Fig. 9(a)). This indicates that the device can be used to apply not only static compression stimuli but also repetitive dynamic compression stimuli.

A structural analysis of the diaphragm was carried out by using finite element methods (FEM) analysis software (ANSYS, ver. 10). The results of the FEM analysis in the case of an applied pressure of 10 kPa are shown in Fig. 10. The analysis parameters of the model are listed in Table I.

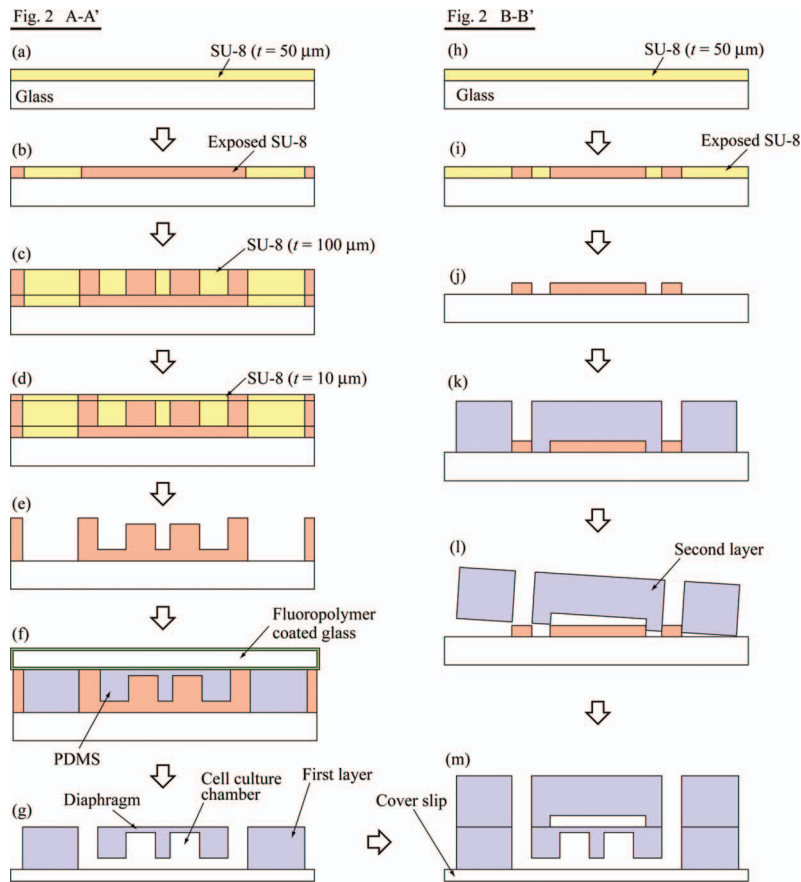


FIG. 4. Process for fabricating the micro cell compression stimulator. (a-g) Fabrication of the first layer. A micro three-dimensional structure fabrication process is used for fabricating a differential thickness SU-8 pattern (a-e). Then, PDMS is cast over the SU-8 mold and cured in a furnace (f). (h-l) Fabrication of the second layer. The second layer is also fabricated by using SU-8 patterning and PDMS molding. Finally, the first and second layers are bonded to each other by self-adhesion (m).

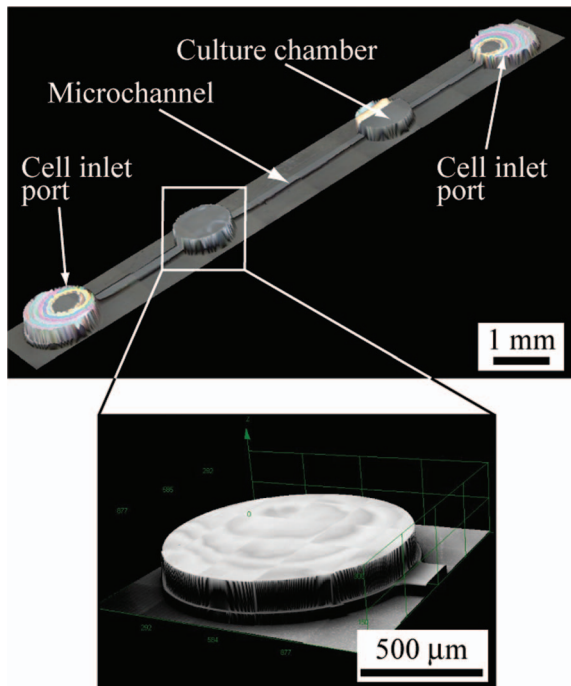
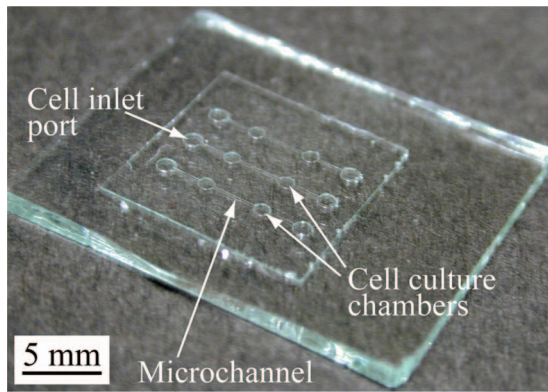


FIG. 5. 3D profile of the fabricated SU-8 mold measured using a 3D measurement laser microscope. The SU-8 mold, which has different thickness patterns such as a microchannel, cell inlet ports, and culture chambers, was fabricated by micro three-dimensional structure fabrication using multiple exposures to SU-8.

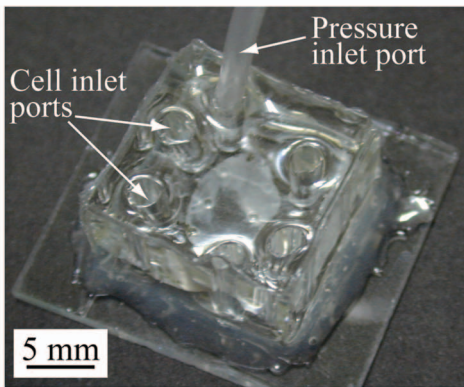
Axis-symmetric analysis was used as the analysis model. The right side of the analysis model was completely fixed and uniformly distributed pressure was applied to the undersurface of the diaphragm. The diaphragm analysis model was deformed 230 μm at 10 kPa from its undersurface. Figure 11 shows the relationship between the displacement of the center of the diaphragm and the applied pressure. The error bars represent the standard deviation of the five experimental runs. The displacement became larger with increasing applied pressure. When the applied pressure was 4 kPa, the diaphragm was deformed by 120 μm . The error between the analysis results and the experimental results was less than 24%, and they showed a similar tendency. These results suggest that the intensity of the stimuli applied to the cells can be controlled by regulating the applied pressure and that they can be modeled by FEM analysis.

TABLE I. Analysis conditions of the model.

Analysis model	Axis-symmetric
Material model	Neo-Hookean (hyperelastic)
Young's Modulus	3.23 MPa
Number of nodes	504
Number of elements	375
Element type	PLANE182 (2-D 4-node structural solid)



(a)



(b)

FIG. 6. Photographs of the fabricated micro cell compression stimulator. (a) Photograph of the first fabricated layer. (b) Photograph of the completed device. The first and second layers were stacked and bonded to each other by self-adhesion. The silicone tube was attached to the pressure inlet port.

C. Dynamic compression stimuli test using the micro cell compression stimulator

A cell compression stimuli experiment was carried out by using the fabricated micro cell compression stimulator. Fibroblastic cells were used as samples in the experiments and prepared as follows. The cells were maintained in culture medium (DMEM, Life Technologies Corp.) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a

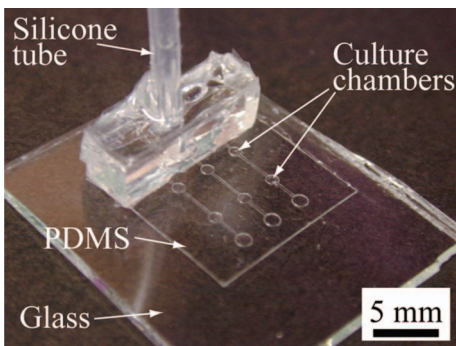


FIG. 7. Photograph of the fabricated micro cell compression stimulator test device. In order to examine the fundamental characteristics of deformation of the diaphragm, only the first layer of the test device was used. The device was connected to the silicone tube to permit pressure to be applied to it.

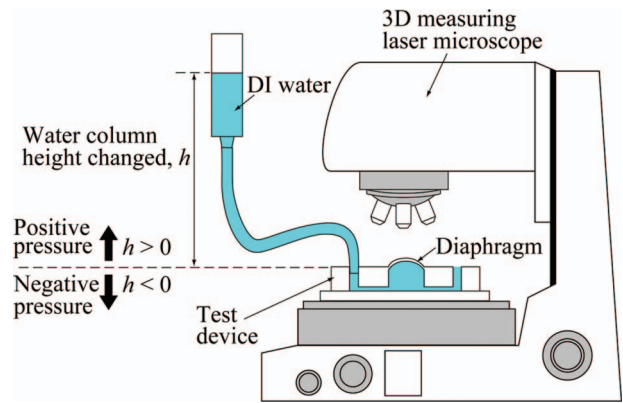


FIG. 8. Experimental setup of the operational testing device. Hydrostatic pressure was used to apply pressure. The degree of applied pressure was changed by adjusting the water column height, h . The extent of deformation of the diaphragm was measured by using a 3D measuring laser microscope.

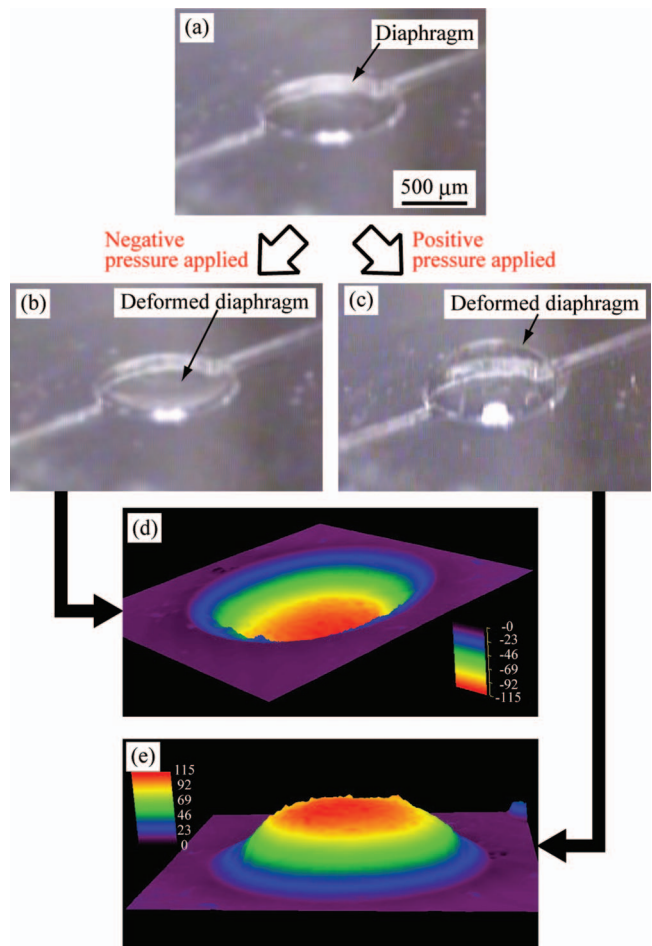


FIG. 9. Photographs and 3D profiles of operational testing using the fabricated test device. (a) Photograph of the initial state of the diaphragm. (b) Photograph of the expanded diaphragm toward the culture chamber caused by applied negative pressure. (c) Photograph of the expanded diaphragm toward the outside caused by applied positive pressure. The diaphragm was expanded immediately when pressure was applied. (d, e) 3D profile and deformation volume of the diaphragm measured by using a 3D measuring laser microscope.

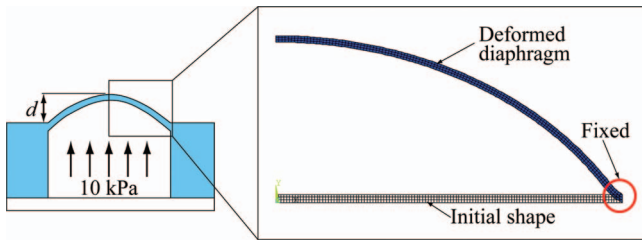


FIG. 10. The boundary condition and the results of FEM analysis. Axis-symmetric analysis was used. The right side of the analysis model was completely fixed and uniformly distributed pressure was applied to the undersurface of the diaphragm.

humidified 95% air, 5% CO₂ atmosphere. In preparation for the test, the cultured cells were injected into the cell culture chamber through the cell inlet port and incubated for 3 h. After pre-incubation, the device was put on an inverted phase-contrast microscope (CKX-41, Olympus Corp.). Figure 12 shows the experimental setup for the cell compression stimuli experiment. The pressure applied from the pressure inlet port was changed by adjusting the water column height, *h*. When positive pressure was applied, the diaphragm immediately deformed from its initial position toward the culture surface. Cells were injected into the culture chamber from the cell inlet port fitted with a silicone tube by using the microsyringe pump. The cultured cells in the culture chamber received the compression stimuli via the deformed diaphragm. Figure 13 shows a time-lapse image of a single compressed fibroblastic cell that was taken at a lens magnification of 40× using an inverted microscope. The photograph in Fig. 13 seems out of focus owing to the optical path difference of the inverted phase-contrast microscope because the light source was located above the fabricated device and the cell was irradiated by the transmitted light that passed through the gasket and the diaphragm of the PDMS. If the cell had been stained with a fluorescent dye, then it would have been clearly visible by using the microscope. The cell is located at the center in the figure and adhered to the culture surface. For sequential compression operation, Fig. 13(a) shows an image of the cell immediately before operation was initiated. When positive pressure was applied to the diaphragm, the fibroblastic cell was

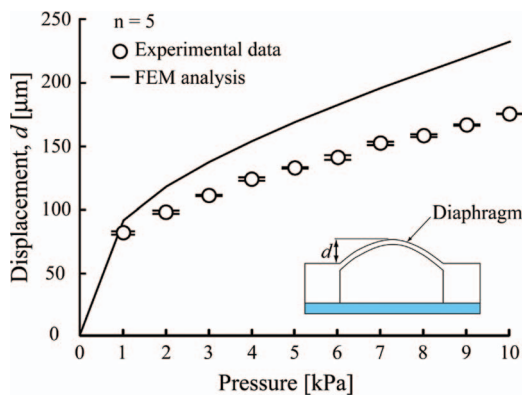


FIG. 11. Relationship between the diaphragm displacement and applied pressure. The experimental results and FEM analysis results became larger with increasing applied pressure and their shapes showed a similar tendency. The experimental errors were less than 24%.

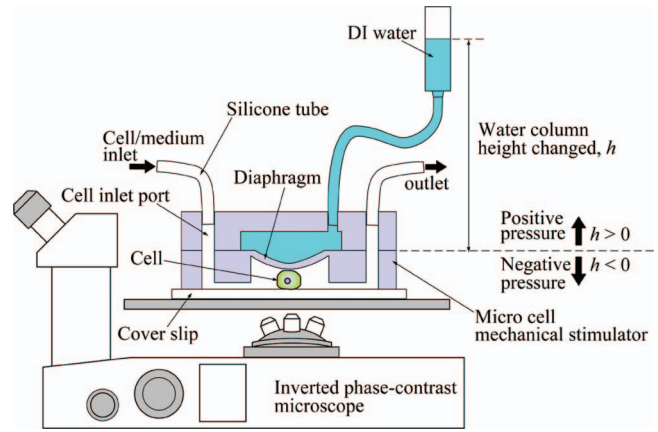


FIG. 12. Experimental setup for the cell compression stimuli test. Cells are injected into the cell culture chamber through the cell inlet port fitted with a silicone tube by using the microsyringe pump. Hydrostatic pressure is used for controlling the deformation of the diaphragm. The degree of applied pressure is changed by adjusting the water column height, *h*. A cultured cell is stimulated by the deformed diaphragm and its behavior is observed by using the inverted phase-contrast microscope.

compressed gradually by the deformed diaphragm depending on the pressure (Fig. 13(b)–13(d)). When the pressure was eliminated, the diaphragm returned to its initial position. In other words, the compression stimuli on the fibroblastic cell were eliminated. As a result, the fibroblastic cell adhered successfully to the culture surface in the fabricated device. This result indicates that the micro cell compression stimulator itself and the fabrication process of the device are biocompatible. Moreover, the observed cell stayed in the field of view and the dynamic deformation behavior of the cell was observed without defocusing occurring while applying compression stimuli. Therefore, the fabricated micro cell compression

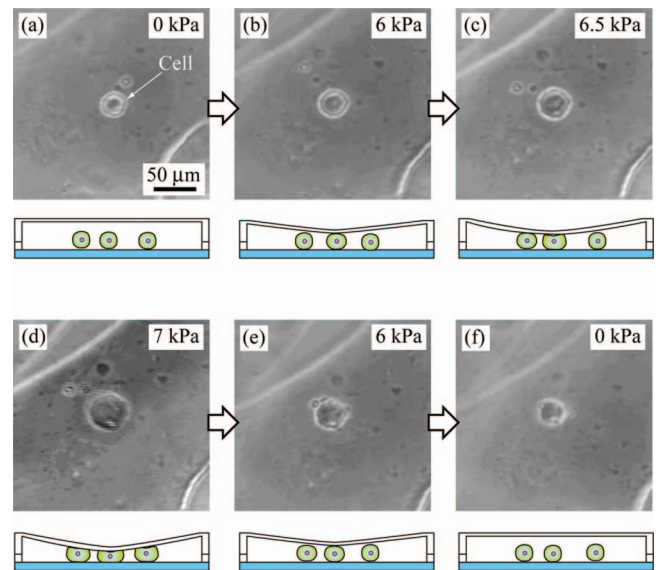


FIG. 13. Sequential time lapse images of cells stimulated by the micro cell compression stimulator. (a-d) The shape of the cultured cell was deformed gradually by compression stimuli via deformation of the diaphragm. (e, f) When the compression stimulus was eliminated, the cell returned to the initial shape. Cell deformation via compression stimuli using the micro cell compression stimulator was observed real-time by optical microscopy. Note that the lines across the image are microscopic linear patterns formed on the diaphragm.

stimulator can be used in real-time experiments for evaluating cellular responses to mechanical stimuli in terms of altered cellular morphology, cell differentiation induction, etc.

IV. CONCLUSIONS

We demonstrated the fabrication and fundamental characteristics of a micro cell compression stimulator and observations of cellular deformation behavior resulting from dynamic cell compression stimuli using a fabricated micro cell compression stimulator. This device can be used to stimulate cells by dynamic compression and evaluate real-time cellular responses. The SU-8 mold was fabricated by micro three-dimensional structure fabrication using multiple exposures to a photoresist. As a result, errors in dimensions and thickness were less than 4%, and therefore, the microdevice can be fabricated accurately. The device can be moved quickly based on the applied pressure and the degree of diaphragm displacement, which is controlled by the applied pressure. Compression stimuli applied to cells can be controlled by regulating the applied pressure. The deformation behavior of cells can be observed successfully in real-time when applying dynamic compression stimuli without defocusing occurring. This system may be used as a basis for other novel experimental systems that require *in situ* observation of cellular behavior or deformation.

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