Microfluidic biomechanical device for compressive cell stimulation and lysis

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Abstract

The physical forces to which living cells are most commonly exposed are fluid shear, pressure, and stretch. These mechanical stimulations influence the physiological and pathological condition of the organism, which induces many aspects of human health and disease. In this study, a new kind of microfluidic biomechanical device has been presented for compressive stimulation and lysis of cells. Mechanical stress is applied to the cells with the deflection of the poly(dimethylsiloxane) membrane between two microchannels, formed by multilayer soft lithography. The membrane functions as an on–off valve for closing the fluid channel and a loading membrane for applying compressive stress. As a demonstration of the feasibility of this microfluidic device, the viability of mammary gland epithelial (MCF7) cells in response to compressive stress is assessed by the change of fluorescence intensity with calcein AM. It is also confirmed that the cells are deformed and lysed under compression by the deflected membrane.

Keywords: Microfluidic device; MCF7 cells; Compressive stress; Cell viability; Mechanical lysis

1. Introduction

Living cells are continually subject to mechanical stresses such as fluid shear, pressure and stretch. For example, cells in the mammary gland are laid open to a dynamic mechanical environment. During pregnancy and lactation, mammary epithelial cells undergo shear (dragging) stress from the flow of milk through the gland, compressive (pushing) stress due to engorgement from the increased fluid in the ductal network, and tensile (pulling) stress from suckling [1]. Since the structure and function of many living cells are mechanically connected to their environments, the mechanical stimulations influence the physiological and pathological condition of the organism, which induces many aspects of human health and disease [2].

To investigate the cellular response against the mechanical stimulations, many researches have been carried out under uniaxial or biaxial tension [3], compression [4], shear [5], hydrostatic pressure [6], bending and twisting [3], and a combination of these. The responses of cells include morphology change, extra-cellular matrix (ECM) production, detachment from the ECM, gene expression, proliferation, intra-cellular calcium signaling, proteoglycan/collagens synthesis, and the alteration of cell motility and orientation [7]. These cellular responses are related to the cell deformation which is important for the understanding of the biomechanical effects and cell lysis [8]. In spite of several designs for cell stimulation, controlling the mechanical environment in precise or well-defined ways has still been difficult due to their large scale compared to the cell size.

Recently, microfluidic systems present a valuable tool for fundamental studies of cell biology and biomedical researches such as cell culture [9,10], cell lysis [11], cell behaviour [12], cell-signaling [13], cell viability assay [14,15], intracellular enzyme reaction [11], and gene expression [16]. Among mechanical stresses, shear stress induced by fluid flow is relatively easy to exploit in a microfluidic device [17,18]. However, to the best of our knowledge, the microfluidic device for applying the mechanical stimulation such as compressive stress has not yet been reported.
Here, we report a new kind of microfluidic biomechanical device using multilayer soft lithography for compressive stimulation and lysis of cells. A compressive stress is directly applied to the cells through the deflected polymer membrane in poly(dimethylsiloxane) (PDMS) between two microchannels. According to the magnitude of the membrane deflection, the device can be operated for two purposes; it is capable of stimulating the cells compressively below a certain extent of the deflection as well as lysing the cells without trypsinizing above the certain extent of the deflection. As a demonstration of the feasibility of this microfluidic device, the cellular damages of MCF7 mammary gland epithelial cells under compression were assessed by viability assay using calcein AM fluorescent dye, according to the compressive stress from the applied pressure. In addition to compressive cell stimulation, cell lysis was achieved by the compressive force. Between the deflected membrane and curved surface, the cells became deformed and lysed. This mechanical lysis method also allows rapid recovery of intracellular contents without introducing lytic agents (chemical lysis) and applying electric field (electrical lysis). The details of this technology are reported herein.

2. Materials and methods

2.1. Design and fabrication of the microfluidic device

The application of stress was accomplished with the deflection of the polymer membrane between two microchannels. The membrane was used as a way of closing the fluid channel as an on–off valve and applying compressive stress to the cells as a loading membrane. As shown in Fig. 1, the microchannel for cell culture was composed of two parallel channels; one for the application of stress and the other for comparison. Each parallel channel was 65 μm in height and 300 μm in width compared to the inlet/outlet channels which were 65 μm in height and 700 μm in width. The height and width of the control channel was 50 and 400 μm, respectively.

The microfluidic device was fabricated from poly(dimethylsiloxane) (PDMS) silicone elastomer using multilayer soft lithography [19]. Briefly, the control and fluidic layers were cast from two different molds fabricated on 4 in. silicon wafers with negative photoresist (SU-8 2025) and positive photoresist (AZ 4903) for thick film according to the manufacturer’s datasheets, respectively. In the case of the mold for the fluidic layer, the patterned photoresist layer was thermally reflowed on a hot plate in order to convert the channel profiles into a semicircular profile. Since a single step of reflow process caused bubble formation on the patterned photoresist due to the thick film, the wafer was particularly subjected to multiple steps of reflow process on a hot plate: initially placed at 85 °C; then at 95 °C; and finally at 110 °C.

After preparation of the molds, the PDMS device was fabricated as shown in Fig. 2a. Briefly, a thin layer (1 mm thick) was formed by pouring the PDMS prepolymer onto the fluidic mold, and a thicker layer (4 mm thick) was formed onto the control mold (Step 1 in Fig. 2a). Also, the PDMS mixture was spun on a silanized silicon wafer at 5500 rpm, resulting in a PDMS thin membrane (15 μm thick) (Step 2). After curing, both the replica peeled from the control mold and the PDMS-coated wafer were oxidized in oxygen plasma for 1 min prior to bring-
Fig. 2. (a) Flow diagram of the fabrication process. The steps to create the masters using UV photolithography were omitted. (b) A photograph of the fabricated device. Control and flow channels are filled with blue and red ink for image capture, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

ing the two surfaces into contact (Step 3). The thin membrane bounded replica was then peeled, trimmed and punched to interface with control and fluid lines. The replica peeled from the fluidic mold was also trimmed, oxidized and bonded to a glass coverslip upside-down. Finally, both the control layer with thin PDMS membrane and the fluidic layer bonded to a glass cover-slip were then oxidized, aligned each other and cured to allow chemical bonding (Step 4). A corresponding photograph of the microfluidic device is presented in Fig. 2b.

2.2. Channel modification for cell attachment

After the fabrication processes, the microfluidic channels were sterilized with 70% ethanol and rinsed thoroughly with phosphate-buffered saline (PBS) prior to cell loading. Because fibronectin-coated PDMS is a suitable substrate for promoting mammalian cell attachment, the channels were then flushed with a solution of human plasma fibronectin (Gibco BRL) (100 μg ml⁻¹ in PBS), followed by physical adsorption of fibronectin onto the channel surfaces under static conditions by overnight incubation at 37 °C [20]. Subsequently, fibronectin-coated microchannels were washed in PBS.

2.3. Cell culture and cell seeding in the microchannels

MCF7 human breast cancer cells (mammary gland epithelial cell) were grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units ml⁻¹ penicillin, and 100 μg ml⁻¹ streptomycin. The cell cultures were maintained under 5% CO₂ on T-75 cell culture flasks, and passaged by trypsinizing approximately 75% confluent layer of cells with 1 ml of 0.25% trypsin/EDTA-4Na and resuspending the cells in 10 ml of FBS-supplemented RPMI. After centrifugation at 1000 rpm for 3 min, the supernatant was removed and the cells were resuspended with 3 ml of FBS-supplemented RPMI.

The device was initially prepared by filling with PBS to remove dead volume and bubbles inside the channel. The cell solution (∼10⁶ cells ml⁻¹) suspended from the culture flasks was loaded into the microchannel via the outlet port using a sterile syringe. Introducing cells through the outlet port prevents
2.4. Experimental setup

The microfluidic device was placed on a warm stage (37 °C) inside a temperature controlled chamber with temperature controller (MC60; Linkam Scientific Instruments Ltd.). The control channels of the microfluidic device were filled with water and connected to an external pressure regulator. The pressure from the control channel deforms the PDMS membrane between the layers, and actuates the opening and closing of the fluidic channel. In addition, as a loading membrane for applying stress to the cells, this polymer membrane can be precisely deformed by varying the pressure with regulator. Valve closing was achieved by applying the pressure of 50 kPa. The pressure for stress loading was varied from 10 to 35 kPa.

Phase-contrast and fluorescence images were taken on an inverted microscope (Axiovert 25; Carl Zeiss, Germany) equipped with a mercury-vapor short-arc lamp (HBO 50) and a CCD camera (Nikon) using ACT-2U software. A filter set for calcein, which was comprised of a 450–490 nm band-pass excitation filter, a 510 nm dichroic filter and a 515 nm long-pass emission filter, was obtained from Carl Zeiss. All images were captured at 100× or 200× magnification. After obtaining the fluorescence images in grayscale, the images were analyzed using ImageJ software [23]. The average fluorescence of each cell region was corrected by subtracting the average local background fluorescence at each point of time. The fluorescence intensity of cells was monitored to investigate the response to the variation of compressive stress.

The deflection of the PDMS membrane was also revealed by fluorescence microscopy when the channel was filled with fluorescein. Since the deflection of the PDMS membrane changes the pathlength through fluorescein solution, it is possible to directly observe the change of the channel height as a function of the applied pressure. The pixel aspect ratio (pixel per length relationship) was used for measuring the width of the channel.

2.5. Cell viability test

Cell viability was determined using calcein AM (calcein acetoxy-methyl ester; green dye; live cells) of the LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Molecular Probes, Inc.). The dye was introduced to the cells by flowing it into the microchannels and then stopping the flow for 30 min for incubation. To ensure that the immobilized cells in microchannels were alive and could respond to applied mechan-
control line for loading membrane varies from 10 to 50 kPa. From the steady-decrease of the fluorescence intensity, it was confirmed that the deflection of the membrane was smoothly increased according to the applied pressures under valve opening. Consequently, the microfluidic device is capable of compressing the cells with direct contact through the tunable membrane deflection.

As shown in Fig. 4, the membrane deflection (height at apex) under pressure control was measured from the optical side-view image using image analysis software (i-solution; iMT Corp., Korea) in order to characterize the membrane deflection. The device having a rectangular membrane with the same dimension as the proposed microfluidic device was additionally fabricated for the membrane deflection measurement. The gap size at apex can be known from both the maximum height of the rounded channel and the height of the deflected membrane. However, the measurements were carried out in a device only having a control layer and 15 μm-thick membrane. Thus, the magnitude of the deflection in a device for measurement may be slightly different.

Fig. 4. Measurements of the membrane deflection at apex for 15 μm-thick membrane. The deflection height was measured from the optical side-view image using image analysis software.

Fig. 5. The change of the fluorescence intensity of MCF7 cells stained with calcein AM in response to applied compressive stress. (a) The fluorescence intensity of the cells was monitored by varying the pressure. The pressure was elevated by the magnitude of 5 kPa every 1 min. All cells directly contacted with the deflected membrane over the pressure of 25 kPa and were particularly burst at 35 kPa. The camera angle of the bright field image was slightly moved to the right. (b) Fluorescence intensity vs. applied pressure. Mean of the entire pixel intensity of all cells in each image were plotted as a function of the applied pressure. As soon as the cells are directly pressed by the deflected membrane, fluorescence intensity began to decrease. At 35 kPa, fluorescence intensity was decreased by 77%.
from that in a real device having a fluidic layer as well. This is because the membrane deflection occurs mainly while pushing out the fluid with which fluidic channel is filled.

3.2. Cellular viability change under compressive stimulation

To monitor cell viability under compressive stress, adherent MCF7 cells were incubated in calcein AM for 30 min, washed in PBS, and then compressed at the elevated pressure ranging from 10 to 35 kPa for loading membrane. Calcein AM is membrane permeant and diffuses into the cells where the acetoxy groups are hydrolyzed by intracellular esterase. The hydrolyzed dye (calcein) is both cell membrane impermeant and fluorescent. The pressure for loading membrane was elevated by the magnitude of 5 kPa every 1 min. It can be seen from Fig. 5a that all cells directly contacted with the deflected membrane over 25 kPa. Particularly, all cells were burst at 35 kPa. In other words, the cells were mechanically lysed through the compressive force. Even though the cells did not contact with the deflected membrane at 20 kPa, all cells were supposed to be subject to both small magnitude of compressive stress and shear stress through the fluid flow. But, at more than 20 kPa, the compressive stress from the direct contact with the deflected membrane is supposed to deform the cells, decreasing the viability. The projected area of the cells was increased owing to the entire deformation of the cells.

As described above, the diminished calcein AM fluorescence due to the cell damage or death caused by compressive stress is clearly visible in these images. In Fig. 5b, the mean pixel intensity of all stained cells of each image remained constant from 0 to 15 kPa and was abruptly decreased over 15 kPa. Since the cell membrane was compromised by the compressive stress from the applied pressure, the calcein was supposed to diffuse out of the cell, reducing the fluorescence signal [15]. Also, compressive stress is assumed to cause severe loss of enzyme activity due to the compromised membrane [11,25]. Therefore, the deformation by the compressive force results in cell damage or death. Any alteration of pushing force is likely to cause a disruption in their normal functioning, thereby producing an unhealthy state, i.e. unprofitable effects. Based on these facts, we can conclude that the microfluidic device for compressive stimulation of cells have a potential to study the effects of compressive stress about the cellular viability.

3.3. Mechanical cell lysis by the compressive force

Among several methods of cell lysis, conventional microfluidic devices for mechanical lysis utilize shear and frictional forces induced by subjecting cells to enter the microchannel with filter structure [26]. Thus, mechanical lysis with pressure-driven cell flow cannot be used to lyse adherent cells directly without trypsinizing. However, this microfluidic device can be useful to simultaneously stimulate the group of cells through the direct contact with the deflected membrane and mechanically perform the lysis of adherent cells for further analysis of their biological contents such as DNA, RNA, and protein.

To monitor cell deformation and lysis with the applied pressure, MCF7 cells were compressed at an elevated pressure ranging from 0 to 29.6 kPa for loading membrane. The cell was gradually ruptured by the elevated compressive force as shown in Fig. 6. With the applied pressure, the membrane deflection increased and small bulges appeared at the cell membrane. Further deflection resulted in an increase of the size and number of the bulges followed by cell bursting. Based on the pressure control of the microchannel with regulator, we can squeeze biological contents out at the appropriate deflection of the membrane. It was also possible to rapidly lyse the adherent cells by momentarily opening an on/off valve in the external regulated pressure line for lysis. A high-speed camera (ultima APX-RS; Photron Inc., USA) connected to an inverted microscope recorded phase contrast images of the cell lysis event to the membrane deflection. The lysis event was recorded with a reso-

![Fig. 6. The radial expansion of cell, the appearance of small bulges and the rupture of the cell membrane according to the elevated pressure. Scale bars are 20 μm.](image-url)
Fig. 7. Images of the cell lysis event captured using a high-speed camera (with a resolution image of 640 × 480, 500 frames/s). The cells were lysed by momentary deflection of PDMS membrane through the opening of an on/off valve in the control line regulated at 35 kPa. All scale bars are 20 μm.

Solution image of 640 × 480 at 500 frames/s (Fig. 7). The cell lysis was assumed to be carried out within 135 ± 37 ms for MCF7 cells (n = 30). The step of the cell lysis is shown as follows: cell deformation, bulges formation on lipid membrane, and cell membrane rupture. Since some enzymatic reaction in cells is fast, the cells must be quickly lysed to accurately measure concentrations of cellular contents [27].

To detect cell burst and leak of cytoplasmic contents in the MCF7 cells, fluorescence measurement was also performed by loading calcein AM. Fig. 8 shows the image-processed fluorescence data of MCF7 cells under compression. These images were processed through the function of “find edges” in ImageJ software. It can be clearly seen from Fig. 8b that small bulges appeared during the lysis event. Fig. 8d shows the number of pixel with the same fluorescent intensity (i.e., gray value) from analysis of the original fluorescent images. The distribution of gray value moved left with the compressive force. The left-hand shifting of maximum gray value means that the larger compressive force is applied, the more the cellular membrane got damaged. Consequently, the calcein signal was decreased with release of calcein due to membrane rupture.

The described microfluidic biomechanical device has three characteristics that make it advantageous over existing macroscopic devices used to study cellular response under compressive stress: (1) because the pressure in the microchannel can be finely controlled with regulator, the magnitude of deflection of the PDMS membrane (loading membrane) is more precisely adjustable compared to macroscopic loading platen; (2) mechanical lysis of adherent cells with the deflected membrane can be performed without trypsinizing compared with conventional mechanical lysis device; (3) the control experiment can be performed simultaneously within a single device. Static compression (i.e., continuous compressive force) in this work was used as the method for applying compressive stress, but the effects of cyclic compression (i.e., intermittent compressive force) on the cells are also important and profitable [7,28]. This microfluidic device is capable of compressing the cells dynamically or statically with help of digitally controlled pneumatic system. In addition to these, the method will offer a new microfluidic platform to study the deformability and viscoelasticity of the group of adherent cells because of the precise controllability of the membrane deflection.

Fig. 8. The fluorescent images (a)–(c) showing MCF7 cells lysis under compressive force. These images are processed using the function of “find edges” in ImageJ software. These images also contain information on the appearance of the small bulges and the leakage of hydrolyzed dye (calcein) according to the elevated pressure during lysis. (d) The pixel number of the each gray value in the original fluorescent images. This graph means that the fluorescent intensity was decreased by the compressive stress due to deformation and lysis of the cells.
4. Conclusions

It was confirmed that the loading membrane fabricated by multilayer soft lithography was finely tunable by the steady deflection according to the applied pressure. We also demonstrated that the cellular response under mechanical stress can be assessed by viability assay such as the change of fluorescence intensity of calcine AM either during or after the application of stress. This device serves as an enabling tool for investigating the cellular response to mechanical stresses. Furthermore, we exhibited that mechanical lysis of cells can be exploited in a microchannel by the compressive force through the membrane deflection. This lysis method could be applied to develop the integrated microfluidic devices for sample preparation and cell-based assays. The ability to stimulate and lyse the cells on the microscale offers many opportunities for improving biomedical and biotechnological researches. Applying direct compression and hydrostatic pressurization, mechanotransduction, cell deformation, viscoelasticity or otherwise, could be explored under this microfluidic biomechanical device.

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