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Method for measuring Young's modulus of cells using a cell compression microdevice

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ABSTRACT

The importance of cell mechanical properties is apparent in many physiological processes; measuring these properties can therefore provide insight into the behavior of cells and tissues in human disease. Here, we describe a method of evaluating cellular deformation of cells by using a cell compression microdevice. The method comprises two steps: 1) observation and evaluation of cell deformation behavior during compression using the microdevice and 2) theoretical calculation of cellular deformation. We constructed the microdevice by stacking two layers of self-adhesive polydimethylsiloxane on a glass plate. The first layer consisted of microchannels, a cell culture chamber, and a diaphragm on the chamber for applying pressure to cells; the second layer consisted of cell inlet ports and a pressure inlet port. The microdevice was made of transparent materials, which permitted in situ monitoring of cellular behavior by optical microscopy. The microdevice was designed to control the magnitude of the pressure applied to cells. We measured the cell compressive strain to evaluate the Young's modulus; the measured values ranged from 3.5 to 4.2 kPa. These results demonstrate that our microdevice is useful for measuring and calculating the mechanical properties of cells under strain.

1. Introduction

The mechanical properties of a cell and tissue are fundamental aspects of their functioning. In particular, cell stiffness is a significant factor in processes such as the cell cycle (Kelly et al., 2011), cellular aging (Berdyyeva, Woodworth, & Sokolov, 2005; Starodubtseva, 2011), and differentiation (Pillarisetti et al., 2011). Therefore, measuring mechanical properties can provide insight into the behavior of cells and tissues. The importance of these properties is also evidenced in human disease; cells affected by cancer, malaria, and sickle-cell anemia show altered shape and stiffness (Brandão et al., 2003; Cross Sarah, Yu-sheng, Jianyu, & Gimzewski, 2007; Efremov et al., 2014; Glenister, Coppel, Cowman, Mohandas, & Cooke, 2002; Li, Lee, Ong, & Lim, 2008; Suresh et al., 2005). Thus, cell mechanical properties can potentially be used as diagnostic criteria for diseases.

There are several ways to measure the mechanical properties of a cell, and atomic force microscopy (AFM) is widely used for this purpose (Berdyyeva et al., 2005; Cross Sarah et al., 2007; Domke et al., 2000; Efremov et al., 2014; Kelly et al., 2011;

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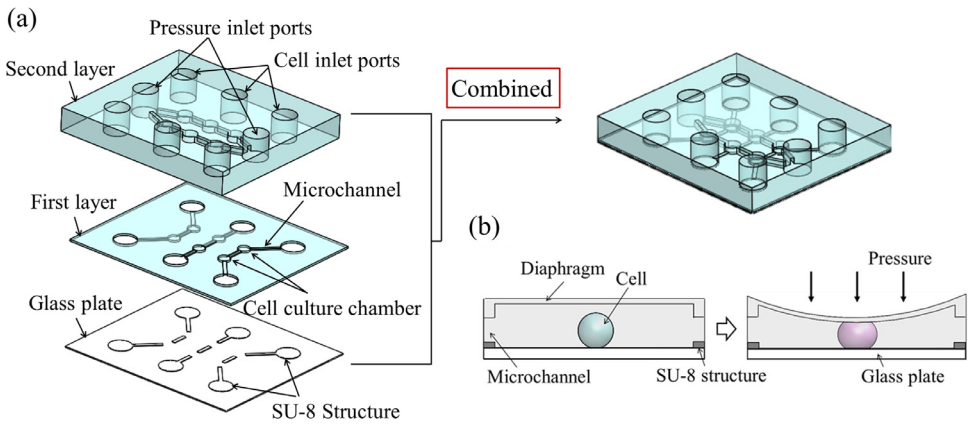

Fig. 1. Schematic illustration of the cell compression microdevice. (a) Microdevice structure. The layers were bonded together by the self-adhesive properties of PDMS. (b) Mechanism of cell compression in the cell culture chamber. When pressure was applied to the diaphragm above the cell culture chamber through the pressure inlet port, the diaphragm was deformed toward the glass plate, directly compressing the cells.

Li et al., 2008; Pillarisetti et al., 2011; Simon et al., 2003; Takai, Costa, Shaheen, Hung, & Guo, 2005). AFM has the capability to measure mechanical properties of living cells under near-physiological conditions. Recently, AFM instruments have been integrated with optical microscopes, permitting the positioning of the AFM tip over a particular region and easy imaging of the regional topology (Richard Bowen & Hilal, 2009).

Other tools such as micropipettes (Glenister et al., 2002), optical tweezers (Brandão et al., 2003; Cuvelier, Derenyi, Bassereau, & Nassoy, 2005; Gerbal et al., 2000; Suresh et al., 2005; Svoboda, Schmidt, Branton, & Block, 1992), and magnetic beads (Bausch, Hellerer, Essler, Aepfelbacher, & Sackmann, 2001; Deng, Fairbank, Cole, Fredberg, & Maksym, 2005; Zeng et al., 2010) are used in parallel according to the specific experimental requirements. However, these instruments are expensive and not easy for many investigators to utilize.

In the present study, we evaluated cellular deformation using our fabricated cell compression microdevice (Nakashima, Yang, & Minami, 2012). The cell-compressing diaphragm is very thin and soft, and therefore, the cell compression microdevice can apply compressive stress to cells with low invasiveness. This method had two steps: observation and evaluation of cell deformation using the microdevice, and simple theoretical calculation of cellular deformation. In our proposed method, we evaluated cellular deformation by estimating the Young's modulus of cells and comparing our estimated values to reported values.

2. Materials and methods

2.1. Cell compression microdevice

The cell compression microdevice (Fig. 1) was designed to control the magnitude of pressure applied to cells and to enable the observation of cell deformation in real time (Nakashima et al., 2012). The device was constructed by a three-dimensional microstructure fabrication process through multiple exposures to SU-8 photoresist. The device was assembled by stacking two layers of self-adhesive polydimethylsiloxane (PDMS; Shinetsu Chemical Co., Ltd. $E = 3.2$ MPa) (Fig. 1(a)). The first layer consisted of microchannels, a cell culture chamber, and a diaphragm on the culture chamber for applying compressive pressure to cells. The second layer consisted of cell inlet ports and a pressure inlet port. The amount of deformation of the diaphragm was controllable by adjusting the air or hydrostatic pressure applied to the diaphragm.

2.2. Evaluation of cell deformation

Cellular deformation was evaluated by observing changes in cell shape. The experimental system consisted of the cell compression microdevice, pressure sensor, micro-syringe pump, and an inverted microscope (ECLIPSE Ti-U; Nikon, Tokyo, Japan) (Fig. 2). MC3T3-E1 osteoblasts were used as target cells, and were cultured in α-Minimal Essential Medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere of 95% humidified air and 5% CO_2. Cultured cells were detached from the culture flask using 0.25 w/v% trypsin-1 mmol/L EDTA solution (Wako Pure Chemical Industries, Ltd.). The detached cells were collected by micropipette, and the cells were injected into the culture chamber of the microdevice from the inlet ports using a micro-syringe pump. Then, the microdevice was held at 37 °C in an atmosphere of 95% humidified air and 5% CO_2 for one hour. Subsequently, air pressure was supplied to the diaphragm through the pressure inlet port and was detected by the sensor on the outlet port. The diaphragm placed on the cell culture chamber was deformed by pressure application; cells were thus directly touched by the diaphragm. The degree of deformation was controlled by adjusting the air pressure. The first contact point between a cell and the diaphragm was defined as the reference point (Fig. 3). The