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Effect of shear stress magnitude on intracellular calcium expression in bone cells

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ABSTRACT

An optically transparent micro cell chip consisting of a polydimethylsiloxane (PDMS) channel structure and cover glass was fabricated to examine the intracellular calcium responses of a single MG-63 cell (human osteoblast-like bone cell) to shear stress with various magnitudes. To supply stable mechanical stimulation to cells seeded on the micro channel structure, a computer-controlled pneumatic system was used to generate pressure-driven fluid flow in the micro channel. Its effectiveness was verified by comparing the measured flow rate of the working fluid induced by the syringe pump and the lab-designed pneumatic system. Intracellular calcium responses in cells were measured using a laser-scanning microscope under varying extracellular mechanical environments. Based on the temporal responses of the intracellular calcium expression, the cells had multiple peaks with a 154 ± 11 s average period. This period was maintained with respect to shear stress of various magnitudes from 0.61 to 2.86 Pa.

1. Introduction

A cell can sense and respond to a wide range of external signals, both chemical and physical, within its microenvironment. By integrating and analyzing this information, the cell can modify its morphology, dynamics, and behavior [1]. There have been a number of studies on the effects of the extracellular physical environment on cells, such as compressive [2], shear [3], and tensile stresses [4]. Additionally, the influences of steady and/or intermittent mechanical stimuli on cellular responses have been investigated, as cellular proliferation and differentiation are highly dependent on the magnitude and frequency of the stimulus [5,6]. The refractory period in cells is recognized as an important factor, and should therefore be considered when determining the proper mechanical stimuli duration [7].

There is increasing interest in using micro system technology to study cell biology. As a result, micro devices have been developed to facilitate both applied and basic research on the biology of cells and tissues [8]. Many different cell chips and cell-based systems have been reported that take advantage of microfluidics technology [9] to overcome some disadvantages of typical methods for cell experiments, such as a large number of cells/media, bulky equipment for cell handling and stimulation, time, and cost. Moreover, as the bulk method in typical biological experiments can be misleading, a reliable and quantitative methodology at the single-cell level [10] is required to investigate the correlation between mechanical stimulation and internal signaling and to determine the optimal duration of mechanical stimulation. These methods provide can micro cell chips with a stable and uniform microenvironment for cell handling and micro fluid manipulation [11–13].

Recently, a cell chip was fabricated using a rapid prototyping (RP) process to study the relationship between the mechanical stimuli pattern to cells and their intracellular calcium expression [14]. Since calcium is an important ubiquitous intracellular messenger that controls a diverse range of cellular processes [15], intracellular calcium levels in cells were measured to study mechanosensory properties, such as the correlation between the applied mechanical physical stimulus and the biochemical signal [16]. Moreover, a study on the effects of shear stress magnitude in cells is necessary to develop an optimized protocol for the magnitude and frequency of mechanical stimuli.

In this study, a micro cell chip was fabricated to investigate the relationship between the mechanical stimuli magnitude and the intracellular calcium responses in MG-63 cells (human osteoblast-like bone cells). A simple and effective micro cell chip was fabricated with a polydimethylsiloxane (PDMS) channel structure and cover glass. Various magnitudes of shear stress were applied to cells seeded on the cell chip and these magnitudes were adjusted using a computer-controlled pneumatic system. Temporal characteristics of the intracellular calcium fluorescence intensity response in a single cell under steady shear stress with various magnitudes were measured and discussed.

2. Experiment

2.1. Micro cell chip

Fig. 1(a) shows a micro cell chip consisting of a PDMS channel structure and cover glass to realize the biomimetic physical

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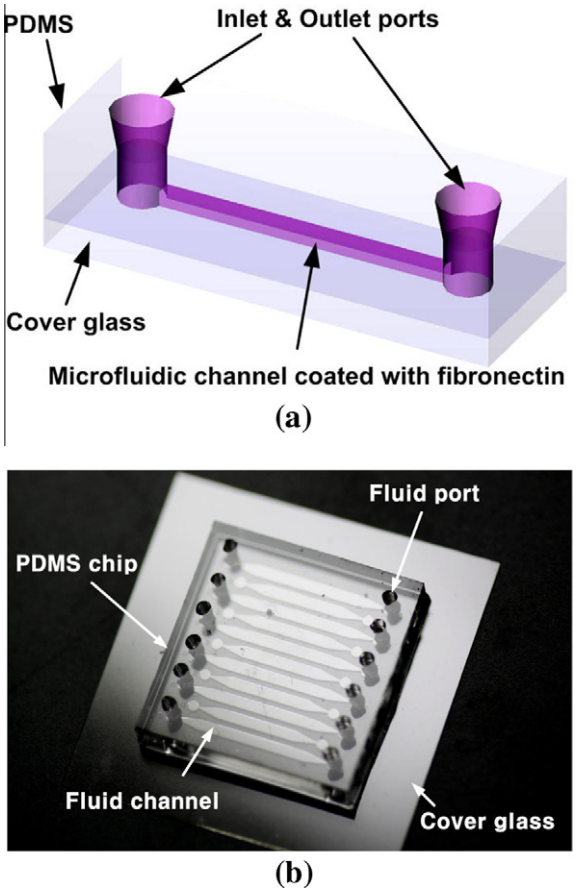

Fig. 1. (a) Schematic view of the micro cell chip, (b) fabricated cell chip.

environment of cells. By taking advantage of microfluidics, the fluid necessary to apply shear stress could be markedly reduced due to the small size of the micro channel structure as well as the small number of cells and uniform extracellular microenvironment.

Fig. 1(b) shows the fabricated cell chip. The typical replica molding method was used for fabricating the cell chip. First, an SU8 mold was prepared for the micro channel array structure. Then, a liquid-type mixture of PDMS was poured onto the prepared mold and cured at 75 °C for 4 h. After the curing process, the micro channel structure was peeled off from the mold. The fluidic ports were then punched out and bonded to a cover glass substrate that was 120 μm thick using the plasma bonding method. The height and width of the microchannel were 88 μm and 1000 μm, respectively. After structural bonding of the prepared PDMS channel structure and the cover glass, the cell chip was sterilized by ultraviolet (UV) irradiation, and the glass surface was coated with fibronectin to promote cell adhesion and growth, as well as enable optical observation. Finally, 1.0×10^5 MG-63 cells/mL were seeded, incubated for 24 h, and stained with the fluorescent calcium indicator Fluo-4/AM (10 μM).

2.2. Measurement system

Fig. 2 shows the experimental setup for applying fluid flow into the micro channel and measuring intracellular calcium expression in cells on the chip. Experiments were performed using our measurement system, which consisted of a pneumatic control system and an observation system. The magnitude and frequency of compressed air were controlled using a lab-designed computer-

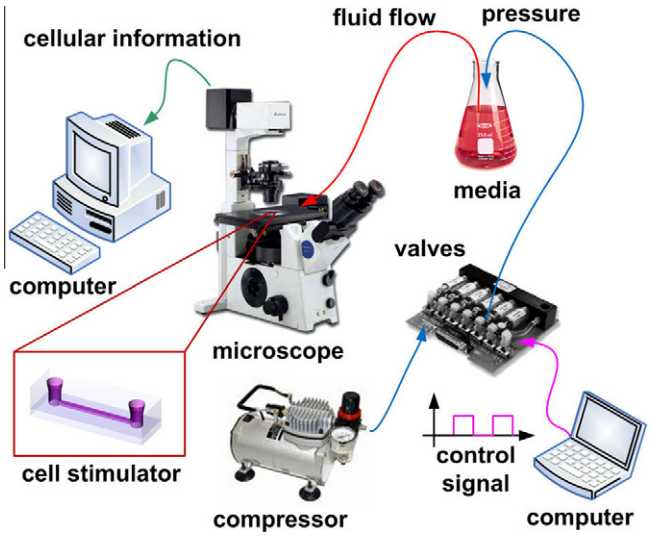

Fig. 2. Schematic view of the measurement system for examining intracellular calcium expression on a micro cell chip.

controlled pneumatic system. For ON/OFF control of the electromagnetic valve, the duty and period of the square wave were programmed using LabVIEW software and the generated electric signal was transferred to the valve through Data Acquisition (DAQ, NI 9264; National Instruments, Austin, TX, USA). Using the programmed valve action, a pneumatic force was applied to the cell medium bottle, and the corresponding fluid flow of the cell medium was introduced into the cell chip. The fluid flow-induced shear stress was adjusted using the flow rate of the cell medium. The supplied fluid flow was monitored and measured using a liquid mass flow meter (Liquid Flow LabKits ASL1600; Sensirion AG, Switzerland). The resolution of the measured data was 3.42 μL/min at 50 Hz. During stimulation of a cell on the chip, the intracellular calcium expression of cells was observed by laser scanning microscopy (LSM 510 META; Carl Zeiss, Oberkochen, Germany). Cellular data, such as fluorescence images, and ASCII data for the fluorescence intensity, were recorded every second and saved on a computer. The region of interest was set to analyze the fluorescence intensity in an individual cell.

3. Results and discussion

3.1. Fluid flow

The fluid flow of the cell medium induced by the syringe pump and computer-controlled fluidic supply system were measured to verify the effectiveness of the computer-controlled fluidic supply system.

Fig. 3 shows the measured liquid flow rates of the cell medium at the outlet port as shown in Fig. 1. Two different working fluid flow rates were set and then supplied using a syringe infusion pump (KDS100: KD Scientific, Holliston, MA, USA). In Fig. 3(a), the flow rate of the working fluid rapidly increased after the syringe pump was operated. An overshoot of the measured fluid flow was first observed and then it became stable. The peak valve flow was 119.14 μL/min and the average flow rate from 12 to 57 s was 77.54 μL/min. This value was much higher than the 60 μL of the setting valve. In contrast, the temporal response of the working fluid flow rate fluctuated under higher fluid flow, as shown in Fig. 3(b). The average flow rate from 13.82 to 77.12 s was 161.32 μL/min. Although the setting value was similar to the measured average value, a ±17.10 μL/min variation was observed in the