Proceedings of the ASME 2013 International Mechanical Engineering Congress and Exposition
IMECE2013
November 15-21, 2013, San Diego, California, USA

IMECE2013-64681

A METHOD FOR APPLYING HIGH CYCLIC STRAIN TO FOCAL ADHESIONS AND MEASURING THE CELL RESPONSE

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ABSTRACT
This paper describes a method by which broadband cyclic strain can be applied to focal adhesions of a cell. In recent years, evidence has been growing that focal adhesions act as mechanosensors of cells which convert mechanical force into biomechanical signaling. However, there are no effective methods by which mechanical stimulation with high frequency can be directly applied to each focal adhesion. Here we develop a micropillar substrate embedding micron-sized magnetic particles and enabling the micropillars to be deflected by external magnetic field. The combination of long and short micropillars produces the difference of deflection between them and enables the micropillars to apply strain to a cell. We verified that the micropillars responded to external magnetic field up to at least 25 Hz without phase difference. Using the magnetic micropillar substrate, we observed the cytoskeletal deformation of an osteoblast cell. The findings indicate that the present micro device can be used for investigating mechanosensing systems of a cell.

INTRODUCTION
Cells respond to various types of mechanical stimulations such as strain or mechanical vibration. It is reported that the proliferation and the bone matrix generation of osteoblasts are promoted by applying mechanical vibration [1]. However, the cellular mechanisms that sense and response to the mechanical stimulation have not been clarified yet. Focal adhesions are a candidate of mechanosensors of a cell. Here we develop a magnetic micropillar substrate to apply broadband cyclic strain to focal adhesions of a cell and observe its deformation.

MATERIALS AND METHODS
A. Fabrication of Magnetic Micropillars:
We used micropillars embedding micron-sized magnetic particles as an "actuator" driven by external magnetic field while the conventional micropillars without the magnetic particles were used as a "sensor" to measure the mechanical properties of cells. If the length of the pillars is all the same, a cell cannot be deformed because there is no difference of the deflection among the pillars. We designed a substrate with the pattern of short and long pillars in order to apply strain to a cell (Figure 1A). According to the previous studies [2][3], we fabricated a magnetic micropillar substrate. A mold of micropillars was made by photolithography. The PDMS (TSE3032, Momentive Performance Materials Japan Co., Ltd., Tokyo, Japan) mixed with carbonyl iron powders of 1.1 μm in diameter and 10% of the total volume (HQ, BASF Japan Co., Ltd., Tokyo, Japan) was poured to the mold, cured in an oven at 100°C for 1 h, and peeled off. Considering the fabrication method and the size of cells, we decided the size of micropillars as 5-μm diameter, 13-μm or 5-μm height, and 11-μm spacing. As the first natural frequency of the magnetic

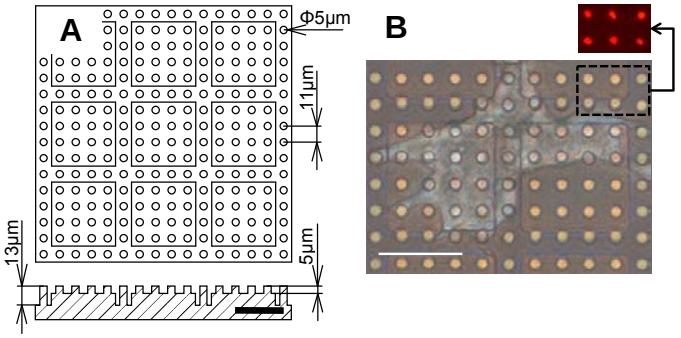

Figure 1. Developed magnetic micropillar substrate. (A) Schematic diagram of the substrate. (B) Micrograph of a cell on the substrate. The cell connected to the tip of micropillars coated with fibronectin (red). Scale bars indicate 30 μm.

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micropillar of 13-μm height was approximately 133 kHz by calculating it as a cantilever, the pillar was expected to response to external magnetic field of high frequency.

B. Cell Cultures:

A mouse osteoblastic cell line, MC3T3-E1 (RIKEN Cell Bank, Tsukuba, Japan), was cultured in alpha-minimum essential medium (α-MEM, Gibco, Grand Island, NY, USA) containing 10% heat-inactivated fetal bovine serum (FBS, Equitech-Bio, Inc., Kerrville, TX, USA) and maintained in 95% air and 5% CO_2 at 37°C. The cells were seeded at the density of 1×10^4 cells/ml on the magnetic micropillar substrate coated with fibronectin to promote focal adhesion formation on the tip of micropillars (Figure 1B).

C. Fluorescent Staining of Actins:

24 h after culture, cells were fixed with 3% paraformaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS, and blocked with 1% BSA in PBS. A fluorescent marker was prepared by mixing Alexa fluor 488 phalloidin (Life Technologies, Inc., Carlsbad, CA, USA) of 2 μl and 0.1% BSA in PBS of 200 μl. The fluorescent marker was added dropwise to the cells and kept in a dark place for 45 min.

D. Broadband Cyclic Strain:

The performance of magnetic micropillars was verified using the experimental setup shown in Figure 2. A micropillar substrate attached to the bottom of a dish. A neodymium magnet was harmonically excited by an electromagnetic exciter to apply time-varied magnetic field to the micropillars. The deflection of micropillars was recorded with a high speed camera through a bright-field microscope. Magnetic flux density was measured with a hall probe. Time series of the pillar deflection and the magnetic flux density were synchronously recorded by trigger input. Frequency of the pillar deflection was in the range from 2 to 25 Hz. After the performance verification, the deformation of actin stress fibers with fluorescent marker in cells on the micropillar substrate was observed with a fluorescent microscope instead of the bright-field microscope in Figure 2.

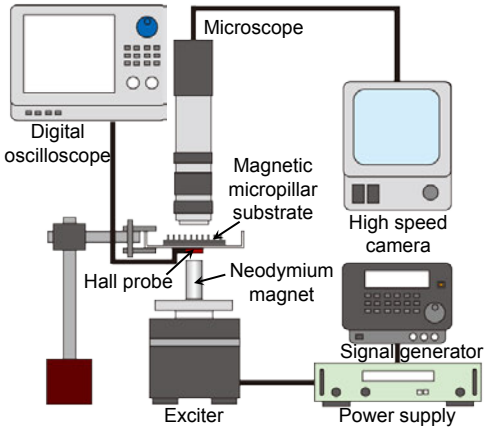

Figure 2. Schematic of the experimental setup for the performance verification of magnetic micropillars.

RESULTS AND DISCUSSION

Time series of the deflection of a micropillar and the magnetic flux density show that a micropillar quickly response to magnetic field (Figure 3). Gain is constant and phase difference is zero between the waveforms of the pillar deflection and the magnetic flux density at 5 and 25 Hz. Figure 4 shows time series of the deformation of each actin stress fiber in a cell during cyclic deflection of a micropillar at 2 Hz. Dynamic deformation of stress fibers can be measured by the proposed method. The deflection of actin stress fibers was higher as their position was nearer to the excited pillar.

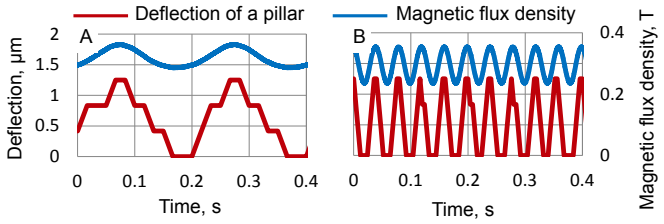

Figure 3. Time series of deflection of a micropillar and magnetic flux density. (A) 5 Hz. (B) 25 Hz.

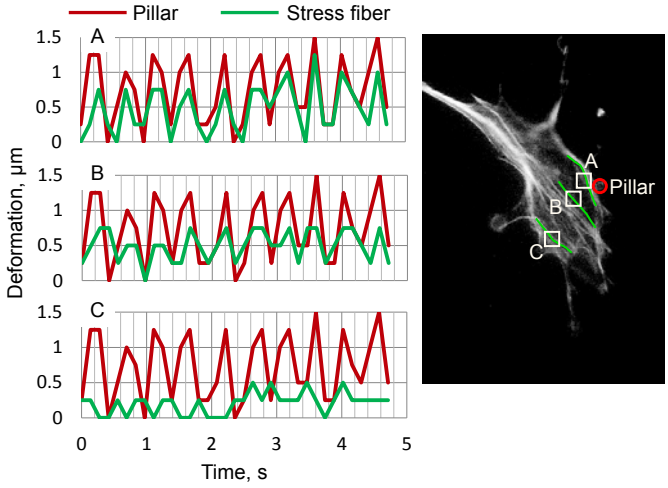

Figure 4. Time series of deformation of each actin stress fiber during cyclic deflection of a pillar at 2 Hz.

CONCLUSIONS

In this study, we have developed a magnetic micropillar substrate to apply broadband cyclic strain to focal adhesions of a cell and experimentally verified its performance. The experimental results indicate that the magnetic micropillars can be used for investigating mechanosensing systems composed of focal adhesions and actin stress fibers in a cell.

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