CELLULAR MECHANICAL IMEPEDANCE MEASUREMENT BY ROBOT INTEGRATED MICROFLUIDIC CHIP WITH WIDTH TUNABLE MICROCHANNEL

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ABSTRACT

This paper presents the on-chip cellular mechanical impedance measurement by OCAIN (On-Chip Impedance ANalyzer). Robochip(robot integrated microfluidic chip) is installed in OCIAN as a disposable microfluidic component integrated with robotic sensing unit. For the high throughput sensing of cellular specimens, they are flown in the microchannel of Robochip and continuous multi-parameter sensing is carried out. For the cellular mechanical impedance measurement, a pair of width tunable wall and force sensor is integrated at the sensing area. One side of the wall is actuated in non-contact by the magnetic force, therefore, the wall is powerful enough to collapse a rigid cell and Robochip is disconnected with the outer magnetic driving system. These are quite advantageous, however, it was quite difficult to improve the position accuracy of the magnetically driven components in the chip. Here we succeeded in nanometric order tuning of the tunable wall width by the displacement reduction mechanism which was previously proposed by our group. Then we achieved cellular impedance measurement of flowing cells in the microchannel by nanometric tuning of the wall.

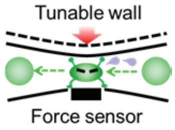
KEYWORDS

MEMS device, robot, impedance analysis, force measurement, oocyte

INTRODUCTION

Recent bioscience research has been focused on the response and quality of cells [1], [2]. In particular, the mechanical stimulation of cells is important for analyzing their mechanical parameters. In general, deformation of cells is required for evaluating its mechanical parameters such as the spring coefficient and viscosity coefficient. Moreover, it is necessary to manipulate a single cell, and to analyze the large number of measured cells as a group because there is variability even among the cells in a same condition. Therefore the high throughput sensing technique is required which is possible to measure a lot of the cells individually. Scanning probe system was employed for the measurement of adhered cells, however, sensing speed is limited and it is difficult to be used for the flowing specimens [3]. Therefore, we studied on the high throughput sensing technique on a chip. We have demonstrated effectiveness of using geometrically-constrained microchannel (Tab. 1, passive) for evaluation of cellular mechanical properties [4]. Passive type is simple and fast, but force sensing is not possible. Herein, we developed an active type by using robochip which was integrated a width tunable wall and a force sensor for cellular mechanical impedance measurement.

Table 1: Classification of continuous cellular force measurement in a micro fluidic chip

Type	Passive	Active
Throughput	High	High
Sensing	Indirect	Direct
Images of cellular force measurement		

METHODS AND MATERIALS

OCIAN and Robochip:

Figure 1 shows the fundamental concept OCIAN for the direct measurement of the cellular force. We employed the robochip as an active type to the disposable part which was integrated of a tunable wall and an implanted force sensor. The tunable wall utilizes the displacement reduction mechanism which is the serially-connected springs with different stiffness to obtain high resolution in positioning [5]. The system consists of three part which is sensing part, actuation part and disposable part. Features of the robochip are (1) high throughput direct sensing by employing the tunable wall and implanted force sensor, (2) in-situ or pre- tuning of tunable wall to the target cell with high resolution, and (3) disposable for biomedical application. Since the chip part is disposable, our concept is suitable for biomedical application.

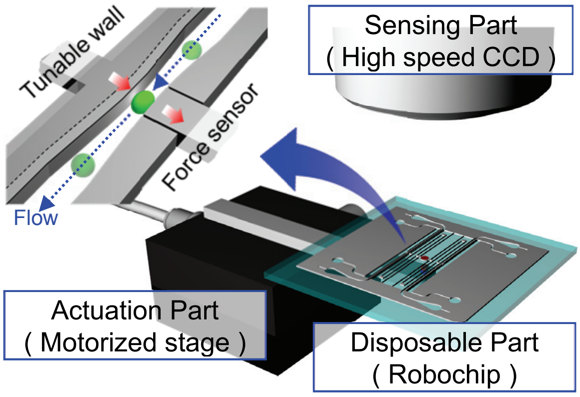


Figure 1: Concept of on-chip impedance analyzer

Fabrication of robochip:

Figure 2 shows the process flow for the fabrication of the robochip. Fabrication process of robochip was based on a simple two-step procedure. First the holder layer and the device layer were fabricated identically. The layers were then assembled and a multi-layer structure was easily achieved.

<Process flow of hold layer>

(a) Cr/Au was patterned as alignment marks for the multi-step exposure of the holder layer.

(b) 1st SU-8 (Nippon Kayaku Co., Ltd.) layer was patterned as a spacer layer. The thickness of the spacer layer defined the gap between the device structure and the substrate surface for reducing the friction force between them. In this case, the gap was designed to be 10 μm in order to avoid contact between the Si structure and substrate surface because of the magnetic force and deflection by its own weight. Then, 2nd SU-8 layer was fabricated as a 220-μm-thick cage structure. This structure holds the device layer.

<Process flow of device layer>

(c) An SU-8 pattern which composed of the tunable wall, the force sensor and the microchannel pattern, was fabricated on the surface of the 200-μm-thick Si.

(d) The Si substrate was etched using a deep reactive ion etching (D-RIE) technique, and the SU-8 was removed.

<Assembly and packaging>

(e) The fabricated device layer was assembled to the hold layer, and a permanent magnet (diameter: 1 mm, height: 0.5 mm, density of magnetic flux: 140 mT) was assembled to the actuation point. Finally, a polydimethylsiloxane (PDMS) cover was bonded onto the hold layer.

A photograph of the fabricated tunable wall and an SEM image of the gap between the device layer and the hold layer are shown in Figure 3.

EXPERIMENT

Figure 4 shows a photograph of the OCIAN. This system is composed of a microscope with a CCD camera (sensing part), a motorized stage (actuation part), and a robochip (disposable part). A permanent magnet (diameter: 1 mm, height: 1 mm, density of the magnetic flux: 176 mT) was placed on the motorized stage to actuate the on-chip probe, and the density of magnetic flux on the glass substrate was 32 mT. Figure 5 shows the demonstration of wall tuning by using the OCIAN. The tunable wall was actuated by the magnetic force and width of the microchannel was tuned by the control of the displacement of the motorized stage. Figure6 shows the evaluation of the repetitive positioning accuracy of the tunable wall. The amplitude of the displacement of the manipulation point and actuation point was measured using the pixels of the images of the CCD camera (1 pix ≅ 0.08 μm) as a function of the amplitude of the displacement of the motorized stage (the drive frequency: 0.25 Hz). In figure 6, horizontal axis shows the displacement of permanent magnet on the motorized stage and vertical axis shows the displacement of actuation point and displacement of manipulation point. The plots showed the average (10 times) of the displacement of them,

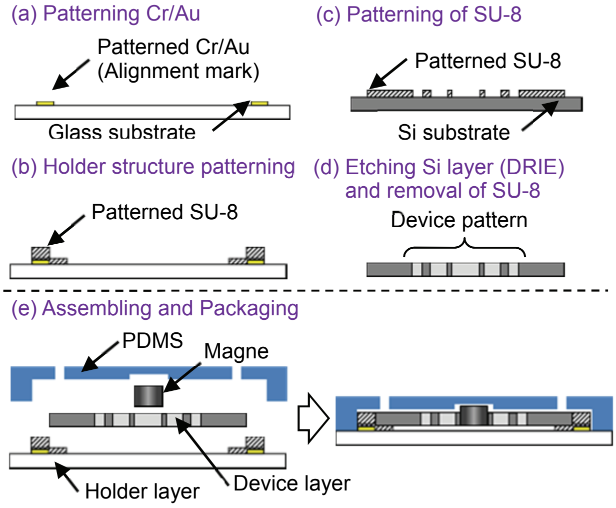
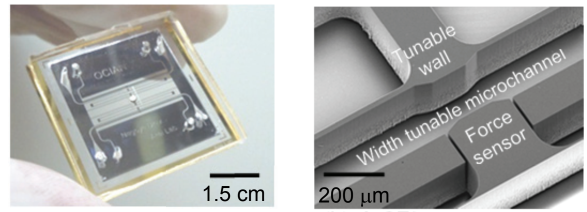


Figure 2: Flow of fabrication process of robochip



(a) A photo of the fabricated robochip

(b) A SEM image of the manipulation point of the robochip

Figure 3: Images of the fabricated microtool

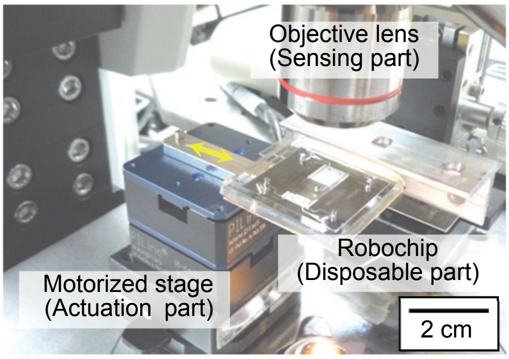


Figure 4: Overall view of experimental setup

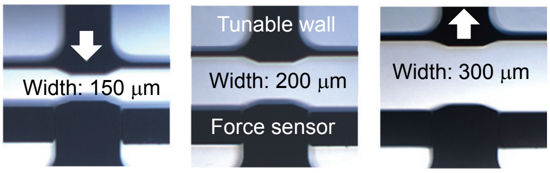


Figure 5: Demonstration of the tunable wall

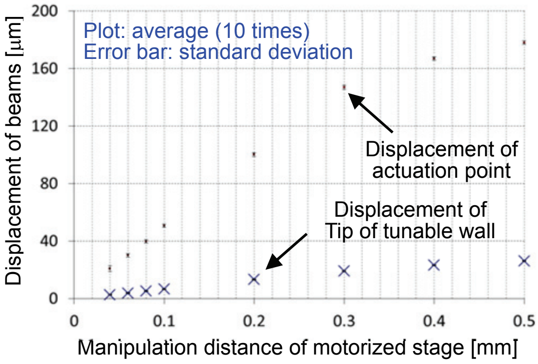
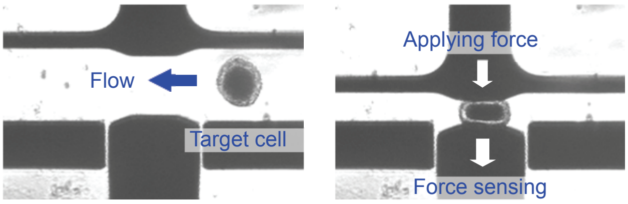


Figure 6: Repetitive positioning accuracy of the tunable wall

and error bar showed each standard deviation of that. The standard deviation of the displacement of the wall was under 0.18 µm, and we concluded that the nanometric order tuning was achieved.

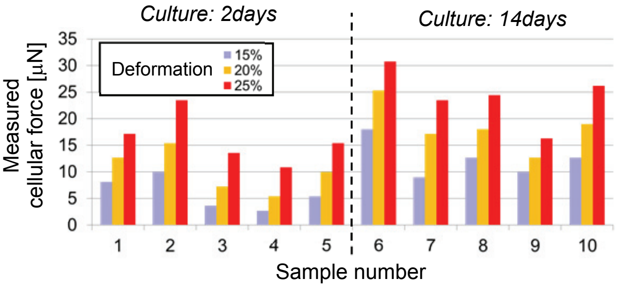
Figure 7 shows the sequence of photographs of the demonstration of the on-chip cellular force measurement. The target cell (oocyte: diameter = 150 µm) was transported (wall width: 180 µm), then it was deformed with the displacement of 65 µm at the tip of force sensor and the force was measured as 8.8 µN. Further, the Young's modulus of the oocyte was measured as 15.4 kPa. Continuous force measurement was also possible by using cell loading unit. Figure 8 shows the measurement result of the Young's module as mechanical impedance. Young's modulus of ten oocytes were measured at OCIAN, 2 days (groupA: sample number 1~5), and 14 days (groupB: sample number 6~10) after harvest. Each oocyte was deformed with displacement of 15, 20, 25 % of original size. GroupB showed relatively higher Young's modulus than groupA. Eventually, quality evaluation of cell will be possible by mechanical impedance measurement using OCIAN.



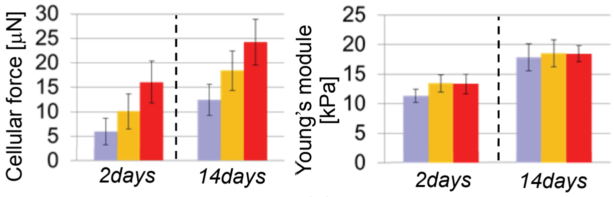
(a) Transportation of the target cell

(b) Measurement of the cellular

Figure 7: Demonstration of the on-chip cellular force measurement



(a) Measured cellular force



(b) Cellular force (Average) (c) Young's module (Average)

Figure 8: Demonstration of the on-chip cellular mechanical impedance measurement

CONCLUSION

In this paper, we presented the on-chip cellular impedance measurement using robochip which consists of an on-chip probe and a force sensor. Here we demonstrated cellular impedance measurement. Oocytes were deformed by the tunable wall, and the cellular force was measured by the force sensor in the flow environment. Unlike conventional measurements using a scanning probe system that can measure adhered cells, the on-chip probe can be conducted as a flow process, and the cells can be supplied to the manipulation area. The performance of the tunable wall was examined, and the result shows that the standard deviation of displacement of the wall was under 0.18 µm and the output force was enough for the deformation of the oocyte. Moreover, the measurement of the Young's module as mechanical impedance was demonstrated and quality evaluation of cell will be possible by mechanical impedance measurement using OCIAN.

The proposed method, which is a microfluidic chip based robochip, is therefore a promising method for realizing high throughput sensing of the mechanical parameters of cell.

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