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Paper:

Evaluation of the Basic Designs of a Micro Device that Provides Vibrational Stimulation to Cells

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It is known that the cells responds to external mechanical stimulations. Although the effectiveness of vibrational stimulation for the osteoanagenesis has been reported, the clarification of detailed mechanism for this phenomenon is insufficient. In this study, a micro device has been developed to evaluate the cell dynamics and responses to vibrations. The micro device has an array of moving micro stages which have transparent 5 µm thick thin film to enable them to observe the cell responses to vibrational stimulations by using an optical microscope. The moving micro stages are moved with a needle actuated by piezo actuator. Microfabrication processes, such as conventional photolithography, lift-off, and sacrificial layer etching, were used to fabricate the micro device. We designed two types of concepts for supporting and vibrating moving micro stages. Prototypes were fabricated and evaluated under vibrational conditions. Proposed design with the moving micro stages vibrating perpendicular to the beams generated simple linear oscillation without rotation. It was verified that the fabricated micro stage could be vibrated at the acceleration amplitude of 0.1 and 0.2 G with frequency 15, 45, and 90 Hz.

Keywords: Bio-MEMS, vibration stimulus, cellular response, moving micro stage, array device

1. Introduction

Cells sense and respond to the surrounding deformation and mechanical stimuli through desmosomes [1]. The elucidation of cellular responses to the surrounding environment is a challenge, which involves not only understanding purely biological cellular functions, but also developing methods to construct tissues and organs from cells for regenerative medicine and to make machines that mimic living organisms in engineering.

Studies have shown that external vibrational stimulation promotes bone formation [2]. This phenomenon is considered to be effective for early healing of bone fractures and prevention or treatment of osteoporosis, and has been put to practical use in certain cases as a treatment method at the clinical level. In these cases, safety guidelines for the vibration exposure of the human body must be considered. It is found that 20–90 Hz vibration below 0.56 G would not cause any acute or chronic complications in [2], which is based on ISO 2631-1:1985.

From the cell biology perspective, the appropriate vibrational conditions for promoting bone formation and the mechanisms of bone regeneration are unclear. Various studies have reported that acceleration amplitudes of 0.15 G (frequency 90 Hz) [3], 0.3 G (frequency 35 Hz) [4], and 0.5 G (frequency 50 Hz) [5] are significant for bone regeneration. However, this has not been clearly established.

Recent studies have reported that even under a constant acceleration amplitude of 0.2 G, cellular response characteristics such as the intensity and velocity of calcium signals of cells differ for different frequencies [6]. In addition, it has been suggested that cell mechanosensing and cell function may be affected by the motion and distortion of the cell nucleus owing to the force transmitted by the cell cytoskeleton [7]. To elucidate the mechanosensing mechanism of cells in response to vibrations, studies have been conducted to measure the displacement of organelles in cells when subjected to vibratory stimuli. The motion and deformation of the nucleus depending on the vibration frequency have been reported [8].

To study of these cellular functions, it is necessary to examine cells under various conditions. Therefore, an experimental device with an array of stimulating units is suitable because it enables several independent experiments to be conducted under different conditions using a single device [9].

In this study, we developed a cell vibration micro device consisting of an array of moving micro stages that could provide vibrational stimulation to adherent cells under microscopic observation. Two basic designs were developed and prototyped. The differences in the driving characteristics of the two basic designs were evaluated by vibrating the moving micro stage of the device with a piezo-driven needle and microscopically observing

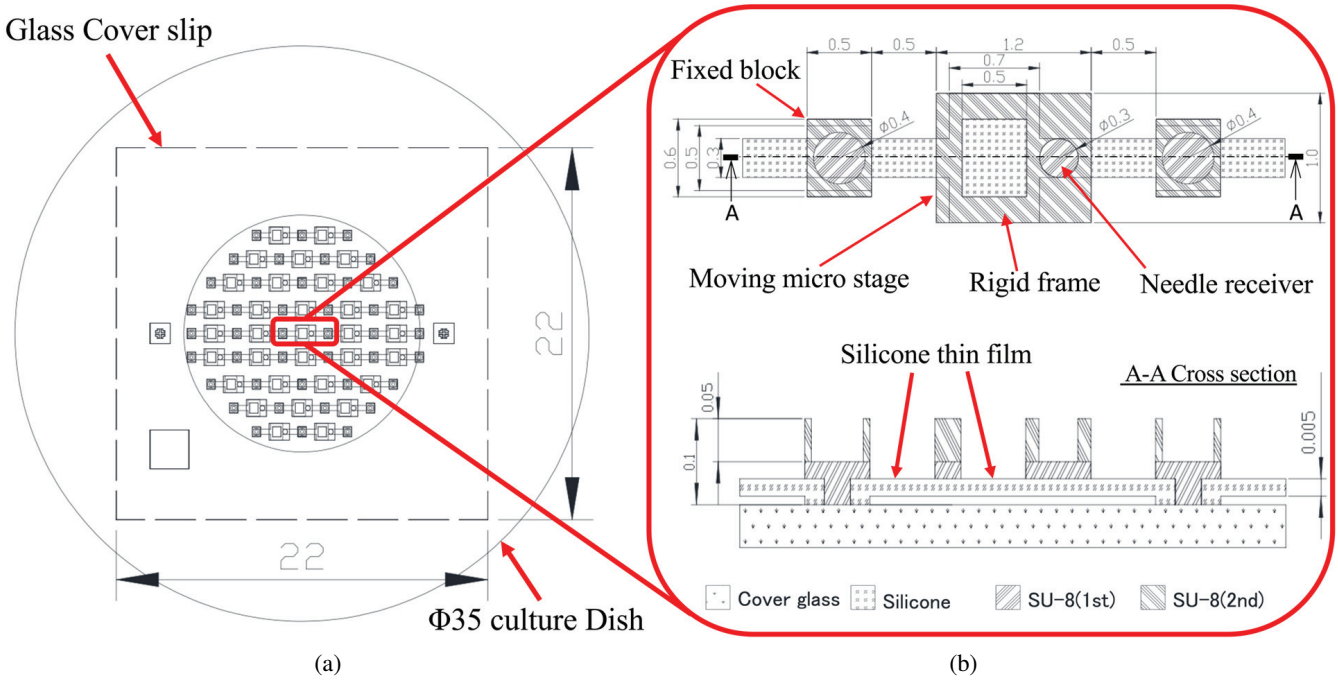

Fig. 1. Conceptual diagram of the first type of micro device for vibrating cells. (a) Micro device. (b) Enlarged view of moving micro stage for vibrating cells.

it using a high speed camera. This is expected to be a key device for further research into mechanosensing and mechanotransduction under vibrational conditions.

2. The First Concept of Micro Device

2.1. Design

Figure 1 shows a conceptual diagram of the first type of micro device proposed for cell vibrational stimulation. **Fig. 1(a)** shows an overall view of the micro device, and **Fig. 1(b)** shows an enlarged view of the moving micro stage.

As shown in **Fig. 1(a)**, the proposed device consists of 33 moving micro stages for cell stimulation placed on a glass cover slip attached to the bottom of a ϕ35 mm cell culture dish with a hole. The moving micro stages shown in **Fig. 1(b)** consist of 5 µm thick transparent thin film of silicone, KE-106 (Shin-Etsu Chemical Co., Ltd.), that serves as the cell adhesion area of 0.5 mm × 0.6 mm, and a rigid frame made of 100 µm thick photoresist, SU-8 (Kayaku Advanced Materials, Inc.), that surrounds the silicone thin film and includes a needle receiver. The 5 µm thick silicone film enables cell observation with high resolution. The rigid frame supports the thin film of silicone to maintain it flat, even under vibration. The fixed blocks bonded to the glass cover slip are connected to the moving micro stage using silicone thin beams, which provide elastic support for the moving micro stage. The moving micro stage and silicone thin beams are not fixed to the glass cover slip, and can move. The needle receiver contacts the needle tip, which provides the driving force. Thus, the moving micro stage and the needle tip moved together.

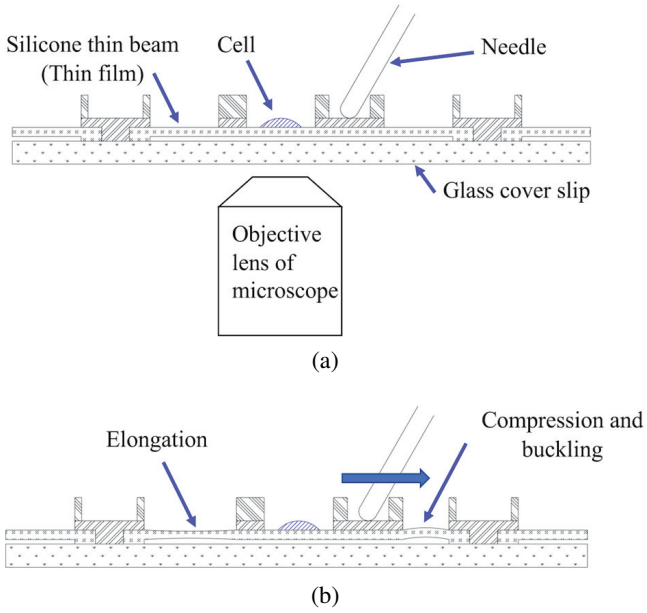

Fig. 2. Working principle of vibrating moving micro stage. (a) Moving micro stage before vibration. (b) At position of the largest displacement of moving micro stage.

Figure 2 shows how the moving micro stage is driven when the cells are stimulated by vibration. **Fig. 2(a)** shows the moving micro stage before driving. The thickness of the silicone thin beam is equal to the direction of the optical axis of the microscope, allowing the microscopic observation of cells through the silicone thin film. The moving micro stage is driven by moving the needle

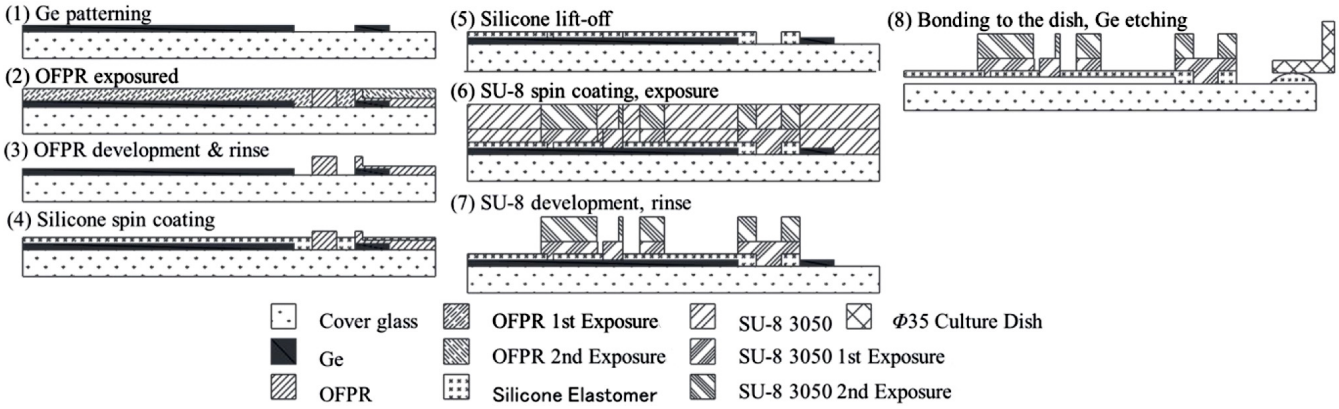

Fig. 3. Fabrication process chart of micro device.

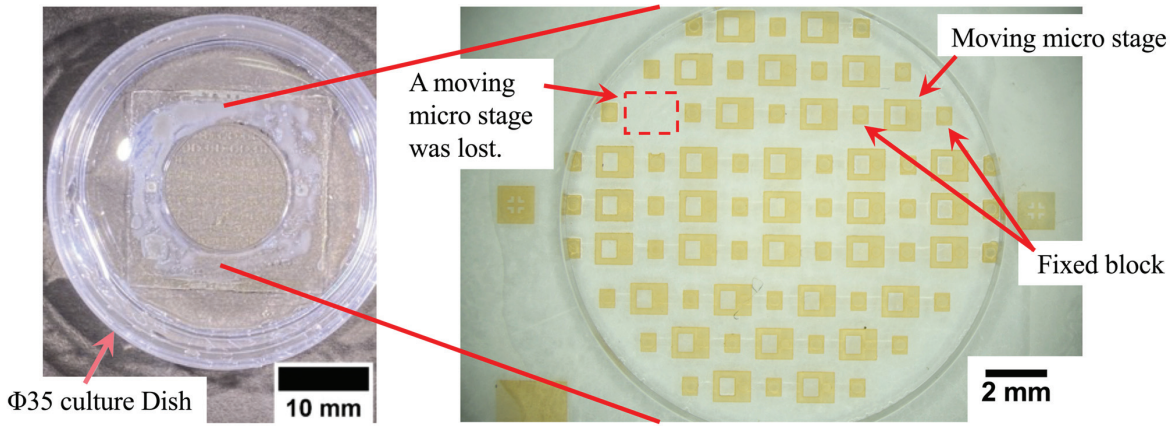

Fig. 4. Photograph of fabricated micro device. (a) Overall view. (b) Enlarged view. A moving micro stage was lost due to a failure in fabrication.

in contact with the needle receiver. A piezoelectric actuator is used to drive the needles. **Fig. 2(b)** shows a case of vibration, where the moving micro stage is displaced linearly in the longitudinal direction of the silicone thin beam until the largest displacement is reached. By vibrating the needle, the micro stage moves back and forth with the needle between the states shown in **Figs. 2(a)** and **(b)**. Therefore, the cells attached to the moving micro stage receive repetitive acceleration, and the organelles in the cells experience repetitive inertial forces. Cells and organelles can be observed under vibrations because they are located in the focal plane of the microscope.

2.2. Fabrication of the Micro Device

Figure 3 shows the fabrication process of the micro device. First, Ge was sputtered as a sacrificial layer on a glass cover slip, and the Ge thin film was patterned using photolithography (**Fig. 3 (1)**). Next, positive photoresist OFPR (OFPR-800, Tokyo Ohka Kogyo Co., Ltd.) is applied by spin-coating with a film thickness of 10 µm. The OFPR film of the area corresponding to the silicone thin film was exposed first, and the OFPR film slightly outside the first exposed area was exposed for a shorter time than the first exposure (**Fig. 3 (2)**). The exposed OFPR was removed by development and rinsing (**Fig. 3 (3)**). Silicone KE-106 was applied by spin-coating onto a mold made of OFPR (**Fig. 3 (4)**), and after curing the silicone of 5 µm thickness, only the necessary silicone thin film structure was left by the lift-off process using the removal of OFPR (**Fig. 3 (5)**). To fabricate the rigid frame of the moving micro stages and the fixed blocks, two layers of the thick photoresist SU-8, which were applied with a film thickness of 50 µm and exposed, were stacked. The total thickness was 100 µm (**Fig. 3 (6)**). SU-8 in the unexposed areas of the two layers was removed by development and rinsing (**Fig. 3 (7)**). Finally, the glass cover slip was bonded to a cell culture dish with a hole using adhesive silicone KE-441-T (Shin-Etsu Chemical Co., Ltd.), and the Ge sacrificial layer was etched to make the moving micro stage and the silicone thin beams movable (**Fig. 3 (8)**). A photograph of the fabricated micro device is shown in **Fig. 4**. **Fig. 4(a)** shows the overall view of the culture dish. **Fig. 4(b)** shows an enlarged view of the area in which the moving micro stages were fabricated. In this device, one moving micro stage was lost because of a rare failure in fabrication.

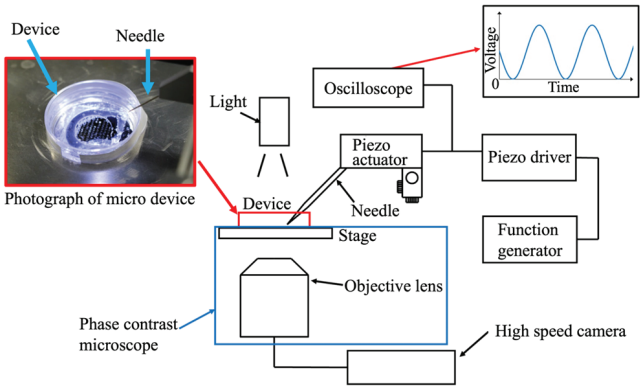

Fig. 5. Experimental setup of evaluation of micro device under vibration.

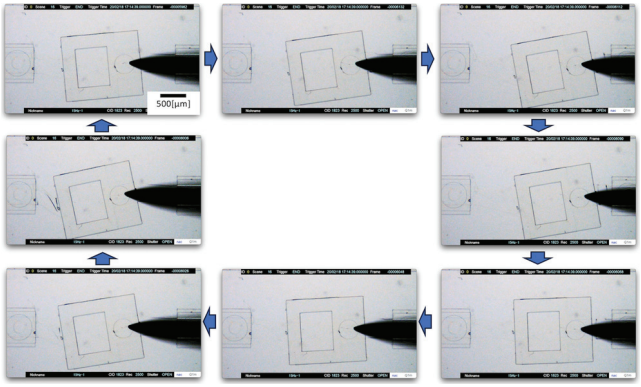

Fig. 6. Snapshot of moving micro stage observed by high speed camera in one cycle under vibration. Frequency and total amplitude were 15 Hz and 440 μm, respectively.

2.3. Evaluation of Dynamic Behavior and Optical Image Under Vibration

The fabricated micro device was evaluated to clarify the dynamic behavior of the moving micro stage and the quality of microscopic images during vibration. **Fig. 5** shows a schematic of the experimental setup used for the evaluation. The micro device, containing DI water, was placed on the stage of an inverted phase contrast microscope. A function generator was used to generate a sinusoidal voltage signal with a DC bias voltage equal to the amplitude, which was amplified by a piezo driver and amplified signal was applied to the piezo actuator. A needle connected to the piezo actuator vibrated the moving micro stage. The inverted phase contrast microscope (CK40, Olympus Corporation) and a high speed camera (MEMRECAM Q1m, nac Image Technology Inc.) were used to observe the vibrational behavior of the moving micro stage at a frame rate of 2500 frames/s. The vibrational conditions were frequency from 15 to 90 Hz and a total amplitude from 12 to 440 μm, which generate acceleration amplitude of 0.1–0.2 G.

The vibration experiments confirmed that the moving micro stage moved normally in a linear reciprocating motion under appropriate conditions. However, when the load of the needle pressing on the moving micro stage was small, the rotational motion of the moving micro stage around the needle contact point was excited, and a swing of the moving micro stage was consequently observed. This phenomenon was independent of frequency and was more prominent when the amplitude was large. **Fig. 6** shows snapshots of one vibration cycle. These data were extracted from a movie captured using the high speed camera. **Fig. 6** shows the swing motion of a moving micro stage. The goal of this study is to provide a single axis of acceleration to cells, and hence, the occurrence of motions other than linear motion is undesirable. One of the causes of rotation is considered to be the distortion of the moving micro stage owing to the internal stress generated during the curing of SU-8 [10]. The distortion of the rigid frame of the moving micro stage made of SU-8 resulted in uneven contact between the glass cover slip and the moving micro stage, and the friction at the contact point far from the needle tip generated torque around the contact point of the needle. We expected that the tensile force of the silicone thin beams would stabilize the movement of the moving micro stage, but it was assumed that the tensile force of the silicone thin beams was not sufficiently large.

However, there was no difference in the resolution or quality of the observed image at rest and under vibration. Therefore, no degradation of the observed image owing to vibration was confirmed. In addition, waves on the water surface in the micro device during the vibration experiments could not be clearly observed because of the low volume of the vibrating parts.

3. The Second Concept of Micro Device

3.1. Design

To resolve the shortcomings of the first type, in the second type of micro device the vibration direction was changed to be perpendicular to the longitudinal direction of the silicone thin beam. **Fig. 7** shows a conceptual diagram of the second type of micro device. **Fig. 7(a)** shows the overall view of the micro device, and **Fig. 7(b)** shows an enlarged view of the moving micro stage. As shown in **Fig. 7(a)**, similar to the first type, the device has 33 moving micro stages for cell stimulation placed on a glass cover slip. The cell adhesion area is 0.3 mm $\times$ 0.6 mm. Because the direction of vibration was changed, the needle receiver is placed on the rigid frame of the moving micro stage at a position equidistant from the fixed blocks.

Figure 8 shows how the moving micro stage is driven when the cells are stimulated by vibration. **Fig. 8(a)** shows the moving micro stage before driving. As in the first type, the vibration of the moving micro stage is performed by moving the needle in contact with the needle receiver; however, the direction of vibration is perpendicular to the longitudinal direction of the silicone thin beam. **Fig. 8(b)** shows the maximum displacement during vibration. By moving the micro stage back and forth between

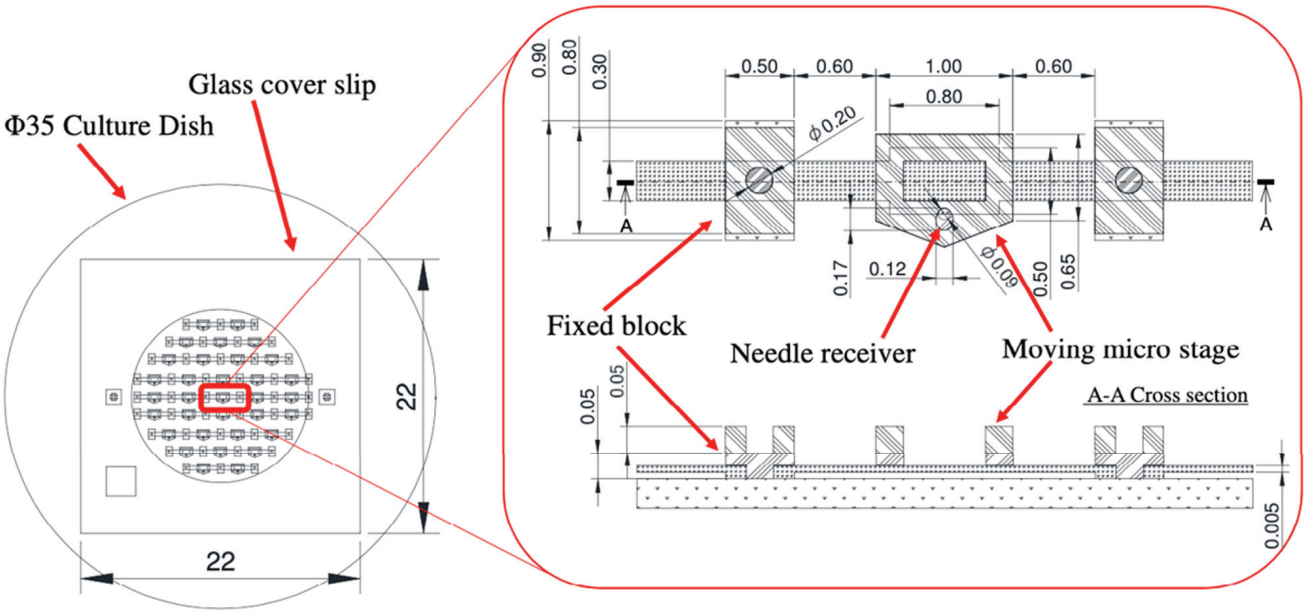

Fig. 7. Conceptual diagram of the second type of micro device for vibrating cells. (a) Micro device. (b) Enlarged view of moving micro stage for vibrating cells.

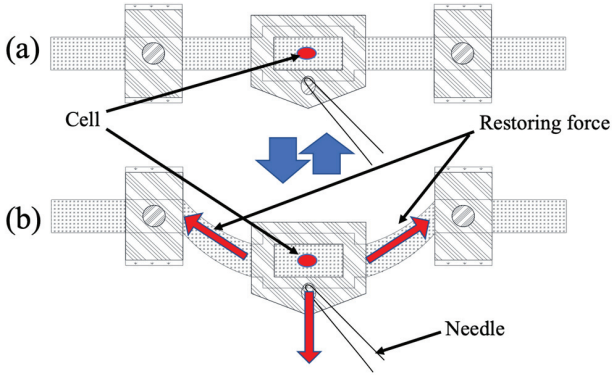

Fig. 8. Working principle of vibrating moving micro stage. (a) Moving micro stage before vibration. (b) Position of largest displacement of moving micro stage.

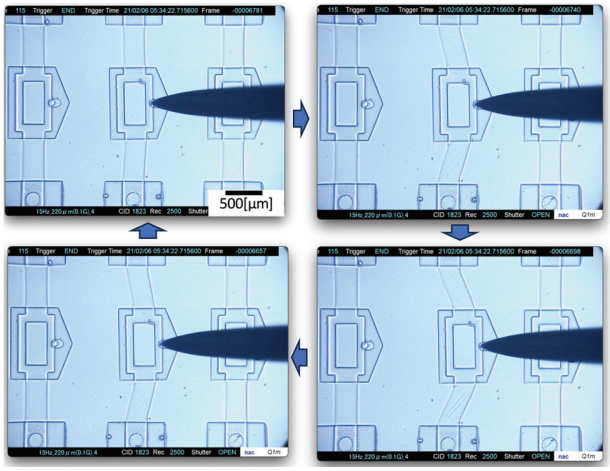

Fig. 9. Snapshot of moving micro stage observed by high speed camera in one cycle under vibration. Frequency and total amplitude were 15 Hz and 220 μm, respectively.

the states shown in **Figs. 8(a)** and **(b)**, the cells attached to the moving micro stage are stimulated, similar to the first type. In contrast to the first type, the silicone thin beams on both sides of the moving micro stage generate a tensile restoring force during vibration. Therefore, when the moving micro stage rotates, the restoring forces of the left and right silicone thin beams are out of balance, and a rotational force in the opposite direction is exerted, thereby suppressing rotation.

3.2. Fabrication and Evaluation of Micro Device

Similar to the first type, the second type of micro device was fabricated using the process shown in **Fig. 3**, and the moving micro stage under vibration was evaluated using the experimental setup shown in **Fig. 5**. **Fig. 9** shows snapshots of a moving micro stage in one cycle of vibration, which were observed at a frame rate of 2500 frames/s. The vibration conditions were a frequency of 15 Hz and a total amplitude of 220 μm, because the narrow layout of moving micro stages in the second prototype did not allow the total amplitude of 440 μm. **Fig. 9** shows that the moving micro stage did not rotate during vibration and synchronized the movement of the needle in a linear motion. Therefore, the moving micro stage vibrated normally.

The same evaluation was performed under the other vibrational conditions. The evaluated vibrational conditions and the results of the observations are summarized in **Table 1**. A stable linear motion was observed under all con-

Table 1. Observation result of moving micro stage of the second type under vibration.

Amplitude of acceleration [G]	Frequency [Hz]	Total amplitude [μm]	Vibration behavior	Quality of the observed image
0.1	15	220	Stable linear motion	Clear and no degradation
	45	24		
	90	6		
0.2	45	49		
	90	12		

ditions. No degradation owing to vibration was observed in the image. Therefore, the second type of micro device fabricated in this study was found to provide vibrational stimulation to cells adherent to the moving micro stage and observe them.

In future studies, vibration tests using microbeads and cultured cells are crucial. Observation of the motion of microbeads on the moving micro stage under vibration will provide essential information about the liquid flow that causes a viscous force on the cell surface. These issues are currently under investigation. These results will be reported in future studies.

4. Conclusions

In this study, we designed, prototyped, and evaluated two types of micro devices with different concepts for vibrational stimulation of cells.

1. We designed and fabricated a prototype micro device having a structure that allows the vibration of a moving micro stage in the longitudinal direction of silicone thin beams that support the moving micro stage, which has 5 μm thick and 0.5 mm × 0.6 mm thin film for cell adhesion. Experiments of vibrating the moving micro stage under conditions such as a frequency of 15 Hz and a total amplitude of 440 μm were conducted, and the dynamic behavior of the moving micro stage was observed with an optical microscope and high speed camera. It was confirmed that the moving micro stage followed the needle in linear motion; however, in addition to linear motion, rotational motion was also observed in the moving micro stage under certain conditions. It was considered to be caused by the generation of a non-uniform contact force owing to the distortion of the moving micro stage induced by the fabrication process.

2. To suppress the rotation of the moving micro stage, we designed and fabricated a micro device with a structure that allows the vibration of the moving micro stage in a direction perpendicular to the longitudinal direction of the silicone thin beams that support the moving micro stage. Vibration experiments were conducted under conditions such as total amplitude from 6 to 220 μm at frequencies from 15 Hz to 90 Hz, which yielded acceleration amplitudes of 0.1 G or 0.2 G. The dynamic behavior of the vibration of the micro stage was observed, and it was verified that the moving micro stage performed linear motion without rotational motion. Therefore, we concluded that this device is useful for observing the behavior of cells while stimulating them with vibrations.

Acknowledgments

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