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DEVELOPMENT OF DISH-ATTACHED MICROCHIP FOR AUTONOMOUS CELL MANIPULATION SYSTEM

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Abstract— **In this paper, we report on the fabrication of a dish-attached microchip applicable to cell culture dishes and the autonomous control of microvalves based on deep reinforcement learning for manipulation of living cells. We have achieved autonomous position control of microparticles in a two-dimensional plane, and the aim of this study is to apply this technology to living cells. To this end, we first fabricated the contact surfaces of the cell manipulation channels of a microfluidic chip in a structure that can be mounted in a dish used for general cell culture. Deep reinforcement learning is applied to the pumping control of this device in order to acquire autonomous cell manipulation behavior. For efficient learning, a simulator representing the behavior of particles in the manipulation area of the fabricated microfluidic chip was constructed using a neural network, and a behavior decision model was trained in this environment. In this task, rewards are given according to the distance between the particle position and a randomly placed target in the virtual environment in the simulator. As a result, it was observed that the model learnt to manipulate the particles to an arbitrary target position, demonstrating the manipulation of live cells in a real environment by this behavioral decision-making model. This technology is promising as a new platform for the realization of automatic cell array techniques.**

I. Introduction

Cell micro-manipulation techniques are an essential core technology in the medical and biotechnological fields in recent years. Large platforms such as micromanipulators have traditionally been used as devices for manipulating cells of several micrometers to several tens of micrometers [1]. However, this manipulation has disadvantages, such as requiring skilled and delicate techniques by the user and low efficiency. On the other hand, microfluidic systems have attracted attention as a high-throughput cell manipulation technique [2]. Microfluidic systems have advantages such as faster reactions, higher efficiency and reagent saving by using microfluidic channels [3, 4], and have recently been integrated with machine learning [5]. Optical tweezers are a typical method of manipulating microparticles using conventional microfluidic systems [6]. However, optical tweezers are expensive and the focused laser light can damage cells [7]. We have applied deep reinforcement learning to the control of devices integrated with multiple microvalves to construct versatile microsystems. In this context, we have successfully manipulated particles in a two-dimensional plane [8]. This technique could be useful for the automatic creation of *in vitro* models of artificially reconstituted cells by arranging them (Fig. 1).

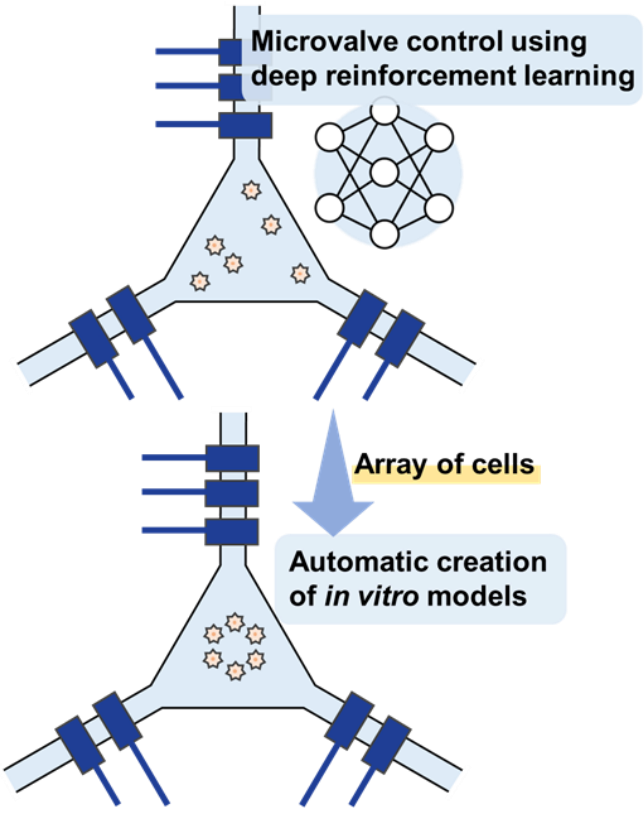

Figure 1. Concept of autonomous *in vitro* model construction.

However, the material of this device is poly-dimethylsiloxane (PDMS), and the properties of the cell-adhesive surface differ from those of common base materials, such as polystyrene of cell culture dish. In this paper, we report on the fabrication of a dish-attached microdevice that allows the selection of the substrate material to be adhered to according to the cells, and its application to the manipulation of single cell on a two-dimensional plane.

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II. Fabrication of Dish-Attached Microchip

The device has a three-layer structure, comprising a channel layer, a pneumatic layer and a roof layer (Fig. 2(a)). Channel layer has microfluidic channels for cell manupulation, and pneumatic layer has the pneumatic cavity to apply pressure for valve closure. Roof layer has channel networks serves to connect the pneumatic cavity to the external pressure source. The plane view of channel design of the device is shown in Fig. 2(b).

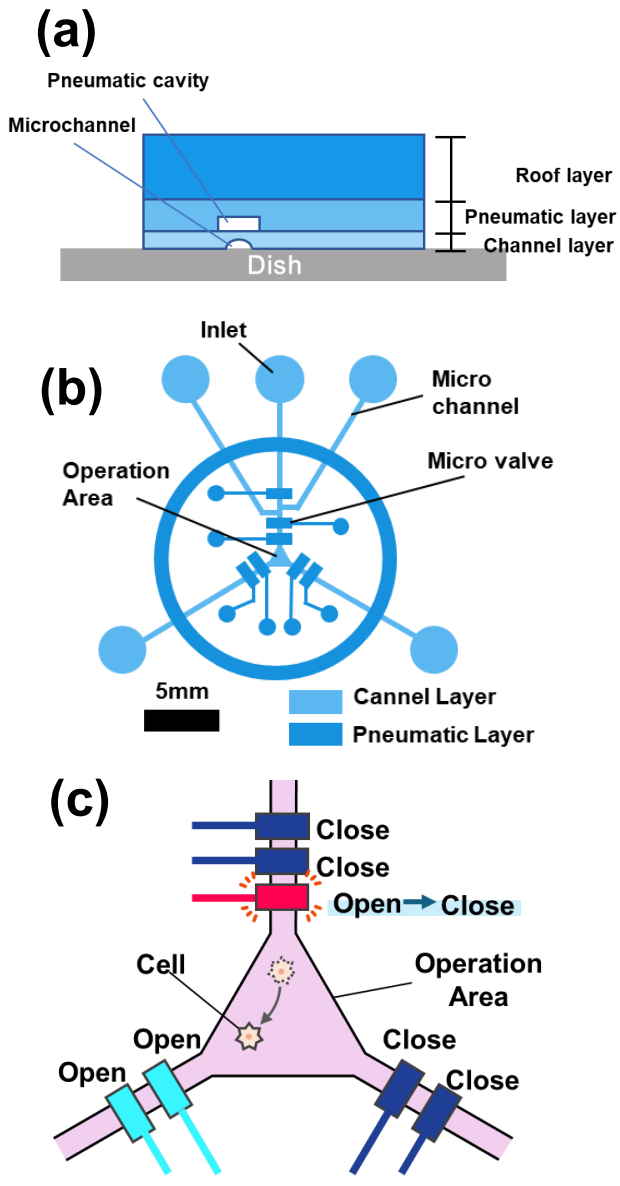

Figure 2. Microfluidic chip for single cell manipulation. a) Structure of microfluidic chip layers. b) Main channel structure of a microfluidic chip. c) Operating principle of single cell manipulation in the manipulation area.

The device consists of a central manipulation area, a flow channel leading to the manipulation area and microvalves that open and close the channel. Manipulation of cells in the channels uses convection currents caused by transitions between the open and closed states of the microvalves, as shown in Fig. 2(c). The valves are integrated in three directions, and the position of the cells is controlled by allowing fluid to flow in and out. Multiple valves were mounted in each microfluidic channel to lead cells into the manipulation area and improve manipulation efficiency. Figure 3 shows the flow path structure near the microvalves. Applying air pressure to the microvalves in the pneumatic layer deforms the valve part of the channel layer, blocking the flow path. For this reason, the mould of the channel layer was fabricated to have both a semi-circular structure to improve sealing as a valve and a rectangular structure to allow smooth passage of cells. For the rectangular structure, negative-tone photoresist SU-8 3025, (Nippon Kayaku Co., Ltd., Japan), which is generally used to make molds for microfluidic chips, was used. For the semi-circular structure, thermoplastic positive-tone photoresist AZ P4903 (MicroChemicals GmbH) was used, and by reflowing, a semi-circular cross-sectional shape suitable for valves was achieved (Fig. 4). After mold fabrication, chip were fabricated by soft lithography. The channel layer was made by spin-coating PDMS (SYLGARD 184, The Dow Chemical Co., USA) onto the mold to create a thin layer. For the pneumatic and roof layers, silicone rubber sheets were attached to the mold as spacers, into which PDMS was poured to adjust layer thickness. The three layers were then joined by vacuum plasma treatment.

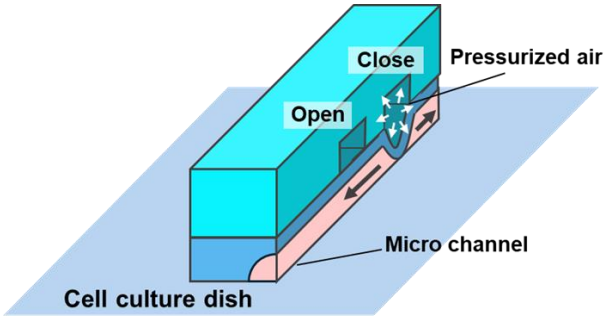

Figure 3. Micropump operating mechanism.

III. Development of Simulator Models and Training of Behavioral Decision Models.

Action decision models (agents) that autonomously manipulate cells are trained by machine learning. Training requires a huge amount of trial and errors. For this reason, a simulator representing the behavior of the device was developed and a fast learning system was constructed. The simulator model for predicting particle positions was trained using supervised learning of a neural network. Figure 5 shows an overview of the learning system for the simulator model. The computer generates flow in the operating area by randomly varying the opening and closing of seven micro-valves. The operating area is photographed by a camera and changes in particle position are obtained by image processing. The valve changes and particle positions thus obtained were used as a dataset for training. The action decision model was trained using a learning method based on deep reinforcement learning. Beads and target areas were placed at random locations in a device constructed virtually on the simulator model. The beads

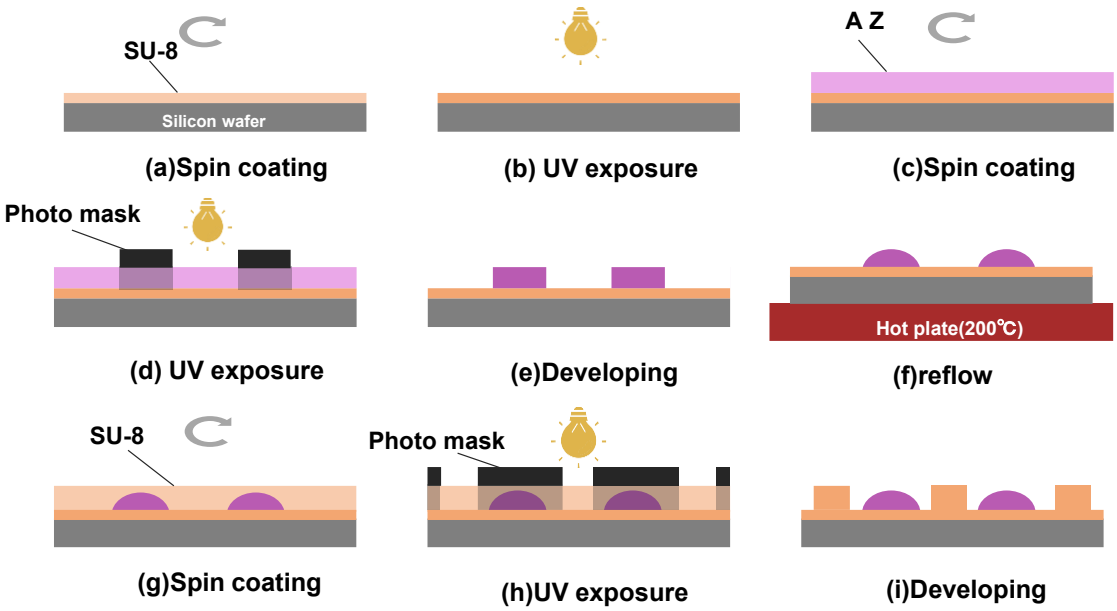

Figure 4. Fabrication process of hybrid mould of resist.
a-b) Fabrication of a layer of SU-8 as a base for AZ. c-f) Fabrication of semi-circular channels with AZ.
g-i) Fabrication of rectangular channels with SU-8.

were placed as a single point and the target area was a single circular region of Φ10μm.The agent operates a virtual valve, which causes the beads to move based on the simulator's predictions. The beads are then manipulated to move them to the target area, and the agent learns through trial and error over many episodes. The reward function during learning was defined as rewarded when the beads reached the target area, and the episode was defined as successful.

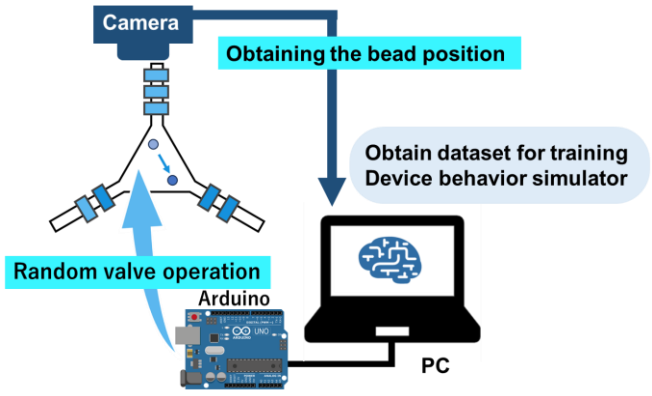

Figure 5. Overview of the simulator model learning system.

IV. RESULT

Figure 6(a) shows the fabricated microfluidic chip; the use of two types of resist and the reflow process enabled the fabrication of a chip with rectangular and semicircular portions within a single microfluidic channel, as shown in Fig. 6(b), with both valve sealing and cell passage properties. Figure 7 shows an image capturing the moment of valve operation. When the control signal was input, the diaphragm was

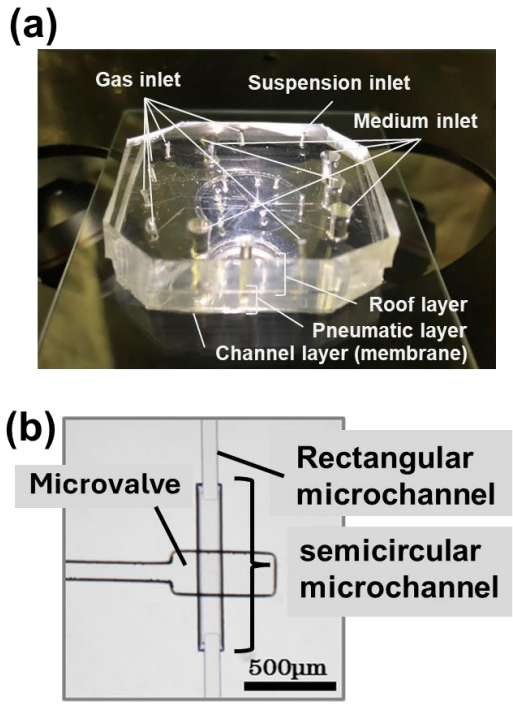

Figure 6. Photograph of a microfluidic chip.
a) Appearance of microfluidic chip b)Flow channel structure of the microvalve section.

completely closed in 70 ms. Similarly, the opening of the valve was completed in 70 ms. This delay is thought to be due to the solenoid valve.Since the valve normally operates at 2 to 3 Hz, it is unlikely that the convection flow is uniquely affected by the combination of the back and forth valve operation. To confirm the closure of the valve, the channel was filled with 1

mol/L fluorescein and the valve was closed by applying an air pressure of 45 kPa at the gas inlet. As shown in Fig. 8, the valve was confirmed to be completely closed by the diaphragm.This chip was used for sampling for simulator model building. Valve operation was stable and no detachment from the substrate was observed. Figure 9 compares the measured and predicted values when trained from 49,500 dataset. The mean square error of the predictions over the measured values for this model was 2 to 3 µm. This confirms that sampling was performed without problems and that we were able to obtain a dataset that could be used to train the simulator model. Figure 10 shows the success rate for each 100 episodes in the training simulator as a moving average. The success rate increases as learning progresses. The results show that the agent has learnt the appropriate valve manipulation task after 200,000 learning episodes. The model, which was well trained on the simulator, was applied to the real environment and manipulated cells in the system shown in Fig. 11. As a result, the learned model was able to properly manipulate the valves and transport the cells to the specified target position as shown in Fig. 12. Manipulation took an average of 10.6 seconds, and the average number of steps was 21.3. In this study, we demonstrated the manipulation of a single cell using deep reinforcement learning. This device does not achieve simultaneous manipulation of multiple cells. However, we believe that by adjusting the reward system based on the specific task, a versatile microfluidic platform can be developed that allows various cell manipulations with a single chip.

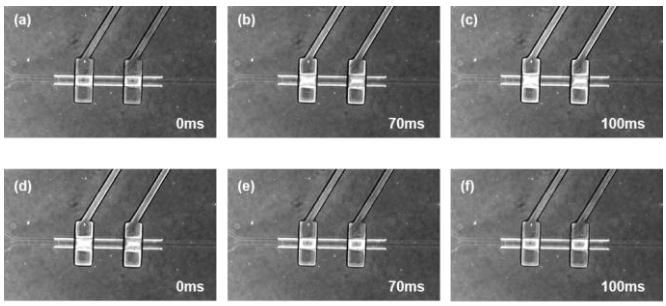

Figure 7: Sequential photographs of diaphragm operation (a-c) Valve closure (d-f) Valve open

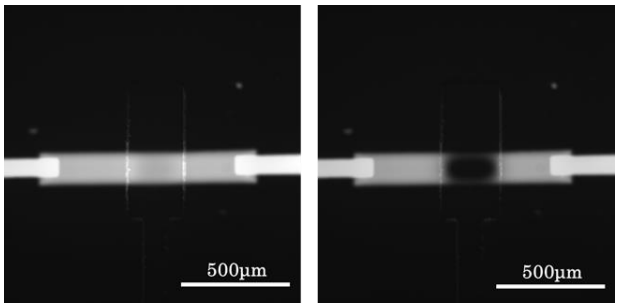

Figure 8. Fluorescence observation of valves

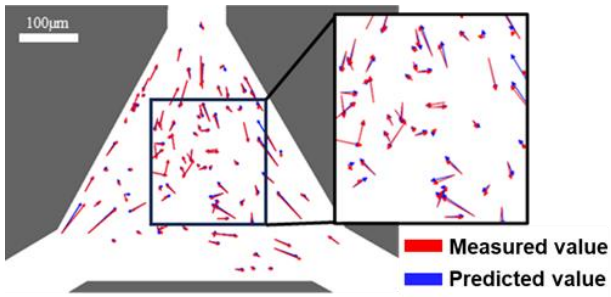

Figure 9. Comparison between simulator predictions and actual measurements

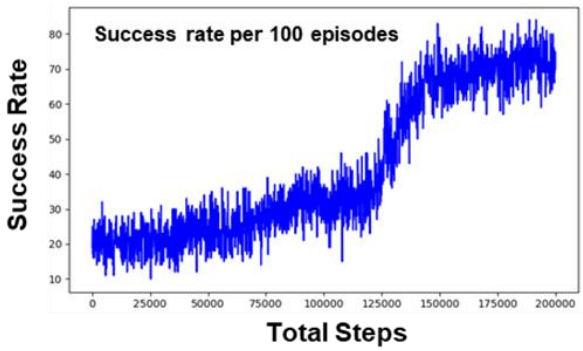

Figure 10. Success rate per 100 episodes over 200,000 training sessions

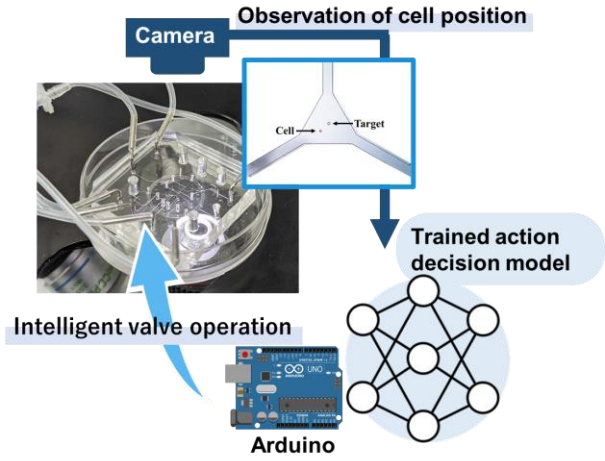

Figure 11. Manipulation system of cells with trained models

V. CONCLUSIONS

In this paper, we demonstrate the fabrication of a microfluidic chip that can be mounted on a typical cell culture dish and the application of reinforcement learning to a micropump integrated on the chip. The microchip was fabricated in a structure that can be directly attached to a cell culture dish. By using two types of photoresists, negative and

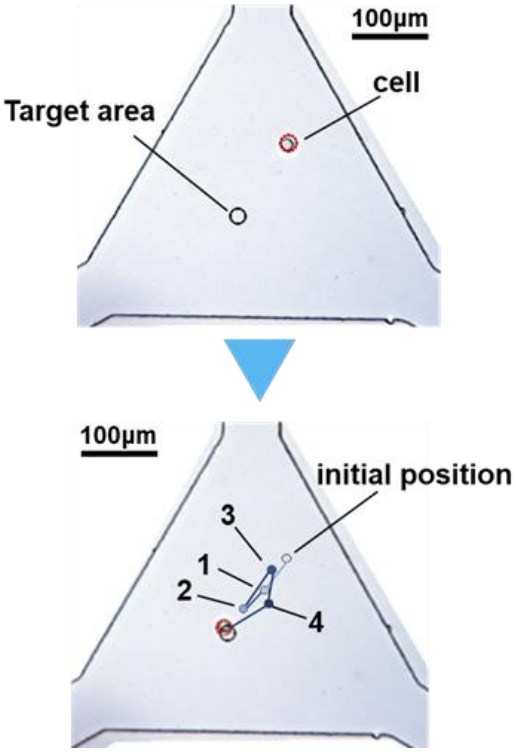

Figure 10. Single cell manipulation results

positive, the microchip has rectangular and semi-circular channel structures. This is considered to be advantageous in that it improves the passage of cells and the sealing property of the valve. Next, we constructed a system for manipulating cells in a two-dimensional plane using this microchip. In this system, particles are manipulated using fluid convection by microvalves mounted in the flow path. Appropriate valve manipulation is learned autonomously by the agent through deep reinforcement learning. To simulate the particle manipulation task, a simulator that reproduces the behavior of the device was trained based on supervised learning. The simulator was then used to train the agent. Training was rewarded to the agent by transporting particles to an arbitrary target area. By applying the trained agent to a real environment, the agent was able to perform single cell manipulations and transport them to the target area. In the future, this technology is expected to be applied as a platform for analysis of cellular networks through automated creation of *in vitro* models.

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