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Untethered Micro-Robot with Gripping Mechanism for On-Chip Cell Surgery Utilizing Outer Magnetic Force

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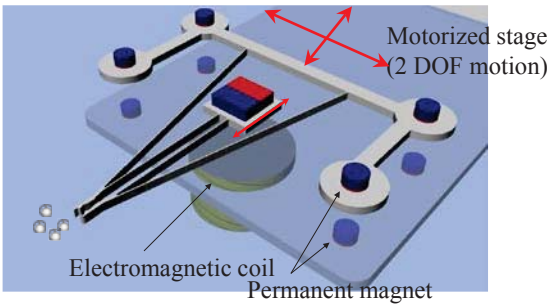
***Abstract* - We have developed a highly functional untethered micro-robot that can manipulate cells with high gripping force in a micro-fluidic chip. The robot has gripping mechanism for grasping and transportation of cells with sufficient power. A permanent magnet is attached at the center of the gripping mechanism, and an electrical magnet controls the position of the magnet from the bottom of the micro-fluidic chip. The distance accuracy of the gripper is 3.0 μm, and it can activate about 50 Hz. The robot has four permanent magnets for positioning, and other four permanent magnets control the robot from the bottom of the micro-fluidic chip. The robot is made very thin and small to allow maneuverability in a micro-fluidic chip. We succeeded in high-power handling and cutting of a cell using this micro-robot.**

I. INTRODUCTION

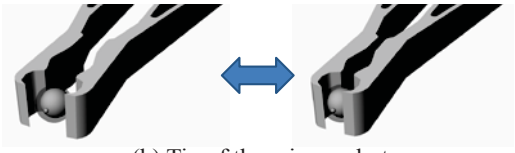
In the field of bio-manipulation, especially cloning or chimera techniques, high-accuracy manipulation (within a few micrometers) is required when investigating a single cell under a microscope, for high-throughput micro-manipulation. On-chip robots have higher accuracy and repeatability compared to manual operation, which is affected by individual variations in skill [1-3]. Magnetic forces are excellent driving forces for controlling micro-robots because of their non-contact nature, minimal invasiveness with respect to a cell, and low production cost. Thus, a considerable amount of research has been carried out on magnetic actuators [4-14].

In a previous study, we developed a Magnetically-driven Micro-Tool (MMT) for cell manipulation, cell loading, cell sorting and droplet generation. [12-15]. The micro-tools are controlled by applied magnetic fields. However, only the on-off control actuation method was carried out and positioning accuracy was not required. Pairs of Helmholtz coils have been used in some studies to achieve positioning control [16]; however, the applied force by an electromagnetic coil is not sufficiently strong to manipulate a relatively large cell such as an oocyte. On the other hand, a permanent magnet has a magnetic field more than ten times as strong as an electromagnetic coil driven by an MMT [17][18]. Our research group proposed MMTs with a minimum resolution of 1.1 μm and response of 0.02 s [19][20]. Using such an MMT, we succeeded in the manipulation of cells [21].

An MMT can rotate, transport and hold a single cell precisely, however in the case of two MMTs, close cooperation between them is needed. Moreover, the holding force is not strong. To solve these problems, we propose a new functional micro-robot incorporating a suction mechanism. The suction mechanism of the micro-robot is activated by magnetic force [22][23]. The aspiration force is enough to fix a cell, however it is difficult to capture a high stiffness cell or micro-object. To solve this problem, we propose a micro-robot which has gripping mechanism. Figure 1 shows the concept of the micro-robot. Normally, mechanical manipulator is not suitable for handling of micro-scale object because of its turbulence. The volume of the on-chip micro-robot, however, is small, so the turbulence is reduced. The micro-robot was made by silicon because of its machining performance, mechanical characteristic and biomedical compatibility. A permanent magnet is attached at the center of



(a) Over view of the micro-robot with gripping mechanism



(b) Tip of the micro-robot

Fig.1 Concept of an on-chip micro-robot with gripping mechanism. A permanent magnet is attached at the center of the gripping mechanism, and an electrical magnet controls the position of the magnet from the bottom of the micro-fluidic chip. The robot has four permanent magnets for positioning, and other four permanent magnets control the robot from the bottom of the micro-fluidic chip. The robot is made very thin and small to allow maneuverability in a micro-fluidic chip.

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the gripping mechanism, and an electrical magnet controls the position of the magnet from the bottom of the micro-fluidic chip. The robot has four permanent magnets for positioning, and other four permanent magnets control the robot from the bottom of the micro-fluidic chip. The robot is made very thin and small to allow maneuverability in a micro-fluidic chip. We use this robot for on-chip cell surgery. In this paper, we designed the gripping mechanism, fabricated the micro-robot, evaluated the gripping mechanism, and performed cell surgery.

II. Design

The conceptual view of micro-robot is shown in figure 1. The micro-robot is actuated by the external magnetic force with the aim of the noncontact drive with high output force. Moreover, four driving points of permanent magnets are arranged in a micro-robot for the purpose of the 2 DOF actuation. These magnets are driven by the external magnetic force of magnets on the motorized stage. In order to achieve the additional 1 DOF gripping motion, a horizontal arranged permanent magnet is assembled center of the micro-robot. The magnet is driven by the magnet filed of the electromagnetic coil placed on the motorized 2 DOF motion in a glass plate, and pick up one by one by gripping motion.

2.2.1 Design of beam of gripping mechanism

The gripping mechanism is achieved using deformation of beams because it is difficult to achieve an ideal microscale hinge and link structure.

Fig. 2 (a) shows the deformation model of the gripping mechanism when the driving force F is applied to the horizontal arranged permanent magnet. First, in order to obtain the gripper's tip deformation δ_x, δ_y caused by applied force F, the gripping mechanism is considered as the half of the frame structure. The point A are tip of the gripper and the point B is driven by the magnetic force, as shown in Fig. 2(b). Where F_x, F_y, and M_A are the virtual load along the X axis for the point A, the virtual load along the Y axis for the point B, the moment around point B, respectively. Here, the structure of the micro-robot is considered as an elastic material, the elastic strain energy U is expressed by the Eq. 1 under the assumption that the deformation due to compression and the torsion of each beams are ignored.

$$\boldsymbol{U} = U_1 + U_2 \qquad (1)$$

$$U_1 = \int_0^{l_1} (M_{AB})^2 dr_1,\ U_2 = \int_0^{l_2} (M_{BC})^2 dr_2 \qquad (2)$$

Where r, l are divided segment along the beams, and the total length of the beams.The gripper's tip deformation δ_x, δ_y are obtained as follows:

$$M_{AB} = (F_x + F/2\sin\theta - f\cos\theta) r_1 + f l_2 - M \qquad (3)$$

$$M_{BC} = -M + f r_2 \qquad (4)$$

$$\left(\frac{\partial U}{\partial M}\right)_{F_x=0} = 0,\ \left(\frac{\partial U}{\partial f}\right)_{F_x=0} = 0. \qquad (5)$$

$$\delta = \frac{\partial U}{\partial F_x},\ \delta_x = \delta\cos\theta,\ \delta_y = \delta\sin\theta \qquad (6)$$

$$\delta = \frac{l_1^3 l_2^3 (l_1 + l_2)\sin\theta F}{3EI\left(l_1^4\cos^2\theta + 4l_1^3 l_2\cos^2\theta - 6l_1^2 l_2^2\cos\theta + 4l_1 l_2^3 + 4l_2^4\right)} \qquad (7)$$

When, the shape of gripper is designed as a linear shape expressed in Eq. 3, the gripper's tip deformation δ_x, δ_y is calculated as Eq. 6 and Eq. 7.

Where, θ, E, I are the angle between the beam and the wall, Young's module of the frame, and the second moment of beam.

Figure 3 shows the graph of the δ, δ_x, and δ_y. For manual handling of the robot by using tweezers, we decided the angle was 13 deg. Then, we need enough length of l_1 and l_2 because the light of the microscope through from bottom of the micro-robot and the electrical magnet interrupts the light. We designed the micro-robot which the l_1 is 6.5 mm and l_2 is 3.3 mm.

In this study, our target cell is oocyte which the size is 150 μm for cloning. So the distance between the tip of the gripping mechanism is decided as 160 μm. To close the tip of the gripper perfectly, the displace δ is needed over 80 μm. From the graph of figure 5, when the angle is 13 deg, l_1 is 6.5 mm and l_2 is 3.3 mm, the δ is over 80 μm.

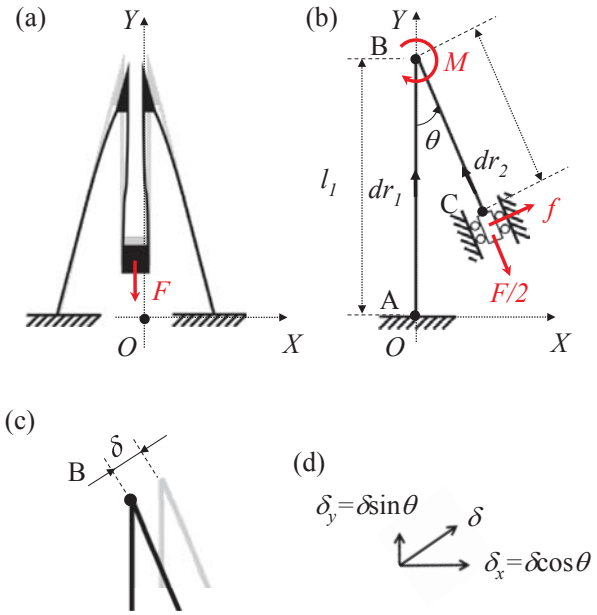


Fig. 2 Analytical model of gripping mechanism. (a) model of deformation, (b) half of frame model of gripping mechanism, (c) enlarged view around point B, and (d) definition of δ_x and δ_y.

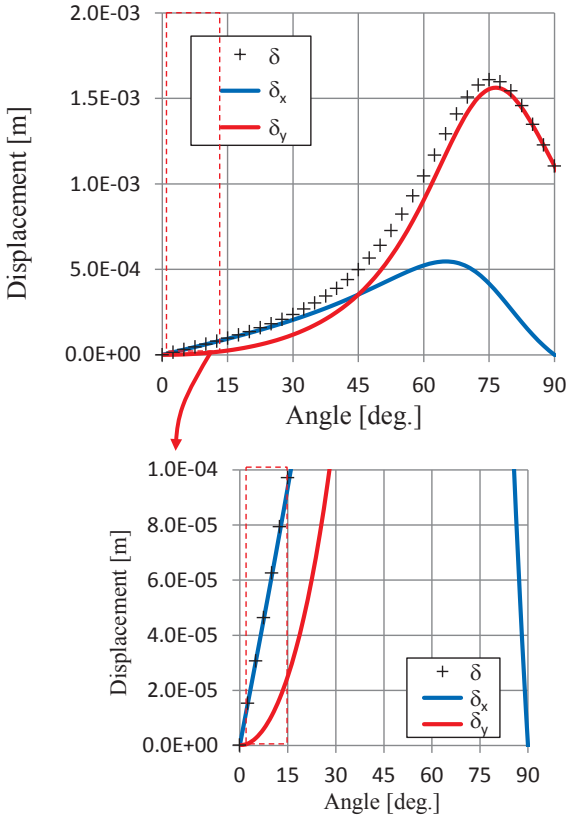
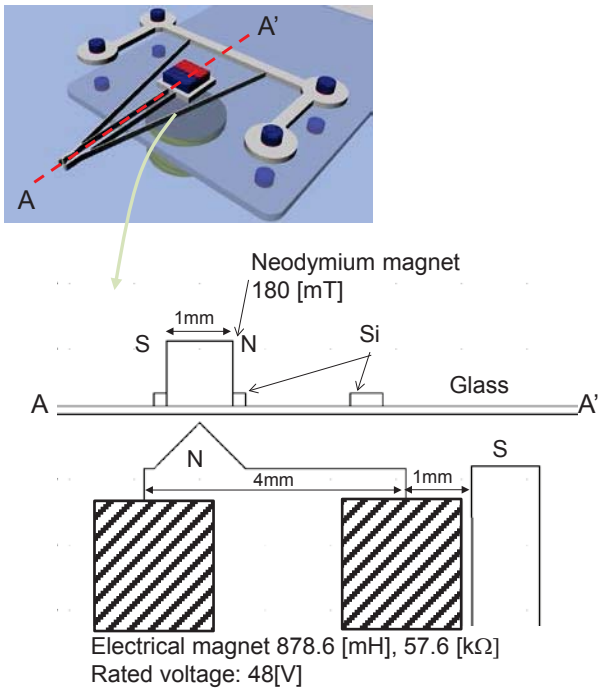
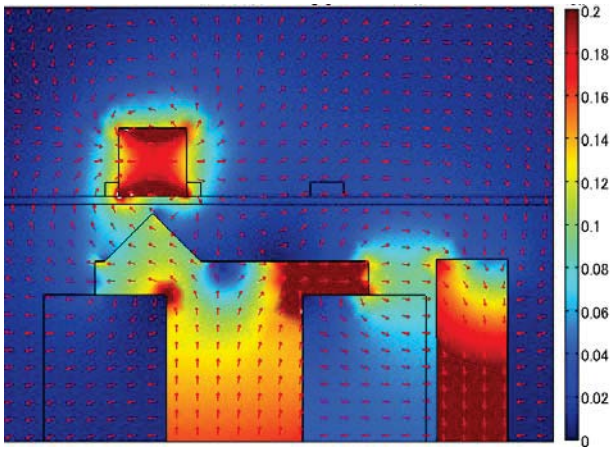


Fig.3 Graph of the δ, δ_x, and δ_y. The upper graph is whole of the graph and the bottom one is the magnification. When the angle is 13 deg, l_1 is 6.5 mm and l_2 is 3.3 mm, the δ is over 80 μm.



(a) Schematic diagram of magnetic flux analysis



(b) Result of the analysis of magnetic analysis

Fig.4 Magnetic flux analysis. (a) The schematic digagram is the cross section of the center of the micro-robot and the electrical magnet. (b) The direction of the magnetic flux around the permanent magnet and the electrical magnet is horizontally.

2.2 Design of magnetic flux of gripping mechanism

The gripping mechanism employs a neodymium magnet and electrical magnet. The neodymium magnet is strong magnet and low cost. We use electrical magnet because the magnetic power can control by the input voltage of the electrical magnet. The size of the permanent magnet 1.0 mm × 1.0 mm × 1.0 mm, and the magnetic power is 180 mT (measured). We use electrical magnet which the inductance is 878.6 mH, resistance is 57.6 Ω was, and the rated voltage is 48.0 V. We analyze the magnetic field using finite element method (FEM). We use ANSYS4.0 (ANSYS co., ltd) as the analytical software.

Figure 4 shows the schematic diagram of the analytical model and result of the magnetic field. The input voltage to the electrical magnet is 25.0 V. We select the electrical magnet which has yoke. To concentrate the magnetic flux, we add an iron needle under the permanent magnet.

We need that the permanent magnet is moved horizontal direction, so we put the permanent magnet horizontally between the electrical magnet. The result of the analysis shows that the direction of the magnetic flux around the permanent magnet and the electrical magnet is horizontally.

III. Fabrication

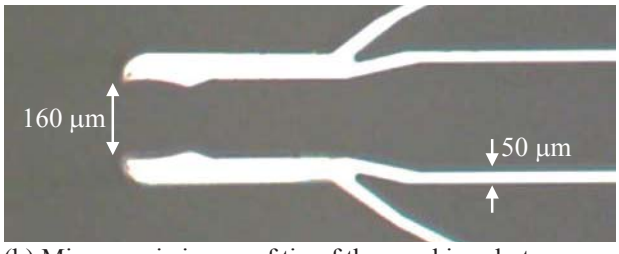
The fabrication process of silicon based micro-griper is shown in Fig. 5. At first, the photoresist OFPR (Tokyo Ohka Kogyo Co.) was coated on the silicon substrate. After the exposure on the OFPR side, the OFPR pattern was developed. Next, deep reactive-ion etching was conducted from the OFPR side, and silicon is etched to a depth of 200 μm until the other side of the silicon substrate. Then acid treatment was conducted to clean away the photoresist OFPR. Finally, in the

center of the micro-griper, a size of 1.0 mm × 1.0 mm × 1.0 mm cube shape of permanent magnet was assembled inside of the square cavity, by control the movement of the magnet the closing of the micro-griper was well achieved. In order to actuate the micro-griper moving in X-Y plane, fourφ 0.5 mm × 0.5 mm permanent magnets are symmetrically assembled inside of the silicon MMT on both sides of the micro-griper.

Figure 6 shows the fabricated micro-robot. The micro-robot is assembled the permanent magnets. In this study, we use this gripper for handling of oocyte, so we design the tip shape of the gripping mechanism as experimentally.

Fig.5 Fabrication process of on-chip robot.

(a) On-chip robot which was assembled neodymium magnets.



(b) Microscopic image of tip of the on-chip robot.

Fig.6 Over view and microscopic image of fabricated on-chip robot.

IV. System

Figure 7 shows the system components of our platform, including a linear stage for the micro-griper actuation in X-Y plane, a microscope with a CCD camera, a joystick for stage control, an electrical magnet with power supply connected to control the closing of griper, a micro fluidic chip. The microscope with CCD camera sends the captured image data to the PC and the stage movement is controlled by joysticks. By changing the power supply, position of the center permanent magnet is controllable, hence, the magnitude of the griper is accordingly control. Therefore, size of oocyte with little bit differences could be captured and delivered inside of the microfluidic chamber. The position of the micro-gripper is controlled by the eight magnets, four magnets are attached the corners of micro-gripper and other four magnets are attached to the X-Y stage.

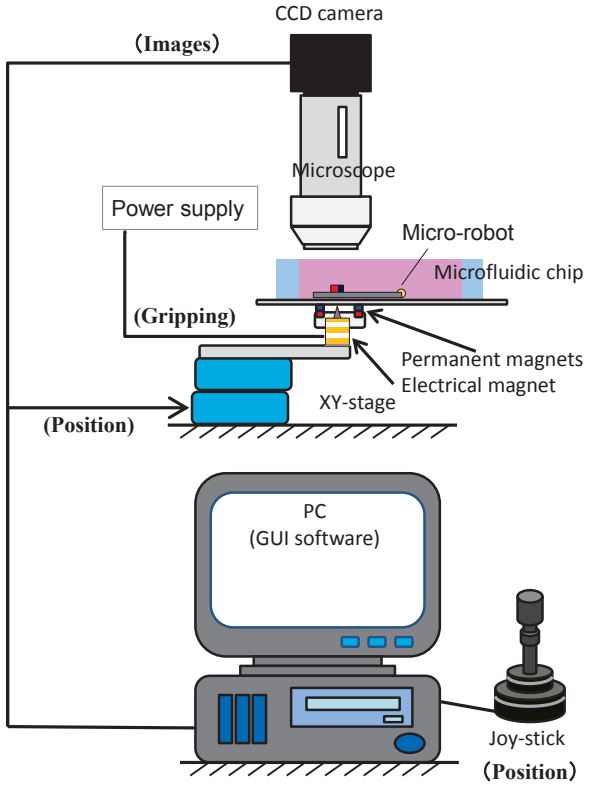


Fig.7 System Schematic diagram of the system. This system is consisted by a linear stage for the micro-robot actuation in X-Y plane, a microscope with a CCD camera, a joystick for stage control, an electrical magnet with power supply connected to control the closing of griper, a micro-fluidic chip.

V. Evaluation of Gripping Mechanism

We measured displacement of the tip of the micro-robot and frequency characteristic. Figure 8 shows the measurement result between the applied voltage of the electrical magnet and displacement of the tip of the gripping

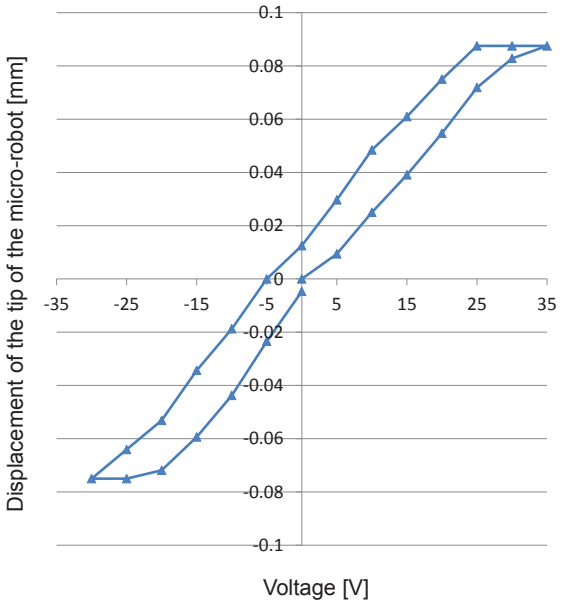
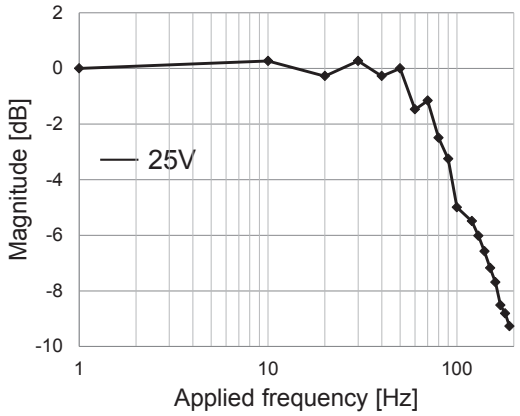
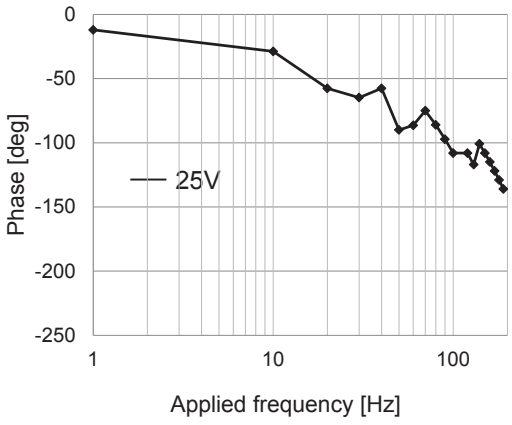


Fig.8 Measurement result between applied voltage of electrical magnet and displacement of the tip of the micro-robot.



(a) Magnitude



(b) Phase

Fig.9 Dynamic characteristics of the gripping mechanism. The magnitude is declined drastically over 50 Hz. The phase is gradually declined.

mechanism. When the voltage is 35.0 V, the gripping mechanism was closed perfectly. The distance accuracy of the gripper is 3.0 μm When we applied negative voltage, the griper was opened. The graph has a hysteresis property. The causes of the hysteresis are friction between the bottom of the gripping mechanism and surface of the glass plate of the micro-fluidic chip, and property of the silicon beam.

Figure 9 shows the dynamic characteristics of the gripping mechanism. We applied frequency from 0 V to 25.0 V to the electrical magnet. We measure the displacement of the tip of the mechanism. The magnitude is declined drastically over 50 Hz. The phase is gradually declined.

VI. Experiment

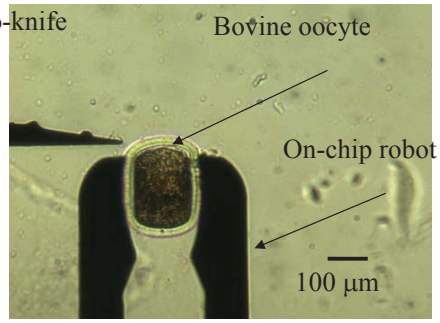
The enucleation of mammalian cells is very important especially for cell cloning technology or stem cell studies. In general, those fields are very hard for researcher to overcome difficulties of many steps of cloning and very limited people easily work on these fields. So far, high efficient control of oocyte orientation is still a hard topic, and is being researched, however our griper shows great capability on oocyte enucleation process. Figure 10 shows the experimental results of potential viable bovine oocyte enucleation process. The oocyte inserted from the inlet flowed to the micro-chamber where the oocyte enucleation be performed here. Oocyte was precisely caught by micro griper, letting the zona pellucida of oocyte revealed a little bit (Fig. 10 (a)). In order to cut a small hole on the oocyte, oocyte was hold and moved toward to the micro-knife (Fig. 10 (b)). A narrow incision was cut by micro-knife, therefore nucleus could have enough space to be squeezed out. In the end the oocyte was released, all the processes are shown in Fig. 10 (c) – Fig.10 (d). From Fig. 11(a) we can see the enucleated oocyte, which is spherical, showing that cell membrane of enucleated cell is remained intact, zona pellucida was still in well shape protected the cytoplast in a good manner.

We performed the enucleation of oocyte(from Ibaraki Meet Center, NILGS). We investigated the viability of oocyte after cutting process manipulated by micro-robot. The enucleated oocytes were cultured in 10% FBS with M199 with 6DMAP for 4 hours, and then transplanted a cloned nucleus and cultured for 5 days. In the end oocytes developed normally to the morula, which could be observed clearly from Fig. 11 (b).

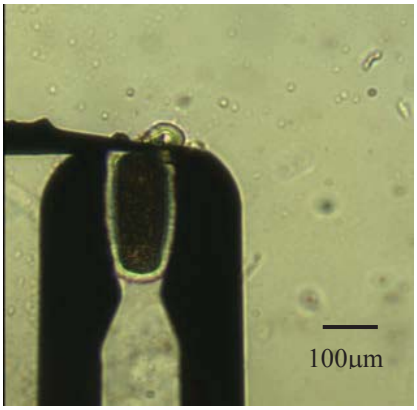
VII. Conclusion

We proposed a highly functional untethered micro-robot that can manipulate cells with high gripping force in a micro-fluidic chip. The robot has gripping mechanism for grasping and transportation of cells with sufficient power. A permanent magnet is attached at the center of the gripping mechanism, and an electrical magnet controls the position of the magnet from the bottom of the micro-fluidic chip. The distance accuracy of the gripper is 3.0 μm, and it can activate about 50 Hz. We made the micro-robot measured the property

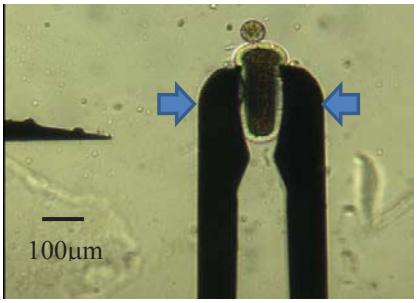
of the gripping mechanism. Finally, we succeeded in high-power handling and cutting, and enucleation of bovine oocytes for cloning method. In near future, we will realize more precise position control measurement of gripping force and control of the gripping force.



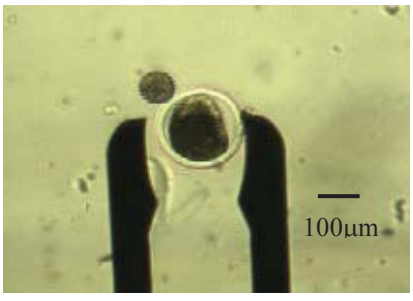
(a) Holding of oocyte



(b) Cutting

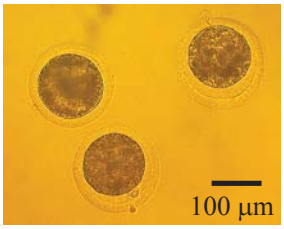


(c) Pushing

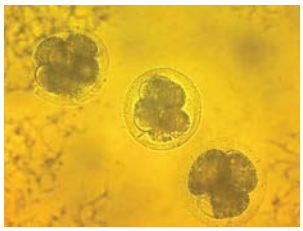


(d) After pushing

Fig.10 Result of enucleation process.



(a) Enucleated oocytes



(b) formation of morula

Fig.11 Growth test of the enucleated oocytes.

ACKNOWLEDGMENT

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