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On-chip Cell Manipulation by Magnetically Modified Soft Microactuators

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***Abstract*—We have developed magnetically modified polymeric soft microactuators in microchip as replacement of conventional manual cell manipulations such as pipetting and centrifugation. In the past papers [1] we have reported novel magnetically driven polymeric microtools for non-intrusive and no contamination experiments on a chip. The main features of them were 1. fabrication of any shape, 2. soft (harmless to cellls) 3. no stiction and 4. mass production at low cost enabling disposability. For the current research, we have demonstrated manipulation of oocyte with produced novel soft microactuators. These magnetic microtools provide a number of functions such as microvalve and microrotor. The potential impact of this technology includes sample preparations, selection and separation, loading and immobilization, genetic operation, tracking, mixing and reaction techniques into portable microfluidic labs-on-a-chip, culture systems.**

I. Introduction

There is great interest in developing the microactuation mechanics in microchip as replacement of conventional cell manipulation. However, many of the preliminary sample preparation such as pipetting, mixing, sample selection and centrifuge have been carried out off chip. It is crucial to handle cells gently and precisely for scientific research and clinical diagnostic applications.

The most popular microactuators that can be applied in the confined space of microchannels are electrostatic microactuators, optical tweezers [2], and magnetic microactuators [3] [4]. The Coulomb force has been often used in manipulating cells of the order of 10 μm, whereas it is necessary to apply a high voltage to manipulate particles of the order of 100 μm, which risks damaging cells by heat generation. The dielectrophoretic force can be adjusted by varying the squared value of the gradient of the electrical field; however, it is controllable only in the limited region adjacent to the electrodes, and requires higher voltages to sort larger objects. Optical tweezers can manipulate cells indirectly by non-contact actuation of microtools, thus reducing the risk of damaging cells during manipulation; however, the generated force is of the order of several pN, which is not suitable for manipulating cells of the order of 100 μm. On the other hand, the magnetic sorting offers a limited risk of cell contamination, and it has been used in many studies because of its low cost [5]-[16].

Conventionally magnetic actuation has been produced by metal or metal membranes, whereas miniaturized hard magnetic materials have not been used until recently due to difficulties in micromachining [17]. The use of magnetic powder and PDMS composites has been applied to local actuation in microchannels that are easily fabricated. Some works on actuation features polymer-bonded magnets on top of a PDMS membrane [18] or ferrofluidic plugs in microfluidic channels [19]. Elastomeric membranes made of a mixture of Silicon polymers and magnetic particles have been used as valves and pumps on biochips [9, 20], but the control of magnetic membranes on the surface of microchannel constrains the area and direction of movement of actuation in microchannels, while raising the potential requirement or flexible control of actuation on biochips.

We have proposed on-chip PDMS-based versatile magnetically driven microtools be produced using photolithography. We use iron oxides (Magnetite, Fe_3O_4) nanoparticle mixed with PDMS. These *magnetically driven microtools* (**MMT**) provide a number of functions such as microrotor, microvalve and microsorter on chip for cell manipulations.

II. Experiments

A. Fabrication Magnetic Soft Microdevices

Figure 1 shows a fabrication process of MMT. The detailed description was described in the past study [1] and which may be summarized as follows: the first (Fig.1(a)) uses thick KMPR-1050 photoresist (Kayaku MicroChem CO., Ltd), and a resist mold for MMTs fabricated by patterning the resist layer on a silicon substrate (resist height = 150 μm)(Fig.1①). The PDMS-magnetite composite is molded into a designed configurations of MMTs and baked at 80℃ for 20 min to cure the composite (Fig.1②). The patterned substrate is then put in a stripper bath (Remover PG at 70℃) with a large

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commercial stirrer made of a permanent magnet that keeps the temperature of the stripper liquid steady (Fig.1③).

KMPR resist is dissolved by the stripper liquid, leaving a gap between the KMPR resist and PDMS-magnetite composite. With dissolving the KMPR resist, the large stirrer can collects a number of patterned PDMS-magnetite composite automatically due to the magnetism of composite (Fig.1③&④). The surface of MMTs are Teflon coated by CF_4 plasma for 20 min after the process of ④ to avoid any stiction in microchip. Figure 2 shows the SEM photos of the produced soft MMTs. The thickness of them were approximately 80 μm. For the simple installations of the MMTs on chip, a single post was mounted on the microchannels in order to fit the ring of MMTs as shown in the Figures 2 (c), (d), (f).

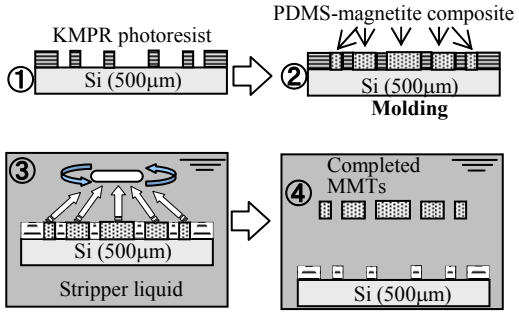


Fig. 1. Fabrication processes of MMT.

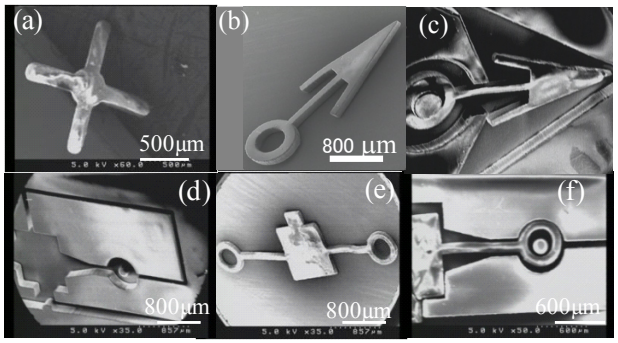
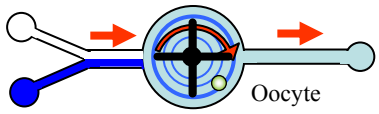


Fig. 2. SEM of MMTs. (a): Microrotor, (b): microsorter, (c) microsorter installed in the microchannel, (d): Patterned PDMS microchannel with post, (e) Microvalve, (f) Microvalve installed in the microchannel.

B. *Actuation of MMT*

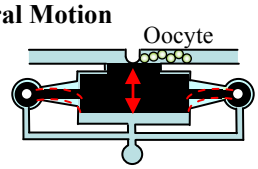
The produced soft MMT provide a number of functions such as microrotor, microvalve and microsorter and so on. Figure 4 shows the concept of the actuation of soft MMTs. Figure 4(a) describes the rotating motion of the MMT applied to microrotor, or microcleaner of cells which are surrounded by debris. Figure 4(b) shows a lateral motion of the MMT applied to use valves for two-phase flow, and which can control the transportation of the cell in microchannel. A couple of arms which are attached on the both side of MMT stabilize the lateral motion of MMT effectively due to the single-degree-of-freedom of MMT. Figure 4(c) describes the deformation model applied to microsorter or microvalve. By stretching suspended fine part of MMT, the positioning of the MMT is controlled to block the microchannel to make a valve due to the elastic nature of the PDMS composite. This sorting function is useful for cell manipulation, for example sorting halved oocytes with and without nuclei—an important technology for cloning—should be attempted.

(a) Rotation



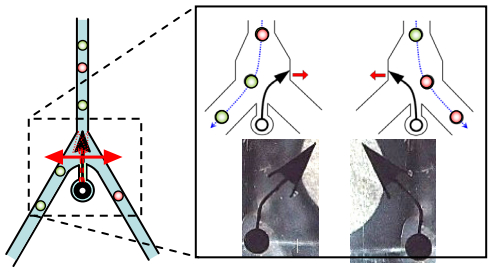
[ex.] Microrotor

(b) Lateral Motion



[ex].Microvalve

(c) Deformation Model



[ex.] Microsorter

Fig.3. Multiple actuation using MMTs. (a) Rotation. (b) Lateral Motion. (c) Deformation.

C. *Amplified Magnetic Microactuation*

Figure 4 shows independent modules of microchannels and magnetic actuation [1]. These are combined when MMTs are actuated and are disconnected when the microchannel module is replaced by a new one to avoid contamination. MMTs were installed on the microchip by tweezers. MMTs were damage-resistant in tweezer operation due to their elasticity. For the present study, the actuation module to produce lateral motion has been improved in order to obtain sufficient actuation power and low power consumption. Figure 5(a) shows the conventional configuration of an MMT [1] that consists of a microchannel module and an actuation module containing a drive unit. Figure 5(b) shows the improved design proposed in this study. The system consists of two modules—an upper module containing a disposable microchannel and a lower actuation module. In the present configuration, the actuation module is composed of a magnetic circuit unit containing an electromagnetic coil and a permanent magnet unit. The magnetic force generated by the electromagnetic coil is amplified by the permanent magnet (neodymium) unit mounted between the microchannel and

the magnetic circuit, and the MMT is moved by non-contact actuation.

Figure 6 shows the mechanism of the magnetic circuit and the permanent magnet unit in the actuation module. The direction of the current in the coil of the magnetic circuit can be switched to reverse the electromagnet's polarity, resulting in translatory motion of the permanent magnet. The magnetic force generated by the electromagnetic coil is amplified, and an adequate magnetic force (maximum = 316 mT, voltage applied to electromagnet = 1.5 V) is transmitted to actuate the MMT in the microchannel. The magnetic force for actuation was approximately 110 times that of the conventional setup without a permanent magnet. It is important to note that the MMT remains in position even after the magnetic circuit is switched off. Thus, it enables lower power consumption, because no energy is consumed in keeping the MMT fixed in the same position.

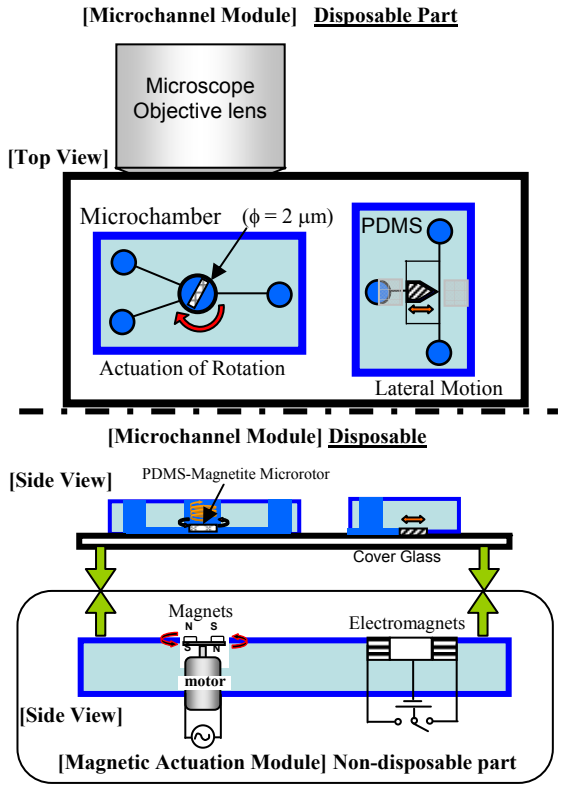


Fig.4. Experimental arrangement to create rotating and lateral movement in a magnetic microtool in a microchannel.

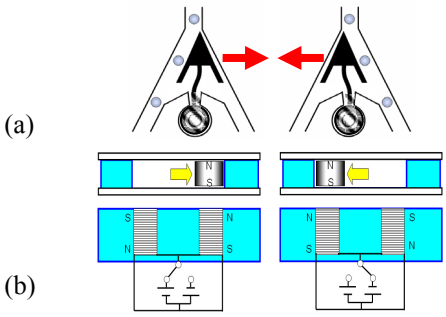


Fig.5. Actuation method of magnet.

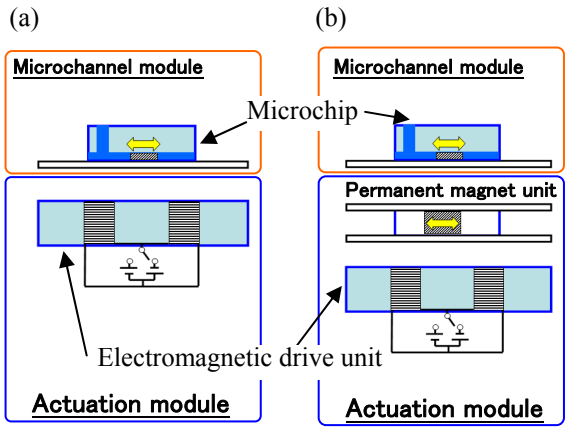


Fig6. Schematic of actuation module.

D. *Evaluations of Softness and design of MMT*

It is important to quantify the displacement and deformation of MMT by magnetic force before designing of MMT and microchannel. A simple model was adopted to evaluate the design of arrow-shaped MMT (Fig3(c)) as an representative example. The Young's Modulus of MMT was measured with a strain gauge and which was E ≈ 5 MPa. We assume that a concentrated mass m is located at the edge of a beam of length *l*. For simplicity, the MMT is approximated as a cantilever beam attached to a column at one end (one end free and the other end fixed; Fig. 7(b)), with a uniform cross-section (Fig. 7(c)). In addition, we assume that there is no flow in the microchannel, and that the viscosity of the fluid can only affect the column. The equation of motion of the MMT can be expressed as

$$m\ddot{x} + c\dot{x} + kx = u(t) \tag{1}$$

where c is the viscous damping coefficient and k is the spring coefficient. Further,

$$c = 8\pi\eta h/(0.5 - 0.558 - \ln(8/\mathrm{Re})) \tag{2}$$

where η is the coefficient of viscosity and h is the height of the column. Consequently, the transfer function for equation (1) can be expressed as

$$G(s) = \frac{X(s)}{U(s)} = \frac{1}{m}\frac{1}{s^2 + 2\varsigma\omega_n s + \omega_n^2} \tag{3}$$

$$\varsigma = c/2\sqrt{mk},\ \omega_n = \sqrt{k/m} \tag{4}$$

To determine k, we assume a concentrated mass m at the edge of the cantilever beam, as shown in Fig. 8. When the Young's modulus of the beam is E, and the section modulus is I, the deflection δx for the static load u(t)const at the edge is expressed as

$$\delta x = u(t)_{const}\left\{l^3 - (l-a)^3\right\}/3EI \quad (5)$$

where

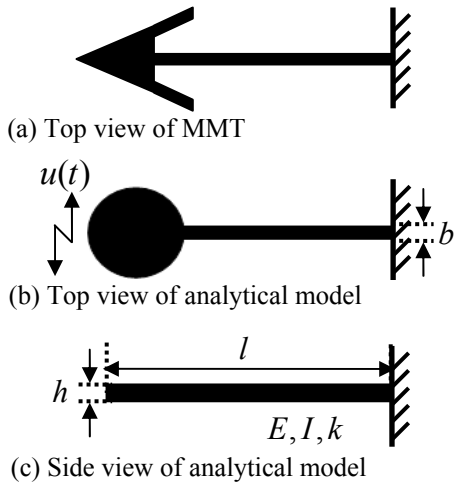
$$u(t)_{const} = k\delta x \quad (6)$$

Combining equations (5) and (6), we obtain the following equation:

$$k = 3EI/\left\{l^3 - (l-a)^3\right\} \quad (7)$$

hus, the natural frequency of the system can be expressed as

$$\omega = \sqrt{1-2\varsigma^2}\,\omega_n \quad (8)$$



(a) Top view of MMT

(b) Top view of analytical model

(c) Side view of analytical model

Fig.7. Analytical model of MMT

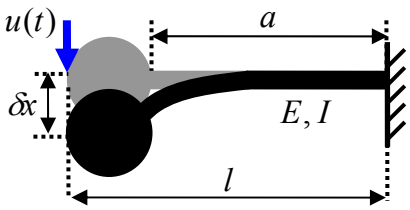


Fig. 8: Top view of analytical model for evaluating k

The length of typical microsorter MMT was 2.25 mm, *a* was 1.03 mm, and its thickness was 80 µm. The spring coefficient k was 0.02 mN/µm (equation 7), and the natural frequency was 66.0 Hz (equation 8).

Figure 9 shows the maximum magnetic force along the center line of the coil as a function of input voltage. The permanent magnetic force (316 mT) produced by an input voltage of 1.5 V was applied to the MMT. The force (3.2 mT) can be calculated from $F = B^2S/(2\mu)$, where B is the density of magnetic flux, µ is the magnetic permeability of air (1.26×10^{-6} H/m), and S is the area of the MMT. The magnetic force can displace the MMT by 159.05 µm, which is sufficient for switching in the microchannel, whose width is 150 µm, thus enabling sorting of particles. During the experiment, we confirmed that the MMT can swing across the full width of the microchannel.

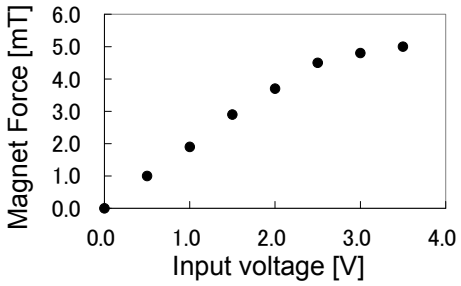


Fig. 9. Measured electromagnetic force

E. Sample Preparation

For the present research a swine oocyte were used for manipulation which were incubated in a medium (TCM199 base) for 44 hours (38ºC, 5% CO2) after the collection of follicle. Figure 10 shows a photo of swine oocyte whose size is approximately 100 µm. The oocyte is composed of black (oocyte) and transparent part (egg zona pellucida). After pipetting to remove the surrounded debris and cumulus cell with an aid of the enzyme, the oocyte has been installed to the microchannel through the inclined inlet of the chip.

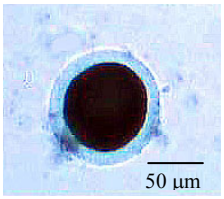


Fig.10. A photo of swine oocyte

F. Magnetic Microrotor

One of the functions of the versatile MMTs is continuous rotation (Fig3 (a)). The microrotor is actuated by placing the microrotor on a rotating couple of disk-shaped ferrite magnet [1]. Figure 11 shows the microscopic views of rotating microrotor in a microchamber. The ceiling of the chamber was sealed with thin PDMS film in order to obtain steady flow and pressure in the microchannel. A photograph of rotating wheel type mirorotor of Figure 11(d) indicated that the axis of rotation is fairly stable. The rotating speed of rotor can be achieved up to approximately 5500 rpm, and which was enough to mix the two liquid sufficient in 2 mm microchamber [1]. The rotating motion of MMT can also contribute to cell manipulations by use of the flexible arm of MMT as cleaner. Figure 12 shows the rotating microrotor which remove the surface debris of an oocyte. The cross-shaped MMT was used with low rotating speed of

about 1000 rpm in order to tap the surface of the oocyte sufficiently. The centre of the microrotor was off-center of the microchamber and hence the microrotor drew a circular orbit in the microchamber. Consequently the microrotor did not touch oocyte continuously during the cleaning process. Hence the debris of the oocyte was removed eventually after delicate touch of the microrotor. It was confirmed that sufficient removal of the debris without any damage of the oocyte due to the softness of the MMT. It is expected that the debris of a group of oocyte can be removed by simply putting the MMT in the Petri dish, and which will reduce the difficulties of preparation process of oocyte dramatically. Because the size of the microrotor was small enough to be inserted in the drop of the culture media on the dish, the microrotor can control the local oocyte in the medium drop on the dish without any damage of the oocyte.

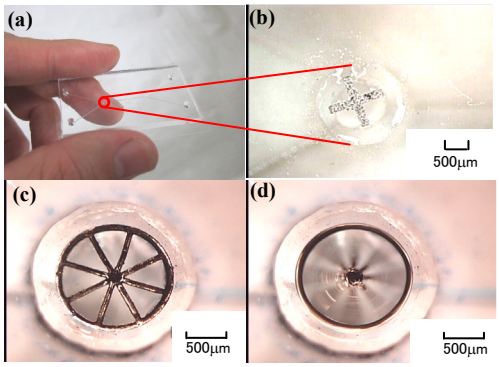


Fig. 11. Microrotor in microfluidic environment (microchannel) (a)PDMS microchip containing double Y channel and chamber (b), (c)Installed cross and wheel-shaped MMT in the microchamber and (d) Operation of rotating wheel-shaped MMT on microchip.

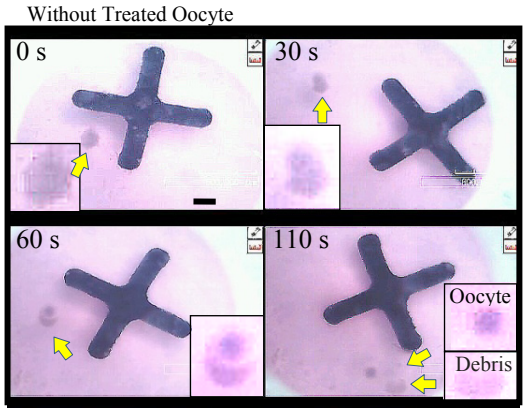


Fig. 12. A Sequential photos of rotating microrotor peeling off the debris which surround a oocyte.

G. *Magnetic Microvalve*

Another important function of MMT is lateral motion (Fig 3(b)). We have demonstrated the operation of the microvalve for the two phase flow of cells and water. In the past study [1] we have developed the microvalve for the polystyrene beads laden two phase flow as shown in Figure 13. However it tend to be wobbling when it try to stop the relatively large objects. For the present study the microvalve has been improved with arms for the stable lateral actuation. Figure 14 shows the operation of microvalve which can control the oocyte transportation in a microchannel by closing and opening the gate. In order to stabilize the motion of actuation, arms are attached to the tool. In order to avoid large displacement of the actuation, the microchannel has a half-circle shape ramp pattern in the middle part of microchannel. Therefore a oocyte can easy to climb up and out and be transported to the downstream smoothly when the microvalve is open.

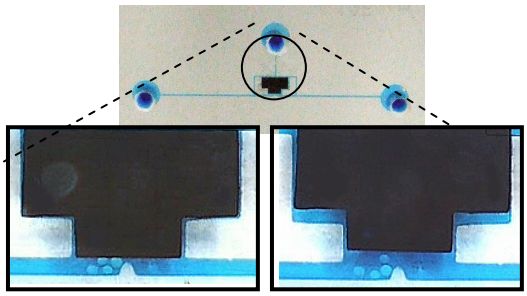


Fig. 13. Operation of conventional microvalve for the polystyrene beads-laden two phase flow.

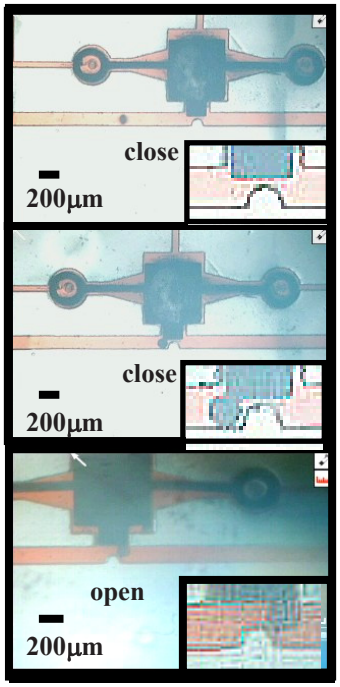


Fig. 14. Operation of microvalve (closing and opening the gate of valve) for oocyte.

H. *Magnetic Microsorter*

The MMTs provide another important function of sorting as shown in Figure 4(c) which can sort the oocyte to the right or left of the microchannel. Figure 15 shows the deformation motion of arrow-shaped MMTs due to its the elastic nature.

By stretching suspended fine parts of MMT, the positioning of the MMT is controlled to block the microchannel to make a valve. Figure 15 shows the picture of actuation of microsorter. In order to control the position of the MMT in the microchannel, a single pole has been patterned in the microchannel so that the ring part of the MMT can fit to the hole. Figure 16 shows the demonstration of sorting of polystyrene beads on chip be transported to the right and left of the microchannel. The sorting speed of the MMTs can be up to 18 Hz. Figure 17 shows the sorting experiment of actual oocyte. It was observed that the oocytes were sorted to the right or left of the microchannel successfully. This function can be applied to the cloning techniques which require to sort halved oocyte by sensing with and without nuclei.

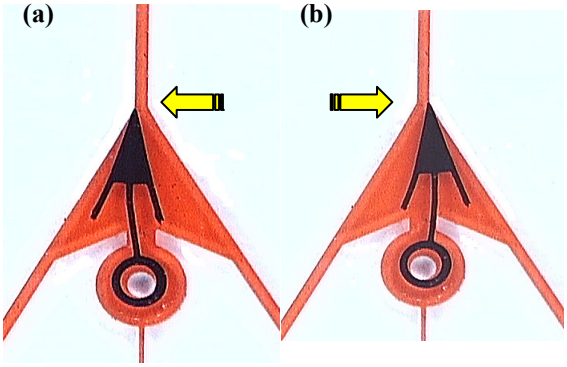


Fig.15. Magnetic microsorter in microchip.

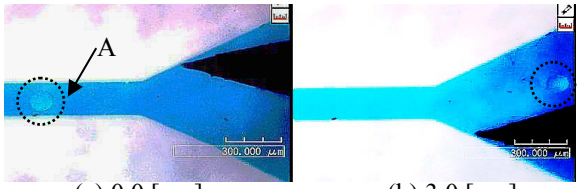


Fig.16. Operation of sorting for the polystyrene beads.

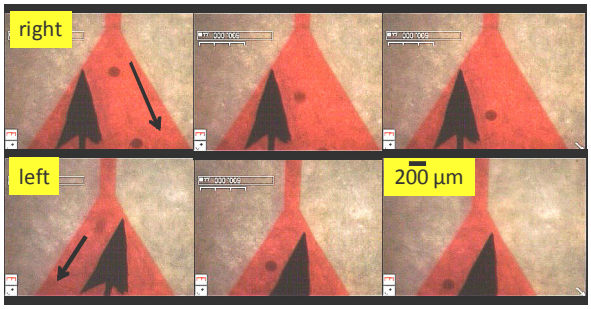


Fig.17. Photos of sorting oocyte by magnetic microsorter .

III. Conclusion

We have developed flexible micromagnetic tools which have a number of functions for cell manipulation in microchip. Many functions such as microrotor, microvalve and microsorter were demonstrated successfully.

In future, sorting halved oocytes with and without nuclei—an important technology for cloning—should be attempted for sorting MMT. Furthermore, our sorting MMT could be combined with MMTs performing other diverse functions, possibly allowing automation of the entire cloning process on a chip.

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