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journal or publication title	International Symposium on Micro-NanoMechatronics and Human Science, 2007. MHS '07
volume	2007
page range	442-447
year	2007
URL	http://hdl.handle.net/10097/46423

doi: 10.1109/MHS.2007.4420896

Magnetically Modified Soft Micro Actuators for Oocyte Manipulation

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Abstract:
We have developed novel magnetically driven polymeric microtool for non-intrusive and no contamination experiments on a chip. The composite is formed by suspending magnetite particles in polydimethylsiloxane. In order to obtain precise and complicated pattern of magnetic microtools, a photolithography techniques has been applied by making good use of thick KMPR-1050 photoresist as sacrifice-mold. The novelties of these tools are 1. fabrication of any 2D shape, 2.softness, 3. no contact actuation, 4. mass production with low cost. These versatile magnetic mirotools can be applied to various functions such as stirrer, valve, loader and sorter and so on. The potential impact of this technology includes sample selection and separation, particle loading and immobilization, genetic operation, tracking, mixing and reaction techniques into portable microfluidic labs-on-a-chip, culture systems.

1. INTRODUCTION

There is great interest in developing the microactuation mechanics in biochip as replacement of conventional bio 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. For last decades, microactuations on chip have been carried out by non-intrusive source of actuations such as optical tweezers, magnet force, electrostatic force, ultrasonic and so on. However it is desired to have actuators which have the ability of enough actuation force to manipulate cells as well as the softness enough to be harmless to actuate cells. Generally, magnetic force has an advantage over electrostatic force to actuate object whose radius is approximately more than 10^{-5} m [1]. Therefore magnetically driven actuation method has been selected for the current study in order to manipulate cells.

Conventionally magnetic actuation has been produced by metal or metal membrane, whereas miniaturized hard magnetic materials have not been used until recently due to the difficulties of micromachining processes. Recently the use of the composite of magnetic powder and PDMS has been applied to local actuation in microchannel with an important feature of easy fabrication. For example, there are some works on actuation by a piece of polymer-bonded magnets on top of PDMS membrane [1] or ferrofluidic plug in a microfluidic channel [2].

Especially, the elastomeric membrane material made of mixture of Silicon polymers and magnetic particles has been used as valve and pump in biochip [3, 4]. However the control of magnetic membrane on the surface of microchannel restrained the area and direction of movement of actuation in microchannel. Therefore there is potential requirement of versatile movement in biochip. Also, the magnetic membrane microvalve is not suitable for the particle laden multiphase flow such as cells-laden flow on a chip. It is important to note that the valve for the two phase flow have additional difficulties to manipulate the cells which tend to stick to the membrane. Also it is tend to be recognized the accumulation of cells in the region of stagnation flow around the membrane, and which has another difficulty to prevent clear observation due to its opaque nature. Hence the development of the valve for two phase flow is indispensable in order to achieve feasible transportation of cells.

One of the important magnetically driven microdevices such as rotor and stirrer provides rotating actuation in microchannel. It is important to obtain sufficient mixing in the laminar sheath flow of microchannel in the field of micro total analysis systems because of the difficulties of the inefficient mixing in low Reynolds number. Also the centrifugal force produced by mixing motion can be a replacement of the initial preparation process of biomedical treatment such as pipetting. Many works have been done to use micromachined magnetic metal rotors enclosed by PDMS channel network in order to obtain sufficient mixing [5], microcirculation or pumps [6, 7], long-term temperature control of microfluidic channels for cell culturing [8]. However the configurations of the rotors are limited to be fabricated due to the difficulties of micromachining processes and complexes of lithography processes.

Another important function in the biochip is loading function. It is long time requirement to have a system of loading one by one particle or cells in the biochip. However it is fairly difficult to complete the system due to the agglomeration of the cells and particle in the microchannel.

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Therefore limited information was obtained in the past decades of study in this field.

Sorting function in the microchannel is another important function of microchip and which are generally by electrostatic force, fluidic force [9] and magnetic force [10]. Especially flow cytometry technique sort cells by electrostatic force after sensing them optically. Although it is difficult to sort cells one by one, it is sufficient to obtain a sorted tendency of a group cells for bioengineering purposes. However there is a requirement to sort cells one by one, especially when the size of cells are larger. Also, magnetic microparticles are widely used in biomedical science, Drag delivery system (DDS) for immunoassays, DNA sequencing and cell analysis. The use of magnetic labels greatly facilitates the separation of the simply by application of an external magnetic field. However it is inefficient to label sufficiently in case the size of the particle is large. It is desired to have a simultaneous detection and separation in the continuous flow on chip sufficiently without any labeling and complicated patterned microchannels.

In the past study [11] we have reported novel magnetically driven polymeric microtool for non-intrusive and no contamination experiments on a chip. The composites were formed by suspending magnetite nanoparticles in polydimethylsiloxane. In order to obtain precise and complicated pattern of magnetically driven mictotools, a photolithography techniques has been applied by making good use of thick KMPR-1050 photoresist as sacrifice-mold [12]. The main features of them are 1.fabrication of any 2D shape, 2.soft and biocompatible, 3.no stiction (Teflon coating), 4.mass production with low cost. These versatile microtools can solve the difficulties of conventional microactuators which are mentioned above. For this thesis, we have demonstrated the microtools with a function of valve, rotor, and sorter and which give an advantage over a conventional microtools. It is important to note that the novelty of magnetically driven microtools is their flexibilities therefore they can actuate very sensitive materials such as cells. 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.

2. EXPERIMENTAL

2.1 Fabrication of Soft Magnetic Microdevices

Figure 1 shows a fabrication method to produce magnetic microtools. The detailed description was described in the past study [12] and which are summarized as follows; the resist mold for magnetic microtools was fabricated by patterning the KMPR 1050 resist layer. Then PDMS-magnetite composite has put in the patterned hole and baked at 80 °C. Finally the patterned substrate is put in the stripper liquid bath in order to collect the magnetic microtools. By using the techniques, high-accuracy magnetic microtools can be fabricated as shown in the series of photos as shown in Figure 2. The thickness of them were approximately 80 µm.

Figure 3 shows the softness of the produced magnetic microtool which are deformed with trying to pierce a jelly which are easy to be penetrated by a metal needle. PDMS-magnetite nanoparticle complexes were soft and rubbery materials depending on the concentration of the magnetite. The composite is formed by suspending magnetite (Fe_3O_4) particles in PDMS at concentrations of 50% by weight. Average diameter of magnetite particles is 0.2 µm. It is required over 30wt% concentration of magnetite enough to support magnetic actuation. The composite is most useful when the weight ratio of magnetite ranges between 30-70wt% and the present work used 50wt% concentration of the magnetite in order to obtain enough magnetic actuation and also have a moderate softness of the elastic condition. The range of the Young's modulus was between 2.7-5.4 MPa for 0-70wt% concentration of magnetite and 4.6 MPa for 50wt%. Young's modulus was estimated by measuring strain vs. stress of macroscopic sample of PDMS prepared under similar conditions as those to form microfluidic system. Consequently, this softness of the magnetic microtool can be applied to treat delicate materials such as cells.

In order to prevent any stiction of produced magnetic microtools to the PDMS biochip, the surface of PDMS was specially Teflon coated with CF_4 gas by plasma ashing method (Discharge Power: 130 W) for 10 minutes.

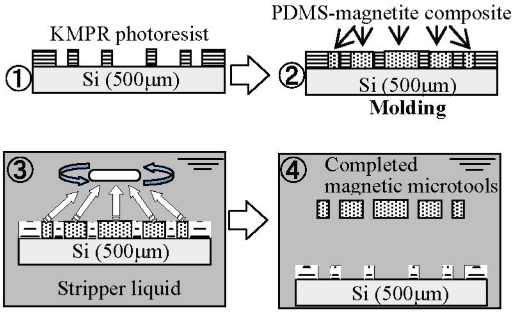


Fig 1: Process flows to fabricate polymeric magnetic microtools.

Fig. 2: Photos of produced magnetic microtools.

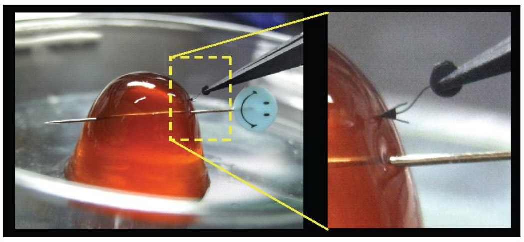
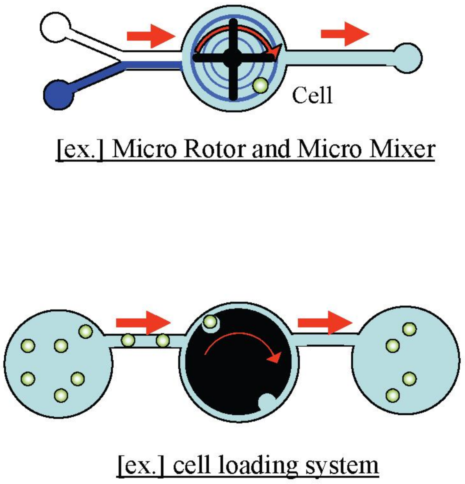


Fig. 3: Softness of the magnetic microtool.

(a) Rotation



(b) Vertical or Lateral Motion

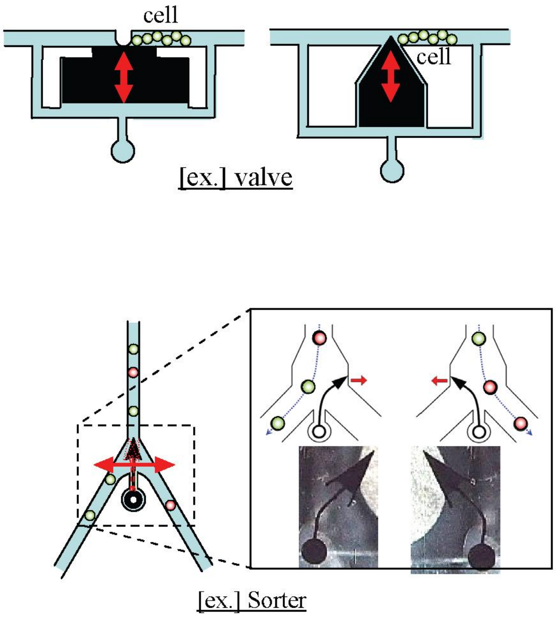


Fig. 4: Illustration of actuation principle of magnetic microtools categorized with actuated motions

2.2 Various Functions of Magnetic Microdevices

The produced soft magnetic microtools provide many functions such as stirrer, valve and sorter and so on. Figure 4 shows the concept of the actuation of soft magnetic microtools. Figure 4(a) describes the rotating motion of the magnetic microtool which can be used as microrotor, mixer and cell loading system. Figure 4(b) shows a lateral motion of the magnetic microtool which has a function to control the transportation of the cell in microchannel. Figure 4(c) describes the deformation actuation which has a function of sorting. By fixing the supporting point of the arrow-shaped magnetic microtool, the top part of the microtool can be bowed in the right and left direction due to its softness. By installing the arrow shape magnetic microtool in the forked-shaped microchannel as show in Figure 4(c), the sorting function can be achieved eventually. This sorting function should be useful to apply to the cloning techniques by sorting the embryo cells by with and without nuclei, combined with a sensor mounted upstream of them.

Figure 5 shows schematic of independent modules of microchannel and magnetic actuation. They are combined when the microtools are actuated and they are disconnected when the module of microchannel is replaced by new one in order to avoid any contamination.

3. MAGNETICALLY DRIVEN MICROTOOL

3.1 Operation of Microrotor

One of the functions of the versatile magnetic microdevice is continuous rotation (Fig4 (a)). The micro-stirrer is actuated by placing the micro stir bars on a rotating couple of disk-shaped ferrite magnet.

Figure 6 shows the microscopic views of rotating micro stir bar in the microchamber of biochip. The ceiling of the chamber of stir bar has been sealed with thin PDMS film in order to obtain steady flow and pressure in the microchannel. A photograph of rotating wheel type stir bar of Figure 6(d) indicated that the axis of rotation is fairly stable. The approximate rotating speed of the microrotor has been measured by the laser tachometer which are shown in Figure 7 (a) (b) which show the rotating speed of 1000-5500 rpm in a microchannel. Figure 8 shows photos of mixing operation of a micro stir-bar with rotating speed of 1000-5500 rpm in a microchannel. The solutions used for this experiment were composed of dyes and DI water. The blue solution contained of methylene blue, and the yellow solution contained yellow food coloring. Before mixing begins, the distinct boundary between blue and yellow sheath streams is observed along the outlet channel. During mixing, two colors of stream were mixed and greenish stream was observed. After mixing finished, the laminar sheath flow condition has been appeared again and hence the effect of mixing by stirrer is evident.

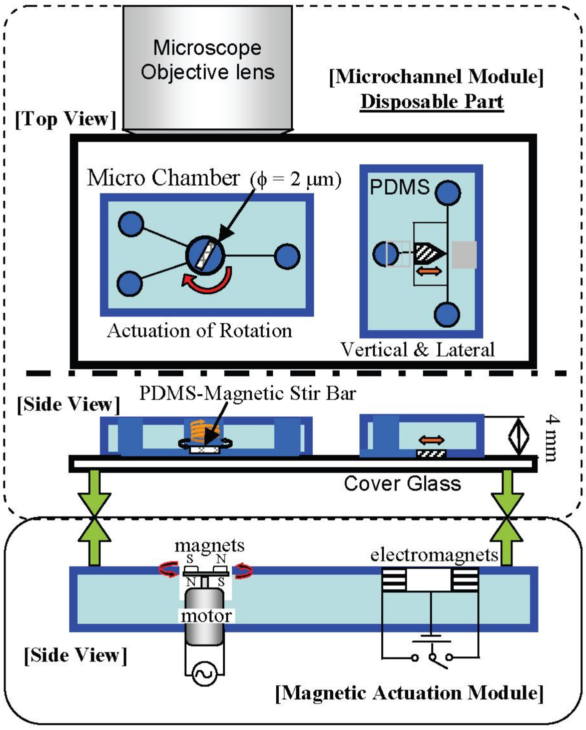


Fig.5. A schematic view of experimental arrangement used to create the rotating and vertical and lateral motion of magnetic microtool in the microchamber of biochip.

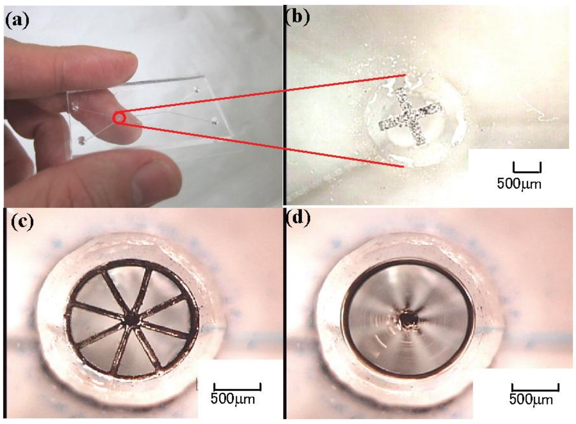


Fig. 6 The microstirrer in microfluidic environment (microchannel). (a)A photograph of the PDMS biochip containing double Y channel and chamber. (b), (c)Microscope view of stir bars in biochip (d) Microscopic view of rotating stir bar in biochip.

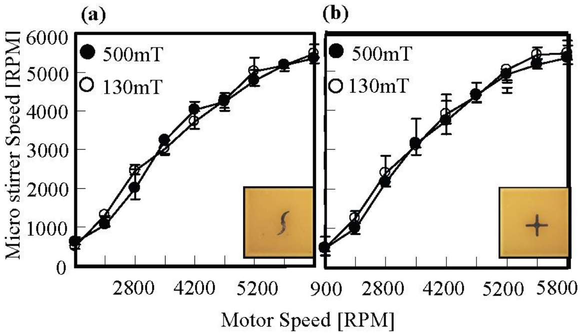


Fig. 7 The microstirrer in microfluidic environment (microchannel). (a) 2-wings and (b)plus shapes. The vertical bars indicate the standard error in the mean value ($\sigma/n^{0.5}$).

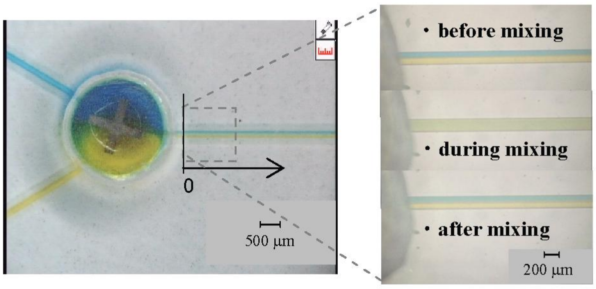


Fig.8. Photos of mixing operation of a micro stir-bar in a microchannel.

3.2 Operation of Microvalve

Another important function of magnetic microdevice is vertical and lateral motion (Fig 4(b)). We have demonstrated the operation of the valve for the two phase flow of cells and water. Figures 8 show one of the examples of the one dimensional actuation of vertical and lateral motion with valve function. Normally membrane type valve are used to control the microchannel of one-phase flow. It is important to note that the valve for the two phase flow have additional difficulties to manipulate the cells which tend to stick to the membrane. Also it is tend to be recognized the accumulation of cells in the region of stagnation flow around the membrane, and which has another difficulty to prevent clear observation due to its opaque nature. Hence the development of the valve for two phase flow is indispensable in order to achieve feasible transportation of cells. For the current study, the polystyrene beads used for the experiment have a size of 100 μm, assuming the size of embryo cell (≈ 100 μm).

Figure 9 shows the operation of microvalve in the microfluidic environment. The convex shaped magnetic microtools move smoothly to narrow the microchannel normal to the magnetic microtools in order to block transportation of cells, whilst blocked water medium find the path to flow toward the downstream by using a fine bypass of microchannel (≈ 50 μm) which is less than the size of polystyrene beads (100 μm). Eventually, the polystyrene beads can be blocked until the magnetic microtools has back to the initial position.

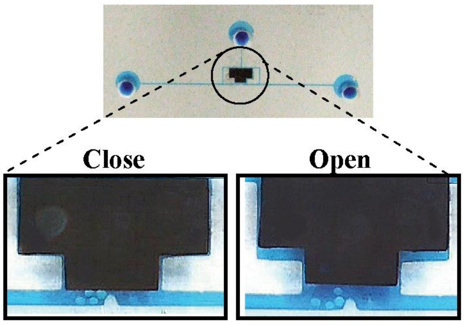


Fig. 9: Operation of magnetic microvalve (closing and opening the gate of valve).

3.3 Operation of Microloader

It is important to note that another important function of the magnetic microdevice is loading system by rotating motion (Fig 4(a)). Figures 10 show a loading system as one of the examples of the revolving type rotation. The each magnetic microdisk has a pocket whose diameter is approximately 100 μm which is the similar size of the oocyte cell.

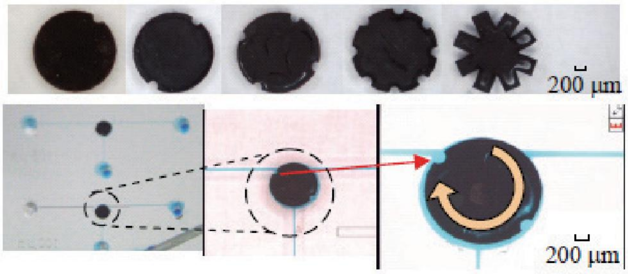


Fig.10 A series of magnetic microtools for revolving type rotation in microfluidic environment. The microchannel was dyed by methylene blue.

Figure 11 shows the sequential shots (a)-(h) of loading a single polystyrene bead (100 μm). First of all, 100 μm sized polystyrene beads have been flowed forward in the microchannel in order to fit the pocket. Then a single polystyrene beads has been loaded toward downstream successfully with a rotating motion of the magnetic microdisk. In order to confirm that this magnetic microtools can be applied to the actual cells, swine oocyte (≈100 μm) cells has been introduced to this loading system. Figure 12 shows the loading actual oocyte cells in the biochip. It was confirmed that a single oocyte cell has been successfully loaded by magnetically driven microtools.

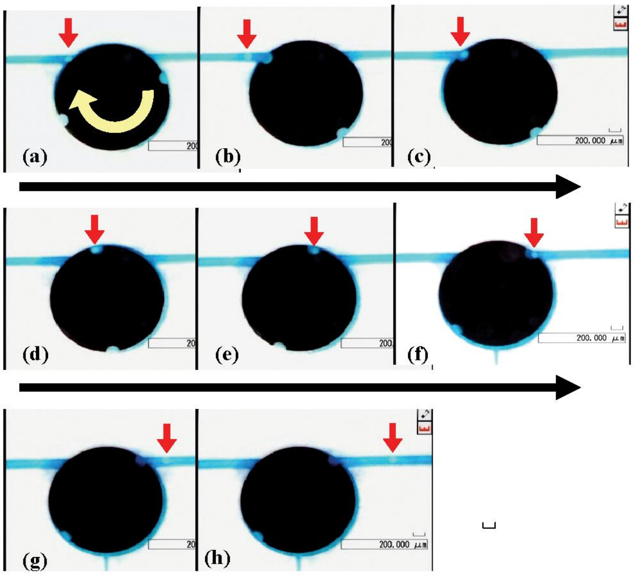


Fig11. Sequential shots (a)-(h) of loading a single polystyrene bead (100 μm). The microchannel was dyed by methylene blue.

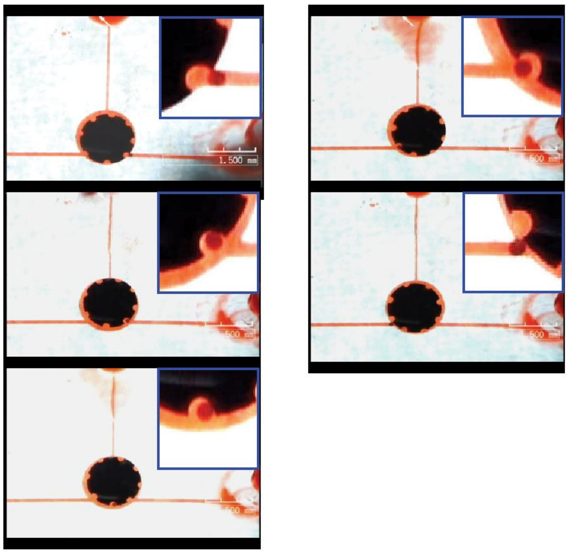


Fig.12. Sequential shots (a)-(h) of loading a single oocyte cell (≈100 μm). The microchannel was dyed by red food coloring.

3.4 Performance of Microsorter

The magnetic microtools 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 13 shows the arrow shaped magnetic microtools which has enough flexibility to seal the microchannel when it is inclined. Figure 13 shows the picture of actuation of sorter. It can be observed that the axis of the arrow can be bended due to its flexibility. In order to control the position of the magnetic microtool in the microchannel, a single pole has been patterned in the microchannel so that the ring part of the microtool can fit to the hole. Therefore the position of the magnetic microtool can be fixed enough to fit the branch of the microchannel.

Figure 14 shows the demonstration of sorting of polystyrene beads on chip to the right and left to the microchannel. The sorting speed of the magnetic microtools can be up to 18 Hz. This function can be applied to the cloning technique which require to sort with and without nuclei of half cut oocyte.

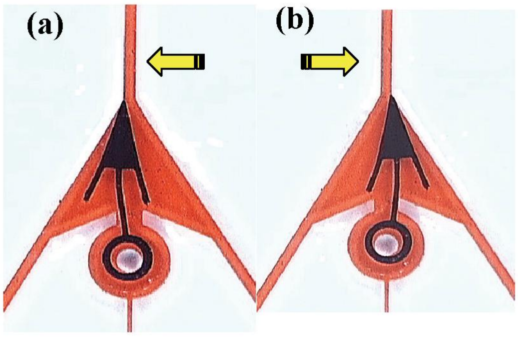
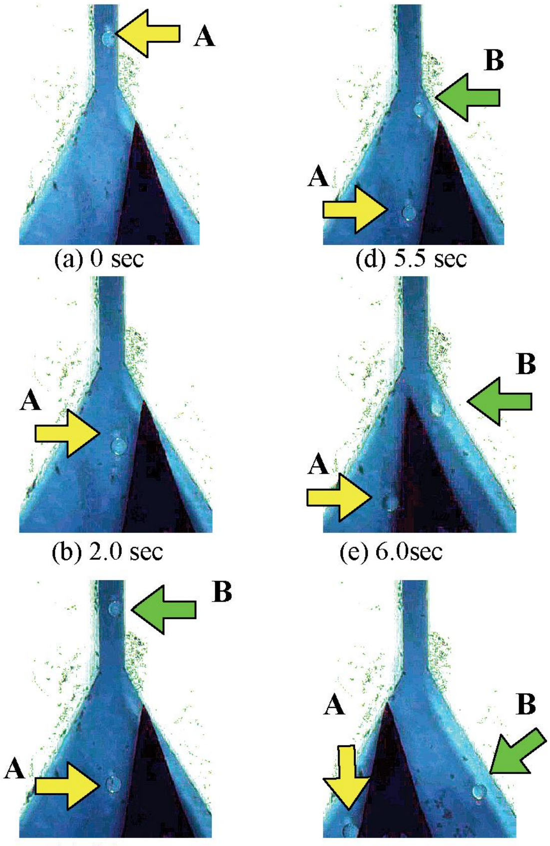


Fig.13 Magnetic microsorter in bio-chip.



(a) 0 sec (d) 5.5 sec

(b) 2.0 sec (e) 6.0sec

(c) 5.0 sec (f) 7.0 sec

Fig.14 Sequential photos of sorting

4. CONCLUSIONS

We have developed flexible magnetic microtools which have various functions in biochip. This versatile microactuator can apply to even delicate oocyte cells due to its flexibilities. Many functions such as stirrer, rotor, valve and soter were demonstrated successfully. The products are important for both commercial and scientific perspectives.

ACKNOWLEDGEMENT

This work has been financially supported by the Research and Development Program for New Bio-industry Initiatives and the ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (17040017), (19016004) and Sasakawa Scientific Research Grant from The Japan Science Society.

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