ON-CHIP CONTINUOUS ENUCLEATION BY HYDRAULIC FORCE CONTROL USING MAGNETICALLY ACTUATED MICROROBOT

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ABSTRACT

We present a continuous enucleation process of bovine oocytes on a microfluidic chip to achieve the continuous cutting of the oocytes and increasing the potential viability of the enucleated oocyte. By combining microfluidic chip and micororobotics, the flow in a channel can be actively controlled and we achieved successive operations of 1) loading oocyte, 2) control cutting volume and 3) removing nucleus. The magnetically actuated microrobot can control local fluid flow by changing its position like gate valve and control fluid force distributions in a microchip which govern oocyte movement in a chip. The optimally designed microchannel for enucleation enables continuous operation and cutting bovine oocyte with smooth manner by hydrodynamic force.

KEYWORDS

Oocyte enucleation, Magnetically driven microtool, Microfluidic chip, Microrobot.

INTRODUCTION

The embryo manipulation shows great potential techniques for the improvement of domestic animals, preservation of genes of rare animal. Especially the oocyte enucleation technique is a primary study for cloning process. The conventional techniques of the enucleation process mainly include manual manipulator operation, chemical treatment methods and femtosecond laser pulses techniques [1]. However, these methods tend to have problems of low success rate, low repeatability and contamination to the cell. Robots on a chip have great advantages for the treatment of biological cell instead of human handling due to its non-skill dependent, high throughput and high repeatability. Magnetic field can be suitable power source for the on-chip robot because of its non-contact drive; low invasiveness with respect to a cell, low production cost. Previously we developed an on-chip microtool called MMT (Magnetically driven microtool) for the micro scale organism manipulation. The MMT driven by permanent magnets can be applied to wide range of cell manipulations such as loader, sorter, droplet generation, etc. [2] [3]. Enucleation by MMT was conducted previously; MMT driven by the horizontally arranged magnets with ultrasonic vibration applied to the microchip could obtain 1.1 μm in position accuracy [4], and also by fabricated riblet type of MMT, which could achieve high performance in speed [5]. However it is difficult to operate continuous process and the enucleated oocyte tends to be misshapen. The key requirement is to achieve higher speed processing with less damage while cutting the area with nucleus in a small volume.

Here, we improved the enucleation system by the cooperation of MMT with fluid control. Figure 1 (a) shows the concept of the enucleation chip. Microfluidic chip is designed to Y shape with two inlet chambers; one for accommodation of the MMT, one for oocyte injection. This design permits MMT have enough space to conduct the enucleation process, and also have enough space to accommodate a large number of oocytes for the continuous enucleation operation. A withdrawal microchannel is connected to the outlet. In order to confine the oocyte position and achieves the accurate cutting of the oocyte in volume.

In the Fig. 1 (b-e), blue arrows show the flow directions in the microchannel, white arrows show the MMT moving directions. At the first stage, oocytes with cell culture medium are injected from the inlet of microchip. Solution in microchannel is continuously sucked out from the outlet; therefore injected bovine oocytes are sequenced by flow in microchannel. Designed microchannel with height difference, withdrawal microchannel is lower than inlet microchannel, is used to confine oocyte with its nucleus located in the withdrawal microchannel (Fig.1 (b)). Then, the lower part of oocyte was sucked into the withdrawal microchannel by hydraulic force (Fig.1 (c)). After the tip of MMT moved right, the nucleus part is torn and flushed away by the flow (Fig.1 (d)). At last, MMT open the port letting enucleated oocyte taken out by the flow, meanwhile the following oocyte is loaded to the operation location for the next enucleation process.

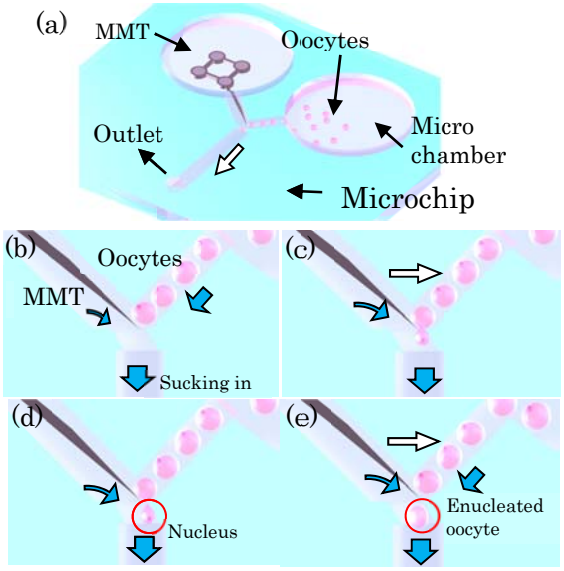

Fig. 1 (a) Overview of the enucleation microchip. (b-e) Oocyte enucleation process.

METHODS AND MATERIALS

Fluid control by MMT: In order to achieve the continuous enucleation process, loading oocyte in sequence is required. The MMT can control the fluid distribution by changing its position at the suction channel. By employing this fluid control by MMT, oocyte can be delivering to the suction port one by one. An electric circuit analogy is used here to specify

parameters of microchannel (Fig. 2). Considering the oocyte is regularly 100μm in diameter, under the same hydraulic resistance of two inlets, therefore, width, height and length of the oocyte inlet microchannel are defined to 150 μm, 300 μm and 1 mm. By using the equation

$$R_h \cong \frac{8\eta L}{r_h^2 A}$$

Where R_h is hydraulic resistance, η is viscosity coefficient, L is the length of the microchannel, A is the cross-sectional area of the channel, r_h is the hydraulic radius of the channel which is given by

$$r_h \cong \frac{2A}{P}$$

Where P is the length of the perimeter of the channel. To balance the hydraulic resistance, R1 is set equally to R2; hence the width, height and length of the other side microchannel are derived to 300 μm, 300 μm and 2343 μm respectively. MMT working like a rheostatic controller governed the distribution of flow letting oocyte loading to the operation location and cutting the nucleus off from the oocyte by increasing the hydraulic pressure.

Volume Control: In order to achieve high precise cutting off of the nucleus from the oocyte with less damage, volume control is significant. MMT can control the volume of sucked oocyte by closing the channel after certain amount of time. Experiments were conducted to find out the correlation of sucking time, MMT position and oocyte volume sucked into the channel. We obtained the correlation between the volume being sucked into the outlet microchannel and the suction time. Due to the different displacement of the MMT, which shown in Fig. 3 (a) represented by length L, the volume, semicircle area in Fig. 3 (a), sucked in the withdrawal microchannel is relatively increased by the time, even though they are not in a linear relationship.

To perform a high speed enucleation process, for instance, cutting time is less than 5 seconds with less than 20 percent of enucleated cytoplasmic volume, displacement of the MMT from 20 μm to 200 μm seem to be optional candidates, but among them 80 μm, 100 μm and 200 μm cases, oocyte was suddenly sucked into the microchannel and flow away immediately, it may inconvenient for operation, because this require the system having a high response speed.

Cutting of oocyte by hydraulic force: A 3D structure was modeled by COMSOL 4.1 to analyze the distribution of the surface traction. According with the experiments, the inlet is wide open; hence the inlet condition is set to 0 Pa in the analysis. MMT angle and out flow rate are set to 150 degree and 6700 μm/s, respectively. Fig.4 shows the Comsol simulation result; it can be seen that the surface traction on oocyte is totally different. The lower part of oocyte which entered in withdrawal microchannel suffering a high traction force with maximum to 73.4 Pa by hydraulic pressure on Y direction, meanwhile the upper part of oocyte is almost 0 Pa protected by the MMT from the impact of the medium. By means of this, we can rely on adjusting location of MMT and control of the outlet flow to conduct the enucleation process.

Fabrication of the hybrid MMT: Nickel (Ni) is ferromagnetic, with magnetic force could be controlled by permanent magnet, however, it has problem of toxicity to biological cells. Therefore we fabricated a hybrid type of MMT composed of Ni and silicon (Si), which is bio-compatible material. The Ni-Si MMT fabrication process is shown in Fig. 7. At first, the Cr-Au was sputtered on the silicon wafer (thickness =200 μm). Then a thick negative photoresist (SU-8, Tokyo Ohka Kogyo Co.) coated and exposed on the silicon substrate as a support layer. Meanwhile on the other

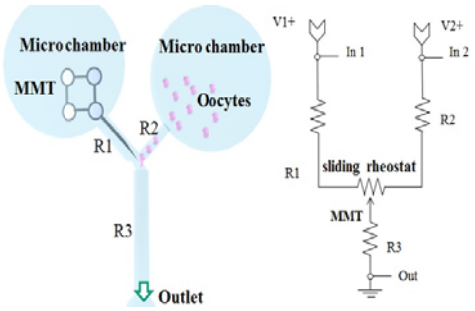

Fig. 2. The electric circuit analogy of the enucleation chip.

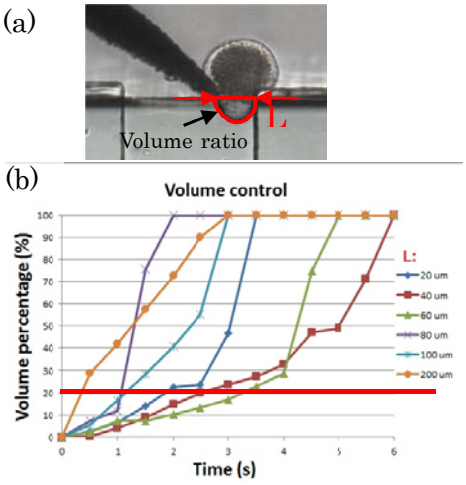

Fig. 3 Correlations of the volume sucked into the outlet microchannel and the time under the different displacement

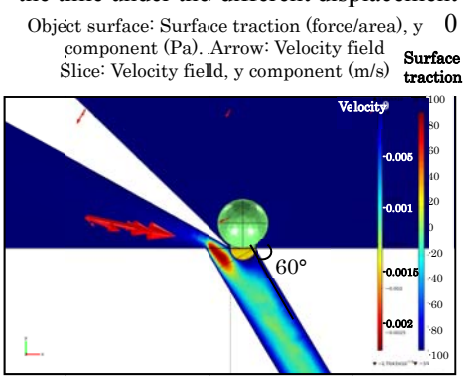

Fig. 4 FEM results of velocity distribution and the surface traction of oocyte.

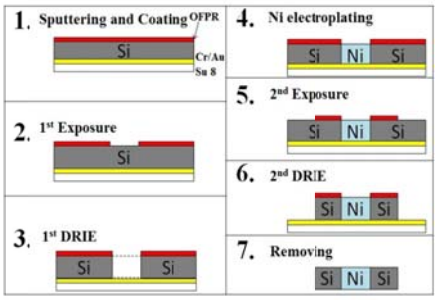

Fig. 5 Fabrication process of the MMT.

side of the silicon wafer, the photoresist OFPR (Tokyo Ohka Kogyo Co.) was coated on the 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 etched the silicon 200 μm depths until the Cr/Au layer. After the wet etching of Cr, Au surface was exposed. Then Nickel was grown on the Au surface by the electroplating (= 200 μm). We again conducted the OFPR coating, exposure and DRIE processes shown in the Fig.5 (4-6) to form the MMT shape, MMT is collected at last by removing the SU-8 layer.

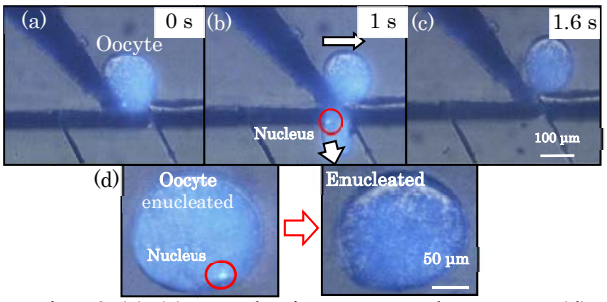

Fig. 6 (a)-(c) Enucleation process by MMT. (d) Contrast diagram before and after enucleation.

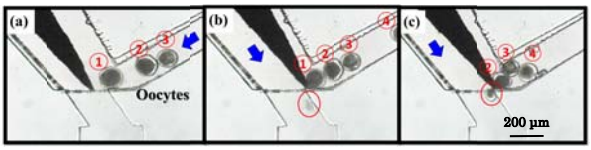

Fig. 7 Continuous cutting process.

EXPERIMENT

Our platform includes a linear stage for the magnet actuation, a microscope with a CCD camera, a joystick, a high response pump and 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. Oocyte is stained by the Hoechst 34580 in the experiment. The nucleus part was florescent when mercury lamp exposed. Figure 6 shows the experimental result of bovine oocyte enucleation process. The oocyte inserted from the inlet flew to the narrow channel and stuck there since the oocyte size was not small enough to get into the microchannel with 50 μm height (Fig. 6(a)). After the nucleus position was confirmed, the downside of the oocyte was aspired by the out flow in the withdrawal microchannel until the nucleus part is sucked in the channel (Fig. 6(b)). Then the tip of the MMT pressed oocyte until the corner of the channel and hold the position there. Then nucleus part was torn by the hydraulic force, and washed away by the out flow (Fig. 6(c)). Figure 6(d) shows the comparison of oocyte shape before and after cutting, it can be seen that the nucleus was successfully removed and the oocyte shape kept circle even after the cutting. The processing time, which is the duration from the time when oocyte reached to the narrow channel to the time when oocyte was cut, was less than 5 seconds and the oocyte removed rate is about 20 % of the original size. After single bovine oocyte enucleation process had been conducted successfully, In order to achieve the multiple oocytes enucleation process with a short period time, we then implemented the multiple oocytes cutting process. Figure 7 shows that we have realized the continuous cutting of oocytes successfully.

CONCLUSION

In this paper, we presented an innovative cutting method using MMT cooperating with the flow control. Currently we can remove the nucleus from oocyte successfully, and this system shows remarkable advantages in following three points: 1) The oocyte could be loaded one by one by flow control with MMT. Continuous cutting process has been succeeded; it shows the superior potential on the continuous enucleation process. 2) The volume of the enucleated parts is controllable. 3) The incision of enucleated oocyte is smooth and neat, this means that damage to the oocyte became very small, and could reduce the influence to the viability of the oocyte.

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