

# Nano Micro Robotics and its Application to Bio Science and Technology

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**Abstract** – The cloning is one of the most promising biotechnologies today. The project aims to automate every process required in the cloning, i.e. supplying, cutting, removing, filtering, assembling and fusing, on the basis of micro robotics technology. The processes are performed on a micro chip consisting of micro channels and chambers in which oocytes and membranes flow and are manipulated. A whole system would be small and compact enough to be fit on desk top to meet various demands in biotechnology. The objectives, the overview and the current progress will be introduced.

**Keywords** – biotechnology, micro chip, cloning, automation

## 1. Introduction

Cloning technology, that allows production of the genetically identical offspring of another organism, has wide variety of applications in agriculture, pharmacy, regenerative medicine, etc. It is one of the most promising technologies in the bioscience and technology. The current cloning technology includes extraction of nucleus, injection or electric cell fusion of donor nucleus. All the processes are performed by skillful manual operation typically using micromanipulator with optical microscope. An operator requires time-consuming training to obtain the required manipulation skills. The success rate in obtaining a normal birth from cloned embryos remains extremely low around a few percent.

We are proposing new embryonic/cell manipulation processes that are fit to automation and mass production for cloning. The research is supported by the Bio-oriented Technology Research Advancement Institution and is conducted in Promotion of Basic Research Activities for Innovative Biosciences. Instead of acquiring a conventional cloning technique we have adopted a new cloning protocol that meets automation and mass production.

## 2. New Protocol and System Configuration

The new protocol consists of several simple processes including (1) removal of zona pellucida, (2) oocyte bisection, (3) separation of enucleated demi-oocyte, (4) donor cell coupling, and (5) electric fusion [1]. All processes are performed in micro flow on small substrate composing what we call a “desk-top bio plant”.

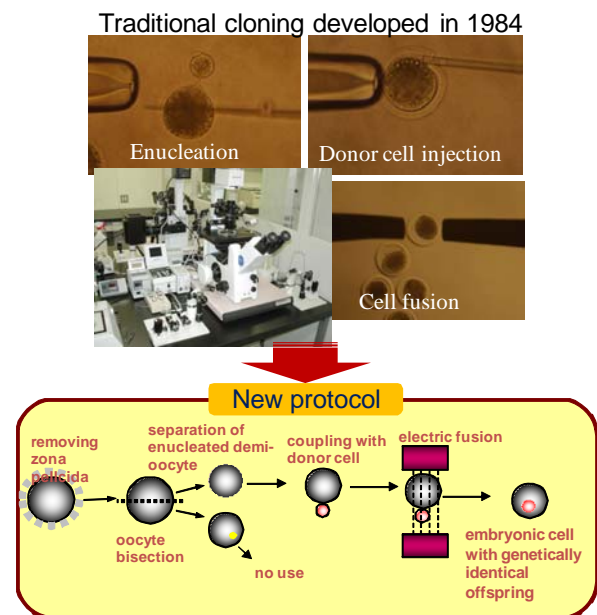


Fig.1 Proposed new protocol for cloning

Each substrate is made from PDMS (polydimethylsiloxane) furnishing with micro flow channels on its surface. The PDMS substrate is widely utilized in micro TAS, bio chips, etc. It is fabricated by utilizing soft lithography technique with original patterned mold.

Currently six modules conducting supplying, cutting & nuclear detection, separation, coupling, and fusion processes have been developed. Sensors and fabrications of PDMS substrate are key technology to achieve automation of each process. A cell detection sensor and a compact microscopic imaging sensor capable of on-board are also proposed. All processes are being integrated on a single chip to perform cloning task.

### 3. Modules and Components

#### 3.1 Supply module

Feeding oocyte and cells one by one into micro channel requires careful and intensive operation. We design supply module composing of Y-shape channel, valves, suction nozzle with 3D stage, microscope, and syringe pump [2]. A container set on the stage is controlled and moved to position target cell to the nozzle by visual feedback. Positioned cells are taken from the nozzle to the sorting area one by one through valve and channel, and sorted with certain intervals. They are then sent into a module through another valve. Fig.2 shows the module configuration.

#### 3.2 Cutting module with nucleus detection

We are proposing a unique cell cutting method that utilizes micro channels with flow control [3]. The cell cutting is performed in two perpendicularly intersected channels having different depths. A 120um diameter oocyte is separated by drawing its half part into 50um deep tributary channel. A drawn part is flat enough to observe fluorescent nucleus (fig.3). Strong flow given in the main channel pulls the rest part of oocyte and removes it. An original oocyte is separated into two demi-oocytes finally.

#### 3.3 Separation and sort module

Enucleated cell part is separated and sorted for use in further cloning process. A separator is composed of a Y-shape channel and micro magnetic tool [4]. The tool is actuated by electromagnetic coil, switches flows, and sorts cell and particle as shown in Fig.4.

#### 3.4 Coupling module

A donor cell (fibroblast) is adhered on the surface of demi-oocyte by dielectrophoresis (DEP). First, a demi-oocyte is caught in the middle of main channel (Fig.5(a)), then a donor cell is fed from tributary channel and drawn to the oocyte surface by DEP (Fig.5(b)).

#### 3.5 Fusion module

A coupled oocyte and donor cell is aligned by DEP (8Vp-p@1MHz) between two parallel micro electrodes separated in 400um distance. The electrodes are made from thin nickel plates and filled in the PDMS surface to compose a channel. Fusion is achieved by applying DC pulse (48V@100us) as shown in Fig.6.

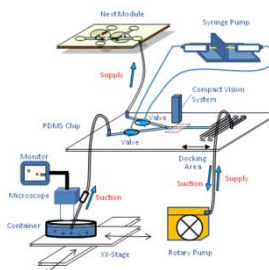


Fig.2 Supply module

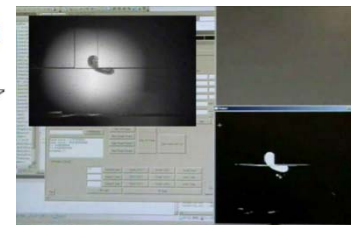


Fig.3 Automated cutting

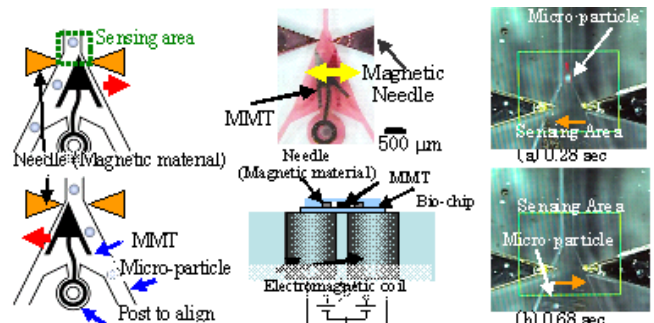


Fig. 4 Separation and sorting

### 3.6 Fabrication and sensing

Every module is fabricated with PDMS by photolithography technique. Male mold is patterned and fabricated by spin-coated SU8 lithography. Mountable particle detection sensor and microscope [5] are also developed for module.

### 4. Conclusions

We have confirmed basic function of every module with biological evaluation, i.e. growth rate and safety. All in one chip (Fig.7) is developed [6] to demonstrate every process of cloning to achieve “desk top bio-plant”.

### References

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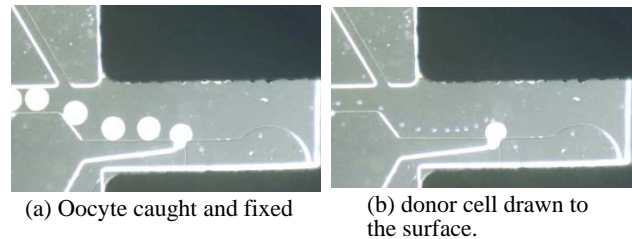


Fig.5 Coupling processes.

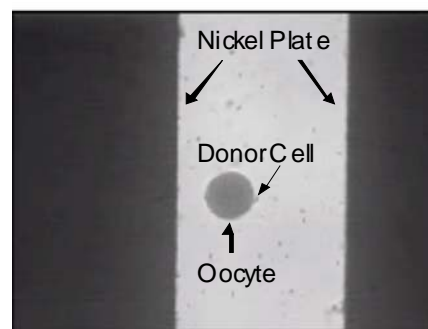


Fig.6 Experiments with fusion module.



Fig.7 All in one chip for desk top plant