# An Integrated Cell Processor for Single Embryo Manipulation

**Jung-Yul Park*, Seng-Hwan Jung**, Young-Ho Kim*, Byung-Kyu Kim †, Seung-Ki Lee*** and Byeong-Kwon Ju***

**Abstract** - In this paper, we present a novel integrated cell processor to handle individual embryos. Its functions are composed of transporting, isolation, orientation, and immobilization of cells. These functions are essential for biomanipulation of single cells, and have been typically carried out by a proficient operator. The purpose of this study is the automation of these functions for safe and effective cell manipulation using a MEMS based cell processor. This device is realized with a relatively simple design and fabrication process. Experimental results indicate that it can act as an efficient substitute for essential but very tiresome and repetitive manual work while contributing significantly to the improvement of speed and success rate of operation by facilitating cell manipulation. The cell viability test for the device is studied through the distribution of mitochondria in mice embryos and cultivation of cells for 86h.

**Keywords**: biomanipulation, cell processor, dielectrophoresis, polypyrrole

## 1. Introduction

Recently, the characterization and manipulation of individual embryo cells are becoming a challenging issue in biomedical applications such as cloning, gene expression analysis, and cell replacement therapy (CRT) [1-4]. These techniques are essential to the agricultural industry [5], and allow individual cell based diagnosis or pharmaceutical testing [6]. Despite great interest on manipulation of single biological cells however, most biomanipulation tasks such as gene injection, in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), etc. are usually carried out by proficient operators relying strictly on visual information provided from a microscope [2-4]. In the case of such embryo cell manipulation tasks in biomedical applications, the operator must take more than a year to perform reliable cell manipulation. Moreover, success rate of the operation is extremely low. Biological cells are irregular in configuration and easily deformable and they can be seriously damaged during manipulation and treatment [7]. Another problem is that classical cell-biology studies carried out on petri-dish cell culture may not be the most suitable methods to study single cells [5]. Since cells are freely suspended in a droplet on a petri-dish, it is very difficult to manipulate an individual cell.

Hence, an automated biomanipulation system is desired to substitute tiresome and repetitive but very essential manual work in the cell manipulation process, which is carried out on a petri-dish. The automated biomanipulation system may include a great deal of mechatronic or MEMS-related equipment, for example, motorized micromanipulators for micro pipette positioning, a visual servoing system for cell tracking, and a cell processor to handle individual cells. Its main advantage is to greatly augment the operational speed and guarantee a kind of repeatability in manipulation outputs. In our previous work, we demonstrated feasibility test results using our autonomous biomanipulation platform [8].

This paper is focused on design, fabrication, and feasibility evaluation of an integrated cell processor for single embryo cell manipulation, which is composed of four sequential functions – transporting, isolation, orientation functions, and immobilization, respectively. Fig 1 summarized the functions of the cell processor that can substitute the manual work. Transporting and retaining embryos in the specific region are the essential and fundamental steps in cell manipulation. A glass micropipette is one of the basic tools used in these procedures. It is specifically used to pick up and place individual embryos, tasks that are required several times for each cell manipulation procedure. This job is not only very tiresome, but requires skill, and if improperly performed can induce physical damage to cells [5]. In this study, microfluidic channels and PPy valves that could eliminate most of this labor handling are employed. Embryos are transported by a pressure driven flow in the microchannel. Then, the isolation of the target cell from other cells is carried out by actuation of the PPy valves.

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The orientation control of single cells is essential for higher fertilization, cleavage, and survival rate [9]. Typically, cell orientation is performed using the motion of a micropipette with expert skills. However, this method is not suitable for autonomous biomanipulation. Cell orientation should be safe, precise, and automation for autonomous biomanipulation should be safe. To satisfy these conditions, the orientation control of cells is carried out using DEP. Cell immobilization is required for stable cell manipulation, because the liquid flow due to the micropipette motion acts as a disturbance to the cell manipulation. Typically it has been performed using the fluid-suction from a holding pipette. In our device, the target embryo cell could be immobilized by the fluid-suction from the micro-hole. Another important feature of this study is that these functions (transporting, isolation, orientation, and immobilization) for biomanipulation are realized with a relatively simple design and fabrication process.

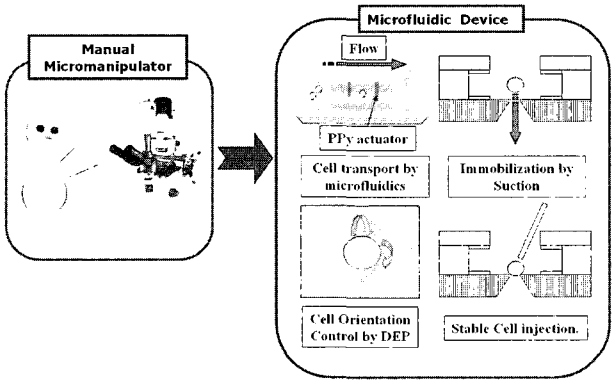


**Fig. 1** The functions of the cell processor.

## 2. Design and Manipulation Process

We designed an integrated cell processor for single cell manipulation. Fig. 2 presents a schematic diagram for the integrated cell processor. This device has a sequence of functions such as transport, isolation, immobilization, and orientation of cells. Researchers have already transported red blood cells, yeast, and other small cells through microchannels [10-12]. In the previous work, mammalian embryo cells were dealt with but only transport was tested [5]. Little has been done about fully handling mammalian embryo cells. This work integrated the functions that are needed for complete cell manipulation. The first step is the separation of a single cell from a group of cells. In conventional approaches, a single cell is isolated from a group of homogeneous cells using optical tweezers [13, 14]. However, the setup is too expensive and too bulky to be integrated with the chip-scale cell processor. In this study, biocompatible PPy actuators were used as active valves in microchannels, and a single cell was separated with a simple MEMS device. In our approach, collected embryo cells are placed in a reservoir in the device. The cells in the reservoir are then moved to an injection port by a pressure driven flow in microchannels whose width is slightly bigger than the cells. As the target cell passes the PPy valve, the valve is closed and the other cells are blocked.

The target cell is further moved to the center of the injection port by the suction in the micro-hole. Once the cell is brought to the vicinity of the micro-hole, orientation is controlled by DEP for the optimal location of the cell injection. DEP has been successful in performing sample sorting, trapping, manipulation, rotation and concentration [15, 16]. Since it is generally accepted that DEP does not lead to any irreversible damage to cells [17], we used it as a method for cell orientation. In this study, a 250 μm gap was formed between a pair of electrodes, and also in an AC wave with a frequency range between 300 kHz and 1MHz and a voltage between 1 and 5V. Once the orientation of the cell is adjusted, it is captured and immobilized at the micro-hole for stable cell manipulation. After biomanipulation tasks such as gene injection, IVF, and ICSI, using a motorized micromanipulator under a microscope, the treated cell is moved to a collection well connected to the microchannels. The above procedures are repeated until the collected cells in the reservoir are used up.

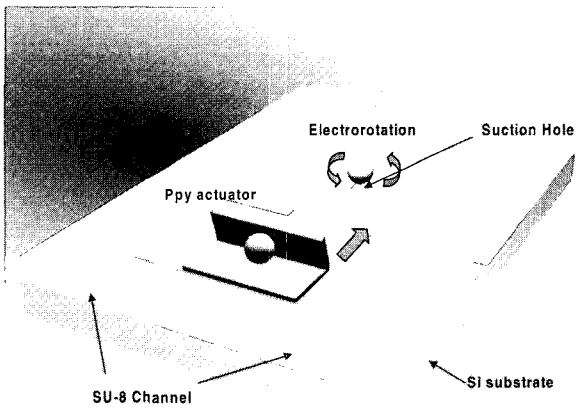


**Fig. 2** A schematic diagram for the integrated cell processor.

## 3. Fabrication Process

The integrated cell processor was fabricated using standard MEMS fabrication procedures on a 4 inch wafer (500 μm thick <100> Si substrate). Fig. 3 shows the schematic diagram of the fabrication process. First, the 1um silicon dioxide layer was deposited with thermal oxidation. Then, the silicon oxide on the back side was anisotropically etched by Tetramethylammoninium

Hydroxide (TMAH) solution at 95°C (Fig. 3(a)) to obtain a microhole (35×35um). The 300Å Cr and 2000Å Au layers were e-beam evaporated on the top surface . The Cr/Au layers were patterned and etched to open a window around the microhole (Fig.3(b)). These windows are necessary for a releasing step in which a method called differential adhesion was used instead of a sacrificial layer [18]. Then, the 2000Å Au layer was deposited (Fig.3(c)) and patterned by etching (Fig.3(d)). The roles of the Au layer include a structural layer in the PPy/Au bilayer, a current collector for the redox reaction that drives the PPy microactuators, and electrodes for DEP. Next, on the Au layer, 170 um thick SU8-100 was coated, soft baked, and patterned (Fig.3(e)). This polymer layer served as the microchannel. The cross section of the microchannels is 235×150 $\mu m^2$. After photopolymerization, we descummed the SU8 structure using reactive ion etching (RIE) (200W, 50 sccm $O_2$ at 50mTorr) to remove the thin polymer residue that remains following the developing process. Without this procedure, Ppy was not electrochemically polymerized as intended. PPy microatuators were electrochemically deposited on the Au surface using the potentiostat system with three electrodes (MEMS potentiostat SC, Advanced micromachining tools). The Au layer was covered with photoresist while windows for PPy microactuators were opened by photolithography. Then, the PPy layer was automatically patterned at a voltage of 0.7V vs. Ag/AgCl(Fig.3(f)). The resist was then removed using ethanol.

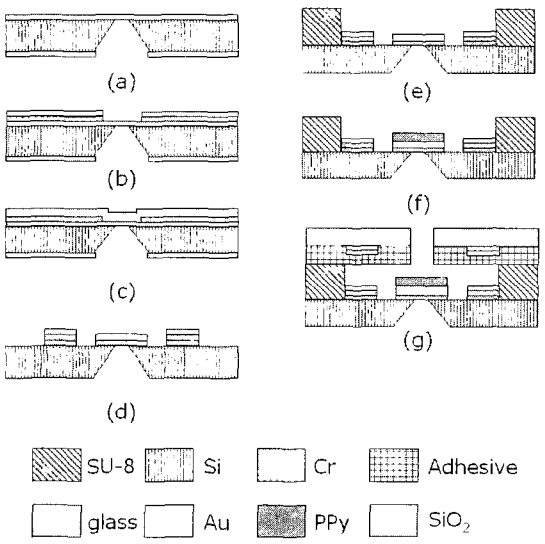


**Fig. 3** A schematic illustration of the process for the fabrication of the cell processor.

Upper electrodes for DEP were patterned on a 0.5 mm thick Pyrex wafer. An access hole for the micromanipulator was drilled with an NdYag micro-laser (M-2000 Laser, Exitech). Finally the microfluidic channel was obtained by bonding the SU-8 channels to the glass cover with a UV-curable adhesive (Type SK-9, Summers optical) (Fig.3(g)).

Fig. 4(a) shows the top view of the fabricated cell processor prior to bonding of the glass cover. The size of the device is 35×35mm. Fig. 4(b) depicts the scanning electron microscope (SEM) image focused on electrodes for DEP and micro-hole for suction. Fig.4(c) shows the image focused on the PPy valve in the microchannel.

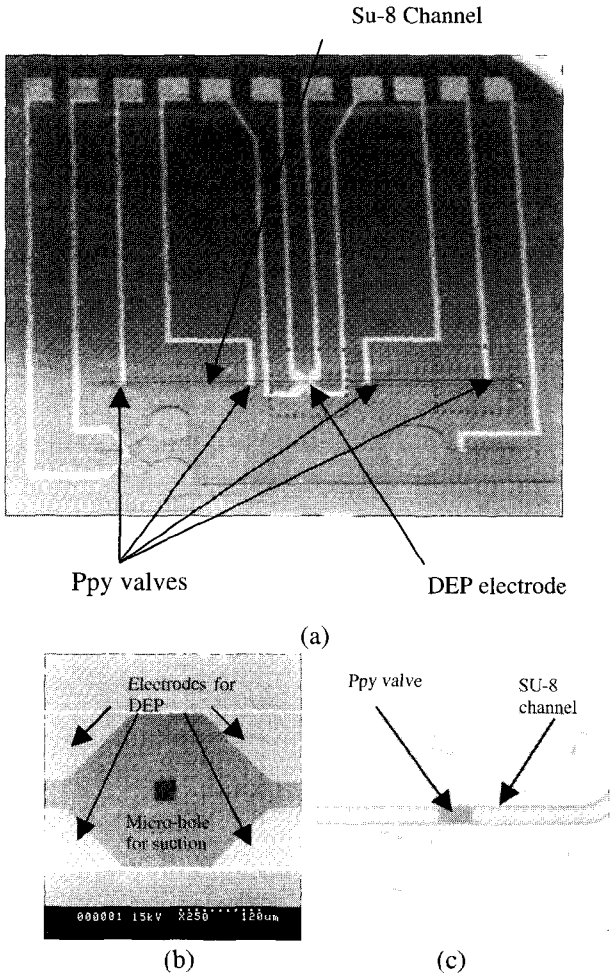


**Fig. 4** The fabricated cell processor: (a) Top view of the device. (b) Detailed top view focused on DEP electrode and micro-hole. (c) Detailed top view focused on Ppy valve.

## 4. Experiment Results and Discussion

### 4.1 Experimental setup

Fig. 5 illustrates the experimental setup for a cell processor. It consists of two function generators, microsyringes (VIT-FIT, Lamda Co.), a stereomicroscope (Leica), and the integrated cell processor. A multi-channeled function

generator (Protek 9302) was used to produce the AC sine wave for DEP. The neighboring electrodes for DEP were driven with a 90°-shifted AC sine wave. In this study, the operating frequency range was between 300 kHz and 1MHz, and the voltage was between 1 and 5V. The other function generator (33250A, Agilent) is used to actuate PPy valves. The flow in the microchannels and at the micro-hole was precisely controlled by microsyringes. The cell behavior in the device is monitored via the microscope and digitally recorded through CCD cameras.

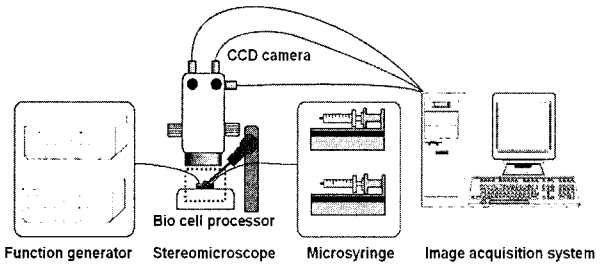


**Fig. 5** Experimental setup.

## 4.2 Operation of the integrated cell processor

### 4.2.1 Transporting and Isolation

Fig. 6(a) illustrates four sequential images that describe the transport and isolation of a cell. At first, a group of cells were moved by a pressure driven flow. Isolation of a cell was achieved when the PPy valve was actuated. The Ppy valve was initially curled up preventing a cell from passing it. It became straight by the bimetal effect of gold-Ppy layers under the voltage of 1-2V, allowing the captured cell to pass once the PPy valve was stretched while the rest of the cells were blocked by another Ppy valve. Any cell that passed the PPy valve was moved to the center of the microchannel by a pressure driven flow (Fig. 6(b)). In our first batch of devices, it was observed that the repeated expansion and contraction of PPy caused the polymer to be peeled off from the Au layer, which leads to reduced strain responses and eventually delamination. We improved surface adhesion between the PPy and Au layer using Thiol treatment [19], and the valve was able to be actuated for several thousand cycles.

### 4.2.2 Cell orientation

The cell was oriented in a desired direction using DEP technique (Fig. 6(c)). As mentioned above, cell orientation should be safe and easy to control and automated. It is known that DEP is safe in biomanipulation, but too high a voltage may lead the joule heating on electrodes. Therefore, we used low voltage ranging from 1V to 2V for the cell orientation, which was enough for cell rotation. Cells were rotated stably with the speed from 0.1 to 0.7 rad/sec at 400 kHz frequency.

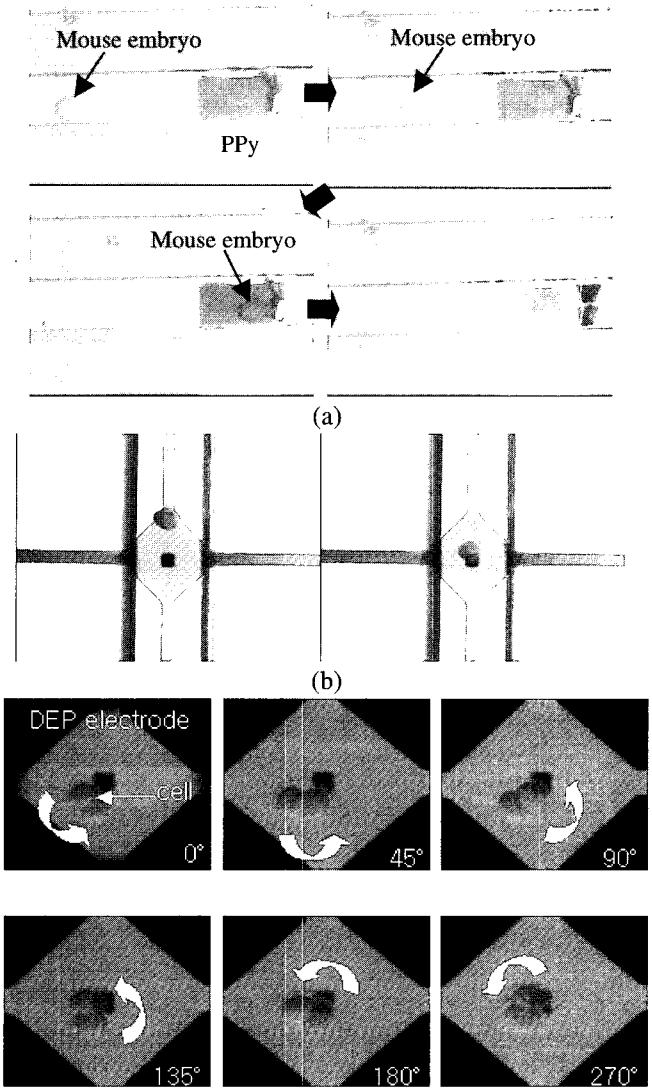


**Fig. 6** Still images from video clips showing the functions of the cell processor: (a) The cell isolation by the actuation of the PPy valve in the microchannel (b) The transporting of the cell to the micro-hole. (c) The cell orientation by DEP.

### 4.2.3 Cell viability test

We investigated the intracellular effects on embryo cells manipulated by DEP. In case of cells *in vivo*, innumerable mitochondria inside the cells can be observed by fluorescent material like Rhodamin123 (Sigma-Aldrich Co.). Fig. 7 shows many mitochondria that appear as a myriad of particles by fluorescence in a healthy cell. If cells are damaged by environmental changes, they enter into the 'Lysis process', and the number of mitochondria will decrease markedly. In order to verify the degree of viability of the cells exposed to the electric field (2V, 400KHz for 60 seconds), we cultured the cells in M16 solution for 150 and 300 minutes. In this experiment, it was observed that the number of mitochondria did not

decrease. In addition, the cells treated by DEP cultured for 86 hours developed normally from 2-cell to blastocyst with a similar ratio in the control group.

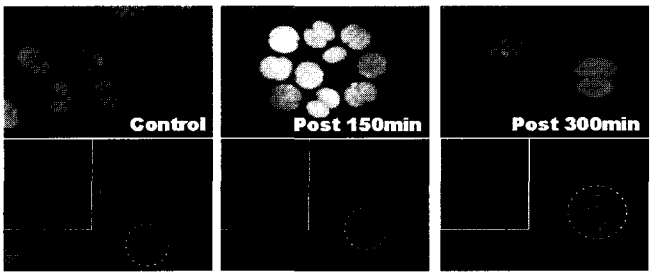


**Fig. 7** Distribution of mitochondria in mice embryo cells after cell was manipulated by DEP.

## 5. Conclusions

This paper presented the design, fabrication and demonstration of an integrated cell processor for single embryo cell manipulation. This device contains multiple functions for handling single cells: transporting, isolation, orientation, and immobilization. These functions were realized with relatively simple and cost-effective MEMS fabrication. The feasibility using the device was successfully demonstrated using mice embryo cells. Cell viability was studied through the distribution of mitochondria in mice embryo cells and cultivation of cells. The developed device will essentially contribute to the automation of the cell manipulation process that is time-consuming and cumbersome using conventional approaches.

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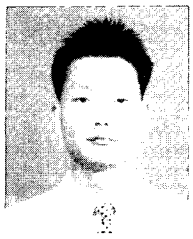

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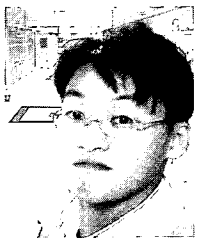

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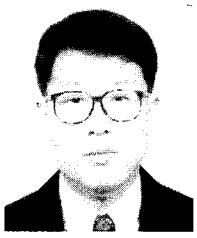

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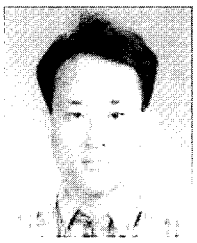

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