A HIGH-THROUGHPUT PLATFORM FOR PATTERNED DIFFERENTIATION OF EMBRYOID BODIES USING AIR BUBBLES

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ABSTRACT

A microfluidic platform for simultaneous patterned differentiation of multiple embryoid bodies of embryonic stem cells using air bubble is developed. Theoretical simulation and experiments using suspension of fluorescent particles or fluorescent solution showed that chemical gradients and little shear existed within the chamber of the devices. EB was pushed by air bubbles into the micro-chambers, after excluding the air bubbles out, EBs are exposed to different media. It was found that patterned differentiation of these EBs was successfully induced. It suggested that the device will help in a wide variety of differentiation using multiple stem cells in biomedical field.

KEYWORDS

Patterned differentiation, air bubble, iPS, high throughput, embryoid body

INTRODUCTION

Patterned differentiation of an embryoid body (so called EB, i.e. cell aggregate) of mouse pluripotent stem cells has been studied successfully by exposing to different factors from a laminar flow pattern, which was formed by two kinds of culture media in a microfluidic device [1, 2]. However, these experiments have applied a culture platform for only one EB; In experiments using microfluidic platforms, it is not easy for seeding, culturing or gathering one EB/derivative cells in the microfluidic device for many analyses. These experimental problems of low throughput and low efficiency have existed for a long time.

In order to study spatiotemporal response of EBs efficiently during their differentiation processes, it is necessary to establish high-throughput ways to analyze them under heterogeneous medium conditions. In the present experiment for that purpose, a new microfluidic device was developed for induction of multiple EBs by using air bubbles. Air bubbles were applied as a tool to manipulate EBs in a microfluidic device. Air bubbles are not easy to be excluded out of the device in many experiments, in fact it is often regarded as one of lethal factors in culturing cell in the microfluidic device, which would affect gas diffusion, media supply, waste discharge etc., correspondingly, cell metabolisms including proliferation and differentiation would be seriously varied. In the experiment, however, we introduced air bubbles into the microfluidic device in safety for controlling EBs, the pressure of the air bubbles is applied to seed EBs into the micro-chambers. After bubbles were excluded out of the microfluidic device, EB differentiation and proliferation can be inspected. By this way, we successfully carried out patterned differentiation of multiple EBs. It would help to understand differentiation experiments in the developmental biology field.

MATERIALS AND METHODS

The microfluidic device as showed in Figure 1 was fabricated using a method of photolithography. At first, the device diagram was drawn by Autocad 3.4, based on which, a GDS file was prepared, then a shadow mask was fabricated using a laser exposing system. The mask is developed for UV exposure. Photosensitive epoxy (SU-8 2100) was spun coated onto a cleaned silicon wafer, followed by being baked at 65℃ for 15 min and 95℃ for 90 min, then it was exposed to UV light through the mask prepared above. After baking, it was developed in SU-8 developer for 15 min and rinsed with IPA (isopropyl alcohol). The wafer was coated with CHF3, then Poly-dimethylsiloxane (PDMS) was poured on the wafer, after degassing, the PDMS block was heated, then it was trimmed to get PDMS model, after adherence onto a slide glass by oxygen plasma and silicon tubes assembling; the microfluidic device can be used for culture experiment.

Before perfusion using the prepared microfluidic device, theoretical simulation using software of Comsol Multiphysics 3.5a and perfusion experiment using suspension of fluorescent particle and fluorescent solution was carried out for determining flowing velocity and chemical concentration in the culture chamber of the microfluidic device. Suspension solution of fluorescent particles (φ0.5um) or fluorescent solution (100mM fluorescein in PBS) was pumped into the device at 2 uL min-1 respectively. After 5 minutes, images of culture chamber were taken by a fluorescent microscope, the fluorescence intensity can be determined by using the software of Image J. Flow velocity of the particles was determined based on the images taken. EB differentiation was induced by perfusing a medium containing FBS (20%) for cardiac differentiation.

Induced pluripotent stem cell (iPS, MEF-Ng-20D-17) was purchased from Kyoto University, cultured on mouse embryo fibroblast (MEF) was maintained in DMEM containing 15% KSR (Knockout Serum Replacement), 0.1% LIF (Leukemia Inhibitory Factor), with medium changed every day. Confluent iPS cells were dissociated and plated onto non-adherent culture plates to form a spheroid (220μm). Spheroids (EBs) were seeded onto the culture chamber of the microfluidic device. Two channels of the microfluidic device were connected to syringe pumps respectively by silicon tubes. Medium containing 20%FBS and/or medium containing LIF was perfused into the device from the two channels at 2ml min-1 respectively. The 3 perfusion methods are scheduled: LIF medium only, FBS medium only, LIF medium and FBS medium perfused simultaneously. After culture, the microphotographs of the EB were taken, EBs can be collected after dismantling the microfluidic device for some analyses.

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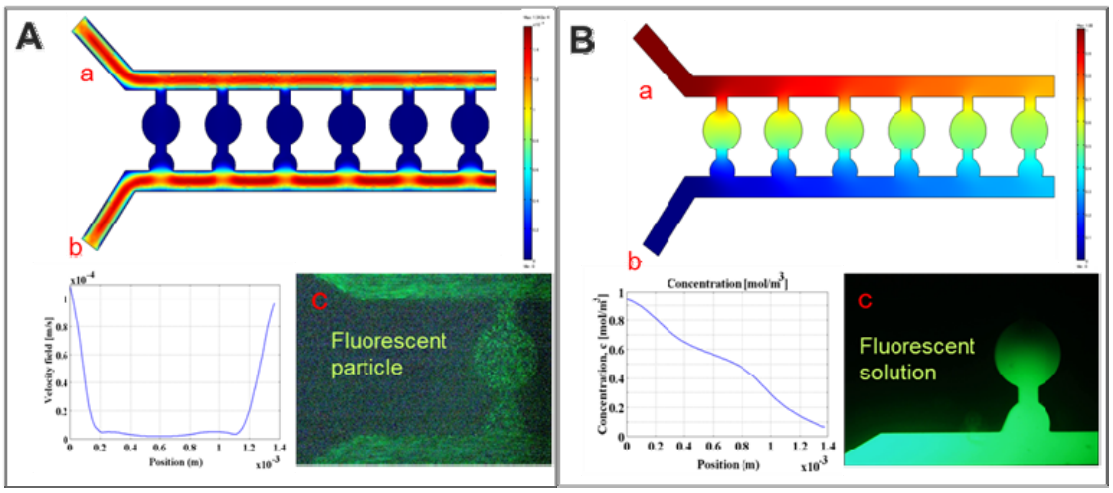

Figure 1. Flowing velocity (A) and chemical gradient (B) in the micro-chamber of the present device estimated by simulation using COMSOL Multiphysics software and perfusion experiments using suspension of fluorescent particles or fluorescent solution. It is shown that flowing velocity of the fluorescent particles is nearly 0 and the chemical gradient is formed within the culture chamber.

RESULT AND DISCUSSION

The present microfluidic device is fabricated as shown in Figure 1, the device has two flowing channels, and some round-shaped culture chambers were located between the channels. In one side of the two channels, there are some trapping cups connected to the chambers, where the EBs are first trapped before being pushed into the culture chamber by air bubbles (Figure 2AB).

The theoretical simulation by COMSOL Multiphysics3.5a indicates that there is little shear flow in the culture chamber (Figure 1A), while chemical gradient can exist stably in the chamber (Figure 1B). By adjusting the flowing velocity, suspension of fluorescent particles was perfused into the microfluidic device, fluorescent particles in the culture chamber could be almost static (flowing velocity is 0) (Figure 1A); gradient fluorescent distribution was also kept after fluorescent solution perfusion into the culture chamber (Figure 1B).

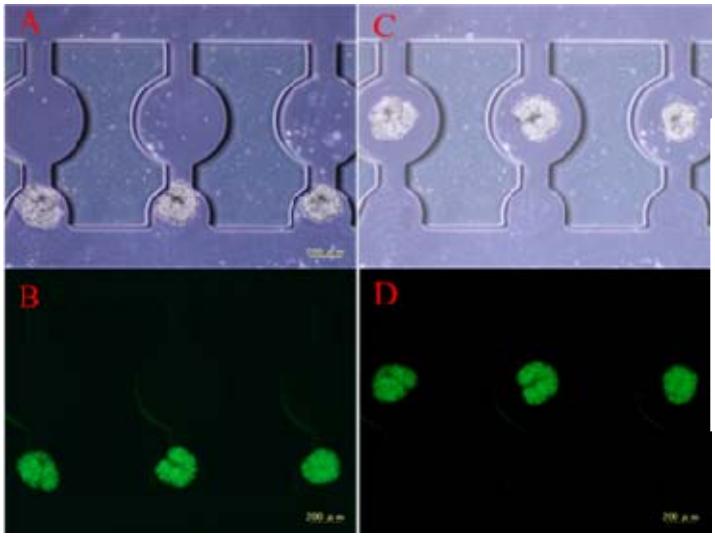

Figure 2. Microphotographs of multiple EBs arrayed in the trapping cups (A&B) and seeded into the microchamber by air bubbles (C&D).
A&C, Microphotographs of photo contrast of EB of Nanog iPS.
B&D, GFP fluorescence microphotographs of EB of Nanog-GFP iPS

After seeding some cell aggregates of Nanog-GFP induced pluripotent stem cells (iPS) into one channel, they would array into the trapping cups by switching on/off some flow inlets and outlets simultaneously (Figure 2AB). Air bubbles were then introduced into the side of the channels with some inlets and outlets on/off. The EBs were then pushed into the culture chambers by the air bubble (Figure 3). After excluding the air bubble out of the microfluidic device by perfusing the medium into the channel (Figure 3), the microfluidic device was connected to pumps via the silicon tube, the differentiation and proliferation of these cell aggregates were studied by persfusing the medium containing 20%FBS and/or medium containing LIF from the flowing channels into the micro-device.

When cultured in medium containing LIF for 5 days in the device, it was found that the fluorescence of iPS cells was kept all the time, it suggested that iPS cells has kept undifferentiated state (pluripotent activity) in LIF media all the time (Figure 4B). After 5 days of perfusion culture in medium containing 20%FBS, it was found that GFP fluorescence intensity decreased then disappeared completely, it suggested that iPS cells were successfully differentiated in 20% FBS medium in the microfluidic device (Figure 4F).

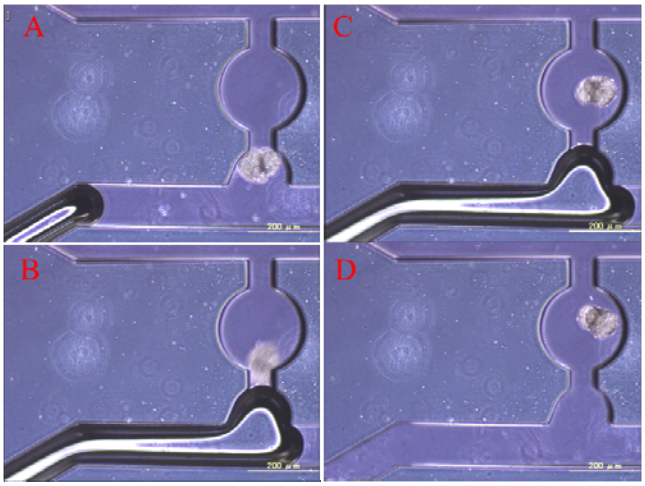

Figure 3. Diagrams showing the procedures of seeding EB into the microchamber using an air bubble
A. Introduction of air bubble into one of the channel of the device from the inlet.
B. EB is entering into the chamber pushed by the air bubble.
C. EB has completely entered in the chamber;
D. Air bubble is excluded out of the device by perfusion of the medium.

If iPS cells were perfusion-cultured with FBS and LIF medium simultaneously, interestingly, it is shown that patterned differentiation of these EBs was induced (Figure 4D), i.e., half of the cell aggregate kept fluorescent, fluorescence of the other half of cell aggregates disappeared, this results suggested that chemical gradient was formed and kept in the culture chamber of the microfluidic device all the time, which regulated the proliferation and differentiation of cell aggregates of iPS.

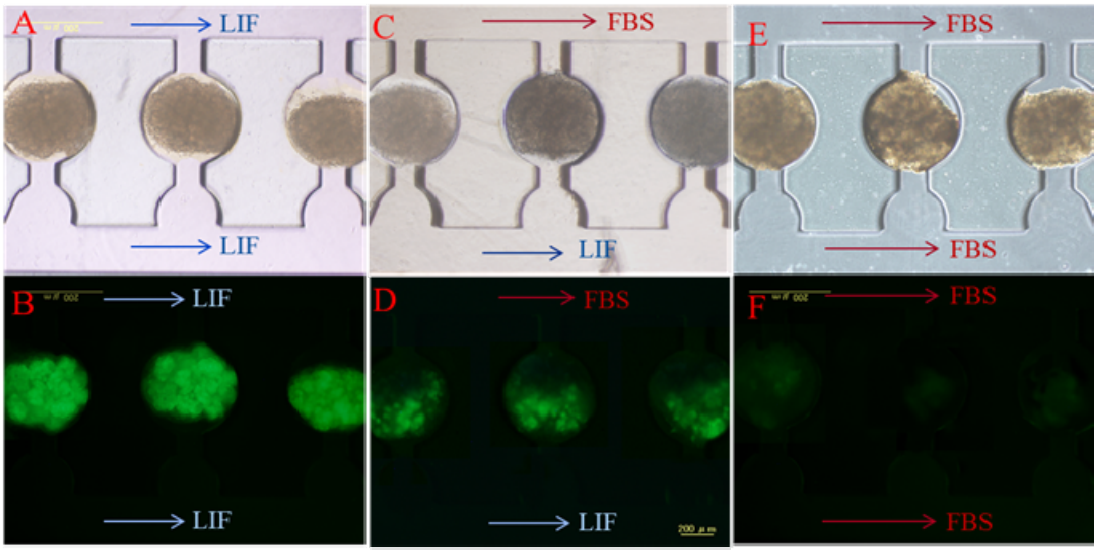

Figure 4. Culture EB in the micro chamber for 5 days using the media containing LIF and/or 20%FBS.
A&B. Perfusion culture in the LIF medium;
C&D Perfusion culture in the FBS medium (upper) and LIF medium (lower) simultaneously;
E&F. Perfusion culture in the FBS medium.

CONCLUSIONS

Because multiple cell aggregates can be seeded and arrayed accurately in the microfluidic device for investigating their differentiation or proliferation with sufficiently small shear acting on them, at the same time, chemical gradient can be formed and kept in the culture chamber of the microfluidic device, which can modulate the cellular metabolism of iPS cells in the culture chamber. Our experimental results suggested that the present method will be useful in a wide variety of experiments in the biomedical field in the future.

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