PAIRING AND FUSION OF HETEROTYPIC CELLS IN A MICROCHANNEL

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ABSTRACT

We present pairing and fusion of somatic cells with embryonic stem (ES) cells in a microchannel. In this study, nuclei of the cells were physically separated with each other during the fusion process in a microchannel. Hemagglutinating virus of Japan envelope (HVJ-E) was used as a fusion reagent. When the slit was smaller than the nuclei of the cells, cell fusion occurred without fusion of their nuclei. The present technique will be utilized to obtain pluripotent cells from somatic cells without genomic contamination.

KEYWORDS: Cell Pairing, Cell Fusion, Embryonic Stem Cells, Reprogramming

INTRODUCTION

Recent studies have revealed that differentiated somatic cells are reprogrammed to pluripotent stem cells under certain conditions *in vitro*. The reprogrammed cells are differentiated again into the desired type of cell and will be utilized for regenerative medicine. Various reprogramming methods such as somatic cell nuclear transfer [1] and induced pluripotent stem (iPS) cells [2] have been reported. Somatic cells are also reprogrammed by fusion with ES cells [3, 4]. However, the fused cells are tetraploid or aneuploid which have both ES cell nucleus and somatic cell nucleus, and will cause rejection if they are transplanted into humans.

Microfluidic devices allow precise positioning of single cells in parallel, by trapping them into weirs under fluid flow in a microchannel [5]. Such a technique has also been utilized to pair and fuse single somatic cell with single ES cell [6]. However, as in the conventional cell fusion, the nuclei of each cell fuse with each other. In this study, we propose a novel method to pair and fuse ES cells with somatic cells through microfabricated structure without fusion of their nuclei. So far, fusion of somatic cells through microfabricated structure have been reported [7]. However, fusion of ES cells with somatic cells through microfabricated structure without fusion of their nuclei has not been reported yet.

PRINCIPLE

Figure 1 shows the concept of this study. In conventional fusion method, both the cytoplasm and the nuclei of the cells are fused. In this study, somatic cells are fused with ES cells through a microslit which is smaller than the nuclei of the cells. As a result, cell fusion occurs without fusion of the nuclei. Figure 2 shows how somatic cells are paired with ES cells through the slit. ES cells and somatic cells are introduced from the separate inlet into the microchannel and trapped into a weir. The weir consists of the trap for ES cells, the trap for somatic cells and a microslit connecting the two traps. The cells which were on the streamlines passing through the traps are trapped and paired while others flow away without trapping. After the trapping, the cells are pushed to the microslit by hydrodynamic force, and kept in contact with each other. After that, HVJ-E solutions are introduced into the channel. HVJ-E adsorbs onto cell membranes at 3 °C, and the HVJ-E on the membranes promotes membrane fusion at 37 °C. If the width of the slit is smaller than the diameter of the nuclei of the cells, the nuclei are expected not to fuse with each other.

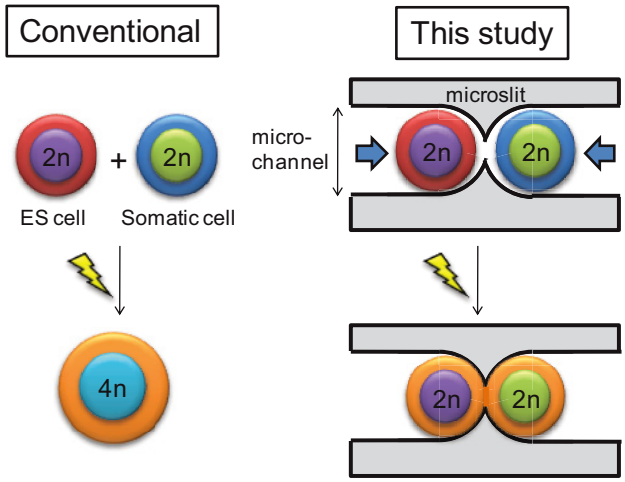

Figure 1. Schematic illustration of cell fusion in conventional method and in this study.

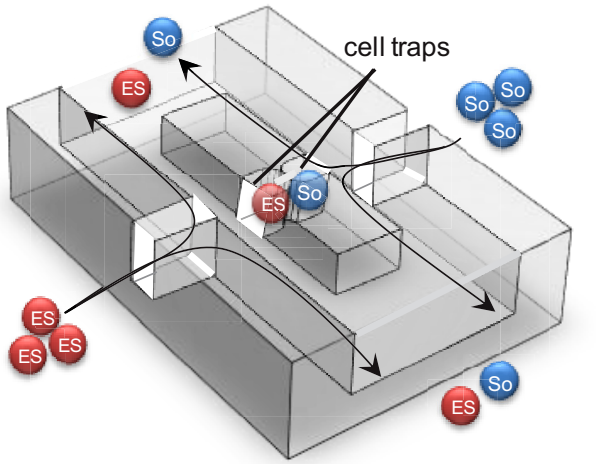

Figure 2. Schematic illustration of cell pairing through microslit in a microchannel.

EXPERIMENTAL

The mouse ES cell line B6G-2 which expressed GFP ubiquitously[8] and a mouse somatic cell line 3T3 were used in this study. Nuclei of the cells were stained with Hoechst 33342 (Invitrogen) when needed. Fusion solution was prepared by mixing 10 μL of HVJ-E with 50 μL of the kit buffer.

A PDMS-glass microchip was fabricated by the standard microfabrication techniques (Figure 3). 3T3 and B6G-2 were introduced into the microchannel by applying negative pressure to the outlet of the channel. After the cells were paired through the slit, HVJ-E [9] solution was introduced to the channel. The temperature of the chip was kept at 3 °C for 10 min to adsorb HVJ-E on the surface of the cells. After that, the chip was heated to 37 °C to initiate cell fusion. Pairing and fusion processes were visualized by a fluorescence microscope. The temperature and humidity of the microscope stage was controlled by a temperature controller (MATS-555RO, Tokai Hit) and cell culture system (INU-ONICS-F1, Tokai Hit).

RESULTS AND DISCUSSION

Figure 4 shows the typical images of cell fusion in the channel when the width of the slit is ~ 10 μm. Nuclei of both cells were stained with Hoechst dye in the experiment. Fluorescence from GFP was located in the B6G-2 side before fusion, suggesting the B6G-2 did not fuse with the 3T3. After heating the cells to 37 °C and incubating them for 30 minutes, green fluorescence was observed from both B6G-2 and 3T3 sides. This means that a part of the GFP was transferred from B6G-2 to 3T3 because of fusion of membranes and cytoplasm of each cell. This experiment was repeated 15 times, and fused pairs were obtained in two trials (fusion rate ~ 0.13). Also, fluorescence from the nuclei was fused as shown in the images, suggesting the width of the slit was too wide to prevent the nuclei from fusion with each other.

Figure 5 shows the images of cell fusion when the width of the slit is ~ 2 μm. In this experiment, the nuclei of B6G-2 were stained with Hoechst dye, while those of 3T3 are not stained. Before the cell fusion, both green and blue fluorescence was observed only from B6G-2. After the cell fusion, green fluorescence was observed from both cells. However, blue fluorescence from Hoechst dye was observed only from B6G-2. This means nuclei of the B6G-2 are kept in their initial location, and do not fused with those of 3T3. In these experiments, fusion rate was lower (~ 0.06) than that in Figure 4. Since the fusion should occur at the location where the HVJ-E connect the membrane of each cell, the contact area of the cells may affect the fusion efficiency.

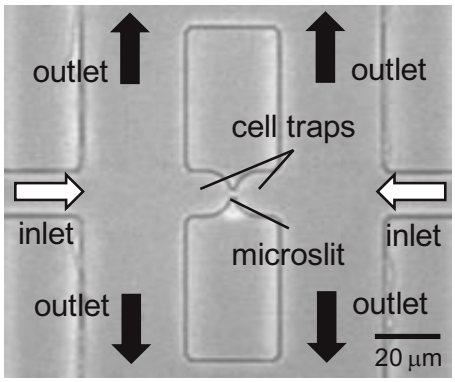

Figure 3. Photo of a microslit and cell traps in the microchannel. Channel depth: 13 μm.

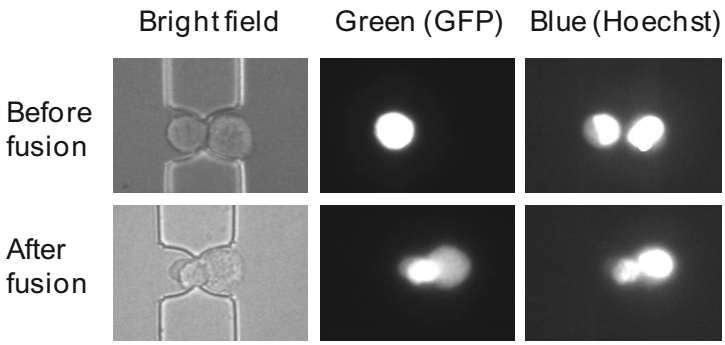

Figure 4. Images of cell fusion in the microchannel. Microslit width: ~ 10 μm.

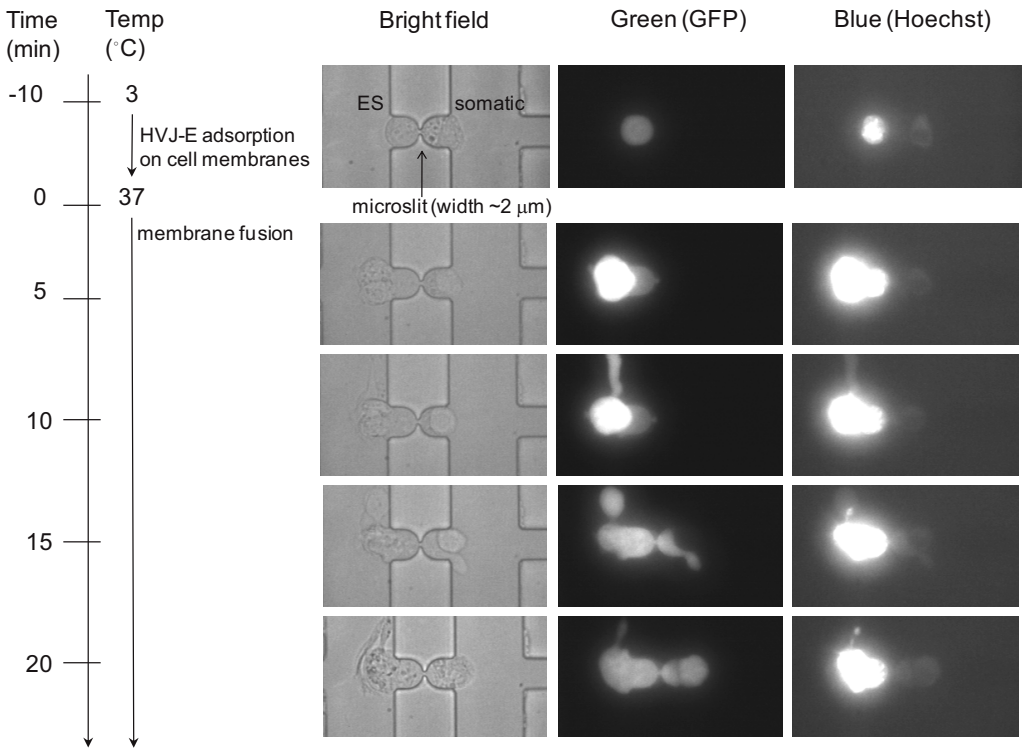

Figure 5. Images of cell fusion in the microchannel. Microslit width is ~ 2 μm.

CONCLUSION

We have presented pairing and fusion of somatic cells with embryonic stem (ES) cells in a microchannel. The present technique will be utilized to obtain pluripotent cells from somatic cells without genomic contamination.

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