HANGING DROP CULTURE DEVICE FOR EMBRYONIC STEM CELL

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ABSTRACT

We invented a microchip for hanging drop (HD) culture of embryonic stem (ES) cells. Our micro HD chip, which is PDMS microchip fabricated from photoresist SU-8 mold on Si-wafer, contained the micro channels open for droplet holes on the surface of device so that the cell suspensions of ES cells or iPS cells formed a droplet with uniform number of the cells. We cultivated both mouse ES cells and iPS cells in this experiment. Our micro HD chip provided an excellent platform for forming the EBs for both mouse ES cells and iPS cells.

KEYWORDS

hanging drop (HD) culture, embryonic stem (ES) cell, iPS cell, cardiomyocyte, mouse ES cell,

INTRODUCTION

In cytological investigation of ES cells or iPS cells, the cells are cultured in medium droplets, called hanging drop (HD) culture, to induce an aggregation to from embryonic body (EB). In the method of HD culture, the embryonic body forms the three dimensional spherical aggregates where the individual ES cells are spontaneously onset the epigenetic alternation and arranged themselves into three dimensional germ layers containing different precursor cells for different organ system.

Conventional HD culture proved to be efficient in EB formation with limitations of medium deprivation, lack of long term culture capabilities, necessity of repeated manual interventions and disturbances at difference stages. The lacking of uniformity of initial number of ES cells in each droplet usually resulted in the undifferentiated cells or cell apoptosis. Our micro HD culture chip, which is made of PDMS microchannel, provides the uniformity of the number of cells in droplet as well as a fresh nutritive medium.

Microfluidic device, which integrated chemical and biological analysis's in micro channel, provides the grater control over the fluid manipulations at micro scale. Several microfluidic approaches have been reported for single cell culture. The purpose in those reports for cell culture is integration of the single cell isolation and single cell culture in the microchannel [1,2]. Those microchannels were designed for the cultivation from the target single cells for a long term culture. Therefore, besides the controlling the microfluid, the microchannel structure that allow the cells to form the spherical aggregation is essential for the culture of the EB formation from ES cells. Moreover the EBs were either put under shear stress from the flowing liquid inside micro channels which inhibit to form the aggregation and orientation with ES or iPS cells to EBs.

In this paper, we are proposing a simple microfluidic HD culture platform that ensures production of EBs of size and shapes in numbers. Our HD platform was based on the centrifugal forces for cell aggregation and the gravity for the formation of sphere droplets. ES or iPS suspensions were injected in the upper injection channel and flew in the microchannel by the centrifugal force and the droplets containing about 200 cells were formed on the well at the end of channel. The channels also provided the fresh medium for the droplet to grow the ES cells. We cultivated both mouse ES cells and iPS cells in this experiment. We compute the sphericity of those EBs cultivated in both our chips and control to evaluate the morphology evolution of EBs. Our micro HD chip provided an excellent platform for forming the EBs for both mouse ES cells and iPS cells.

EXPERIMENTAL

The working principle for our microchip HD chip is a gravity and centrifugation. Our micro HD chip, which is PDMS microchip fabricated from photoresist SU-8 mold on Si-wafer, contained the micro channels open for droplet holes on the surface of device so that the cell suspensions of ES cells or iPS cells formed a droplet with uniform number of the cells. (Figure 1)

The same cell suspension was introduced into the chips manually for the formation of droplets of desired sizes (20 to 30 μl in volumes) containing desired number of cells (500, 1000, 1500 or 2000 cells). The pumping, in these experiment were done manually after taking the amount of cell suspension needed to the droplet formation into the syringe, but in ultimate use of the chip these pumping can be easily automated with the help of a syringe pump or can be integrated into the chip by using valve controls. After the formation of droplets, chip containing lid was kept on the lower part of the tissue culture dish in order to form hanging drops. The lower dish was filled with sterile Milli-Q water to check evaporation. In the future designs, the measures for controlling evaporation can be incorporated into the stand

alone chips.

The medium was stoked though the same channel resulting the long term culture. After the formation of droplet, the chip was kept in the incubator at 37 degree in a 5% CO_2 environment for 72 hour for cells to form the EB.

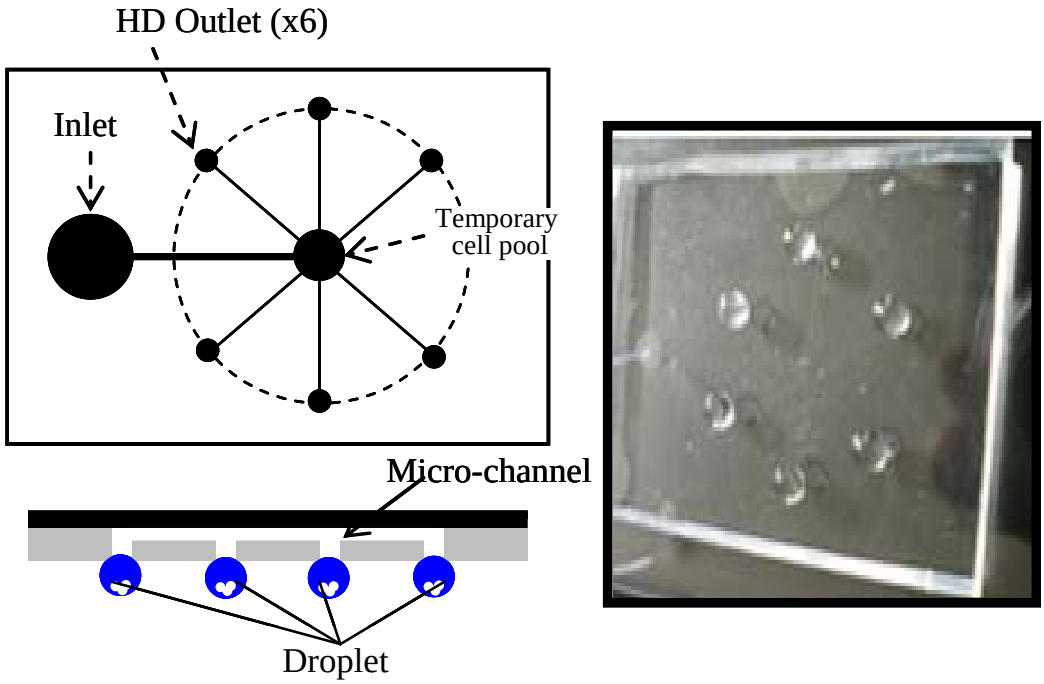

Figure 1: Our microchip hanging drop (HD) culture chip for ES cells and iPS cells

RESULT and DISCUSSION

We tried four, five and six drop out let design with the outlet channels forming different angles with each outlet channel. We found that the chip containing the 6 outlet with 60 degree was the best for forming the droplet. The cells suspension was introduced into the chip for the formation of droplets desired size of 20 μl containing desired number of cells. The initial number of cells in droplet formed by the cell suspension flowed through the channel was depended on the number of cells in suspension but the initial number of cells was predictable.

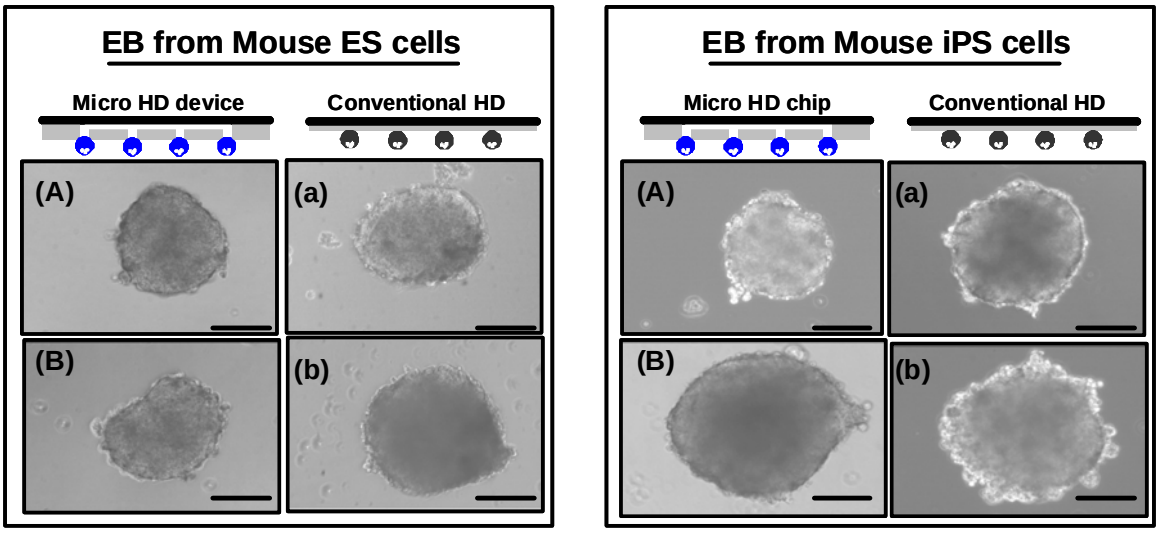

Figure 2. Images of mouse iPS (left) and EBs incubated 72hours after making hanging droplet with our microHD device (left column in each figure), and conventional HD in deferent initial number of cells in each droplet. EBs upper 2 images, (A) and (a), are stated at 1000 cells. EBs in (B) and (b) are grown from 1500 cells.

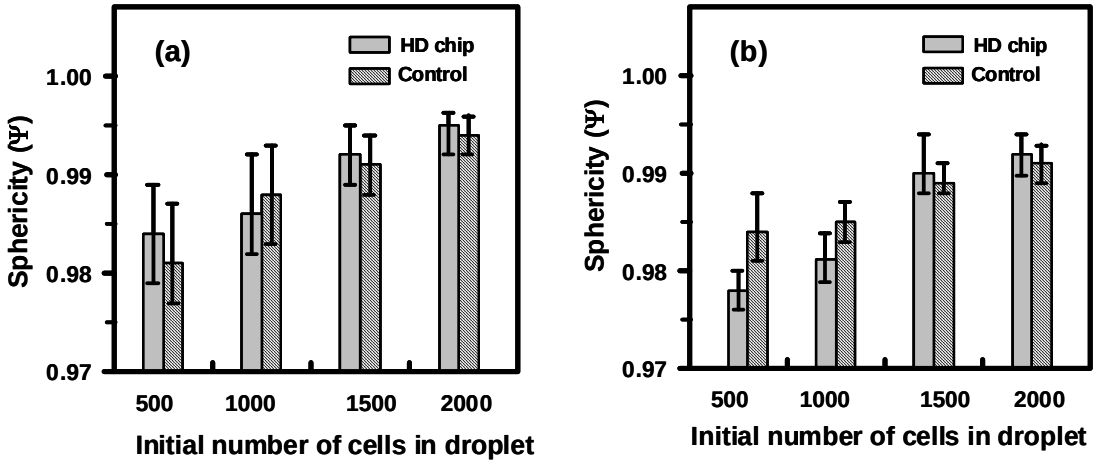

Figure 3. The sphericity of each EB cultured by our micro HD device. (a)EBs cultured from mouse ES cells, and (b)EBs cultured from mouse iPS cells.

EBs of varying sizes were formed from varying cell density in droplets in our micro HD chip. We cultivated both mouse ES cells and iPS cells in this experiment. After 72 hours we obtained EBs of comparable size and shapes to those from control platform. (Figure2).

We compute the sphericity of those EBs cultivated in both our chips and control to evaluate the morphology evolution of EBs.(Figure 3) We observed that the sphericity of EBs were satisfactory while the sphericity in the EB formed from mouse iPS cells were strongly depended on the initial number of cells in droplet. Our micro HD chip provided an excellent platform for forming the EBs for both mouse ES cells and iPS cells.

We observed that the EBs cultured in our HD device was more round than the EBs of the cells by conventional HD system. The sphericity of the EBs from ES cells in our HD culture system is slightly more round than that of the EBs in conventional HD system. In both ES and iPS EB by our HD culture device, the roundness of EBs, which in initial number of cells was more than 1500 cells in droplet, was more spherical because the large number of cells consumed more medium, thus the droplet in conventional HD system shrank gradually by the cell metabolism.

Our HD microchip successfully created the ES and iPS EBs after three to four days of culture. We cultivated both mouse ES cells and iPS cells in this experiment. After 72 hours cultivation, we obtained EBs of comparable size and shapes to those from control platform. We compute the sphericity of those EBs to evaluate the morphology evolution of EBs and we observed that the sphericity of EBs were satisfactory while the sphericity in the EB formed from mouse iPS cells were strongly depended on the initial number of cells in droplet. Our micro HD chip provided an excellent platform for forming the EBs for both mouse ES cells and iPS cells.

CONCLUSIONS

We report our simple but effective microfluidic microchip to produce mouse ES and mouse iPS cell aggregates of desired sizes and shapes in this research. Our microfluidic platform in this paper was designed based on the hanging drop culture system with the microchannel structure to provide a fresh medium to keep the spherical shapes of the EBs in the droplet. Our HD microchip provided the uniform droplet and the number of cells with small variability in droplet. Our HD microchip has the potential for medium supplementing for long term EB culture and for being appended into an integrated EB and differentiation platform in the future.

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