MICROFLUIDIC PERFUSION CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELL IN MICROCHAMBER ARRAY CHIP

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ABSTRACT

This paper reports on the microfluidic perfusion culture of human induced pluripotent stem cells (hiPSCs) under fully defined culture condition. By screening various ECMs we determined that fibronectin and laminin are appropriate for microfluidic devices made out of the most popular material, polydimethylsiloxane (PDMS). We found that the growth rate of hiPSCs under pressure-driven perfusion culture conditions was higher than under static culture conditions in the microchamber array. We believe that our system will be a platform technology for future large-scale screening of fully defined conditions for differentiation cultures on integrated microfluidic devices.

KEYWORDS: Human induced pluripotent stem cells, Microchamber array, Perfusion culture

INTRODUCTION

Human induced pluripotent stem cells (hiPSCs) are capable of self-renewal and pluripotency [1, 2], and are expected as a promising cell source for regenerative medicine and drug screening. For these applications, undefined factors supplemented in conventional culture conditions are not desirable, because these undefined factors contain a variety of growth factors, hormones, and integrin receptors, which maintain hiPSCs in an undifferentiated state. Generally, an appropriate defined culture condition for maintenance and differentiation culture could potentially been found through large scale screening of culture conditions [3, 4].

The use of conventional culture vessels for large-scale screening of culture conditions is labour intensive and cost prohibitive. Furthermore, screening of differentiation conditions in such vessels is intrinsically limited by poor spatiotemporal control of the microenvironment, because it is difficult to determine whether exogenous factors act directly or through paracrine-dependent mechanisms. To overcome these limitations, we and other research groups have applied a microfluidic device to high-throughput cellular microenvironment screening [5-7]. In this study, we applied this microfluidic perfusion culture device to cultivation of hiPSCs under fully defined culture condition for the first time.

EXPERIMENTAL

The perfusion culture microchamber array chip had an 8 × 8 array of microchambers. To fabricate a photoresist pattern as a master template, we used a multilayer photolithography method and SU-8 negative photoresists (SU-8 3005, 3025, and 2075, MicroChem, Corp., Newton, MA) [5, 6, 8]. A sequence of four steps, consisting of spin-coating, soft-baking, exposure, and postexposure baking, was repeated for three cycles. Then the photoresist pattern was developed in ethyl lactate and washed with isopropanol. The master template was treated with tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane at 25 °C for 3 h. PDMS prepolymer and curing agent, Sylgard 184 (Dow Corning), were thoroughly mixed at a 10:1 weight ratio and poured onto the master template. After curing in an oven at 120 °C for 2 h, the micropatterned PDMS plate was peeled from the master template. The macroscopic liquid reservoirs and a flat PDMS plate were fabricated from PDMS with an acrylic resin template and glass plates. After the surface was oxidized by O_2 plasma with the use of a PR500 plasma reactor (Yamato Scientific Co. Ltd. Tokyo, Japan), the microchamber array chip was assembled with a flat PDMS plate and two macroscopic liquid reservoirs [5, 6].

We designed a perfusion culture microchamber array chip that was suitable for ECM coating, cell loading, and hiPSC perfusion culture based on our previous reports. ECM coating solution and cell suspensions are loaded into all 64 microchambers from a cell-inlet/medium-outlet port

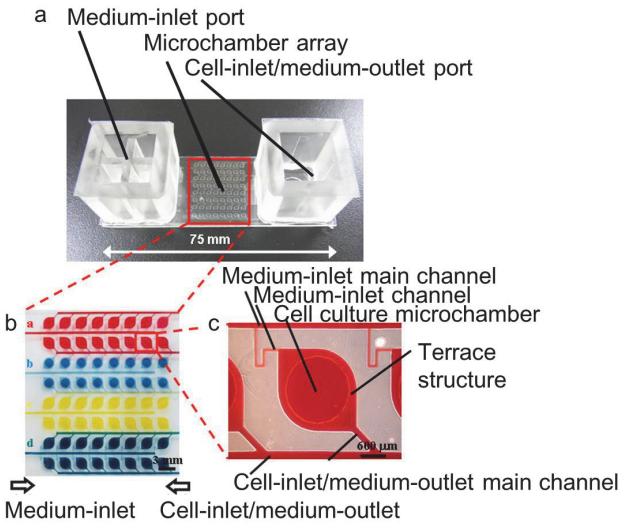

Figure 1: Structure of the perfusion culture microchamber array chip. (a) Overview of the perfusion culture microchamber array chip. (b) Enlargement the microchamber array. To visualize the microfluidic networks on the perfusion culture microchamber array chip, dye solutions (red: new coccine, blue: gardenia blue, yellow: gardenia yellow, green: fast green) were introduced into the microchamber array. (c) Enlargement of the microchamber. Note that the color density designates three different depths of microstructure.

(Fig. 1a, right) via five cell-inlet/mediumoutlet main channels (Fig. 1b and 1c). The culture media are supplied from a medium-inlet port (Fig. 1a, left) via four mediuminlet main channels (Fig. 1b and 1c). We screened appropriate extracellular matrix (ECM) for microfluidic device made out of PDMS, which is most popular material for fabrication of microfluidic device, and then we found fibronectin is an appropriate ECM.

For all experiments, hiPSCs were cultured in the fully defined culture conditions based on hESF-9a medium [4]. The assembled microchamber array chip was filled with PBS, coated with 1 μg/cm^2 fibronectin solution in PBS for more than 12 h at 37 °C, and used without any washing process. hiPSCs were harvested and suspended in hESF-9a medium. The cell suspension (4.2 × 10^5 cells/mL) was added to the cell-inlet/medium-outlet port by using a micropipette, and the cells were loaded into the microchambers by applying 5 kPa of pressure to the same port.

The cell-loaded array chips were first incubated under static culture conditions in a CO_2 incubator to induce cell adherence on the microchamber surface. After 24 h, intermittent perfusion of hESF-9a medium was started by applying sequenced pressure (Fig. 2) with a sequenced pressure control system (Engineering System Co. Ltd., Matsumoto, Japan) equipped with an S100 air pump (Atem Corp., Tokyo, Japan) and PR-4102 pressure regulators (GL Science, Tokyo, Japan). Controlling the range of applied pressure at 5 to 50 kPa enabled intermittent perfusion culture with the desired medium flow-rate range of 10 to 50 μL/h.

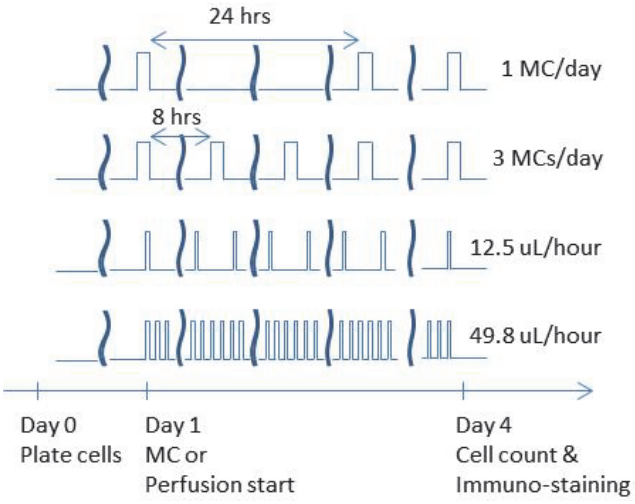

Figure 2: Schematics of medium change protocols under static culture and perfusion culture. hiPSCs were loaded at day 0 and placed to adhere in microchamber for overnight. From day 1 after plating, medium were changed with one medium change per day (1 MC/day) and three medium changes per day (3 MCs/day), or perfused at indicated flow rate.

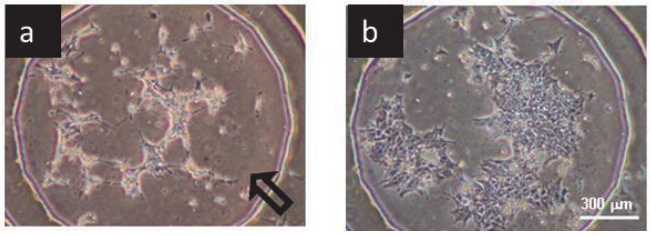

Figure 3. Cultivation of hiPSCs in the perfusion culture microchamber on days 1 (a) and 3 (b). Medium perfusion was started on day 1. Scale: 300 μm

RESULTS AND DISCUSSION

After cell loading into microchamber array coated with fibronectin, hiPSCs were cultured in static culture condition for 24h, and then perfusion culture with fully defined culture medium for hiPSCs (hESF-9a) [4] was carried out at various medium flow rates (Fig. 2). We observed adhesion and extension of hiPSCs on the microchamber surface after 24h static culture (Fig. 3a), and we confirmed growth of hiPS cells in the microchamber array for 2 days of perfusion culture at 12.5 μL/hour of perfusion rate (Fig 3b). After 2 days of perfusion culture, the cells formed normal hiPSC colonies (Fig. 3b), which were tightly packed, and flat colonies consisting of cells with large nuclei and scant cytoplasm [1], suggesting that we had successfully cultured hiPSCs in the microchamber array under fully defined conditions.

Culturing cells in a conventional culture dish requires that the medium be changed every one to three days to supply nutrients and remove waste products, and it is recommended to change the culture medium of hiPSCs every day [4]. Moreover, autocrine or paracrine factors or both affect proliferation and differentiation of hiPSCs. Thus hiPSCs self-renewal could be affected by an excessive perfusion flow rate as well as a lack of perfusion. To reveal the effects of medium perfusion on culturing hiPSCs, we cultured the cells under four perfusion conditions for 3 days after 1 day of static culture (Fig. 2 and 4). As a control, the cells were cultured in ESF9a medium on fibronectin coated normal tissue culture dishes. The number of cells at static culture with 1 MC/day was the smallest, that with 3 MCs/day was middle, and that with the two perfusion culture conditions and the control condition were the same and the largest (Fig. 4), suggesting that perfusion culture is suitable for culturing hiPSCs in microchamber array chip.

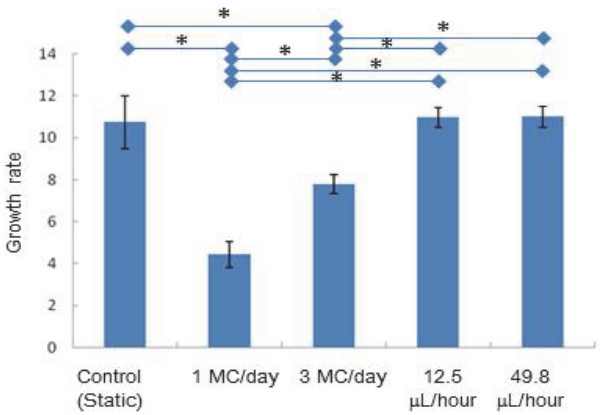

*Figure 4. Proliferation of hiPSCs in the perfusion culture microchamber array. Growth rate was defined as number of cells at day 4 was divided by that plated at day 0. mean ± SE, n = 3. * P < 0.01 Tukey-Kramer multiple comparison.*

CONCLUSION

We have designed and fabricated a PDMS microchamber array chip suitable for ECM coating, controlled cell-loading, and microfluidic perfusion cultures. We applied microchamber array chip to perfusion culture of hiPSCs in defined culture medium, hESF-9a. We used intermittent perfusion driven by sequenced applied pressure in the microchamber array and found that the growth rate of hiPSCs under perfusion culture conditions was higher than that under static culture conditions in which the medium was replaced one or three times a day. We believe that the system developed here will be a platform technology for future use in drug discovery and differentiation analysis using fully defined culture media.

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