Biochemical and Biophysical Research Communications 453 (2014) 131–137

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Single-cell cloning and expansion of human induced pluripotent stem cells by a microfluidic culture device

Taku Matsumura [a], Kazuya Tatsumi [b], Yuichiro Noda [a], Naoyuki Nakanishi [a], Atsuhito Okonogi [a], Kunio Hirano [a,*], Liu Li [c], Takashi Osumi [a], Takashi Tada [d], Hidetoshi Kotera [e,*]

[a] Research and Development Division, ARKRAY, Inc., Kyoto 602-0008, Japan
[b] Mechanical Engineering and Science, Faculty of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8501, Japan
[c] Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan
[d] Stem Cell Engineering, Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-cho, Shogo-in, Sakyo-ku, Kyoto 606-8507, Japan
[e] Micro Engineering, Faculty of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto 606-8501, Japan

ARTICLE INFO

Article history:
Received 12 September 2014
Available online 27 September 2014

Keywords:
Microfluidic culture
Single-cell cloning
Microenvironment
Culture conditions
Human induced pluripotent stem cells

ABSTRACT

The microenvironment of cells, which includes basement proteins, shear stress, and extracellular stimuli, should be taken into consideration when examining physiological cell behavior. Although microfluidic devices allow cellular responses to be analyzed with ease at the single-cell level, few have been designed to recover cells. We herein demonstrated that a newly developed microfluidic device helped to improve culture conditions and establish a clonality-validated human pluripotent stem cell line after tracing its growth at the single-cell level. The device will be a helpful tool for capturing various cell types in the human body that have not yet been established *in vitro*.

1. Introduction

Microfluidic culture systems have many advantages for analyzing and manipulating cells, such as a confined culture area, precise control of medium flow, low consumption of reagents, and the ability to perform high-throughput analyses by designing parallel experiments on a single chip [1]. These features allow the microenvironments of cells, which consist of cellular and non-cellular biochemical, bioelectrical, and biophysical factors, to be mimicked [2,3]. These systems have been developed over the past two decades in many fields including drug screening [4], cell engineering [5], and developmental biology [6] where they have been used to analyze underlying mechanisms. Additionally, conventional static culture systems cannot avoid an accumulation of metabolites like ammonia and lactate, and are also unable to maintain consistent levels of nutrients. Constant culture medium composition surrounding the cells is important when investigating the cellular response to a specific factor.

Although the properties of a cellular population are generally analyzed as an average, many studies have indicated that each cell in a culture exhibits different behaviors [7]. To approach such clonal differences, each single cell and the progeny should be traced and separated from others within an analysis [8]. Furthermore, it is favorable for the cells being analyzed to be recovered and expanded as a cell line. Many studies using microfluidic devices have focused on analyzing cellular responses, whereas only a few have established clonal populations [9]. Generally it is difficult to recover cells from a microfluidic device after analyzing, because of its complicated structure. Therefore, we herein attempted to construct a simple microfluidic perfusion culture device that could culture cells for more than two weeks, and that could be removed from the base plate in order to recover the clonal population. All functions to maintain proliferation of cells were designed in a simple transparent polydimethylsiloxane (PDMS)-chip, except for a pump and medium reservoir. The clonality of the cells was validated by microscopic lineage tracing. We also attempted to optimize the culture conditions in order to improve cell-survival rates.

We chose human induced pluripotent stem cells (hiPSCs) as a model of adherent cells to be cultivated in the microfluidic device. hiPSCs hold promise as tools for regenerative medicine and drug discovery because of their unique abilities to proliferate and differentiate into all cell types in the human body [10,11].

* Corresponding authors. Fax: +81 (0)75 753 5291 (H. Kotera).
E-mail addresses: hiranok@arkray.co.jp (K. Hirano), Kotera_hide@me.kyoto-u.ac.jp (H. Kotera).

http://dx.doi.org/10.1016/j.bbrc.2014.09.081
0006-291X/

2. Materials and methods

2.1. Cell culture

hiPSCs were established from human fetal lung fibroblasts (TIG1, JCRB Cell Bank) through the retroviral induction of *Oct4*, *Sox2*, *Klf4*, and *c-Myc* and stably maintained in mouse embryonic fibroblast (MEFs)-conditioned medium (CM) {DMEM/F12 (Sigma–Aldrich, Madison, WI, USA) supplemented with 20% knockout serum replacement (Life Technologies, Carlsbad, CA, USA), L-glutamine, non-essential amino acids, 2-mercaptoethanol, and 10 ng/ml bFGF (Peprotech, Rocky Hill, NJ, USA) on Matrigel-coated dishes. We used hiPSC-conditioned CM (cCM) for single-cell cloning.

2.2. Fabrication of the microfluidic culture device

Microchannels were constructed by grafting a PDMS layer onto a poly-L-lysine (PLL)-coated glass slide (Matsunami Glass Ind., Ltd., Kishiwada, Japan). The PDMS layer was fabricated using molding masters made with aluminum. Thermally curable PDMS (Silpot 184; Dow Corning Toray, Tokyo, Japan) was poured onto the mold to achieve a thickness of 3 mm and cured at 80 °C for 1 h in an oven. Two turndown microchannels (width, 0.5 mm; length, 20 mm; height, 0.5 mm) were formed in the PDMS layer when it was released from the mold. The device was then clamped together with the lid using retainer plates and bolts. Tubes (PTFE tube TUF-100 series AWG-30, Chukoh Chemical Industries, Japan) were attached to the inlet holes in the PDMS layer and connected to a peristaltic pump (Aquatech Japan, Inc., Osaka, Japan). The cell isolation device was constructed by adding a PDMS layer between the microchannel-formed PDMS layer and PLL-coated glass slide. The cell isolation PDMS layer formed 27 wells and was fabricated using the same method, except at a final thickness of 500 μm. The diameter of the wells was designed to be 1 mm.

2.3. Single hiPSC cloning in the microfluidic culture device or in dishes

The microchannel was coated with human recombinant laminin 521 (Veritas, Tokyo, Japan). hiPSCs were dissociated using 0.25% trypsin (Gibco, Carlsbad, CA, USA)/0.04% ethylenediaminetetraacetic acid (EDTA) and the cells were suspended in 400 μl of fresh medium at a density of 5.0×10^2 cells/ml. The cells were introduced into the inlets of the microchannels using a peristaltic pump at a flow rate of 21 μl/min. The cells were cultured in cCM at a flow rate of 5000 nl/min in a CO_2 incubator; on day 0, the cells were cultured without flow. To trace the clonal population in the dish, an adhesive tape with a grid pattern (AGC TECHNO GLASS CO., LTD, Tokyo, Japan) was placed on a laminin-coated 35 mm dish. hiPSCs were dissociated and seeded at 50 cells/cm^2 density.

2.4. Recovery and expansion of the clonal cell line

To extract the expanded colony, the PDMS layer was first removed in prewarmed DMEM from the glass plate. The single hiPSC-derived colony in the microchannel was picked up using a glass capillary tube (Drummond Scientific Co., Broomall, PA, USA) under phase contrast microscopy. The cells were reseeded on a laminin 521-coated 35 mm culture dish in CM. The cells were subcultured every 4 days by TrypLE Express (Gibco), and plated on laminin 521-coated dishes.

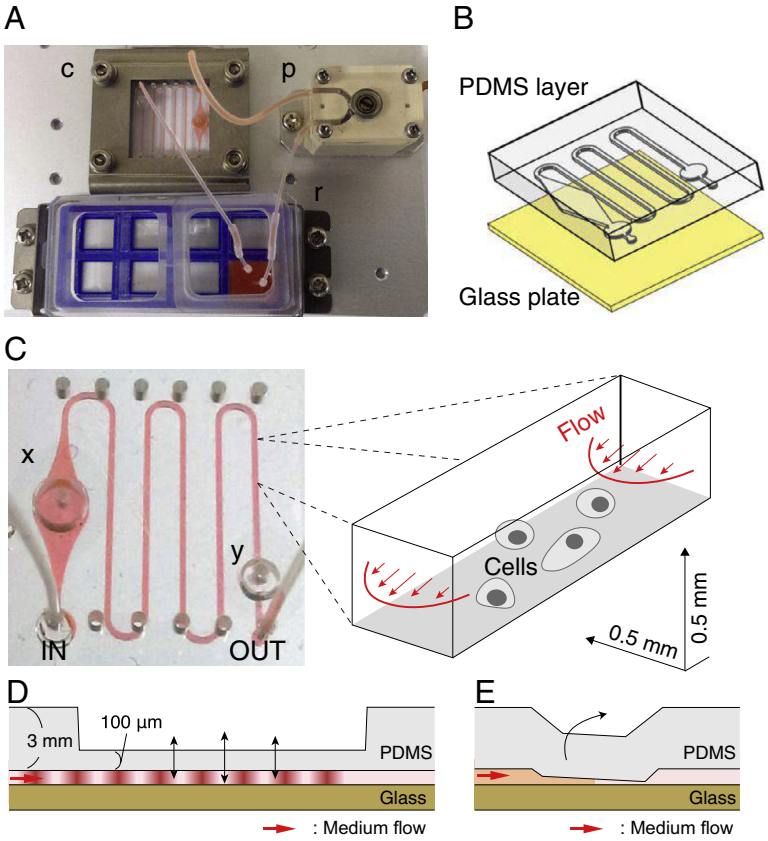

Fig. 1. Microfluidic culture device for single hiPSC cloning. (A) The microfluidic culture device contained a peristaltic pump (p), microchannel chip (c), and medium reservoir (r). (B) Structure of the microchannel chip. The PDMS layer containing the microchannel was placed on a glass plate. (C) Design of the microchannel chip. The microchannel containing the growth area was flanked by a diaphragm damper (x) and pressure valve (y). The right panel shows a scheme of microfluidic flow and the size of the microchannel. (D) Diaphragm damper. The thin PDMS layer constituting the roof of microchannel expanded and contracted to maintain a uniform flow rate. (E) Pressure valve. The PDMS pillar on the PLL-coated glass plate upregulated the pressure in the microchannel.