Biochemical and Biophysical Research Communications 487 (2017) 344–350

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Closed-channel culture system for efficient and reproducible differentiation of human pluripotent stem cells into islet cells

Kunio Hirano [a, **, 1], Shuhei Konagaya [b, 1], Alexander Turner [a], Yuichiro Noda [a], Shigeru Kitamura [a], Hidetoshi Kotera [c], Hiroo Iwata [b, *]

[a] Research and Development Division, ARKRAY, Inc., Kyoto, 602-0008, Japan
[b] Institute for Frontier Medical Sciences, Kyoto University, 53 Kawahara-cho, Shogo-in, Sakyo-ku, Kyoto, 606-8507, Japan
[c] Faculty of Engineering, Kyoto University, Yoshida Honmachi, Sakyo-ku, Kyoto, 606-8501, Japan

ARTICLE INFO

Article history:
Received 3 April 2017
Accepted 12 April 2017
Available online 13 April 2017

Keywords:
iPS cells
Induced differentiation
Microfluidics
Pancreatic islet cells
Microwells
Cellular aggregates

ABSTRACT

Human pluripotent stem cells (hPSCs) are thought to be a promising cell-source solution for regenerative medicine due to their indefinite proliferative potential and ability to differentiate to functional somatic cells. However, issues remain with regard to achieving reproducible differentiation of cells with the required functionality for realizing human transplantation therapies and with regard to reducing the potential for bacterial or fungal contamination. To meet these needs, we have developed a closed-channel culture device and corresponding control system. Uniformly-sized spheroidal hPSCs aggregates were formed inside wells within a closed-channel and maintained continuously throughout the culture process. Functional islet-like endocrine cell aggregates were reproducibly induced following a 30-day differentiation protocol. Our system shows an easily scalable, novel method for inducing PSC differentiation with both purity and functionality.

1. Introduction

Human embryonic stem cells (hESCs) [1] and human induced pluripotent stem cells (hiPSCs) [2] have attracted a great deal of attention from the medical sciences due to their self-renewal capabilities and pluripotency, and have been examined as cell sources for regenerative medicine and for drug screening. It has been reported that various types of somatic functional cells can be obtained by differentiating hESCs/hiPSCs *in vitro* by mimicking the process of embryonic development [3]. Some clinical trials for the treatment of age-related macular degeneration, Parkinson's disease, spinal cord injuries, myocardial infarction, and type I diabetes have already started [4].

In some cases, however, it has been difficult to reproduce published results, even when the reported procedures were strictly followed. These discrepancies might be caused by differences in cell strains or culture media, or the failure may simply be the result of inexperience with regard to cell culture techniques. In our previous study, we developed a method for the differentiation of hiPSCs to pancreatic endocrine cells [5]. Cell aggregates were prepared from hiPSCs in an agarose-gel microwell plate and maintained in series of culture media containing different factors and chemicals. We were able to successfully derive pancreatic endocrine cells from three different strains of iPS cells. However, differentiation efficiency was both technician and lot dependent. Additionally, it was difficult to maintain sterile conditions in the microplate over the 30-day culture period due to the frequent replacement of culture media. In this study, we developed a closed channel-based culture system to reduce the risks described above. Human iPSCs were differentiated into pancreatic islet cells as the working example.

2. Materials and methods

2.1. Cell line

The human induced pluripotent stem cell (hiPSC) line 253G1 was the primary cell line used in this study. The 253G1 cell line was stably maintained in DMEM/F12 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 20% (v/v) knockout serum replacement (Life Technologies, Carlsbad, CA, USA), 0.1 mM nonessential amino

* Corresponding author.
** Corresponding author.
E-mail addresses: hiranok@arkray.co.jp, kuniohirano@gmail.com (K. Hirano), iwata@frontier.kyoto-u.ac.jp, hiwata00@gmail.com (H. Iwata).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2017.04.062

acid (Life Technologies), 2 mM l-glutamine (Sigma-Aldrich), 0.1 mM 2-mercaptoethanol (Nacalai Tesque, Kyoto, Japan), and 5 ng/mL basic fibroblast growth factor (Wako Pure Chemical Industries, Osaka, Japan) with a feeder layer of SNL 76/7 cells (ECACC, Salisbury, UK) [6]. The other hiPSC line used in this study, the 771-2 cell line, was cultured in StemFit AK02N medium (ReproCELL Inc., Kanagawa, Japan) using Geltrex (Thermo Fisher Scientific, Waltham, MA)-coated dishes. The 771-2 was genome integration-free hiPSC line that was reprogrammed by microRNA cocktail and polycistronic self-replicative RNA.

2.2. Fabrication of the closed channel culture device

A disposable, polydimethylsiloxane (PDMS) chip with an open channel and 382 microwells was used as the primary component of the cell culture device. Thermally curable PDMS (Silpot 184; Dow Corning Toray, Tokyo, Japan) was molded once to create a PDMS master and then again to replicate the geometry of the original aluminum master. PDMS curing was carried at 125 °C for 30 min and the final PDMS chip was coated with Lipidure^{R}-CM5206 (NOF Corporation, Tokyo, Japan) by dipping. The PDMS chip was exposed to 0.5% of 2-methacryroyloxyethylphosphorylcholine polymer (MPC) in ethanol and dried to prevent surface cell adhesion. After sterilization by autoclaving in water, the chip was set on an aluminum base plate with a supporting polycarbonate plate below and sealed from above with a separate polycarbonate block, thereby creating a closed-channel culture device. Peristaltic pumps (Aquatech Japan, Inc., Osaka, Japan) were set on the base plate and connected to the culture device to control culture medium delivery.

2.3. Culture system

Pumps were driven by a control unit (Arkray, Inc., Kyoto, Japan) containing a programmable driver for the pump. Flow rate for culture medium delivery to the device was programmed using a PC connected to the unit. Fresh and spent culture media were stored in conventional 50 ml tubes with sealing caps (Arkray, Inc.).

2.4. Starting culture with the channel device

At one day before inducing differentiation, hiPSCs were dissociated using TrypLE (Life Technologies) and suspended in E8 medium containing 10 μM Y-27632 (Wako Pure Chemistry) at certain densities. The cells were introduced via a luer port on the upper polycarbonate block using a 1 ml syringe at a flow rate of about 200 μl/s. After introduction of the cells, the device was left for 10 min at room temperature to achieve uniform sedimentation of cells into the microwells. About 1 ml of E8 medium was then introduced at a rate of 20 μl/s to flush out any cells outside of the microwells. The entire culture device unit was then connected to the control unit and left in a CO_2 incubator. Fresh E8 medium containing 10 μM Y-27632 was injected 10 times a day at a flow rate of 30 μl/min for 15 min (every 2.4 h). The formation of spheroidal cellular aggregates was confirmed 24 ± 3 h after the application of hiPSCs into the channel device, at which point the differentiation protocol was initiated (day 0).

2.5. Inducing pancreatic islet cells

The human iPSCs aggregates were induced to differentiate into pancreatic islet cells using media previously described with some modifications [7]. Briefly, MCDB131 medium (Thermo Fisher Scientific) containing 1x Glutamax (Life Technologies), 1.5 mg/mL sodium bicarbonate, and 1x Pen-Strep was used with following supplements: day 0–1: 150 ng/mL activin A (R&D systems, Minneapolis, MN), 4.5 μM CHIR99021 (Wako Pure Chemistry), 10 mM Glucose, 0.5% fat-free BSA (Wako Pure Chemistry); day 1–3: 150 ng/mL activin A, 1/50000 ITS-X supplement (Life Technologies), 10 mM Glucose, 0.5% fat-free BSA; day 3–5: 75 ng/mL FGF-7 (PeproTech, Rocky Hill, NJ), 0.25 mM Ascorbic Acid (Sigma-Aldrich), 1/50000 ITS-X supplement, 10 mM Glucose, 0.5% fat-free BSA; day 5–7: 75 ng/mL FGF-7, 0.25 mM Ascorbic Acid, 0.38 μM SANT-1 (Wako Pure Chemistry), 0.30 μM TPB (Merck Millipore, Billerica, MA), 0.15 μM LDN 193189 (Wako Pure Chemistry), 1.5 μM retinoic acid (Sigma-Aldrich), 1/200 ITS-X supplement, 20 mM Glucose, 2.0% fat-free BSA; day 7–10: 3.0 ng/mL FGF-7, 0.25 mM Ascorbic Acid, 0.38 μM SANT-1, 0.15 μM TPB, 0.30 μM LDN 193189, 0.15 μM retinoic acid, 1/200 ITS-X supplement, 20 mM Glucose, 2.0% fat-free BSA; day 10–13: 0.38 μM SANT-1, 0.15 μM LDN 193189, 0.075 μM retinoic acid, 1.50 μM T3 (Sigma-Aldrich), 15.0 μM ALK5 inhibitor (Enzo Life Science, Farmingdale, NY), 10.0 μM ZnSO4, 10.0 μg/ml Heparin (Nacarai Tesque), 1/200 ITS-X supplement, 20 mM Glucose, 2.0% fat-free BSA; day 13–20: 0.15 μM LDN 193189, 1.50 μM T3, 15.0 μM ALK5 inhibitor, 0.15 μM GS inhibitor XX (Merck Millipore), 10.0 μM ZnSO4, 10.0 μg/ml Heparin, 1/200 ITS-X supplement, 20 mM Glucose, 2.0% fat-free BSA; day 20~: 1.50 μM T3, 15.0 μM ALK5 inhibitor, 15.0 μM Trolox (Enzo Life Science), 3.0 μM R428 (Selleckchem, Houston, TX), 1.0 mM N-Cys (Sigma-Aldrich), 10.0 μM ZnSO4, 10.0 μg/ml Heparin, 1/200 ITS-X supplement, 20 mM Glucose, 2.0% fat-free BSA. Images of cellular aggregates were taken at the end of each stage and size analysis was performed using ImageJ software.

2.6. Quantitative PCR

Primer sequences used in this work are listed in Supplementary Table 1. Cells were collected at days 0 (undifferentiated hiPSCs), 4, 10, 20, and 27 of the differentiation process. Complementary DNA templates were synthesized from about 20 aggregates from each sample by Power SYBR Green Cells-to-Ct Kit (Life Technologies) according to the manufacturer's instruction. Quantitative PCR (qPCR) was carried out using the StepOne™ Real-Time PCR System (Life Technologies) with the Power SYBR Green PCR Master Mix (Life Technologies). The expression level of GAPDH gene was used for normalization of each sample.

2.7. Immunostaining

All antibodies used in this work are listed in Supplementary Table 2. Cellular aggregates were taken out from the culture device and seeded onto Geltrex-coated plastic dishes. On the following day, adherent cells were fixed with 4% paraformaldehyde/PBS (PFA) for 15 min at room temperature, and washed with PBS three times for 10 min each. Fixed cells were permeabilized with 0.2% Triton X-100 in PBS, washed with 0.05% Tween 20 in PBS (PBST) three times for 10 min each, and then treated with Blocking One Reagent (Nacarai Tesque) for 90 min at room temperature. First antibodies were applied with Blocking One Reagent and incubated for 90 min at room temperature. The cells were then washed with PBST and stained with fluorescence-conjugated secondary antibodies. The cell nuclei were counterstained with 1 μg/mL Hoechst 33258 (Dojindo Laboratories, Kumamoto, Japan).

2.8. Flow cytometry

For analyzing the expression of marker proteins, cellular aggregates were dissociated by TrypLE into single cells and fixed with PFA. Cells were permeabilized and labeled by antibodies following the method described above. Labeled cells were analyzed by a