Journal of the Taiwan Institute of Chemical Engineers 45 (2014) 2096–2105

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Capillary electrophoresis of induced pluripotent stem cells during differentiation toward neurons

Cheng-Chin Wang, Yung-Chih Kuo *

Department of Chemical Engineering, National Chung Cheng University, 168, University Rd., Min-Hsiung, Chia-Yi 62102, Taiwan

ARTICLE INFO

Article history:
Received 10 March 2014
Received in revised form 26 June 2014
Accepted 29 June 2014
Available online 22 July 2014

Keywords:
Induced pluripotent stem cell
Nerve growth factor
Zeta potential
Electrophoretic mobility
Fixed charge density

ABSTRACT

Understanding of electrical properties of neurons, derived from induced pluripotent stem cells (iPSCs), is an emerging issue in treating human neurological disorders. This study presents the variation in zeta potential and electrophoretic mobility of iPSCs during differentiation toward neurons. The fixed charge density of differentiating iPSCs was also evaluated using Ohshima's soft particle theory. The optical images demonstrated that in passage 12, a cluster of iPSCs emerged at day 5. The colonial expansion enlarged the cluster size and an increasing culture period led to a mature multiplication. In addition, immunochemical staining with stage-specific embryonic surface antigen-1, Oct4, Sox2, and Nanog assured the starting iPSCs of phenotypic pluripotency. A longer period of induction with nerve growth factor (NGF) produced a higher quantity of neuron-like cells. Moreover, a high concentration of NGF yielded short migration time of differentiating iPSCs, suggesting a raised surface charge and accelerated neuronal differentiation. An increasing NGF concentration and inductive span enhanced the absolute value of zeta potential, electrophoretic mobility, and fixed charge density of differentiating iPSCs. The propagated colony of embryonic phenotype and membrane charge of differentiating iPSCs can be identified and controlled as a biophysical foundation for future clinical application.

1. Introduction

Induced pluripotent stem cells (iPSCs), derived from somatic cells with ectopic expression of transcription factors (Oct4, Sox2, Klf4, and c-Myc), displayed embryonic feature, including gene expression, self-renewal, and differentiation pluripotentiality [1,2]. Recent studies have recommended that the use of iPSCs could be a potent therapeutic strategy to treat a variety of neuronal diseases, such as stroke, spinal cord injury, amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease [3–6]. However, the regeneration of neural tissue from iPSCs relied on controllable differentiation to neuron-like cells in optimized conditions. In a study on genetic regulation, it has been drawn that neurotrophin-3 (NT-3)-loaded polybutylcyanoacrylate nanoparticles could stimulate iPSCs to differentiate toward neurons and enhance the expression of NT-3, NT-3 growth factor receptor, neurofilament-200, neuron-specific enolase, and postsynaptic density protein 95 [7]. In addition to genetic approaches, the biochemical techniques, such as nerve growth factor (NGF) and brain-derived neurotrophic factor, have been employed to guide the neuronal differentiation of iPSCs [8–10].

NGF, one of the neurotrophin family proteins, can mediate cell bioactivity and regulate neuronal survival, growth, and differentiation [11–13]. It has been concluded that the cellular receptors, TrkA and p75 (NTR), could recognize NGF [14,15]. TrkA mainly expresses in the central nervous system, human monocytes, and keratinocytes, while p75 (NTR) is widely distributed in neurons, fibroblasts, keratinocytes, lymphocytes, and tumors [16]. In a study on the differentiation of iPSCs in NGF-incorporated biomedical scaffolds, an increase in the concentration of NGF has been demonstrated to efficiently promote the neuronal production in cultivated constructs [17].

The membrane charge of neurons plays an important role in discerning physiological, pathological, and biochemical

Abbreviations: CE, capillary electrophoresis; DPBS, Dulbecco's phosphate-buffered saline; ECM, extracellular matrix; FBS, fetal bovine serum; HESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; LIF, leukemia inhibitory factor; NeuN, neuronal nuclei; NGF, nerve growth factor; NT-3, neurotrophin-3; SSEA, stage-specific embryonic surface antigen.

* Corresponding author. Tel.: +886 5 272 0411x33459; fax: +886 5 272 1206.
E-mail address: chmyck@ccu.edu.tw (Y.-C. Kuo).

http://dx.doi.org/10.1016/j.jtice.2014.06.022
1876-1070/

Nomenclature

C_{NGF}	concentration of nerve growth factor for differentiation (ng/mL)
t	migration time of iPSCs in capillary electrophoresis (min)
t_{ind}	induction time for neuronal differentiation (h)
ζ	zeta potential of iPSCs (mV)
μ	electrophoretic mobility of iPSCs ($m^2/(s\,V)$)
ρ_{fix}	fixed charge density of iPSCs (C/m^3)

characteristics of nervous system. This is because the expressions of membrane proteins, lipids, and glycocalyx molecules are regulated by cell signaling. In addition, capillary electrophoresis (CE) is an efficacious method for analyzing particulate mobility, which can be a critical index for identifying electrical and mechanical properties of cell surface [18,19]. In a study on electrophoretic mobility, μ, of cerebellar granule neurons, it has been observed that the theoretical model espoused could accurately predict the density of dissociable functional groups and the equilibrium constant of dissociation reaction [20]. Moreover, it has been found that amino acids could regulate the charge behavior and alter electrophoretic mobility of PC-12 cells [21].

This study aimed to investigate the electrical properties of iPSCs during their differentiation toward highly charged neurons. Understanding of the electrokinetic traits is crucial to clinical application of stem cells [22]. Thus, it is ineluctable to analyze the membrane charge of differentiating iPSCs. We examine the influences of culture period, fluorescent staining, NGF concentration, and inductive period on the variation in membrane charge of differentiating iPSCs.

2. Materials and methods

2.1. Propagation of iPSCs

Mouse iPSCs (System Biosciences, Mountain View, CA) were propagated by a feeder-free culture protocol described in the previous study [7]. Briefly, iPSCs at a density of 2×10^4 cells/cm^2 were seeded on tissue culture flask (75 cm^2, Techno Plastic AG, Trasadingen, Switzerland) pretreated with 0.1% surface gelatin (type A from porcine skin, Sigma, St. Louis, MO) and cultivated with 15 mL of ESGRO complete PLUS clonal grade medium containing leukemia inhibitory factor (LIF; SF001-500P, Millipore, Billerica, MA) in an incubator (Forma 310, Thermo Fisher Scientific, Waltham, MA) with gas phase of 5% CO_2 and 95% relative humidity at 37 °C. The culture medium containing LIF could maintain an undifferentiated state of iPSCs and was replaced every 2 days during cultivation. When the cell confluence reached 80–90% in the culture, the expanded iPSCs were washed with 15 mL of Dulbecco's phosphate-buffered saline (DPBS; Sigma), detached from the flask with 3 mL of ESGRO complete accutase (Millipore), and centrifuged at $160 \times g$ for 5 min. After removal of supernatant, the bottom pellet was resuspended in 10 mL of ESGRO medium and equally dispensed to 3 gelatin-coated flasks. The proliferated iPSCs in ESGRO medium containing 10% (v/v) dimethyl sulfoxide (J.T. Baker, Phillipsburg, NJ) and 10% (v/v) fetal bovine serum (FBS; Sigma) were cryopreserved in liquid nitrogen. iPSCs in passage 12 were applied to CE. The optical images of multiplied iPSCs were visualized using an inverted phase-contrast fluorescent microscope (Eclipse TE300, Nikon, Tokyo, Japan).

2.2. Identification of iPSCs

Phenotypic iPSCs were identified by immunocytochemical staining described as follows. The cultured iPSCs were fixed with 4% paraformaldehyde (Sigma) in DPBS at room temperature for 10 min, washed with DPBS, and treated with 0.5% (v/v) Triton X-100 (Acros Organics, Geel, Belgium) in DPBS for 10 min, washed with DPBS, and immersed in serum blocking reagent (Zymed Laboratories, South San Francisco, CA) at room temperature for 10 min. The pretreated iPSCs were reacted with primary antibodies against stage-specific embryonic surface antigen-1 (SSEA-1; 1:200, Abcam, Cambridge, MA), Oct4 (1:1000, Abcam), Sox2 (1:1000, Abcam), and Nanog (1:700, Abcam) at 4 °C overnight and incubated with fluorescein isothiocyanate-conjugated secondary antibody (1:100, Millipore) at room temperature for 1 h. Nuclei of iPSCs were counterstained with 0.5% (w/v) 4′,6′-diamidino-2-phenylindole (DAPI; 5 mg/mL, Sigma) in 0.1% (v/v) Triton X-100 at room temperature for 3 min. The immunofluorescent images were obtained using a fluorescent microscope (Eclipse E600, Nikon).

2.3. Differentiation of iPSCs into neuron-like cells with NGF

The recombinant human beta-NGF (PeproTech, Rocky Hill, NJ) was applied to induction of iPSCs differentiating toward neurons. The cultured iPSCs at a density of 8×10^4 cells/well were seeded on gelatin-pretreated 12 well plate (Corning Costar, Cambridge, MA) with ESGRO medium, and incubated in the humidified CO_2 incubator at 37 °C overnight. After cell adhesion, the culture medium was replaced with inductive medium containing ESGRO medium (without LIF) and NGF of 5, 10, and 20 ng/mL. iPSCs were cultured for a prescribed period. The morphological variation in differentiating iPSCs was obtained using the inverted phase-contrast fluorescent microscope (Eclipse TE300, Nikon). The average neurite lengths were evaluated using ImageJ software (version 1.47, NIH, Bethesda, MD) [23].

2.4. Immunochemical staining of differentiating iPSCs

The propagated iPSCs at a density of 4×10^4 cells/well were seeded on a gelatin-coated microscope cover glass in a 24-well plate (Corning Costar) and cultured in the humidified CO_2 incubator at 37 °C overnight. After adhesion, iPSCs were induced with ESGRO medium containing NGF of 5 and 20 ng/mL for neuronal differentiation. Afterward, iPSCs were fixed with 4% (v/v) formalin (Sigma) at 25 °C for 10 min, permeabilized with 0.5% (v/v) Triton-X-100 at 25 °C for 10 min, and reacted with serum blocking reagent at 25 °C for 10 min. The specimens were reacted with mouse anti-neuronal nuclei (NeuN) with Alexa Fluor 488 conjugates (1:100, Millipore) and SSEA-1 at 25 °C for 1 h, and counterstained with DAPI in 0.1% (v/v) Triton X-100 at 25 °C for 3 min. The fluorescent images of differentiating iPSCs were visualized using a confocal laser scanning microscope (LSM 510, Zeiss, Oberkochen, Germany) at emission wavelengths of 600 nm (red), 545 nm (green), and 460 nm (blue).

2.5. Measurement of zeta potential and electrophoretic mobility

Phenotypic and differentiating iPSCs were severed with 0.5 mL of ESGRO complete accutase and suspended at a density of 3×10^4 cells/mL in tris hydroxymethyl aminomethane (Tris; 0.1 M, Riedel-de Haen, Seelze, Germany) at pH 7.4. The zeta potential, ζ, of iPSCs were analyzed using a Zetasizer 3000 HS_A (Malvern, Worcestershire, UK) with a photon correlation spectroscope and a laser Doppler velocimeter at 25 °C.

The electrophoretic mobility of phenotypic and differentiating iPSCs was examined using a high performance CE with Gold data