Biomaterials 33 (2012) 9179–9187

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Efficient neuronal differentiation and maturation of human pluripotent stem cells encapsulated in 3D microfibrous scaffolds

Hong Fang Lu*, Sze-Xian Lim, Meng Fatt Leong, Karthikeyan Narayanan, Rebecca P.K. Toh, Shujun Gao, Andrew C.A. Wan*

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669, Singapore

ARTICLE INFO

Article history:
Received 24 August 2012
Accepted 4 September 2012
Available online 19 September 2012

Keywords:
Neural cell
Stem cell
Scaffold
Nerve regeneration

ABSTRACT

Developing an efficient culture system for controlled human pluripotent stem cell (hPSC) differentiation into selected lineages is a major challenge in realizing stem cell-based clinical applications. Here, we report the use of chitin-alginate 3D microfibrous scaffolds, previously developed for hPSC propagation, to support efficient neuronal differentiation and maturation under chemically defined culture conditions. When treated with neural induction medium containing Noggin/retinoic acid, the encapsulated cells expressed much higher levels of neural progenitor markers SOX1 and PAX6 than those in other treatment conditions. Immunocytochemisty analysis confirmed that the majority of the differentiated cells were nestin-positive cells. Subsequently transferring the scaffolds to neuronal differentiation medium efficiently directed these encapsulated neural progenitors into mature neurons, as detected by RT-PCR and positive immunostaining for neuron markers βIII tubulin and MAP2. Furthermore, flow cytometry confirmed that >90% βIII tubulin-positive neurons was achieved for three independent iPSC and hESC lines, a differentiation efficiency much higher than previously reported. Implantation of these terminally differentiated neurons into SCID mice yielded successful neural grafts comprising MAP2 positive neurons, without tumorigenesis, suggesting a potential safe cell source for regenerative medicine. These results bring us one step closer toward realizing large-scale production of stem cell derivatives for clinical and translational applications.

1. Introduction

Neurodegenerative diseases represent a large group of heterogenous disorders characterized by progressive degenerative loss of functional neural cells [1]. While primary neuron transplants show promising therapeutic effects, these are limited by the availability of donor tissues for transplantation. Thus, *in vitro* generation of neuronal populations represents an attractive alternative source for neuronal transplantation. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (iPSCs) may provide an inexhaustible reservoir of diverse neural types, offering an attractive approach for *in vitro* studies, drug screening and cell transplantation [1]. Specially, these iPSC–derived neural cells offer not only a promising approach to circumvent issues pertaining to the supply of clinical cadaveric neurons, but also the possibility of providing an unlimited cell source for personalized therapy [2,3]. In particular, the transplantation of hPSC-derived neural cells led to effective integration into the animal brain and ameliorated disease symptoms [4–6]. However, the tendency for tumorigenesis resulting from poorly differentiated hPSCs and proliferative progenitors presents a significant issue for the clinical application of these cells [3,4,7–10]. Thus, clinical hPSC-based therapy requires the development of efficient culture systems which can provide stringent differentiation of hPSCs into large numbers of neuronal cells with clinical utility.

Several laboratories have demonstrated efficient neural generation from hPSCs *in vitro* [6,11–18]. Traditional two-dimensional (2D) adherent cultures including the use of ECM coating, co-culture with feeder cell layers or conditioned medium showed promising results for neural generation [6,11–14]. However, 2D culture systems fail to recapitulate *in vivo* three-dimensional (3D) stem cell niches. Moreover, its lack of scalability hinders its biomedical applications. In addition, undefined foreign/animal biological supplements or feeders used for cell culture and differentiation limit the direct use of such cell derivatives for possible clinical applications. Another efficient approach is a five-stage

* Corresponding authors. Tel.: +65 6824 7134; fax: +65 6478 9082.
E-mail addresses: hflu@ibn.a-star.edu.sg (H.F. Lu), awan@ibn.a-star.edu.sg (A.C.A. Wan).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.09.006