ABSTRACT

In the past decades, neural stem cell has been studied to increase the understanding of neurogenesis and brain injuries, which may have implication on development of effective treatments for neurodegenerative diseases. In this study, the characterization of human neural stem cells in cell expansion and neurogenesis has been conducted. The neural stem cells were induced from H9 human embryonic stem cell line. Cultivated in conventional adherent 2D monolayer and neurosphere culture, the multipotency and the differentiation state was examined through protein markers expression. Immunofluorescence was performed and images were made with LSM. Varied methods and conditions were performed to investigate the effect of medium, neurotrophins and additional substrates used; initial cell density and confluency; and cultivation time on neural stem cell cultures.

Differentiation of neural stem cells under 2D culture was induced with dibutyrl-cAMP and generated immature neurons which were identified through expression of ß3-tubulin. Optimized neurosphere cultivation produced MAP2-positive neurons. This demonstrates the method developed in this study to be superior as the model of neuronal development in comparison to 2D culture. At the end, electrical activities of the generated neuron from the 2D differentiated NSCs grown on MEA chips were observed. However no peaks could be seen, indicating that the generated neurons were not mature enough.

Keyword: 2D monolayer, 3D neurosphere, db-cAMP neuronal differentiation, human neural stem cells, immunofluorescence, laser scanning microscopy, neural stem cell culture, neurogenesis, MEA measurement