BrainPhys™ Neuronal Medium Supports the Electrical Activities of Neurons Derived from Human Pluripotent Stem Cells and Primary CNS Tissues in Long-term Cultures


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Abstract

Action potential firing and synaptic activity are fundamental properties of neurons in the brain. Bardy et al. (2015) have recently reported that Neurobasal® Medium and DMEM/F-12 support neuron survival but suppress their synaptic activities in culture. To solve this problem, we developed BrainPhys™ Neuronal Medium (BrainPhys™), based on the formulation published by Bardy et al., to support growth and synaptic function of neurons in long-term cultures. Here we describe the effect of BrainPhys™ and DMEM/F-12 based media on neuronal electrical activity of human pluripotent stem cell (hPSC)-derived and primary E18 rat cortical neurons in 18 and 6 weeks cultures, respectively. For hPSCs, neuronal progenitor cells derived from induced pluripotent stem cells (iPSCs) were differentiated in BrainPhys™ instead of DMEM/F-12 (control) with supplements, and cultured for 18 weeks. We performed half-medium changes every 4-5 days and measured the neuronal electrical activity twice a week using the multielectrode array (MEA) system. Our data showed that the mean firing rate of hPSC-derived neurons (n = 1; 128 electrodes) in BrainPhys™ increased from <0.17 Hz at week 10 to 1.60 Hz by week 18. In contrast, the mean firing rate of neurons in DMEM/F-12 (mean: 0.15 Hz) over the same 18 weeks was lower in all conditions using primary tissues. E18 rat cortical cells were plated in Neurobasal® Medium with NeuroCult™ SM1 neuronal supplement, 0.5 mM L-glutamine and 25 mM L-glutamic acid. After half-medium changes every 3-4 days for 6 weeks. Electrical activities were measured twice a week throughout the culture period. Our data showed that the mean firing rate of neurons in BrainPhys™ medium increased over time, from 0.03 Hz at week 2 to 1.4 Hz by week 6 (n = 1, 128 electrodes). The percentage of active electrodes (>0.005 Hz) also increased from 24% at week 2 to 69% by week 3, and then remained stable at 70-70% for weeks 3-6. In contrast, the mean firing rate remained low (~0.14 Hz) in the control cultures, in which the electrodes were active over a 6-week period. Our data confirm that BrainPhys™ Neuronal Medium with appropriate supplements supports the synaptic function of hPSC-derived and primary neurons by providing a physiological in vitro condition that closely mimics the environment of the brain.

Methods

(A) Culture of hPSC-derived Neurons

Neural progenitor cells derived from hPSCs (iPSCs) were cultured in STEMdiff™ Neuronal Differentiation Medium supplemented with 0.5 mM L-glutamine in Neurobasal® Medium + supplements: 1% N2 Supplement-A, 2% NeuroCult™ SM1 Neuronal Supplement, 20 ng/mL GDNF, 20 ng/mL BDNF (100 mM), 200 µM ascorbic acid. Half changes were performed every 3-4 days throughout the culture period.

(B) Culture of Primary E18 Rat Cortical Neurons

Alkaline phosphatase (AP) positive neurons were cultured in STEMdiff™ Neuronal Medium + supplements: 1% N2 Supplement-A, 2% NeuroCult™ SM1 Neuronal Supplement, 20 ng/mL GDNF, 20 ng/mL BDNF (100 mM), and 200 µM ascorbic acid. Half changes were performed every 3-4 days throughout the culture period.

(C) Multi-Electrode Array (MEA) Recording and Analysis

Cells in each culture condition were plated in duplicate wells of a 96-well plate (STEMdiff Medium + supplements or Neurobasal® Medium + supplements) 3-5 days before recording with 10,000 cells/cm2. After 5 days, half of the cultures were transitioned to BrainPhys™ Neuronal Medium with 2% NeuroCult™ SM1 and 0.5 mM L-glutamine. Half-medium changes were performed every 4-5 days. Electrical activities were measured twice a week throughout the culture period. Our data showed that the mean firing rate of hPSC-derived neurons (n = 1; 128 electrodes) in BrainPhys™ increased from <0.17 Hz at week 10 to 1.60 Hz by week 18. In contrast, the mean firing rate of neurons in DMEM/F-12 (mean: 0.15 Hz) over the same 18 weeks was lower in all conditions using primary tissues. E18 rat cortical cells were plated in Neurobasal® Medium with NeuroCult™ SM1 neuronal supplement, 0.5 mM L-glutamine and 25 mM L-glutamic acid. After half-medium changes every 3-4 days for 6 weeks. Electrical activities were measured twice a week throughout the culture period. Our data showed that the mean firing rate of neurons in BrainPhys™ medium increased over time, from 0.03 Hz at week 2 to 1.4 Hz by week 6 (n = 1, 128 electrodes). The percentage of active electrodes (>0.005 Hz) also increased from 24% at week 2 to 69% by week 3, and then remained stable at 70-70% for weeks 3-6. In contrast, the mean firing rate remained low (~0.14 Hz) in the control cultures, in which the electrodes were active over a 6-week period. Our data confirm that BrainPhys™ Neuronal Medium with appropriate supplements supports the synaptic function of hPSC-derived and primary neurons by providing a physiological in vitro condition that closely mimics the environment of the brain.