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/F12 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/F12-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 hPSC cultures, neuronal progenitor cells were derived from induced pluripotent stem cells (iPSCs) and differentiated in BrainPhys™ or DMEM/F12 (control) with supplements, and cultured for 18 weeks. We performed half-medium changes every 4 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 <0.1 Hz at week 10 to 3.8 ± 0.2 Hz at week 18. In contrast, the mean firing rate of neurons in DMEM/F12 (control) remained low (<0.15 Hz) over the same 18 weeks. Our results using primary tissues, E18 rat cortical cells were plated in Neurobasal® Medium with NeuroCult® SMI Neuronal Supplement (SMI). After 5 days, cultures were either transitioned to BrainPhys™ with SMI or maintained in the Neurobasal® control by performing 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 2.1 ± 0.3 Hz by week 12 (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 remained stable at 60 - 70% from 3 weeks on. In contrast, <20% of electrodes were active in DMEM/F-12 for the same 18-week period. The MFR of neurons cultured in BrainPhys™ increased over time, from 0.1 ± 0.04 Hz at week 6 to 2.1 ± 0.2 Hz at week 18 (n=1, 128 electrodes). In contrast, the MFR of neurons cultured in DMEM/F-12/Neurobasal® and BrainPhys™-12 remained low (<0.1 Hz) over the same 18 week period. The percentage of active electrodes (>0.005 Hz) also increased from 24% at week 2 to 69% by week 3, and remained stable at 60 - 70% from 3 weeks on. In contrast, <20% of electrodes were active in DMEM/F-12 for the same 18-week period. The MFR of neurons cultured in BrainPhys™ increased over time, from 0.1 ± 0.04 Hz at week 6 to 2.1 ± 0.2 Hz at week 18 (n=1, 128 electrodes). In contrast, the MFR of neurons cultured in DMEM/F-12/Neurobasal® and BrainPhys™-12 remained low (<0.1 Hz) over the same 18 week period. To measure neuronal activity, MFR was calculated based on the activity recorded from 128 electrodes for each culture (cultures grown up to duplicate wells, 644 electrodes per well).

Methods

(A) Culture of hPSC-derived Neurons

Initial Plating

Weeks in Culture

0.0 0.5 1.0 1.5 2.0 2.5 3.0

Weeks in Culture

Network bursts were first detected at week 3 in the BrainPhys™ culture. The number of network bursts detected in a 5-minute recording increased from 38 to 79 from week 3 to week 6, showing that a synchronous neuronal network was developed in the BrainPhys™ culture over time. The number bursts also increased from 2 (week 3) to 33 (week 6) across week 3 to 6 in the BrainPhys™ condition. In contrast, no network burst activity was detected in the DMEM/F12-12 condition (sensor plots not shown).

(B) Primary E18 Rat Cortical Neurons

Summary

BrainPhys™ Neuronal Medium: BrainPhys™ Neuronal Medium supports the maturation of hPSC-derived and primary neurons. Neurons cultured in BrainPhys™ Neuronal Medium exhibit improved electrical activity and develop synchronous neuronal activity over time based on MEA data.