BRAINPHYS™ NEURONAL MEDIUM: A MEDIUM THAT PROMOTES THE MATURATION AND SYNAPTIC FUNCTION OF HUMAN PLURIPOTENT STEM CELL (hPSC)-DERIVED NEURONS IN LONG-TERM CULTURES

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Introduction

BrainPhys™ Neuronal Medium was developed based on a published formulation (Bardy *et al.*, PNAS 2015) to support maturation and synaptic function of neurons in long-term cultures. Here, we describe the effect of BrainPhys™ on the electrophysiology of hPSC-derived neurons. Neural progenitor cells derived from the hPSC XCL-1 line were differentiated in BrainPhys™ with NeuroCult™ SM1 Neuronal Supplement and other growth factors (BP/SM1). Cells were cultured on microelectrode array (MEA) plates and activity was measured twice per week. Our data show that XCL-1-derived neurons cultured with BP/SM1 gradually became electrically active over an 18-week period. The mean firing rate (MFR) of neurons (n = 1; 128 electrodes) progressively increased from 0.1 Hz on day 30 to 1.6 Hz by day 125. The percentage of active electrodes (> 0.005 Hz) also increased from 30% on day 30 to 59% by day 125. Network bursts, a measure of synaptic connectivity, increased from 2 during a 10-minute interval on day 30 to 97 on day 125, indicating synchronous firing was enhanced as neurons matured in BP/SM1. We performed the same experiment on H9 hPSC line, and we found that BP/SM1 consistently supports the electrical activity of neurons. Similar to the XCL-1 culture, there was an increase in MFR and network bursts when the H9 neurons were cultured in BP/SM1 over a 10-week maturation period. In summary, these results demonstrate that BrainPhys™ supports the physiological maturation and synaptic function of hPSC-derived neurons in long-term culture.

Methods

Culture of hPSC-derived Neurons

Neural progenitor cells derived from an iPS cell line (XCL-1) and an ES cell line (H9) were cultured in STEMdiff™ Neuron Differentiation Medium on a poly-L-ornithine (PLO) / laminin-coated 6-well plate for 5 days. On Day -1, neural progenitor cells were dissociated and cells were re-plated onto a PLO / laminin-coated multielectrode array (MEA) plate at 30,000 cells/cm^2 in the same differentiation medium. On Day 0, half of the medium was replaced with different neuron maturation media. For the XCL-1 culture, cells were matured in either DMEM/F-12/NB-A (DMEM/F-12 and Neurobasal®-A Medium mixed in a 1:1 ratio) + Supplements or BrainPhys™ Neuronal Medium (BrainPhys™) + Supplements, where Supplements are: 1% N2 Supplement-A, 2% NeuroCult™ SM1 Neuronal Supplement, 20 ng/mL GDNF, 20 ng/mL BDNF, 1mM db-cAMP, and 200 nM Ascorbic Acid. For the H9 culture, cells were matured in BrainPhys™ + Supplements or in an alternate serum-free medium (Competitor Medium), which was prepared according to the supplier's recommended protocol. Half-medium changes were performed every 3 - 4 days throughout the maturation stage of the culture period.

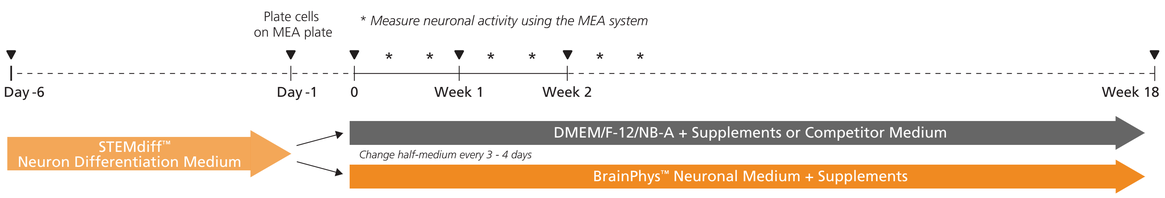

FIGURE 1. Workflow for Neural Progenitor Cell Differentiation and Neuronal Maturation

Multi-Electrode Array (MEA) Recording and Analysis

Cells in each culture condition were plated in duplicate wells of a 12-well plate (Axion Biosystems; M768-GL-1-30Pt200), with 64 recording electrodes in each well. Spontaneous neuronal activity was acquired at 37°C under a 5% CO_2 atmosphere using an MEA system (Maestro, Axion Biosystems) at a sampling rate of 12.5 kHz/channel. For recording, a Butterworth band-pass filter (200 Hz - 3000 Hz) was applied and the adaptive threshold spike detector was set at 6X standard deviation. A 15-minute recording was taken twice a week. Only the last 10 minutes of each recordings were exported for analyses using the AxIS (2.3.3) analysis software.

Results

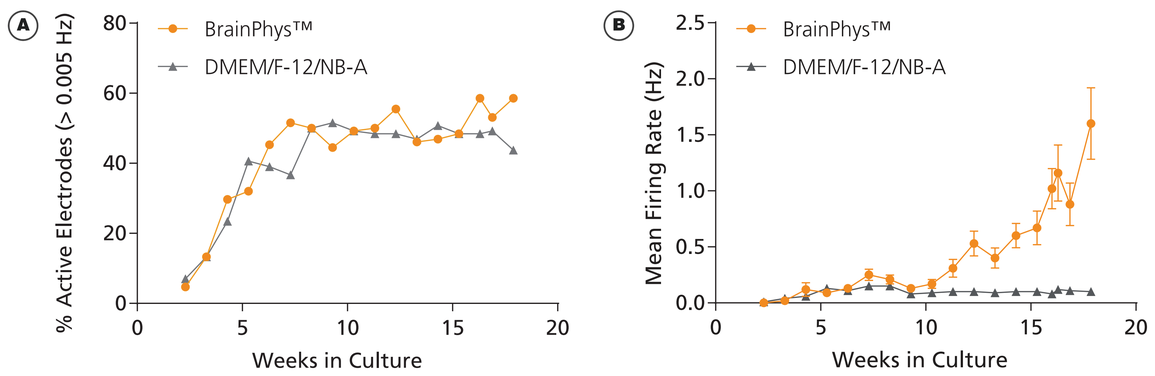

FIGURE 2. BrainPhys™ Neuronal Medium Supports Cell Survival and Promotes Synaptic Activities of Neurons Derived from an iPS Cell Line (XCL-1) in Long-term Cultures

(A) The percentage of active electrodes (> 0.005 Hz) in BrainPhys™ and DMEM/F-12/NB-A progressively increased to ~ 60% by week 8 and remained stable thereafter until the end of the culture, indicating that both media support survival of active neurons in long-term culture. **(B)** Neurons in BrainPhys™ became electrically active over an 18-week period, with a gradual increase in MFR from 0.21 ± 0.04 Hz at week 8 to 1.60 ± 0.32 Hz (n = 1; mean ± SEM, 128 electrodes) at week 18. In contrast, the MFR of neurons in DMEM/F-12/NB-A remained low (≤ 0.15 Hz) throughout the same culture period.

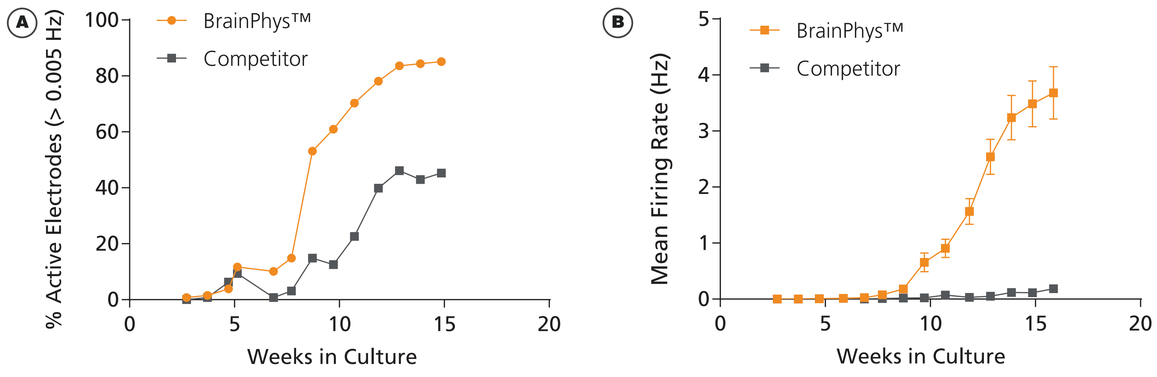

FIGURE 3. BrainPhys™ Neuronal Medium Showed Improved Cell Survival And Enhanced Synaptic Activities of Neurons Derived from an ES (H9) Cell Line in Long-term Cultures

(A) The percentage of active electrodes (> 0.005 Hz) in BrainPhys™ reached ~ 80% by week 16. In contrast, only ~ 45% of electrodes were active in Competitor Medium by the end of the culture, suggesting that Competitor Medium is suboptimal in supporting long-term growth of neurons. **(B)** In addition, neurons in BrainPhys™ became electrically active over a 15-week period, with a gradual increase in MFR from 0.18 ± 0.05 Hz at week 8 to 3.68 ± 0.47 Hz at week 16 (n = 1; mean ± SEM, 128 electrodes). Conversely, the MFR of neurons in Competitor Medium remained low (≤ 0.2 Hz) throughout the same culture period.

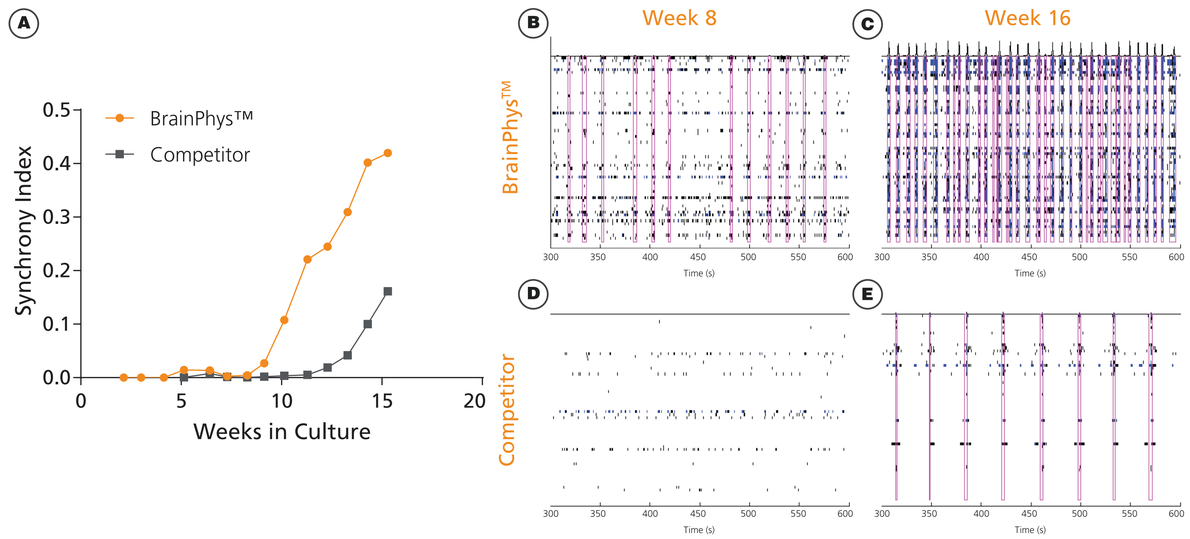

FIGURE 4. H9-derived Neurons Cultured in BrainPhys™ Neuronal Medium Developed Strong Synchronous Neuronal Activity Over Time

(A) Synchronous neuronal networks are established when neurons become mature and develop strong synaptic connections within the network. Relative to the Competitor Medium, a higher degree of synchrony was achieved early in the BrainPhys™ culture, suggesting that BrainPhys™ is more efficient in promoting the growth of mature neurons in vitro. **(B-E)** Raster plots showing the firing patterns of H9-derived neurons across 64 electrodes at different time points. Each black line represents a detected spike. Each blue line represents a single channel burst - a collection of at least 5 spikes, each separated by an inter-spike interval (ISI) of ≤ 100 ms. Each purple box indicates a network burst - a collection of at least 10 spikes from a minimum of 25% participating electrodes across the entire well, each separated by an ISI of ≤ 100 ms. **(B-C)** Neurons cultured in BrainPhys™ demonstrated improved electrical activity as shown by the increased number of spikes over time. In addition, there was an increase in network bursting frequency in the BrainPhys™ condition, which suggests that neuronal firing gradually organized into synchronized network bursts as the neurons matured. **(D-E)** In contrast, the number of spikes and network bursts in Competitor Medium remained relatively low throughout the culture, showing that Competitor Medium is suboptimal in supporting network-level activities in vitro.

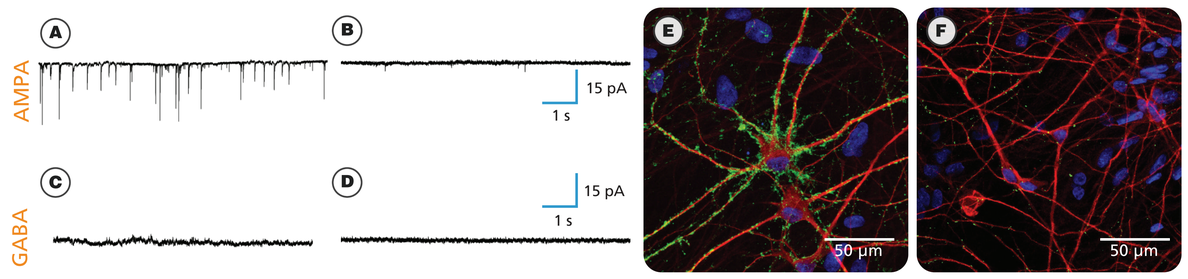

FIGURE 5. XCL-1-Derived Neurons Matured in BrainPhys™ Neuronal Medium Showed Improved Synaptic Activity

The resting membrane potential recorded for XCL-1-derived neurons cultured in BrainPhys™ and Competitor Medium on day 65 were -49.9 ± 1.5 mV and -30.0 ± 2.8 mV (n = 1; mean ± SEM; 5 neurons for each condition), respectively, indicating that neurons were not fully mature at this stage. **(A-B)** Neurons cultured in BrainPhys™ showed improved excitatory (AMPA-mediated) activity. The frequency and amplitude of AMPA-mediated events were greater in neurons cultured in BrainPhys™, compared to neurons matured in Competitor Medium. **(C-D)** Inhibitory (GABA-mediated) synaptic events were not detected in either culture conditions, likely because neurons were immature as indicated by the resting membrane potentials. Traces are representative. **(E-F)** XCL-1-derived neurons cultured in BrainPhys™ for 77 days are phenotypically more mature than neurons cultured in Competitor Medium, as indicated by the expression and localization of pre-synaptic marker, Synapsin 1. In the BrainPhys™ culture **(E)**, Synapsin 1 (green) labeling is distributed along the soma and dendritic processes, as defined by MAP2 labeling (red). In contrast, significantly less Synapsin 1 staining was detected in the Competitor Medium culture **(F)**.

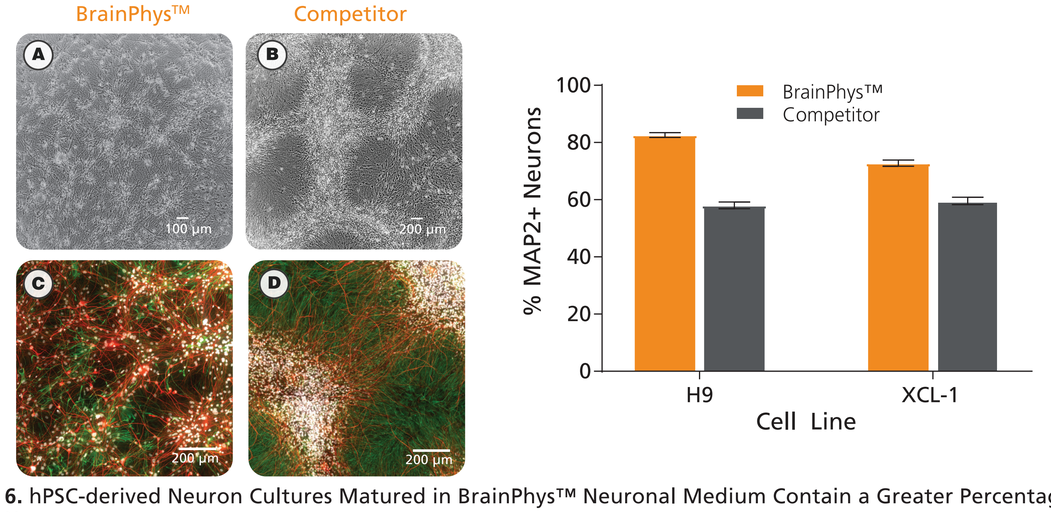

FIGURE 6. hPSC-derived Neuron Cultures Matured in BrainPhys™ Neuronal Medium Contain a Greater Percentage of MAP2+ Neurons

(A-B) XCL-1-derived neurons matured in BrainPhys™ for 77 days exhibited less clumping than neurons cultured in Competitor Medium. **(C-D)** BrainPhys™ condition contains significantly fewer GFAP+ cells (green), resulting in fewer cell clumps and a purer neuronal population as indicated by MAP2 staining (red). **(E)** MAP2+ neurons were quantified at day 14 in both H9- and XCL-1-derived cultures. BrainPhys™ cultures showed a significantly higher percentage of MAP2+ neurons (MAP2+/total DAPI+ cells) when compared to Competitor Medium (H9: 82.6 ± 0.8% vs 58.0 ± 1.2%, XCL-1: 72.8 ± 1.1% vs. 59.6 ± 1.3%) (n = 1; mean ± SEM; is of 36 images per condition).

Summary

- BrainPhys™ Neuronal Medium supports the maturation of neurons derived from ES cell- and iPS cell-derived neurons.
- Neurons matured in BrainPhys™ Neuronal Medium exhibit improved synaptic activity and develop synchronous network activity over time based on the patch clamp and MEA data.