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BrainPhys™ Neuronal Medium: A Medium Optimized to Support the Synaptic Activity of Neurons Derived from Human Pluripotent Stem Cells and Primary CNS Tissues

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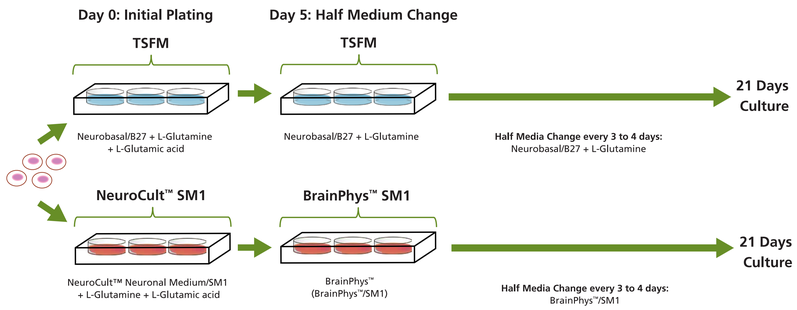
Abstract

Neuronal cultures derived from human pluripotent stem cells (hPSCs) and primary central nervous system (CNS) tissues are useful models for studying neurological development and disease. To increase the relevance of these studies, it is important that neurons are cultured in medium that closely resembles the normal physiological conditions of the CNS. Traditional media, such as DMEM/F-12 and Neurobasal, were designed to promote neuronal survival in culture, without extensive functional activities. Consequently, these media do not support normal physiological neuronal functions. Furthermore, researchers need to replace these media with artificial cerebrospinal fluid (ACSF) immediately prior to electrophysiological evaluation to maintain synaptic function *in vitro*.

A novel medium, BrainPhys™ was designed to circumvent these issues (Bardy et al. 2015). We have developed BrainPhys™ Neuronal Medium based on the published formulation and show that it not only supports the growth and differentiation of hPSC-derived neurons (thus confirming the findings of Bardy et al.) but also mature primary rat neurons. Neural progenitor cells (NPCs) derived from hPSCs cultured in BrainPhys™ Neuronal Medium supplemented with growth factors and differentiated in BrainPhys™ displayed characteristic neuronal morphology and appropriate expression of the neuronal markers MAP2, class III β-tubulin (TuJ-1) and Synapsin1. Primary E18 rat cortical neurons initially plated in NeuroCult™ Neuronal Basal Medium supplemented with NeuroCult™ SM1 Supplement and then with BrainPhys™ Neuronal Medium with NeuroCult™ SM1 Neuronal Supplement for 21 days expressed TuJ-1 and co-expressed MAP2 and Synapsin1, suggestive of a pure population of mature neurons. Primary CNS neurons cultured in BrainPhys™ Medium demonstrated AMPA and GABA receptor currents, exhibiting normal stimulatory and inhibitory synaptic activities. These data demonstrate that BrainPhys™ Neuronal Medium supports the growth and maturation of hPSC-derived and primary neurons under conditions that are truly physiologically compatible to that of the CNS. BrainPhys™ is a new cutting-edge medium for the study of neurons *in vitro*.

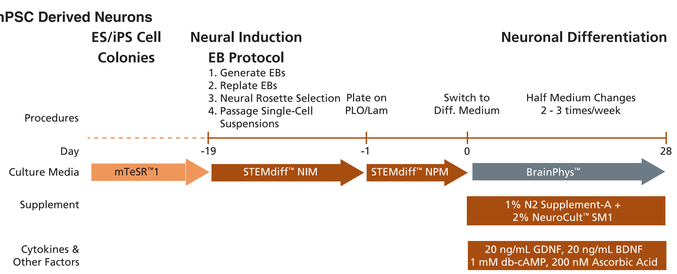
Methods

(A) Primary Neuronal Cultures



Single-cell suspensions of E18 rat cortical cells were plated on PLO/Laminin coated surfaces in either traditional serum-free medium (TSFM: Neurobasal, 2% B-27) or NeuroCult™ SM1 (NeuroCult™ Neuronal Basal Medium, 2% NeuroCult™ SM1) with 0.5mM L-glutamine and 25μM L-glutamic acid. After 5 days, half of the medium from each well was replaced with the same volume of TSFM (Neurobasal, 2% B-27 and 0.5mM L-glutamine) or BrainPhys™ SM1(BrainPhys™ Neuronal Medium, 2% NeuroCult™ SM1). Half-medium changes were performed every 3 - 4 days throughout the duration of the culture period. Cells were cultured for a total of 21 days. Neurons were detected by β III Tubulin and DAPI double staining and were qualified in eight random fields per well, in triplicate. Neurons were also characterized for the expression of dendritic spine (MAP2) and synaptic marker (Syapsin1).

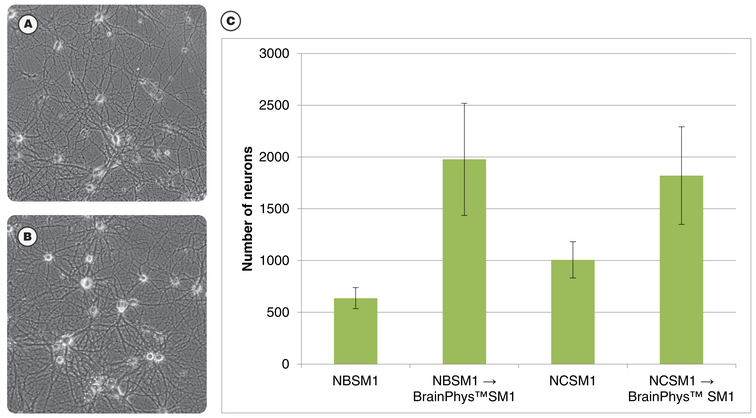
(B) hPSC Derived Neurons



Neural Induction: The embryoid body (EB) protocol for neural induction uses STEMdiff™ Neural Induction Medium and Aggrewell™800 plates, while the neural rosette selection uses STEMdiff™ Neural Rosette Selection Reagent (see full protocol in STEMCELL website Doc #28782). **Neural Differentiation:** The neuronal differentiation protocol uses BrainPhys™ basal medium with NeuroCult™ SM1 Supplement, N2 Supplement-A and specific cytokines.

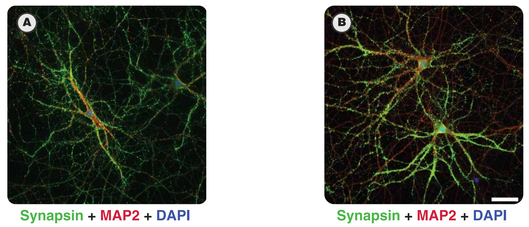
Primary Neuronal Cultures in BrainPhys™ + SM1

Figure 1. Morphology of primary neurons in TSFM and BrainPhys™ cultures at 21 days *in vitro*.



Primary rat E18 cortical neurons were cultured for 21 days in TSFM or BrainPhys™ SM1. Cells were first plated in either Neurobasal (NB) or NeuroCult™ Neuronal Basal Medium (NC) with NeuroCult™ SM1 Neuronal Supplement (SM1). After 5 days in culture, half of the plating medium was replaced by the same volume of Neurobasal (A) or BrainPhys™ Neuronal Medium (B) with NeuroCult™ SM1 Neuronal Supplement. Higher number of neurons were obtained when BrainPhys™ Neuronal Medium was used as the maturation medium (C); (n = 2, mean ± SE [triplicate wells were set up for each experiment]).

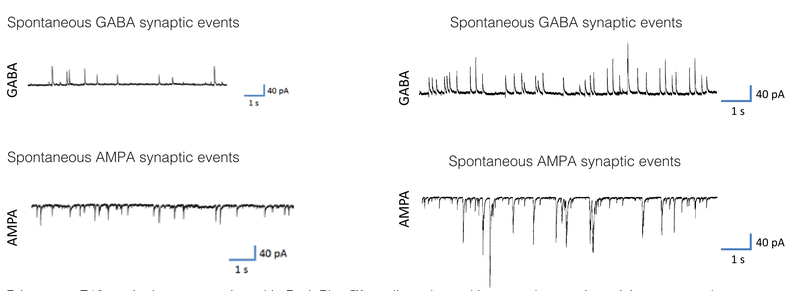
Figure 2. Expression of synaptic marker for neurons cultured in TSFM and BrainPhys™ Medium.



Primary rat E18 cortical neurons cultured in TSFM (A) or BrainPhys™ medium (B) for 21 days are phenotypically mature as indicated by the presence of an extensive dendritic arbor and appropriate expression of synaptic marker (Synapsin1). Synapsin1 (**green**) staining is concentrated in discrete puncta distributed along the soma and dendritic processes, as defined by MAP2 (**red**).

Figure 3. BrainPhys™ Medium supports synaptic activity of mature neurons *in vitro*.

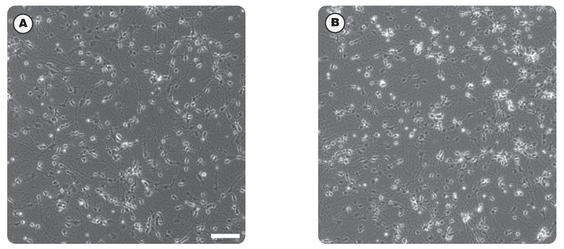
(A) TSFM **(B) BrainPhys™ Medium**



Primary rat E18 cortical neurons cultured in BrainPhys™ medium showed improved synaptic activity compared to neurons cultured in TSFM (A). Spontaneous synaptic events mediated by GABA receptors (top) and AMPA receptors (bottom). For TSFM (A), cells were cultured in Neurobasal/ B-27 throughout the duration of the culture period. For the BrainPhys™ condition (B), cells were cultured in NeuroCult™ Neuronal Basal Medium with NeuroCult™ SM1 for the first 5 days and switched to BrainPhys™ Neuronal Medium with NeuroCult™ SM1 until the end of the culture period. This indicates that switching to BrainPhys™ improves neuronal maturation compared to continuous culture using the traditional serum-free medium and protocols.

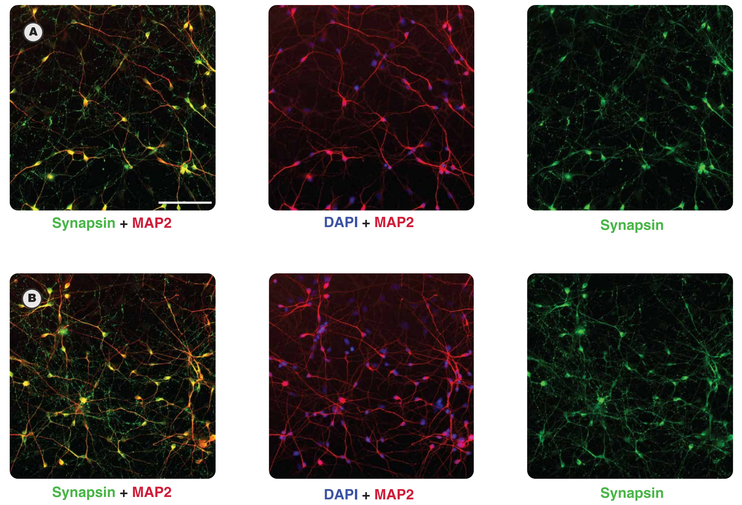
hPSC derived Neurons in BrainPhys™

Figure 4. Morphology of hPSC-derived neurons cultured in BrainPhys™ for 14 days.



hPSCs were used to generate neural progenitor cells using an EB protocol. Neural progenitor cells were then differentiated in (A) DMEM/F12 or (B) BrainPhys™ with additional growth factors for 14 days. Phase contrast images show a high number of viable neurons with extensive neurite outgrowth and branching in both conditions tested. Scale bar = 50 μm.

Figure 5. iPSC derived neurons express synaptic markers after 21 days in BrainPhys™ Medium.



hPSC-derived neurons express synaptic markers (Synapsin1) and the mature neuronal marker MAP2. Neurons were matured for 14 days in (A) DMEM/F12 or (B) BrainPhys™ and were subsequently fixed and stained for Synapsin1 (**green**) and MAP2 (**red**).

Summary

BrainPhys™ Medium:

- BrainPhys™ can be used to culture neurons derived from primary CNS tissues and hPSCs. Neurons exhibit mature neuronal morphology and express Synapsin1 and MAP2.
- Primary neurons cultured in BrainPhys™ exhibit spontaneous GABA synaptic and AMPA synaptic activities.
- BrainPhys™ and growth factors can support the maturation of hPSC-derived neurons that express Synapsin1 and MAP2.
- BrainPhys™ is a novel media based on Bardy et al. 2015 for the maintenance and maturation of primary and hPSC-derived neurons that is physiologically relevant.