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ACCELERATED MATURATION AND IMPROVED FUNCTIONALITY OF HUMAN IPSC-DERIVED NEURONS WITH THE B-27™ PLUS NEURONAL CULTURE SYSTEM

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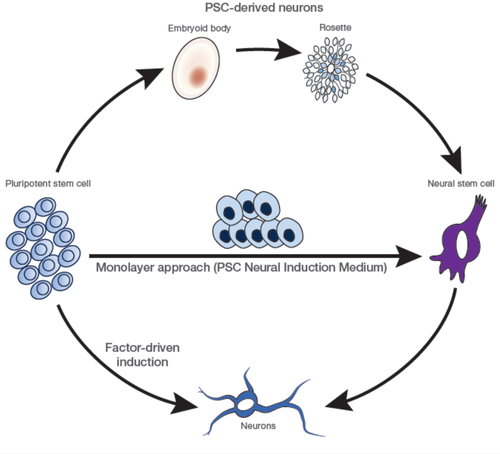
INTRODUCTION

Human iPSC-derived neurons have increasingly become a valuable system for the study of neurological disorders. Robust cell reprogramming and improved differentiation protocols enable scientists to generate patient-specific, disease in a dish models for disorders such as Parkinson's, Alzheimer's, and Autism, among others. These human models tend to be flexible, scalable and maintain many of the characteristics of found in these disorders, which are key requirements for their use in mechanistic and drug discovery studies. Further, the development of gene editing technology has spawned intense interest in the use of gene-edited, patient-specific iPSC-derived neurons in cell therapy applications for the treatment of neuro-degenerative disorders.

A critical step in generating useful iPSC-derived neurons is neuronal maturation. During maturation neurons extend neurites to form highly connected networks, express synaptic markers, and become electrically active. Typical maturation conditions are inefficient, generating poorly matured neurons with low levels of functionality over extended periods of time. Recently we developed a new neuronal neuronal maturation and maintenance system, (B-27™ Plus and Neurobasal™ Plus) and showed significantly improved neuronal survival, maturation, and functionality of primary rodent neurons compared to other culture systems.

Here we expand our studies to PSC-derived neurons, utilizing multiple human lines PSC and different approaches for neural stem cell derivation. Diverse endpoints were used to interrogate maturation; neurite outgrowth, neuronal maturation marker expression (through quantitative imaging), and functionality through Multi-Electrode Array (MEA) analysis. We found that human PSC-derived neurons matured in the new "Plus" system showed both accelerated neurite outgrowth and improved activity as compared to other approaches. Additionally our studies highlight the importance of optimizing several key parameters, including extracellular matrix coating concentrations and delivery conditions for improved reproducibility and quality of stem cell derived neural cultures.

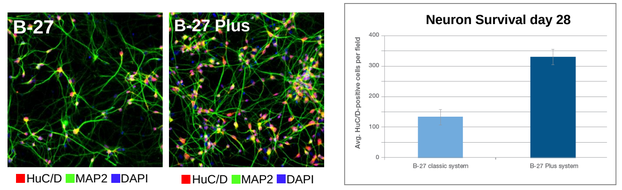
Figure 1. Schematic outlining three common methods for generating PSC-derived neurons



RESULTS

Figure 2. Increased survival of PSC-derived neurons cultured in the B-27™ Plus Neuronal Culture System

a) Neurons matured from rosette NSCs



b) Neurons matured from monolayer NIM NSCs (PSC Neural Induction Medium)

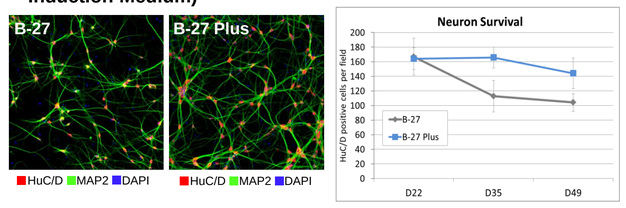
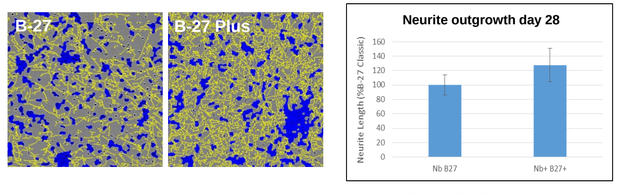


Figure 3. Superior maturation of PSC derived neurons with the B-27™ Plus culture system

a) Neurons matured from rosette NSCs



b) Neurons matured from monolayer NIM NSCs (PSC Neural Induction Medium)

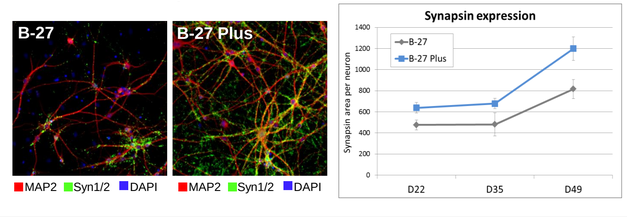
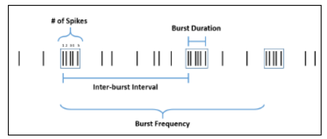
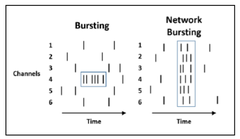


Figure 4. Improved Functionality: Multi-Electrode Array Analysis (MEA)

a) MEA 101: Spontaneous activity parameters- mean firing rate, bursting, network bursting

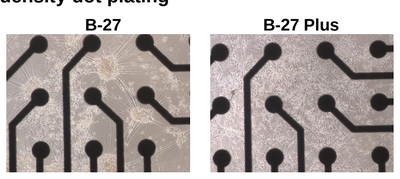


Activity analysis from single electrode over time



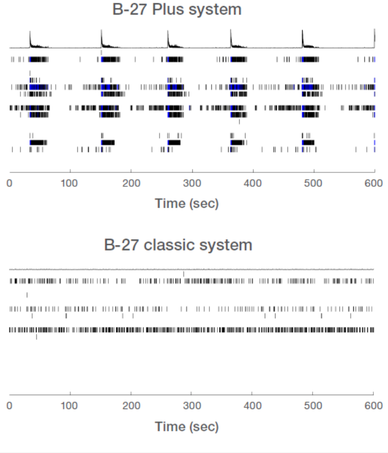
Multiple electrodes enable spatial and temporal analysis (Network activity)

b) High-density dot plating



- Clumping observed in "classic" B-27 medium
- Neurons maintain a uniform monolayer in B-27 Plus medium

c) Activity raster plots at Day 36

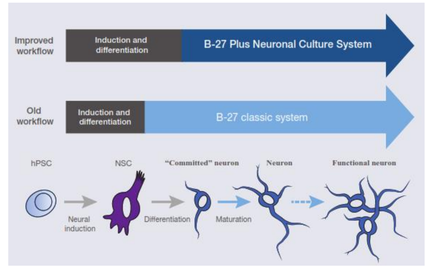


- Medium activity
- High synchrony
- Consistent network bursting

- Low activity
- Low synchrony
- No network bursting

Figure 5. General guidance: Using B-27™ Plus Neuronal Culture System for stem cell-derived neuron culture

B-27™ Plus is for maturation and long-term maintenance of PSC-derived neurons



When differentiating NSCs that were created using a monolayer or rosette method, we recommend switching to the B-27 Plus system after 3–7 days or when the cell population has adopted a neuronal-like morphology.

CONCLUSIONS

The Gibco™ B-27™ Plus neuronal culture system provides significant improvement in survival, maturation, and functionality of PSC-derived neurons

1. NSCs derived from multiple methods show increased survival in cell number at 4 weeks and beyond
2. Enhanced maturation: neurite outgrowth and synapsin staining
3. Electrophysiological analysis by MEA shows a remarkable improvement in overall activity and the formation of synchronous bursting networks using the B-27™ Plus system
4. Transition to the Plus system is recommended after NSCs "commit" to neuronal fate, a process that can vary depending on the cell background and method of derivation

ACKNOWLEDGEMENTS

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