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Regulated control of glial cell outgrowth in primary rodent neuronal cultures by CultureOne™ supplement

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ABSTRACT

Primary neurons derived from rodent fetal brain are used extensively throughout neuroscience research providing scientists with dynamic and convenient model systems to study basic neuronal function and morphology, disease modeling, drug development, and neurotoxicity. Cryopreserved primary neurons significantly reduce the resources, variability, and time, necessary to isolate these cells from embryos and are routinely used for electrophysiological recording, excitability experiments, and high-throughput and High-Content Screening (HCS) studies. A common complication observed when culturing primary neurons is the overgrowth of glial cells. Levels of glial cells can vary widely with different isolation methods, age of embryos used, the composition of different media systems, and species of origin. Glial cell contamination and overgrowth can affect assay sensitivity, resolution, and reproducibility. Current methods for reducing glial cell populations in primary neuronal cultures involve treatment with anti-mitotic molecules, such as Cytosine Arabinoside (Ara-C), which have been shown to be toxic to neurons in culture.

Gibco CultureOne™ supplement was developed for the differentiation of human pluripotent stem cell-derived neural stem cells (NSCs) into neurons by suppressing the proliferation of NSCs and accelerating the maturation of differentiating neurons. Here we show that addition of CultureOne™ supplement to neuronal culture medium can suppress the outgrowth of glial cells (astrocytes and oligodendrocytes) in primary rat (E18) cortical neurons, and mouse (E17) cortical and hippocampal neurons. We also demonstrate CultureOne™ supplement does not impact neuron numbers, morphology, or function. To quantify glial cells and neurons we employed Immunocytochemistry (ICC) and quantitative image analysis with a High-Content Analysis platform. Multi-Electrode Array (MEA) analysis were used to assess neuronal function.

Treatment of primary neuron cultures with CultureOne™ supplement at time of cell plating resulted in nearly complete elimination of contaminating glial cells assessed following 14 and 21 days in culture with no significant impact on neuron numbers or morphology. In contrast, delaying the addition of CultureOne™ 4, 6, and 8 days following plating, resulted in increasing levels of glial cells. The results suggest that glial cell levels can be controlled or optimized by adjusting CultureOne™ treatment schedule.

INTRODUCTION

The goal of this work is to provide a solution for suppressing and controlling glial cell levels in primary neuronal cultures without impacting the number of neurons or morphology. We demonstrate that CultureOne™ effectively suppresses glial cell outgrowth without impacting neuron number or morphology. We also show delaying the time of addition of CultureOne™ results in a "tuning" effect on the levels of Astrocytes. Finally, we look at functional activity of neuronal cultures treated with CultureOne™ employing Multi-electrode array studies.

MATERIALS AND METHODS

Neuronal cell culture and treatment

Rodent neurons were plated on Poly-D-Lysine coated plates and cultured in the **B-27™ Plus neuronal culture system** for 3 to 4 weeks and treated with CultureOne™ Supplement at 1x concentration as indicated. Image capture and quantitation was performed on the CellInsight™ CX5 High Content Screening (HCS) Platform.

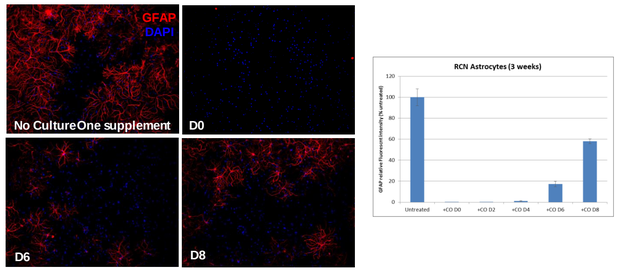
Electrophysiology Studies: Multi-Electrode Array (MEA)

Rat cortex neurons were "dot" plated on Poly-D-Lysine coated 48 well CytoView™ (Axion BioSystems) MEA plates at a density of 80,000 cells per 8ul. Cells were cultured in the B-27™ Plus neuronal culture system for 24 days with or without CultureOne™ supplement. A third condition of standard Neurobasal B-27™ media was included for comparison. Electrophysiological activity was recorded and analyzed using the Maestro MEA™ System from Axion BioSystems.

www.axionbiosystems.com

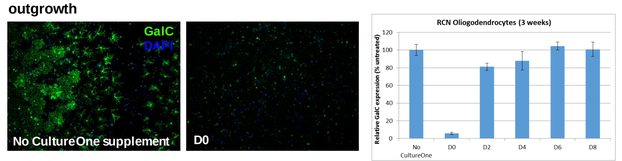
RESULTS

Figure 1. CultureOne™ Supplement controls astrocyte outgrowth and proliferation in neuronal cultures



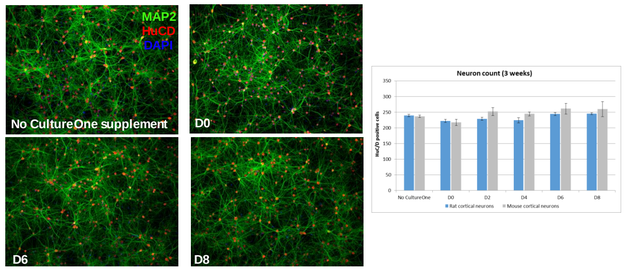
Treatment of primary neurons with CultureOne™ at the time of plating (D0), or starting at day 2 (D2), showed complete suppression of astrocytes when cultures were assessed after 3 weeks. Delaying the addition of CultureOne™ to days 4, 6, and 8 after plating resulted in increasing levels of astrocytes in the cultures.

Figure 2. Treatment with CultureOne™ Supplement suppresses oligodendrocyte outgrowth



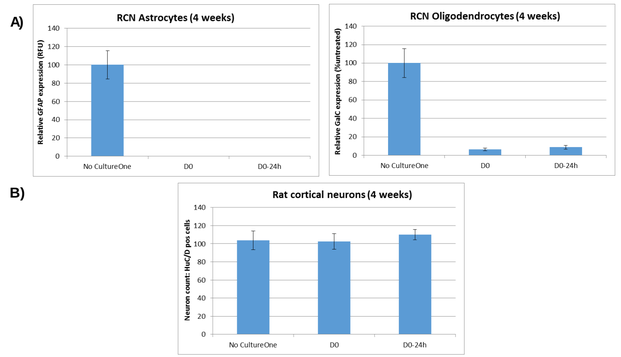
Similarly, oligodendrocyte outgrowth was completely inhibited (Figure 2) in neuronal cultures treated with CultureOne™ Supplement at the time of plating (D0). Delayed treatment resulted in similar levels compared to untreated condition.

Figure 3. CultureOne™ treatment does not impact neuron number or morphology



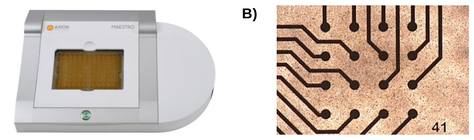
The data here show that CultureOne™ Supplement has no effect on the number or morphology of cultured neurons

Figure 4. Short-term (24h) "pulse" treatment and long-term culture (4 weeks)



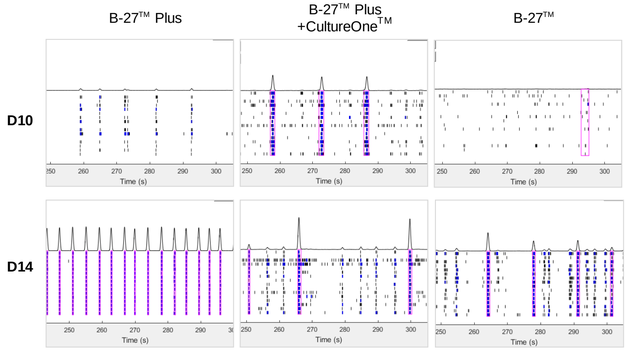
A) Short-term (24h) treatment with CultureOne™ is effective for suppression of glial cells.
B) Long-term treatment with CultureOne™ (4 weeks) does not impact the number of neurons.

Figure 5. Electrophysiological analysis, Multi-Electrode Array (MEA)



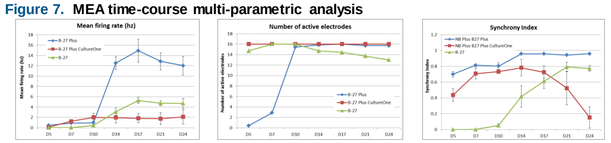
A) Maestro MEA™ System from Axion BioSystems was used for MEA experiments.
B) 10x phase image of a well from a 48 well CytoView™ MEA plate containing 16 electrodes

Figure 6. Raster plotted MEA data from Rat cortical neurons treated with CultureOne™



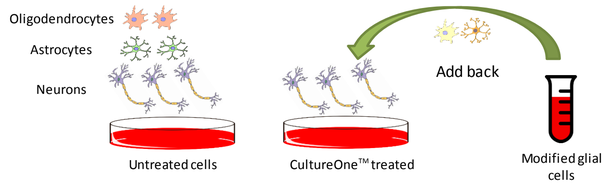
Raster plotted activity show CultureOne™ treated neurons form synchronous bursting activity at early time points.

Figure 7. MEA time-course multi-parametric analysis



CultureOne™ treated neurons are physiologically active, show early networked activity and distinct spiking characteristics. Mean firing rate is decreased compared to the untreated condition and synchronous activity decreases over time. Differences in activity reflect the lack glial cells

Figure 8. Potential co-culture model system for modified glial cell studies



CONCLUSIONS

- The addition of CultureOne™ Supplement can completely suppress the outgrowth of glial cells (astrocytes and oligodendrocytes) in primary rat and mouse neurons with no impact on neuron numbers or morphology, tested out to 4 weeks.
- Delaying the addition of CultureOne™ up to 8 days following plating resulted in increasing levels of astrocytes when assessed after 3 weeks. These results suggest that glial cell levels can be optimized by adjusting CultureOne™ treatment schedules.
- We show that short term (24h) CultureOne™ treatment is effective for suppression of glial cells.
- Functional studies demonstrate CultureOne™ treated neurons are active and demonstrate a distinct firing pattern.
- Pure neuronal cultures generated by suppression of glial cells with CultureOne™ may be a valuable model system for studying glial cell dysfunction.

REFERENCES

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TRADEMARKS/LICENSING

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