Cell culture

Improve the purity of primary neurons with the CultureOne Supplement

Introduction

The Gibco™ CultureOne™ Supplement helps achieve superior neuronal cultures, whether working with human stem cell–derived neurons or primary rodent neurons. CultureOne Supplement efficiently controls the proliferation of both neural progenitor cells and glial cells, resulting in neurons of the highest purity. Furthermore, these neurons can be maintained in the Gibco™ B-27™ Plus Neuronal Culture System for extended periods to achieve desired maturation and function.

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. While glial cells play important roles to support various neuronal functions [1,2], a common complication observed when culturing primary neurons is the presence of proliferating glial cells in the cultures [3]. The population of glial cells in neuronal cultures can vary widely with different isolation methods, age of embryos used, composition of different media systems, and species of origin. Varying degrees of glial cell growth can affect assay sensitivity, resolution, and reproducibility. Traditional 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 [4].

In this study, we show that addition of CultureOne Supplement to the B-27 Plus Neuronal Culture System can suppress the outgrowth of glial cells (astrocytes and oligodendrocytes) in primary rat (E18) and mouse (E17) cortical and hippocampal neurons without impact to the number of neurons or their morphology.

Materials and methods

The materials used for primary neuron cell culture, immunostaining, and cell imaging assays are listed in Table 1.

Table 1. Materials used in this study.

Product	Cat. No.
Neurobasal Plus Medium	A3582901
B-27 Plus Supplement (50X)	A3582801
CultureOne Supplement (100X)	A3320201
GlutaMAX Supplement	35050061
Primary Mouse Cortical Neurons	A15585
Primary Mouse Hippocampal Neurons	A15587
Primary Rat Cortical Neurons	A1084001
Gentamicin (10 mg/mL)	15710064
DPBS, calcium, magnesium	14040133
HuC/HuD Monoclonal Antibody (16A11)	A21271
MAP2 Polyclonal Antibody	PA517646
GFAP Monoclonal Antibody (2.2B10)	130300
Anti-Galactocerebroside Antibody, Clone mGalC (EMD Millipore)	MAB342
HCS Studio Cell Analysis Software	Inquire
CellInsight CX5 High Content Screening Platform	CX51110

Neuronal cell culture and treatment

Gibco™ Primary Rat Cortical Neurons (E18), Primary Mouse Cortical Neurons (E17), and Primary Mouse Hippocampal Neurons were plated at 60,000 cells/cm^2 (rat cortical neurons) and 90,000 cells/cm^2 (mouse cortical and hippocampal neurons) onto poly-D-lysine–coated 96-well plates. Cells were maintained for 3 weeks in the B-27 Plus Neuronal Culture System (Gibco™ Neurobasal Plus Medium and B-27™ Plus Supplement) with Gibco™ GlutaMAX™ Supplement (1:400). The cells were maintained in a humidified incubator at 37°C with 5% CO_2. To suppress the outgrowth of glial cells (astrocytes and oligodendrocytes), CultureOne Supplement was added to the complete Neurobasal Plus Medium (1:100) at the time of plating (D0), or treatment was delayed 2, 4, 6, and 8 days after plating.

Immunocytochemistry

Cells were fixed at day 21 and then stained with primary and secondary antibodies. Neurons were stained with Invitrogen™ HuC/HuD (5 µg/mL) and MAP2 (1:400) primary antibodies; astrocytes and oligodendrocytes were stained with Invitrogen™ GFAP antibody (1:100) and galactocerebroside (GalC) antibody (EMD Millipore, 1:200), respectively. Following incubation, cells were washed (3x) with DPBS and stained with the appropriate secondary antibody conjugated to Invitrogen™ Alexa Fluor™ dye according to the manufacturer's instructions. Cell nuclei were counterstained with DAPI solution (3 ng/mL) for 5–10 minutes prior to image acquisition and analysis.

Analysis and quantification of glial cell outgrowth and neuron survival

The Thermo Scientific™ CellInsight™ CX5 HCS Platform was used for automated image acquisition and quantitative analysis of fluorescently labeled cells. For quantitative image analysis, the target activation assay in the Thermo Scientific™ HCS Studio™ Cell Analysis Software was used to quantify the relative fluorescence from labeled cells and the total number of neurons per image field (HuC/HuD-positive cells). To quantify glial cells, the general intensity protocol in HCS Studio software was used to quantify relative fluorescence intensities per image field for GFAP- and GalC-stained wells.

Results

CultureOne Supplement has been shown to effectively suppress the proliferation of neural stem cells (NSCs) and accelerate the maturation of differentiating neurons [5]. The current experiments were designed to determine the ability of CultureOne Supplement to control glial cell levels in primary neuronal cell cultures. Data here show that the addition of CultureOne Supplement to complete Neurobasal Plus Medium (Table 2) suppresses the outgrowth of glial cells in primary rat and mouse neuronal cultures (Figures 1 and 2). Treatment of primary neurons with CultureOne Supplement at the time of plating (D0), or starting at day 2 (D2), showed complete suppression of astrocytes when cultures were assessed after 3–4 weeks (Figure 1).

Table 2. Complete Neurobasal Plus Medium with CultureOne Supplement.

Reagent	Volume
Neurobasal Plus Medium	98 mL
B-27 Plus Supplement (50X)	2 mL
CultureOne Supplement (100X)	1 mL
GlutaMAX Supplement	250 µL
Gentamicin (10 mg/mL)	100 µL

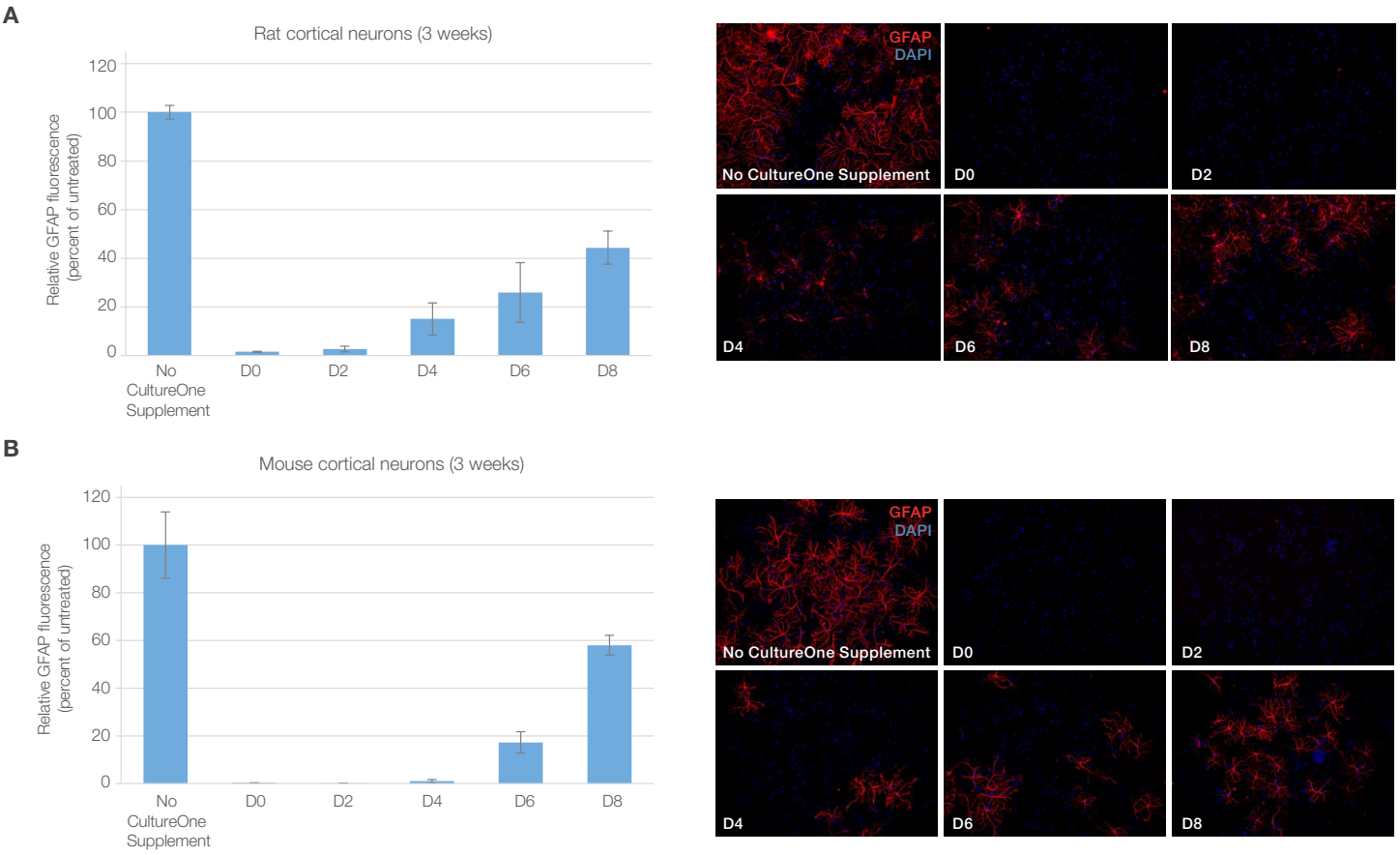

Figure 1. CultureOne Supplement controls astrocyte outgrowth and proliferation in neuronal cultures. (A) Primary rat cortical neurons and **(B)** mouse cortical neurons were cultured for 3 weeks in B-27 Plus Neuronal Culture System complete medium. Cells were treated with CultureOne Supplement at the time of plating (D0) or treatment was delayed to day 2 (D2), day 4 (D4), day 6 (D6), and day 8 (D8). Cells were fixed at day 21 and stained (images, A and B) with astrocyte-specific GFAP antibody (1:100, red). Nuclei were counterstained with DAPI (blue). Images were captured on the CellInsight CX5 HCS Platform. HCS Studio Cell Analysis Software was used to quantify the relative fluorescence from GFAP-labeled cells (graphs, A and B). The data represent the average GFAP fluorescence per field from 3 wells; 15 fields were captured per well. Data are reported as the mean ± SEM. Similar results were observed for mouse hippocampal neurons (data not shown).

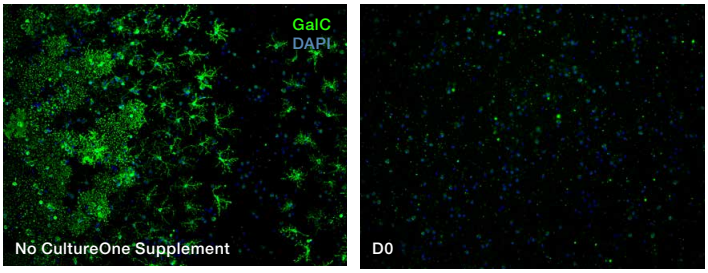

Figure 2. Treatment with CultureOne Supplement suppresses oligodendrocyte outgrowth. Primary rat cortical neurons were cultured for 3 weeks in the B-27 Plus Neuronal Culture System and treated with CultureOne Supplement at the time of plating (D0). Cells were stained with oligodendrocyte-specific GalC antibody (1:200, green). Nuclei were counterstained with DAPI (blue) prior to imaging. Images were captured on the CellInsight CX5 HCS Platform. Similar results were observed for mouse cortical and hippocampal neurons (data not shown).

Delaying the addition of CultureOne Supplement to days 4, 6, and 8 after plating resulted in increasing levels of astrocytes in the cultures. Similarly, oligodendrocyte outgrowth was completely inhibited in neuronal cultures treated with CultureOne Supplement at the time of plating (D0) (Figure 2). However, in contrast to the “tuning” effect observed with astrocyte levels using timed addition of CultureOne Supplement, delaying treatment of CultureOne Supplement to days 2, 4, 6, or 8 had minimal or no effect on the oligodendrocyte levels (data not shown).

Traditional methods used to reduce glial cell populations in primary neuronal cultures have shown to be toxic to neurons. The data here show that CultureOne Supplement has no effect on the number or morphology of cultured neurons (Figure 3).

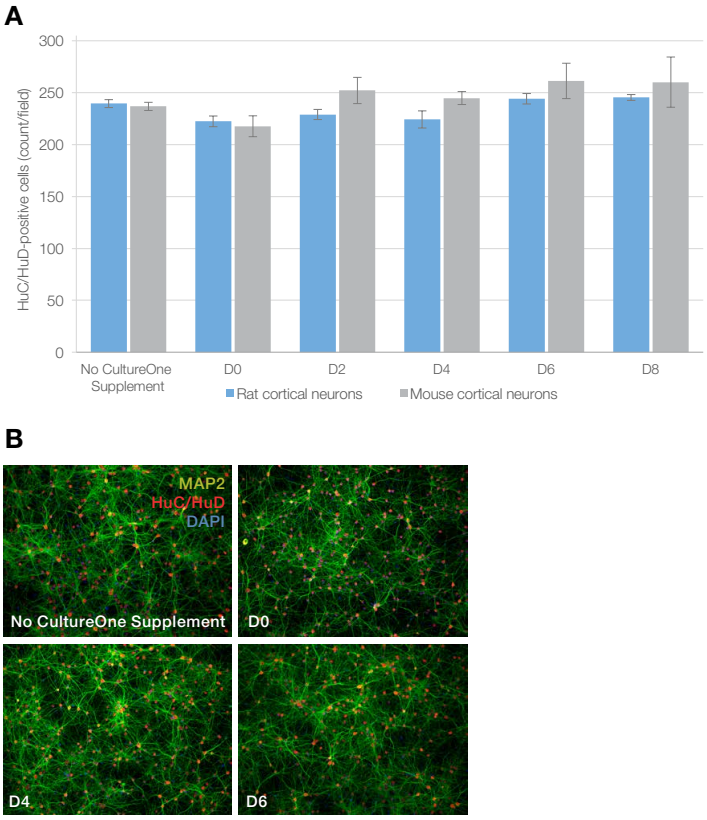

Figure 3. No effect on number or morphology of neurons. Rat cortical neurons or mouse cortical neurons were plated on poly-D-lysine–coated plates and cultured in the B-27 Plus Neuronal Culture System. **(A)** Cells were treated with CultureOne Supplement at the time of plating, D0, or the onset of treatment was delayed to days 2 (D2), 4 (D4), 6 (D6), and 8 (D8). **(B)** Rat cortical neurons were fixed at day 21 and neurons were stained with MAP2 (green) and HuC/HuD (red) antibodies. HCS Studio Cell Analysis Software was used to quantify the number of neurons and number of HuC/HuD-positive cells. Nuclei were labeled with DAPI (blue). The data in panel A represent the average number of HuC/HuD-positive cells per field from 3 wells; 6 fields were captured per well. Data are reported as the mean ± SD. Similar results were observed with mouse cortical and hippocampal neurons (data not shown).

Conclusions

The addition of CultureOne Supplement to the B-27 Plus Neuronal Culture System 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. Delaying the addition of CultureOne Supplement up to 8 days following plating resulted in increasing levels of astrocytes when assessed after 3 weeks. These results suggest that CultureOne Supplement can provide highly pure neuronal cultures when used early with the B-27 Plus Neuronal Culture System.

References

1. Banker GA (1980) Trophic interactions between astroglial cells and hippocampal neurons in culture. Science 209(4458):809–810.
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4. Meberg PJ, Matthew MW (2003) Culturing hippocampal and cortical neurons. Methods Cell Biol 71:111–127.
5. User guide: B-27 Plus Neuronal Culture System. Thermo Fisher Scientific, Pub. No. MAN0017319.

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