Complete serum-free culture kit and protocols for culturing high yields of functional mature neurons from primary embryonic mouse CNS tissues

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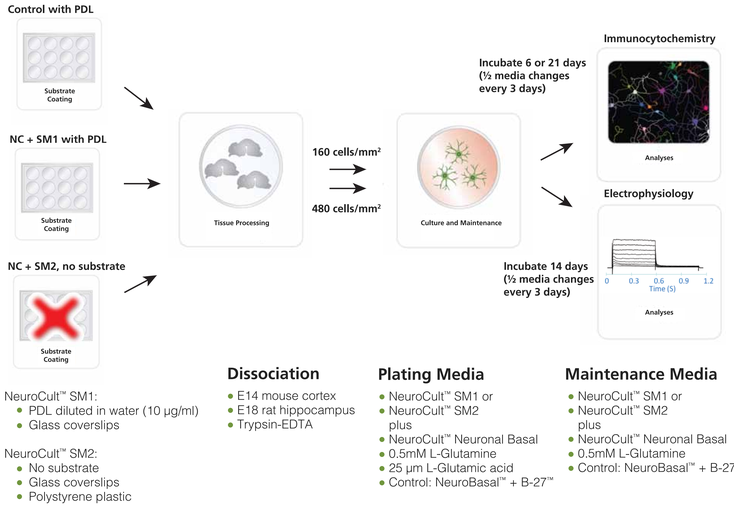
Introduction

Neurons from normal and neurodegenerative central nervous system (CNS) tissues can be cultured *in vitro* to study the properties of neuronal sub-types by measuring neurite outgrowth. However, a traditional serum-free media (NeuroBasal™ plus B27™; Life Tech: Control) and substrate (e.g. poly-D-lysine (PDL)) used for these cultures is often variable. We developed a basal medium (NeuroCult™ Neuronal Basal medium: NC) and two supplements, SM1 and SM2-Substrate Independent, which in combination consistently produce higher yields of neurons. SM2 provides a unique system for culturing neurons without substrate coating.

Methods

FIGURE 1: Experimental design



Dissociation
- E14 mouse cortex
- E18 rat hippocampus
- Trypsin-EDTA

Plating Media
- NeuroCult™ SM1 or
- NeuroCult™ SM2 plus
- NeuroCult™ Neuronal Basal
- 0.5mM L-Glutamine
- 25 µm L-Glutamic acid
- Control: NeuroBasal™ + B-27™

Maintenance Media
- NeuroCult™ SM1 or
- NeuroCult™ SM2 plus
- NeuroCult™ Neuronal Basal
- 0.5mM L-Glutamine
- Control: NeuroBasal™ + B-27™

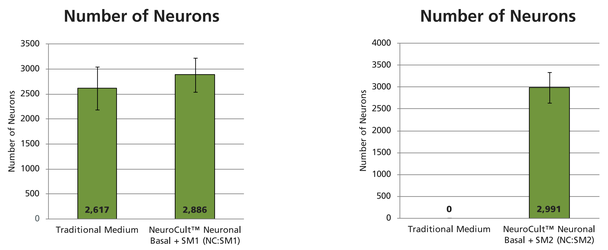
NeuroCult™ SM1:
- PDL diluted in water (10 µg/ml)
- Glass coverslips

NeuroCult™ SM2:
- No substrate
- Glass coverslips
- Polystyrene plastic

Cortices from E14 mice or hippocampi from E18 rat were dissected, mechanically dissociated, and diluted into three separate media: Control, NC plus SM1 (SM1) or NC plus SM2 (SM2). Cells in Control or SM1 were then plated in wells containing PDL-coated cover-slips either at 160 cells/mm^2 for immunocytochemical (ICC) analysis or 480 cells/mm^2 for electrophysiology, while cells in SM2 were plated at the same cell densities in wells containing uncoated cover-slips. Cells were cultured for either 6 or 21 days for ICC analysis or electrophysiology studies, respectively. Neurons were detected by β III Tubulin and DAPI double staining and scored for quantitation analyses.

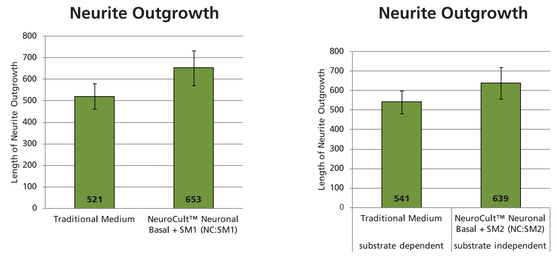
Results

FIGURE 2: Growth of primary neurons in NeuroCult™ Neuronal Basal and SM1 or SM2 compared to a traditional media (control) (mean ± SE; n =11)



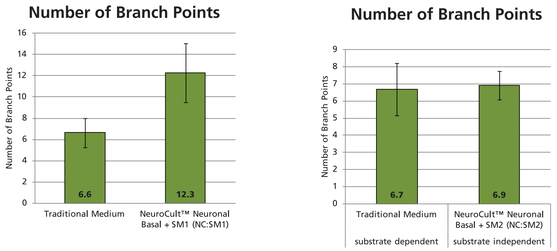
A) SM1 combined with new NeuroCult™ Neuronal Basal medium gave slightly higher numbers of neurons (2886 ± 339) compared to a traditional medium (2616 ± 428). B) Neurons cultured in NeuroCult™ Neuronal Basal medium in combination with SM2 promotes neuronal growth in the absence of a substrate coating. Neurons cultured in a traditional medium do not support the survival of neurons in the absence of poly-D-lysine coating.

FIGURE 3: Mean neurite outgrowth of neurons cultured in NeuroCult™ Neuronal Basal and SM1 or SM2 compared to a traditional media (control) (mean ± SE; n =11)



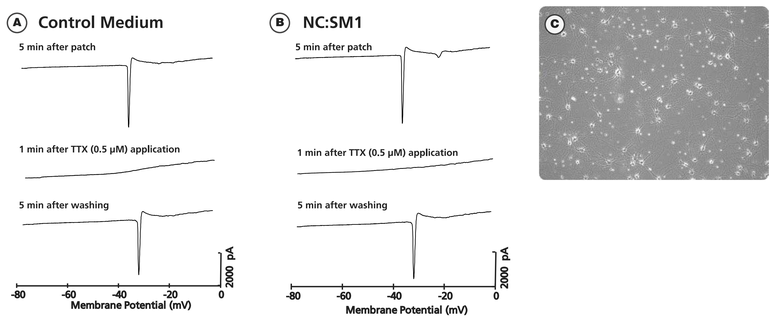
A) The mean neurite outgrowth of the neurons cultured in NC:SM1 was significantly higher (653 ± 80; p<0.03) compared to neurons cultured in traditional medium (521 ± 58). B) Neurons cultured in NeuroCult™ Neuronal Basal medium and SM2 in the absence of a substrate coating show extensive neurite outgrowth (639 ± 56), comparable to neurons cultured in a traditional medium on poly-D-lysine coated plates (541 ± 67).

FIGURE 4: Average number of branch points of neurons cultured in NeuroCult™ Neuronal Basal and SM1 or SM2 compared to a traditional media (control) (mean ± SE; n=11)



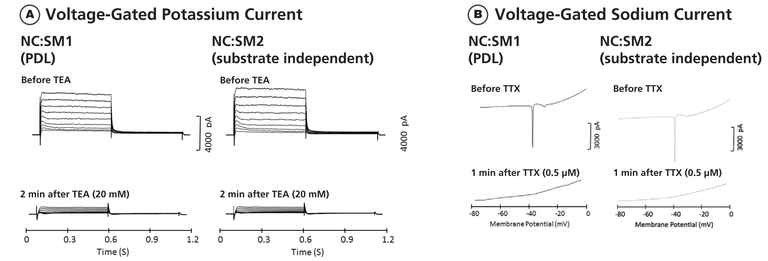
A) In addition, neurons cultured in NC:SM1 were significantly more mature as determined by their average branch point compared to neurons cultured in traditional medium (12.3 ± 2.8 versus 6.6 ± 1.4; p<0.01 respectively). B) Comparable neurite branching are obtained when neuronal culture is performed with substrate independent NeuroCult™ Neuronal Basal medium and SM2 (6.9 ± 0.8) versus a substrate dependent traditional medium (6.7 ± 1.5).

FIGURE 5: Electrophysiology analyses of neurons cultured in NeuroCult™ Neuronal Basal and SM1 showing normal reversible action potentials with TTX treatment



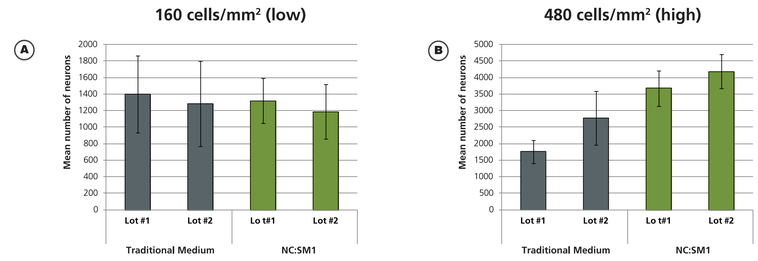
Neurons cultured in the NC:SM1 (B) had an average resting potential of -60 mV comparable to neurons cultured in the traditional medium cultures (A). The action potential traces induced by a ramp test (depolarize from -80 to 0 mV) revealed normal action potentials. These action potentials could be blocked by application of 0.5 µM TTX, and this blockage could be quickly reversed by washing out the TTX (n=3) (A and B). C) Between 40-50% of the neurons in NC:SM1 displayed optimal cell morphology for whole-cell patch clamping.

FIGURE 6: Detailed electrophysiology analyses of neurons cultured in NeuroCult™ Neuronal Basal and SM1 or SM2 showing potassium, sodium, and GABA$_A$ receptor current recordings



Neurons cultured in substrate dependent NC:SM1 and substrate independent NC:SM2 are functionally mature as indicated by normal electrophysiology profiles. A) Potassium currents were blocked by the application of 20mM tetraethylammonium (TEA). B) Sodium currents were recorded by a ramp test (depolarizing cell) from -80 to 0 mV in 200 ms. Currents were blocked by application of 0.5 µM tetrodotoxin (TTX). C) GABA$_A$ receptor currents were induced by the application of 3 µM neurotransmitter gamma-aminobutyric acid (GABA).

FIGURE 7: Neurons cultured at low and high cell densities in NeuroCult™ Neuronal Basal and SM1 for 21 days (mean ± SE; n=4)



Neurons can be cultured at low (A) or high (B) cell plating densities in Neuronal Basal and SM1 for 21 days. Two separate lots of SM1 tested gave comparable total number of neurons.

Summary

The complete kits containing NeuroCult™ Neuronal Basal Medium plus SM1 or SM2 Neuronal Supplement is highly efficient at generating functionally mature neurons compared to a traditional neuronal media formulation. The new NeuroCult™ Basal Medium, SM1 and SM2 media efficiently supported neuronal survival. The SM2 supplement allows neuronal differentiation in the absence of a substrate coating.

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