

A new defined serum-free medium formulation to culture mature neurons at higher yields from primary embryonic mouse CNS tissues.

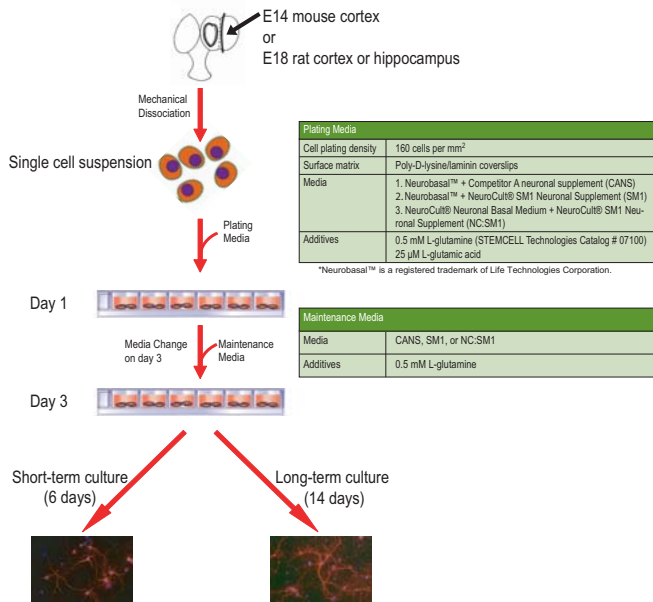
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Introduction

Mature neurons isolated from normal and neurodegenerative tissues of the mammalian CNS are routinely cultured in vitro to study the basic biology of neurogenesis. The neurons produced in these cultures are also used in drug screening for factors that promote or inhibit neurogenesis. The yield and viability of neurons in these serum-free cultures is often variable and so low that subsequent studies are limited. As a result, we sought to develop a serum-free basal medium (NeuroCult® Neuronal Basal Medium) and supplement (NeuroCult® SM1 Neuronal Supplement) which in combination would consistently produce higher yields of neurons from primary CNS cells compared to traditional formulations.

Methods

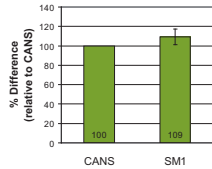
Figure 1: Experimental Design



- Label with anti-β-tubulin (red) antibody and DAPI (blue).
- Count β-tubulin⁺DAPI⁺ cells in six random areas using a software analysis program.

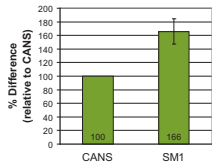
Results

Figure 2: Total number of neurons derived from E14 mouse cortex cells cultured in Neurobasal™ supplemented with Competitor A neuronal supplement (CANS) or NeuroCult® SM1 Neuronal Supplement (SM1).



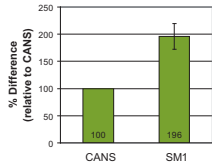
The SM1 supplement combined with NeuroBasal™ gave comparable numbers of neurons to CANS (mean ± SE; n=15).

Figure 3: Mean neurite outgrowth of E14 mouse cortex neurons cultured in Neurobasal™ supplemented with Competitor A neuronal supplement (CANS) or NeuroCult® SM1 Neuronal Supplement (SM1).



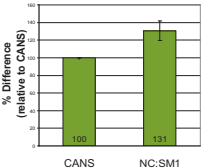
The mean neurite outgrowth of neurons cultured in NeuroCult® SM1 Neuronal Supplement was significantly higher compared to neurons cultured in CANS (mean ± SE; n=15).

Figure 4: Total number of branch points of E14 mouse cortex neurons cultured in Neurobasal™ supplemented with Competitor A neuronal supplement (CANS) or NeuroCult® SM1 Neuronal Supplement (SM1).



Neurons cultured in NeuroCult® SM1 Neuronal Supplement were significantly more mature as determined by their average number of branch points compared to neurons cultured in CANS (mean ± SE; n=15).

Figure 5: Comparison of the total number of E14 mouse cortex neurons cultured in Neurobasal™ supplemented with Competitor A neuronal supplement (N:CANS) or NeuroCult® Neuronal Basal Medium supplemented with NeuroCult® SM1 Neuronal Supplement (NC:SM1).



The complete NeuroCult® medium formulation showed a significantly higher number of neurons compared to the control cultures.

Figure 6: Neurons derived from E18 rat cortex (A,B) or hippocampus (C,D) cultured for 14 days in CANS (A,C) or NC:SM1 (B,D).

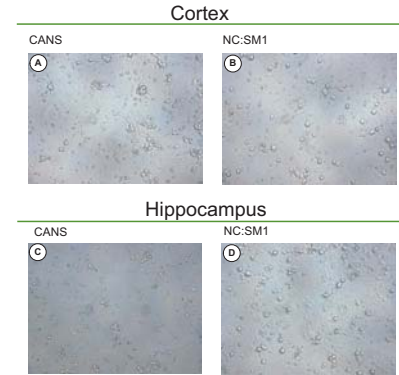
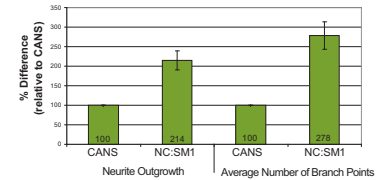


Figure 7: Higher neurite outgrowth and branch points of neurons cultured in NC:SM1 compared to CANS (mean ±SE; n=10).



Summary

A new medium, NeuroCult® Neuronal Basal Medium plus NeuroCult® SM1 Neuronal Supplement is more efficient at generating a higher number of mature neurons from fresh CNS cells compared to a traditional neuronal medium formulation. Studies are currently on-going to evaluate the electrophysiology potential of neurons cultured in NeuroCult® Neuronal Basal Medium plus NeuroCult® SM1 Neuronal Supplement.



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