A New Defined Serum-Free Medium Supplement to Culture Mature Neurons From Primary Embryonic Mouse and Rat CNS in the Absence of an Extracellular Matrix

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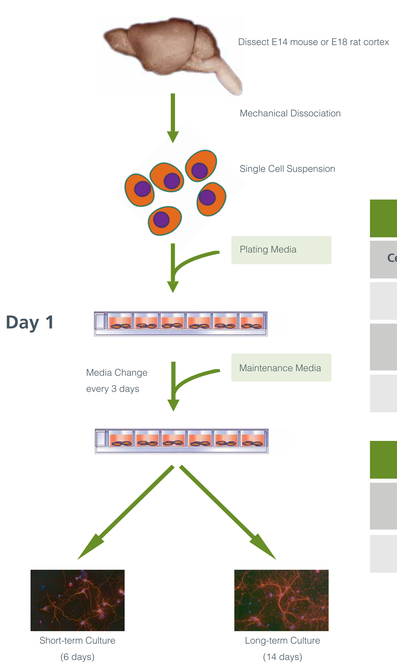
Introduction

Mature neurons isolated from normal and neurodegenerative mammalian CNS tissues are routinely cultured *in vitro* to study the basic biology of neurogenesis, as well as to discover or test pharmaceutical agents that promote or inhibit neurogenesis. Unfortunately, the yield of neurons in serum-free cultures is often variable and is attributed to inconsistencies in preparations of extracellular matrices (ECM), such as poly-D-lysine (PDL) or laminin (L) used in these cultures. Therefore, using cultures devoid of ECM products, we sought to develop a serum-free supplement to improve the culture of mature neurons derived from E14 mouse cortices. We have called our resulting culture supplement NeuroCult® SM2 Neuronal Supplement (SM2), and here compare it to a traditional supplement.

Methods



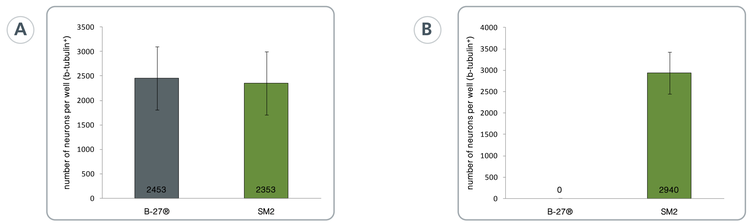
Plating Media	
Cell plating density	160 or 480 cells per mm²
Matrix	PDL/Laminin (PDL/L) or no matrix
Media	NeuroBasal™ + B27® NeuroBasal™ + SM2 (STEMCELL Cat. #05721)
Additives	0.5 mM L-glutamine (STEMCELL Cat. #07100) 25 µM L-glutamic acid

Maintenance Media	
Media	NeuroBasal™ + B27® NeuroBasal™ + SM2 (STEMCELL Cat. #05721)
Additives	0.5 mM L-glutamine (STEMCELL Cat. #07100)

- Label with anti-ß-tubulin antibody and DAPI.
- Count ß-tubulin⁺DAPI⁺ cells in six random areas using a software analysis program.
- Electrophysiology analyses.

Results

FIGURE 2: Growth of Primary Neurons in SM2 and B-27® Medium in Matrix-Dependent and Matrix-Independent Conditions (mean ±SE; n=5)



A) SM2 gave comparable numbers of neurons to B-27® in the presence of PDL/L.

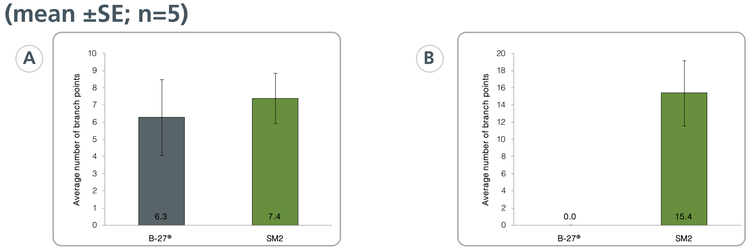
B) In matrix-independent conditions neurons were detected in SM2 cultures, while no neurons were detected in B-27®.

FIGURE 3: Mean Neurite Outgrowth of Neurons Cultured in Matrix-Dependent (B-27®) and Matrix-Independent Conditions (SM2) (mean ±SE; n=5)

A) The mean neurite growth of neurons cultured in SM2 with PDL/L is comparable to neurons cultured in B-27® with PDL/L.

B) The mean outgrowth of neurons cultured in SM2 without PDL/L was higher compared to neurons cultured in B-27® with PDL/L (A).

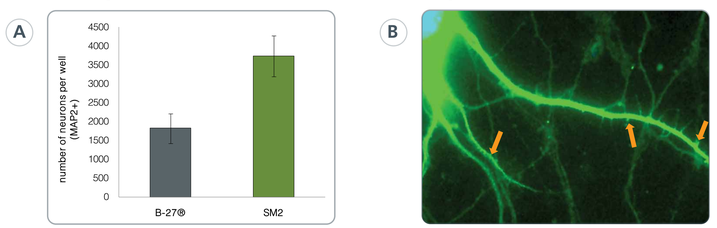
FIGURE 4: Average Number of Branch Points of Neurons Cultured in Matrix-Dependent (B-27®) and Matrix-Independent Conditions (SM2) (mean ±SE; n=5)



A) The mean number of branch points of neurons cultured in SM2 with PDL/L is comparable to neurons cultured in B-27® with PDL/L.

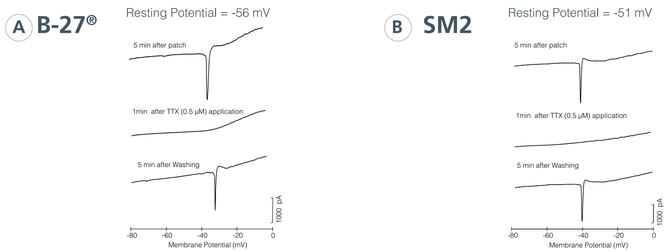
B) The mean number of branch points of neurons cultured in SM2 without PDL/L was higher compared to neurons cultured in B-27® with PDL/L (A).

FIGURE 5: Characterization of Neurons Cultured in B-27® Matrix-Dependent and SM2 Matrix-Independent Cultures



(A) Neurons cultured in SM2 without PDL/L express high levels of MAP2. (B) Neurons cultured in SM2 also express markers for synaptic formation and dendritic spines (arrows) (Millipore Milli-Mark™ FluoroPan Neuronal Marker Cat. #MAB2300X).

FIGURE 6: Electrophysiology Analyses of Neurons Cultured in B-27® Matrix-Dependent and SM2 Matrix-Independent Cultures



Neurons cultured in SM2 without PDL/Laminin (B) are functionally active and capable of producing action potentials similar to those cultured in B-27® matrix-dependent conditions (A).

Summary

- NeuroCult® SM2 Neuronal Supplement is highly efficient at generating mature neurons and stimulating neurogenesis in the absence of a matrix.
- Use of NeuroCult® SM2 Neuronal Supplement eliminates the variability associated with matrices in neuronal cultures.