

Optimized Reagents for the Reproducible Expansion and Differentiation of Adult and Embryonic Mouse Neural Stem Cells in Neurosphere and Adherent Cultures



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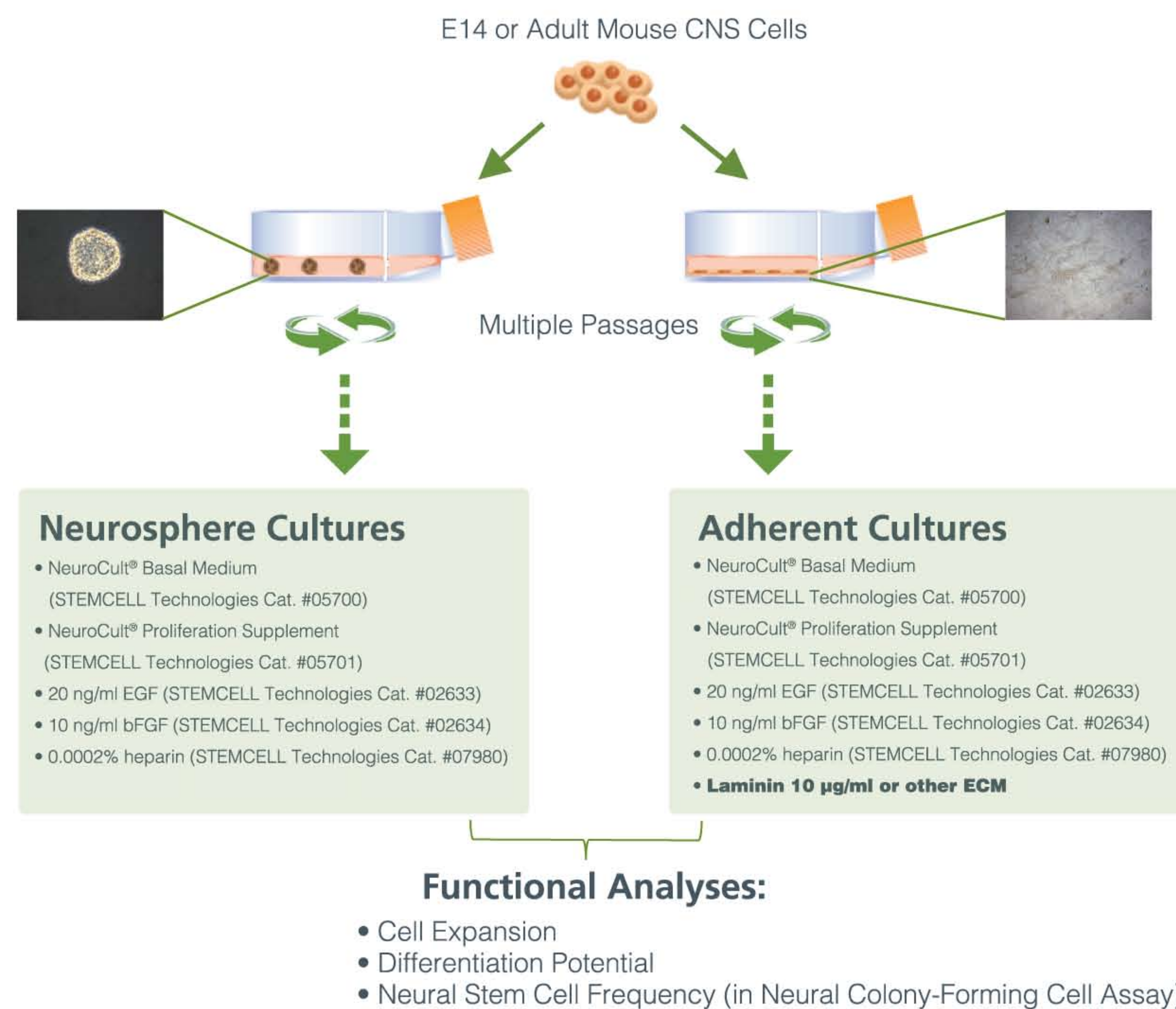
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Introduction

The neurosphere suspension culture system is widely used to isolate neural stem cells (NSC) and progenitors from the adult and embryonic mammalian CNS. A rare population of cells proliferate in the serum-free neurosphere cultures containing EGF and FGF to form aggregates called neurospheres, which have the ability to: 1) self-renew, 2) produce a large number of progeny, and 3) maintain multi-lineage differentiation, thus meeting the criteria of being a NSC. Recently, several reports have suggested that culturing CNS cells in neurosphere cultures does not efficiently maintain NSC and produces a heterogeneous cell population. In contrast, it is reported that culturing cells in serum-free adherent culture conditions in the presence of extracellular matrices (ECMs) such as poly-D-lysine (PDL)/laminin or other matrices does maintain NSC (Conti *et al.*, 2005). However, none of the reports directly compared neurosphere and adherent cell culture in the same medium or matrix evaluating NSC numbers, proliferation, and differentiation potential. We, therefore, sought to test if serum-free NeuroCult® media could support the proliferation of subventricular zone (SVZ) cells in both neurosphere and adherent cultures and then to directly compare NSC number, proliferation, and differentiation potential of SVZ cells in these two culture systems.

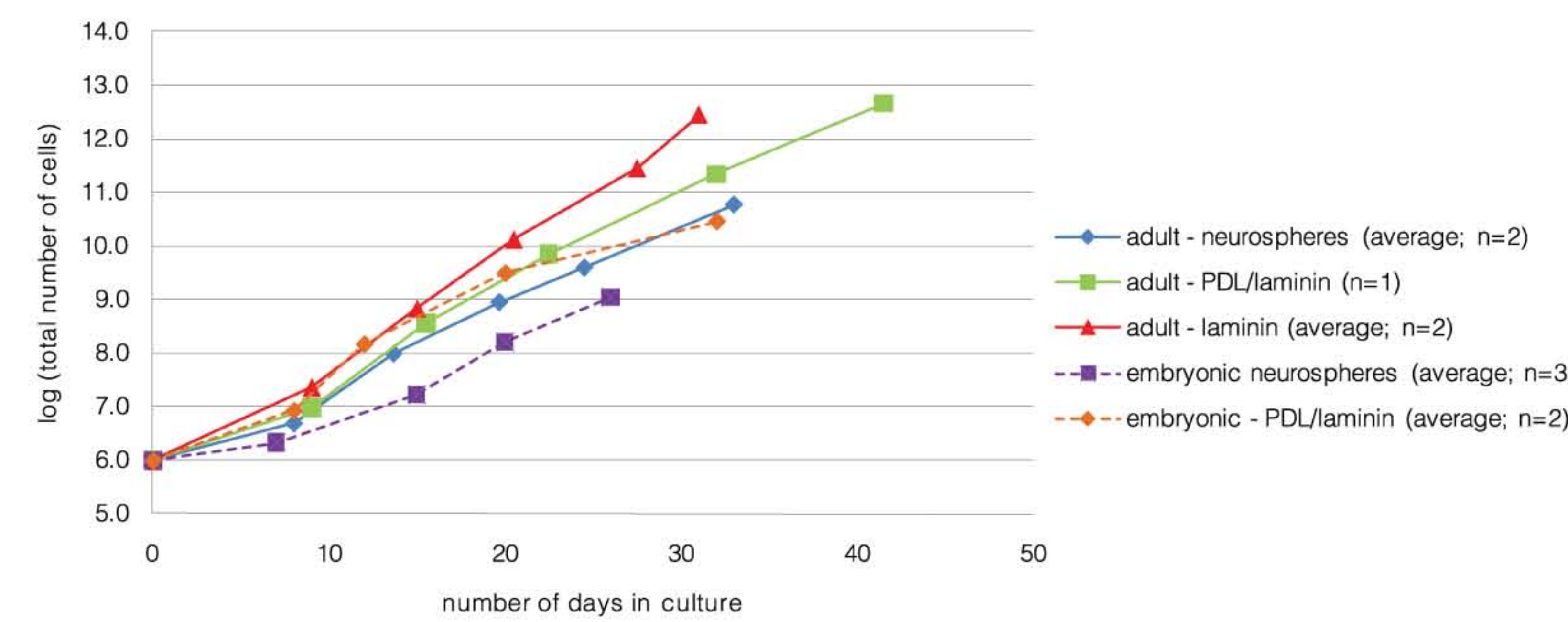
Methods

FIGURE 1: Experimental Design



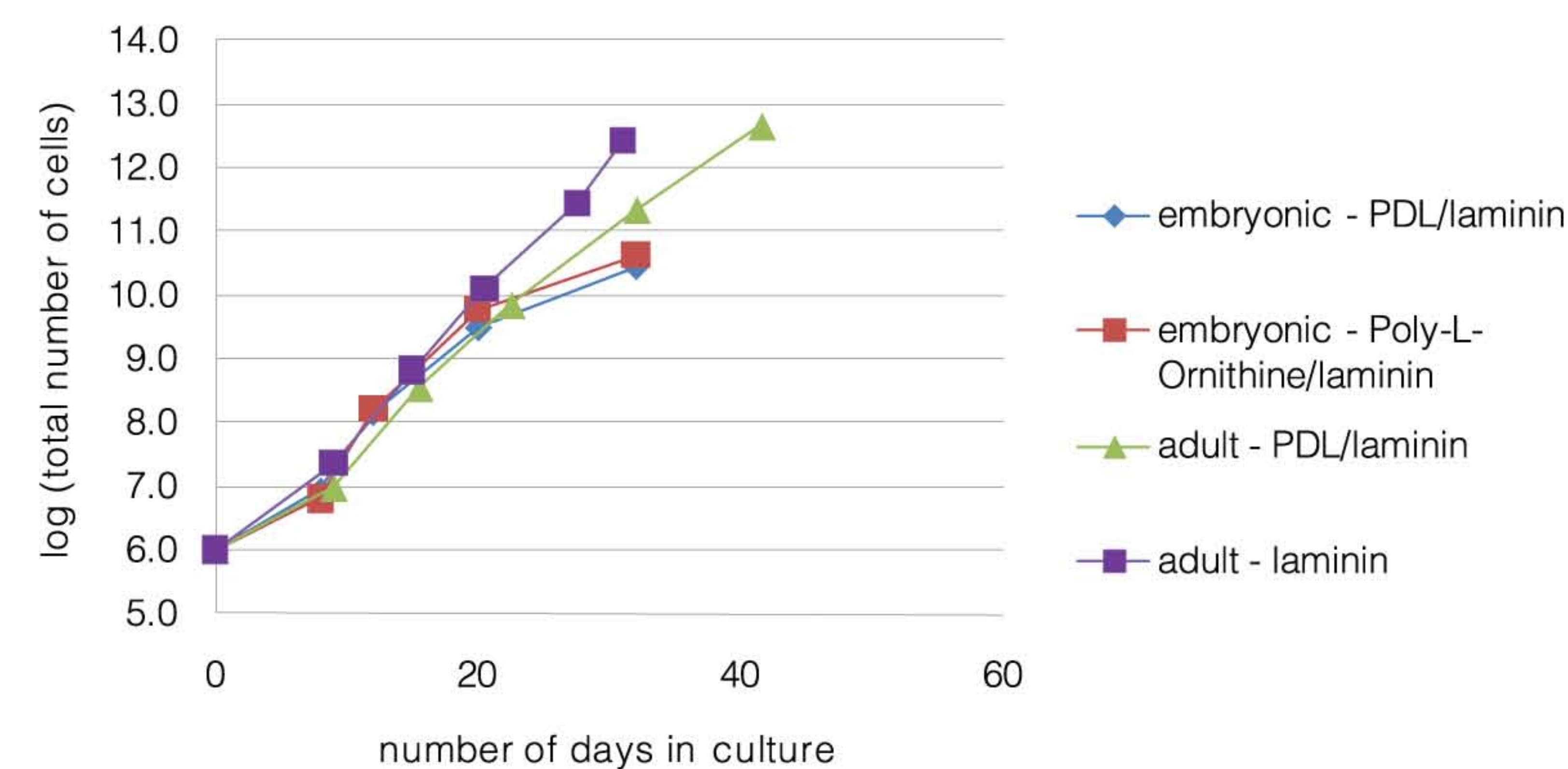
Results

FIGURE 2: Total Cells Expansion of E14 Cortical and SVZ Cells (Adult or Embryonic) Cultured in the Neurosphere Versus Adherent Cell Culture Systems (PDL/Laminin or Laminin Matrix)



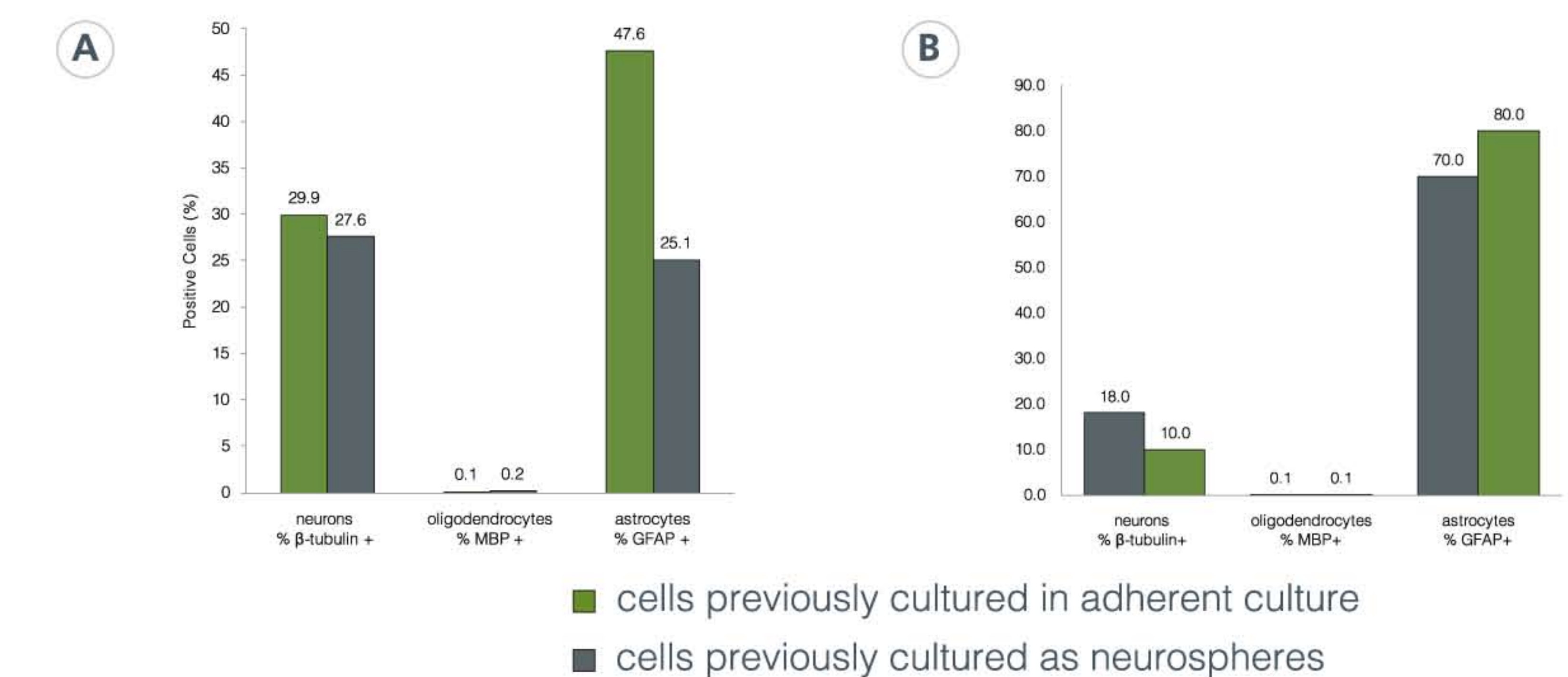
• Total cell expansion is higher in adherent cultures compared to neurosphere cultures for both E14 and SVZ cells.

FIGURE 3: Total Cells Expansion of E14 and SVZ Cells Cultured in Adherent Cultures Containing Various ECMs



• Cells can be cultured in NeuroCult® media in the presence of different matrices (n=1).

FIGURE 4: Multi-Lineage Potential of SVZ Cells Obtained from the Neurosphere Versus Adherent Culture Systems



• Cells obtained from neurospheres or adherent cultures retain multi-lineage potential at passage 1 (A) and passage 5 (B) (n=1).

FIGURE 5: Frequency of NSCs in Neurosphere Versus Adherent Cultures of Adult SVZ Cells After Passage 5 (n=2)

Frequency*	Culture System	Primary Cells		Cells from Passage 5	
		NCFC-Progenitor Colonies <2mm	NCFC-NSC Colonies >2mm	NCFC-Progenitor Colonies <2mm	NCFC-NSC Colonies >2mm
Neurosphere Culture		1.09 ± 0.02	0.02 ± 0.01	2.4 ± 0.6	0.1 ± 0.03
Adherent Culture				4.7 ± 1.0	not detected

* Frequency = # of colonies/cells plated x 100%

• 0.1% of the cells derived from neurosphere cultures at passage 5 formed colonies > 2 mm in diameter; these colonies are derived from cells that meet all the functional criteria of a NSC in the NCFC assay.
 • Colonies >2mm were not detected in these studies for cells from adherent cultures at passage 5.

Summary

- NeuroCult® media can be used in both the neurosphere and adherent culture systems.
- Both culture systems are capable of supporting the isolation of a population of cells capable of self-renewal and multi-lineage differentiation.