Methylcellulose-Laminin Hydrogel Supports Migration and Influences Differentiation of Neural Stem Cells

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Statement of Purpose: Neural transplantation for traumatic brain injury is a promising treatment modality, yet most transplantation studies have reported poor donor cell survival. We hypothesize that a 3-D matrix decorated with bioadhesive moieties will improve the survival and differentiation of the donor cells. The aim of this study was to examine neural stem cell (NSC) differentiation and migration within a methylcellulose (MC) -based hydrogel covalently tethered to laminin-1 (LN).

Methods: *MC-x-LN Hydrogel* – Tethering of LN to MC was accomplished via the photocrosslinker sulfo-SANPAH (Pierce Biotech). Briefly, LN was incubated in the dark with sulfo-SANPAH solution. LN-SANPAH was mixed at 4ºC with MC (Sigma Aldrich). A thin layer of the mixture was cast onto glass and exposed to UV light (100W, 365nm).

NSC culture – NSCs were harvested from the germinal zone of E14.5 mice (C57BL/6 strain β-actin +GFP). Tissue was mechanically dissociated into a single cell suspension and cultured in serum-free DMEM/F12-based media containing insulin (25 μg/ml), transferrin (100 μg/ml), putrescine (60 μM), sodium selenite (30 nM), progesterone (20 nM), and glucose (33 μM) supplemented with human recombinant FGF2 (20 ng/ml). NSC neurospheres were passaged every 7-10 days and used at passage 3-5 by seeding either within MC (~300μm thick) or on control plates. Experimental groups included MC, MC with soluble LN (MC+LN), and MC tethered to LN (MC-x-LN). Control groups included 2-D cultures with NSC media +FGF, media -FGF, and LN coated plates with no FGF (LN).

Migration – Migration away from the neurospheres was quantified at 7 days *in vitro* with confocal z-stacks of ~150μm. Projections of each z-stack were analyzed for differences in neurosphere area and morphology.

Quantitative RT-PCR – The expression of nestin, β-tubulin III, glial fibrillary acidic protein (GFAP), and β-actin were analyzed using previous methods (1). Briefly, RNA was isolated and cDNA synthesis was performed on DNaseI-treated total RNA by oligo(dT) priming (Superscript™ First Strand Synthesis System; Invitrogen). qRT-PCR with SYBR Green dye was performed with an ABI Prism 7700 System.

Statistics – One-way ANOVA followed by Student-Newman-Keuls Method post-hoc test ($p<0.05$) was used to determine statistical significance.

Results/Discussion: Migration data are presented as the normalized migration distance from a neurosphere. At 7 days *in vitro*, the greatest migration was observed on the LN coated wells (Figure 1). Notably, the migration in the MC groups containing LN in either tethered or soluble form (MC+LN, MC-x-LN) was significantly greater than MC, +FGF, and -FGF. Therefore, the presence of LN in the soluble or tether form enhanced NSC migration.

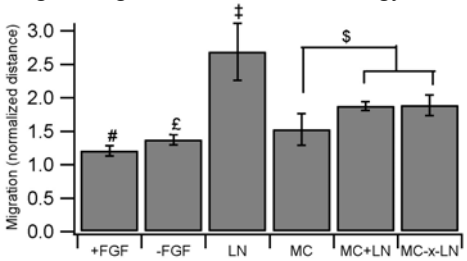

Figure 1: NSC migration; n=3-5; mean±standard dev. (‡ $p<0.01$ LN vs. all groups; $ $p<0.05$; £ $p<0.05$ –FGF vs. MC+LN and MC-x-LN; # $p<0.05$ +FGF vs. all MC groups)

Expression of nestin, β-actin, β-tubulin III, and GFAP were examined after 7 days *in vitro*. The results are presented as the fold increase normalized to immature NSCs levels (+FGF; Figure 2). The most prominent increase in expression was observed with the astrocyte marker GFAP, which correlates with previous phenotypic analysis of NSCs plated on LN (2). Notably, GFAP expression in all MC groups was significantly lower than LN, but statistically higher than +FGF and -FGF. Examination of β-tubulin III (neuronal marker) demonstrated a significant difference in expression between MC and MC-x-LN. The elevated expression in MC-x-LN was similar to LN and -FGF groups. Nestin expression decreased in -FGF and LN groups. Yet, nestin expression remained near +FGF control levels in all MC groups. This result suggests that culturing NSCs within MC maintains a subset of the NSCs in an undifferentiated state. Mechanisms for this activity may be due to increased endogenous paracrine signaling of the NSCs within MC as the diffusion of soluble factors is limited.

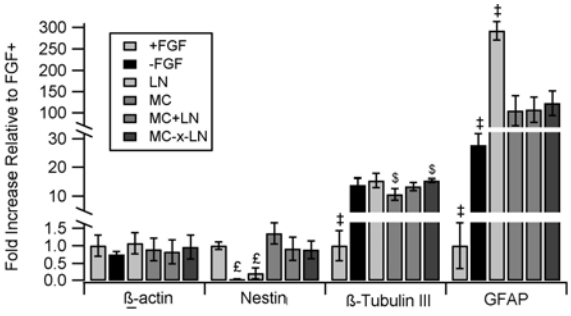

Figure 2: Phenotype expression; mean ± standard dev; n=3-5. (‡ $p<0.01$ all groups; $ $p<0.05$ MC vs. MC-x-LN; £ $p<0.01$ –FGF and LN vs. all other groups)

Conclusions: Collectively, the results suggest that MC is a material that supports NSC migration and provides an environment that enables NSCs to either remain in an immature state or differentiate into neuronal or astrocytic lineages. Furthermore, LN tethered to MC supported an increase in neuronal differentiation compared to MC, thereby upholding the initial hypothesis.

References:

1. Byers BA et al. J Bone Miner Res. 2002;17:1931-44.
2. Tate MC et al. Mol Cell Neurosci. 2004;27:22-31.