

# Activated Microglia Differentially Regulates Neural Stem Cell Phenotype and Neurite Outgrowth Within Co-cultures

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## Introduction

- Microglia compose 10-15% of the total cell population within the brain and spinal cord, residing as neurological macrophages primarily involved in immunological responses to various diseases and trauma<sup>1</sup>
- Animal models, while beneficial in validating the utility of NSCs after disease or trauma, may not specifically uncover stand-alone microglia-NSC biochemical interactions due to the multitude of variables surrounding the in vivo microenvironments<sup>2</sup>
- In this study, murine NSCs within 2D and 3D hyaluronan based hydrogels were co-cultured with a spontaneously derived microglial cell line<sup>3</sup> and evaluated at critical endpoints for survival, differentiation, gene expression and neurite outgrowth
- Results suggest that the combination of cytokines and chemokines produced by activated microglial cells regulate NSC neurogenesis.

## Methodology

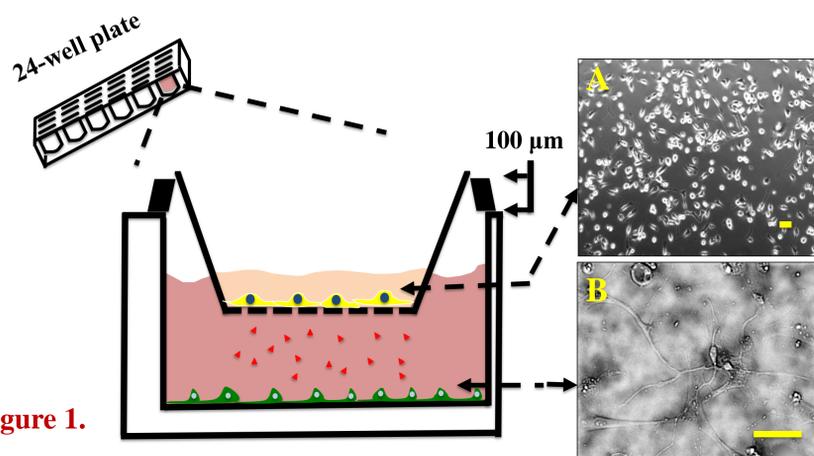


Figure 1.

- A commercially available co-culture system was modified to study the behavior of NSCs and microglial cells, under 2D and 3D co-culture conditions (Fig. 1). Scale bar = 50 μm.
- Endpoints were evaluated using the following techniques:
  - Antibody staining for differentiation including MBP (Oligodendrocyte), GFAP (Astrocyte), TUJ1 (Pre-neural),
  - LIVE/DEAD assay for cellular viability
  - RT-PCR to evaluate the NSC expression
  - 31-Plex Mouse Cytokine/Chemokine Array
  - SEM imaging, TUJ1 Staining, and post-processing via NeuronJ software was used to evaluate neurite outgrowth

## Conclusions

- The spontaneously immortalized microglial cell line cloned from mouse cerebral tissue (SIM-A9) used in this study allows for simultaneous exposure to several inflammatory mediators which may better mimic the native neural milieu and its subsequent effect on NSC behavior
- This work provides a comparison on the conventional (2D) culture and the more physiologically-relevant (3D) microenvironment.
- Microglial cells produce requisite cues which can elicit distinct morphological features accentuated by extensive axonal outgrowth within NSCs, cultured in both 2D and 3D.
- The beneficial and detrimental effects of cytokines and chemokines on NSC behavior can provide important clues which could guide regenerative repair strategies aimed at awakening lost neuronal function.

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## Results & Discussion

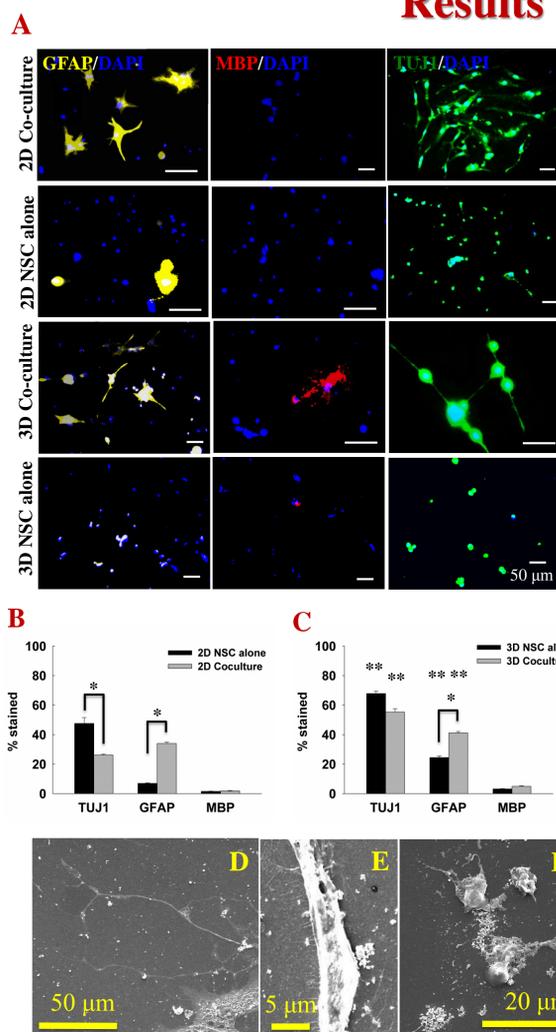


Figure 2. (A) IF images of NSC differentiation under respective culture conditions. Quantification of lineages on day 10 in 2D (B) and 3D scaffolds (C). \* indicates  $p < 0.05$ , \*\* indicates  $p < 0.05$  2D vs. 3D. SEM images of NSCs in 2D microglial co-cultures (D,E) and by themselves (F).

- TUJ1 was observed in all culture conditions, however, a more distinct cellular morphology was noted in co-culture conditions (A).
- 2D TUJ1 staining was significantly down-regulated in co-culture conditions compared to stand-alone NSC conditions (B).
- Conversely, in the same 2D environment GFAP lineage commitment was up-regulated in co-culture conditions vs. stand-alone conditions (B).
- For both the stand-alone and co-culture conditions, 3D microenvironments were found to have increased TUJ1 lineage commitment compared to 2D (B,C).
- Minor MBP commitment observed in both 2D and 3D cases with no significant difference between co-cultures and NSC alone (B,C).
- Real-time PCR was used to analyze changes in NSC genotype across samples, validating the changes in TUJ1 expression (data not shown).
- SEM images of co-culture conditions (D) promotes extensive axonal outgrowth as well as increased attached (E) whereas NSC cultured by themselves are more rounded and have significantly shorter axonal outgrowth (F).

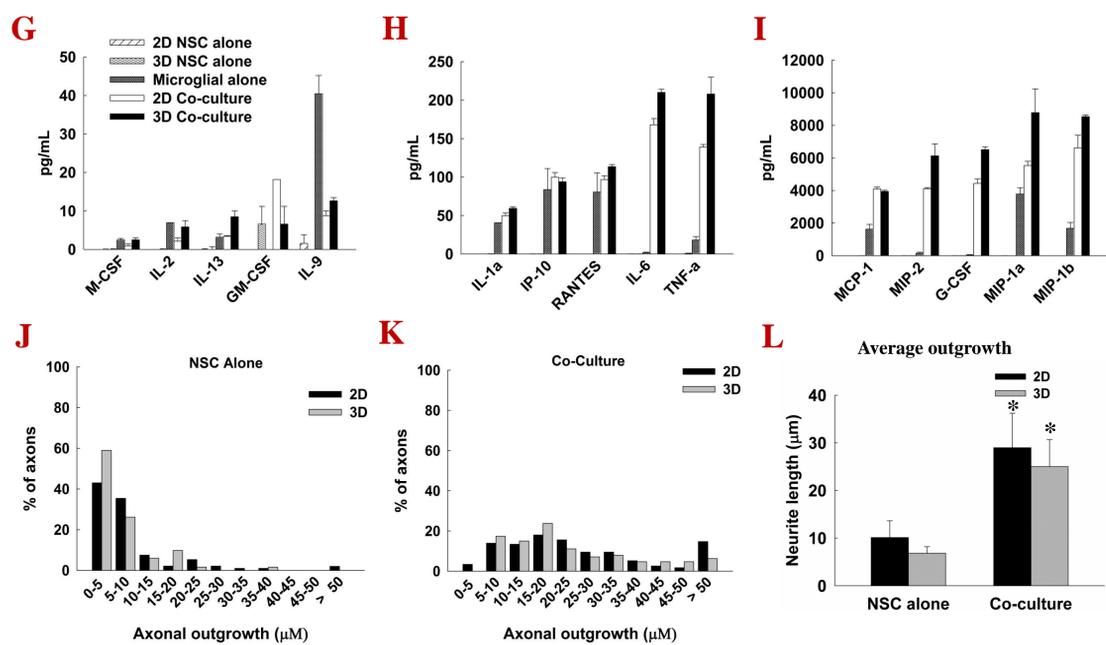


Figure 3. Cytokine and Chemokine production levels split into low (G), mid (H) and high (I) concentrations (n=2). Distribution of axonal outgrowth in NSCs cultured stand-alone (J), and within co-culture conditions (K). Averages (L) indicated significant outgrowth in co-culture conditions (\* indicates  $p < 0.05$ ).

- IFN- $\gamma$ , IL-1b, IL-3, IL-4, IL-5, IL-7, IL-10, IL-12(p40), IL-12(p70), IL-15, IL-17, LIF, LIX, MIG, Eotaxin, KC, and VEGF were tested with no significant levels recorded.
- NSCs were tested by themselves and did not produce any significant concentrations of any of the listed cytokines or chemokines.
- Further analysis of the individual effect of cytokines and chemokines produced in this study and their role in neurite elongation and NSC phenotype is ongoing.