The Effect of Fiber Size on the Neuronal Differentiation of Mouse Embryonic Stem Cells

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Statement of Purpose: Peripheral nerve injuries can arise from trauma, cancer, or congenital defects, and are challenging clinical issues to address. While the peripheral nervous system (PNS) has the ability to regenerate, the process is slow, rarely completes, and fails if the defect is too large. Neural tissue engineering is a promising approach to creating a novel graft for the functional regeneration of nerve injuries. The two arguably most important aspects of neural tissue engineering are the scaffold and cells. On the cell side of the equation, there is a significant need to establish a clinically viable cell source. The commonly used cells in neural tissue engineering, including neural stem cells and Schwann cells, are difficult to harvest and expand. Embryonic stem cells (ESCs), on the other hand, have great potential for neural tissue engineering and tissue engineering in general. They can differentiate into any cell type and are capable of indefinite proliferation, yet their pluripotency makes controlling their fate to high degree difficult. Additional layers of induction and control would be highly desirable, and thus we investigated the effect matrix fiber size has on the neuronal differentiation of ESCs.

Methods: Solutions of poly(L-lactic acid) (PLLA) in hexafluoroisopropanol (HFIP) of varying concentration (5%, 8%, 15%, 20% w/v) were electrospun to create fibrous matrices with controlled diameters. Thermally-induced phase separation (TIPS) was also used to fabricate PLLA nanofibers[1]. The fibers were imaged using an SEM and the diameters were calculated with ImageJ. D3 mouse ESCs were seeded on the PLLA fibers at a density of $3x10^4$ cells/cm^2 and cultured in either non-directional medium or neural permissive medium for 1 week. Afterwards, the cells were either fixed for immunofluorescence or the RNA was isolated for real-time PCR gene expression analysis. For immunofluorescence, cells were labeled with the neuronal marker TUJ1. For PCR, the neuronal marker βIII-tubulin was assayed.

Results: The fiber diameters from electrospinning and TIPS are summarized in Table 1.

Table 1. Summary of fiber diameters from electrospinning and TIPS.

Sample	Fiber Size (nm)
Electrospun 5% PLLA in HFIP	129±27
Electrospun 8% PLLA in HFIP	529±141
Electrospun 15% PLLA in HFIP	901±214
Electrospun 15% PLLA in HFIP	1889±216
TIPS	135±65

In the neural permissive medium, there was a gradual increase in TUJ1 labeling as the fiber size increased (Figure 1). The gene expression of βIII-tubulin mirrored that result (Figure 2).

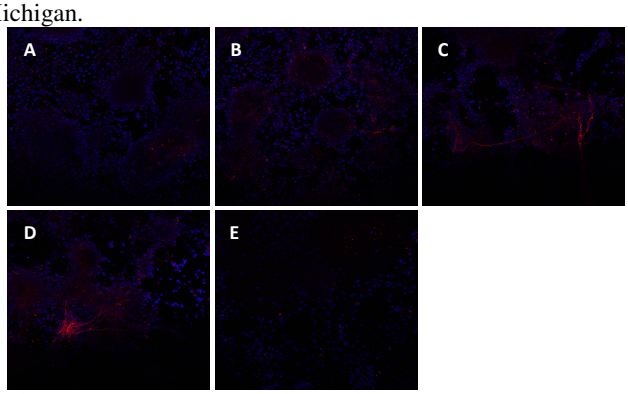

Figure 1. Immunofluorescence of TUJ1 (red) and DAPI (blue) of ESCs cultured in neural permissive medium on 129nm (A), 529nm (B), 901nm (C), or 1889nm (D) electrospun nanofibers, or 135nm TIPS nanofibers (E) for 1 week. There is a noticeable increase in TUJ1 expression and neurite outgrowth on the larger fibers.

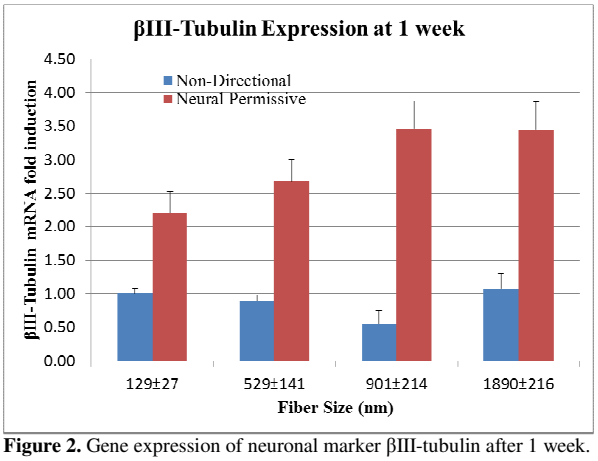

Figure 2. Gene expression of neuronal marker βIII-tubulin after 1 week. There was no significant difference in non-directional medium (blue bars), but there was a steady increase βIII-tubulin gene expression in neural permissive medium (red bars) as the fiber size increased up to 901nm.

Conclusions: The fiber diameter had a clear effect on the neuronal differentiation of mouse ESCs. There was a greater degree of neuronal differentiation as fiber size increased, which plateaued above approximately 900nm. This can provide invaluable guidance to designing an optimized neural tissue engineering scaffold. In addition to using media conditions to guide ESCs differentiation, it is possible to tailor the architecture of the scaffold to add another layer of induction with the hope of increasing the efficiency of differentiating ESCs to a single cell type. This would be a significant step towards being able to reach the potential of ESCs in tissue regeneration. However, the mechanism behind this interaction needs to be established to fully understand the cell-biomaterial interaction for applications in both tissue engineering and developmental biology.

References

1. Ma PX. J Biomed Mater Res. 1999;46:60-72