Laminin-Polycaprolactone Blend Nanofibers Promote Neurite Extension

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Statement of Purpose: Laminin has shown great promise as a surface for cell attachment, growth, and differentiation, especially in the peripheral nervous system due to its inherent neurite-promoting ability. Currently most laminin is used in a two-dimensional film. In a recent publication by our group, we discovered laminin nanofibers created by electrospinning used as a surface for cell attachment and differentiation augment the beneficial properties of laminin, causing greater cell attachment and driving differentiation without culture additives (Neal RA Tissue Eng Part C Methods. 2009;15:11-21). Unfortunately, the isolation process to purify laminin from the mouse Englebreth-Holm-Swarm (EHS) tumor is an arduous and expensive process. To this end, we have begun investigating synthetic polymer-laminin blends. Polycaprolactone (PCL) was chosen as the synthetic polymer for its mechanical strength and repeatability of the electrospinning process, and it holds the bonus of FDA approval for use in humans. Initial studies used laminin-PCL blend films to determine the ideal composition to promote neurite extension equivalent to laminin films alone. Using 10% (wt) laminin films, with the remaining 90% (wt) being PCL, no significant difference was seen between neurite extension here and on pure laminin films. When transitioning to electrospun nanofibers, this trend still holds. Nanofibers containing 10% (wt) laminin are sufficient to reproduce cell attachment and neurite extension seen on 100% laminin nanofibers.

Methods: Laminin was isolated from the murine EHS tumor and used in lyophilized form for these studies. PCL was purchased from Sigma. To make blend films or fibers, both polymers were dissolved simultaneously at appropriate concentrations in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, purchased from Fisher). To make films, the solution was allowed to evaporate overnight at 4°C onto 5mm coverslips. For electrospinning, the solution was placed into a syringe, pumped out at a designated flow rate between 0.5 and 3mL/hr onto a grounded collector 10-15cm from the tip of the needle using 10-30kV driving voltage. Samples of both film and fibers were imaged using scanning electron microscopy. For cell studies, either PC12 cells or dorsal root ganglia (DRG) were used. PC12 cells were purchased from ATCC and cultured in DMEM/F12 supplemented with 5% FBS and 10% horse serum. Attachment assays were done in serum free conditions. DRG were isolated from E18-N4 mice and allowed to attach to the substrate for 30 minutes. After 30 minutes, the samples were flooded with media containing 50ng/mL nerve growth factor (NGF). Samples were imaged using confocal microscopy after 4 days of growth and analyzed for length and density of neurites using Image J (NIH).

Results: Laminin-PCL blend films of 10%, 30% and 50% laminin were compared to pure laminin and pure PCL films using DRG neurite outgrowth length and number as metrics for success. Unfortunately, attachment of the DRG to PCL films was impossible, so the pure PCL group was removed from the film study. Measuring outgrowth length from the edge of the body of the DRG to the visible end of the neurite, we found no significant difference in neurite outgrowth among any of the laminin contents we examined, as well as between the blend films and the pure laminin film. This suggests as low as 10% (wt) laminin within a PCL film is sufficient to saturate the active sites on the growth cones of the neurons and promote neurite extension.

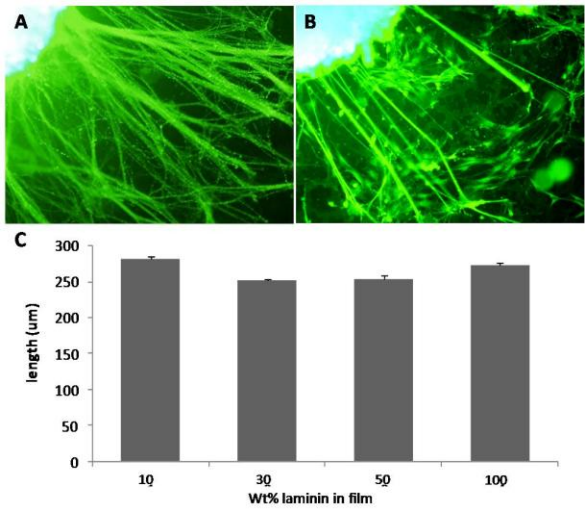

Figure 1. Neurite extension on laminin-PCL blend films Representative fluorescent micrographs of DRGs (top left corner of each image) extending neurites on (A) 10% laminin film and (B) 100% laminin film. (C) Average neurite length from 3 DRGs showed no statistical difference among varying wt% laminin in blend films.

In addition, we have successfully electrospun laminin-PCL blend nanofibers with 10% laminin incorporation. We can manipulate fiber diameter via process parameters in electrospinning; with these blends we can achieve 130nm +/- 40nm diameter. PC12 cells plated on these fibers show significantly greater attachment to fibers containing laminin, but do not differentiate between 10% and 100% laminin.

Conclusions: Laminin-PCL blend nanofibers, with at most 10% (wt) laminin content, retain attachment and neurite extension promoting properties indistinguishable from 100% laminin nanofibers. This blend dramatically reduces the cost of these nanofiber substrates, and allows us to manipulate the orientation of the fibers for enhanced guidance in conduit repair of peripheral nerve regeneration, as well as applications in other areas of tissue engineering.