Basement Membrane-Polycaprolactone Blend Nanofibers as a Scaffold for Tissue Engineering

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Statement of Purpose: Bridging critically-sized peripheral nerve defects greater than 3 cm in humans remains a clinical challenge. Mimicking one or more components of the basement membrane (BM) is a popular technique for improving peripheral nerve regeneration after traumatic injury. While simple systems may be sufficient to form an idealized BM-like substrate for a specific tissue, the combination of all components and structure of the *in vivo* BM may provide the optimal substrate for mimicking *in vivo* cell behavior and modulating cell fate. To that end, we have investigated whole BM isolated from a murine Engelbreth-Holm-Swarm (EHS) tumor and fabricated nanofibers from these biological materials alone, as well as blended with polycaprolactone (PCL). We then analyzed the ability of these nanofibers to promote adhesion and growth of neuron-like PC12 cells and primary neurons.

Methods: BM components were isolated from the murine EHS tumor and blended with PCL for electrospinning. Nanofibers were either randomly oriented or aligned using an insulating gap technique. PC12 cells and primary neurons dissociated from the dorsal root ganglia of neonatal mice were used for cell attachment and neurite extension studies. Both cell types were maintained in a basal medium supplemented with serum, and stimulation with nerve growth factor (NGF, 50-100µg/mL) was used to encourage process extension. Electron microscopy was performed on a JEOL 6400 SEM, and ImageJ was used for all image processing and measurements. Minitab statistical software was used for hypothesis testing with significance asserted at $p < 0.05$.

Results: We fabricated RBM and RBM-PCL blend nanofibers that mimic the geometry and functionality of the peripheral nerve BM. Manipulating the concentration of RBM in solution as well as the flow rate of the solution exerts control over RBM fiber diameter. Mean nanofiber diameters ranged from 127nm to 219nm, with the smallest average diameters found using the highest initial concentration (3% w/v) and the lowest flow rate (0.5mL/hr). Although RBM fibers swell within the first 15 minutes of hydration, no significant swelling is observed thereafter, and fiber diameter remains within the physiological range. Since the isolation process for RBM is difficult and expensive, we sought to blend this natural matrix with the synthetic polymer PCL. We successfully fabricated blended nanofibers containing 1% or 10% RBM by weight, and were able to modify parameters to maintain no significant differences among the mean fiber diameters (135nm to 145nm), regardless of the amount of RBM incorporated. 10% RBM incorporation was determined to be an appropriate limit based on previous work.[1] Bioactivity of these nanofibers was quantified using neuron-like PC12 cell attachment and extension studies. Cell attachment on 1% RBM-PCL and 10% RBM-PCL nanofibers was found to be significantly greater than attachment on PCL nanofibers ($p < 0.001$). To address peripheral nerve regeneration, we investigated the ability of these meshes to promote neurite outgrowth from PC12 cells and primary neurons. In comparison to PCL and 1% RBM-PCL blends, 10% RBM-PCL promoted a significantly greater percentage of neurite extending cells ($p < 0.001$ and $p < 0.015$, respectively). Lastly, to engineer directionality into the meshes, we fabricated aligned RBM-PCL nanofibers that display average angular deviations with 6° of an axis of alignment. The neurite extension study was repeated on these nanofibers. 10% RBM-PCL showed a significantly greater percentage of neurite extending cells than PCL alone ($p < 0.005$), suggesting that aligned morphology plays a critical role in the directed outgrowth of peripheral nerves.

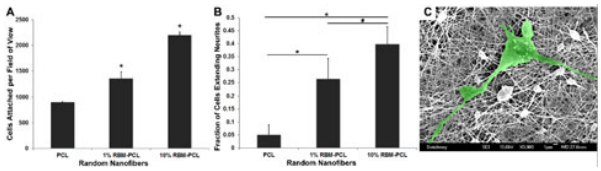

Fig. 1. PC12 Cell Attachment and Neurite Extension
(A) PC12 cells seeded on varying concentrations of RBM-PCL random nanofibers. After 2 hours of incubation, attachment on 10% RBM-PCL nanofibers was significantly greater than all other groups, and attachment on 1% RBM-PCL nanofibers was significantly greater than that on PCL. (B) Neurite extension of PC12 cells seeded on varying concentrations of RBM-PCL random nanofibers. After 5 DIV, the percentage of neurite-extending cells on 1% and 10% RBM-PCL nanofibers was significantly greater than that on PCL nanofibers. Extension was also significant between the RBM-PCL groups. Error bars display standard deviation (*, $p < 0.001$ and #, $p < 0.015$). (C) Representative SEM micrograph illustrating process extensions of pseudocolored primary neuron dissociated from murine DRG on 10% RBM-PCL nanofibers.

Conclusions: We have successfully fabricated nanofibers of reconstituted basement membrane, as well as RBM-PCL blend nanofibers in both random and aligned orientations. RBM fibers show promise as a substrate for cell adhesion; however, aligned RBM-PCL blend fibers are ideal for applications where directional outgrowth is desired such as peripheral nerve regeneration or tendon repair. We expect these materials to prove useful in a variety of tissue engineering applications as both their composition and structure closely mimic that of native basement membrane.

References: 1. Neal RA. J Biomed Mater Res A. 2011; 100A:406-423

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