Neurite Outgrowth on Nanofiber Scaffolds with Different Structures, Orders, and Surface Coatings

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Statement of Purpose: Electrospinning is an enabling technique that has been widely employed to fabricate various1-D materials including nanofibers, nanobelts, and nanotubes for enormous applications. Electrospun nanofibers can be easily assembled into a variety of 2-D structures, which have great potential for applications in neural tissue engineering. In the present study, the main objective is to develop various 2-D assemblies composed of electrospun nanofibers and to examine neurite outgrowth by culturing dorsal root ganglias (DRGs).

Methods: The electrospinning setup and the collector used for fabricating and collecting 2-D nanofiber assemblies are similar to those used in our previous studies (Li D. Nano Lett 2004; 4: 933.; Li D. Adv. Mater. 2004, 16, 361.). The polymer solution used for electrospinning contained 20% poly(□-caprolactone) (PCL) (w/v) in a mixed solvent of dichloromethane and dimethylformaldehyde with a volume ratio of 80:20. The PCL fibers were sputter-coated with gold before imaging with scanning electron microscope. Immunohistochemistry was performed to visualize the spatial distribution of neurites

Results: Figure 1 shows various 2-D assemblies of electrospun PCL nanofibers. We performed DRG culture on random PCL nanofibers and found that DRG could not adhere on them well and easily fall off the fiber mat. By contrast, DRG can adhere well on disordered PCL nanofibers after laminin coating. The neurites grew radially outward from the main body without preference to any specific direction. Aligned PCL nanofiber samples were obtained by putting glass cover slips between two stripes of grounded aluminum foil on a nonconductive substrate. When DRG were cultured on aligned PCL fibers, we found that DRG adhered well on aligned PCL nanofibers even without laminin coating and neurites preferred to grow along long axis of the fiber. Part of the neurites initially did not grow along the fiber alignment direction and eventually turned and succeeded their extension along the direction of fiber alignment. Similarly, DRG adhered very well on aligned PCL nanofibers with laminin coating and neurite outgrowth was guided by the fiber alignment direction. We also found that neurites turned to the direction of fiber alignment much more sharply than the aligned fiber without coating laminin, indicating that laminin coating could enhance the guidance of neurite outgrowth. Therefore, aligned fibers could promote DGR adhesion and enhance the neurite extension and the guidance of neurite outgrowth comparing to random fibers. Laminin coating could further promote the neurite extension and guidance of neurite outgrowth.

We examined typical morphology of DRG cultured on the border between random and aligned electrospun nanofibers. The neurites grew without any preference on the site of disordered fibers and grew along the long axes of fiber alignment on the site of ordered fibers, showing Janus properties. Similarly, we observed the enhancement of neurite extension and guidance of neurite outgrowth for laminin-coated fiber samples.

We also demonstrated DRG culture on two layered fiber mesh with stacking angle of 90º, respectively. When DRGs were cultured on crossed fiber mat, DRG adhered to the fiber mat. Only few neurites were observed and they grew along each direction of crossed fibers. Comparing with the crossed fiber mat without laminin coating more neurites were seen and neurites seemed to form crossed network. By manipulation of electrospun nanofiber assemblies and coating with contact-attractants such as laminin, we might be able to control neurite orientation and the formation of complex neural architecture. We also investigated DRG culture on two layered fiber mats with randomly-oriented fibers on the top and aligned fibers on the bottom or vice versa. The fiber mat on the bottom layer played some role on DRG neurite outgrowth and laminin coating could amplify the role of fiber mat on the bottom layer.

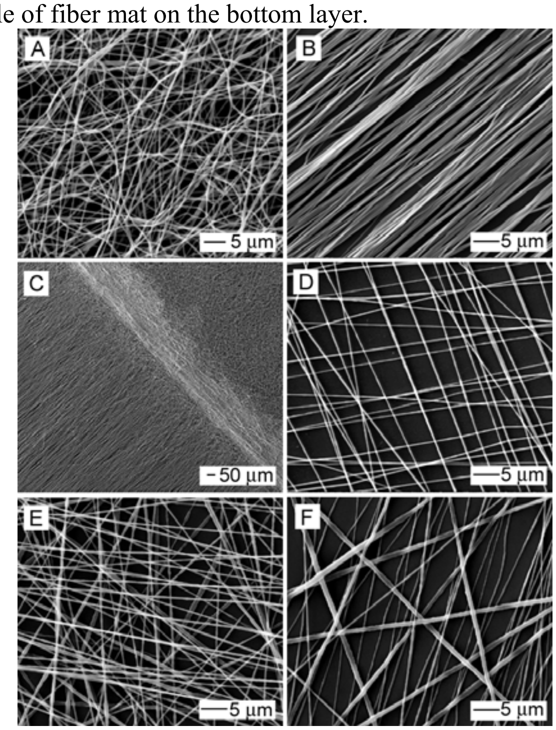

Figure 1. Scanning electron microscopy images of electrospun fibers with different 2-D structures: random (A), aligned (B), aligned-to-random (C), aligned/aligned cross (D), aligned/random multi-layered (E), and random/aligned multi-layered (F).

Conclusions: We have demonstrated that electrospinning could provide a variety of nanofiber assemblies as a nanoplatform for studies of neurite outgrowth behavior. All the results could contribute to a better design of fiber scaffold for nerve repair or more insights on neurite outgrowth behavior.