Characterization of Neural Scaffolds by Atomic Force Microscopy

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Abstract

The nervous system is built on a network of interconnected neurons which are capable of connecting to one another by following chemical, electrical, and mechanical cues. Neurons possess an appendage, the axon, which is responsible for seeking out other neurons in its environment to which it can connect and form the wonderfully complex networks that are characteristic of mammalian nervous systems. To better understand how neurons follow these cues neurons are placed on patterned nanofiber substrates where it is possible to study the mechanical interactions between the cells and their environment. Utilizing atomic force microscopy (AFM) it is possible to simultaneously characterize the mechanical properties of the surface and track both the motion and forces involved in axon movement and network growth on the surface. This technique is coupled with fluorescence microscopy to increase the visibility of neurons. The patterned nanofiber substrates successfully guided neuron growth resulting in aligned neural networks.

Experimental Section

Sample Preparation: PVA (Polysciences, Inc.) with molecular weight of 78,000 g/mol was electrospun, cut into disks, and placed into a 24-well plate. After electrospinning, resulting fibers were heat treated at 160°C for 15 min and made insoluble in water.

Cell Culture: The PVA substrates were soaked in a solution of Poly-D-Lysine in order to promote the adhesion of neurons. Substrates were then soaked in solutions of Fibronectin or Laminin, which are known growth factors that increases viability and strength of axon growth. Lastly, samples were sterilized under UV light and rat cortical neurons were seeded onto the substrate in a solution of growth media, penicclin/streptomycin, B27 serum, and Glutamax (both growth factors).

Scanning Electron Microscopy (SEM): SEM (Phenom) gave images of fiber appearance and allowed for the measurement of fiber diameters. Samples were sputter coated with Au-Pd before imaging.

Atomic Force Microscopy (AFM): AFM (Asylum Research MFP-3D-BIO) was used to characterize the topography of fiber mats and to test their stiffness. Scans were taken using Asylum Research and Veeco tips.

Fluorescent Microscopy: Fluorescent imaging (Asylum Research MFP-3D-BIO: Optical Component) was used to gain greater resolution of neurons against background fibers. Neurons were fixed to the fiber substrates using glutaraldehyde and DAPI was used to stain nucleic acid in the neurons.

Electrospinning Fibers

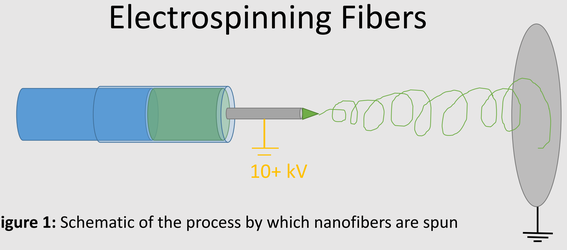


Figure 1: Schematic of the process by which nanofibers are spun

To electrospin fibers, conductive polymer solutions are loaded into a syringe which is fitted with a narrow, metal needle tip. The syringe is pumped at constant rate while high voltage is applied. The droplet on the needle's tip is deformed by the electric field and forms a cone from which a stream of fibers is ejected. The electric field pulls the stream of fibers to the grounded collector, leaving a fibrous mat on its surface. For my research project the polymer of choice was Poly(vinyl alcohol), or PVA. It was chosen because it's biocompatible, water soluble, and non-petroleum based.

Anatomy of a Neuron

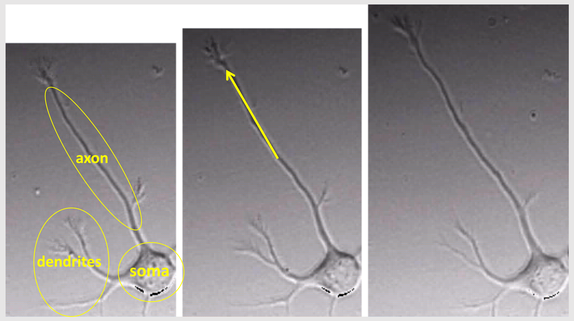
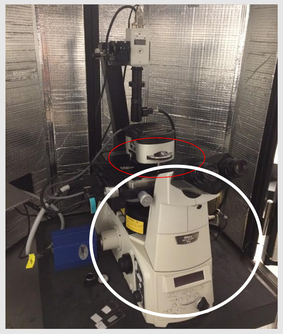


Figure 2: Optical images of a growing neuron. Taken by Marc Simon, PhD.

- Soma: the main body of the neuron that contains all traditional parts of the cell, including the nucleus, mitochondria, ribosomes, and other organelles.
- Axon: long extension from the soma that is tipped by a growth cone, a web of interconnected microtubules that responds to and exerts forces on its environment. The axon is the channel down which the neuron fires its electrochemical signal
- Dendrites: shorter extensions that receive chemical signals from other neurons

Atomic Force Microscopy



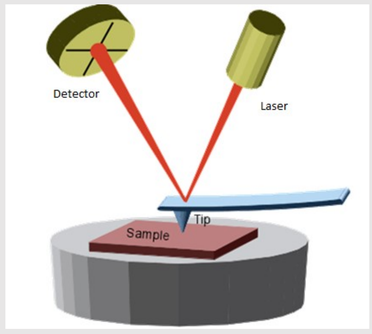
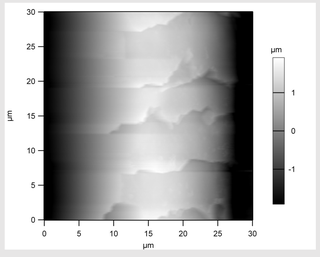


Figure 3A (left): Our lab's AFM, an Asylum Research MFP-3D. The AFM is circled in red, while the optical microscope used for fluorescent microscopy is circled in white.
Figure 3B (right): A schematic portraying the action by which an AFM scans a surface.

The Atomic Force Microscope works by dragging a small tip across a sample. The features on the sample's surface deflect the AFM tip, and these deflections are recorded by a detector which tracks changes in the reflected laser. By sweeping up and down over the sample this allows for a full 3D image of the surface to be made This action is analogous to the process by which a record player's needle converts the bumps on a record into sound. The AFM is sensitive to incredibly small forces and can resolve features down to a few nanometers.



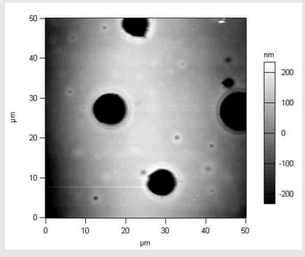
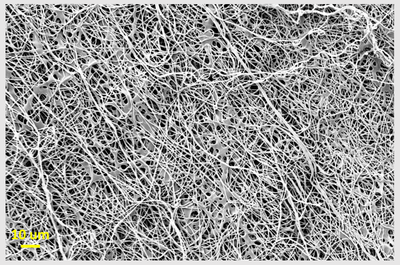
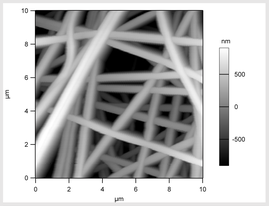


Figure 4A (left): An AFM scan showing the topology of a human hair.
Figure 4B (right): An AFM scan of a silk film covered in micropores.

Characterizing Nanofibers





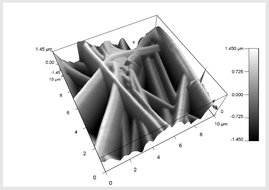


Figure 5A (top): an SEM scan of heat-treated nanofibers
Figure 5B (top right): AFM scan of nanofibers
Figure 5C (bottom right): 3D image generated from an AFM scan of nanofibers

AFM and SEM are the two major tools used to characterize the surfaces for neuron growth. SEM images are useful because of the ease which large regions of the sample can be resolved allowing for automated software analysis. Fiber directionality and diameters were calculated from SEM images. Samples are coated in gold palladium as SEM requires a conductive coating whereas AFM scans can be done on plain fibers. This, as well as the sensitivity of the AFM, means the fibers can be resolved in incredible detail. These AFM scans can then be used to produce three dimensional images, meaning AFM scans can give incredible insight into what the contours and features of a sample's surface are.

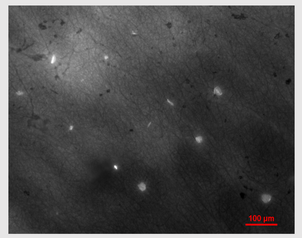
Conclusions

PVA provides a viable substrate for healthy neuron growth

Electrospinning proves to be an effective way to produce tunable nanopatterned substrates for aligned neuron growth

Heat treating the fibers stabilizes them and prevents the normally water soluble polymer from dissolving in the water based media used over the four days of surface treatment and cell culturing

Fibronectin and laminin were successfully bound to the surfaces of the fibers. These proteins are found in the extracellular matrix and cell membranes and are known to be important in neuron growth.



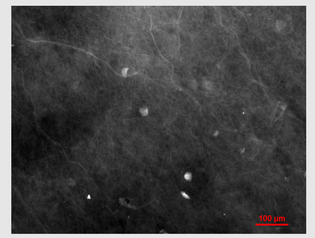


Figure 6: Fluorescent images show the cell bodies of neurons (bright areas) attached to Poly(vinyl alcohol) fibers after 42 h in the culture medium.

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