Electric field enhances axon length and directionality of dorsal root ganglia embedded in hydrogel on a conductive polypyrrole substrate

Hieu T Nguyen[1], Shawn Sapp[2], Silvia Luebben[2], Christine E Schmidt[1]
[1]Department of Biomedical Engineering, University of Texas at Austin, Austin TX 78712 USA
[2]TDA Research Inc, Wheat Ridge CO 80033 USA

Statement of Purpose: A thin film polypyrrole (PPy) conductive substrate was created to provide an electric field (EF) across dorsal root ganglia (DRG) embedded in extracellular matrix hydrogel.

Endogenous EF originating from extracellular fluid ionic gradients are known to present growth cues to various cell types in live animals[1]. Simulated EFs generated in vitro by applying a voltage across conductive fluid (i.e. buffered salt solution) is able to direct cell growth and migration[2], however, it is unknown if applying a voltage across a cell substrate can also direct cell behavior. Advantages of using a conductive substrate include the ability to create a nerve guidance channel, provide scaffolding for protein and growth factors, and to localize and control EF parameters.

Methods: Conductive PPy films supplied by TDA Research Inc (Wheat Ridge, CO) were cut into rectangular strips 1.5 x 0.4 cm, and edges were secured to a glass slide with copper tape. High vacuum silicon grease (Dow Corning, Midland, MI) was used to adhere polycarbonate wells to the film and glass, isolating the cell culture from the copper tape and electrodes. A 20ul strip of Matrigel (BD Biosciences, Franklin Lakes, NJ) was injected onto the PPy completely covering the film immediately before embedding 2 DRGs extracted from a Sprague Dawley P2 rat pup (Charles River, Wilmington, MA). The DRGs were placed 1/3 of distance from the film edge and far enough from each other so that they do not interact (Figure 1). The well was then filled with 300ul of RPMI media with 10% FBS and 50ng/ml NGF (2.5s murine, Promega, Madison, WI). The DRG was given 1 day to acclimate and grow within the gel before experimentation.

For experimental groups, electrodes were clipped to the copper tape in contact with the PPy film (50 kOhm) and stimulated with 200mV (approximately 100mV/cm) for 2 hours at 37 C. The control group had an identical setup, but was not stimulated with an EF. The unit was fixed with 4% paraformaldehyde 3 days after stimulation and stained against beta-III-tubulin (Abcam, Cambridge, MA). DRGs were imaged with fluorescent microscopy and axon length and direction were analyzed using ImageJ (available through NIH). The thickest axons were measured yielding n=330 axons from 24 DRGs for each group.

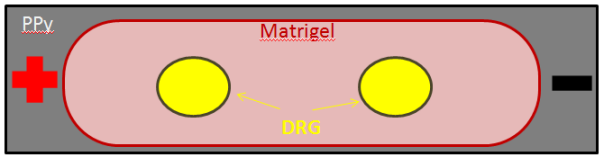

Figure 1. Illustration of setup. DRGs are embedded in Matrigel on top of a PPy film. Electrodes are attached to copper tape adhered to the edges of the film.

Results: DRGs grown on substrates stimulated with electricity exhibited longer axons with average 930um compared to controls with 820um. Student t-test reveals this 11% increase in axon length is significant, $p<0.001$. Upon determining directionality of axons in an EF, it was discovered that DRGs extended axons further towards the nearest electrode parallel with the EF (Figure 2). The average axon length parallel to the EF was 15% longer towards the electrode. Axons facing the center, growing perpendicular to the EF, and measured in the control group did not show a similar trend.

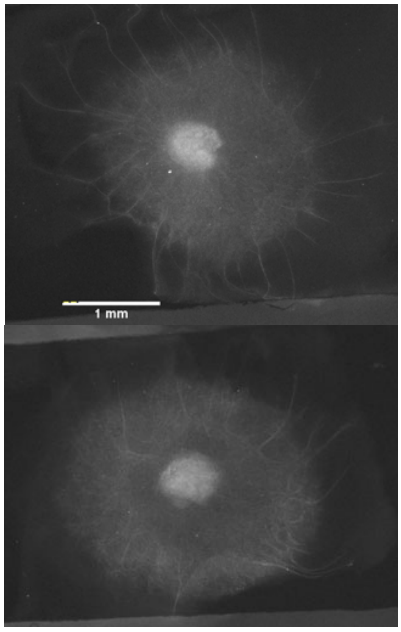

Figure 2. The top image is a fluorescent image of a control DRG and equal distribution of axons. The bottom image shows the axon growth preference towards the edge closest to an electrode when stimulated (bar = 1mm).

Conclusions: The results indicate that applying an EF through the cell substrate may be an effective means for controlling cell growth and behavior. Furthermore, the effect of the EF was able to penetrate into the hydrogel, demonstrating that the effect of the electric current extends beyond the surface of the substrate. However, the mechanism associated with EF effects on cell behavior is not well understood. Future work will include measurement of ionic and protein gradients within the gel and on the substrate, which may help elucidate this phenomenon.

References:

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