**Tissue Engineered Model of the Inner Neural Retina.**

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**Statement of Purpose:**

The inability of the adult mammalian retina to replace or regenerate retinal ganglion cells (RGCs) following injury motivates the study of cell and tissue engineering approaches. However, it is not sufficient to merely create a scaffold capable of supporting cell growth, it is also necessary to recreate the organization of the inner retina including the x-y organization of the nerve fiber layer (NFL) as well as the different cell lamina in the z direction. Here we describe an electrospinning (ES) method used to radially aligned ES scaffolds that direct axon growth towards a central point, mimicking retinal axon growth towards the optic nerve head. We further re-created the 3D organization of the retina, by recreating the inner plexiform layer (IPL) and forming a surface capable of supporting the growth of the RGCs synaptic binding partners, amacrine cells (AmCs).

**Methods:** Polylactic Acid (a generous gift of Purac Biomaterials) was dissolved in hexafluoroisopropanol and electrospun under various conditions using a radial collector consisting of a grounded central pole combined with a grounded collecting ring. Fibers were analyzed for diameter and alignment using scanning electron microscopy. RGCs and AmCs were isolated from early postnatal rats or mice (GFP+) and purified by sequential immuno-panning. RGCs were seeded onto scaffolds and cultured for 3 days. AmCs were seeded at a 10x density in matrigel to match their in vivo ratio to RGCs and cultured in the presence of astrocytes. Samples were fixed and blocked. Cells were stained for neuronal β3 tubulin, Synaptophysin and PSD-95, imaged by confocal microscopy and analyzed using Fiji.

**Results:** ES scaffolds under various flow rates and electric potentials produced PLA fibers ranging from 400 to 800 nm in diameter. In culture, 82% of RGC axons aligned radially, a significantly higher orientation of axon growth along the radial orientation of the scaffold fibers compared to that in control cultures and statistically indifferent from the measured orientation of the NFL of a native retina. IPL formation was created using an in situ gelling hydrogel, scaffolds showed survival and neurite outgrowth in both the RGC and AmC cell layers with processes extending through the hydrogel (figure 2).

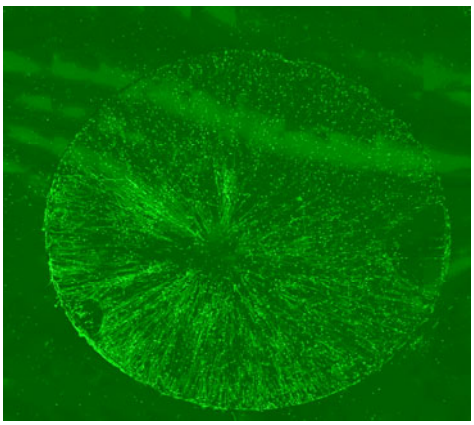

**Figure 1** RGCs seeded on ES scaffold direct axons radially

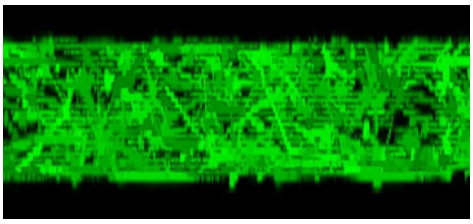

**Figure 2** Matrigel formed IPL seeded with AmCs.

**Conclusions:** Seeding RGCs onto a radially aligned scaffold directs axon growth towards a central point, potentially creating a model to study optic nerve formation as well as axon bundle fasciculation. The introduction of the AmCs into this model allows for the further re-creation of the inner retina in three dimensions and the potential for studying synapse formation. Together these studies combine to form a model for the study of cellular transplantation and synapse formation as well as a possible translatable tissue implant for the injured inner retina.