

# Immobilized Netrin-1 but not BDNF or L1cam Polarizes Retinal Ganglion Cell Growth

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## Introduction

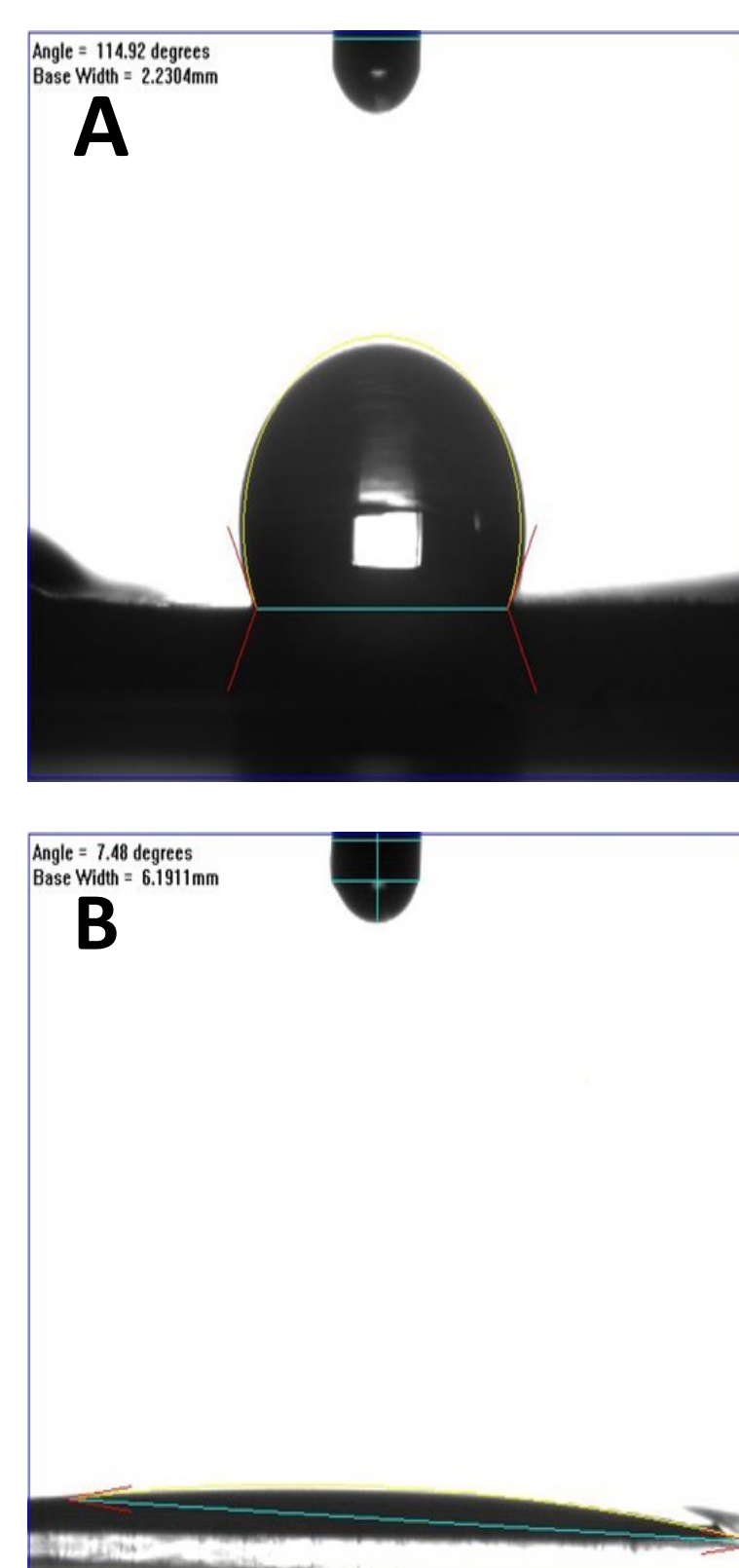
Retinal neurons, like other neurons in the central nervous system, are unable to regenerate following injury or disease. This has led to the development of cell transplantation therapies including for glaucoma with the transplantation of retinal ganglion cells (RGCs). Previous studies have shown bioengineered scaffolds can increase transplantation efficiency as well as direct their growth to mimic the structural components of the retina.<sup>1</sup> However, RGC polarization towards the optic nerve, and by extension the brain, was not able to be achieved during these early transplantation studies as cells will either grow randomly across the retina or grow both in and out when transplanted via scaffold.<sup>1,2</sup>

During development, RGCs are polarized through several soluble or ECM bound factors which guide their growth toward the optic nerve head including BDNF, semaphorins, Netrin-1, NCAM, L1 Cam and Heparin Sulphate with the immobilization of some of these factors having been used to direct neuron growth *in vitro*.<sup>3</sup>

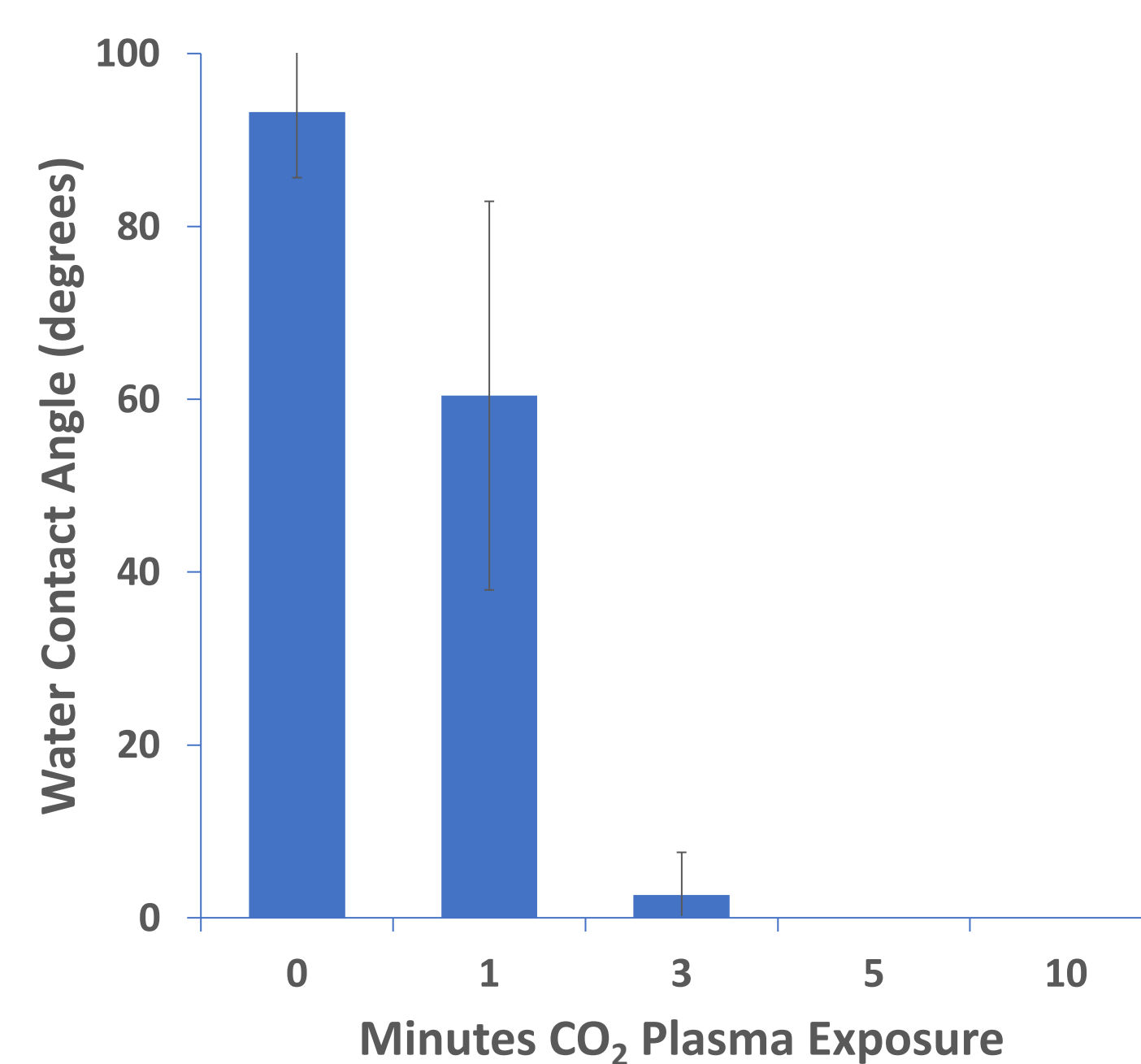
Poly lactic acid (PLA) is a FDA approved biomaterial which slowly degrades over time. PLA has been widely used in the process of electrospinning for implants throughout the body. However, PLA is inert and as a result difficult to chemically modify. Studies have shown that the use of plasma is able to chemically modify the surface through free radical reactions with the surface modified with chemical groups dependent on the plasma type. In the case of CO<sub>2</sub> plasma, carboxylic acid functional groups are added to the PLA surface.<sup>4</sup> This addition not only allows for the scaffolds to become more reactive but also for them to become more hydrophilic.

In this study, we will chemically modify radial PLA scaffold with CO<sub>2</sub> plasma for different lengths of time in order to identify the optimum length to create a reactive surface without degrading the scaffold. We will then demonstrate that we can immobilize protein on these scaffolds using a fluorescently labelled albumin protein. Finally, we will study what factors are able to polarize RGC growth on our radial scaffolds.

## CO<sub>2</sub> Plasma Modification of Scaffolds



**C Hydrophobicity of PLA Samples After Plasma**



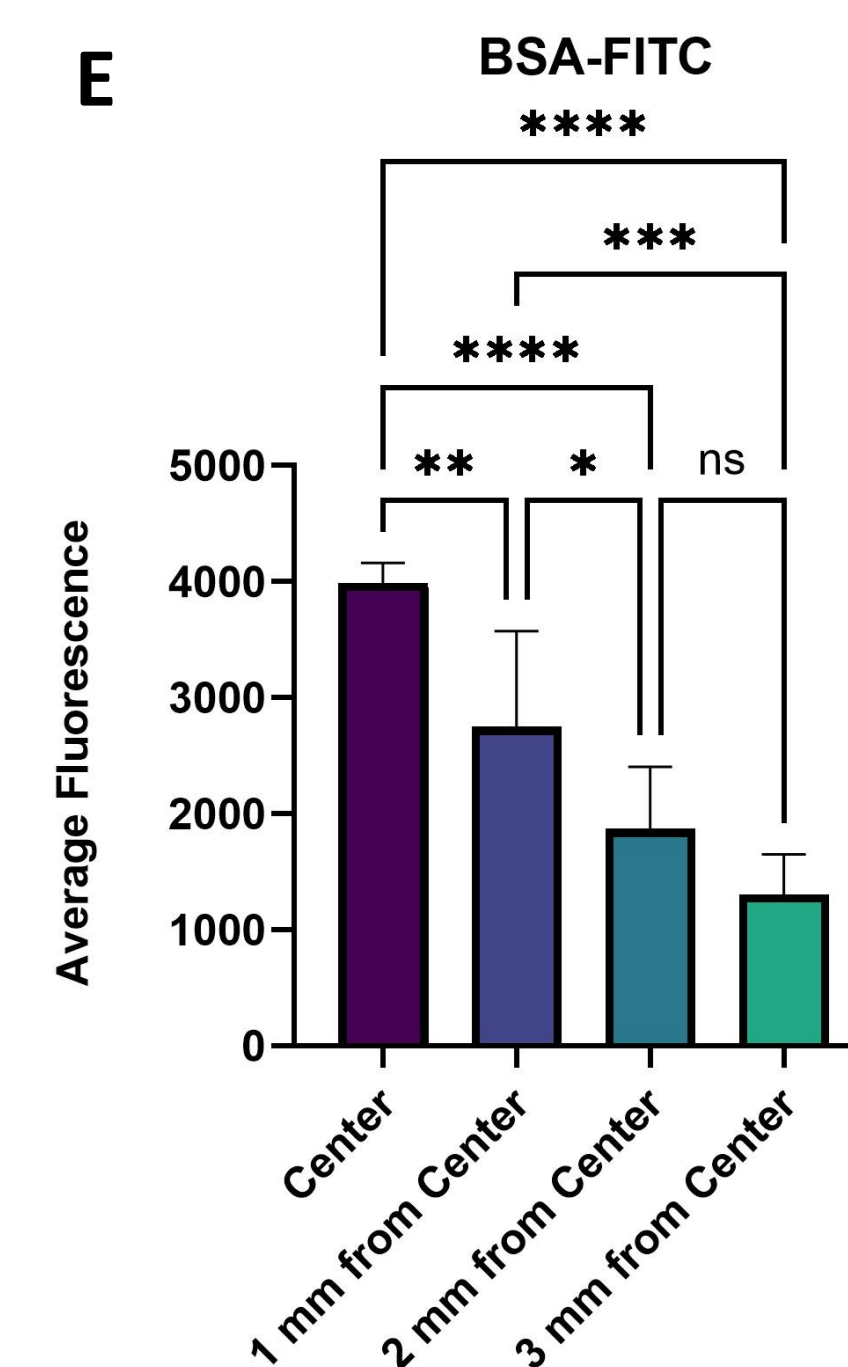
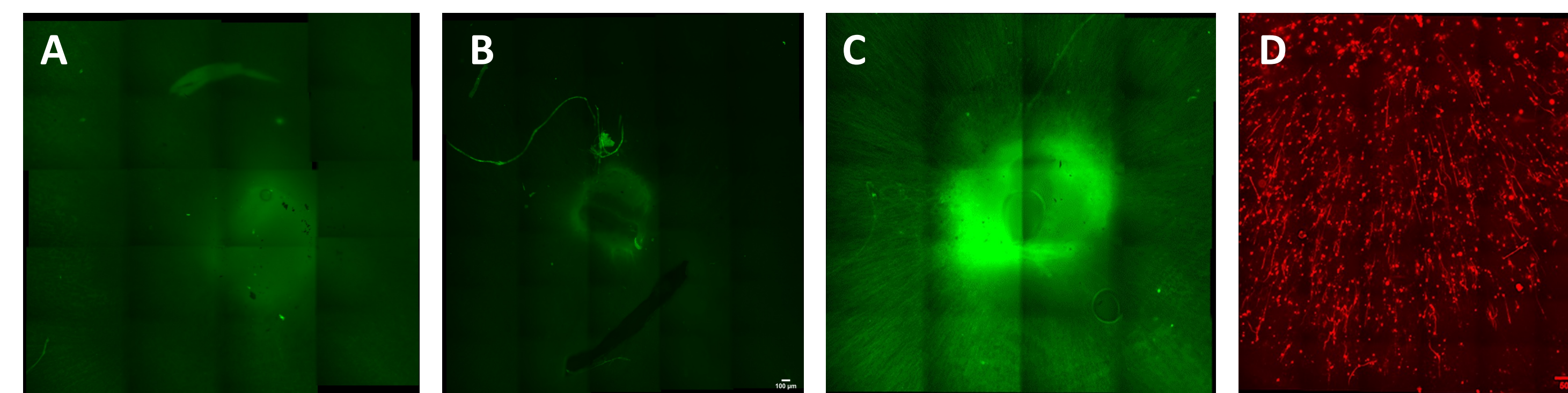
**Water Contact Angle of Unmodified PLA and Plasma Treated PLA Electrospun Scaffolds**

Electrospun PLA samples were reacted with CO<sub>2</sub> plasma for different lengths of time in order to create a more reactive surface containing surface carboxylic acid groups. Because these groups are more hydrophilic than PLA, the water contact angle decreased with longer reaction times. While unmodified PLA has a hydrophobic surface (A), surfaces reacted for three minutes or longer resulted in the adsorption of the water drop (B). Samples that were reacted for five and ten minutes were observed to have material damage caused by the free radicals formed during the plasma reaction.

## Acknowledgements

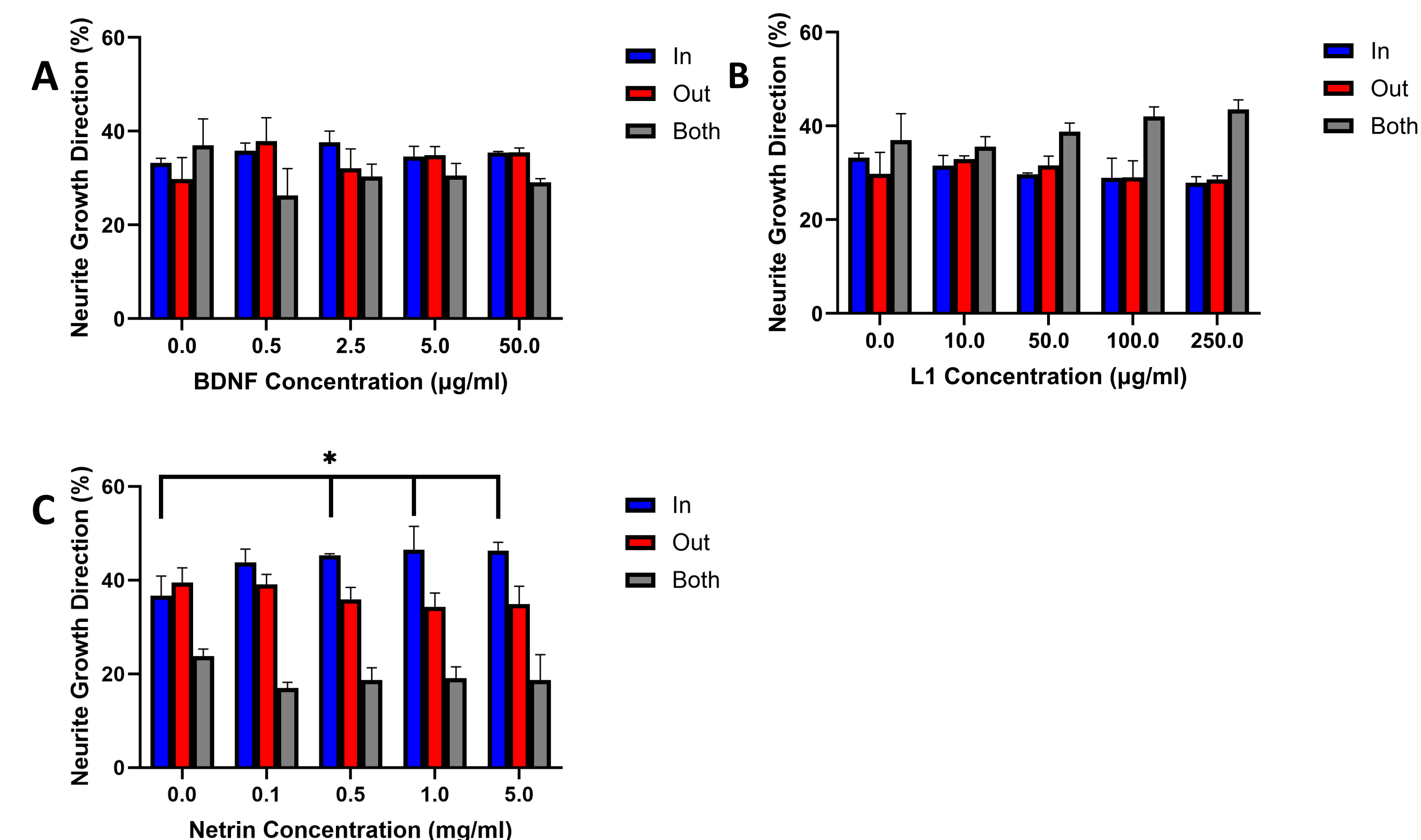
We would like to acknowledge the support from the National Eye Institute (R01-EY028946 KEK). Research reported in this publication was supported in part by a grant from the National Center for Research Resources and the National Institute of General Medical Sciences (RR027093) of the National Institutes of Health. For detailed protocols please email Dr. Karl Kador (kador@umkc.edu).

## Protein Immobilization on Scaffolds



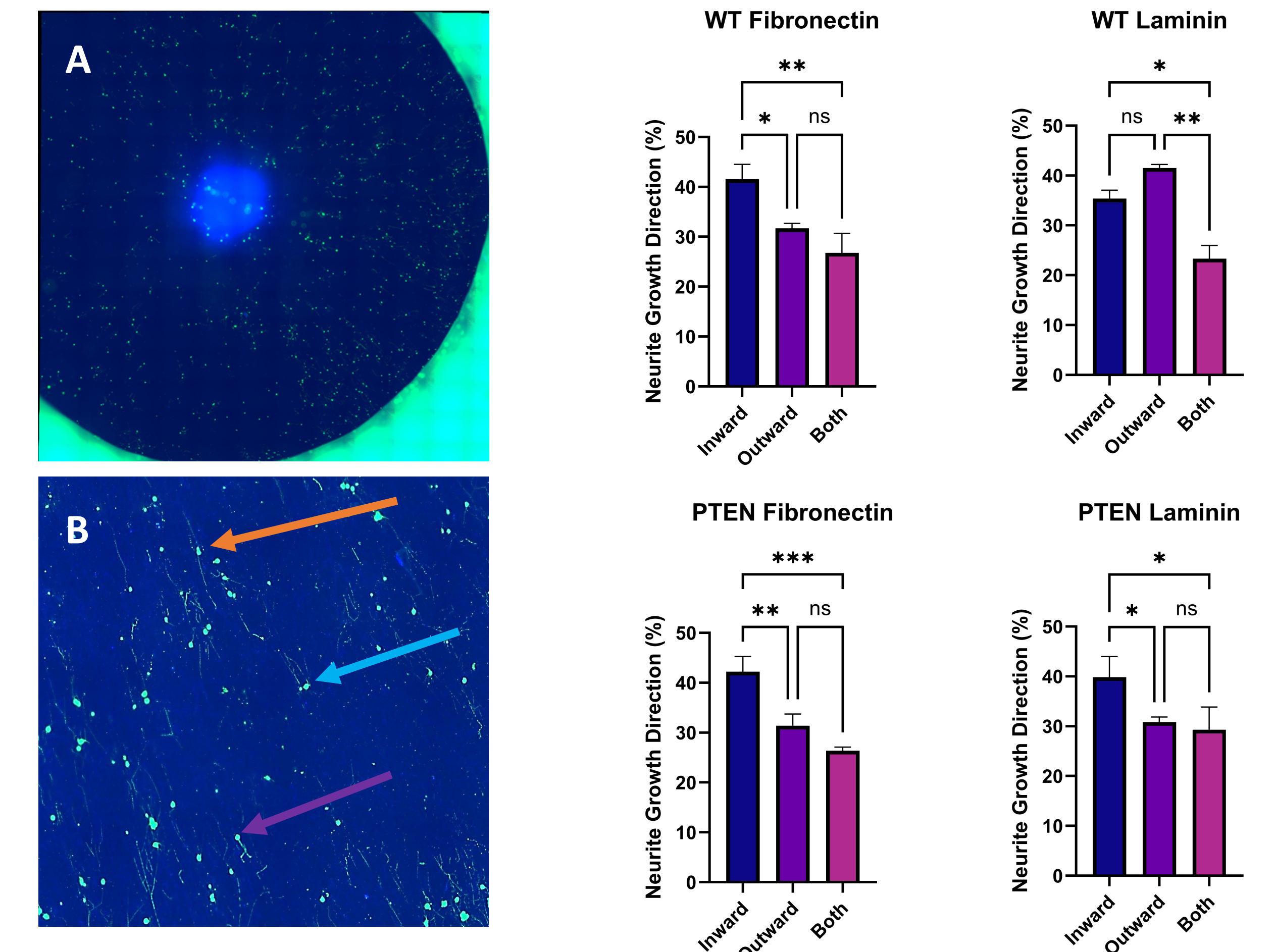
Unmodified scaffolds incubated with EDC/NHS (A), plasma treated scaffolds incubated with EDC/NHS (B) and plasma treated scaffolds incubated with EDC/NHS (C) all had a 50  $\mu$ L drop 0.1 mg/mL BSA-FITC placed at the scaffold center and the drop allowed to wick across the scaffold surface prior to washing. Samples were imaged and compared for total fluorescence. Plasma modified samples that had been incubated with EDC/NHS showed significantly more fluorescence than either unmodified samples incubated with EDC/NHS or plasma samples without EDC/NHS. To demonstrate that these modifications would not be cytotoxic or impede RGC growth, RGCs were seeded on plasma treated samples incubated with EDC/NHS and BSA-FITC that were then coated with laminin. RGCs were cultured for 3 days and then fixed and stained with  $\beta$ 3-tubulin antibody (red, D). RGCs showed similar growth to RGCs seeded on control samples. Fluorescence of immobilized BSA-FITC extending from the center showed the formation of a gradient extending away from the scaffold center (E).

## Which Immobilized Factors Can Polarize RGCs?



BDNF (A), L1cam (B) and Netrin-1 (C) were immobilized onto radial scaffold in a dose dependant manner with the lowest concentration matching previous reported concentrations for soluble guidance. Following immobilization samples were coated with PDL and Laminin (or Fibronectin for Netrin-1 samples) and RGCs isolated from postnatal rats (P3-P5) seeded and cultured for 2 days. Samples were fixed and stained with  $\beta$ 3-tubulin with all cells with neurites greater than two cell bodies in length counted for directionality. Cells were counted as growing in towards the center, out away from the center or in both directions. Only cells cultured on scaffolds immobilized with Netrin-1 showed a significant increase in RGCs with growth polarized towards the scaffold center and along the biochemical gradient.

## Does PTEN Affect RGC Guidance?



## RGCs Cultured on Netrin-1 Immobilized Scaffolds Exhibit Different Polarization Based Upon ECM Coating and PTEN Expression

RGCs cultured on scaffolds immobilized with Netrin-1 and coated with fibronectin (A, with zoomed image B) showed an increase in polarization of RGCs compared to control scaffolds without Netrin-1. This result was reversed for wildtype RGCs on Netrin-1 immobilized scaffolds coated with laminin on which Netrin-1 exhibits a repulsive effect.<sup>5</sup> RGCs treated with PTEN siRNA to knockout expression showed a similar growth pattern on fibronectin coated scaffolds but did not show a repulsive effect when scaffolds were coated with laminin. A and B staining  $\beta$ III tubulin (RGC neurites), and DAPI (nucleus). Arrows indicate neurites growing inward, growing outward and growing in both directions (B).

## Conclusions

- CO<sub>2</sub> plasma can be used to modify the inert PLA scaffold surface to make it more hydrophilic and chemically reactive.
- Protein gradients can be immobilized on plasma modified scaffolds using established EDC / NHS chemistry.
- Netrin-1 immobilized scaffolds but not BDNF or L1cam immobilized scaffolds are capable of polarizing Retinal Ganglion Cell neurite guidance.
- Wild type RGCs follow the Netrin-1 gradient on Fibronectin coated scaffolds but are repulsed on Laminin-1 coated scaffolds while PTEN KO RGCs polarize along the Netrin-1 gradient on both Fibronectin and Laminin-1 coated scaffolds.

## References

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