Towards unravelling the mechanisms by which topographical cues enhance neurite extension

Vivek Mukhatyar, Sumon Rudra, Shoumit Mukhopadhaya, Samir Panjwani, Richard Huber, Ravi Bellamkonda

1The Wallace H. Coulter Department of Biomedical Engineering Georgia Tech/Emory University, Atlanta, GA, USA

Introduction

The clinical “gold standard” for bridging peripheral nerve gaps is autografts. However, their usefulness is limited by several issues including the requirement for multiple nerve segments, mismatch between injured nerve and nerve grafts, and the loss at the donor site [1]. Empty hollow conduits created from natural and synthetic materials have been used to bridge nerve gaps. These scaffolds have shown promising outcomes in small gaps but fail when the nerve gap is longer than 10 mm in rats or more than 30 mm in humans. Tissue engineering strategies that involve the use of luminal fillers have been used to augment the rate as well as efficacy of nerve regeneration across long gaps. But the mechanisms by which they affect nerve regeneration are not completely understood.

In this study, we design an oriented fiber based scaffolds to understand how protein adsorption as well as cell behaviour are altered based on the underlying topography.

Materials and Methods

Ten micron thick oriented nanofiber films were fabricated by electrospinning poly(acrylonitrile-co-methylacrylate) (PAN-MA) on a high-speed rotating metal drum. Random fibers were fabricated using a flat plate target compared to a rotating mandrel for aligned fibers. Polymer film was casted by solvent evaporation. The morphology of these fibers and the film were evaluated using scanning electron microscope.

Serum was incubated on 6 mm diameter substrates overnight and amount of Fibronectin adsorption on each substrate was quantified using an enzyme linked immunosobent assay (ELISA).

We also incubated purified Schwann cells on these substrates and evaluated how the underlying topography help in the organization of Fibronectin laid down by these cells. Cell were fixed with Histochoice and stained with anti-FN antibody.

Results

Fig 1. demonstrates the amount of Fibronectin adsorbed from serum on the different topographies. We observe that fiber based topographies stimulate the absorption of FN from serum compared to smooth films of the same chemistry. In figure 2 we show that the underlying topography influences the organization of FN laid down by Schwann cells.

Discussion and Conclusions

Several strategies to enhance peripheral nerve regeneration have been proposed. In order to develop scaffolds that augment PNS repair, it is vital to understand how they interplay with microenvironment at the injury site and alter the function of supporting cells. In this study we suggest that aligned fibers enable enhanced adsorption of Fibronectin from serum and can therefore affect the attachment and behavior of infiltrating glial cells during nerve regeneration. Topographical cues have shown to create contact guidance as well as chemical patterns which can influence the differential adsorption of proteins from serum.[2] We also observe that oriented fibers promote ECM organization and subsequently lay the foundation for neurite outgrowth during the nerve regeneration process. Hence we suggest that 3D scaffolds created from oriented nanofiber films will enhance nerve regeneration in vivo by providing topographical cues necessary to induce the regenerative phenotype of Schwann cells and aid in neurite bridging peripheral nerve gaps.

References


Acknowledgments

The authors gratefully acknowledge funding from NIH NS44409 and GTEC NSF EEC-9731643.