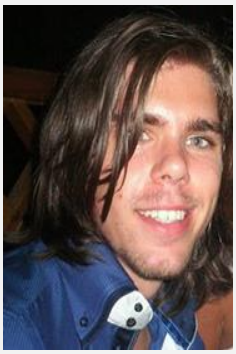
ImmunoTools *special* Award 2014



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Synergistic or Antagonistic Signalling? The Interaction between Topography Induced Signalling and Growth Factor Signalling in Regenerating Dorsal Root Ganglia

Injury to the peripheral nerve system (PNS) is relatively common and can have a devastating impact on quality of life. Despite being able to heal naturally without assistance, the outcome is never fully satisfactorily due to nerve cell death, axons remaining disconnected and neuroma formation. Currently, autologous nerve grafts provide the best outcome although this procedure requires collateral damage to the areas supplied by the nerves sourced. Consequentially, research into alternative treatment systems, capable of enhancing the intrinsic process of PNS regeneration is of vital importance. Success of treatment is dependent on the ability to provide a sufficiently connected neural network by encouraging axonal growth as well as guiding the direction of the regenerating neurons. The former is supported by drug and cytokine treatment the later by a nerve repair scaffold, that supports guidance using surface topography. Our initial results using nerve growth factor (NGF) and recent publications indicate that growth factor (GF) activated signalling and signalling initiated by scaffold properties may interact leading to unexpected responses (reduced/increased response to NGF, fibroblast GF, …).

The initial signal that a nerve is damaged leads to interruptions to the normal flow of retrograde transport of neurotrophins from the previously innervated organ/ neighbouring Schwann cells to the soma. This results in the phenotypic switch to a regenerative state and an activation of transcription factors responsible for regulation of neurotrophic factors e.g. NGF, BDNF and NT-3.

Initiating the regenerative process is a necessity, and providing the optimal environment to promote axon growth with the ideal morphology is critical to success of treatment. This is largely dependent on substrate surface properties and the

selection of neurotrophic GFs used. Micro- and nano-structured surface topography has been shown to have a profound influence on nerve cell behaviour with grooved surface providing directional guidance, leading to a higher amount of axonal bridges. Research suggests that the topography does not lead to longer axonal growth. Instead, regenerating axons need to be stimulated by GFs. Although it is known that GFs are required for regeneration, it is not fully understood at which concentrations outcomes are optimal. Current research also suggests that GFs may even have a detrimental effect on axonal outgrowth. Topography influences the direction of the growth cone and GFs stimulate axonal growth however our group has shown that these aspects are not active separately with topographical cues having an impact on GF efficacy and on resulting morphology.

I will use the ImmunoTools GFs to gain a deeper understanding of the influence substrate surface topography has on GF signalling in regenerating rat dorsal root ganglia (DRG). Whole DRG are used to provide a complex in vitro model for analysis. Extracted DRG will be cultured on microstructured and flat polydimethyl-siloxane (PDMS) substrates with a selection of different growth factors known to have an effect on neurogenesis. The GFs to be investigated- BDNF, platlet derived GF, basic fibroblast GF and NGF, as well as Neuregulin and CXCL12 are known to either have a specific effect on neurons, or have been implicated as being differentially regulated by topography (BDNF/PDGF, FGF). The effect that these GFs have on DRG will be analysed at varying concentrations (10-100ng/ml). Through monitoring the resultant network and axon morphology on and off the topographical pattern as a function of GF concentrations, an insight into the interaction between topographical cues and GF will be gained which will help understanding topography induced signalling and GF efficacy for neural regeneration. The effectiveness of human and rat specific cytokines will be compared (FGF, CXL12, TNF-a, VEGF) as the interesting ones apart from CXCL12 are not part of the current Immunotools rat selection.