In vitro assay systems in the development of therapeutic interventions strategies for neuroprotection and repair

Summary

The aim of this thesis was to analyze and improve *in vitro* assays systems used for the characterization of cell-substrate interactions as well as for donor cell-tissue interactions in the development of strategies in neuroregenerative medicine and neuroprotection.

Chapter 2 briefly reviews the pathophysiology of SCI. It further explains approaches taken on the development of biomaterials and tissue engineering strategies intended to promote axon regeneration following traumatic injury. While this section focuses primarily on injury to the CNS, the basic principles explained extend to the PNS.

Chapter 3 evaluates the interactions of single nanofibers with single cells/neurites. For this, an *in vitro* assay which takes advantage of the collection of orientated electrospun nanofibers onto glass coverslips at low densities was used. Neurite-nanofiber interactions were studied using the cell line SH-SY5Y after differentiation with BDNF and RA into neurite bearing cells. The U373 astrocytic cell line and primary astrocytes derived from neural stem cells, helped reveal the differences on the behavior of cell lines versus primary cells. Results showed that topographical cues play an important role on guiding neurite and process extension as demonstrated by the ability of a single nanofiber to support morphological changes on multiple cells. Furthermore, the poor response of the U373 cell line to the substrate functionalization contrasted with that of the neural derived astrocyte progenitors and indicated the poor value cell lines have for the evaluation of bioengineered substrates.

Chapter 4 shifts the focus of this research from 2D *in vitro* assays to more complex 3D *in vitro* assays. Regeneration of motor axons originating from organotypic spinal cord slice preparations into explanted spinal nerve roots as well as by 3D bioengineered scaffolds is evaluated. The validity of the nerve root reconstruction model *in vitro* and the correlation of *in vivo* events associated with Wallerian degeneration, axon regeneration and re-myelination is explored in the first part of this chapter. The model was then used to assess axonal growth and cell migration into 3D bioengineered constructs. Confronting the slice cultures on its ventral side with a microstructured collagen scaffold and a fibrin hydrogel block, showed the response axons and cells from the OSC had to the different materials and topographies. Axonal growth and cell migration through the microstructure collagens scaffold indicated the importance topographical cues have for the development of bioengineered scaffolds. Using transmission electron microscopy we further showed the complex cell-cell, cell-axon and cell-biomaterial interactions occurring within the collagen scaffold.

Chapter 5 moves the focus from neuroregeneration to neuroprotection. We use the organotypic spinal cord slice preparation to investigate aspects of motoneuronal excitotoxicity in complex 3D tissues. The excess glutamate slice culture model of motor neuronal degeneration was subsequently established using tissue from different levels of the spinal cord (cervical, thoracic and lumbar). The observation that MN in the different levels were protected from excitotoxicity by different neuroprotective agents targeting either NMDA or AMPA/KA receptors, shed a light on the differential rostro-caudal localization of specific sub-populations of motor neurons within the spinal cord. These observations are valuable for the refinement of the organotypic slice culture model of excess glutamate.

Chapter 6 focuses on the characterization of an immortalized human fetal spinal cord stem cell line SPC-01; its ability to induce astrocytic differentiation by treatment with BMP4/LIF, and their potential to provide neuroprotection to MN in the of organotypic spinal cord slice excitotoxicity model. We showed that the cell line SPC-01 in its undifferentiated state possesses powerful glutamate uptake machinery compared to those differentiated into an astrocytic phenotype. We also demonstrate that the OSC excitotoxicity model is a strong tool capable of detecting the neuroprotective effects of cells, as demonstrated by the increased MN survival provided by the SPC-01 line.

Chapter 7 discusses the findings from the preceding chapters in regard to the refinement and development of new *in vitro* models that would aid the scientist in the field of neurosciences.

Adapting the principles of "reduction, replacement and refinement" in science signifies a shift in the way we make research. Laboratory pre-screening will require improved *in vitro* models capable of better characterizing the potential new therapies have for further development. To accelerate this process, it is necessary for old methods to be revised and updated, and new methods to be developed. To succeed in this endeavor, dynamic renewal mechanisms should be put in place, such as the continuous assimilation of new discoveries and their effects on the *in vitro* models, in order to maintain the validity of the developed methods.