

INVESTIGATING THE REQUIREMENT FOR DUAL CELL CO-CULTURE PLATFORMS IN CREATING REGENERATIVE CELL THERAPIES FOR CNS INJURY

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Injuries to the central nervous system (CNS) can be devastating. CNS injuries include those to the spinal cord, where there can be a complete loss of function below the point of injury. Spinal cord injury impacts up to 500,000 people worldwide every year and where function is lost, quality of life can be severely limited.

Olfactory ensheathing cells (OECs) are a candidate cell therapy for spinal cord injury as they can promote neuronal survival and facilitate regeneration of severed axons. Despite their unique properties, OECs are very challenging cells to work with because they are difficult to isolate, difficult to sustain in culture for prolonged periods and there is still controversy around how to characterize their identity and potency.

The overall aim of this project is to identify methods to enhance the survival, growth and function of OECs. It has been reported that for OECs to be truly functional they require interaction with fibroblasts. Therefore, we sought to investigate whether it is necessary to use fibroblasts as a feeder layer to support OECs via physical cell-cell contact, or whether paracrine soluble factors in the conditioned media from fibroblasts would provide the trophic support necessary to enhance OEC survival and growth.

A human mucosal fibroblast cell line was used as a feeder layer. Primary rat OECs were cultured for 14 days on the feeder layer or control substrate (laminin-coated dishes). After 14 days, the morphology of cells was assessed and an algorithm generated using ImageJ was used to ascribe a mathematical value to OEC morphology to determine whether a correlation of morphology to expression of markers could be made.

Cells cultured on feeders adopted a more spindle-like appearance compared with cells cultured on laminin, which adopted an enlarged morphology. The algorithm was used to analyse the circularity of cells that labelled positive for candidate identity marker S100 β . It was found that cells cultured on feeders had a lower circularity, and therefore more elongated shape compared to those cultured on laminin ($p=0.037$). Additionally, a significant increase in p75^{NTR} expression (a second candidate OEC marker) was observed ($p=0.01$) on feeders.

To further investigate the relationship between the OECs and the feeders, cells were cultured in the presence of conditioned media from the fibroblasts. When cells were cultured in conditioned media there was a significant ($p=0.002$) upregulation of Thy1, an undesirable marker, and the significance of this will be investigated further with work underway to compare different feeder types and their impact on marker expression.