09.P15 Human dental pulp stem cells have a positive influence on neural regeneration

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Stem cell-based therapies can be a future novel strategy to repair peripheral nerve injury, based on their multilineage differentiation potential and ability to produce and secrete (neuro) trophic factors. Recently, a possible transdifferentiation of MSC into SC have been demonstrated which had a positive effect on neuronal survival and neurite outgrowth. In this study, dental pulp stem cells (DPSC) are differentiated toward SC via a mix of growth factors. In addition the influence of secreted neurotrophic factors by DPSC and SC-DPSC on neuronal outgrowth and survival is investigated. After 21 days of differentiation, the expression of SC-markers GFAP, p75 and S100 was observed together with a decreased expression of nestin and Str6-1. Ultrastructurally, SC-DPSC displayed a spindle-shaped bipolar morphology with numerous organelles spread throughout the cell cytoplasm. Furthermore, collagen fibers were observed in the extracellular matrix. DPSC and SC-DPSC produced and secreted several neurotrophic factors which promoted the survival and neurite outgrowth in DRG cultures, with SC-DPSC yielding a significantly better effect than control DPSC. The results of this study indicate that DPSC are capable of differentiating toward SC. Moreover, differentiated DPSC had a better neuroprotective and neurotrophic effect than naïve stem cells suggesting that DPSC can be good candidates for cell-based therapies as treatment for peripheral nerve injuries.

09.P16 Effect of neurotrophic factors on Schwann cell differentiation prior to transplantation

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Schwann cells (SCs) secrete neurotrophic factors (NTs) that promote neuronal survival and guide axons during regeneration. The addition of SCs to nerve grafts is a promising strategy for enhancing peripheral nerve regeneration. To obtain a sufficient number of cells for transplant, SCs must be expanded in vitro. However, in our lab, we have shown that after long-term in vitro expansion SCs de-differentiate into an immature state. In vivo as axons grow, cues from the environment guide SC differentiation into mature myelinating SCs to support functional recovery. The goal of this study was to determine the effect of NTs on SC differentiation. SCs were harvested from motor and sensory branches of rat femoral nerves and expanded in culture until the cells were confluent (~30 days). Cells were then seeded in media with 0, 50, or 100 ng/ml of nerve growth factor (NGF) or glial-derived neurotrophic factor (GDNF). SC differentiation was evaluated by qRT-PCR to determine the levels of S100 (mature SC) and nestin (dedifferentiated SC) compared to fresh nerve and SCs that were freshly passaged (Day 0). The addition of NGF or GDNF increased S100 and decreased nestin expression in motor and sensory-derived SCs at 3 and 7 days. This change in gene expression suggests that NTs may provide cues to guide SC differentiation. Culturing SCs with NTs prior to transplantation may promote differentiation into mature SCs, which may increase myelination of nerves to restore function more quickly.

09.P17 Antioxidant and neuroregenerative properties of chondroitin sulfate-coated scaffolds in an in vitro oligodendroglial cell model

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Radical oxygen species are metabolism waste products that in excess lead to oxidative stress involved in several neurodegenerative diseases. In the CNS, oligodendrocytes are especially vulnerable to ROS. Here we studied the potential of chondroitin sulfate as antioxidant and neuroregenerative biomaterial for nerve tissue regeneration. HOG cells reach mature phenotype by replacing growth media with differentiation media. Cells are cultured and differentiated on CS- coated scaffolds or other ECM components. Oxidative stress is H2O2. Cell proliferation was analysed by spectrophotometry, viability and apoptosis by annexin staining and flow cytometry. ORAC method reported antioxidant capacity. HOG cells maintain their proliferative capacity up to moderate levels of oxidative stress but once differentiated moderate concentrations of H2O2 affect viability. Oligodendroglial cells cultured on ECM components-coated surfaces have differential cell proliferation profile which shows that GAGs facilitate cell growth while ECM proteins. ORAC test reports that CS has antioxidant properties lasting longer than ascorbic acid. Finally, CS exerts immediate mild neuroprotective effect on HOG cells and also promotes proliferation up to 48 h. CS presents relevant properties for oligodendrocyte protection and regeneration. This GAG acts as antioxidant and enhances HOG cell proliferation. Therefore, CS has potential value as a component of a biomaterial designed to promote remyelination.

09.P18 Extracellular-matrix mimetic peptide nanofibers for neural regenerative medicine

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Regenerative medicine studies rely on mimicking the natural extracellular matrix for promoting new tissue formation by host cells. Extracellular matrix contains an abundant variety of signals that are received by cell surface receptors contributing to cell fate, via regulation of cellular activities such as proliferation, migration and differentiation. Neural extracellular matrix (ECM) is rich in axonal growth inducer proteins and by mimicking these permissive elements in the cellular environment, neural differentiation as well as neurite outgrowth can be induced. In this study, we used a synthetic peptide nanofiber system that can mimic not only the activity of laminin, an axonal growth promoting constituent of the neural ECM, but also the activity of heparan sulfate proteoglycans in order to induce neuritogenesis. Heparan sulfate mimetic nanofibers with heparan sulfate mimetic and laminin derived epitopes significantly promoted neurite outgrowth by PC-12 cells. In addition,
these nanofiber systems were even effective in the presence of chondroitin sulfate proteoglycans (CSPG), which are the major inhibitory component of central nervous system. In the presence of these nanofibers, cells could overcome CSPG inhibitory effect and extend neurites on peptide nanofiber scaffolds.

**9.0.P19**

**Design of PCL and PCL/gelatin electrospin conduits for in vivo evaluation in rat sciatic nerve model**

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Material choice plays a crucial role in ensuring the success of neural tissue engineering strategies, minimizing inflammatory response and providing the required support and guidance to regenerating axons. Here, poly(ε-caprolactone) (PCL) or PCL and gelatin solutions were processed by electrospinning to fabricate fibrous conduits as artificial grafts for sciatic nerve repair after transection. We demonstrated that the integration of gelatin in PCL fibers reduced the characteristic net-work size scale – average diameter of 0.59 ± 0.15 μm - compared to micrometric PCL fibers (5.61 ± 0.80 μm), interacting more effectively with cells in vitro, due to the higher scaffold surface area. The presence of gelatin also affected the stiffness of conduits, with PCL/gelatin conduits having a smaller compressive modulus than PCL conduits in longitudinal compression tests. Conduits were also compared in vivo when implanted in the 5 mm rat sciatic nerve defect in an 18-week study. Animals implanted with PCL conduits showed better recovery of the injured muscle weight and electrophysiological signal, as well as more mature nerve morphology as compared to PCL/gelatin conduits. In vivo data suggested that bioactive signals induced by gelatin are countered by unfavorable mechanical properties of PCL/gelatin conduits. Hence, PCL electrospin conduit appears to be the more promising device to support the in vivo regeneration of peripheral nerve.

**9.0.P20**

**Intraluminal fiber based scaffolds: a platform for cell migration and axonal regrowth and increased targeted nerve regeneration**

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Introduction: This study investigates the ability of intraluminal collagen fibres (ICFs) to improve existing nerve guidance conduits (NGCs). It is hypothesized ICFs increase the surface area available for cell adhesion and migration, and provide structural cues to regenerating axons for improved nerve repair.  

Method: ICFs were produced in a multi-step process and their surfaces characterised using SEM-FIB analysis. Neuronal interaction and cell migration were assessed in vitro using both neural and migratory cells. ICFs were enclosed within a hollow NGC, and implanted in a rat sciatic nerve model for 16 weeks. After 16 weeks, retrograde tracing and nerve morphometric analyses were carried out.  

Results: In vivo assessment on the ICFs, showed a significant increase in neurite length and higher alignment versus controls, with cells successfully migrating across the fibres. After implantation, all ICF groups demonstrated increased nerve regeneration across a 10 mm nerve gap. ICFs showed a significant decrease in the number of misdirected axons versus autograft treatment.  

Conclusion: NICFs increase aligned nerve growth and act as a platform for cell migration in vivo. In vivo ICFs significantly reduce the numbers of axons incorrectly re-innervating distal targets versus autograft repair.

**Acknowledgements:** Science Foundation Ireland, Grant No. 07/SRC/B1163 and Enterprise Ireland - Proof of Concept Grant (PC/2008/399)

**9.0.P21**

**Activated Schwann-like cells guided by fibrin structures enhance Axonal Regeneration**

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Introduction: The gold standard in peripheral nerve regeneration is the autologous nerve transplant, which is limited by source. New approaches include not only nanoscaled guiding structures but also activated Schwann cells (SCs) forming bands of Bungner for enhanced axonal outgrowth. In this study we characterise rat Schwann-like cells (SCs) differentiated from adipose derived stem cells (ASCs) concerning SC markers for the use in vivo.

Materials and methods: SCs were evaluated morphologically, with flow cytometry (P75, S100, MAG, P0), PCR (ATF3, cJun, PAX3) and Western Blot (ATF3, cJun, PAX3) concerning differentiation and activation status. SCs were cultured with/without forskolin to trigger both, proliferation and potential myelination. Seeded on an electrospun fibrin matrix, SCs were used to bridge an 8 mm dissected rat sciatic nerve.

Results: Cultured with forskolin SCs appeared spindle-like and expressed P75, S100, ATF3 and cJun- indicating an activated status. Cultured without forskolin SCs appeared more flat and round, expressing S100, MAG, P0 and PAX3 - indicating a promyelinating status. Grafting a sciatic nerve in vivo, animals treated with SCs showed enhanced axonal regeneration.  

Conclusion: It is possible, to trigger SCs in activated or promyelinating status, similar to native SC. Activated SCs seeded on fibrin guided structures are promising for axonal regeneration. Financial support from FFG (#818412) and City of Vienna is gratefully acknowledged.

**9.0.P22**

**A living replacement tissue for peripheral nerve that can enhance regeneration in vitro and in vivo**

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Introduction: A peripheral nerve repair device with the ability to enhance regeneration would be a promising alternative to nerve autograft repair. The growth of axons across a lesion is most effective when supported by columns of aligned Schwann cells that provide cell-level guidance, as found in an autograft. Here we report the development and testing of engineered neural tissue (ENT): aligned Schwann cells in a 3D collagen environment, which supports and guides neuronal growth.

Methods: Collagen gels containing F7 Schwann cells were tethered for 24 h to permit cellular self-alignment and then stabilised by rapid removal of interstitial fluid. This process generates sheets of ENT, which are stable tissue-like gels with cells organised within a 3D matrix. Cell alignment was monitored before and after stabilisation. Dissociated dorsal root ganglia neurons were cultured on the surface of the material for 3 days and neurite growth assessed. Sheets of ENT were rolled into columns and packed together to form the core of a

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DOI: 10.1002/term.1586