Schwann Cell Gene Expression Profiles After Injection into Acellular Cold-Preserved Nerve Grafts

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Statement of Purpose: The extent of peripheral nerve regeneration depends on environmental cues and trophic support present after injury. Schwann cells (SCs) secrete trophic factors and extracellular matrix molecules that promote neuronal survival and help guide axons during regeneration. Previous studies have shown that SCs may have different phenotypes (sensory or motor) based on their source, which may influence the pathway that an axon chooses (motor vs. sensory branch) during regeneration. The purpose of this study was to determine if the injection of different types of SCs into acellular nerve grafts leads to enhanced expression of trophic factors that promote nerve regeneration. We hypothesized that in a rat sciatic nerve injury model (1) the addition of the different types of SCs to acellular grafts will increase the expression of nerve growth factor (NGF) and glial-derived neurotrophic factor (GDNF) that have been previously shown to enhance nerve regeneration[1, 2] (2) transplanted SCs will increase the extent of nerve regeneration.

Methods: Twenty male Lewis rats were divided into 5 experimental groups *(n = 4 per group)* for the different graft treatments used to repair a 14mm sciatic nerve gap. Three groups received acellular (cold-preserved) grafts injected with cultured rat SCs obtained from either sciatic *(Sc)*, femoral motor *(M)*, or femoral sensory *(S)* nerve. An isograft *(Iso)* group served as the positive control, with an untreated cold-preserved *(CP)* nerve graft group as the negative control. Grafts were prepared by immersing freshly harvested nerve grafts in University of Wisconsin solution over a period of 7 weeks[3]. Gene expression profiles were analyzed prior to transplantation for each SC group and 2 weeks post-transplantation using quantitative real time polymerase chain reaction (qRT-PCR) analysis. The fold difference in gene expression levels was calculated using the delta crossover threshold (C_t) method (delta-deltaC_t)[4] comparing to the expression in grafts with no SCs. Histomorphometric analysis was conducted in order to obtain the total fiber counts through each graft after 8 weeks post-transplantation.

Results: Gene expression levels were analyzed to evaluate levels of trophic support and the phenotype of the SCs present in the acellular grafts after transplantation. When expanded in culture, the SCs show disregulation of some genes, which were also disregulated in the grafts after transplantation (data not shown). The grafts injected with sensory SCs and motor SCs showed a significant increase in NGF mRNA levels when compared to the grafts injected with sciatic SCs and the isograft groups (Fig. 1). The grafts injected with sensory SCs showed a significant increase in GDNF expression when compared to the other 3 groups (Fig. 1) and all 3 cell transplantation groups showed increased GDNF expression compared to grafts without cells (relative to 1 on the fold difference scale). After 8 weeks, the isografts and the acellular grafts injected with SCs showed a significant increase in the total fiber number when compared to the graft devoid of transplanted SCs (Fig. 2).

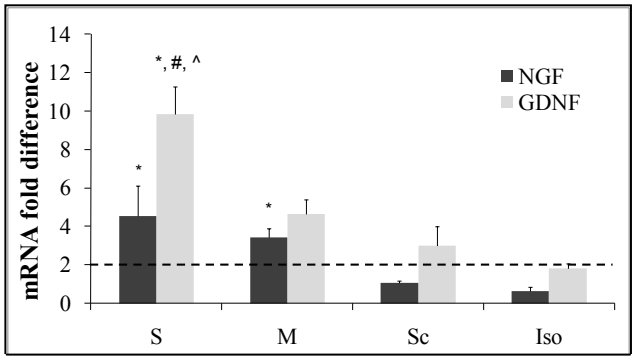

*Figure 1: Expression levels of NGF and GDNF. Error bars represent the standard deviation (n=3). Dotted line represents threshold value for upregulation. * - p< 0.05 when compared to Iso, # - p< 0.05 when compared to CP nerves with M, ^ - p< 0.05 when compared to CP nerves with SC.*

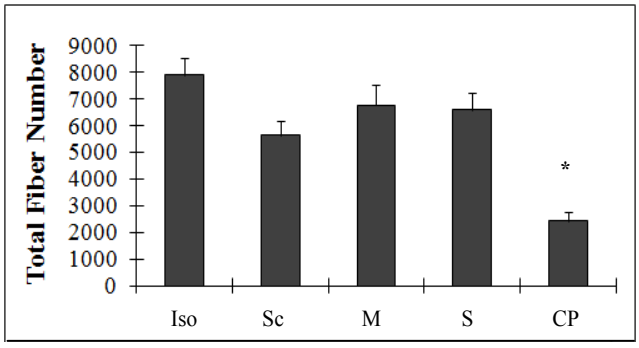

Figure 2: The total fiber counts present in each graft 8 weeks post-transplantation. Error bars represent standard error (n=8)
** - p< 0.05when compared to Iso group.*

Conclusion: The transplantation of phenotype specific SCs derived from the motor and sensory branches of the femoral nerve provide increased levels of the trophic factors to enhance peripheral nerve regeneration. This to promote nerve regeneration that is better than the clinical standard, of autografts (similar to isografts in our study) and could be used in conjunction with current commercially available acellular nerve grafts (Avance, Axogen).

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