Culturing De-differentiated Schwann Cells on Fibrin Scaffolds Promotes Differentiation into Mature Schwann Cells

Nithya J. Jesuraj, Laura M.Marquardt and Shelly E. Sakiyama-Elbert.
Department of Biomedical Engineering, Washington University, St. Louis, MO

Statement of Purpose: Schwann cells (SCs) secrete trophic factors and extracellular matrix (ECM) molecules that promote neuronal survival and help guide axons during regeneration. The addition of SCs to acellular nerve grafts is a promising strategy for enhancing peripheral nerve regeneration. Recent studies have successfully transplanted 10^6 cells into acellular grafts to promote peripheral nerve regeneration[1]. Currently, the seeding efficiency of the SCs into grafts is ~10%. A potential improvement would be to incorporate the cells into a scaffold, such as fibrin, to improve cell retention in the grafts. Prior to injection of SCs with fibrin into acellular grafts, it is important to study the effects of the fibrin scaffolds on SCs. The purpose of this study was to determine if the SCs are viable in the fibrin scaffolds and also to determine the effects of culturing SCs on fibrin scaffolds on SC differentiation over 4 weeks.

Methods: Sciatic nerve SCs were harvested from Lewis rats and expanded in culture on poly-L-lysine coated (pLL) plates (Sigma) until the cells were confluent. Cells were seeded onto 4 mg/mL fibrin scaffolds (Calbiochem, prepared as previously described[2]) and pLL-coated tissue culture polystyrene (TCPS) (control) in expansion culture *(n = 3 for each condition)*. After 1, 2 and 4 weeks, the viability and differentiation of SCs were analyzed. Calcein AM was used to stain for the live cells and visualized by fluorescence microscopy. The differentiation of the SCs was evaluated using quantitative real time polymerase chain reaction (qRT-PCR) analysis for S100 (mature SC maker) and nestin (undifferentiated SC marker). The fold difference in gene expression levels was calculated using the delta crossover threshold (C_t) method (delta-deltaC_t)[3] comparing the expression to the SCs that were freshly passaged (Day 0).

Results/Discussion: To ensure that the fibrin scaffolds provided a hospitable environment for the SCs, the scaffolds were stained with Calcein AM to visualize live cells. The SCs migrated into the scaffold over 2 weeks and SC growth continued over 4 weeks (Fig. 1). Expression of S100b and nestin was monitored over 4 weeks. The mRNA fold difference for the SCs grown on fibrin scaffolds (3D culture) and on pLL coated (2D culture) TCPS was compared back to day 0. After 2 weeks, the SCs that were grown on fibrin scaffolds exhibited increased S100 expression, but by 4 weeks S100 expression decreased. The expression of nestin in SCs grown on fibrin scaffolds was lower at 1week but increased by 4 weeks. Additionally, we observed degradation of the fibrin scaffold over 4 weeks resulting in less contact of the SCs with the fibrin scaffolds. This degradation may account for the decrease in S100 expression and increase of nestin expression at 4 weeks.

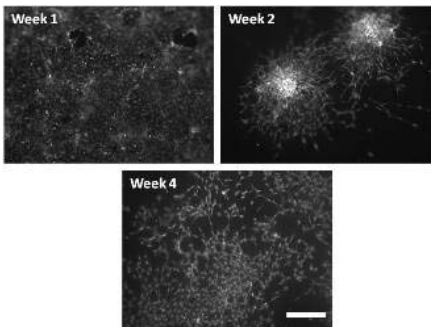

Figure 1: Calcein AM staining shows good viability of SCs cultured on 4mg/mL fibrin scaffolds for 1, 2 and 4 weeks. At 1 week the cells were still on top of the scaffold. After 2 weeks, the cells have migrated into the scaffold and by 4 weeks, significant scaffold degradation has occurred and the cells have continued to extend and connect with each other. Scale bar = 250 μm.

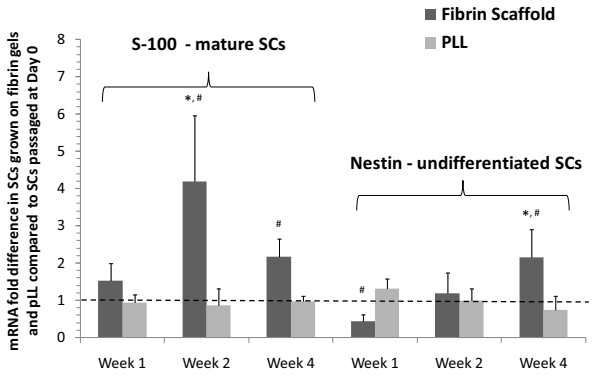

*Figure 2: Effect culture substrate on S100 and nestin expression. Error bars represent standard deviation (n=3). Dotted line represents threshold value for differential expression vs. Day 0. * - $p < 0.05$ vs. other time points, # - $p < 0.05$ vs. pLL at the same time point.*

Conclusion: To determine the effects of culturing SCs on the fibrin scaffolds, the viability and differentiation of the SCs were monitored over 4 weeks. By week 2, the SCs had migrated into the scaffolds and showed increased expression of S100, which suggests that the SCs received cues to differentiate in a 3D culture (fibrin scaffold) rather than in a 2D culture (pLL) over 4 weeks. The fibrin scaffold mimics the 3D *in vivo* environment (minus the axons) by allowing the SCs to migrate in 3D, which may promote differentiation into mature SCs. In general the transplantation of SCs requires the cells to be expanded in culture, due to limited autologous sources. This expansion culture causes SCs to de-differentiate. Culturing the cells on fibrin scaffolds prior to transplantation may promote the differentiation into mature SCs, which may help increase growth factor and ECM secretion to support nerve regeneration.

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References: 1. Brenner M. Microsurgery 2005 2. Wood MD. J Biomed Mater Res A 2008. 3. Schmittgen TD. Nature Prot., 2008.