Realignment of Cells and Matrix Following Release from Constrained Compaction for Neural Engineering

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Statement of Purpose: The purpose of this work is to develop and characterize a 3-D, aligned, Schwann cell (SC) loaded collagen I-Growth factor reduced Matrigel™ (col-GFRMat) scaffold to serve as a platform for studying the effects of multiple cues (cell, matrix alignment, external forces) in directing neurite outgrowth in 3D. PNS guidance scaffolds of aligned collagen I are attractive but SC in collagen I adopt a spherical morphology.[1] The first aim was to develop a scaffold supportive of both glial and neural components. Composite col-GFRMat were used to support SC spreading in 3D; GFRMat was chosen due to the high amount of laminin and collagen IV, main constituents of SC basal lamina. A second aim was to align SC in the scaffold. Scaffold and subsequent cell alignment were achieved using fibroblast (FB) mediated constrained compaction[2; 3] since SC do not readily compact the composite scaffolds. Constrained compaction alignment, however, introduces tension to the system and it is difficult to decouple effects of tension, matrix and cell alignment, and cell density. Likewise constrained models are not easily transferable to an in vivo model. In this work, we characterized SC and col-GFRMat scaffolds and alignment following constrained and unconstrained compaction to create a stable, aligned matrix, with no external tension with the long-term goal to examine subsequent effects on neurite outgrowth.

Methods: Constructs were created by subsequently combining 5X DMEM, SC growth medium(cite), FBS, 1 N NaOH, and 4 mg/ml acid solubilized bovine collagen type I at 2:1:1:1:5 on top of a SC pellet. GFRMat was then added at 10%-50%/vol and SC density was fixed at $1x10^6$ cells/ml. Cell number and morphology were assessed using a DNA assay and via microscopy to select an optimal GFRMat concentration. Next, using 35% GFRMat constructs to examine compaction, FB were incorporated at $1x10^6$-$2x10^4$ cells/ml (1:1-1:0.02 SC:FB ratio, respectively). Construct solution was pipetted into well plates and constructs were imaged and analyzed daily to quantify compaction. For a constrained model, 500 µl of construct solution with a 1:0.25 SC:FB ratio was pipetted into rectangular wells containing two pieces of porous polypropylene to constrain the matrix. Compaction proceeded for 1-7 d and constructs were fixed or released and cultured for an additional 1-7 d. Following fixation and immunostaining, cell and matrix alignment were visualized using a multiphoton laser scanning confocal microscope.

Results: Col -35% and -50% GFRMat composites supported the greatest number of SC after 14 d in culture and the most elongated morphology. To minimize GFRMat and maximize SC number, Col-35% GFRMat was selected for compaction studies. Constructs exhibiting high degrees of constrained compaction result in a high degree of matrix and cell alignment. Varying the SC:FB ratio resulted in changes in gel compaction (Fig 1) but no significant differences were observed for 1:1, 1:0.5, and 1:0.25 ratio constructs from 3-7 d. To maximize compaction and minimize FB in the scaffolds, a 1:0.25 SC:FB ratio was chosen. Both SC and FB exhibited alignment along the axis of constraint following 1-7 d of constrained compaction (Fig 2A). Release from constraint initially resulted in disorganization (Fig 2B) but reorganization of cell and matrix alignment perpendicular to their original direction of constraint occurred over 7 days in unconstrained culture. This reorganization appeared strongest following 7 d and 4 d of constrained then unconstrained compaction, respectively (Fig 2C).

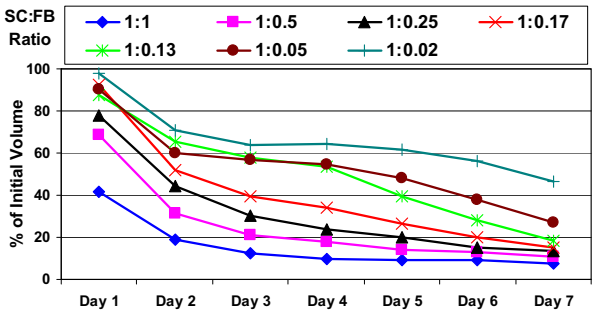

Figure 1. Gel Compaction: Variable SC:FB Ratio

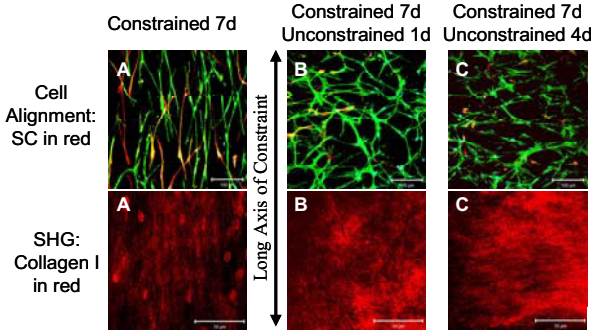

Figure 2. Cell and Matrix Alignment

Conclusions: Aligned col-35% GFRMat constructs were created using FB mediated constrained compaction. Following release from 1D constraint, the constructs reorganize likely due to FB mediated compaction perpendicular to the constrained compaction. While in constraint, constructs are only allowed to compact in 2D, but upon removal, constructs compact in the 3rd dimension with little subsequent change in the first 2 dimensions, reorienting cells and matrix. The addition of GFRMat maintained SC number and supported cell spreading in the col-35% GFRMat constructs likely due to the addition of the basal lamina proteins laminin and collagen IV present in Matrigel™. In this study, aligned cellular composite matrices under no external forces have been created to investigate neurite outgrowth in a 3D aligned cellular construct.

References:

[1](Rosner, BI. Experimental Neurology. 2005:195:81-91.)
[2](Phillips, JB. Tissue Eng. 2005:11:1611-7.)
[3](Voge, CM. J Biomed Mater Res A. 2008:86:269-77.)