Creating 3D Aligned Collagen I Scaffolds for Peripheral Nerve Repair

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Statement of Purpose: Each year, peripheral nerve injury affects ~13-23 out of every 100,000 people, worldwide (Wang *et al.*, 2017). Type I collagen scaffolds have been increasing in interest and testing with applications in peripheral nerve repair. Several studies analyzing the effect of axonal direction in these scaffolds have shown their advantage in treatment of peripheral nerve injury (Bozkurt *et al.*, 2007). Embedding adipose-derived stem cells (**ASCs**) in collagen I scaffolds has been shown to create degradation pores and positively influence invasion of other cell types (Song *et al.*, 2016). Additionally, studies have shown that collagen embedded ASCs will secrete pro-angiogenic and neurotrophic factors (Huang *et al.*, 2020). Our goal is to create an aligned collagen I scaffold with embedded ASCs that will allow for better aligned neurite outgrowth, as well as more neurotrophic and pro-angiogenic signaling for effective peripheral nerve repair.

Methods: Aligned collagen scaffolds were created using our custom stretching device (**Figure 1L**). Silicone-based molds were stretched to 1.75x their original length and the collagen I pre-gel solution with or without ASCs was cast. After thermal gelation, the mold was then released to align the fibers. Collagen fiber alignment was visualized with Quantitative Polarized Light Imaging (**QPLI**) and compared to non-stretched controls. Multiphoton imaging was used to visualize cell-laden scaffolds, and the images were analyzed for cell orientation within the hydrogels. ASCs cultured in stretched and non-stretched gels were stained with fibronectin and DAPI to assess orientation of ASC-deposited extracellular matrices (**ECM**). Before staining, conditioned media from gels was also collected and concentrated for Luminex analysis of cell secretome. To assess neurite outgrowth in our scaffolds, dorsal root ganglia (**DRGs**) were harvested from 3-week-old Sprague Dawley rats and seeded on the aligned and non-aligned gels in the direction of collagen fiber orientation. After 7 days of culture, the gels were stained with βIII tubulin, phalloidin, and DAPI for immunofluorescence assessment of neurites, ASCs and cell nuclei, respectively.

Results: QPLI showed significant increase in collagen fiber alignment in the stretched collagen gels (**Figure 1A-C**, $p<0.05$). Multiphoton imaging of the embedded ASCs showed increased cell alignment in the direction of collagen fiber alignment in the stretched 2M cells/mL samples compared to the 200k/mL samples (**Figure 1D-F**). The collected conditioned media showed higher βNGF secretion in the stretched samples with higher cell concentration (**Figure 1I**, $p<0.05$). Immunofluorescence staining revealed fibronectin deposition in the cell laden collagen I gels (**Figure 1G, H**). Furthermore, the aligned scaffolds showed aligned fibronectin deposition, even in the bulk of the scaffold, compared to random fibronectin deposition in the bulk of the unaligned scaffolds. DRG culture and staining showed randomly oriented neurite outgrowth on the surface of the stretched gels with (**Figure 1J**) or without (**Figure 1K**) embedded ASCs, possibly indicating a surface barrier to neurite infiltration. Further studies are needed to assess DRG neurite infiltration into the bulk of the gels.

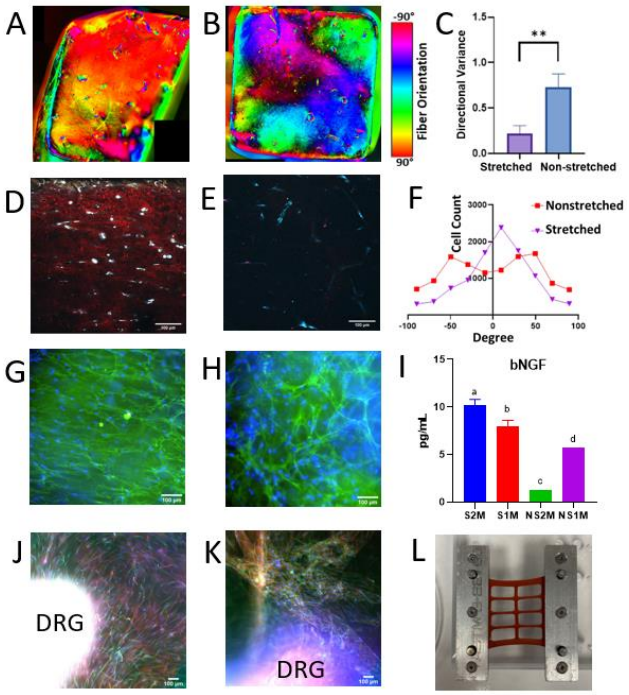

Figure 1. A, B. QPLI for stretched (A) and non-stretched (B) collagen gels. **C.** Directional variance of collagen fibers in A & B ($p<0.05$). **D, E.** Multiphoton images of stretched (D) and non-stretched (E). **F.** Analysis of multiphoton imaging. **G, H.** Fibronectin (green) and DAPI (blue) in stretched (G) and non-stretched (H) gels. **I.** βNGF concentration, all values significant ($p<0.05$). **J, K.** DRG outgrowth on cellular (J) and acellular (K) scaffolds, βIII tubulin (green), phalloidin (red), DAPI (blue). **L.** Mold in stretching device.

Conclusion: We have demonstrated that our method produces aligned collagen I scaffolds with aligned embedded ASCs. Additionally, our scaffolds provide cell-secreted ECM and growth factors, which are beneficial in nerve repair. Future directions involve investigation into improving neurite infiltration into the bulk of the gels and *in vivo* testing.

References:

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