Controlling the Local Environment to Improve Schwann Cell Response

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Introduction: It is known that the peripheral nervous system (PNS) is a complex environment with multiple cell types and tissue of varying stiffness. Consequently, designing a biomaterials based tissue engineering scaffold to promote peripheral nerve regeneration while mimicking the in vivo environment remains challenging. The main cells in the PNS, neurons and Schwann cells, prefer different mechanical environments, 0.1-1 kPa [1] and about 7.5 kPa [2], respectively. Our lab has designed a three-dimensional 3kDa molecular weight poly(ethylene glycol) (PEG) based modular scaffold that spans the stiffness range of the peripheral nervous system and provides decoupled biochemical and mechanical properties [3]. The 3D modular scaffold is composed of crosslinked PEG microgels, spherical hydrogels about 1µm in diameter, which can be easily functionalized with surface peptides or other molecules of interest [4]. As it is formed under mild conditions, it is suitable for including cells. Using this scaffold, we are interested in modulating the binding of cells to the scaffold to improve Schwann cell response in scaffolds of non-optimal stiffness.

Methods & Results: We used 8-arm PEG vinyl sulfone crosslinker concentration to develop a direct relationship between concentration and the scaffold stiffness as shown in Figure 1. The shear modulus was measured via oscillatory shear rheometry with 5% strain and at 10 rad/second and converted to elastic modulus assuming isotropic material. We first examined human fibroblast proliferation, viability (>90%), and morphology (Figure 2) within the scaffolds to characterize cell response. Preliminary results with fibroblasts indicate minimal differences in morphology with stiffness. We then seeded Schwann cells into the scaffolds to investigate viability, proliferation, and gene expression of neurotrophins. Preliminary results indicate good viability in gels with or without collagen, noting that the encapsulation procedure is mild (Figure 2). No significant differences were found in gene expression of nerve growth factor or glial derived growth factor with stiffness on 2D substrates. Growth factor gene expression from 3D scaffolds is still being collected.

Conclusions: We have developed a 3D scaffold that can be used to study how the stiffness can be manipulated in a range relevant to neural cells while maintaining the biochemical nature of the gels. Using these gels, we have begun to study how to improve the response of cells that, typically, do not respond well to soft scaffolds. Current work includes quantifying the growth factor expression of Schwann cells in the soft scaffolds, with the goal of using these interaction points to improve the cellular outcomes.

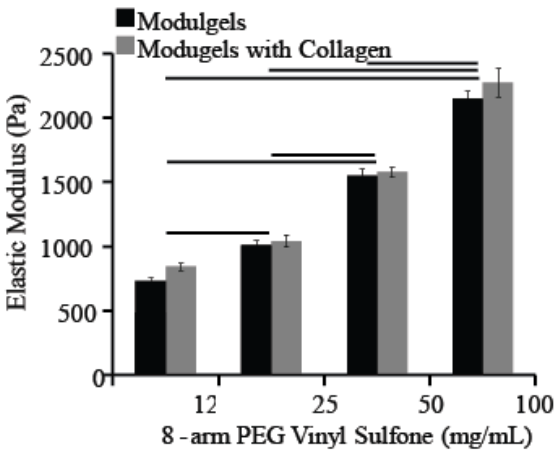

Figure 1. Effect of crosslinker concentration on the elastic modulus of modular scaffolds with or without 100 µg/mL collagen. Statistical differences were observed between each crosslinker concentration. There were no significant differences between the blank scaffolds and those containing collagen for each crosslinker concentration with $p<0.05$.

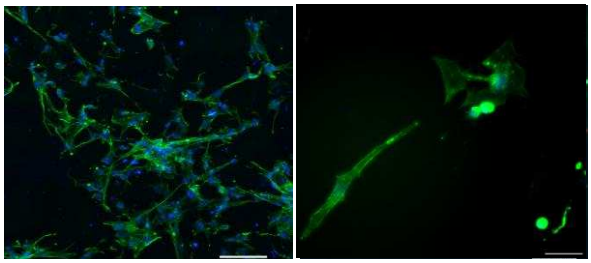

Figure 2. Cells seeded within a modular scaffold stained with Hoechst 33342 and Phalloidin staining nuclei and F-actin, respectively. (A) Fibroblasts (scale bar = 100 µm) and (B) Schwann cells (scale bar = 20 µm).

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References: [1] Engler et al., Cell; 2006. [2] Gu et al., Biomater; 2012. [3] Scott et al., Acta Biomater; 2011. [4] Thompson et al., Jove; 2013.