Neurite Growth in PEG Gels: Effect of Mechanical Stiffness and Laminin Concentration

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Introduction: Within a 3D environment, the chemical and mechanical properties of a scaffold can significantly influence nerve behavior. How these properties interact with nerve cells is important for optimizing neurite extension within a scaffold. The purpose of this study was to investigate the effect of poly(ethylene glycol) (PEG) with added laminin (LN) on 3D growth of dorsal root ganglia. Due to its high affinity for neurite adhesion and extension promotion[1], LN was conjugated to PEG, as well as mixed in the gel, to provide chemical cues for the growing cells. The mechanical microenvironment has previously shown to significantly alter cellular behavior[2], thus various mechanical stiffnesses of PEG gels were studied for their impact on neurite extension.

Methods: Oscillatory shear rheometry was used to determine the mechanical properties of plain PEG gels, PEG with LN (mixed in), and PEG with PEG-LN conjugate. The mechanical stiffness of each gel (G*) was measured using a 25 mm parallel plate configuration, a frequency sweep of 1-100 rad/s, and constant strain of 5%. Dissociated E9 chick dorsal root ganglia (DRG) were used to study neurite extension. PEG gels were made of PEG-DA (3, 4, 5 wt %), PEG-LN conjugate or LN (0, 1, 10, and 100 ⌈g/ml), Irgacure 2959, nerve growth factor (NGF), and F12K; cells were seeded into the PEG solution at $1.2x10^5$ cells/ml. Competitive inhibition study gels were fabricated as described above with 4 or 5% PEG with 100 μg/ml of PEG-LN conjugate and 1 mM of IKVAV, YIGSR, or RGDS. Statistical differences were determined by single-factor ANOVA ($p<0.05$).

Results: The mechanical stiffness, or G*, was sampled at 10 rad/s and average stiffness for plain 3, 4, and 5% gels were 69.23 ± 4.27 Pa, 479.49 ± 9.79 Pa, and 992.24 ± 36.07 Pa, respectively, each statistically different. The addition of LN mixed within the gel did not alter the mechanical properties of the plain gel, but increased PEG-LN significantly decreased mechanical stiffness. Neurite extension was examined within gels with added LN or PEG-LN conjugate and compared with plain gels as control. A model for neurite extension[3] fit growth rate as a function of mechanical stiffness and chemical adhesion properties (Figure 1). Average neurite extension statistically increased with increasing LN or PEG-LN at all concentrations of PEG. No significant difference was noted between the addition of LN versus PEG-LN conjugate. Neurite growth was significantly reduced due to competitive inhibition in 5% PEG gels with the addition of any of the peptide sequences, IKVAV, YIGSR, and RGDS; extension in 4% PEG gels was only significantly reduced in gels with added IKVAV(Figure2).

Conclusion: Mechanical testing demonstrated increased stiffness with increased [PEG] and decreased stiffness with increased [PEG-LN]. Further testing confirmed this decrease was an effect of PEG conjugate and not the addition of LN, similar to other results.[4] Cell testing showed increased neurite extension with increasing [LN] and [PEG-LN]; no statistical differences between these conditions indicated that conjugation of LN to PEG does not alter its bioactivity. A model of neurite extension demonstrated that the chemical adhesion cues provided by LN appeared to have a more significant influence on neurite extension than mechanical properties of the scaffold. Via competitive inhibition, the interaction between neurites and LN in a 3D environment was further delineated indicating that IKVAV has a greater influence over neurite extension over of the peptide sequences found on the LN chain. These results demonstrate that the microenvironment properties are important when developing a 3D scaffold to optimize neural cell behavior.

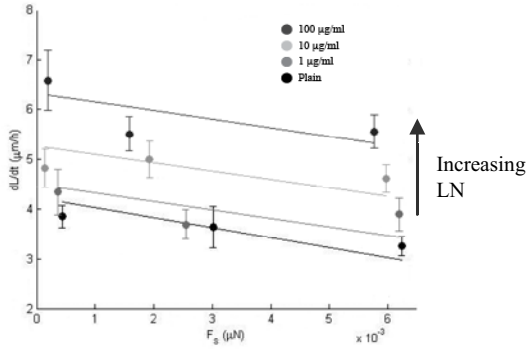

Figure 1. Rate of neurite extension as a function of scaffold stiffness (F_S) and force of interaction (F_N). (N≥100). Error bars are ±SEM.

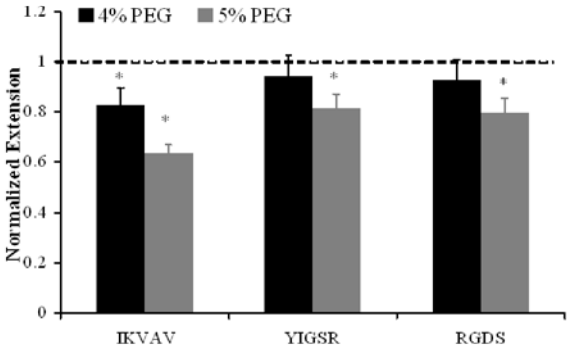

Figure 2. Average extension in 100 μg/ml PEG-LN gels with added peptide was normalized with the average extension of unsuplemented gels. Statistical significance from unsupplemented gels indicated via stars ($p<0.05$). N≥ 53.

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Reference: 1.Douville, P. J of Bio Chem. 1988. **263**(29): 14964-69. 2. Peyton, S. Biomaterials. 2006. **27**(28): 4881-93. 3. Balgude, A. Biomaterials. 2001. **22**(10): p. 1077-84. 4. Scott, R. J Biomat Res A. 2010. **93**(3): p. 817-823. (3): p. 817-823.