Characterization of Poly(Ethylene Glycol) Gels with Added Collagen for Neural Tissue Engineering

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Statement of Purpose: Disruption of the signaling in the nervous system can result in impaired muscle function, loss of sensation, and pain. Functional regeneration may be possible through tissue engineering, where a 3D scaffold would support and guide neuronal growth through various mechanical and chemical cues. Design of the scaffold requires investigation into how neurite growth is influenced by the chemical and/or mechanical environment. Varying the chemical nature of the scaffold can be accomplished using extracellular matrix (ECM) proteins, such as collagen, to influence cell attachment, proliferation, and expression [1]. While collagen supports nerve growth, scaffolds composed primarily of ECM can be broken down naturally by the body, altering their ability to support nerve growth. Alternatively, a synthetic polymer, such as poly(ethylene glycol) (PEG), could be used. Using PEG, the mechanical environment can be readily manipulated via the concentration of PEG [2], and molecules, such as ECM proteins or peptide sequences [3], can be conjugated into the gel to influence cell growth [4].

Methods: PEG-diacrylate (PEGDA) was synthesized using published methods [5] and reaction completion was analyzed via NMR. Calculated amounts of acryl-PEG-NHS (Nektar) and collagen I, were mixed overnight at 25°C and stored in -20°C. Irgacure 2959 was obtained from CIBA; F12K and serum were obtained from Sigma; nerve growth factor (NGF, 2.5s) was obtained from Millipore. To test the mechanical properties, gels were made with varying concentrations of PEG (3, 4, 5% w/w), PEG-collagen or PEG-NHS conjugate (0.1, 1, 10, or 100 µg/mL), Irgacure, and DI H_2O, crosslinked via UV light (3.2 mW/cm^2, 365 nm). The mechanical stiffness of each gel (G*) was measured via oscillatory shear rheometry, using a 25 mm parallel plate configuration, a frequency sweep of 1-100 rad/s, and constant strain of 5%. Cell studies were performed using PC12 cells on PEG gels with 0, 10, 100, and 500 µg/mL collagen or PEG-collagen conjugate. Percent neurite extension for PC12 was measured and analyzed as in [6]. ANOVA was utilized ($p<0.05$) for statistical significance.

Results: Examination of G*, at 10 rad/sec, demonstrated significant differences between 3, 4, and 5% PEG gels, with respective average stiffness of 69.23±4.27 Pa, 479.49±9.79 Pa, and 992.24±36.07 Pa. The addition of PEG-collagen conjugate decreased the stiffness compared to plain gels (Figure 1). This phenomenon was confirmed to be an effect of the conjugate, and not the protein itself, as G* of gels containing PEG-NHS conjugate was not significantly different than G* of gels with similar amounts of conjugated protein. Percent neurite expression of PC12 cells was analyzed on PEG gels with added collagen and PEG-collagen conjugate. Percent neurite expression increased as the concentration of PEGDA decreased and the concentration of collagen or PEG-collagen conjugate increased (Figure 1). The greatest percent expression was seen on 3% PEG gels with 100 µg/mL, where 81.4±4.6% of cells expressed neurites.

Conclusions: Mechanical evaluation of the PEG gels demonstrated that as the concentration of PEGDA increased, gel stiffness increased, which is consistent with previous tests on PEG gels in tensile testing [7]. The addition of protein conjugate to the PEG gels reduced the stiffness of the gel, even at the lowest concentration of added protein. A decrease is not unexpected, as the protein conjugate terminates the growing PEG chain, which, in turn, lowers stiffness. This study found that 3% PEG gels exhibited increased neurite expression at any concentration of conjugate over 5% PEG gels, further indicating that lower concentration gels better promote neurite expression. In addition, 3% PEG gels with 100 µg/mL PEG-collagen conjugate were found to have statistically increased expression compared to 3% PEG gels with added 100 µg/mL collagen, indicating a possible difference in how the collagen is presented to the cells. The use of low concentration PEG gels is promising for neural tissue engineering applications as the neurite expression on these gels was significantly increased over higher concentration PEG gels and approached that of tissue culture plastic [6]. This result encourages further exploration of these low concentration gels, either with or without added ECM components; our current work is focused on examining neurite growth from dissociated dorsal root ganglia.

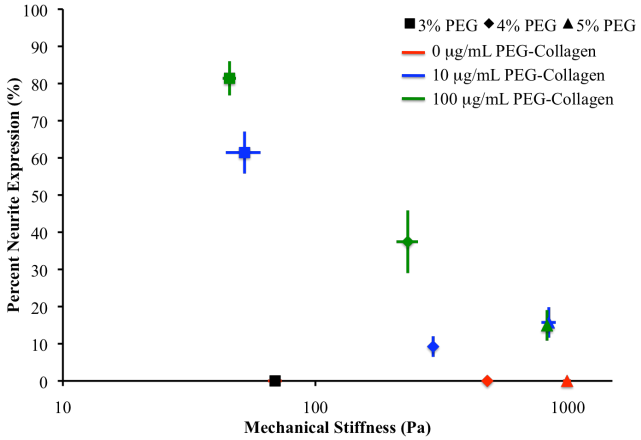

Figure 1. Mechanical stiffness of PEG-collagen conjugate gels as compared to the percent neurite expression of PC12 cells on the gels. Error bars represent standard error. N≥4 for all samples.

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