The Effect of Fibronectin and Collagen IV on Neurite Extension in 3D Collagen Gels

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Statement of Purpose: Collagen I provides a suitable three-dimensional scaffold for the survival and neurite expression of neurons *in vitro*[1,2]. Within such a scaffold, extension of neurites is dependent upon both the abundance of adhesion sites and the mechanical properties of the collagen[3]. Previous studies show that extension varies with the collagen gel concentration having optimal extension at 0.6 mg/ml[4]. Other extracellular matrix (ECM) molecules may, in conjunction with collagen I, effect neurite extension. This study focuses on fibronectin, a large glycoprotein involved in cell attachment[5], and collagen IV, the primary protein constituent of the basement membrane[6].

Methods: E9 chick dorsal root ganglia (DRG) (Sunrise Farms, Catskill, NY) were extracted, dissociated in 1X trypsin (Sigma, St. Louis), and reconstituted in unsupplemented 1X F12K media (Sigma, St. Louis) at $2.4x10^6$ cells/ml. Gels were prepared by adding 5% $NaHCO_3$ (Sigma, St. Louis) , H_2O, collagen, 0.1 M NaOH (Sigma, St. Louis), 10X F12K media (Sigma, St. Louis), 250 mM HEPES (Sigma, St. Louis), and cells[4]. Fibronectin (Sigma, St. Louis) or collagen IV was added prior to gelation at concentrations of 1, 10 or 100 µg/ml to test the effect on neurite extension. Solutions were allowed a 30 min gelation period at 37°C, 5% CO, and 100% humidity. Gels were supplemented with 1X F12K (Sigma, St. Louis) media containing 20% FBS (Cambrex, Walkersville, MD) and 50 ng/ml NGF (BD Biosciences, San Jose). After 24 hr incubation the gels were washed and fixed with 10% formalin (Sigma, St. Louis) for one hour at room temperature. Neurite extensions were measured using inverted light microscopy and analyzed based on average length per concentration of collagen gel. Statistical tests were administered using ANOVA with a level of significance of $p<0.05$.

Results / Discussion: Results show that average neurite extension varies with respect to the collagen concentration in the gels [Fig. 1]. The average extensions in gels varied in a biphasic manner with the collagen concentration. Previous work agrees that there is an optimal point of neurite outgrowth in gels at 0.6 mg/ml after a 24h growth period[4]. Preliminary results suggest that the concentration of fibronectin in the collagen gels does not affect the extension of neurites [Fig. 2]. Extension varied significantly at only one concentration of fibronectin for only one collagen gel concentration (0.001 mg/ml Fn in 1.0 mg/ml collagen gel).

Conclusions: Previously published results show that the extension of neurites within collagen gels varies in a biphasic manner with the concentration of collagen having an optimal growth concentration between 0.4 mg/ml and 0.8 mg/ml[4]. While preliminary results for gels containing fibronectin do not suggest an effect on neurite extension more data is needed to form accurate conclusions. Data has yet to be collected for gels containing collagen IV.

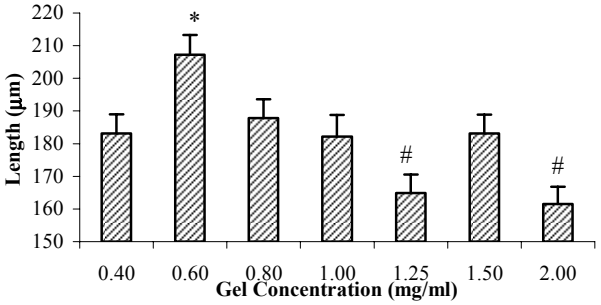

Fig. 1: Graph of neurite extension versus collagen gel concentration. Error bars represent the standard error of the mean (SEM) ($n\geq219$). 0.6 mg/ml gels neurites were significantly longer (*) than all other concentrations ($p\leq0.05$). The 1.25 mg/ml and 2.0 mg/ml gel neurites were significantly shorter (#) than all other concentrations ($p\leq0.05$).

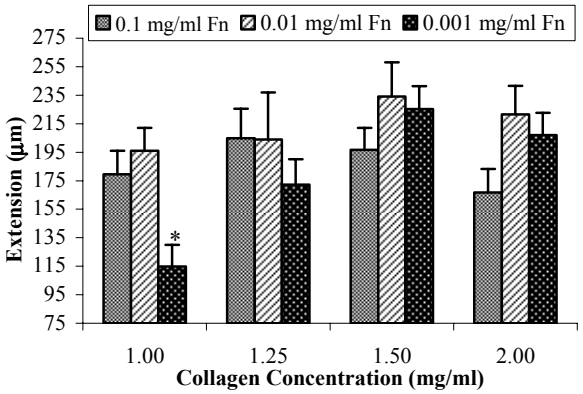

Fig. 2: Graph of neurite extension versus collagen gel concentration for three concentrations of fibronectin (Fn). Error bars represent SEM ($n\geq7$). Neurites in gels containing 0.001 Fn extended significantly less (*) than other 1.0 mg/ml gels ($p\leq0.05$).

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