

The effect of soluble peptide sequences on neurite extension in three-dimensional collagen gels

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Statement of Purpose: The extension of neurites within 3D collagen gels is based on both the mechanical strength of the gel and the concentration of adhesion sites. Neurite extension varies with concentration of collagen with optimal extension between 0.4 mg/ml and 0.8 mg/ml. Soluble peptide sequences can be used to inhibit neurite outgrowth. Three soluble peptide sequences were tested: glycine-arginine-glycine- aspartic acid-threonine-proline (GRGDTP), cyclo(RGD-D-Phe-Val), and aspartic acid-glycine-glutamic acid-arginine (DGEA). The peptides were added to the media atop collagen gels with E9 chick dorsal root ganglion (DRG) cells. DRGs grew and extended for 24h before the gels were fixed and imaged. Linear sequences showed little affinity for inhibiting neurite extension. The cyclo(RGD-D-Phe-Val) sequence was the best inhibitor of neurite extension within collagen gels and is probably more effective because it has a rigid conformation^{1,2}. The ability of cyclic forms of RGD to affect neurite outgrowth within three-dimensional collagen gels exhibits both the importance of peptide sequence components and conformation in neurite growth.

Methods: E9 chick dorsal root ganglia were extracted, dissociated in 1X trypsin, and reconstituted in unsupplemented 1X F12K media at 1.2×10^5 cells/ml. Gels were made by adding 5% NaHCO₃, H₂O, collagen, 0.1 M NaOH, 10X F12K media, 250 mM HEPES, and the 1X F12K media with the reconstituted cells³. After gelation 1X F12K media supplemented with 20% FBS, 50 ng/ml NGF and 1.0 mM of a soluble peptide (GRGDTP, cyclo(RGD-D-Phe-Val), DGEA, control GRGESP) was added atop the gels. After a 24 hr incubation the gels were washed with 1X PBS and fixed with 10% formalin for two hours 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 without peptide supplementation, as expected, varied in a biphasic manner with the collagen concentration. Previous work agrees that there is an optimal point of neurite outgrowth in gels around 0.6 -0.8 mg/ml after a 24h growth period³. The average extension of gels supplemented with cyclo(RGD-D-Phe-Val) was statistically different ($p < 0.05$) from the gels with no peptide added for all three concentrations [Fig. 2]. The other experimental peptides, GRGDTP and DGEA, and the RGES control did not show any significant differences in extension from gels with no peptide added.

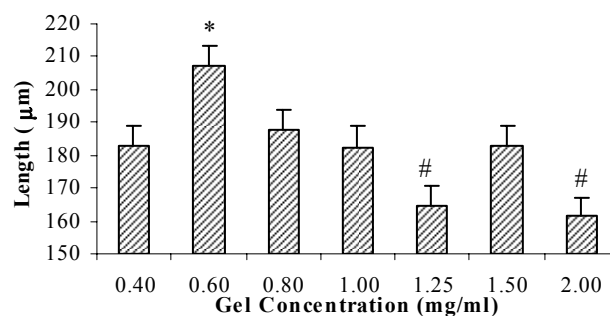


Fig. 1: Graph of neurite extension versus collagen gel concentration. Error bars represent the standard error of the mean (SEM) ($n \geq 219$). 0.6 mg/ml gels neurites were significantly longer (*) than all other concentrations ($p \leq 0.05$). The 1.25 mg/ml and 2.0 mg/ml gel neurites were significantly shorter (#) than all other concentrations ($p \leq 0.05$).

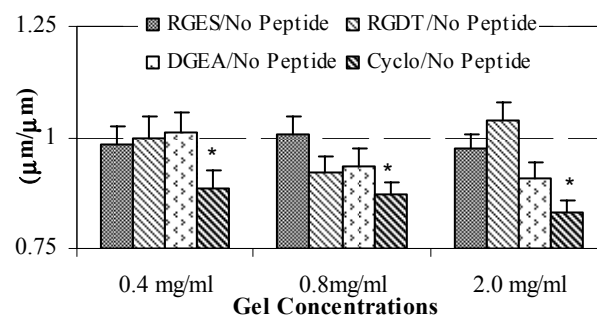


Fig. 2: Graph of neurite extension in peptide supplement versus no peptide. Error bars represent the SEM ($n=100$). The cyclic RGD neurites were statistically reduced (*) from the control no peptide in all concentrations ($p \leq 0.05$).

The current study focused on the ability of certain soluble peptide sequences to inhibit neurite binding in 3D collagen gels. The linear sequences of RGD were not able to inhibit neurite extensions from binding to the collagen I within the gels. The cyclic form of RGD can inhibit neurite binding because its rigid structure forces the sequence to mimic a natural RGD site^{1,2}.

Conclusions: In conclusion, the results obtained have agreed with the hypothesis of this study. 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. The cyclic form of RGD has shown the highest inhibitory affinity while the linear peptides have not been able to significantly inhibit binding.

References:

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