EFFECTS OF CONCENTRATION ON DRG NEURITE EXTENSION IN THREE DIMENSIONAL COLLAGEN GEL MATRICES

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INTRODUCTION

Traumatic injury to the central nervous system can result in a loss of the quality of life. Depending on the age of the victim, a lifetime of therapeutic measures can cost in excess of a million dollars. In the United States an estimated 10,000-12,000 injuries to the spinal cord occur yearly [1]. For injuries where the gap between the proximal and distal stumps of the spinal cord is too large to be repaired with tension and suturing, alternative methods of repair are being investigated.

Different three-dimensional models have previously been assessed for their ability to reinforce recovery. Agarose gels, synthetic polymers, and extracellular matrix (ECM) protein gels are a few of the commonly used materials [2-4]. Collagen, the most commonly occurring extracellular matrix molecule, is found both in the adult and developing mammal and has been determined to be a suitable model for neurite outgrowth [4]. While collagen has been frequently used as a model matrix, a range of concentrations has been used with varying results. This study was initiated to determine how concentration and mechanical properties of collagen gels affect the length of neurite extension.

MATERIALS AND METHODS

In this experiment, dorsal root ganglia (DRGs) were excised from day 9 SPF chicken eggs using standard procedure and placed into Hank's balanced salt solution. The DRGs were centrifuged and resuspended in 1% trypsin for 30 min. to separate the DRG clusters and isolate the cells [5]. Once the cells were trypsinized, they were stained with Vybrant DiI (Molecular Probes) for 30 min. in order to more readily image once suspended in gels. Collagen gels were formed by mixing, in order: $NaHCO_3$, sterile filtered water, type I collagen isolated from rat tail tendons, NaOH, 10X F12K (Sigma), Hepes, and 30 µL of cells in 1X F12K unsupplemented media at a concentration of $1X10^6$ cells/mL. The total volume for each gel was 250 µL and placed into a 48 well plate. The plates were incubated at 37°C in order for the collagen solution to gel. A range of concentrations of collagen (0.4, 0.6, 0.8, 1.0, 1.25, 1.5, and 2.0 mg/mL) was used. The gels were covered with 500 µL of F12K supplemented with 20% fetal bovine serum (Invitrogen) and 50 ng/mL of nerve growth factor (Chemicon Int.). The cultures were grown in 5% CO_2 at 37° C and the media was changed every 2 to 3 days. Cells were fixed at days 1 and 4 using 10% formalin (Fisher). Gels were then imaged using a Zeiss inverted microscope and Axiovision software. Ungrouped neurites in the gels were imaged, and measurements of neurite lengths were obtained in two dimensions. The average values were evaluated using ANOVA to determine statistical differences between concentrations.

Gel response to dynamic strain frequency sweep was obtained using a RFSIII rheometer (Rheometric Scientific). A 375 µL collagen solution was made and loaded onto the plate. The cone, with an approximate angle of 0.04 radians, was lowered onto the solution leaving a gap distance of 0.051 mm and the solution was allowed to gel. After approximately 30 min., the gel was subjected to shear. Both storage modulus (G') and loss modulus (G'') were measured as a function of angular displacement from 0.1 rad/s to 100 rad/s. The diameter for the cone and plate was 25 mm for all gels except 0.4 mg/mL. The 0.4 mg/mL required a 50 mm cone diameter. Four or more tests were run for each concentration.

RESULTS AND DISCUSSION

The number of samples, average values and standard error of the mean of neurite length for each gel from days 1 and 4 are presented in Table 1. Each set of gels had at least 94 samples. Results from ANOVA demonstrated statistically lower average lengths between 0.6 mg/mL and 0.4 mg/mL gels at day 1 ($p<0.05$), between 0.6 mg/mL and 0.8 mg/mL at day 4 ($p<0.02$), and between 1.5 mg/mL and 2.0 mg/mL gels for both days 1 and 4 ($p<0.005$). These results, with the maximal outgrowth in 0.6 mg/ml at day 4, are slightly different than those for neurite lengths of differentiated PC12 cells versus collagen concentration, where 0.4 mg/mL gels had the longest neurites [4]. However, both results demonstrate that concentrations lower than those typically used for these applications may be more appropriate for encouraging longer neurites.

The complex shear modulus (G*, dyn/cm^2) for each collagen gel concentration was 8.8±1.1, 78.0±10.8, 114.2±18.9, 162.3±66.2, 254.1±41.1, 327.1±89.3, 395.0±55.1 for each increasing concentration respectively (Figure 2). The shear modulus for gels increased with concentration, indicating a mechanical strengthening of the gel. More tests will be done to determine statistical differences in shear moduli between gel concentrations. Future tests also include determining G* for all gel concentrations using the 50mm diameter cone and plate. These tests will help to conclude if results obtained from 25mm and 50mm geometries are the same.

Ultimately, mechanical strength and neurite length will be correlated to aid in the development of a neural tissue engineered scaffold.

Conc. (mg/mL)	# samples	Average± SEM (µm)	Conc. (mg/mL)	# samples	Average± SEM (µm)
1 day			**4 day**		
0.4	113	168.4106±7.016	**0.4**	100	217.0139±12.64
0.6	94	188.9345±7.468	**0.6**	139	240.9143±14.53
0.8	122	193.8741±7.903	**0.8**	151	199.6928±10.20
1	121	184.6995±8.339	**1**	172	207.6803±8.744
1.25	119	169.9615±7.703	**1.25**	151	198.3417±9.814
1.5	120	181.1251±6.639	**1.5**	177	186.1968±9.767
2	120	149.7165±5.694	**2**	134	136.2701±7.816

Table 1. Results for measured lengths of neurite extensions obtained from Zeiss microscopes combined with Axiovision software.

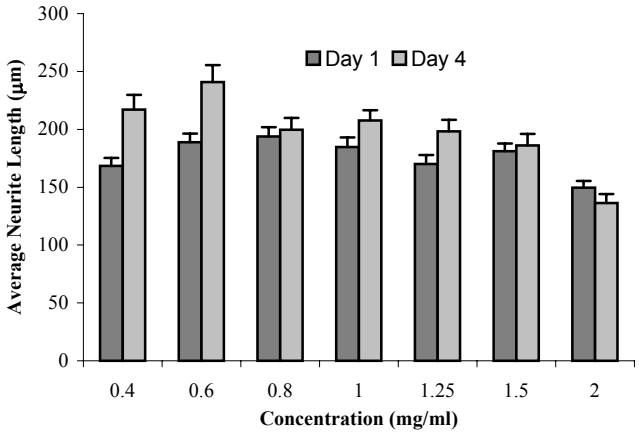

Figure 1. Average values for neurite lengths for collagen gels from 0.4mg/mL to 2.0mg/mL with the standard error of the mean for day 1 and day 4.

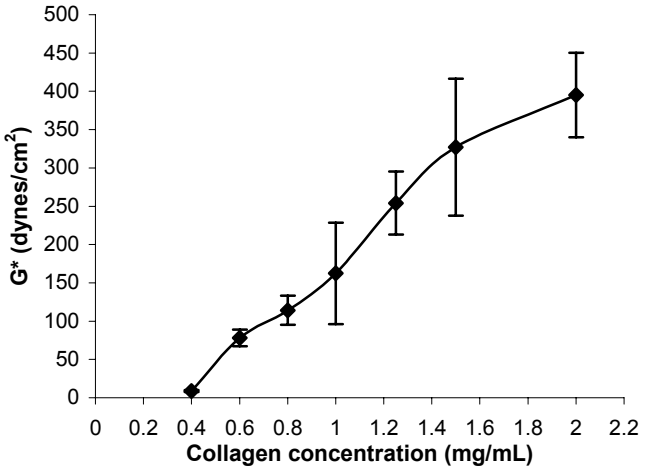

Figure 2. Complex shear modulus of varying concentrations of collagen gels determined using rheology. Error bars are standard deviation from the mean where n≥4.

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