Addition of Chitosan to Mechanically Adjustable Thermoresponsive Hydrogel Blends to Enhance Neural Adhesion and Neurite Extension

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1. Statement of Purpose: Thermoresponsive hydrogels are currently being investigated for applications in regenerative medicine. These hydrogels are fluidic at room temperature, but when heated to temperatures near body temperature, gelation occurs. Previously an injectable, thermoresponsive hydrogel blend was created to allow for the regeneration of the nervous system. This first generation blend, composed of methylcellulose (MC) (Dow Midland, MI) and agarose (Lonza Rockland, ME) [1], had adequate gelation properties (gelation around 10 minutes at 37°C), but was relatively stiff and did not allow for acceptable neural adhesion and neurite extension. To reduce the hydrogel stiffness, the concentration of MC was lowered, and dextran (Dex) (Sigma St. Louis, MO) was added in its place. Further, addition of chitosan (Ch) (Sigma St. Louis, MO) may help elicit neuronal attachment and neurite extension, since a previous report showed that chitosan incorporation into an agarose gel promoted cortical cell neurite extension [2]. By including different polysaccharides into a hydrogel, desirable mechanical and surface properties can be incorporated to encourage neuronal infiltration following nervous system injury.

2. Methods: 2.1. ***Creation of Hydrogel Blends*** A Ch solution was heated, and then agarose was added and allowed to dissolve under stirring. MC was added next and stirred until wetted. A chilled Dex solution was added, and this was stirred on ice. The hydrogel blend was then centrifuged, and placed in a 4°C refrigerator until needed.

2.2. ***Gelation Time Test*** The gelation time of the different blends was determined using the inverted test tube test at 37 ºC [3].

2.3. ***Rheology*** All rheological data was obtained at 37 ˚C using an Anton Paar Physica MCR 301 rheometer.

2.4. ***Neuronal Response Study*** Dorsal root ganglia (DRG) explants were obtained from 9 day old (E9) chicken embryos and dissociated. Isolated neurons were then placed on top of the hydrogel blends. They were allowed to incubate for 4 days, and then imaged using Calcein AM.

2.5. ***Statistics*** Statistical analysis was performed using JMP 8 (SAS). A one-way ANOVA test and a Tukey-Kramer HSD pairs test ($p < 0.05$) were performed to determine significance.

3. Results: 3.1. ***Elastic Modulus and Gelation Time of Hydrogel Blends*** Replacing the MC in the hydrogel blends with Dex significantly increased gelation time (Figure 1A), but also lowered the elastic moduli of the hydrogel blends (Figure 1B)

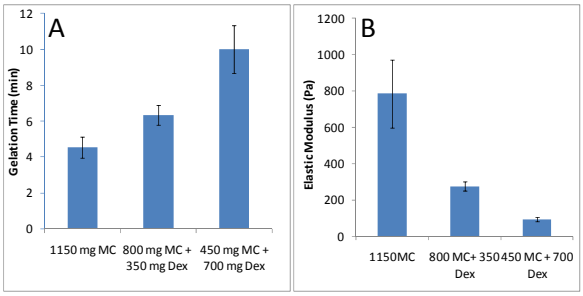

Figure 1: A) Average gelation times of different hydrogel blends **B)** Average elastic moduli of different hydrogel blends (All groups are significantly different in both A and B).

3.2. ***DRG Cell Adhesion/Neurite Extension*** Results of the DRG cell culture studies show that blends with the highest content of MC supported less DRG cellular adhesion (Figure 2A) than hydrogels (Figure 2B) with more Dex and a subsequently lower elastic modulus. Blends with Ch (Figure 2C) were more conducive to neuronal adhesion and neurite extension than the blends with higher elastic moduli or no Ch.

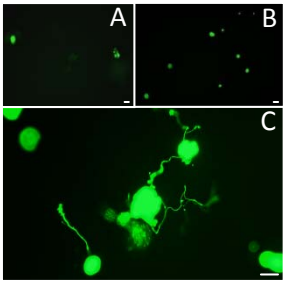

Figure 2: DRG neurons cultured on hydrogels containing: **A)** 800 mg MC 350 mg Dex. **B)** 450 mg MC 700 mg Dex. **C)** 450 mg MC 700 mg Dex 75 mg Ch.

4. Conclusions: Decreasing the elastic modulus of our hydrogel blends allowed for an increase in neuron adhesion. This shows that the response of the DRGs to a three-dimensional scaffold is dependent on the mechanical properties of the scaffold. Lowering the amount of MC in the hydrogel blends and replacing it with Dex allowed for a decrease in the elastic modulus of the hydrogel. However, the reduction of MC also increased the gelation time of our blends. Also, the addition of Ch allowed for an increase in neurite extension. The fact that hydrogel blends without Ch had little or no neurite extension shows that Ch induces neurite outgrowth. In future studies, we will be using rheology to completely characterize the different hydrogel blends. The degradation properties of these blends will be determined both *in vitro* and *in vivo*. Also, cortical neurons will be cultured with our gels to determine their response to the different blends. These exciting results show the promise of thermoresponsive hydrogels for the purpose of regenerating the injured nervous system.

5. References:

1. Martin BC. J Neural Eng. 2008; 2; 221-31
2. Cao Z. Biomacromolecules. 2009; 10; 2954-9
3. Gupta D. Biomaterials. 2006; 27; 2370-9