Test Methodology of Characterizing the Behavior of Injectable Hydrogels: An In Vitro Model

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Statement of Purpose: The development of synthetic models for the prediction of swelling and mechanical behavior is crucial for the development of new biomaterials due to an inability to test every candidate material in an in vitro model. Commonly phosphate-buffered saline (PBS) is used as a model swelling solution for candidate nucleus replacement materials in the intervertebral disc (IVD); this however does not take into account the gradient through the annulus fibrosus (AF). The aim of this study was to evaluate the swelling behavior and compressive moduli of polyvinyl alcohol/polyvinyl pyrrolidone/polyethylene glycol hydrogel system in synthetic osmotic and in vitro model systems.

Methods: Polyvinyl alcohol (PVA) was chemically stabilized with polyvinyl pyrrolidone (PVP) to form a solution in deionized water. Barium sulfate was added to the PVA/PVP solution as a slurry. Polyethylene glycol (PEG) was stirred into the solution to form a gel. Heat Distribution: Five human spinal segments were dissected and posterior elements removed. The nucleus was cored and removed by drilling through superior vertebral body and halfway through the disc. The nuclear material and bone plug were removed. The anterior column units (ACU) were placed in 1x PBS with protease inhibitor (PI) and placed in refrigerator overnight. Thermocouples were placed around the circumference of the annulus in three locations: posterior, anterior and lateral. Each region had three thermocouples placed at three different locations along the thickness of the annulus. The bone plug was replaced and the ACU was submerged in a 37°C heated water bath. The hydrogel was heated to 90°C and injected into the nuclear cavity. Time versus temperature graphs are compiled from thermocouple output data for annular wall and hydrogel thermocouples.

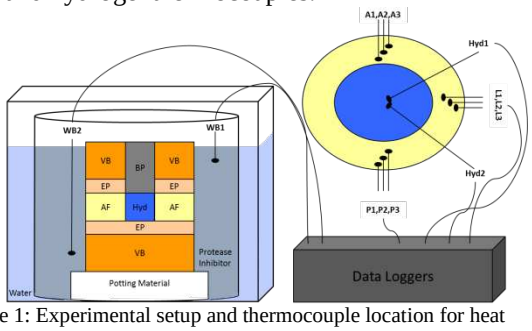

Figure 1: Experimental setup and thermocouple location for heat distribution study.

Mechanical Properties: Each ACU is placed in protease inhibitor with a 5kg weight on top of superior vertebral body and kept at 5°C overnight (12-18 hours). Upon removal, the ACU is kept in PI with weight for 1 hour and allowed to reach room temperature. The cored ACU is fixed into a mechanical tester and surrounded by a water bath with a small access port. A special plunger was fabricated to have a line-to-line fit with the cylindrical hole in ACU. The plunger is lowered down so that the bottom is flush with the top of the IVD. A 5kg weight is placed on top of the superior vertebrae around the Instron plunger. An LVDT is placed so that it is against the posterior section of the annulus and attached via the water bath wall. Through the access port in the water bath, a small needle is used to puncture a hole in the annulus through to the cavity. Hydrogel (volume calculation based on disc height and diameter of core) is injected into the nuclear cavity. The access port closed and protease inhibitor is allowed to fill the water bath. The mechanical testing protocol is then allowed to begin.

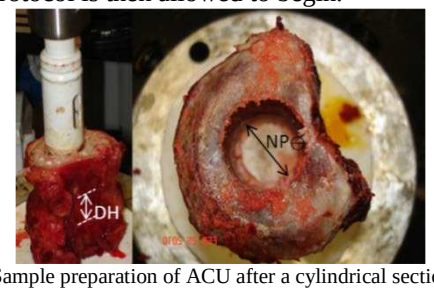

Figure 2: Sample preparation of ACU after a cylindrical section of nucleus and the superior vertebral body is removed.

The test protocol consisted of 30 minute sequences of a quasistatic test at the beginning and end, with 29 minutes of swelling data in between. This sequence can be repeated for X-number of times, until equilibrium is reached. The effective modulus over time was investigated to determine when the gel is fully setup in vivo and the equilibrium effective confined modulus. The 30 minutes of holding by the plunger allows the axial swelling to be determined from the change in position of the plunger. This data can be filtered and the equilibrium axial swelling determined and also swelling over time. Similarly, the output data from the LVDT gave radial swelling of the hydrogel in vivo.

Results:

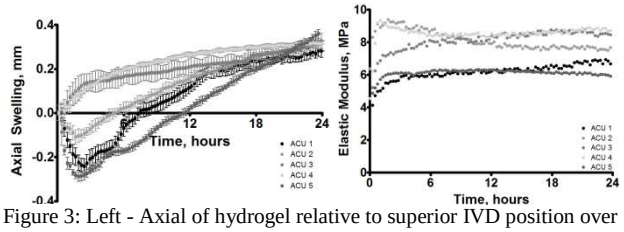

Figure 3: Left - Axial of hydrogel relative to superior IVD position over 24 hours. Average and standard deviation of three time points within each 15 minute hold were plotted for each ACU. Right - Elastic modulus from 5th quasi loading cycle of hydrogel over 24 hours. Note time zero is the beginning of the mechanical test, but it is actually *t*=5.5minutes since hydrogel injection.

Results shown are representative curves for specific hydrogel tested.

Conclusions: A new method was designed that evaluates the swelling behavior and modulus of a hydrogel system in *in vitro* model systems.