Effects of Long-Term Nutrient Deprivation on the Activity and Viability of Notochordal Nucleus Pulposus Cells of the Intervertebral Disc

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Disclosures:

Introduction: The intervertebral disc (IVD) is the largest avascular structure in the body. Due to this, nutrient transport becomes difficult, especially to the center of the disc containing the Nucleus Pulposus (NP) (Jackson 2012). The cells of the NP are derived from the notochord and maintain a notochordal phenotype until the early teenage years when they start becoming chondrocytic and the first signs of degeneration occur (Urban 2003). Analyzing how NP cells behave under various glucose conditions could elucidate what level of nutrients is critical in sustaining a healthy population, as well as provide a better understanding of the disc degenerative process at the earliest stages. Glucose is under investigation due to the fact that it is the more critical nutrient in cell viability, as IVD cells can function without oxygen but start to die off immediately in glucose lacking environments (Grunhagen 2006). In this study, we investigated the glucose consumption rate of notochordal NP cells cultured in low (1mM) and normal (5mM) glucose levels for 1, 5, and 10 days, in order to determine how nutrient availability (or deprivation) affects the activity and viability of these cells.

Methods: Pigs (n=3) in the weight range of 190-250lbs and ranging between 4-7months of age were obtained from a local slaughterhouse within an hour of death. After the spine was isolated, the discs were cut open and the NP tissue was harvested. The notochordal NP cells were then isolated by enzyme digestion in a solution containing Dulbecco’s Modified Eagles Media (DMEM)(Gibco), protease(Sigma), and collagenase II (Sigma) for 16 hours, after which the remaining tissue was agitated and the solution was filtered through a 70µm sieve to remove large tissue debris. Cell-agarose constructs were prepared by mixing a solution containing 2x10^7cells/mL DMEM with 4% agarose gel in a 1:1 mixture and aliquoted 100µL at a time into 8mm diameter molds. Final constructs had 1x10^6cells/gel in 2% agarose gel. The gels were then placed into 6-well plates and 5mL of either 1mM or 5mM glucose DMEM solutions were added and stored in an incubator with 5% CO2 and atmospheric (21%) oxygen. The gels were cultured at these conditions for 1, 5, or 10 days, with the media being changed daily. For the consumption rate experiment the gels were quartered and placed into their own well of a 96 well plate alone with a stir bar and 200µL of each gels respective culture media; experiments were carried out on a magnetic stir plate inside an incubator. 1µL of media was collected ever two hours and the glucose concentration was measured using a custom modified blood glucose meter (Accu-Chek Aviva, Roche Diagnostics, Inc) and sourcemeter (Model 2401, Keithley, Inc.). These data were then used to determine the Vmax and Km of the cells by curve-fitting an analytical solution of the Michaelis-Menten equation using Matlab. At the end of consumption rate experiments, cell viability was assessed using a LIVE/DEAD® Viability / Cytotoxicity Assay Kit (Invitrogen) and fluorescent imaging with an inverted confocal microscope.

Results: The results for Vmax and Km are shown in Table 1 and Figures 1 and 2; for all groups, n=5. At days 5 and 10, the value of Vmax for the 5mM group was significantly higher than that of the 1mM group (p<0.05). For the 5mM group, there was no difference in Vmax from day 1 to day 5, while the value at day 10 was significantly lower than at day 1 (p<0.05). For the 1mM group, there was a decrease with increasing culture period for Vmax; the value was significantly higher at day 1 as compared to days 5 of 10 (p<0.05). The values for Km for both 1mM and 5mM groups showed no significant trend throughout the duration of the experiment. Cell viability images (Figure 3) showed a decrease in the cell viability during culture for the 1mM group, while the 5mM group maintained the same viability throughout the experiment.

Discussion: The results found here for Vmax ~ are similar to those found in the literature for NP cells, which ranged from 20 to 120 nmol/million cells/hr for bovine NP cells (Bibby 2002), to 205 nmol/million cells/hr for notochordal NP cells (Guehring 2009). The drop in consumption rate by cells over the culture period could be the result of changes in cellular activity due to the difference in culture conditions. Additionally, the decrease in the number of viable cells over the course of cell culture would also cause this decrease; it is not apparent which factor was more significant in these studies. For days 5 and 10, the maximum reaction rate was lower for the low glucose group as compared to the high glucose group. This is expected since there is a reduced nutritional supply to the cells for the lower glucose group; furthermore, cells may have altered their activity in order to accommodate this reduced nutrition. One important finding, although only qualitative, was the much lower viability of the 1mM group when compared to the 5mM group. Though needing further analysis, the viability of the 5mM group seemed to stay consistent over the culture period. This should not affect the K-m values but should have a significant impact on the Vmax of the experiments. This is consistent with the literature indicating that glucose is most important in maintaining NP viability (Grunhagen 2006). Glucose concentrations of an intermediate range will need to be tested to see if viability over time can be improved, for the low glucose group, to determine the minimal sustainable glucose concentration to maintain a high viability.
Moreover, future experiments will vary the oxygen tension to see how differing oxygen will affect glucose consumption.

Significance: Since the disappearance of notochordal cells from the nucleus corresponds to the earliest degenerative changes in the disc, the findings of this investigation will provide important insight into the pathophysiology of disc degeneration, as well as aid in the development of new strategies for the treatment of disc degeneration and related low back pain.

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References:

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Figure 2: Result for $K_m$ for two groups during the culture period; n=5 for each group.
Figure 3: Live/Dead images of the agarose gels over the culture period. Images a, b, and c are 1mM days 1, 5, and 10, respectively. Images d, e, and f are 5mM days 1, 5, and 10, respectively. The images show the higher viability of the 5mM gels by the 10th day of culture.
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<tr>
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<th>Vmax (nmol/million cells/hr)</th>
<th>Km (mM)</th>
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<tbody>
<tr>
<td></td>
<td>5 mM</td>
<td>1 mM</td>
</tr>
<tr>
<td>Day 1</td>
<td>75.4 ±5.40</td>
<td>105 ±66.1</td>
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<tr>
<td>Day 5</td>
<td>79.7 ±42.7</td>
<td>24.5 ±12.3</td>
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<tr>
<td>Day 10</td>
<td>37.0 ±14.2</td>
<td>16.3 ±8.51</td>
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Table 1: The V_{max} and K_{m} for each group during the culture period; n=5 for each group.

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