THE APPARENT PERMEABILITY OF THE NUCLEUS PULPOSUS IS INCREASED BY IONIC OSMOTIC EFFECTS

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SUMMARY
Experimental data on bovine nucleus pulposi has been acquired that show that the apparent permeability of the tissue’s matrix depends on the ionic concentration of the permeating fluid. These results provide evidence for an ionic concentration gradient mechanism that increases the fluid flow within the tissue, thereby increasing the apparent permeability. Our analysis suggests that 50% of the fluid flow in the tissue may be as a result of the ionic concentration gradients.

INTRODUCTION
The intervertebral disc (IVD) is the largest avascular structure in the body and relies on fluid flow to and from the disc for nutrient supply [1]. Although diffusion is the primary mechanism for the transport of small molecules such as oxygen and glucose, convection may enhance the movement of larger metabolites [10] with the tissue’s hydraulic permeability playing an important factor in convective transport [9]. To measure tissue permeability directly, a pressure differential technique together with Darcy’s law can be applied [3]. When a fluid pressure gradient is applied to a sample in confined compression commonly utilised models, such as the linear biphasic theory [7] or the nonlinear finite deformation biphasic theory [4] predict that, at equilibrium, under a constant fluid velocity, there is a variation in strain through the tissue. A strain distribution will result in a permeability gradient through the tissue. Thus, direct permeation tests determine the apparent permeability of the sample, not a localised permeability value. Furthermore, assuming an equal distribution of negatively charged proteoglycans (PG) in the sample, a strain gradient will also result in a PG concentration gradient [5] which, in turn, causes an ionic osmotic pressure gradient affecting fluid flow. Such effects could be included in electromechanical tri/quadruphasic theory [5,6] but the relative magnitude of these effects should be demonstrated before the extra complexity of such models is routinely demanded.

The aim of this study is to determine the apparent permeability of the nucleus pulposus whilst changing the salinity of the permeating fluid. Changes in permeability would indicate the relative importance of ionic osmotic gradients in driving fluid flow.

METHODS
Intervertebral discs were harvested from bovine tails (aged 14-30 months). A total of 16, ø10mm, 1020 ± 122 µm (mean ± S.D) thick plugs of NP tissue, orientated in the axial direction, were harvested with a cork borer prior to placement in a direct permeation rig, used previously [3]. Aqueous NaCl solution was permeated through each sample at zero applied mechanical strain. The flow rate was adjusted to maintain a pressure gradient of either: 30, 45 or 60 kPa in a random sequence. Eight samples were infused with 0.15M NaCl (isotonic) and eight with 3M NaCl (hypertonic, negates the tissues ionic osmotic swelling pressure).

For each sample, a simple Darcy’s law was used to determine the apparent permeability, k, of the sample at equilibrium, defined by constant fluid velocity through, and constant fluid pressure difference across, the sample:

\[ k = \frac{\mu v}{\Delta P} \]  

(1)

where \( v \) is the fluid velocity through the sample, \( \Delta P \) the applied fluid pressure gradient and \( h \) the thickness of the sample. The viscosity of the permeating solution, \( \mu \), is given by \( \mu_{0.15M} = 0.001015 \text{ Pa.s} \) and \( \mu_{3M} = 0.001375 \text{ Pa.s} \) at 20°C [2]. The permeability in the hypertonic solution may be deemed representative of the intrinsic apparent permeability of the tissue, since ionic osmotic and concentration effects are minimised in such external solutions [8]. An effective pressure difference can then be defined as:

\[ \Delta P_{eff} = \frac{\mu_{0.15M} v_{0.15M} h_{0.15M}}{k_{3M}} \]  

(2)

The effective pressure includes an ionic osmotic effect and, therefore:

\[ \Delta \pi = \Delta P - \Delta P_{eff} \]  

(3)

Velocity and apparent permeability (equation 1) were each analysed using repeated measures ANOVA, with the applied fluid pressure being a within-sample factor and external salt concentration being a between-sample factor. Where significant differences with the applied fluid pressure were found, subsequent t-tests with bonferroni adjustment were conducted to identify differences between specific pressures. Significant differences were assumed when \( p \leq 0.05 \).
RESULTS AND DISCUSSION

A significant increase in fluid velocity and decrease in $k$ was observed with increasing fluid pressure gradient (Figure 1) ($p \leq 0.001$). There was also a significant effect of permeating solution on fluid velocity ($p < 0.001$) and $k$ ($p = 0.017$). Tukey HSD post hoc tests revealed that a significant reduction in $k$ was observed when 3M NaCl was permeated compared to 0.15M NaCl ($p = 0.014$). The results of this experiment suggest that if a fluid pressure gradient exists across a tissue then, in the physiologic condition, there exists an ionic osmotic pressure gradient that is approximately 50% of the fluid pressure gradient (Table 1). This ionic osmotic pressure gradient acts to increase the fluid velocity within the tissue. Assuming an initial homogenous distribution of PG in the tissue, this suggests significant strain gradients across the sample as a result of the applied fluid pressure. Quadriphasic theory [5] predicts that the ionic effects arise due to a concentration gradient of the fixed charges and a concomitant gradient of mobile ions. Thus this theory predicts an increased fluid flow associated with this mechanism, in keeping with our results.

CONCLUSIONS
A relationship between tissue permeability and ionic osmotic swelling pressure has been established, and has demonstrated a significant augmenting mechanism which aids fluid flow through the disc by approximately 50%. Existing theory suggests that this mechanism is associated with the ionic gradients of the fixed charges and mobile ions in the tissue which occur due to the strain distribution associated with fluid flow. These results highlight the importance of using a constitutive equation for permeability that includes ionic effects when modelling cartilaginous tissue.

REFERENCES

Table 1: The effective fluid pressure difference and osmotic pressure difference across the sample as a function of the applied fluid pressure (mean ± SD).

<table>
<thead>
<tr>
<th>$\Delta P$ (kPa)</th>
<th>$\Delta P_{df}$ (kPa)</th>
<th>$\Delta \pi$ (kPa)</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>46.6 ± 9.6</td>
<td>-16.6 ± 9.6</td>
<td>55</td>
</tr>
<tr>
<td>45</td>
<td>68.6 ± 24.4</td>
<td>-23.6 ± 15.7</td>
<td>52</td>
</tr>
<tr>
<td>60</td>
<td>92.4 ± 22.8</td>
<td>-32.4 ± 22.8</td>
<td>54</td>
</tr>
</tbody>
</table>