**Introduction:** The intervertebral disc (IVD) is one of the body's most important load-bearing structures, and daily activities expose the IVD to dynamic oscillatory loads which have a vast spectrum of frequencies and amplitudes. These forces arise from a combination of gravity and muscle tension during movement and result in various types of pressure being experienced within the IVD. Abdominal and back muscles stabilize the spine and also resist gravitational forces to allow spinal movement, but by doing this they exert additional compressive forces on the lumbar discs. These combined forces result in a wide variety of mechanical stimuli including compression, shear, torsion and flexion of the spine, electro-kinetic effects and volumetric changes. The biological response to these stimuli varies according to the region of the IVD, type of loading experienced and the duration. In the non-degenerate IVD the nucleus pulposus (NP) behaves like a fluid and thus experiences hydrostatic pressure whereas the Annulus fibrosus (AF) experiences radia omni-directional forces resulting in tensile stress.

The effects of hydrostatic pressure have been thoroughly investigated in articular chondrocytes but to a lesser extent in the IVD. Hydrostatic pressure is predominantly experienced in the fluid-like NP region of the IVD and it has been hypothesized that different magnitudes of pressure will alter the NP cells metabolic response. Studies to date have involved investigations with a number of cell sources including non-degenerate animal NP cells and degenerate NP cells from herniated samples, and have used a number of loading regimes and culture conditions. Results of such studies suggest that physiological levels of hydrostatic pressure have an anabolic effect on IVD matrix metabolism whereas high pressure has a catabolic effect. However to date, no studies have investigated the response to hydrostatic loading of non-degenerate human disc cells and degenerate human disc cells from non-herniated discs. Here we investigate the response of NP and AF cells derived from non-degenerate and degenerate discs to physiological hydrostatic loading using our novel loading rig.

**Materials and Methods:** Human normal cadaveric IVD tissue (n=4) was obtained with informed consent from relatives and local ethical committee approval and degenerate human IVD tissue (n=6) were obtained from patients undergoing spinal surgery with informed consent from the patients and local ethical committee approval. Tissue was separated into nucleus pulposus (NP) and annulus fibrosus (AF), finely minced and cells extracted using collagenase. Following expansion in monolayer culture cells at low passage (<2) were resuspended in 1.2% medium viscosity alginate in 0.15M NaCl and 300μl constructs formed in a 24 well plate by polymerization with 200mM CaCl2. Following 48hrs of culture in the alginate construct samples were transferred to bags and complete HEPES media added, air was removed and bags sealed. Control (unloaded samples) were placed in a 37°C water bath and test (loading) samples placed into the water filled hydrostatic loading chamber. Dynamic loading was then applied at 0.8 – 1.7MPa 0.5Hz for 2hrs using the loading rig described previously. Following loading samples were returned to a 37°C incubator for 1hr and then harvested. Cell viability was assessed using Carboxyfluoresein Diacetate Succinimidyl Ester and Propidium iodide staining. RNA was also extracted from triplicate alginate constructs per patient sample, reverse transcribed to cDNA and real time PCR performed for the house keeping gene (18s), the early response gene (c-fos), matrix genes (Sox-9, Collagen type II and Aggrecan) and the matrix degrading enzyme (MMP 3).

**Results:** All alginate constructs maintained cell viability at >80% with no difference observed between loaded and unloaded controls. In NP cells derived from non-degenerate discs, hydrostatic loading significantly increased c-fos and Aggrecan gene expression (P<0.05), and to a lesser extent Sox-9 and Collagen type II (P<0.1) (Fig 1). No significant increase was observed for MMP 3. In contrast application of hydrostatic load to NP cells derived from degenerate discs had no effect on the gene expression of any target gene (Fig 1). Hydrostatic loading on AF cells from either non-degenerate or degenerate discs had no effect on gene expression for any of the genes investigated (Fig 2).

**Discussion:** This is the first study to investigate the effect of hydrostatic pressure to NP and AF cells obtained from non-degenerate and degenerate human IVDs, demonstrating clear differences in the response to hydrostatic loading between disease states. This study has highlighted that AF cells derived from either non-degenerate or degenerate discs do not in our system respond to hydrostatic loading. In vivo the cells of the AF are subjected to tensile strain rather than hydrostatic loading and our study suggests that these cells are unable to respond to the loading environment observed in the NP in vivo. Interestingly this study highlighted a clear difference in the response of NP cells derived from non-degenerate discs to those derived from degenerate discs: NP cells from non-degenerate discs were the only cell type to generate significant changes in gene expression following hydrostatic loading suggesting the response to loading in degenerate discs is altered, a feature also reported in diseased articular cartilage.

**References:**

**Acknowledgements:** The authors wish to the AO Foundation and Backcare for funding this project.

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**Figure 1:** Relative gene expression in NP cells loaded at 0.8-1.7MPa at 0.5Hz for 2hrs (**P<0.05, *P<0.1**).

**Figure 2:** Relative gene expression in AF cells loaded at 0.8-1.7MPa at 0.5Hz for 2hrs (**P<0.05**).