Background: Low back pain is a common problem in the United States and throughout the world with a lifetime prevalence estimated at 80-90% of the population. Disc degeneration has been implicated as a major source of low back and radicular pain. However, understanding of the factors that may initiate and perpetuate disc degeneration is limited. Epidemiological studies have shown that excessive loading, particularly vibration in the 4-6 Hz range, can stimulate the degenerative process. It is thought that mechanical loading can cause disc changes through direct cellular effects on tissues around the spine. However, the mechanism of how cells sense and respond to mechanical strain is unknown. Adenosine 5’-triphosphate (ATP) is a ubiquitous intracellular source of energy. In addition, ATP release may serve as an extracellular paracrine and/or autocrine signal via cell surface purinoceptors (1). ATP activation acts to potentiate annulus cell response to mechanical stimulation and may serve as an intracellular signaling messenger to themselves and adjacent cells. Exogenous ATP can induce phosphorylation of protein such as ion channels that may regulate the cell’s response to vibratory load.

Methods: Human annulus tissues were obtained from material discarded at surgery. Adult New Zealand white rabbits were used for collection of annulus tissue. The tissue was dissected free of nucleus pulposus and adherent connective tissue, and annulus cells were isolated by collagenase digestion. Cells were grown in Medium 199 containing 10% fetal bovine serum with 20 mM HEPES pH 7.2 and antibiotics. At passages 3-6, cells were seeded at 25k cells/cm²/well of 12-well plastic plates in complete medium and grown to quiescence by halving the medium with serum-free medium on days 3 and 5. On day 6, the medium was changed to M199 without serum and phenol red. A special vibratory jig was used to apply loads to the cells in culture. The vibration loading device utilized an eccentric cam drive by a DC motor to produce the sinusoidal motion of the follower/actuating rod/culture plate assembly (3). The cam rotated against a bearing interfaced to a piston delivering vibratory motion to the table where the culture plate was fixed. Cells were rested in the system for 4 hours and stimulated with vibration at 6 Hz. hAN cells were vibrated for 1, 5, 10, 15, 20, 30 and 60 minutes. rAN cells were vibrated for 1, 5, 15, and 30 minutes. The culture supernatant fluid was collected carefully without touching the cell layer at 0, 1, 5, 10 min after loading for hAN cells and 1, 5, 10 min. for rAN cells, then boiled for 1 min to inhibit ATPase activity. [ATP] in the culture supernatant fluids was measured by a firefly luciferase assay. Samples were added with luciferin and luciferase in reaction buffer and the reactive emission was read in a 96-well plate using a luminometer (WALLAC Inc. AutoLumat LB953). The sample collected from the control plate (no vibration) was measured as a negative control and cell lysates as a positive control.

Results: The concentration of endogenous ATP in resting culture supernatant fluids indicated that both cultured human and rabbit annulus cells released ATP continuously at a baseline concentration in the range of 1-2 nM (Figure 1,2). Vibratory loading of hAN cells stimulated ATP release by two-fold with time, reaching a net maximum concentration by 10 minutes. Shortly thereafter, ATP concentration declined and returned to below baseline level. For vibration times longer than 15 min, the [ATP] in the culture supernatant fluid became significantly lower than control in hAN cells. In rAN cells, [ATP] increased above control; however, the difference was not significant until the cells were vibrated for 30 minutes.

Discussion: This study has shown that resting annulus cells secrete ATP continuously at levels that are approximately that of published values for airway epithelial cells and chondrocytes. Vibratory loading stimulates ATP release by at least two-fold after 10 minutes in hAN cells and 30 minutes in rAN cells. It is possible that the actual values may be higher given that extracellular NTPases are known to quickly degrade ATP. Human cells in particular exhibited a decline in detectable [ATP] after 15 minutes of vibration to 60 minutes indicating either inhibition of ATP release or destruction by ATPase. ATP can act in a paracrine/autocrine fashion as a signaling molecule. We have demonstrated that intervertebral disc cells express cell surface purinoceptors and respond to 1 µM ATP by increasing intracellular [Ca²⁺]c (1). Exogenous ATP has also been shown to transiently inhibit the response of annulus cells to direct mechanical stimulation (poke) (2). These results show that annulus cells secrete ATP and likely utilize this nucleotide as a signaling messenger to themselves and adjacent cells. Exogenous ATP can induce phosphorylation of protein such as ion channels that may regulate the cell’s response to vibratory load.
