INTRODUCTION: Proteoglycan loss is one of the first signs of disc degeneration; there is increasing interest in developing biological methods for its replacement both by in vivo repair and through tissue engineered constructs. The most important problems in disk regeneration medicine are to supply nutrients to cells activated by grafting or growth factors and to maintain a healthy extracellular environment. Regeneration of disk tissue with sufficient mechanical strength particularly requires the production of glycosaminoglycan (GAG), which accounts for 7-10% of healthy disk tissue. However, it has been shown that the extracellular osmotic pressure decreases with disc degeneration in candidates for treatment. In the present study, we evaluated the influence of bone morphogenetic protein-7 (BMP-7), which are involved in the activation of cell metabolism, on the metabolism of proteoglycans by nucleus pulposus (NP) cells cultured under low-osmotic conditions as seen the degenerated disc.

METHODS: Cells were isolated from the nucleus pulposus of 18-24 month bovine caudal discs. They were cultured for 5 days in alginate beads in DMEM containing 6% FBS under 21% O2 at cell densities of 4 million cells/ml. Medium osmolality was altered by addition of 5 mol/l sodium chloride (NaCl) and was monitored using a freezing point osmometer (Semi-micro osmometer, Knaue, Germany). NP cells were cultured in culture fluid with a normal osmotic pressure (370 mOsm) as seen the healthy disk or a low osmotic pressure (270 mOsm) as seen the degenerated disc. The medium was changed every day and BMP-7 (100 ng/ml) was added to both groups every day. The cell viability profile was determined by manual counting using trypan blue staining. Lactate production was measured enzymatically as a marker of energy metabolism. Rate of sulfate GAG synthesis was measured using a standard 35S-sulfate radioactive method. GAG accumulation (as a measure of proteoglycan) was measured using a DMB assay.

RESULTS: Lactate production was decreased by about 30-40% under 270 mOsm compared with that at 370 mOsm, showing that cell metabolism was impaired despite OP-1 being added to cultures. The rate of lactate production per live cell was significantly higher for cells cultured at 370 mOsm with BMP-7 than those at 270 mOsm after 5 days (Fig.1).

DISCUSSION: The intervertebral disc is avascular, and the metabolic activity of its cells is regulated by various factors in the extracellular matrix, such as oxygen, osmolality, and pH. In aging, the tide mark (calcification) in the end-plate acts as a barrier to nutrients transport and is thought to be a major factor in the development of disc degeneration. The osmolality of the extracellular matrix is regulated by negatively charging the GAG chains of proteoglycans which adjust ionic composition. Urban and Maroudas et al. 17 assessed the osmolality was decreased in degenerated disc. Takeno et al. 21 demonstrated that GAG production was largest in the 370mOsm, and the capacity for GAG production and cell metabolism (lactate production) was low under hypo-osmolality and hyper-osmolality, and cell deaths were observed on electron microscopy. Thus, it may be said that osmotic pressure gradient disturbance associated with reduced proteoglycans is an important factor contributing to the development of disc degeneration.

The interrelationships between cell density, cell viability and activity, and diffusion distance resulting from nutrient supply constraints, limit the rate at which GAG can be accumulated in three-dimensional constructs. GAG accumulation depends on GAG production per cell and on cell density. 3,17 In this study, calculated times to produce a concentration equal to the in vivo concentrations of 7% GAG per wet weight (viz. 70 mgs/ml) assuming initial rates were maintained and there was no loss of GAG, were > 1 year using BMP-7 (100 mg/ml/day) in 370 mOsm medium. An increase in GAG production rate per cell can be induced by addition of BMP-7, but the relative increase which can be achieved is limited (usually two–threefold under optimal conditions) and the consequent increase in metabolic demand can lead to a fall in pH in the construct center41 and thus severely limit growth factor efficacy. In this study, addition of BMP-7 to constructs was found to have big effect on the concentration of accumulated GAG under low osmolarities. Thus, increasing cell metabolism potentially should increase GAG deposition, but leads to a more nutrients demands.

SIGNIFICANCE: Incubation with BMP-7 enhances GAG production during culture at a normal osmotic pressure, but cell function is decreased in degenerated discs. Thus, the clinical application of disc regeneration medicine needs to be advanced by providing appropriate physiological conditions with consideration of age-related disc changes.

REFERENCES:

The Effect of Bone Morphogenetic Protein-7 under Low Osmotic Conditions As Seen in Degenerated Discs: The Rate of Glycosaminoglycan Accumulation by Disc Cells in Vitro.