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**THE EFFECT OF DYNAMIC MECHANICAL COMPRESSION ON NITRIC OXIDE PRODUCTION BY THE KNEE MENISCI**

**Introduction:** The menisci are intraarticular fibrocartilaginous structures essential to the normal function of the knee joint. Numerous studies have determined their capacity for load transmission, shock absorption, stabilization, and lubrication.1-3 While there is overwhelming evidence that NO plays a role in cell signal transduction and there is growing evidence that NO plays an important role in arthritis,6-7 where a major pathophysiological consequence may be the inhibition of chondrocyte matrix production. Therefore, alterations in the mechanical environment of the knee joint may have an important influence on the production of NO in meniscal fibrochondrocytes.

The purpose of this study was to determine if meniscal fibrochondrocytes can respond to mechanical stress by increasing NO production. Because of variations in fibrochondrocyte arrangement and density with distance from the tissue surface, the response of the superficial and deep zones of the meniscus to dynamic compression was also investigated separately.

**Material and Methods:** Medial and lateral menisci from knees of 2 year old female pigs were obtained from a local slaughterhouse and processed within 4 hours following sacrifice. Ten cylindrical explants (5 mm in diameter and approximately 2 mm millimeters in thickness) were harvested aseptically from the femoral surface of each meniscus using a biopsy punch. The samples were incubated in culture medium (DMEM, 0.1 mM non-essential amino acids, 10 mM HEPES Buffer solution, 100 U/ml penicillin/streptomycin [Gibco, Gaithersburg, MD] and 10% heat inactivated fetal bovine serum [Sigma Chemicals, St. Louis, MO]). Test and control explants were paired at harvest and originated from adjacent sites on the meniscus.

Compression loads were applied to 20 samples simultaneously using a modified version of the Biopress™ system (Flexcell International, Hillsborough, NC). Compression experiments were performed for 24 h at a frequency of 0.5 Hz (square wave with one second on, one second off) at a compressive stress of 0.1 MPa. Control explants were maintained unloaded for the same duration. Additionally, the effect of the NOS2 specific inhibitor 1400W (Alexis Biochemicals, San Diego, CA) and the NO response of superficial versus deep zones of the meniscus samples were evaluated. Nitric oxide was assessed by measuring the concentration of nitrite and nitrate (the stable NO metabolites) in the conditioned media using the “Total NO” Assay (R & D Systems, Minneapolis, MN). Immunoblots were performed using anti-NOS1, anti-NOS2 or anti-NOS3 monoclonal antibodies (Transduction Laboratories, Lexington, KY) and detected using the enhanced chemiluminescence reagents from Amersham (Arlington Heights, IL). Cell viability was assessed immediately following the mechanical loading regimens by a fluorescent live/dead assay (Molecular Probes, Eugene OR). A two-factor ANOVA and Newman-Keuls post hoc test were used for statistical calculation (α=0.05).

**Results:** Since spontaneous NO release from the explants remained constant after 2 days in culture medium, all compression experiments were performed 72 h following sample harvest (Fig.1). Twenty-four hr compression at 0.1 MPa caused a significant (p<0.01) increase in total NO. There was no significant (p>0.05) difference between samples from the medial and lateral menisci (Fig.2). Addition of the NOS2 specific inhibitor 1400W (2 mM) to the culture medium decreased compression induced NO production approximately 50% (720.1 ± 11.7 µmol/g/24h to 336.3 ± 40.4 µmol/g/24h; p<0.05). Supernatant medium from dynamically compressed explants of the superficial zones of the menisci contained more NO than that from discs harvested from deep zones (Fig.3).

Immunoblot analysis of two separate samples showed induction of NOS2 antigen by compression. There was no NOS2 antigen detected in uncompressed samples (Fig.4). NOS1 and NOS3 were not detected.

Cell viability was found to be 99-100% in control and compressed samples, except for a region of cell death of approximately one to two cell layers thick near the cut edges of the explant.

**Discussion:** Our findings provide direct evidence that dynamic mechanical stress influences the biological activity of fibrochondrocytes. NO production was significantly increased by dynamic loading in both the medial and lateral menisci and significant zonal differences were detected. These findings suggest that NO production in vivo may be regulated by mechanical stress acting upon the menisci. Since NO can affect matrix metabolism in various intra-articular tissues, alterations in the distribution and magnitude of stress in the menisci (e.g., those encountered in injury, inflammation, degeneration, or surgery) have important metabolic as well as biomechanical consequences on the physiology and function of the knee joint. Meniscal NO and NOS may represent novel treatment targets in degenerative, inflammatory and traumatic disorders of the knee joint.

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**Poster Session - Knee - Hall E**

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