Osteoarthritis and Cartilage (2008) 16, 1213–1219
© 2008 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.joca.2008.02.019

Osteoarthritis and Cartilage

ICRS International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

IL-1 and *iNOS* gene expression and NO synthesis in the superior region of meniscal explants are dependent on the magnitude of compressive strains

T. Gupta Ph.D., Research Assistant in Mechanical Engineering†, B. Zielinska Ph.D., Research Assistant in Mechanical Engineering†, J. McHenry M.S., Research Assistant in Mechanical Engineering†, M. Kadmiel M.S., Laboratory Assistant‡ and T. L. Haut Donahue Ph.D., Associate Professor of Mechanical Engineering†*

† *Department of Mechanical Engineering – Engineering Mechanics, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, United States*

‡ *Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, NC 27599, United States*

Summary

Objective: Partial meniscectomy is known to cause osteoarthritis (OA) of the underlying cartilage as well as alter the load on the remaining meniscus. Removal of 30–60% of the medial meniscus increases compressive strains from a maximum of approximately 10% to almost 20%. The goal of this study is to determine if meniscal cells produce catabolic molecules in response to the altered loading that results from a partial meniscectomy.

Method: Relative changes in gene expression of *interleukin-1* (*IL-1*), *inducible nitric oxide synthase* (*iNOS*) and subsequent changes in the concentration of nitric oxide (NO) released by meniscal tissue in response to compression were measured. Porcine meniscal explants were dynamically compressed for 2 h at 1 Hz to simulate physiological stimulation at either 10% strain or 0.05 MPa stress. Additional explants were pathologically stimulated to either 0% strain, 20% strain or, 0.1 MPa stress.

Results: *iNOS* and *IL-1* gene expression and NO release into the surrounding media were increased at 20% compressive strain compared to other conditions. Pathological unloading (0% compressive strain) of meniscal explants did not significantly change expression of *IL-1* or *iNOS* genes, but did result in an increased amount of NO released compared to physiological strain of 10%.

Conclusion: These data suggest that meniscectomies which reduce the surface area of the meniscus by 30–60% will increase the catabolic activity of the meniscus which may contribute to the progression of OA.
© 2008 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Key words: Fibrochondrocyte, Osteoarthritis, Mechanotransduction, Unconfined compression.

Introduction

Partial meniscectomy is a common treatment for meniscal tears which do not heal due to the lack of vascularity in the inner two-thirds of the tissue. Partial meniscectomy has been shown to lead to osteoarthritis (OA) in the long term[1,2]. The amount of tissue removed has a bearing on the severity of the onset of OA[2], the loads carried by the meniscus[3], and the cellular environment of fibrochondrocytes within the tissue[4]. Hellio Le Graverand *et al.* [5] used ACL transection as an experimental model of OA to capture early changes in meniscal tissue and articular cartilage. This study showed that extracellular matrix deterioration and changes in cell and collagen distribution occurred in the menisci prior to any changes in articular cartilage. This suggests that meniscal tissue degeneration may be a precursor to the development of OA. Although the mechanism by which articular cartilage degrades has been widely studied, the mechanism of meniscal tissue degeneration and its association with OA are not clear. However, two biomolecules, nitric oxide (NO)[6,7] and interleukin-1 (IL-1)[8–10], are believed to regulate catabolic activity in both articular cartilage and meniscus.

NO is an important intracellular and extracellular messenger serving different functions in a variety of tissues[11]. NO causes tissue degeneration[12,13] and apoptotic cell death in cartilage and meniscus[6,7]. NO is produced by a family of enzymes called nitric oxide synthases (NOSs), two of which, NOS1 and NOS3, are constitutive forms and the third, NOS2 or inducible nitric oxide synthase (iNOS) is an inducible form. NO production within cartilaginous tissues such as articular cartilage and meniscus has been previously shown to be primarily of the inducible form and is regulated by chemical and mechanical stimuli[11–15,18]. While it has previously been shown that meniscal cells can make both NOS2 and NOS3[19], it is the inducible form of NOS that can be activated by inflammatory mediators such as IL-1 and tumor necrosis factor[11].

*Address correspondence and reprint requests to: Tammy L. Haut Donahue, Michigan Technological University, Department of Mechanical Engineering, 1400 Townsend Drive, Houghton, MI 49931, United States. Tel: 1-906-487-2078; Fax: 1-906-487-2822; E-mail: thdonahu@mtu.edu

Received 24 May 2007; revision accepted 22 February 2008.

IL-1, an inflammatory cytokine, has been shown to increase the production of NO in meniscal and articular cartilage[11,12] and also reduce synthesis of the major articular cartilage matrix protein (type II collagen), independently of NO at a transcriptional or post-transcriptional level[12]. IL-1 also causes a decrease in the incorporation of proteins specific to collagen[13]. Exogenous IL-1 has been shown to regulate the production of prostaglandin E_2 (PGE_2). PGE_2 is an inflammatory mediator which inhibits synthesis of collagen [15], *via* an NO dependant pathway and decreases proteoglycan synthesis[15,17]. Taken together, this suggests a catabolic role for IL-1 as collagen and proteoglycans constitute the majority of the solid constituents of meniscal tissue.

Mechanical forces such as tension and compression are essential for maintaining tissue homeostasis by balancing the catabolic and anabolic activities of biomolecules[20]. Mechanically loading cartilaginous tissue has been shown to both inhibit[15,18] and upregulate[16,17] NO production in articular cartilage and meniscus. This discrepancy may be due to a dose dependent response of the tissue to loading or tissue type. Although mechanical loading is a potent mediator of matrix synthesis and degeneration, it is not clear which magnitudes of mechanical loading are catabolic and which are anabolic in their overall effect on meniscal tissue. It is our hypothesis that there exists an optimal level of compression that will cause the lowest production of IL-1 and NO and hence be least damaging to meniscal tissue. This level may be $\sim$10% compression which is predicted to be the upper limit of the healthy range of strains within intact meniscus[3]. We also hypothesize that superior and deep tissues will respond differently to the same loading by producing different amounts of NO and IL-1. These hypotheses will be tested by measuring *iNOS* and *IL-1* gene expression and NO production *in vitro* in response to unconfined compression at levels below physiological loading (0%), physiological levels ($\sim$10%) and supraphysiological loading (20%).

Methods

COMPRESSION TESTING

Stifle joints (knees) were obtained from 18-week-old pigs 24 h after death (Mayo Foundation, Rochester, MN) giving a sample size of six animals. For a given animal, both the medial and lateral menisci were excised using sterile techniques and 24 explants (six from each of the four menisci in an animal) measuring 6 mm in diameter and 5 mm in height were cored using a biopsy punch (6 mm Biopunch, Fray Products Corp., Buffalo, NY). The bottom face of each explant was trimmed to be parallel to the superior surface of the explants. The explants were cored perpendicular to the superior surface of the meniscus and were taken primarily from the outer region. Explants were then allowed to equilibrate for 48 h at 37°C with 5% CO_2 in media constituting 44.5% Dulbecco's modified Eagle medium (DMEM), 44.5% Ham's F12, 10% fetal bovine serum (FBS) and 1% penicillin streptomycin. Media were changed after 24 h of incubation.

Mechanical compression was achieved with a previously validated bioreactor[21]. Briefly, the bioreactor was designed to test six explants simultaneously in unconfined compression. Six frictionless Delrin© (DuPont, Willmington, DE) compression rods were driven by a stepper motor to compress explants in individual wells while housed in a sterile casing. A static pre-stress of 0.0076 MPa was applied prior to dynamic compression. Dynamic tests were then carried out at 37°C at 0%, 5%, 10%, 15% or 20% strain and also at 0.05 MPa or 0.1 MPa stress for 2 h at 1 Hz. Therefore, 0% strain actually refers to the amount of dynamic strain superimposed on top of the static 0.0076 MPa of pre-stress, and 0 MPa stress is actually no dynamic stress superimposed on top of the static 0.0076 MPa of pre-stress. The bioreactor used in the current study was previously presented with details of the stress and strain waveforms[21]. Tables I and II present a brief summary of the stress and strain tests. Neither confined or unconfined compression exactly replicates the *in vivo* environment of meniscal tissue. While previous computational modeling has documented the strain levels in the normal and meniscectomized menisci, unconfined compression to similar strain levels leads to abnormally large stresses on the tissue explant. Therefore, since the loading history is quite different between a stress controlled test and a strain controlled test, stress controlled experiments were also completed to determine differences between these two different mechanical effects. The stress levels were chosen such that they represent approximately the equilibrium values found during stress relaxation studies of meniscal tissue. For the 10% stress relaxation studies, equilibrium stress was approximately 0.05 MPa. For 20% stress relaxation studies, 0.1 MPa was the equilibrium stress. Hence, 0.05 MPa is considered to be physiologically normal, whereas 0.1 MPa is considered to be supraphysiological, or overloading[21].

Following mechanical loading, explants were removed from the bioreactor, cut into half to distinguish between superior and deep portions, weighed and incubated for 24 h in 1 ml of media similar in composition to that used for equilibration but containing 2% FBS. Explants were then removed from the media and stored in 200–300 μl of RNALater (Ambion Inc., Austin, TX) at 4°C for 1 day. RNALater was then removed and the samples were stored at −20°C. Post-compression media were stored in 1.5 ml vials at −80°C for subsequent analysis.

Table I
Change in pressures over the duration of meniscal tissue stress–relaxation tests. Data represent mean ± standard deviation. n = *6 for all groups*

Strain (%)	Pressure (MPa)	
	Start	End
5	0.166 ± 0.108*,†	0.038 ± 0.010†
10	1.141 ± 0.103†	0.046 ± 0.010†
15	2.185 ± 0.827	0.035 ± 0.026†
20	3.548 ± 0.429	0.128 ± 0.020

*Significantly different than 15% ($P < 0.05$).
†Significantly different than 20% ($P < 0.05$)[21].

RNA ISOLATION

Total RNA was isolated from superior and deep sections of the explants using a commercial kit (SV Total RNA Isolation System, Promega, Madison, WI). Three explants were combined and crushed to a fine powder using liquid nitrogen. Preliminary data showed that combining three explants provided sufficient RNA for subsequent polymerase chain reaction (PCR) analyses. The powder was immediately added to lysis buffer and then stored at 4°C. A homogenizer was used to lyse the cells further. RNA was extracted and DNase-treated on the column according to the manufacturer's instructions. RNA was eluted in 100 μl of nuclease free water, analyzed with a spectrophotometer and run on a 1.5% native agarose gel to check for integrity by observing the 18S rRNA and 28S bands.

PRIMERS DESIGN

Specific primers were design based on *Sus scrofa* sequence (either partial or complete mRNA) available from the National Center for Biotechnology Information (NCBI). Amplicon sizes ranged from 150 bp to 260 bp and primers were approximately 20 bp long (Table III). PCR was performed using gene-specific primers and the products were verified by sequencing.

SEMI-QUANTITATIVE REVERSE TRANSCRIPTION-PCR (RT-PCR)

RT-PCR was performed using a two step process. First-strand RT was synthesized using Superscript II (Invitrogen Corporation, CA) and random primers. PCR was carried out for 28 cycles at an annealing temperature of 58°C using cDNA equivalent to 300 ng of RNA. cDNA used for amplifying *18S rRNA* was diluted 1/40 to allow both *iNOS* and *18S rRNA* to be amplified to similar levels, within the linear range of amplification, using the same cycle number and annealing temperature. PCR products were run on a 2.5% agarose gel and a digital image was taken. MATLAB (The MathWorks Inc.,

Table II
Change in strains over the duration of meniscal tissue creep tests. Data represent mean ± standard deviation. n = *6 for all groups*

Pressure (MPa)	Strain (%)	
	Start	End
0.05	2.6 ± 0.53	11.6 ± 1.36
0.1	3.0 ± 0.12	20.7 ± 1.45*

*Significantly different than 0.05 MPa ($P < 0.05$)[21].

Download English Version:

https://daneshyari.com/en/article/3380711

Download Persian Version:

https://daneshyari.com/article/3380711

Daneshyari.com