Osteoarthritis and Cartilage (2009) **17,** 754–760
© 2008 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.joca.2008.11.018

Osteoarthritis and Cartilage

ICRS International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Meniscal tissue explants response depends on level of dynamic compressive strain

B. Zielinska†, M. Killian†, M. Kadmiel†‡, M. Nelsen† and T. L. Haut Donahue†*

† *Biotechnology Research Center, Department of Mechanical Engineering-Engineering Mechanics, Michigan Technological University, Houghton, MI 49931, United States*

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

Summary

Objective: Following partial meniscectomy, the remaining meniscus is exposed to an altered loading environment. *In vitro* 20% dynamic compressive strains on meniscal tissue explants has been shown to lead to an increase in release of glycosaminoglycans from the tissue and increased expression of interleukin-1α (IL-1α). The goal of this study was to determine if compressive loading which induces endogenously expressed IL-1 results in downstream changes in gene expression of anabolic and catabolic molecules in meniscal tissue, such as MMP expression.

Method: Relative changes in gene expression of MMP-1, MMP-3, MMP-9, MMP-13, A Disintegrin and Metalloproteinase with ThromboSpondin 4 (ADAMTS4), ADAMTS5, TNFα, TGFβ, COX-2, Type I collagen (COL-1) and aggrecan and subsequent changes in the concentration of prostaglandin E_2 released by meniscal tissue in response to varying levels of dynamic compression (0%, 10%, and 20%) were measured. Porcine meniscal explants were dynamically compressed for 2 h at 1 Hz.

Results: 20% dynamic compressive strains upregulated MMP-1, MMP-3, MMP-13 and ADAMTS4 compared to no dynamic loading. Aggrecan, COX-2, and ADAMTS5 gene expression were upregulated under 10% strain compared to no dynamic loading while COL-1, TIMP-1, and TGFβ gene expression were not dependent on the magnitude of loading.

Conclusion: This data suggests that changes in mechanical loading of the knee joint meniscus from 10% to 20% dynamic strain can increase the catabolic activity of the meniscus.
© 2008 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Key words: MMP, IL-1, Meniscus, Knee, Osteoarthritis, ADAMTS.

Introduction

Patients who undergo partial meniscectomy, a common surgical treatment for meniscal tears, often experience osteoarthritis (OA) in the underlying cartilage in long-term follow-up studies[1–5]. The meniscus plays a major role in load distribution and transmission in the knee joint[6–8], and it has been shown to be mechanically sensitive[9,10]. Recently, 20% dynamic compressive strain on meniscal explants has been shown to lead to increased glycosaminoglycan (GAG) content in the culture media, an upregulation of interleukin-1 (IL-1) gene expression and increased release of nitric oxide (NO)[11,12]. IL-1 is a pro-inflammatory cytokine involved in the etiology of OA[13,14]. It has been shown to increase levels of NO[13] which in turn can induce gene expression of other catabolic molecules such as metalloproteinases (MMPs matrix cleavage proteins) and inhibit cell proliferation[15–17]. IL-1 has been shown to increase cyclooxygenase-2 (COX-2) synthesis leading to increased production of prostaglandin E_2 (PGE_2) in osteoarthritic cartilage[14].

*Address correspondence and reprint requests to: Tammy L. Haut Donahue, Department of Mechanical Engineering, Michigan Technological University, 815 R. L. Smith Building, 1400 Townsend Dr., Houghton, MI 49931, United States. Tel: 1-906-487-2078; Fax: 1-906-487-2822; E-mail: thdonahu@mtu.edu
Received 18 March 2008; revision accepted 28 November 2008.

Certain matrix metalloproteinases (MMP-1, MMP-3, MMP-9, and MMP-13) and aggrecanases-1 and -2 (ADAMTS4 and ADAMTS5) have been shown to be responsible for the breakdown of collagens and proteoglycans in cartilaginous tissues[18–23]. MMP-1 and MMP-13 represent collagenases, and are involved in degradation of native collagen fibers[24]. MMP-3 is a representative of the stromelysin group and is partially responsible for degradation of proteoglycans and type IX collagen. The last group of MMPs-gelatinases, includes MMP-9. Gelatinases degrade denatured collagen, proteoglycan and fibronectin[24]. MMP-1 and MMP-13 are partially responsible for the breakdown of the helical region of fibrillar collagens[25,26] and cleavage of the triple helices[25], respectively. Tissue inhibitor of metalloproteinases (TIMPs) are elevated in OA synovial fluid and may indicate a natural attempt by the body to counteract the action of MMPs[27]. In a rat iodoacetate model of OA, both A Disintegrin And Metalloproteinase with ThromboSpondin (ADAMTS) and MMP neoepitopes were present, suggesting that specific cleavage of aggrecan by ADAMTS and MMP may be responsible for the degradation of aggrecan[21]. ADAMTS4 and ADAMTS5 have been shown to cleave aggrecan *in vitro* in the interglobular domain between $Glu^{373}–Ala^{374}$ and $Glu^{1871}–Leu^{1872}$ sites, respectively[28–30]. Overexpression of these aggrecanases results in cartilage matrix degradation[31–33], and inhibition of these enzymes can prevent aggrecan degradation *in vitro*[34].

In articular cartilage, both cytokines and growth factors have a role in tissue homeostasis. Both IL-1 and tumor necrosis factor α (TNFα) appear to be responsible for pathological processes in articular cartilage[35,36,27]. While previous studies have added exogenous IL-1 to meniscal tissue and noted degradative downstream effects[37,38], it is unclear if compressive mechanical loading with endogenously expressed IL-1 results in changes of anabolic and catabolic genes in meniscal tissue. It has not yet been determined whether mechanical loading alters TNFα gene expression in meniscal tissue. Furthermore, growth factors such as transforming growth factor β (TGFβ) have an important role in articular cartilage biosynthesis[27], but whether mechanical loading of meniscal tissue alters expression of such genes is unclear.

This *in vitro* study was designed to measure gene expression of catabolic molecules (MMPs, COX-2, TNFα, and ADAMTS), release of PGE_2 to the media, and gene expression of matrix proteins (Type I collagen (COL-1) and aggrecan) following various levels of dynamic mechanical compression of meniscal tissue. Additionally, gene expressions of molecules whose function in repair and degradation of matrix molecules are unclear (TGFβ and TIMP-1) were studied.

Methods

MECHANICAL STIMULATION

Mechanical stimulations were performed according to the previously introduced protocol[11,12]. Briefly, six explants (5 mm tall and 6 mm diameter) were cut from each of 12 porcine menisci (6 animals, age 18 weeks). Each explant was cut perpendicular to the femoral surface of the meniscus to preserve as much superficial surface as possible and was primarily taken from the outer zone. Explants were approximately 7–8 mm tall and were placed in a custom device which cut the explants to 5 mm tall while preserving the superficial surface. Each explant was incubated separately for 48 h in growth media (89% Dulbecco's Modified Eagle Medium/Ham's F12, 10% Fetal Bovine Serum (FBS), 1% Penicillin Streptomycin) at 37°C with 5% CO_2 to equilibrate. Media were changed every 24 h. A total of 72 explants were harvested and 54 were randomly used for the study. Nine explants from each animal were randomly selected and exposed to 1 of 3 loading protocols (0%, 10% or 20% dynamic compression strain, with three explants per group). Mechanical stimulation was performed with a custom built bioreactor[11,12]. All explants were allowed to free swell for 48 h during which time no visual swelling was noted. Therefore, all strain measurements assumed no swelling of the tissue. Following this period of free swelling, a preload of 0.0076 MPa was applied by the platen, and six explants were simultaneously loaded to either 10% or 20% dynamic compressive strain or 0% compressive loading (tare weight of the platen) for 2 h at 1 Hz to simulate walking. It has previously been shown that when using this bioreactor, 10% dynamic strain results in a peak stress of 1.41 ± 0.10 MPa and an equilibrium stress of 0.046 ± 0.01 MPa, while 20% dynamic strain results in peak and equilibrium stresses of 3.55 ± 0.43 MPa and 0.13 ± 0.02 MPa, respectively[11]. The equilibrium stress was indicated as the measured value after 2 h of dynamic loading, at which the stress did not significantly change with each cycle. Following mechanical stimulation, explants were first cut in half to separate superficial and deep zones tissue and then weighed and placed in media for 24 h at 37°C with 5% CO_2. The explants were then treated with RNALater (Ambion Inc., Austin, TX) for 24 h prior to storage. The superficial explants represent the top 2.5 mm of the meniscus that contacts the femur while the deep explants represent the middle 2.5 mm of the tissue.

MOLECULAR BIOLOGY TESTS

To isolate an adequate amount of RNA for gene analysis three explants from a given animal that received the same loading treatment were combined for total RNA isolation using a commercially available kit (SV Total RNA Isolation System, Promega, Madison, WI) as previously described[12]. The RNA quality was verified by running ~200 ng of RNA on a 1.5–2.5% ethidium bromide-stained agarose gel, and visualizing the intact large and small ribosomal subunits under ultraviolet light. Reverse Transcription (RT) and real-time Polymerize Chain Reaction (PCR) were carried out to measure gene expression in mechanically stimulated meniscal tissue as well as calibration (control) tissue.

RT reactions began with a 12 μL reaction, consisting of 300 ng RNA, 100 ng random primers and 0.83 mM dNTPs. Samples were heated to 65°C for 5 min and then placed on ice. At this time, a 7 μL mix consisting of 4 μL of the 5× buffer provided with the SuperScript II (Invitrogen Corporation, Carlsbad, CA, USA), 0.029 M dithiothreitol (DTT) and 40 units of RNase Out (Invitrogen Corporation, Carlsbad, CA, USA) were added to each reaction. The reaction was incubated at 25°C for 2 min and again placed on ice while 50 units of SuperScript II (Invitrogen Corporation, Carlsbad, CA, USA) were added to each reaction. Tubes were then incubated at 25°C for 10 min, followed by 42°C for 50 min, 70°C for 15 min and 4°C for 5 min. Samples were placed on ice, and 2 units RNase H (New England BioLabs, Ipswich, MA, USA) were added, bringing the final reaction volume to 20 μL. Samples were incubated at 37°C for 20 min, followed by 20 min at 65°C, and 5 min at 4°C. All reactions were performed in an Eppendorf Mastercycler Gradient machine (Westbury, NY, USA).

Real-time PCR was performed in 25 μL reaction volumes using gene specific primers designed from partial or complete *Sus scrofa* cDNA sequences available from National Center for Biotechnology Information (NCBI) (Table I), or by using primers from the literature[39]. Newly designed primers were created using the PrimerQuest and OligoAnalyzer 3.1 software (Integrated DNA Technologies, Coralville, IA, USA). The ribosomal 18s RNA was used as a housekeeping gene and was run on each plate alongside the gene of interest. Reactions for most genes (MMP-1, MMP-3, MMP-9, MMP-13, COX-2, COL-1 and 18s [when used a as a housekeeper for these genes]) contained 7.5 ng of cDNA, 0.2 μM of each primer, 12.5 μL SYBR Green Fluorescence Mix (Absolute QPCR SYBR Green Mix, Abgene, Inc., Rockford, IL, USA) and 2.5×10^{-3} μL of carboxy-X-rhodamine (ROX). For the remaining genes, 15 ng of cDNA (Aggrecan, ADAMTS4, ADAMTS5 and 18s [when used a as a housekeeper for these genes]), 2.5 ng (TGFβ, TNFα and TIMP-1) or 2.5×10^{-3} ng (18s when used as housekeeper for these four genes) of cDNA was used in each reaction, with 0.1 μM of each primer, 12.5 μL SYBR Green Fluorescence Mix and 7.5×10^{-4} μL ROX. All qPCR reactions were run on a Stratagene MX3000P QPCR System (La Jolla, CA, USA), and began with an initial denaturation of 15 min at 95°C. This was followed by 40 cycles of: 95°C for 15 s, 60 s at 55–61°C (55°C: ADAMTS4, ADAMTS5; 57°C: TIMP-1; 59°C: MMP-1, MMP-3, MMP-9, MMP-13, COX-2; 60°C: COL-1; 61°C: Aggrecan, TGFβ and TNFα) and 40–60 s at 72°C (40 s: ADAMTS4, ADAMTS5, MMP-1, MMP-3, MMP-9, MMP-13, COX-2 and COL-1; 60 s: Aggrecan, TGFβ, TNFα and TIMP-1). This was followed by a dissociation curve analysis to verify the specificity of the amplification. All samples were run in duplicate and data were analyzed using Stratagene MXPro QPCR Software (La Jolla, CA, USA). A dissociative curve was run with each plate setup to confirm regularity of the tests. Samples were analyzed using the Pfaffl method by which the ratio of the target gene to the housekeeping gene is quantified with respect to the calibrator using the following formula:

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta C_t \text{target(control}-\text{treated)}}}{(E_{\text{ref}})^{\Delta C_t \text{ref(control}-\text{treated)}}}$$

where C_t – cycle number in the linear range of amplification, E – efficiency of the process for each gene, target – gene of interest, ref – housekeeping gene, control – calibrator, treated – sample.

Quantification of PGE_2 release from the explants to the media was performed. PGE_2 release was measured using a commercially available Prostaglandin E_2 Biotrak EIA assay kit (Amersham Biosciences, NJ, USA). This assay is based on competitive binding of PGE_2 and mouse monoclonal antibody. Absorbance was read at 450 nm. Data were normalized to the wet weight of the explant.

DATA ANALYSIS

All data are presented as mean ± standard deviation (SD). A repeated measures ANOVA with Fisher's protected least significant difference method was used to calculate statistical differences ($P \leq 0.05$ was considered significant) between different loading conditions for both zones. Paired *t*-tests were used to measure differences in cell response of pair-wise superficial and deep zones for each loading conditions ($P \leq 0.05$ was considered significant).

Results

The expression of four MMP genes and two ADAMTS genes, following various levels of mechanical compression, was studied (Fig. 1). For the superficial explants, MMP-1, MMP-3 and MMP-13 gene expression were upregulated following 20% strain compared to no dynamic strain and 10% strain tests (Fig. 1). Additionally, there was a statistically

Table I
Specific primers sequences: MMP-1, MMP-3, MMP-9 and MMP-13, COX-2, COL-1, ribosomal 18s RNA, Aggrecan, TGFβ, TNFα, TIMP-1, ADAMTS4, ADAMTS5

Gene	FWD 5′-3′	REV 5′-3′
MMP-1	GGACCTGGAGGAAACCTTGCT	GCCTGGATGCCATCAATGTC
MMP-3	AGAAGTTCCTTGGGTTGGAGGT	TCTTGGAGAATGTAAGCGGAGT
MMP-9	CAGGCAGCTGGCAGAGGAATA	CCAGTAGGTGATGTCGTGGTGG
MMP-13	GATCCCCATTTTGATGATGATGAA	GTCTTCATCTCCTGGACCATAGAGAGA
COX-2	TCAACCAGCAATTCCAATACC	ATTCCTACCACCAGCAACC
COL-1	TCCGGCTCCTGCTCCTCTTA	GGCGCTGGGACAGTTCTT
18s	GCAAATTACCCACTCCCGAC	CGCTCCCAAGATCCAACTAC
Aggrecan	ACAGGTGAAGACTTTGTGGAC	AGTCAGTGAGTAGCGGGAGG
TGFβ	GGAGTGGCTGTCCTTTGATGT	AGTGTGTTATCTTTGCTGTCA
TNFα	TCTGCCTACTGCACTTCGAGG	TGGCTACAACGTGGGCG
TIMP-1	CGCCTCGTACAAGCGTTATGA	TAGATGAACCGGATGTCAGGG
ADAMTS4	AGGAGGAGATCGTGTTTCCAGAGA	AAAGGCTGGCAAGCGGTACAACAA
ADAMTS5	TTCGACATCAAGCCATGGCAACTG	AAGATTTACCATTAGCCGGGCAGG

significant increase in both MMP-1 and MMP-3 gene expression for the deep explants compressed to 20% strain, compared to no dynamic strain (Fig. 1). The only difference between no dynamic strain tests and 10% dynamic strain was in MMP-9 gene expression for deep explants, with a two-fold decrease in MMP-9 expression under no dynamic strain (Fig. 1).

Similar to MMP-9 expression, ADAMTS5 showed a significant upregulation with 10% dynamic compressive strain compared to no and 20% dynamic strain tests for deep explants. On the other hand, ADAMTS4 displayed trends similar to MMP-1, MMP-3 and MMP-13 where 20% strain upregulated ADAMTS4 expression for superficial explants compared to 10% strain. In contrast to any other genes of interest in this study, ADAMTS4 was significantly upregulated at no dynamic strain compared to 10% dynamic strain tests for superficial explants. For the MMPs and aggrecanases studied, only MMP-13, in the 20% dynamic strain test showed a significant difference between superficial and deep explants (Fig. 1).

While COX-2 expression was downregulated by two-fold following no dynamic strain (Fig. 2) compared to 10% dynamic strain, PGE_2 release showed no significant differences between strain levels (Fig. 2). No significant differences were found between superficial and deep zones for COX-2 expression or PGE_2 release.

Expression of COL-1 was not significantly affected by exposure to different magnitudes of dynamic strain for 2 h (Fig. 3). Aggrecan gene expression was, however, significantly downregulated approximately three-fold with no dynamic strain for both superficial and deep explants compared to 10% strain (Fig. 3). No significant differences between loading levels or superficial vs deep explants were found for TGFβ, TIMP-1 or TNFα (Fig. 4).

Discussion

This study was designed to investigate the response of meniscal explants to various magnitudes of dynamic compressive strain while measuring changes in gene expression of matrix molecules, as well as the expression of both catabolic and anabolic genes. The results suggest that 2 h of dynamic loading at 20% strain increase expression of catabolic enzymes involved in the degradation of proteoglycans, namely aggrecanases (ADAMTS4) and MMPs (MMP-1, MMP-3 and MMP-13) whereas 10% dynamic strain increases aggrecan, COX-2, MMP-9 and ADAMTS5 compared to no dynamic loading.

The role of aggrecanases and MMPs in mediating articular cartilage and fibrocartilage (intervertebral disc) degeneration has been well established. Goupille *et al.* and Jacques *et al.* suggest that MMP-13 is activated in the early stage of degeneration while MMP-1 is activated at later stages of OA[25,24]. This contradicts the findings of Aigner *et al.*, who showed no activation of MMP-1 in any stage of degeneration and significant increases in gene expression of MMP-13 in the late-stage of degeneration[40]. The influence of IL-1β in these studies is suggested, as Goupille *et al.* used intervertebral disc cells stimulated with IL-1 while Aigner *et al.* measured gene expression in osteoarthritic articular cartilage without any exogenous stimuli[25,40]. Recently Hennerbichler and others have shown that the meniscus may serve as an intra-articular source of pro-inflammatory mediators, specifically NO and PGE_2, and that alterations in the magnitude or distribution of joint loading could significantly influence the production of these mediators[37,38,41,42].

Previous computational studies by our group suggest that removing 30% or more or the meniscus increases the maximum compressive strains in the remaining meniscal tissue to approximately 20%[43], compared to 10% strain in the intact meniscus. Hence, dynamic strain values of 10% and 20% were compared in this study. It was expected that both underloading (0% dynamic strain) and overloading (20%) would increase catabolic gene expression compared to 10% dynamic strain. Overloading did result in increased gene expression for catabolic enzymes (MMPs and ADAMTS) compared to 10% dynamic strain. However, ADAMTS4 was the only enzyme upregulated following underloading compared to 10% strain, which may indicate that the period of no dynamic loading in this study (2 days) is insufficient to induce degradative pathways. Previous studies have shown that exposing meniscal explants to cyclic hydrostatic pressure for 4 h prevented upregulation of COX-2, IL-1, MMP-1, MMP-3, and iNOS that was seen in unloaded explants[44]. Djurasovic *et al.* showed that 4 weeks of immobilization in a canine model resulted in a decreased aggrecan gene expression in the menisci[45]. Further long-term studies are underway to investigate if long-term lack of dynamic loading adversely affects meniscal tissue composition and potential mechanisms.

The current study only considered gene expression following 24 h of post-incubation. Aigner *et al.* detected an increase in the expression of MMP-9 only in late-stages of OA[46]. Therefore, measurement of this gene following only 2 h of altered loading may not clearly demonstrate the

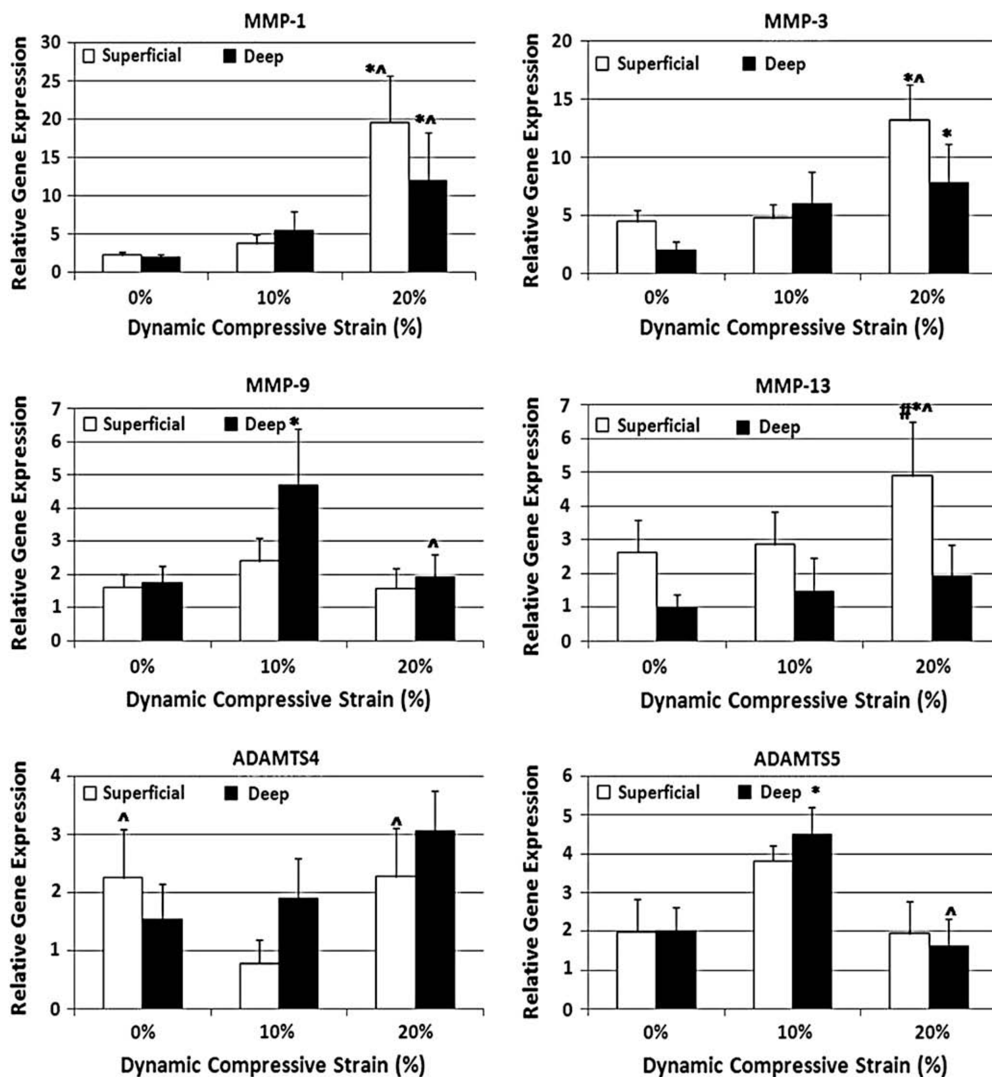

Fig. 1. Gene expression following 0%, 10% or 20% dynamic compressive strain administered for 2 h at 1 Hz relative to calibrator gene expression. Expression normalized to 18srRNA gene expression for each sample. (A) MMP-1, (B) MMP-3, (C) MMP-9, (D) MMP-13, (E) ADAMTS4, (F) ADAMTS5, $n = 6$. * Significantly different than 0% for the same zone; ^ significantly different than 10% for the same zone; and # significantly different than deep zone for same strain level.

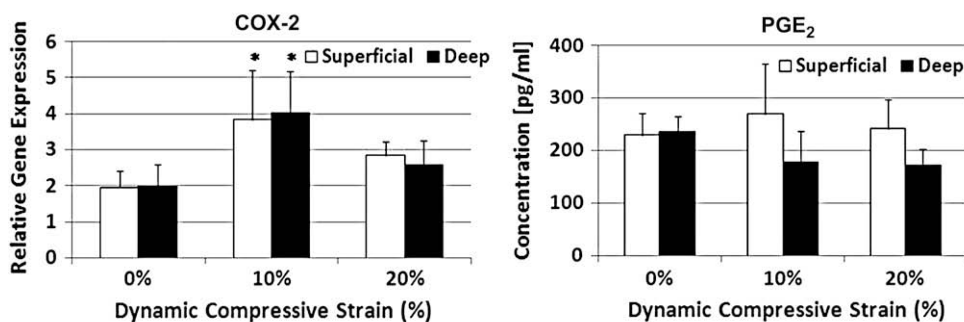

Fig. 2. (A) Gene expression of COX-2 relative to calibrator gene expression normalized to 18srRNA gene expression for each sample and (B) PGE_2 concentration in the conditioned media following dynamic compressive strains of 0%, 10% or 20%, $n = 6$. * Significantly different than 0% for the same zone.

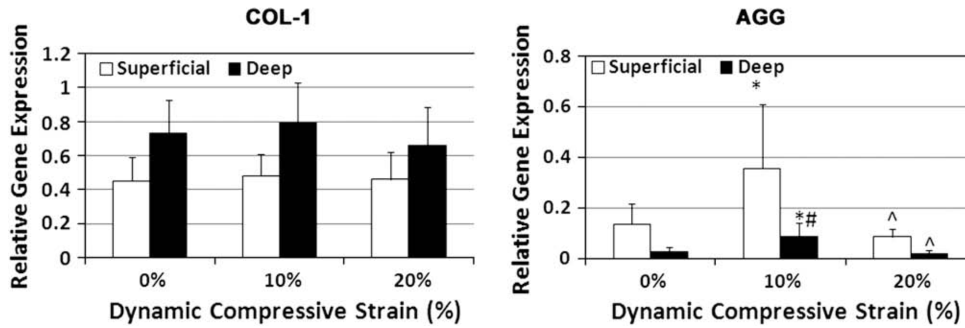

Fig. 3. Gene expression of A) COL-1 and B) aggrecan following dynamic compressive strains of 0%, 10% or 20% relative to calibrator gene expression. Expression normalized to 18srRNA gene expression for each sample. * Significantly different than 0% for the same zone; ^ significantly different than 10% for the same zone; and # significantly different than deep zone for same strain level.

potential role of this molecule in meniscal tissue degeneration. Similar to MMP-9, ADAMTS5 was downregulated following no dynamic compressive strain compared to 10% strain, whereas the remaining MMPs and ADAMTS4 were upregulated following overloading dynamic strain. As this study is a single time-point of 2 h of dynamic loading with 24 h post-incubation, it will be important to investigate the expression of such molecules over longer periods of time in order to better understand the temporal expression patterns following mechanical loading. Although increased COX-2 levels in OA joints have been demonstrated, the origin is still unclear. COX-2 can be produced by cartilage and/or the meniscus, or it may be released by other tissues in the knee joint like the synovium. It may also accumulate as a result of the immune response and be carried to the joint by blood circulation. In fact, Hardy *et al.* showed the highest concentration of PGE_2 paralleled with significant activation of COX-2/PGE synthase gene in cells from osteophytes compared to cells from other knee structures[14]. No significant changes in release of PGE_2 were detected in this study. Surprisingly, COX-2 was significantly downregulated under 0% and 20% strain for both the superficial and deep zone explants compared to 10% strain. This may be an artifact of the time-point used to quantify expression of COX-2. Previously, Dixon *et al.* have demonstrated the COX-2 mRNA half-life of human lung fibroblasts to be approximately 90 min[47]. In the present study, all genes and PGE_2 release were measured at the same post-incubation time point based on previous work by McHenry *et al.*[11] Previous observations of cytokine and anabolic gene expression profiles following mechanical loading demonstrated a maximum expression of ADAMTS4, MMP-3 and MMP-9, as well as COL-1 after 24 h of mechanical loading[17,48]. Nonetheless, changes in COX-2 expression, as well as other genes of interest may be influenced by both the *in vitro* approach and/or the choice in post-incubation time period.

Because the inner region of the meniscus is typically removed during partial meniscectomy, only outer meniscal

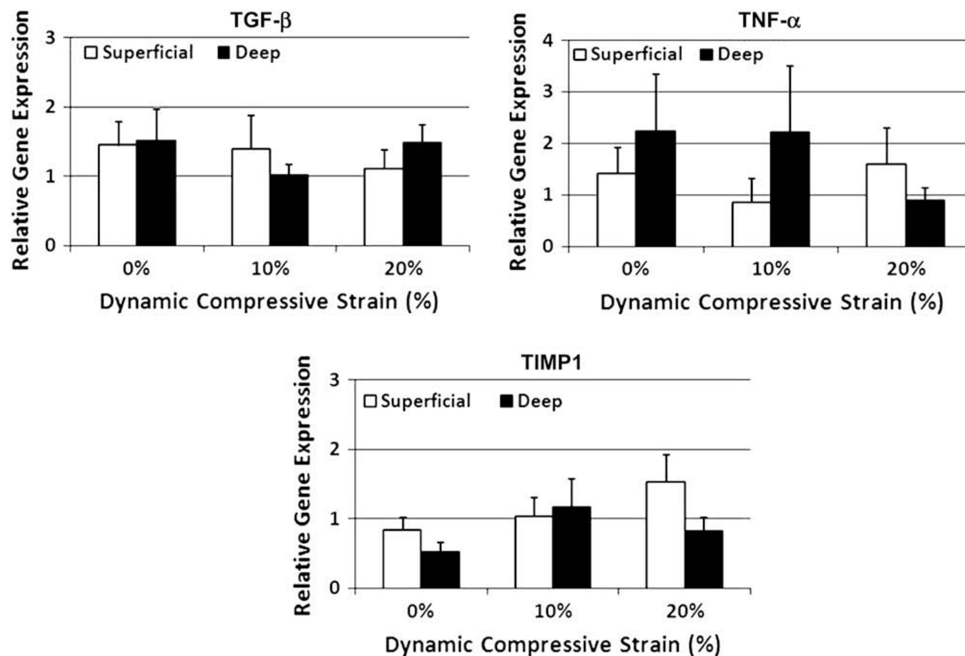

Fig. 4. Gene expression of A) TGFβ B) TNFα and C) TIMP-1 following dynamic compressive strains of 0%, 10% or 20% relative to calibrator gene expression. Expression normalized to 18srRNA gene expression for each sample. No statistical differences.

explants were studied. Meniscal tissue is comprised of at least three relatively distinct cell populations[49] and cells from different regions of the meniscus have been shown to exhibit varying cell morphology and gene expression[50–52]. As in previous experiments from our lab, there were limited statistical differences between superior and deep explants[11,12], possibly due to an inefficient separation of the different cell populations. Previous investigations have demonstrated differences between superficial and deep zones of the tissue following mechanical stimulation[53]. Additionally, the inner region has been shown to produce more NO and PGE_2 in response to mechanical stimulation than the outer region[37,38,41]. Hence, an explanation for the lack of statistical changes in PGE_2 production could be that only outer explants were used in this study. Future studies should include collection and compression of explants from both inner and outer regions, as well as immunostaining of explants to determine spatial expression and production of genes and molecules to account for potential ineffectiveness of isolation between different cellular populations.

Bisection of the explants into superior and deep zone has previously been shown to release many growth factors which may present misleading expression results[54]. However, all samples in this study were bisected, and therefore the relative differences are still significant. It is possible that the measured response may be due to both mechanical stimuli and cutting the samples, and absolute values should be interpreted with caution.

The potential for complete unloading of the explants may arise due to platen lift-off during dynamic compression. In this study, we measured loading stresses during compression and recovery of each cycle during the 2 h period. However, it is possible that platen lift-off from the explants occurred, especially during several of the initial cycles for 20% strain. Regardless, measured stress at or above the measured tare-load returned following roughly 5–10 cycles (data not shown). The recovery of the explants to original height during dynamic compression may vary depending on the native anatomical location of the explant. For example, *in vivo* MRI imaging of meniscal thickness reflects differences in rate of recovery following distance running in humans[55]. Variation in thickness recovery, and therefore duration of platen lift-off, may depend on rate of compression as well as whether the explant is of lateral or medial meniscal origin.

A finite element model of the knee has demonstrated that 20% axial strains may be indicative of strain levels seen following removal of 30% or more of the meniscus[43]. However, it is important to note this model used an intact meniscus, not an isolated meniscal explants, which may lead to different stresses due to lack of confinement of the surrounding tissue. The current study shows that strains of 20% initiate expression of several catabolic enzymes that have been previously found to degrade proteoglycans in the solid matrix of meniscal tissue[28,31,14]. Thus, the remaining meniscal tissue may be vulnerable to degeneration following certain partial meniscectomies. Future *in vivo* studies should investigate changes in morphology, gene expression, and matrix molecules of the remaining meniscal tissue following partial menisectomy. It is unknown if the gene expression found in this study would translate to protein expression and eventually a change in the material properties of the remaining meniscal tissue. This would likely further exacerbate the degeneration of the underlying articular cartilage.

This data, in conjunction with our previous studies, suggests that dynamic loading of 20% increases IL-1, NO, matrix metalloproteinases, and ADAMTS genes which could lead to meniscal tissue degeneration. It remains to be determined whether mechanically induced IL-1 in the meniscus is responsible for changes in gene expression.

Conflict of Interest

The authors have no conflict of interest.

Acknowledgement

The authors would like to acknowledge the Whitaker Foundation for funding this research under grant #RG-03-0047. The authors would like to thank Mr. Steve Krage from Mayo Foundation for supplying us with tissue.

References

1. Cox JS, Nye CE, Schaefer WW, Woodstein IJ. The degenerative effects of partial and total resection of the medial meniscus in dogs' knees. Clin Orthop Relat Res 1975;109:178–83.
2. Baratz ME, Fu FH, Mengato R. Meniscal tears: the effect of meniscectomy and of repair on intraarticular contact areas and stress in the human knee. A preliminary report. Am J Sports Med 1986;14(4):270–5.
3. Rangger C, Klestil T, Gloetzer W, Kemmler G, Benedetto LP. Osteoarthritis after arthroscopic partial meniscectomy. Am J Sports Med 1995; 23(2):240–4.
4. Schimmer RC, Breulhart KB, Duff C, Glinz W. Arthroscopic partial meniscectomy: a 12-year follow-up and two-step evaluation of the long-term course. Arthroscopy 1998;14(2):136–42.
5. Wyland DJ, Guilak F, Elliott DM, Setton LA, Vail TP. Chondropathy after meniscal tear or partial meniscectomy in a canine model. J Orthop Res 2002;20(5):996–1002.
6. Walker PS, Erkman MJ. The role of the menisci in force transmission across the knee. Clin Orthop 1975;109:184–92.
7. Fukubayashi T, Kurosawa H. The contact area and pressure distribution pattern of the knee. A study of normal and osteoarthrotic knee joints. Acta Orthop Scandanavia 1980;51(6):871–9.
8. Ahmed AM. A pressure distribution transducer for in-vitro static measurements in synovial joints. J Biomech Eng 1983;105(3):309–14.
9. Cao M, Stefanovic-Racic M, Georgescu HI, Miller LA, Evans CH. Generation of nitric oxide by lapine meniscal cells and its effect on matrix metabolism: Stimulation of collagen production by arginine. J Orthop Res 1998;16(1):104–11.
10. Kobayashi K, Mishima H, Hashimoto S, Goomer RS, Harwood FL, Lotz M, *et al.* Chondrocyte apoptosis and regional differential expression of nitric oxide in the medial meniscus following partial meniscectomy. J Orthop Res 2001;19:802–8.
11. McHenry JA, Zielinska B, Donahue TL. Proteoglycan breakdown of meniscal explants following dynamic compression using a novel bioreactor. Ann Biomed Eng 2006;34(11):1758–66.
12. Gupta T, Zielinska B, McHenry JA, Kadmiel M, Haut Donahue TL. IL-1 and inos gene expression and no synthesis in the superior region of meniscal explants is dependent on magnitude of compressive strains. Osteoarthritis Cartilage 2008;16(10):1213–9.
13. LeGrand A, Fermor B, Fink C, Pisetsky DS, Weinberg JB, Vail TP, *et al.* Interleukin-1, tumor necrosis factor alpha, and interleukin-17 synergistically up-regulate nitric oxide and prostaglandin E2 production in explants of human osteoarthritic knee menisci. Arthritis Rheum 2001; 44(9):2078–83.
14. Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, *et al.* Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum 2002;46(7):1789–803.
15. Blanco FJ, Ochs RL, Schwarz H, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Pathol 1995;146(1):75–85.
16. Clancy RM, Amin AR, Abramson SB. The role of nitric oxide in inflammation and immunity. Arthritis Rheum 1998;41(7):1141–51.
17. Sasaki K, Hattori T, Fujisawa T, Takahashi K, Inoue H, Takigawa M. Nitric oxide mediates interleukin-1-induced gene expression of matrix metalloproteinases and basic fibroblast growth factor in cultured rabbit articular chondrocytes. J Biochem (Tokyo) 1998;123(3): 431–9.
18. Imai K, Hiramatsu A, Fukushima D, Pierschbacher MD, Okada Y. Degradation of decorin by matrix metalloproteinases: identification of the cleavage sites, kinetic analyses and transforming growth factor-beta1 release. Biochem J 1997;322(Pt 3):809–14.

19. Vincenti MP, Brinckerhoff CE. Transcriptional regulation of collagenase (mmp-1, mmp-13) genes in arthritis: Integration of complex signaling pathways for the recruitment of gene-specific transcription factors. Arthritis Res 2002;4(3):157–64.
20. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. Mol Cell Biochem 2003; 253(1–2):269–85.
21. Janusz MJ, Little CB, King LE, Hookfin EB, Brown KK, Heitmeyer SA, *et al*. Detection of aggrecanase- and MMP-generated catabolic neoepitopes in the rat iodoacetate model of cartilage degeneration. Osteoarthritis Cartilage 2004;12(9):720–8.
22. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. Front Biosci 2006;11:529–43.
23. Majumdar MK, Askew R, Schelling S, Stedman N, Blanchet T, Hopkins B, *et al*. Double-knockout of ADAMTS-4 and ADAMTS-5 in mice results in physiologically normal animals and prevents the progression of osteoarthritis. Arthritis Rheum 2007;56(11):3670–4.
24. Jacques C, Gosset M, Berenbaum F, Gabay C. The role of IL-1 and IL-1ra in joint inflammation and cartilage degradation. Vitam Horm 2006; 74:371–403.
25. Goupille P, Jayson MI, Valat JP, Freemont AJ. Matrix metalloproteinases: the clue to intervertebral disc degeneration? Spine 1998; 23(14):1612–26.
26. Shingleton WD, Ellis AJ, Rowan AD, Cawston TE. Retinoic acid combines with interleukin-1 to promote the degradation of collagen from bovine nasal cartilage: matrix metalloproteinases-1 and -13 are involved in cartilage collagen breakdown. J Cell Biochem 2000;79(4):519–31.
27. Hedbom E, Hauselmann HJ. Molecular aspects of pathogenesis in osteoarthritis: the role of inflammation. Cell Mol Life Sci 2002;59(1):45–53.
28. Tortorella MD, Burn TC, Pratta MA, Abbaszade I, Hollis JM, Liu R, *et al*. Purification and cloning of aggrecanase-1: a member of the ADAMTS family of proteins. Science 1999;284(5420):1664–6.
29. Zeng W, Corcoran C, Collins-Racie LA, Lavallie ER, Morris EA, Flannery CR. Glycosaminoglycan-binding properties and aggrecanase activities of truncated ADAMTSS: comparative analyses with ADAMTS-5, -9, -16 and -18. Biochim Biophys Acta 2006;1760(3): 517–24.
30. Durigova M, Soucy P, Fushimi K, Nagase H, Mort JS, Roughley PJ. Characterization of an ADAMTS-5-mediated cleavage site in aggrecan in OSM-stimulated bovine cartilage. Osteoarthritis Cartilage 2008;16(10):1245–52.
31. Tortorella MD, Malfait AM, Deccico C, Arner E. The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation. Osteoarthritis Cartilage 2001;9(6):539–52.
32. Bau B, Gebhard PM, Haag J, Knorr T, Bartnik E, Aigner T. Relative messenger RNA expression profiling of collagenases and aggrecanases in human articular chondrocytes *in vivo* and *in vitro*. Arthritis Rheum 2002;46(10):2648–57.
33. Yamanishi Y, Boyle DL, Clark M, Maki RA, Tortorella MD, Arner EC, *et al*. Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. J Immunol 2002;168(3):1405–12.
34. Malfait AM, Liu RQ, Ijiri K, Komiya S, Tortorella MD. Inhibition of ADAM-TS4 and ADAM-TS5 prevents aggrecan degradation in osteoarthritic cartilage. J Biol Chem 2002;277(25):22201–8.
35. Pelletier JP, DiBattista JA, Roughley P, McCollum R, Martel-Pelletier J. Cytokines and inflammation in cartilage degradation. Rheum Dis Clin North Am 1993;19(3):545–68.
36. Martel-Pelletier J, Alaaeddine N, Pelletier JP. Cytokines and their role in the pathophysiology of osteoarthritis. Front Biosci 1999;4:D694–703.
37. Hennerbichler A, Fermor B, Hennerbichler D, Weinberg JB, Guilak F. Regional differences in prostaglandin E2 and nitric oxide production in the knee meniscus in response to dynamic compression. Biochem Biophys Res Commun 2007;358(4):1047–53.
38. Hennerbichler A, Moutos FT, Hennerbichler D, Weinberg JB, Guilak F. Interleukin-1 and tumor necrosis factor alpha inhibit repair of the porcine meniscus *in vitro*. Osteoarthritis Cartilage 2007;15(9): 1053–60.
39. Donati I, Stredanska S, Silvestrini G, Vetere A, Marcon P, Marsich E, *et al*. The aggregation of pig articular chondrocyte and synthesis of extracellular matrix by a lactose-modified chitosan. Biomaterials 2005; 26(9):987–98.
40. Aigner T, Hemmel M, Neureiter D, Gebhard PM, Zeiler G, Kirchner T, *et al*. Apoptotic cell death is not a widespread phenomenon in normal aging and osteoarthritis human articular knee cartilage: a study of proliferation, programmed cell death (apoptosis), and viability of chondrocytes in normal and osteoarthritic human knee cartilage. Arthritis Rheum 2001;44(6):1304–12.
41. Hennerbichler A, Moutos FT, Hennerbichler D, Weinberg JB, Guilak F. Repair response of the inner and outer regions of the porcine meniscus *in vitro*. Am J Sports Med 2007;35(5):754–62.
42. Wilusz RE, Weinberg JB, Guilak F, McNulty AL. Inhibition of integrative repair of the meniscus following acute exposure to interleukin-1 *in vitro*. J Orthop Res 2007.
43. Zielinska B, Haut Donahue TL. 3D finite element model of medial meniscus meniscectomy; changes in contact behavior. J Biomech Eng 2006;128(1):115–23.
44. Natsu-Ume T, Majima T, Reno C, Shrive NG, Frank CB, Hart DA. Menisci of the rabbit knee require mechanical loading to maintain homeostasis: cyclic hydrostatic compression *in vitro* prevents derepression of catabolic genes. J Orthop Sci 2005;10(4):396–405.
45. Djurasovic M, Aldridge JW, Grumbles R, Rosenwasser MP, Howell D, Ratcliffe A. Knee joint immobilization decreases aggrecan gene expression in the meniscus. Am J Sports Med 1998;26(3):460–6.
46. Aigner T, Fundel K, Saas J, Gebhard PM, Haag J, Weiss T, *et al*. Large-scale gene expression profiling reveals major pathogenetic pathways of cartilage degeneration in osteoarthritis. Arthritis Rheum 2006; 54(11):3533–44.
47. Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3′-untranslated region. J Biol Chem 2000;275(16): 11750–7.
48. Fitzgerald JB, Jin M, Dean D, Wood DJ, Zheng MH, Grodzinsky AJ. Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic amp. J Biol Chem 2004;279(19):19502–11.
49. Ghadially FN, Thomas I, Yong N, Lalonde JM. Ultrastructure of rabbit semilunar cartilages. J Anat 1978;125(3):499–517.
50. Upton ML, Chen J, Setton LA. Region-specific constitutive gene expression in the adult porcine meniscus. J Orthop Res 2006.
51. Spindler KP, Mayes CE, Miller RR, Imro AK, Davidson JM. Regional mitogenic response of the meniscus to platelet-derived growth factor (PDGF-AB). J Orthop Res 1995;13(2):201–7.
52. Hellio Le Graverand MP, Ou Y, Schield-Yee T, Barclay L, Hart D, Natsume T, *et al*. The cells of the rabbit meniscus: their arrangement, interrelationship, morphological variations and cytoarchitecture. J Anat 2001;198(Pt 5):525–35.
53. Fink C, Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Guilak F. The effect of dynamic mechanical compression on nitric oxide production in the meniscus. Osteoarthritis Cartilage 2001;1–8.
54. Vincent TL, Hermansson M, Bolton M, Wait R, Saklatvala J. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. Proc Natl Acad Sci U S A 2002;99(12):8259–88264.
55. Kessler MA, Glaser C, Tittel S, Reiser M, Imhoff AB. Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of *in vivo* investigation based on 3-dimensional magnetic resonance imaging. Am J Sports Med 2008;36(5):966–70.