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Dynamic Loading Enhances Integrative Meniscal Repair in the Presence of Interleukin-1

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Abstract

Objective—Meniscal tears are a common knee injury and increased levels of interleukin-1 (IL-1) have been measured in injured and degenerated joints. Studies have shown that IL-1 decreases the shear strength, cell accumulation, and tissue formation in meniscal repair interfaces. While mechanical stress and IL-1 modulate meniscal biosynthesis and degradation, the effects of dynamic loading on meniscal repair are unknown. The purpose of this study was to determine the effects of mechanical compression on meniscal repair under normal and inflammatory conditions.

Experimental Design—Explants were harvested from porcine medial menisci. To simulate a full-thickness defect, a central core was removed and reinserted. Explants were loaded for 4 hours/day at 1Hz and 0–26% strain for 14 days in the presence of 0 or 100pg/mL of IL-1. Media were assessed for matrix metalloproteinase (MMP) activity, aggrecanase activity, sulfated glycosaminoglycan (S-GAG) release, and nitric oxide (NO) production. After 14 days, biomechanical testing and histological analyses were performed.

Results—IL-1 increased MMP activity, S-GAG release, and NO production, while decreasing the shear strength and tissue repair in the interface. Dynamic loading antagonized IL-1-mediated inhibition of repair at all strain amplitudes. Neither IL-1 treatment nor strain altered aggrecanase activity. Additionally, strain alone did not alter meniscal healing, except at the highest strain magnitude (26%), a level that enhanced the strength of repair.

Conclusions—Dynamic loading blocked the catabolic effects of IL-1 on meniscal repair, suggesting that joint loading through physical therapy may be beneficial in promoting healing of meniscal lesions under inflammatory conditions.

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Conflict of interest

None of the authors have any financial or personal relationships with other people or organizations that influence this work.

Keywords

meniscus; tissue repair; mechanical loading; interleukin-1; matrix metalloproteinase; aggrecanase; nitric oxide

Introduction

The menisci are C-shaped fibrocartilaginous tissues situated between the femoral condyles and tibial plateau. These tissues are required for normal biomechanical function of the knee, including load bearing, shock absorption, joint congruity, and joint stability¹⁻³. The intact meniscus has been suggested to carry approximately 50% of the load applied to the joint⁴, resulting in approximately 10% strain in the medial meniscus⁵. Tears in the meniscus are a common knee injury with nearly 15% of all athletic knee injuries involving the meniscus⁶. In addition to the pain and loss of function associated with the initial meniscal injury, damage or loss of meniscal tissue is associated with progressive degenerative changes in the joint that ultimately lead to osteoarthritis (OA)⁷⁻¹¹. Long term studies have shown that 50% of patients with a meniscal injury develop OA within 10 – 20 years¹². Therefore, current clinical therapies for meniscal injury seek to preserve and repair damaged tissue.

The pathogenesis of osteoarthritic degeneration following knee injury appears to involve the anti-anabolic and pro-catabolic activity of inflammatory cytokines, such as interleukin-1 (IL-1), which are upregulated in injured and degenerated joints^{13, 14}. In patients without rheumatic disease or joint effusion, less than 20 pg/mL IL-1 β has been measured in the joint at necropsy¹⁵. On the other hand, in patients undergoing an arthroscopy following a meniscal tear, the concentration of synovial fluid IL-1 α is between 25 – 175 pg/mL¹⁶. IL-1 β levels are also elevated in patients with rheumatoid arthritis (RA; 115 pg/mL–193 pg/mL) and OA (21 pg/mL–146 pg/mL)^{15, 17}. Additionally, full thickness meniscal tears show elevated IL-1 α staining for at least 14 days¹⁸, suggesting that IL-1 α may be produced for several weeks after the initial meniscal tear.

IL-1 upregulation in the joint induces many degradative and pro-inflammatory pathways^{13, 14, 19, 20}. IL-1 increases the production of the inflammatory mediators nitric oxide (NO)²¹⁻²⁵ and prostaglandin E₂ (PGE₂)^{21, 24, 26} in a dose-dependent manner. Additionally, IL-1 upregulates matrix metalloproteinases (MMPs)^{23, 24, 27, 28}, increases proteoglycan release^{22, 24}, suppresses collagen synthesis²⁴, and upregulates tumor necrosis factor (TNF) expression²³ in meniscal cells and explants. IL-1 also upregulates aggrecanase-1 (ADAMTS-4) activity but not aggrecanase-2 (ADAMTS-5) activity in bovine cartilage explants²⁹ and porcine chondrocyte-agarose cultures^{30, 31}. Both acute³² and chronic^{33, 34} exposure to IL-1 inhibits integrative meniscal repair *in vitro* by decreasing the shear strength of repair and suppressing cell accumulation and tissue formation in the interface. In an explant model of meniscal repair, treatment with IL-1 receptor antagonist (IL-1ra)³³, MMP inhibitors²⁸, or the anabolic growth factor TGF- β ¹³⁵ has shown promise in overcoming some of the degradative effects of IL-1.

In addition to changes in the biochemical environment of the joint, trauma or arthritis can also alter the biomechanical environment of the joint. In articular cartilage, many studies have shown that mechanical stress in the joint is an important factor in the regulation of chondrocyte activity³⁶. The general consensus of these studies is that static compression suppresses matrix biosynthesis, while cyclic or intermittent loading at specific frequencies can stimulate chondrocyte metabolism (36 for review). However, little information is available regarding the response of meniscal cells to mechanical stress. Meniscal explants subjected to static compression showed decreased expression of type I and II collagen³⁷, increased MMP-1

expression³⁷, and decreased biosynthetic activity, as measured by ³⁵S-sulfate and ³H-proline incorporation³⁸. Dynamic mechanical compression increases proteoglycan and total protein synthesis²² and NO and PGE₂ production by meniscal explants^{39, 40}. Additionally 20% compressive strain of porcine explants caused increased expression of the following catabolic mediators: inducible nitric oxide synthase (NOS2), IL-1 α , MMP-1, MMP-3, MMP-13, and a disintegrin and metalloproteinase with thrombospondin 4 (ADAMTS-4)^{41, 42}. On the other hand, in the presence of IL-1, mechanical compression inhibits NO production²². Biaxial cyclic tensile stretch of isolated meniscal cells inhibited the catabolic and pro-inflammatory effects of IL-1^{23, 43} by inhibiting NOS2, MMP expression, and NO production. Biaxial stretch also counteracted IL-1-dependent stimulation of NOS2, RANK, and RANKL in meniscal cells, potentially via inhibition of NF- κ B^{23, 43}. These findings suggest that mechanical loading can overcome many of the inflammatory effects of cytokines on meniscal cells; however, the effects of mechanical compression on repair of meniscal lesions are not known.

The goal of this study was to investigate the effects of dynamic mechanical compression on meniscal repair in the presence or absence of the inflammatory cytokine IL-1. We hypothesized that mechanical compression at lower magnitudes (<10%) would enhance meniscal repair under both normal and inflammatory conditions, but higher magnitudes (>10%) of dynamic compression would inhibit repair. We used an *in vitro* model system^{28, 32-34, 44} to examine the effects of 0–26% dynamic compressive strain on the integrative repair of meniscal explants treated either with or without IL-1. Explants were dynamically loaded for 14 days and the media was assessed for MMP activity, aggrecanase activity, sulfated glycosaminoglycan (S-GAG) release, and NO production. Meniscus healing was investigated by mechanical testing of the explants to determine the interfacial shear strength, and histology was performed to visualize tissue repair.

Materials and methods

MENISCAL REPAIR MODEL SYSTEM

An explant model system was used to study integrative repair of the meniscus⁴⁴. Medial menisci were harvested from 2–3 year old skeletally mature female pig knees obtained from a local abattoir. Cylindrical explants were harvested from the femoral surface of the middle one-third of the meniscus, using a 5 mm biopsy punch (Miltex, Inc, York, PA) oriented perpendicular to the meniscal surface. Using a custom built cutting device, explants were cut to an average thickness of 2.4 mm. A central 3 mm core was punched completely through each explant, using a biopsy punch (Miltex, Inc), and immediately reinserted in the same orientation to simulate a full thickness meniscal tear. Explants were placed in Costar ultra-low attachment surface 24 well plates (Corning Inc.; Corning, NY) in the center of polyacetal retaining rings. Samples were incubated in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA), containing 1000 U/mL penicillin/streptomycin (Invitrogen) for 1 hour at 37° C. This medium was removed, and explants were cultured in DMEM containing 10% heat inactivated fetal bovine serum (HyClone, Logan, UT), 0.1 mM non-essential amino acids (Invitrogen), 10 mM HEPES buffer solution (Invitrogen), 100 U/mL penicillin/streptomycin, and 37.5 μ g/mL L-ascorbic acid 2-phosphate (Sigma-Aldrich, St. Louis, MO). Explants were cultured in a mechanical compression bioreactor for 14 days at 37°C and 5% CO₂. Media were collected and replaced every 3 days.

MECHANICAL COMPRESSION BIOREACTOR

A closed-loop displacement controlled bioreactor was designed and manufactured to apply dynamic, unconfined compressive deformational loading to 24 specimens simultaneously. The instrument consists of a rotary stepper motor (Oriental Motor, Torrance, CA) set to microstep at 4000 steps/rev (0.09°/step) with an optical encoder for closed-loop feedback control. The

stepper motor drives a linear stage (Danaher Motion, Wood Dale, IL) that is connected to a loading platform housing 24 individual polyacetal pistons. The pistons were designed such that the posts slip freely through the loading platform if not locked in place, ensuring that all pistons are in contact with their respective explant prior to being locked to the plate. The design of the instrument and the written code (LabView 6.1, National Instruments, Austin, TX) allow for precise displacement of the loading platform with sub-micron resolution.

Explants ($n \geq 9$ per treatment group, each from different porcine menisci) were cultured in the mechanical compression bioreactor at 37°C/5% CO₂ and loaded sinusoidally at 1 Hz to strains of 1% ± 0.2% (mean ± standard deviation), 10% ± 1%, or 26% ± 4% for 4 hours/day for 14 days. Unstrained samples were cultured in the bioreactor in parallel with strained samples; however, the pistons were locked into place approximately 1 mm above the explants. For each strain, explants received either control media or media containing 100 pg/mL recombinant porcine IL-1 α (R & D Systems, Minneapolis, MN).

MMP ACTIVITY ASSAY

Total specific MMP activity was measured in media samples from day 3 using the quenched fluorogenic substrate Dab-Gly-Pro-Leu-Gly-Met-Arg-Gly-Lys-Flu (New England Peptide; Gardner, MA) as described previously^{28, 32, 45}.

AGGRECANASE ACTIVITY ASSAY

Aggrecanase activity was measured in media samples from day 3, using a commercially available fluorescent substrate (Anaspec; San Jose, CA) specific for ADAMTS-4, as described previously⁴⁶. Samples were run in duplicate and incubated at 37°C for 30 minutes in the dark. Fluorescence was measured at 340 nm excitation and 405 nm emission. The data is expressed as fluorescence units (measured fluorescence minus the fluorescence in the blank media) corrected for the wet weight of the tissue. Recombinant human ADAMTS-4 (5 μ g/mL; Anaspec) was included as a positive control for the assay. Additionally, porcine cartilage explants that were treated for 72 hours with 1 ng/mL IL-1 α were used to confirm cross-reactivity of the substrate with porcine ADAM-TS4.

S-GAG RELEASE

Total S-GAG release was measured in day 3 media, using the 1,9-dimethylmethylene blue (DMB) assay⁴⁷. For this assay, standards ranging from 0 – 100 μ g/mL bovine trachea chondroitin-4-sulfate type A (Sigma, St. Louis, MO) were prepared in control media. Forty microliters of standards and samples and 125 μ l/well of DMB reagent were added to a 96 well plate in duplicate. Absorbance was read at 540 nm within 5 min of DMB addition and the total μ g of S-GAG released from each sample was corrected for the wet weight of the tissue.

NO RELEASE

Total NO production was assessed by measuring the concentration of total nitrate and nitrite (or NO_x) in day 3 media, as detailed previously⁴⁸. All media samples and standards (0 – 320 μ M NaNO₃ in culture media) were filtered through Micron Ultracel YM-10 filters (Millipore, Bedford, MA) and diluted 1:2 in dH₂O to eliminate interference of protein and media components. All nitrate was then converted to nitrite, using nitrate reductase (Roche Diagnostics; Mannheim, Germany), and total nitrite concentrations were determined using the Greiss reagent. Absorbance was read at 540 nm and total μ mol NO_x were normalized to the wet weight of the tissue.

BIOMECHANICAL TESTING

Interfacial shear strength of repair between the inner core and outer ring of meniscal repair model explants was determined after 14 days in culture, using a push-out test as described previously⁴⁴. The peak force at failure was normalized by the area of the interface between the inner core and outer ring to determine interfacial shear strength. Measured sample thickness was used to determine the actual surface-to-surface compressive strain applied to each explant during deformational loading.

HISTOLOGICAL STAINING

Meniscal repair model explants were processed for histology after 14 days in culture. Samples were fixed overnight in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA), containing 100 mM sodium cacodylate trihydrate (Electron Microscopy Sciences) at pH 7.4 and 4°C. Samples were dehydrated in EtOH, infiltrated with xylene, and paraffin embedded. Sections were stained with Harris Hematoxylin with glacial acetic acid (Poly Scientific, Bay Shore, NY), 0.02% aqueous fast green (Sigma-Aldrich), and Accustain Safranin O solution (Sigma-Aldrich).

STATISTICAL ANALYSES

Statistical analyses were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK). A two-factor ANOVA and the Newman-Keuls *post hoc* test were performed to determine significant differences ($\alpha=0.05$) and the interactive effect of strain and IL-1. The relationship between interfacial shear strength and actual applied strain was determined using linear regression.

Results

MMP activity in the media of meniscal repair model explants was increased by IL-1 treatment in the absence of strain (Figure 1; $p < 0.0005$). However, application of strain at 1%, 10%, and 26% blocked the increase in MMP activity due to IL-1 ($p < 0.05$). Inhibition of IL-1 mediated MMP activity was most pronounced at lower strains ($p < 0.0005$ for 1% and 10%), as compared to 26% strain ($p < 0.05$). Additionally at both 1% and 10% strains, there was an interactive effect of strain and IL-1 on MMP activity ($p < 0.001$). In the absence of IL-1, there was no change in MMP activity at any of the strains tested.

Aggrecanase activity was measurable in all treatment groups (Figure 2). However, neither IL-1 treatment nor strain altered aggrecanase activity, as compared to control samples. The combination of IL-1 and strain also did not affect aggrecanase activity.

In the absence of strain, S-GAG release into the media was enhanced approximately 5-fold by IL-1 treatment (Figure 3; $p < 0.0005$). Strains of both 1% and 10% were effective in reducing IL-1 mediated release of S-GAG ($p \leq 0.005$). However, 26% strain had no effect on the S-GAG release in the presence of IL-1. At both 1% and 10% strains there was an interactive effect of strain and IL-1 ($p < 0.05$). For all strains there was a significant effect of IL-1 that contributed to S-GAG release into the media from the meniscal repair explants ($p < 0.00001$). None of the strains tested altered the S-GAG release into the media in the absence of IL-1.

NO release into the media was increased by IL-1 treatment in the absence of strain (Figure 4; $p < 0.0005$). However, all tested strains reduced the IL-1 mediated NO release ($p < 0.005$). At all strains, there was an interactive effect of strain and IL-1 that contributed to NO release into the media ($p < 0.05$). In the absence of IL-1, none of the strains tested altered the NO release into the media from meniscal repair explants.

In the absence of strain, IL-1 decreased the integrative shear strength of repair in meniscal repair model samples (Figure 5; $p < 0.05$). Dynamic compression at 1%, 10% or 26% strain increased the shear strength of IL-1 treated explants to strengths that were comparable to that measured in unstrained explants ($p \leq 0.01$). Additionally, there was a positive correlation between increasing shear strength in the presence of IL-1 and actual applied strain ($r^2 = 0.25$; $p < 0.00005$). At 1% and 26% strains, there was a significant effect of cytokine ($p < 0.05$) with an average 47 % decrease in strength of repair, and at all strains there was a significant effect of loading ($p < 0.005$) that contributed to the integrative shear strength of repair. In the absence of IL-1, at 26% strain, there was a 2-fold increase in the shear strength of repair compared to unstrained samples ($p < 0.005$), but there was no effect of either 1% or 10% strains alone. Furthermore, there was also a positive correlation between increasing shear strength of repair and actual applied strain ($r^2 = 0.14$, $p < 0.005$).

Histological analysis of unstrained and all strained meniscal repair model explants revealed healing of the meniscal defect at the interface over 14 days (Figure 6). The interface was filled with an extracellular matrix that stained strongly with fast green, revealing the presence of collagen fibers, and moderately with safranin O, indicating the presence of proteoglycans. On the other hand, no visible tissue repair was detected in IL-1 treated explants that were not strained. Additionally, IL-1 treatment resulted in loss of proteoglycan staining in the meniscal extracellular matrix. However, IL-1 treated explants that received 1%, 10%, or 26% strain showed increased tissue repair at the interface.

Discussion

Our results demonstrate that dynamic mechanical loading can overcome some of the degradative effects of IL-1 on meniscal repair. Pathophysiologic concentrations of IL-1 caused increases in MMP activity, S-GAG release, NO production, decreases in tissue formation in the interface, and consequently decreases in the shear strength of repair. However, dynamic compression at 1%, 10%, or 26% strain blocked the IL-1 mediated inhibition of meniscal repair. Interestingly, mechanical loading under noninflammatory conditions did not alter the healing of meniscal lesions. These findings support our hypothesis that, in an inflammatory environment, meniscal repair was enhanced by low magnitudes of dynamic loading, but refute our hypothesis that high magnitudes of strain would inhibit meniscal repair. Additionally, our results refute the hypothesis that dynamic loading enhances repair under control (non-inflammatory) conditions, with the exception of the highest applied strain, a level that led to increased integrative shear strength of repair in the absence of IL-1. Our findings suggest that dynamic loading of the knee following a meniscal injury may be a beneficial physical therapy regime to promote healing of meniscal lesions and to combat the degradative effects of inflammation. However, further studies are necessary to translate this work *in vivo* and to elucidate the exact nature of the therapy.

The effects of biomechanical factors on cartilaginous tissues have been shown to be dependent on the type of load, magnitude, and frequency of loading. It has been estimated that *in situ* compressive strains range from approximately 10 – 15% in normal menisci⁴ and increase to 20% following 30 – 60% meniscectomy⁵. Using a similar compression device, McHenry *et al.* have shown that the application of 0.05 MPa or 0.1 MPa of dynamic compressive stress to porcine meniscal explants resulted in final measured strains of 10% or 20%, respectively⁴⁹. At higher magnitudes of loading, meniscal explants appear to exhibit a pro-inflammatory response, characterized by increased production of NOS2 and prostaglandin E2⁴⁰. Thus, to examine a range of physiologic and hyper-physiologic conditions, we assessed the effects of dynamic compression at strains ranging from 0 – 26% at a frequency of 1 Hz.

In this study, loading of meniscal repair explants in the absence of IL-1 had no effect on MMP activity. Experiments on isolated chondrocytes and fibrochondrocytes have also shown that 6% cyclic tensile strain alone does not alter MMP activity^{50, 51}. Other studies in superficial porcine meniscal explants have shown that expression of MMP-1, MMP-3, and MMP-13 was increased by 2 hours of 20% strain⁴². Additionally, bovine cartilage explants loaded at 0.5 MPa and 1 Hz for 3 hours showed increased MMP-2 and MMP-9 expression, activation, and activity⁵². Dynamic compression of porcine tibial plateau cartilage explants at 0.1 MPa and 0.5 Hz for 6 hours increased expression of both MMP-3 and its inhibitor the tissue inhibitor of metalloproteinase 2 (TIMP-2)⁵³. Differences in our results and previous studies may be due to intrinsic tissue differences, duration of loading, type of loading, actual applied strain, frequency, and/or assessed outcome measures (enzyme activity versus gene expression).

In this study, dynamic compressive loading blocked IL-1 mediated increases in MMP activity in the media of meniscal repair model explants. In agreement with our results, 2% cyclic biaxial tensile strain of isolated rheumatoid synovial cells reduced the mRNA and protein levels of MMP-1 and MMP-13 and decreased the activity of these enzymes in the presence of IL-1 β and use of a transcriptional inhibitor suggested that mechanical loading reduced the activity of the MMPs at a post-transcriptional level⁵⁴. Biaxial cyclic tensile strain has also been shown to antagonize IL-1 β effects in chondrocytes and temporomandibular fibrochondrocytes by reducing IL-1-mediated increases in MMP-1 mRNA and protein levels and increasing TIMP-2 expression^{50, 51}. Loading-induced decreases in MMP expression and increases in TIMP expression are consistent with our results demonstrating a net decrease in MMP activity with loading in the presence of IL-1. However, the specific MMPs involved in IL-1 mediated degradation of meniscal repair explants are currently unknown. In a previous study, we have shown that the inhibition of individual MMPs was not sufficient to overcome the effects of IL-1; however, there was no specific inhibitor of MMP-1 activity available at the time²⁸. In contrast, broad spectrum inhibition of MMP activity increased repair in the presence of IL-1. Thus either MMP-1 is driving the degradation due to IL-1 or a combination of MMPs are necessary to mediate these effects.

IL-1 increased S-GAG release into the media, presumably through the upregulation of MMPs, aggrecanases, and other degradative enzymes. ADAMTS-4 activity was not upregulated by IL-1, suggesting that this enzyme is not mediating the increase in S-GAG release. Our data is consistent with a recent study in immature bovine meniscus showing that inhibition of MMPs but not ADAMTS-4 and ADAMTS-5 was effective at blocking IL-1 mediated release of S-GAGs and decreases in mechanical properties⁵⁵. However, bovine meniscus has been shown to upregulate aggrecanases in response to TNF- α treatment⁵⁶. Previous studies have shown that ADAMTS-4 is the major aggrecanase upregulated by IL-1 in porcine chondrocytes³⁰. Therefore, in the presence of IL-1, MMPs are likely the predominant degradative enzymes causing the release of S-GAGs. However, it is possible that other aggrecanases or degradative enzymes may also be involved in the IL-1 mediated catabolism of the extracellular matrix.

Physiologic magnitudes (1% or 10%) of dynamic compressive strain blocked the IL-1-mediated release of S-GAG, while hyperphysiologic strain (26%) did not have a significant effect on GAG release in the presence of IL-1. High magnitudes of strain have previously been shown to be catabolic⁵⁷, suggesting that 26% strain may be activating additional pathways that prevent loading from antagonizing the IL-1 mediated release of S-GAGs. Other studies have shown that 10% dynamic strain at 1 Hz for 3 hours/day of young bovine chondrocyte-seeded agarose constructs was not sufficient to block the IL-1 induced GAG loss⁵⁸. The differences in our results are likely due to assessment of meniscal explants versus isolated chondrocytes in agarose. In our study, dynamic loading alone had no effect on S-GAG release into the media. However, other studies have shown that 2 hours of 20% strain at 1 Hz is sufficient to increase S-GAG release from porcine meniscal explants⁴⁹. This difference may

be due to the short term loading regime (only 1 day for 2 hours versus 14 days for 4 hours/day), as previous studies have shown that under normal culture conditions, meniscus explants show significant loss of S-GAG, which may obscure additional increases caused by mechanical loading or other stimuli³³.

Dynamic loading of meniscal repair explants also had no effect on NO release. Experiments performed by Agarwal and colleagues using a variety of different cell types have consistently shown no effect of dynamic tensile strain on NOS2 expression^{23, 51} or NO production⁵⁰. Furthermore, porcine meniscal explants subjected to 0 – 20% strain have shown no change in NO release⁴¹. However, other studies have shown conflicting results from decreased NO release by 15% strain of chondrocyte-agarose constructs^{59, 60} to increased NO production by porcine meniscal explants subjected to dynamic compressive stresses of 0.0125 – 0.5 MPa at 1 Hz for 24 hours^{39, 40}. These differences are likely attributable to the different model systems and magnitudes of the applied stress or strain, particularly the overall duration of loading. In this study, dynamic loading suppressed the IL-1-mediated upregulation of NO release. In agreement with our results, many other studies have also demonstrated that dynamic compression and dynamic strain antagonize IL-1-induced NOS2 expression^{23, 51} and production of NO^{22, 50, 59, 60}. Strain-induced suppression of the catabolic effects of IL-1 prevents degradation of meniscal extracellular matrix following meniscal injury, perhaps providing an opportunity for the intrinsic repair capacity of the tissue^{33, 34} to heal the meniscal lesion.

Interestingly, loading alone increased the shear strength of repair at the highest strain tested. In addition, the strain applied to each meniscal repair explant correlated with increased shear strength, suggesting that higher strains would be most beneficial to promote integrative meniscal repair. In the absence of strain, meniscal explants exhibited an intrinsic repair response that is consistent with previous studies in meniscus^{33, 44, 61} and articular cartilage^{62–65}. The integrative repair of cartilage is dependent upon the migration and proliferation of viable cells into the defect⁶² and cell-mediated biosynthesis, including collagen production^{62, 64} and cross-linking⁶⁵.

Many studies have analyzed the effects of mechanical loading and/or IL-1 on the expression and synthesis of both collagen and proteoglycans with mixed results. In explants of cartilage or meniscus, dynamic loading alone has been shown to have a wide range of effects from no change in aggrecan transcript levels³⁷ or proteoglycan synthesis^{50, 51} to upregulation in their expression^{42, 53, 59} and synthesis^{60, 66}. A similar range of effects have been reported with respect to collagen. Dynamic loading alone has been shown to cause no change in collagen I mRNA levels⁴², decreased type II collagen transcript levels³⁷, no effect on collagen II transcription^{51, 59}, increased collagen II expression⁵³, and enhanced [³H]-thymidine incorporation^{60, 66}. However, in the presence of IL-1, dynamic loading is generally anabolic, resulting in an overwhelming increase in expression⁵⁹ and synthesis of aggrecan^{50, 51, 60, 66}. For collagen, dynamic loading in the presence of IL-1 had no effect on collagen II transcript levels in one experiment⁵⁹, but showed an increase in collagen II expression⁵¹ and [³H]-thymidine incorporation^{60, 66} in several other studies. All of these results suggest that loading alone can have disparate effects depending on the experimental parameters.

In this study, we demonstrated that dynamic loading blocks the pro-inflammatory effects that are mediated by IL-1 and promotes integrative meniscal repair in an inflammatory microenvironment. Mechanical loading of articular cartilage can also inhibit IL-1 induced matrix degradation⁶⁷. Interestingly, mechanical loading of meniscal repair model explants was more effective than IL-1ra³³, MMP inhibitors²⁸, or the anabolic growth factor TGFβ1³⁵ in promoting integrative meniscal repair in the presence of IL-1. The mechanism(s) by which dynamic loading antagonizes the effects of IL-1 need to be investigated further. Mechanical

loading may regulate IL-1 expression by meniscal fibrochondrocytes. A slight induction of IL-1 α but not IL-1 β transcript levels have been measured in porcine menisci subjected to 20% strain⁴¹ but cyclic hydrostatic pressure suppresses IL-1 β expression in rabbit menisci⁶⁸. It is also possible that mechanical loading caused a downregulation in the IL-1 type I receptor and/or an upregulation in the type II IL-1 decoy receptor^{66, 69}. Under 50% static compression, IL-1ra blocks decreases in cartilage explant proteoglycan synthesis⁷⁰, suggesting a role for the IL-1 receptors in mediating the effects of load. Preloading for 1 hour with cyclic tensile strain was unable to significantly decrease NOS2 expression and NO production in chondrocytes treated with IL-1, suggesting that strain alone is unable to downregulate the cell surface IL-1 receptors⁵¹. However, these experiments did not directly assess receptor number and did not address the idea that a synergism between mechanical loading and IL-1 may be required to diminish the cell surface receptors. Alternatively, mechanical loading may regulate IL-1 signaling mediators that are downstream of the IL-1 receptor, such as NF- κ B⁵⁷, RANK, and RANKL⁴³. Dynamic loading and IL-1 have been shown to be anti-inflammatory by suppressing not only the catabolic effects of IL-1 but also by diminishing expression of other pro-inflammatory factors, such as TNF- α ²³.

In summary, our data suggest a beneficial effect of dynamic loading in promoting repair of meniscal lesions in an inflammatory microenvironment, such as a knee joint following meniscal injury, meniscal repair surgery, or arthritis. Our data further suggest the potential usefulness of physical rehabilitation treatment for meniscal lesions, but more research is needed to fully elucidate the mechanism(s) of IL-1 inhibition and translation of this work into clinical practice.

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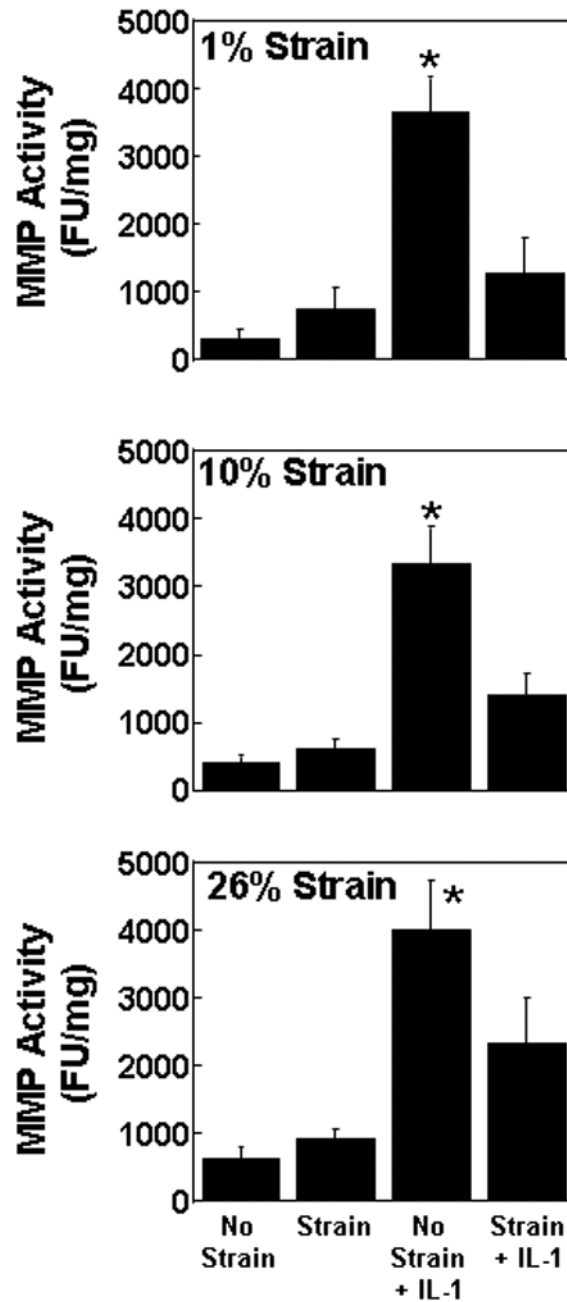


Fig. 1. Matrix metalloproteinase (MMP) activity in the media of meniscal repair model explants that were exposed to no strain, strain, no strain + IL-1, or strain + IL-1. The strains tested were 1% (top panel), 10% (middle panel), and 26% (bottom panel). The MMP activity is expressed as fluorescence units (FU) per mg wet weight + standard error. *: $p < 0.05$ compared to all other treatments.

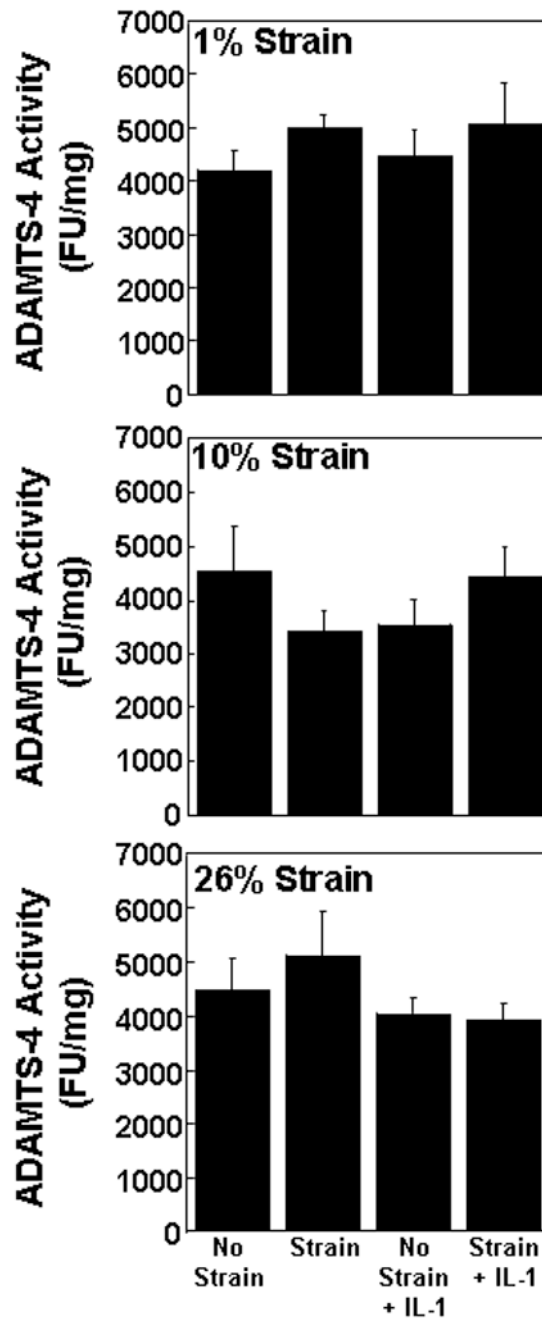


Fig. 2. ADAMTS-4 activity in the media of meniscal repair model explants that were exposed to no strain, strain, no strain + IL-1, or strain + IL-1. The strains tested were 1% (top panel), 10% (middle panel), and 26% (bottom panel). The aggrecanase activity is expressed as fluorescence units (FU) per mg wet weight + standard error.

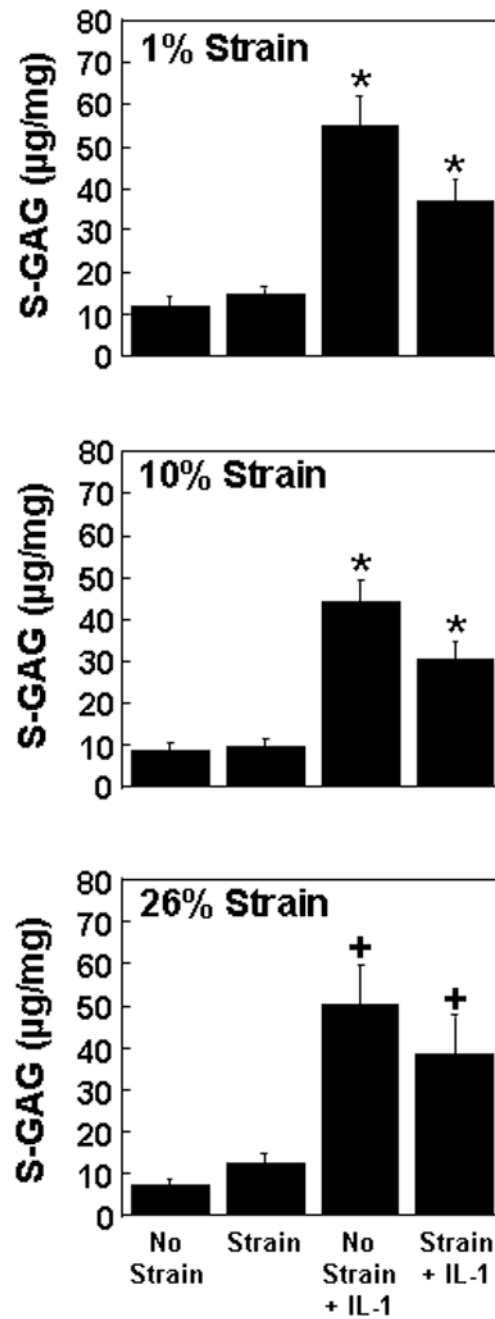


Fig. 3. Sulfated glycosaminoglycan (S-GAG) release into the media of meniscal repair model explants that were exposed to no strain, strain, no strain + IL-1, or strain + IL-1. The strains tested were 1% (top panel), 10% (middle panel), and 26% (bottom panel). The S-GAG release is expressed as µg S-GAG per mg wet weight + standard error. *: $p \leq 0.005$ compared to all other treatments. +: $p < 0.01$ compared to no strain and strain.

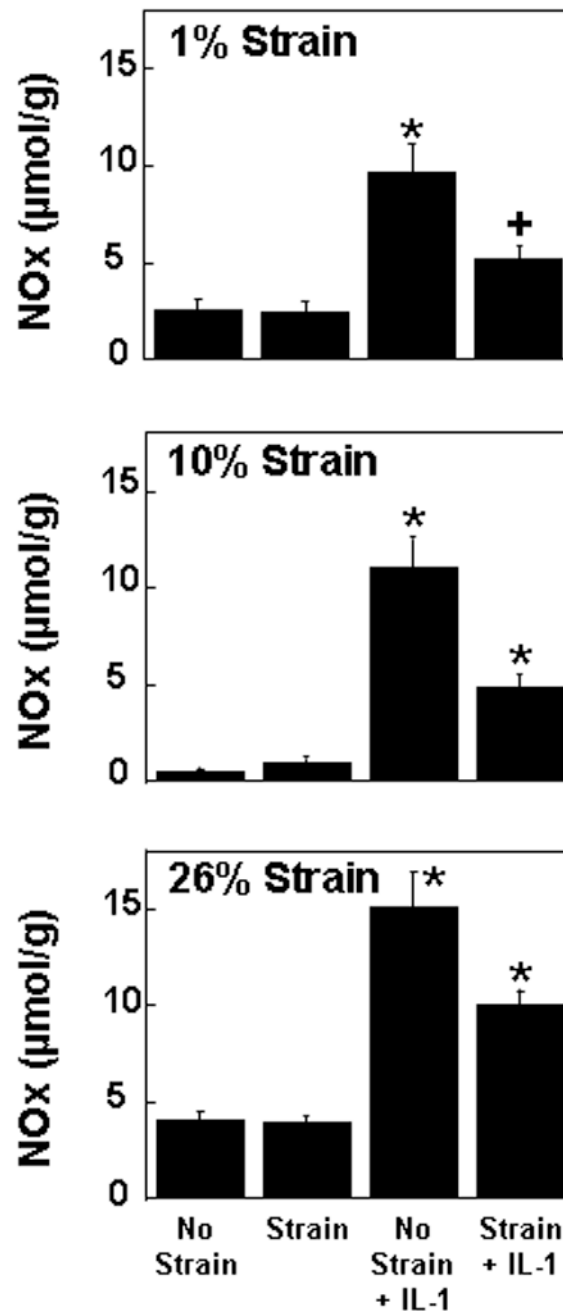


Fig. 4. Nitric oxide (NO) release into the media of meniscal repair model explants that were exposed to no strain, strain, no strain + IL-1, or strain + IL-1. The strains tested were 1% (top panel), 10% (middle panel), and 26% (bottom panel). The NO release is expressed as μmol per g wet weight + standard error. *: $p < 0.005$ compared to all other treatments. +: $p < 0.05$ compared to no strain.

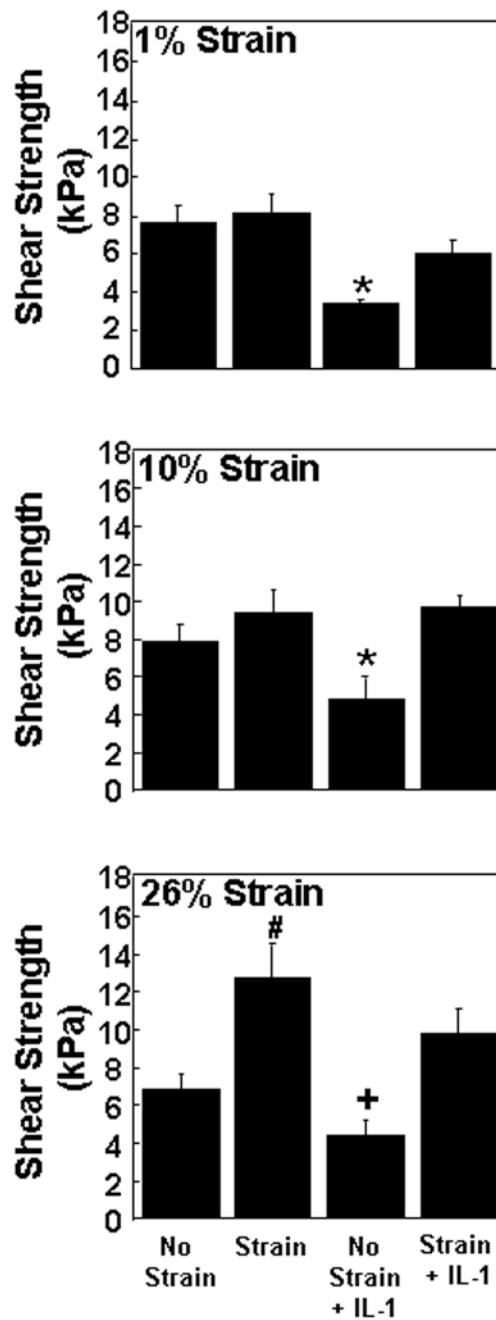


Fig. 5. Integrative shear strength of repair for meniscal repair model explants that were exposed to no strain, strain, no strain + IL-1, or strain + IL-1. The strains tested were 1% (top panel), 10% (middle panel), and 26% (bottom panel). The shear strength of repair is expressed as kPa + standard error. *: $p < 0.05$ compared to all other treatments. #: $p < 0.005$ compared to no strain and no strain + IL-1. +: $p < 0.01$ compared to 26% strain + IL-1.

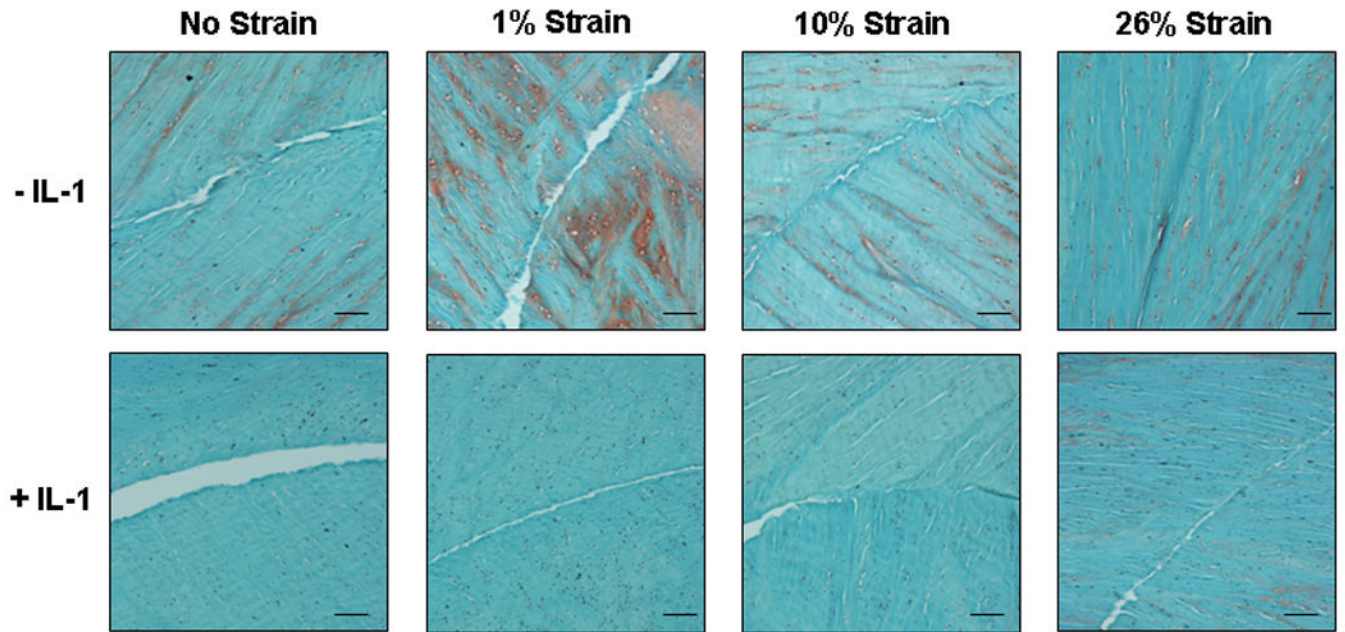


Fig. 6. Histological images of paraffin embedded meniscal repair model explants stained with hematoxylin to identify the cell nuclei (black), fast green (green) to identify the collagen fibers, and safranin O (red) to stain proteoglycans. Samples were incubated with no IL-1 or 100 pg/mL IL-1 and subjected to 0%, 1%, 10%, or 26% strain. Scale bar is equal to 100 μ m.