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Cells from Degenerative Intervertebral Discs Demonstrate Unfavorable Responses to Mechanical and Inflammatory Stimuli: A Pilot Study

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Abstract

Objective—Mechanical forces and inflammatory signaling influence intervertebral disc matrix homeostasis. We hypothesized that annulus fibrosus cells from degenerative discs would have altered responses to mechanical and inflammatory stimuli compared with cells isolated from normal discs.

Design—Annulus fibrosus cells were isolated from New Zealand White rabbits with normal and magnetic resonance imaging-confirmed degenerative discs created by annular stab. Cells were cultured with and without inflammatory and mechanical stimuli (tensile strain). After 4 or 24 hrs, the mRNA expression of inflammatory, catabolic, and anabolic genes was measured by reverse transcription polymerase chain reaction.

Results—Baseline gene expression differences were noted between cells from normal and degenerative discs. Degenerative cells demonstrated a more proinflammatory response profile to inflammatory and mechanical stimuli and loss of the beneficial effects of mechanical signaling. Decreased expression of catabolic and anabolic genes was observed in degenerative cells under conditions of inflammatory and mechanical stimuli.

Conclusions—These data demonstrate that degenerative cells have a decreased capacity to respond positively to beneficial levels of mechanical strain and demonstrate an exaggerated response to an inflammatory stimulus. This may, in part, help to explain differential responses to motion-based therapies in patients with intervertebral disc degeneration.

Keywords

Intervertebral Disc; Degeneration; Tensile Strain; Inflammation; Gene Expression

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Intervertebral disc degeneration (IDD) and its sequelae are among the most common reasons for chronic back pain.¹ IDD is characterized by progressive loss of extracellular matrix proteoglycans, causing disc dehydration, alterations in disc structure, and impaired disc function.² A critical component in IDD is the imbalance of the synthesis and degradation of extracellular matrix components, which involves inflammatory cytokines and matrix proteases, both of which are regulated by mechanical loading.³ Current nonoperative treatment for disc degeneration and associated axial low back pain addresses two main contributors to degeneration and matrix loss: the inflammatory component (via nonsteroidal anti-inflammatory drugs, oral steroids, and epidural steroid injections) and the mechanical component (physical therapy, chiropractic care) of the disease process. However, there is a lack of understanding of the optimal loading paradigms for motion-based treatments and the key interactions between inflammatory and mechanical signaling that govern the response to these treatments. Matching specific nonoperative intervention with the specific disc pathology (e.g., using an anticatabolic therapy for an inflamed disc without significant matrix loss vs. using an anabolic therapy for a burned out disc with severe degeneration and matrix loss, but little inflammation) has the potential to lead to improved outcomes. However, this approach requires an in depth understanding of the mechanisms responsible for the effects of nonoperative therapies such as anti-inflammatory treatments and mechanical-based treatments such as physical therapy and exercise because understanding the mechanisms has the potential to lead to rational design of treatment protocols. Variability in the response to exercise-based treatment is further complicated by the fact that the tissues of degenerated discs have differences in their mechanical properties. Degenerative discs have less ability to resist compression,⁴ and the pericellular matrix, which is altered in the course of degeneration, affects biochemical and biomechanical responses.^{5, 6} Thus, it is clear that the tissues of degenerated discs have differences in their mechanical properties. However, it is not clear how these changes in mechanical properties relate to the biochemical changes stimulated by mechanical loading and inflammation.

Our work and those of others have demonstrated a threshold effect for the response of the normal healthy intervertebral disc to loading. Both beneficial and traumatic effects have been observed. Dynamic loading of the spine in animal models has demonstrated an anabolic effect of compressive force on the production of matrix structural proteins.⁷ Similarly, physiologic levels of hydrostatic pressure stimulate the production of proteoglycans and tissue inhibitors of metalloproteases, which slow matrix degradation.⁸ However, degenerate nucleus pulposus cells have demonstrated differences in their gene expression response to compression compared with normal cells.⁹ Similarly, degenerative cells from the annulus fibrosus (AF) respond to tensile strain with differences in catabolic and anabolic gene expression compared with normal cells.¹⁰ In particular, tensile strain at 1 Hz demonstrated decreased expression of collagen I and aggrecan in degenerative cells compared with normal cells and increased catabolic matrix metalloprotease (MMP)-13 expression in degenerative cells at 0.33 Hz. Similarly, in a study comparing the responses of mature and aged disc cells, older cells demonstrated lower anabolic gene expression (collagen II and aggrecan) and greater catabolic expression (MMP-1) in response to tensile strain.¹¹ However, these studies did not explore the effects on inflammatory gene expression or the interaction of inflammation and mechanical signaling. Because there are key

interactions between inflammatory and mechanical signaling pathways that coexist in vivo, and overall intervertebral disc matrix homeostasis is a result of the balance between catabolic activity and anabolic activity, we found it important to examine the responses of degenerative disc cells to these combined stimuli. In fact, in addition to the effects on matrix structure, we have demonstrated an anticatabolic and anti-inflammatory effect of applied tensile strain on normal cells from the AF of the disc,¹² as well as an interaction between inflammatory and mechanical signaling, with catabolic responses at high magnitudes and durations of strain.¹³ It was therefore the goal of this work to establish the responses of degenerative disc cells to the stimuli to which the cells are exposed in vivo, namely, inflammatory, mechanical, and combined stimuli. We hypothesized, similar to previous studies, that degenerative cells would have a less anabolic response to mechanical stimuli. However, we also hypothesized that a more catabolic and proinflammatory response would be observed in response to both mechanical and inflammatory stimuli.

METHODS

Overview of Study Design

AF cells were isolated from New Zealand White rabbits with normal or magnetic resonance imaging–confirmed degenerative discs created by annular stab (see below). Cells were cultured under four conditions: control, inflammatory stimulus (1 ng/ml interleukin [IL]-1[β]), 6% tensile strain at 0.1 Hz, or combined inflammatory stimulus and tensile strain. After 4 or 24 hrs, the gene expression was measured, and comparison was made between normal and degenerative cells. IL-1[β] was chosen as the inflammatory stimulus because of its previous use in this mechanically active culture system.¹³ Tensile strain regimens were chosen based on previous data that demonstrated anti-inflammatory and anti-catabolic effects of 6% 0.1 Hz tensile strain for 4 hrs but loss of these effects after 24 hrs, with increases in catabolic and inflammatory gene expression.¹³ In addition, the estimates of fiber strains in vivo are approximately 6% under physiologic loading,¹⁴ further justifying the choice of regimens.

Creation of IDD

Disc degeneration was created using a well-validated approach.¹⁵ This method was used to isolate the cells used for the degenerative cell experiments. Briefly, the left anterolateral aspect of discs L2-3, L3-4, and L4-5 in skeletally mature New Zealand White rabbits was punctured with a 16-gauge hypodermic needle to a depth of 5 mm under an institutionally approved Institutional Animal Care and Use Committee protocol. T2-weighted sagittal images of the rabbit lumbar spines were obtained at 0 and 12 wks using a 3-T Trio (Siemens) magnetic resonance scanner through the University of Pittsburgh Magnetic Resonance Research Center to confirm at least 50% degeneration based on the magnetic resonance imaging index, calculated as the product of the T2-weighted signal intensity and the disc area.

Isolation of Rabbit Intervertebral Disc Cells

Intervertebral discs from skeletally mature normal New Zealand White rabbits or rabbits with induced degeneration as described above were dissected out immediately after killing.

For experiments using normal disc cells, all lumbar discs from a single rabbit were combined to create independent normal cell populations, and for the degenerative cells, two stabbed discs from a single rabbit were combined to create independent degenerative cell populations used for the experiments. Under sterile conditions, the AF and nucleus pulposus were separated and minced, washed, and digested in 5% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA), 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA), 0.2% pronase (CalBiochem, San Diego, CA), followed by 5% fetal bovine serum, 1% penicillin streptomycin, 0.02% Collagenase-P (Roche, Indianapolis, IN) and expanded in monolayer.

Preparation of Flexcell Plates

First passage AF fibrochondrocytes were plated onto six-well culture plates with a silicone membrane coated with collagen I (Bioflex, Hillsborough, NC) at a density of 50,000 cells per well and grown to 90% confluence in a standard tissue culture incubator under standard conditions of 37°C, 5% CO₂ in F12 (Invitrogen), 1% penicillin/streptomycin, and 10% fetal bovine serum. Media was changed to F12 1% penicillin/streptomycin and 1% fetal bovine serum 12 to 15 hrs before applying tensile strain as previously described and was found to be appropriate for fibrochondrocyte and chondrocyte mechanobiology testing.^{13,16}

Inflammatory Stimulation

Cells in the inflammatory stimulation conditions were activated by exposure to recombinant human IL-1[beta] at a final concentration of 1 ng/ml immediately before application of tensile strain. We have chosen this concentration of IL based on previous studies as well as our experience that this level of inflammatory stimulus results in consistent stimulation of the cells.¹² Adding IL before mechanical stimulation has been established previously to provide the most consistent results and greater observed effects compared with addition during mechanical stimulation.¹⁶

Application of Tensile Strain

AF cells in the mechanically stimulated groups were subjected to tensile strain using a Flexercell Strain Unit FX-4000 (Flexcell International Corp, Hillsborough, NC). Using this system, a deformation of the surface of the plate is created via vacuum beneath the plate. The cells adherent to the membrane experience uniform circumferential strain at a preset magnitude and duration. Minimal (<1%) cell detachment occurs in response to all regimens tested. AF cells were exposed to tensile strain at 6% elongation at a frequency of 0.1 Hz for 4 or 24 hrs. These regimens were chosen based on previous experiments demonstrating that mechanical stimulation at this level demonstrated a beneficial effect at 4 hrs but a detrimental effect at 24 hrs.¹³ This mechanically active system has been previously demonstrated to result in maintenance of the AF cell phenotype.¹⁷

Outcome Measures

Gene Expression of Matrix Genes—After exposure to inflammatory or mechanical stimuli, the cells were lysed, and mRNA was isolated using a Qiagen mini-prep kit including a DNase-I step to remove genomic DNA. The mRNA expression was measured by

quantitative real-time reverse transcription polymerase chain reaction using customdesigned and validated primers (Table 1) for inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX- 2), MMP-1 (collagenase), MMP-3 (stromolysin), collagen I, collagen II, and tissue inhibitor of MMP-1 (TIMP-1) with the SYBR Green (Applied Biosystems, Carlsbad, CA) detection system, using an annealing temperature of 62°C and melting temperature of 95°C. Melt curve analysis was performed on all samples to ensure specificity of primer products. After normalization to the constitutively expressed gene glyceraldehyde phosphate dehydrogenase, which has been shown to be an appropriate housekeeping gene for mechanobiologic testing in disc cells,¹⁸ relative quantitative gene expression was calculated using the delta-delta Ct method¹⁹ and the SYBR Green detection system, which has the advantage of accounting for any differences in different cell isolates, thus providing a specific measure of relative changes in gene expression that can be attributed to the conditions compared. Relative gene expression was converted to percentage change compared with unstimulated samples to reflect relative increases or decreases in gene expression (i.e., $(1 - \text{relative gene expression}) \times 100$). For cells exposed to inflammatory stimuli, percentage change in gene expression was calculated compared with cells from the same isolate not exposed to inflammatory stimuli. Similarly, gene expression of cells exposed to mechanical stimuli was calculated relative to cells from the same isolate not exposed to mechanical stimuli. To examine the effect of mechanical stimulation in an inflammatory environment, cells exposed to combined inflammatory and mechanical stimuli were compared with cells from the same isolate exposed to inflammatory stimulus alone.

Statistical Analysis

Values represent the mean $\pm$ SE of three to six trials from independent cell isolations from separate rabbits, with 95% confidence intervals calculated from the percentage change values to determine statistical significance at the $P < 0.05$ level. The confidence intervals were calculated based on the t distribution because of the small sample size. Computations were performed using Microsoft Excel and R statistical software.

RESULTS

Baseline Differences in Gene Expression (in Unstimulated Cells)

To establish baseline differences in normal and degenerative cells before stimulation with inflammatory or mechanical stimuli, the gene expression of unstimulated cells was examined. The expression of inflammatory genes was dramatically decreased in degenerative cells compared with normal cells, with iNOS decreasing by $91\% \pm 12\%$ and COX-2 decreasing by $94\% \pm 5\%$. In addition, MMP-3 decreased by $83\% \pm 27\%$ in degenerative cells compared with normal cells. Changes in MMP-1 and TIMP-1 gene expression showed large variability, decreasing by $25\% \pm 39\%$ and $26\% \pm 157\%$, respectively. Collagen gene expression also demonstrated large variability, with trends toward decreased expression in degenerative disc cells by $47\% \pm 30\%$ and $52\% \pm 96\%$ for collagen I and II, respectively.

Differences in Response to Inflammatory Stimulus

Degenerative disc cells demonstrated a greater increase in expression of inflammatory genes (iNOS and COX-2) at both 4 and 24 hrs (Figs. 1A, B). Collagen expression was not significantly altered in degenerative cells compared with normal cells. Although MMP expression did not demonstrate higher expression in degenerative cells, TIMP-1 expression, which would act to limit catabolic activity, was significantly increased at both 4 and 24 hrs in degenerative cells. TIMP-1 expression, which would act to limit catabolic activity, was significantly increased at both 4 and 24 hrs in degenerative cells.

Differences in Response to Mechanical Stimulus

After 4 hrs, with the regimen previously shown to be anticatabolic in normal disc cells, the inhibitory effects of tensile strain on iNOS expression were lost in the degenerative cells (Fig. 2A), with degenerative cells actually demonstrating an increase in iNOS expression compared with nonstimulated degenerative cells, although these differences did not reach statistical significance. In addition, whereas iNOS and COX-2 were stimulated after 24 hrs in normal cells, statistically significantly less stimulation of both iNOS and COX-2 was noted in degenerative cells after 24 hrs (Fig. 2B). Collagen I gene expression at both 4 and 24 hrs was also significantly lower in response to mechanical stimuli in degenerative cells compared with normal cells, and a similar trend was noted for collagen II, although this did not reach statistical significance. The change in MMP-1 and MMP-3 gene expression was slightly lower in degenerative cells exposed to mechanical stimuli compared with normal cells exposed to mechanical stimuli, at both 4 and 24 hrs. However, the stimulation of TIMP-1 expression seen in normal cells in response to mechanical stimuli was lost in degenerative cells at both 4 and 24 hrs.

Differences in Response to Mechanical Stimuli in an Inflammatory Environment

Under conditions of combined inflammatory and mechanical stretch, the protective effect of mechanical stimulation in limiting iNOS expression at 4 hrs seen in normal cells was lost in degenerative cells (Fig. 3A). In addition, a more robust response to 24 hrs of mechanical stretch in an inflammatory environment was observed in degenerative cells, with an increase in iNOS expression of 137% in stretched degenerative cells compared with unstretched cells vs. only 9% increase in stretched normal cells compared with unstretched cells (Fig. 3B). No significant differences were noted in COX-2 gene expression. Interestingly, change in MMP expression showed greater inhibition in degenerative cells exposed to combined inflammatory and mechanical stimuli compared with normal cells at 4 hrs and less stimulation at 24 hrs, although this difference was significant only for MMP-1 at 24 hrs. However, change in TIMP-1 expression in degenerative cells showed loss of the stimulation observed in normal cells by applied strain at 4 hrs, with no differences in normal and degenerative cell response at 24 hrs. The stimulatory effect of applied strain on collagen I expression seen in normal cells was also lost in degenerative cells at 4 hrs, and at 24 hrs, applied strain actually resulted in inhibition in degenerative cells, as opposed to stimulation observed in normal cells. Collagen II also demonstrated a trend toward decreased expression in response to stretch in degenerative cells, although this was not statistically significant.

DISCUSSION

We noted an overall decrease in the baseline gene expression of cells isolated from animals with annular puncture-induced degeneration compared with normal animals, particularly the expression of the catabolic factors iNOS and COX-2, which were expressed at much lower amounts than cells from normal discs. Importantly, because these values were all normalized to the housekeeping gene expression, glyceraldehyde phosphate dehydrogenase, in the respective cells, these data demonstrate a decrease in metabolic activity in degenerative cells in genes responsible for matrix remodeling, both catabolic and anabolic. However, although the baseline inflammatory gene expression was lower, degenerative cells exhibit greater response to inflammatory stress and mechanical load, suggesting that degenerative disc cells are not only more quiescent at baseline but also more reactive to exogenous stimuli. Interestingly, there was greater variability in degenerative disc cell gene expression of matrix-related genes (as evidenced by the large errors associated with these measurements). This may be related to slightly different levels of degeneration, which could not be assessed by imaging within cell populations isolated from different animals. Although not exhaustive, these data demonstrate that phenotypic differences exist between cells of normal vs. degenerative disc tissue.

Overall, degenerative cells demonstrate a more proinflammatory response profile to inflammatory stimuli compared with normal cells, with greater increases in inflammatory mediators iNOS and COX-2. In addition, the beneficial effect of mechanical signaling in response to short-duration (4 hrs) tensile strain that was observed in normal cells after exposure to inflammatory stimuli is lost in degenerative cells (see Fig. 3A). For example, a decrease in iNOS was observed in response to short-duration tensile strain in normal cells, whereas an increase in iNOS was observed in degenerative cells. It should be noted that although the trends were similar for cells in a noninflammatory environment, this finding was not statistically significant. Much greater differences between control and degenerative cells were noted in iNOS compared with COX-2 gene expression. Although they are both inflammatory mediators, differences in iNOS vs. COX-2 responses may be related to different signaling pathways involved. In particular, the nuclear factor-[kappa][beta] and p38 kinase pathways are both involved in inflammatory signaling in disc cells^{20,21} and could be differentially regulated in normal and degenerative cells. This represents an important topic for future investigations to determine the mechanism behind the observed differences in responses of normal and degenerative cells to both inflammatory and mechanical stimuli.

In contrast to the inflammatory gene expression response, degenerative cells demonstrated higher TIMP-1 (which limits metalloprotease activity) gene expression in response to inflammatory stimuli, without a corresponding increase in MMP expression in cells exposed to inflammatory stimuli. Similarly, the gene expression of MMPs demonstrated a decreased expression in degenerative cells under mechanically stimulated conditions. However, TIMP-1 expression was also decreased under conditions of mechanical stimulation. It is possible that under inflammatory conditions, the decreased metalloprotease expression, coupled with the increased TIMP-1 expression, could act to limit catabolism of disc matrix, although this effect was not observed in mechanically stimulated conditions. Under conditions of combined inflammatory and mechanical stimuli, TIMP-1 expression was

decreased in degenerative cells after 4 hrs, and MMP-1 expression decreased after 24 hrs. The relationship between TIMP expression and MMP expression is critical to maintaining matrix homeostasis and, when out of balance, can result in net matrix loss or gain. The greater increase in TIMP-1 in degenerative cells under inflammatory conditions despite no difference in MMP expression could act to limit the catabolic activity in degenerative cells. However, it is also possible that differences in other steps that modulate MMP enzymatic activity, such as enzyme activation, could result in a change in MMP activity despite no change in gene expression. Similarly, differences in what steps are most critical in regulating matrix homeostasis may occur in normal and degenerative disc cells. For example, relationships between TIMP and MMP expression have previously been identified in human disc degeneration, in which TIMP-1 and TIMP-2 expression levels correlated moderately with MMP-1, whereas TIMP-1 expression correlated with MMP-2. However, no significant correlations between TIMP-1 and TIMP-2 and MMP-3, MMP-7, MMP-8, MMP-9, and MMP-13 were observed.²² A causal effect is less clear, although such imbalances are likely to be involved in loss of matrix homeostasis. However, because neither protein nor enzymatic activity was examined in this study, it is unclear how these gene expression level changes would result in a net effect on disc matrix homeostasis, and this point warrants further study. This is important because AF cells exposed to moderate level of tensile strain (6%) have been shown to demonstrate decreased MMP-3 gene expression and enzymatic activity compared with unstimulated cells, whereas high level of tensile strain stimulated increased MMP-3 activity despite a persistent decrease in gene expression.¹³ Although MMP-1 gene expression was not affected as significantly in these experiments, MMP-1 enzymatic activity has been shown to increase with increasing magnitude of tensile strain,¹³ underscoring a potential disconnect between gene expression and enzymatic activity. However, because of the small sample size in this study and the inherent variability of biologic responses, many comparisons did not yield statistically significant results but may have been underpowered to demonstrate meaningful differences. Similarly, statistical significance cannot always confer biologic significance, and therefore, these results need to be confirmed with both a larger number and measurement of protein end products. For example, small changes in inducible gene products or genes with low basal expression may confer great biologic consequences, such as iNOS, whereas large changes in constitutively expressed genes may have minimal biologic effects. Future experiments on net matrix homeostasis, that is, the balance between matrix degradation and synthesis, are needed to further interpret the biologic consequences of these observed changes. Although differences between normal and degenerative cell collagen gene expression were not noted in response to inflammatory stimulus alone, mechanical stimulation resulted in decreased collagen I gene expression in degenerative cells, under both noninflammatory and inflammatory conditions. This has the potential to have a negative impact on matrix homeostasis.

Limitations exist in extrapolating the current results to human cellular responses. We chose to study cells from an animal model for this study to eliminate environmental and other variables, such as medications, that would affect the cellular responses. In addition, in an animal model, we were able to carefully control the degree of degeneration and control for age-related changes. Our results differ to that found in human cells by Gilbert et al.,¹⁰ in which cells isolated from degenerated discs were exposed to tensile strain and compared

with nondegenerated cells isolated postmortem. Unlike in our study, no difference was observed between control and degenerative cells in collagen I expression. In addition, the expression of MMP-3 was lower in nondegenerate cells exposed to tensile strain than in degenerative cells, unlike our findings of decreased MMP expression in degenerative cells in response to tensile strain. However, the differences in gene expression between nondegenerated and degenerated cells exposed to the analogous stimuli did not reach statistical significance in the study by Gilbert et al.,¹⁰ which was likely related to the greater variability of human cells. In addition, in the human study, higher frequencies were used (0.33 Hz, compared with 1 Hz used in our study), and cells were not exposed to inflammatory stimuli. Thus, the differences in the results between the current study and that by Gilbert et al.¹⁰ may relate to the different types of stimuli applied, to the differences in human and animal cell responses, or in the degree of degeneration of the cells. Because cells used in the current study were obtained from a single time point in the course of disc degeneration in the animal model, it is possible that responses to inflammatory and mechanical stimuli could differ at different stages of degeneration, which could not be assessed in the current study design. An additional limitation exists in the use of an in vitro culture environment. Although previous studies have demonstrated gene expression changes in intervertebral disc cells in culture,²³ these data used third passage cells. Therefore, to minimize any potential phenotypic changes, we used first passage cells for all experiments. In addition, the gene expression changes that we have examined are in reference to control cells also in identical culture systems, including low serum conditions, which should help to correct for any slight changes in outcome measures resulting from culture conditions, allowing the conclusion that the observed changes are a result of the condition tested (i.e., inflammation or tensile strain) and not of the culture system. In addition, previous mechanobiology studies using intervertebral disc cells as well as chondrocytes used similar culture conditions that did not prestimulate cells and did not result in appreciable cell overgrowth or death and facilitated protein analysis, which was the basis for our choice of culture conditions.^{13, 16} Although it is certainly possible that synergy exists between low serum concentrations and inflammatory or mechanical stimuli, this was not explicitly studied currently. However, because the disc is in a low nutrient state in vivo, this may have important biologic relevance. It should also be noted that the inflammatory stimulus used here is a simplification of what the intervertebral disc cells are exposed to in vivo, which can include additional cytokines, such as IL-6 and tumor necrosis factor-[alpha], as well as other mediators of inflammation, including nitric oxide and prostaglandins.^{24, 25} Similarly, although cells of the intervertebral disc are certainly not exposed to isolated types of loading, examining AF cell responses to inflammatory stimuli and tensile strain represent the initial building blocks for understanding the mechanisms behind the observed effects. Therefore, although it is clear that the absolute magnitudes of the observed in vitro effects cannot be translated to in vivo changes, because of the effects of matrix interactions and loading patterns of greater complexity than those examined here, identification of the important variables for governing the biologic responses represents the key contribution of this work.

In addition, these data demonstrate that degenerative cells have a decreased capacity to respond positively to beneficial levels of mechanical strain and demonstrate an exaggerated

response to an inflammatory stimulus. This may in part explain the mechanisms behind the inability of degenerative disc cells to facilitate repair and may help to explain differential responses to motion-based therapies in patients with IDD. Through obtaining a greater understanding of the beneficial effects of loading, these mechanisms can be incorporated into the future study of motion-based therapies. This has the potential to lead to studies that incorporate the concept of biologic differences in tissue responses when developing novel nonoperative therapies, with a goal of limiting catabolic activity and promoting anabolic activity, and actually reverse the disease process instead of simply treating the symptoms. Thus, future targeted motion-based therapies may be developed and tested based on basic science mechanisms to decrease degeneration and facilitate healing of tissues. The key to translation of this knowledge to improved clinical treatment paradigms lies in unraveling the mechanisms behind the effects, allowing novel clinical studies to be designed with mechanism in mind. Importantly, optimally beneficial mechanical loading conditions for degenerative cells were not identified in this study. However, it is possible that beneficial conditions do exist for degenerative cells, and this represents an important area for further exploration. Clearly, the in vitro results presented here cannot be used directly to design motion-based therapies, but it is hoped that these identified differences between normal and degenerative cell responses will lead to consideration of more individualized programs for patients. Future studies are needed to identify the specific thresholds by which normal and degenerative cells respond to mechanical loading and how these may be exploited to facilitate beneficial effects on tissue matrix homeostasis.

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FIGURE 1.

Percentage change in gene expression in normal and degenerative annulus fibrosus cells exposed to interleukin- 1A for (A) 4 hrs and (B) 24 hrs compared with unstimulated cells, where no change would be reflected as 0% change. *Statistical significance compared with control, unstimulated cells. #Statistically significant comparison between cell types. iNOS indicates inducible nitric oxide synthase; TIMP-1, tissue inhibitor of matrix metalloprotease-1; Col-1, collagen I; Col-2, collagen II; MMP-3, matrix metalloprotease-3 (stromolysin); COX-2, cyclooxygenase-2; MMP-1, matrix metalloprotease-1 (collagenase).

FIGURE 2.

Percentage change in gene expression in normal and degenerative annulus fibrosus cells exposed to 6% 0.1 Hz tensile strain for (A) 4 hrs and (B) 24 hrs compared with unstimulated cells. iNOS indicates inducible nitric oxide synthase; TIMP-1, tissue inhibitor of matrix metalloproteinase-1; Col-1, collagen I; Col-2, collagen II; MMP-3, matrix metalloproteinase-3 (stromolysin); COX-2, cyclooxygenase-2; MMP-1, matrix metalloproteinase-1 (collagenase). *Statistical significance compared with control, unstimulated cells. #Statistically significant comparison between cell types.

FIGURE 3.

Percentage change in gene expression in normal and degenerative annulus fibrosus cells exposed to both interleukin-1A and 6%0.1 Hz tensile strain for (A) 4 hrs and (B) 24 hrs compared with cells stimulated with interleukin-1A alone. iNOS indicates inducible nitric oxide synthase; Col-1, collagen I; Col-2, collagen II; TIMP-1, tissue inhibitor of matrix metalloprotease-1; MMP-3, matrix metalloprotease-3 (stromolysin); COX-2, cyclooxygenase-2; MMP-1, matrix metalloprotease-1 (collagenase). *Statistical significance compared with control, unstimulated cells. #Statistically significant comparison between cell types.

TABLE 1

Primer sequences used for RT-PCR

Gene	Sequence
GAPDH	Rev: 5'-GCTGAGATGATGACCCTTTTGG-3' For: 5'-GATGCTGGTGCCGAGTAC-3'
Collagen I	Rev: 5'-GCCATCGACAAGAACAGTGTAAGT-3' For: 5'-ATGGATGAGGAACTGGCAACT-3'
Collagen II	Rev: 5'-CAGTCCCCGTGTCACAGACA-3' For: 5'-AAGAGGTATAATGATAAGGATGTGTGGAA-3'
COX-2	Rev: 5'-CAGGCACCAGACCAAACACTT-3' For: 5'-CACGCAGGTGGAGATGATCTAC-3'
iNOS	Rev: 5'-TCTGTGACGGCCTGATCTTTC-3' For: 5'-CCCCTTCAACGGCTGGTA-3'
MMP-1	Rev: 5'-GCCTGTCACCTCGCAAACC-3' For: 5'-GACCTACGCACCCACACAC-3'
MMP-3	Rev: 5'-CCAGTGGATAGGCTGAGCAA-3' For: 5'-AGCCAATGGAAATGAAACTCTTC-3'
TIMP-1	Rev: 5'-CCACAACTTGGCCCTGATG-3' For: 5'-AGCAGAGCCTGCACCTGTGT-5'

GAPDH, glyceraldehyde phosphate dehydrogenase; Rev, reverse; For, forward; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; MMP-3, matrix metalloprotease -3 (stromolysin); MMP-1, matrix metalloprotease-1 (collagenase); TIMP-1, tissue inhibitor of matrix metalloprotease-1.