A three-dimensional collagen matrix as a suitable culture system for the comparison of cyclic strain and hydrostatic pressure effects on intervertebral disc cells

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**Object.** To study intervertebral disc cell mechanobiology, the authors developed experimental systems that allow the application of cyclic strain and intermittent hydrostatic pressure (IHP) on isolated disc cells under equal three-dimensional (3D) culture conditions. The purpose of the study was to characterize disc cell proliferation, viability, morphology, and gene expression in 3D collagen matrices.

**Methods.** The effects of cyclic strain (1, 2, 4, and 8% strain; 1 Hz) and IHP (0.25 MPa, 0.1 Hz) on gene expression (real-time polymerase chain reaction) of anabolic and catabolic matrix proteins were investigated and compared with those derived from mechanically unstimulated controls. Intervertebral disc cells proliferated in the collagen gels (mean viability 91.6%) and expressed messenger RNA for collagen I, collagen II, aggrecan, matrix metalloproteinase (MMP)–2, and MMP-3. Morphologically, both spindle-shaped cells with longer processes and rounded cells were detected in the collagen scaffolds. Cyclic strain increased collagen II and aggrecan expression and decreased MMP-3 expression of anulus fibrosus cells. No significant difference between the four strain magnitudes was found. Intermittent hydrostatic pressure tended to increase collagen I and aggrecan expression of nucleus cells and significantly decreased MMP-2 and -3 expression of nucleus cells, whereas aggrecan expression of anulus cells tended to decrease.

**Conclusions.** Based on these results, the collagen matrix appeared to be a suitable substrate to apply both cyclic strain and IHP to intervertebral disc cells under 3D culture conditions. Individual variations may be influenced by the extent of degeneration of the disc specimens from which the cells were isolated. This experimental setup may be suitable for studying the influence of degeneration on the disc cell response to mechanical stimuli.

**Key Words** • intervertebral disc cell • cyclic strain • hydrostatic pressure

The IVD has a complex histological structure that consists of the central pulpy nucleus pulposus area that is enclosed by the concentric collagenous lamellae of the anulus fibrosus. Intervertebral disc cells exhibit spatial variation in phenotype and morphology that may be related to differences in their local mechanical environment. In the outer anulus cells fibroblast-like phenotypes are present, whereas in the inner anulus and nucleus pulposus chondrocyte-like cells are predominant.

Dynamic loading is important for IVD physiology and prevention of disc degeneration. Mechanical loads vary across the disc, exposing IVD cells to complex physical stimuli including compressive, tensile, and shear stresses, fluid flow, hydrostatic pressure, and osmotic pressure.

Cells of the nucleus and inner anulus are predominantly exposed to hydrostatic pressure, whereas those in the outer anulus are influenced by tensile strains during flexion, extension, and torsion of the disc. It has been suggested that these different mechanical stimuli influence disc matrix synthesis and turnover. Both in vivo and in vitro studies of IVD mechanobiology have been performed to examine the biological responses of IVD cells to loading. Mechanical deformations alter the biochemical environment of the disc via tissue compaction and fluid movement.

The authors of previous studies of mechanical effects on isolated IVD cells have demonstrated that disc cell metabolism is influenced by hydrostatic pressure, tensile strain, and shear stress. All of these different stimuli affected cell cycle and matrix biosynthesis of disc cells. The stimulation of anabolic or catabolic factors of disc matrix turnover depended on the level of the mechanical stimulus. These data support the hypothesis that loading directly influences disc cell metabolism and that abnormal loading, either too high or too low, may accelerate disc degeneration.
Because different cell sources (bovine, ovine, canine, and human discs) and experimental models have been used in these studies, comparison of their results is difficult. To our knowledge, no studies have been undertaken to examine the effects of different mechanical stimuli in equivalent culture conditions. Most studies on the effects of cyclic strain or fluid-induced shear strain on disc cells have been conducted using monolayer culture systems.\textsuperscript{7,24,30,31} Experiments on the effects of hydrostatic pressure have been performed with disc cells cultured in monolayer cultures or alginate culture systems.\textsuperscript{15,21} Thus, in addition to the differences of the mechanical stimulus, the culture conditions have been very diverse (two-dimensional monolayer cultures compared with 3D beads). Because the culture system has a strong influence on in vitro cell reactions, we used the same substrate—a 3D collagen gel—for studying the influence of both cyclic strain and IHP on IVD cells derived from anulus fibrosus and nucleus pulposus. We investigated the hypothesis that cyclic strain and hydrostatic pressure have different effects on gene expression of anulus and nucleus cells cultured at equivalent substrate conditions. To support this hypothesis, we sought to answer the following questions. 1) Does the collagen substrate allow the application of both cyclic strain and hydrostatic pressure at comparable experimental conditions? 2) Are the in vitro reactions of IVD cells influenced by the different mechanical stimuli? 3) Do anulus and nucleus cells respond differently to mRNA expression of anabolic and catabolic matrix proteins?

To answer these questions we cultivated IVD cells in collagen Type I gels with or without mechanical stimulation and investigated cellular parameters such as morphology, viability, proliferation, and mRNA expression of anabolic (collagen Types I and II, and aggrecan) and catabolic proteins (MMP-2 and -3).

**Materials and Methods**

**Cell Isolation and Maintenance**
We obtained biopsy samples from the disc space of 18 patients (Table 1) who were undergoing surgery for disc herniation and separated the specimens into anulus and nucleus cells intraoperatively. Cells were isolated from the fresh tissue samples within 2 to 3 hours after removal by using collagenase/pronase digestion (in standard DMEM [Gibco, BRL, Karlsruhe, Germany] with 25 mM HEPES, 1 mM sodium pyruvate, 1000 mg/L glucose). The DMEM had a concentration of approximately 400 mOsm with the addition of 5 M NaCl and 0.4 M KCl and is designated as DMEM-Osm. Digestion medium for nucleus biopsy samples consisted of 0.8 mg/ml collagenase-I (Sigma, Mannheim, Germany) and 2.6 U/ml DNA (Sia), and for anulus cells of 2 mg/ml collagenase-I, 0.6 mg/ml protease (Type I), and 2.6 U/ml DNA in DMEM-Osm. Digestion media for anulus and nucleus cells were supplemented by antibiotics (500 U/ml penicillin, 500 μg/ml streptomycin sulphate and 25 μg/ml fungizone [Biochrom, Berlin, Germany]). After 17 to 19 hours of digestion (37°C, 100% humidity, 5% CO₂, 6% O₂), the cell suspension was filtered (40μm pore size) to remove nondigested fragments, and cells were washed by two repetitions of centrifugation (5 minutes, 1000 G) and resuspension in culture medium. Cell viability was determined by trypan blue exclusion. Cell cultures with a viability of greater than 95% were used for the experiments. Disc cells were characterized by analysis of mRNA expression of collagen I and II, aggrecan, and MMP-2 and -3 expression by quantitative real-time PCR. Fragments of both anulus and nucleus samples were fixed in 4% formalin for histological examinations.

Depending on the experimental protocol (cyclic strain or hydrostatic pressure experiments), primary cultures of IVD cells were subcultured using trypsin/ethylenediamine tetraacetic acid treatment (0.05%/0.02% [Biochrom]) for further increase of cell number or plated directly into 3D collagen gels (Ars Artho AG, Esslingen, Germany). Subcultured cells were used for the experiment in the second-to-third passage. Cell cultures were maintained in standard culture conditions in DMEM-A (DMEM-Osm supplemented by 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 25 μg/ml fungizone, fetal bovine serum 5% [vol/vol], nonessential amino acids 1% [vol/vol] and L-glutamine 1% [vol/vol] [Biochrom]) with a reduced O₂ atmosphere during the entire culture period and the mechanical stimulation experiments (37°C, 100% humidity, 5% CO₂, and 6% O₂). The medium was changed every 3 days to fresh medium of the same composition (DMEM-A). Because of limited cell numbers obtained in the cell isolation and subculturing procedures, only part of the proceeding experiments could be conducted with the cell populations of all 18 donors. Table 1 provides a summary of the cell populations used in the different experiments.

**Three-Dimensional Collagen Gel Cultures**
Cell suspensions of IVD cells in culture medium were mixed in a ratio of one to one with a rat tail collagen Type I solution (Ars Artho AG) to obtain a final concentration of 3 mg/ml collagen. For each of the different experiments 3 ml of the cell-loaded collagen solution (1.5 × 10⁵ cells/ml) were either poured into the specially designed silicone dishes (cyclic strain experiments) or into standard culture dishes (diameter 3 cm) for determination of proliferation and morphology and for the hydrostatic pressure experiments. Gelation of the cell-loaded collagen matrices occurred within 30 minutes at 37°C in an incubator. Afterward, 2 ml of cell culture medium DMEM-A were added to each sample. Medium was replaced every 3 days during the entire culture period.

**Substrate Influence on Cell Proliferation and Viability**
To test the suitability of the collagen gel substrate for disc cell cultures, cell-loaded collagen gels (150,000 cells/ml) were maintained during a period of 4 weeks (28 days). After each 7-day interval, cell number and viability in each three parallel cultures were determined. Two additional cell-loaded gels were fixed in formaldehyde, embedded in paraffin, and prepared for microscopic examination with H & E.

**Determination of Cell Number and Viability**
Cell-loaded collagen gels were dissolved by 30 minutes of collagenase digestion (25 mg/ml collagenase Type IA in PBS [Sigma]) at 37°C. Cell numbers were determined by counting the cells with a Coulter Counter (Z1; Coulter Electronics, Krefeld, Germany). Trypan blue exclusion was used to determine cell viability by microscopic hemocytometer counting of stained and unstained cells. Cell
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Mechanical Stimulation Experiments

Specially designed devices allowed the application of cyclic strain (Fig. 1) and IHP (Fig. 2). The cell culture substrate in the cell-stretching device were specially developed rectangular silicone dishes (Wacker Chemie, Munich, Germany) that were deformable and optically transparent to allow microscopic observation of the cells. Six dishes with cells could be stimulated simultaneously in a six-station stimulation apparatus that was driven by an eccentric motor.3 Strain magnitude could be modified by the eccentricity of the cam on the rotating motor axis and frequency was controlled by the motor speed. The silicone dishes had knobs on their surface to anchor the cell-loaded collagen gels in the dishes as previously described.19 Cells were maintained for 1 week in the collagen gels with DMEM-A to adapt to the culture conditions. Twenty-four hours before beginning of cell stimulation, DMEM-A was replaced by serum-reduced DMEM-B of the same composition but with reduced fetal bovine serum (2%). Twenty-four hours after serum reduction, cyclic strain or IHP was applied to the cultures.

During the cell-stretching experiments, the dishes were placed into the cell stimulation apparatus (Fig. 1) that could be maintained in the incubator at run time of the experiments. We used strain magnitudes of 1% (equivalent to 10,000 µm of strain), 2, 4, and 8% strain at a frequency of 1 Hz for 24 hours. Cells cultured at equal conditions but without any mechanical stimulation served as controls. Each three parallel cultures of anulus cells obtained in four patients were tested at all loading conditions. To compare cyclic strain effects on anulus and nucleus cells, we repeated these experiments with cells obtained in seven donors at one distinct loading condition (2% cyclic strain). For determination of mRNA expression of anulus and nucleus cells acquired in nine patients were poured into the culture dishes and maintained in the aforementioned fashion for the silicone dishes. The dishes were placed into the pressure chamber for mechanical stimulation. The chamber was kept in a cell incubator connected to the pressure-generating cylinder outside the incubator via a small tube. Intermittent hydrostatic pressure was generated by the motion of a pressure cylinder, which itself was driven by two lateral working cylinders. During stimulation, the air inside the pressure chamber was compressed. The actual hydrostatic pressure was measured by a pressure gauge connected to the chamber. We tested the effects of 0.25 MPa IHP with a frequency of 0.1 Hz for 30 minutes per day on disc cell reactions.

During the duration of the pressure experiments, control cultures were maintained in a second chamber of equivalent design that was also placed into the incubator but without compression. Twenty-four hours after the end of mechanical stimulation, cells were harvested and mRNA expression of anabolic and catabolic matrix proteins was determined.

Analysis of RNA and cDNA Synthesis

To determine mRNA expression of IVD cells, cell-loaded gels were dissolved by 30 minutes of collagenase digestion (25 mg/ml collagenase Type IA in PBS [Sigma]) at 37°C. Cell pellets were washed by one repetition of centrifugation (5 minutes, 1000 G) and resuspension in PBS. Total RNA was prepared using the RNeasy purification system (Qiagen, Hilden, Germany) according to the manufacturer’s instructions as recently described.19 The RNA concentration was calculated by absorbance measurements at 260 nm and assessment of the ratio of absorbance at 260 and 280 nm. Total RNA was transcribed into cDNA by using Omniscript RT Kit (Qiagen).

Analysis of Factors for Disc Matrix Biosynthesis and Degradation

The expression of RNA of collagen Types I and II, aggrecan (all anabolic matrix proteins), and the matrix degrading enzymes MMP-2 (gelatinase-A) and MMP-3 (stromelysin) was determined by quantitative real-time PCR by using the iCycler analysis system (BioRad, Munich, Germany). We designed specific primers based on published sequences (NCBI Entrez search system). All primer sequences are shown in Table 2. For quantification of the amplified target sequences, standards of all amplification products were obtained by cloning the fragments into the pCR4-TOPO vector (TOPO TA Cloning Kit; Invitrogen, Karlsruhe, Germany). Serial 10-fold dilutions of these samples were used for the standard curve calculation in real-time PCR. Expression of the amplified products was calculated relative to the house-keeping gene GAPDH in each sample. Normalized values were compared with mechanically unstimulated controls.

FIG. 1. Schematic drawing of the apparatus for cell stimulation by cyclic strain demonstrating two of six deformable silicone dishes. The dishes have silicone knobs on their surface to anchor the cell-loaded collagen gels.
Statistical Analysis

Data regarding real-time PCR were determined by calculating the relative expression of all amplified products to the GAPDH expression in each sample. The results of the mechanically stimulated cultures (by IHP or cyclic strain) were related to the respective unstimulated control cultures. Standard statistical methods were used. Descriptive statistics including means and SDs were calculated. Differences between stimulated cultures and controls were analyzed using distribution analysis. The relationship of percentage difference between stimulated cultures and controls were calculated. The null hypothesis was 1 for no difference between stimulated cultures and controls, less than 1 if decreased effects were detected, and greater than 1 if increasing effects occurred. A probability value of less than 0.05 was considered statistically significant (Wilcoxon signed-rank test).

Results

Our collagen gel scaffold for IVD cells supported disc cell proliferation and differentiation. Both anulus and nucleus cells proliferated in the collagen gels during a culture period of 4 weeks (mean viability 91.6%). Most proliferation occurred during the first 2 weeks of the culture period during which cell number doubled in the first 7 days and only slightly increased after 21 and 28 days. There was no difference in proliferation of anulus and nucleus cells. Histologically, different cell morphologies were found. Cells isolated from the anulus exhibited predominantly a spindle-shaped cell phenotype with long processes (Fig. 3a and b). Nucleus cells initially exhibited a spindle-shaped morphology (Fig. 3c), but an increasing number of cells with a more rounded phenotype were observed over time in the cell-loaded collagen gels (Fig. 3d).

During a 4-week culture period, both anulus and nucleus cells expressed the matrix-forming proteins collagen Types I and II, as well as aggrecan and the matrix-degrading enzymes MMP-2 and -3. Collagen I expression of nucleus cells of three donors was higher than the mRNA expression of the other factors (Fig. 4 left). Collagen I and MMP-3 expression tended to decrease during the 4 weeks of culture duration. Collagen II expression was lower and could only be detected if nucleus cells were seeded with no or only short subculturing in monolayer before initiation of the gel cultures. Aggrecan expression of nucleus cells increased during the culture period (Fig. 4 left).

Because of higher cell numbers at the beginning of the experiment, more time periods could be analyzed during a 4-week culture period of anulus cells in collagen gels (Fig. 4 right). We found a continuous increase of collagen Type I mRNA expression. All other factors were detected; however, individual differences were rather large and no preferred tendency could be seen. Collagen II expression was very inconsistent and failed to be detected in cell cultures that required subculturing in monolayer to increase cell number. Thus, the variation of collagen Type II expression was high.

Proliferation, viability, morphology, and gene expression of disc cells in the collagen gels suggested that the substrate was suitable for in vitro studies on disc cell mechanobiology. Therefore, this substrate was used for both experiments of cyclic strain and IHP effects on disc cell reactions.
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**Results of Mechanical Stimulation Experiments**

In both nucleus cells exposed to IHP and controls not submitted to mechanical stimulation, increased proliferation was observed during the 4-week period of culture. There was no difference between stimulated or nonstimulated cells. Anulus cell proliferation was also not significantly influenced by hydrostatic pressure (data not shown).

Intermittent hydrostatic pressure influenced mRNA expression of anabolic and catabolic matrix components of both anulus and nucleus cells obtained in nine donors (Fig. 5 left). Nucleus cells tended to increase collagen Type I and aggrecan expression in response to IHP and to decrease significantly gene expression of MMP-2 (p = 0.016) and MMP-3 (p = 0.016). Anulus cells significantly increased collagen I (p = 0.047) expression and tended to decrease MMP-3 expression. In contrast to the nucleus cells, aggrecan expression of anulus cells tended to decrease in response to IHP. Matrix metalloproteinase–2 expression of anulus cells and collagen II expression of both anulus and nucleus cells was very inconsistent with high individual differences. In anulus cultures from three of nine donors, no collagen Type II expression was found.

Cyclic strain significantly increased aggrecan expression (p = 0.031) and increased collagen Type II (p = 0.062) expression of anulus cells (Fig. 5 right). Matrix metalloproteinase–3 expression samples obtained in seven donors was decreased significantly (p = 0.03). Nucleus cells strongly tended to increase expression of the anabolic matrix protein collagen I (p = 0.055) and tended to decrease MMP-2 and -3 expression, but these results failed to achieve significance. Cyclic strain of different magnitudes (1, 2, 4, and 8%) applied for 24 hours with a frequency of 1 Hz influenced mRNA expression of anulus cells of four donors. Anabolic gene expression (collagen I, II, and aggrecan) tended to increase and catabolic gene expression (MMP-2 and -3) tended to decrease in response to cyclic strain, but there was no significant difference between the effects of the four strain magnitudes (data not shown). If anulus cells were subcultured in monolayer before initiation of the collagen gel cultures, no collagen Type II expression was detected.

**Discussion**

**Influence of the Culture System on IVD Cells**

Analysis of the results of the present study demonstrates that IVD cells cultured in Type I collagen matrices proliferate, express both anabolic and catabolic proteins of disc matrix, and exhibit viability rates of more than 90% during a 4-week culture period. This 3D substrate appears to be suitable for the in vitro maintenance of the disc cell phenotype. In the disc tissue, anulus fibrosus and nucleus pulposus cells are embedded in a 3D matrix. Histologically, both cell types are morphologically different and exhibit different collagen production patterns as shown by immunostaining. In monolayer cultures, anulus and nucleus disc cells have a spindle-shaped phenotype similar to fibroblasts. When placed in a 3D culture environment, disc cells become a more rounded phenotype and form multicelled colonies. These findings confirm that the culture system is a critical factor influencing in vitro disc cell behavior. Many studies have been performed to investigate isolated IVD cells in 3D systems such as alginate beads, or Matrigel. These culture systems have been reported to be suitable for the in vitro maintenance of the disc cell phenotype. Alginate culture systems were also used to test the effect of the application of hydrostatic pressure on IVD cells, because alginate beads are unsuitable for the application of cyclic strain, an alternative 3D culture system was used in the present study in both mechanical loading conditions.

The collagen Type I gel that we have used as substrate for IVD cells supported the in vitro maintenance of a disc cell–specific phenotype. In this scaffold both anulus and nucleus cells expressed anabolic (collagen Types I and II and aggrecan) and catabolic factors (MMP-2 and -3) that are involved in disc matrix formation or degradation. Three-dimensional collagen gels have also been used by other investigators as a substrate for both anulus and nucleus cell cultures. Using collagen scaffolds, several authors have reported mRNA expression of proteoglycans and collagens (Types I and II) when IVD cells were cultured during a longer period. Our data support these findings. We found, however, that subculturing of IVD cells in monolayer (necessary to increase cell number) reduced the capability of disc cells to express collagen Type II. This finding is consistent with those reported in other studies investigating the influence of the culture system on matrix expression and cell phenotype of IVD cells. Horner, et al., found that proteoglycan biosynthesis was lower in monolayer cultures than in collagen gels. In monolayer cultures, only weak immunostaining for Types I and II collagen could be detected. Gruber, et al., found extensive immunohistochemical evidence of Type I and II collagens only in 3D cultures and not in monolayer. In 3D collagen scaffolds, expression of a number of genes that are important for extracellular matrix formation have been reported. Our present results of Types I and II collagen and aggrecan expression of disc cells in collagen Type I

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**Table 2**

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gels support these findings. Morphologically, shortly after initiation of the collagen gel cultures we found that anulus and nucleus region cells of human discs formed processes, as has been described by other authors. With increasing culture duration, however, we also observed cells with a rounded phenotype, especially in collagen gels seeded with nucleus cells. Gruber, et al., also reported cells with rounded morphology in collagen gels seeded with nucleus cells, and they showed by in situ hybridization that only these rounded cells—not the spindle-shaped ones—expressed Types I and II collagen and aggrecan. These findings suggest that disc cell cultures are not homogeneous and that there are cells with different phenotypes in cultures isolated from either anulus or nucleus biopsy samples. In the present study, we analyzed gene expression of all cells within the gel cultures by isolation of total mRNA and real-time PCR in which specific primer sequences were used. This method does not allow separation between cells of different phenotypes in one specific culture sample because mRNA of all cells within one sample is used for further analysis. Therefore, the rather significant individual differences in gene expression that we found could be due to the varying number of cells of different phenotypes in the gel culture samples. Another limitation is the use of cells isolated from disc material that was removed because of disc herniation. Disc tissue degenerative changes could influence disc cell reactions. Human disc material without any degeneration, however, is only rarely available and cell numbers are too limited for extensive experiments. In vitro studies involving disc cells obtained in a herniated disc might allow investigation of the influence of age and degeneration on disc cell responses to mechanical stimulation.

Mechanical Stimulation of IVD Cells

The 3D Type I collagen gel has proven to be suitable for both hydrostatic pressure and cyclic strain experiments. With our specially developed mechanical stimulation devices, the same volume (3 ml) and cell density (1.5 × 10^5 cells/ml) of cell-loaded collagen gels could be used for both cyclic strain and hydrostatic pressure experiments. Thus, the effects of different mechanical stimulants could be analyzed under equivalent culture conditions. Most studies involving in vitro stimulation of disc cells by physical forces have been performed by either application of hydrostatic pressure in alginate culture systems or by cyclic strain in monolayer cultures. Therefore, in addition to the different physical stimuli, the culture systems were also diverse. Because the culture system has a strong influence on cell reactions it may be better for the comparison of different physical stimuli to study the cell reactions in equivalent culture conditions. In the disc tissue the nucleus pulposus has a lower collagen content than the anulus fibrosus. Therefore, a collagen scaffold might be less physiologically sound for nucleus than for anulus cells. This could influence cell reactions and might explain the higher variations that we found in experiments involving nucleus cells compared with anulus cells.

Effects of Cyclic Strain

We found that cyclic strain—the principal mechanical load in the anulus lamellae—increased gene expression of the matrix-forming proteins collagen II and aggrecan and decreased expression of the matrix-degrading enzyme MMP-3 of anulus cells. Nucleus cells tended to increase anabolic and to decrease catabolic gene expression, but
these results failed to be significant. The effects of cyclic strain on IVD cells have also been studied in monolayer systems of rabbit anulus and nucleus cells. Rannou et al. did not detect Type I collagen expression of anulus cells cultured on flexible-bottomed culture plates but did find Type II collagen expression. The production of neo-synthesized proteoglycans did not exhibit differences in response to the applied mechanical stimulus. Matsumoto et al. reported increased growth rates and collagen synthesis of nucleus cells in response to cyclic mechanical stress. This finding is supported by our results of a tendency-related increase of anabolic matrix protein expression (Types I and II collagen and aggrecan) of nucleus cells in response to cyclic strain. The differences between our results and those of other studies may be caused by the different cell types and culture systems.

In the present study, the cyclic strain experiments were performed within a strain magnitude range that occurs in the anulus lamellae during loading exercises of the spine as calculated by finite element modeling. Analysis of models suggested that the anulus fibers have maximum tensile strains between 6.8 and 8%. These strain magnitudes have been used as the upper strain limit in our cyclic stretching experiments. The cyclic strain experiments were conducted at a physiological walking frequency of 1 Hz. We found no significant differences between the four strain magnitudes, and the results of experiments involving cells from different donors varied widely. The large variations in our data may be due to variations in the cell populations that were isolated from the donor tissue and to the degree of degeneration of the disc samples. In addition, strain conditions in our 3D collagen gels were not absolutely homogeneous throughout the collagen gels. The strain of cells embedded in the 3D collagen matrices may be lower than that of the entire silicone dish. The applied mechanical stimuli had no detrimental effect on IVD cells as demonstrated in preexperiments; lactate-dehydrogenase activity and gene expression of apoptosis-inducing protein p53 showed no difference between stimulated cultures and controls (data not shown).

Effects of Hydrostatic Pressure

Intervertebral disc cell gene expression was influenced by IHP. Nucleus cells predominantly exposed to hydrostatic pressure in the disc tissue tended to increase Type I collagen and aggrecan expression and decreased expression of the matrix-degrading enzymes MMP-2 and -3. Additionally anulus cells increased collagen Type I expression but their effects on other anabolic and catabolic genes varied in a wide range.

In the present study the hydrostatic pressure experiments were performed within a low-pressure range that was estimated by in vivo measurements of intradiscal pressure. The chosen pressure conditions correspond to in vivo intradiscal pressure ranges that occur in the nucleus when a person is lying down or sitting relaxed in a chair as previously described. Because this loading magnitude is rather low, the effects on mRNA expression might be...
greater at higher pressure magnitudes or with further repetitions of the applied loading cycles. In other studies, hydrostatic pressure influenced matrix production of disc cells, depending on the magnitude of the applied stimulus. Hutton, et al., found a differential response of canine anulus and nucleus cells in response to higher-magnitude hydrostatic pressures. Some of these results were confirmed by our results. In agreement with our findings, collagen and proteoglycan syntheses were stimulated in the nucleus and inhibited in the anulus cells. In contrast to their results, we found increased collagen Type I expression of anulus cells in response to IHP. Hutton, et al., reported no significant IHP-induced increase in cell proliferation. These findings are supported by the results of our present study in which we also failed to detect IHP-related significant influence on disc cell proliferation. Handa, et al., suggested that physiological levels of hydrostatic pressure (0.3 MPa) may be an anabolic factor for stimulation of proteoglycan and tissue inhibitor of metalloproteinase-1, whereas very high (3-MPa) and very low (<0.1-MPa) pressures may cause a catabolic effect with reduction of proteoglycan-synthesis rate and increase of MMP-3 production. Other investigators reported an increased expression of matrix-degrading metalloproteinases associated with IVD degeneration. Our findings indicated that mechanically stimulated disc cells, either stimulated by cyclic strain or hydrostatic pressure, tended to decrease mRNA expression of MMP-2 and -3. These results might suggest that moderate mechanical loading may prevent disc matrix degradation by suppressing cellular production of MMPs.

Conclusions

The results of the present study suggest that collagen matrices provide a suitable culture system for mechanical stimulation of IVD cells by both cyclic strain and IHP under equivalent culture conditions. We demonstrated that both types of mechanical stimuli influenced disc cell reactions, whereas anulus cells appeared to be more responsive to cyclic strain and nucleus cells to hydrostatic pressure. We found that cyclic strain and IHP within the applied moderate loading range stimulated disc matrix turnover by alterations of gene expression of proteins involved in matrix formation or degradation. The individual variations of cells obtained in different donor patients were high and could be influenced by the degree of degeneration of the disc samples. These methods may be suitable for studying the influence of degeneration on disc cell responses to mechanical loading.

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References

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