Effects of Controlled Dynamic Disc Distraction on Degenerated Intervertebral Discs
An in Vivo Study on the Rabbit Lumbar Spine Model

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Study Design. An in vivo study on the rabbit lumbar spine model.

Objectives. Effects of temporary dynamic distraction on intervertebral discs were studied on the lumbar spine rabbit model to characterize the changes associated with disc distraction and to evaluate feasibility of temporary disc distraction to previously compressed discs in order to stimulate disc regeneration.

Summary of Background Data. Studies have shown that accelerated degeneration of the intervertebral disc results from altered mechanical loading conditions. The development of methods for the prevention of disc degeneration and the restoration of disc tissue that has already degenerated are needed.

Methods. New Zealand white rabbits (n = 32) were used for this study. The rabbits were randomly assigned to one of five groups. In 12 animals, the discs were first loaded for 28 days using a custom-made external loading device to stimulate disc degeneration. After 28 days loading time, the discs in six animals were distracted for 7 days and in six animals for 28 days using the same external device, however, modified as dynamic distraction device. In six animals, the discs were distracted for 28 days without previous loading; and in six animals, the discs were loaded for 28 days and afterwards the loading device removed for 28 days for recovery without distraction. Six animals were sham operated. The external device was situated; however, the discs remained undistracted and they also served as controls. After 28 to 56 days loading and distraction time, the animals were killed and the lumbar spine was harvested for examination. Disc height, disc morphology, cell viability, relative neutral zone, and tangential modulus were measured.

Results. After 28 days of loading, the discs demonstrated a significant decrease in disc space. Histologically, disorganization of the architecture of the anulus occurred. The number of dead cells increased significantly in the anulus and cartilage endplate. These changes were reversible after 28 days of distraction. The disc thickness increased significantly as compared with the specimens from the 28 days loading group without distraction. Histologically, the discs showed signs of tissue regeneration after 28 days of distraction. The number of dead cells decreased significantly in comparison with the loaded discs without distraction. The flexibility of compressed discs was higher than of compressed/distracted discs.

Conclusions. The results of this study suggest that disc regeneration can be induced by axial dynamic distraction in the rabbit intervertebral disc. The decompressed rabbit intervertebral discs showed signs of tissue recovery on a biologic, cellular, and a biomechanical level after 28 days of distraction.

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The intervertebral disc, in accordance with its mechanical requirements, is an organized, independent cell unit. One of its main functions consists of dampening compressive loads. During normal activities, the disc is compressed, thereby producing a loading pressure, which is balanced in the disc with a suction pressure, designated as hydrophilic pressure, whereby concentrated solutions pull water through semipermeable membranes. The interplay between hydrostatic and inhibition pressure is important for nutrition of the disc tissue as well as for the functionality of the moving segments. The pressure-dependent fluid exchange represents a pump mechanism, which moves the water and lower molecular metabolic substances at the disc boundary back and forth. This mechanism improves both the supply of reagents to the disc cells and the removal of metabolic waste products.

When the tissue environment deviates from its physiologic set point, biologic remodeling will cause changes in tissue architecture, and consequently, material properties. This is particularly true during disc compression, where the mechanical demands on skeletal tissues are greatly increased. The disc tissue reacts to the increased compression with remodeling. Depending on the duration and extent of the loading, this can lead to significant degeneration. The disc degeneration has often an impact on the facet joints, which suffer loss of articular space, deformation of the facets, hypertrophic osteophytes, and finally segment instability. Destabilization and the resulting unphysiologic movements can lead to accelerated disc degeneration and may consequently lead to pain and neurologic deficit.

A relatively new surgical treatment option for chronic disc degeneration or degenerative instability is dynamic...
neutralization of one or more intervertebral segments. A dynamic neutralization system for the spine allows stabilization with a controlled range of motion through dynamic neutralization absorbing the nonphysiologic loads in compression and flexion-extension, and suppressing pathologic movements. With the dynamic neutralization system, the spinal segments can be repositioned in a more anatomic condition in which healing of the lesions could take place. The first multicenter study proved dynamic neutralization to be an efficient procedure for the treatment of degenerative lumbar disease. In early stages, as well as in more advanced stages of disc degeneration of the motion segment, dynamic neutralization relieved pain and improved neurologic deficit of unstable conditions of the lumbar spine presenting with neurocompression.

However, relatively little is known about how controlled dynamic disc distraction alters the biomechanical function and tolerance of the intervertebral disc on a cellular level. To investigate the biomechanical and biologic response of the intervertebral disc to hyper- and hypo-physiologic spinal loading, we have developed an in vivo rabbit model and with it demonstrated that load-mediated remodeling is proportional to the magnitude and duration of the mechanical perturbation.

The purpose of the current study was to use this animal model to explore the ability of the disc to recover through reduced loading by using a custom-made dynamic disc distraction device. During the application of the device, spinal compression is largely removed, which could lead to altered spinal mechanics and increased disc nutrition. The tissues of the disc are hypothesized to remodel in response to these changes, which, depending on the duration of the exposure, may create significant potential for recovery. Precise knowledge of the cascade of biologic events characteristic of this modeling, and the means by which they are coupled to physical loading, would be invaluable for developing countermeasures for maintaining disc health and for developing paradigms for directing tissue repair. Unfortunately, current animal models are limited in the extent by which they may be used to accomplish these goals. This is the gap, which our current research is intended to fill.

Materials and Methods

Animals. A total of 32 New Zealand skeletal mature white rabbits weighing an average of 3.6 kg were used for this study (conducted under a protocol approved by the Institutional Review Board of the Animal Experimentation Committee, Regierungspräsidium Karlsruhe, Germany). The rabbits were randomly assigned to one of five groups. In 12 animals, the discs were first loaded for 28 days using an custom-made external loading device to stimulate disc degeneration as described earlier. After 28 days loading time, the discs in six animals (G1) were distracted for 7 days and in six animals (G2) for 28 days using the same external device, however, modified as dynamic distraction device (Figure 1). In six animals (G3), the discs were distracted for 28 days without previous loading; and in six animals (G4), the discs were loaded for 28 days and afterwards the loading device was removed for 28 days for recovery without distraction. These 12 animals served as controls. Finally, six animals were sham operated (G5). The external device was situated; however, the discs remained undistressed and they also served as controls.

Surgical Procedure. Under general anesthesia, through a dorsal approach to the lumbar spine, the custom-made external device was attached using four stainless steel pins (80 mm length, 3.5 mm diameter). The pins were placed percutaneously and attached to two K-wires (1.5 mm diameter) inserted into the vertebrae body L4 and L5 parallel to the adjacent study disc by use of a variable-speed electric drill. After the wound was closed, in 18 animals (G1, G2, and G4), axial compression to the disc was first created by a calibrated spring within the device to produce a disc compression force of 2.4 MPa to stimulate disc degeneration.

In G3, axial distraction to the disc was created by a calibrated spring immediately after the distraction device was attached, applying a relative distraction force similar to a distraction force by dynamic neutralization system to a human disc. In G5, a sham operation was carried out. These animals underwent surgical pin placement attached to the two K-wires. However, the external device was situated without loading/distraction. In the sham-operated animals, adverse effects of pin placement and surgery on the intervertebral disc were investigated. After surgery, all animals were allowed free unrestricted weight bearing and activity in cages and monitored daily.

Tissue Preparation. After 28 to 56 days loading and distraction time, the animals were killed and the lumbar spine was harvested for examination. First, the external device and the percutaneous pins were removed. The instrumented intervertebral disc with adjacent vertebral bodies and the cranial adjacent segment were then dissected for histology, terminal nick-end labeling (TUNEL) reaction, and biomechanical studies using sterile technique.
A control radiography of each harvested segment was then taken in the lateral view. Next, control and instrumented intervertebral discs with adjacent vertebral bodies were either subjected directly to mechanical testing or placed immediately in 4% paraformaldehyde and fixed overnight. Decalcification in 19% EDTA up to 21 days was followed by dehydration in a graded series of ethanol before paraffin embedding. Sagitally oriented sections were made at 5- to 7-μm intervals and placed on silane-coated slides. Sections were stained using standard histochemical staining procedures including giesma and hematoxylin and eosin to demonstrate cell density as well as general morphologic structures.

Four mid-sagittal sections of each disc were stained for dead cells using the TdT-DUTP TUNEL reaction (In Situ cell death Detection Kit, Fluorescein; Boehringer-Mannheim, Mannheim, Germany).

Radiology. All harvested specimen were used for radiologic examination. Lateral radiographs were taken from each experimental intervertebral disc with adjacent vertebral bodies and the cranial adjacent segment (Figure 2). The radiographs were scanned using a flatbed scanner with backlighting and passed to a computer on which images were magnified. Under lateral view, the disc height was marked and measured in each segment for all different loading conditions. Measurement for disc thickness was calculated using the radiograph to make two linear and angular measurements of calibration standards. The average disc thickness of each specimen was then calculated.

Disc Morphology. The disc histology and architecture were analyzed by standard light microscope (20X Olympus). After preparation of control, loaded and distracted intervertebral discs for histology as previously described, the specimens were graded using a morphologic scale proposed by Boos et al (2002) modified for rabbit discs. This modified schema classifies rabbit disc degeneration into four grades.

Cell Viability. Quantitative analysis was used to correlate the extent of cell death (TUNEL-positive cells) to the magnitude and duration of loading. The number of cells that were positive by the TUNEL reaction were counted in each section with fluorescence microscope (Vanox). The TUNEL-positive cells were bright green and unaffected cells appeared light red. In combination with the nucleus color, also the morphologic character of the cells was considered. Typical morphologic features of TUNEL-positive cells are condensation of chromatin, cell nucleus DNA fragmentation, and blebbing. The cells were reported as total number of cells in the examined disc sections (Figure 3).

Cell viability was additionally analyzed by releasing the cells from the ventral part of fresh disc specimen by combined collagenase B (2 mg/mL)/hyaluronidase (10 mg/mL) digestion overnight. In all loaded and unloaded discs, the total number of released cells/100 mg disc tissue and the percentage of dead cells were determined by trypan blue staining. To test the proliferation capacity of the obtained living cells, their DNA synthesis was measured by 3H-thymidine incorporation during the first 2 days in culture. Viable cells (3000/well) were seeded in triplicates in 96-well microtiter plates in DMEM culture medium containing 10% fetal calf serum. Immediately after seeding, 0.25 μCi of [3H]-thymidine was added per well for 2 days and the incorporated activity was determined after extensive washing of lysed cells in a beta counter.

Biomechanical Testing. For biomechanical testing the lumbar spine segment L4-L5 was harvested as described previously. Muscular and ligamentous structures were detached from these spinal segments, while facet joints, transverse processes, and posterior elements were left intact. Each vertebral body/disc/vertebral body complex was then embedded in polymethylmethacrylate and x-rayed in the dorsal-ventral plane at two points in time 1) after embedding and 2) during specimen preparation. Radiographs were used to facilitate and confirm the placement of an appropriate moment-arm rod (surgical
RNZ is defined as the segment’s motion from a characteristic load of 0.1 Nm in flexion through the unloaded, neutral position to that at a characteristic load of 0.1 Nm in extension. The tangent modulus was calculated at 10°, 12°, 14°, 16°, and 17° in flexion and at −3°, −5°, and −7° in extension. The measured biomechanical parameters (RNZ, TM) of all 15 specimens were tabulated and compared using a two-way analysis of variance to account for specimen type: control, compressed only, or compressed and distracted. Post hoc (SYSTAT, V. 5.2, Evanston, IL) multiple pairwise comparison tests were performed to quantify any observed differences with a significance of $P < 0.05$.

### Results

**Animals**

A total of 30 of 32 animals survived the complete experiment. Two animals were killed before the completion of the study protocol because of neurologic deficits after surgery. All animals tolerated the application of the external fixateur, and no wound infection or other complications were observed. The average animal weight was 3.550 g at the beginning of the study, 3.750 g after 4 weeks, and 3.650 g at the end of the protocol, indicating the animals kept their normal average weight. The external device weighed 25 g and was tolerated well by the animals, as evidenced by their ability to move in the cages.

**Increased Disc Height After Distraction**

The discs thickness was measured through a radiograph in lateral view. The distance from a horizontal line parallel to the endplate of L4 and L5 was measured for each specimen. Axial loading indicated disc thickness was generally related to axial force. Intervertebral disc thickness with no load (G5) averaged 1.87 mm in all specimens. There was a significant decrease in disc thickness after 28 days of compression and 28 days recovery (G4) ($P = 0.005$). In the specimens with 28 days compression and 28 days of distraction (G2), the disc thickness increased significantly compared with the specimens from the 28-day loading group without distraction (G4) and reached disc thickness equivalent to the sham-operated
controls (G5) (Figure 5). Discs adjacent to the loaded/distracted discs did not change the disc height.

Physiologic Organization of Nucleus, Anulus, and Cartilage Endplate After Distraction

The intervertebral discs of the unloaded control animals consisted of proteoglycan-rich nucleus pulposus cells with round cell shapes, fishbone-layered anulus fibrosus lamellae, and a cartilaginous endplate with vertical chondrocytic cell lines. After 28 days of loading at 5 times body weight, histologic studies of the loaded discs showed typical qualitative morphologic changes (Grades 2 and 3) in comparison with the unloaded discs according to the Boos classification. After 28 days of distraction of previously loaded discs, however, the disc showed signs of tissue regeneration. Histologically, the nucleus pulposus showed a decrease of fibrous tissue and an increase of volume. Disappearance of tissue shrinkage was noted. The nucleus pulposus cells became less separated by proteoglycan matrix with more round cell shapes. The lamellair architecture of the inner, middle, and outer anulus became more organized with increasing distraction. The disorganized lamellair structure of the degenerated anulus fibrosus changed into the typical physiologic fishbone structure after temporary distraction. Also, the proliferation of cartilaginous tissue decreased in the anulus with distraction. With increasing duration of distraction, these changes became more pronounced with disappearance of clefts or fissures in the anulus fibrosus and less herniation of disc materials or osteophyte formation (Figure 6A–D). Intervertebral discs from the adjacent levels maintained their normal morphology.

Less TUNEL-Positive Cells in the Disc After Distraction

In the animals of the control group without loading (G3 and G5), few TUNEL-positive cells were observed in the anulus. In the animals that were loaded and not distracted (G4), a significantly increased number of dead cells in comparison with the unloaded controls were noted ($P < 0.05$). In the discs that were distracted up to 28 days after 28 days of loading (G1 and G2), the number of dead cells decreased significantly in comparison with the loaded animals (G4) without distraction and reached similar dead cell numbers as the unloaded controls (G5). The discs of the animals, which were only distracted without previous loading (G3), showed no different number of dead cells compared with the unloaded controls (G5) (Figure 7).

Biomechanical Differences

The mean (SD) angular range of motion (ROM) for the control specimens in flexion was 35.69°. The mean (SD) angular ROM for the compressed specimens in flexion was 24.87°. The mean (SD) angular ROM for the compressed/distracted specimens in flexion was 21.72°. Neutral zone (NZ) was on average 60% of ROM for the motions studied (Figure 8).

Discussion

We have developed an in vivo New Zealand rabbit model in which changes similar of human intervertebral disc degeneration were created by axial compressive loading. Compressive stress led to changes on a macroscopic and a cellular level. Results in previous animal studies showed changes in disc structure and composition under complex loading conditions. Mechanically, complex loading conditions in these experiments may be considered to be comprised of a combination of compression and shear forces and bending moments. Ex-
Experiments using animal models in which compressive forces were applied to the intervertebral disc resulted in altered composition on a cellular level.\textsuperscript{11}

Taken together these findings, the current results from these studies support the concept that, in intervertebral disc tissue, there appears to be threshold at which forces stimulate tissue degeneration, presumably due to a complex pathomechanism initiated through a change in cell shape from a change in the mechanical stresses or an adverse biochemical environment produced by water loss. During the degenerative process as consequence of overload, there is early proliferation of cells within the annulus,\textsuperscript{4,12} followed by metaplasia of these cells from fibrocyte-like cells into chondrocytes. With progressive degeneration, however, diffusion of substances through the disc declines and, as has been previously described, the cells become deprived of oxygen. This leads to anaerobic metabolism to an extent that cell viability is compromised. Cell density within the disc then decreases; consequently, synthesis and maintenance of the matrix are affected. Synthesis of matrix macromolecules decreases, which results in decreased water content in the disc.\textsuperscript{13} Inadequate matrix maintenance cause the accumulation of degraded macromolecules. These products are most likely to arise in tissues with marginal diffusion rates. The accumulated breakdown products impair diffusion through the disc by physically obstructing the flow of substances, as well as by decreasing the water content of the medium through which diffusion occurs. A vicious circle is created, with progressive deterioration in oxygen, nutrient, and waste transport leading to further cell death and depletion of the matrix.

This remodeling concept of disc degeneration, however, needs further exploration for implication strategies to stimulate direct tissue repair. Therefore, we modified our in vivo animal model to allow on a macroscopic and microscopic level the investigation of intervertebral disc regeneration initiated through dynamic disc distraction. The axial unloading apparatus in our model is an external fixateur, which guarantees due to its free external position constant dynamic decompression over the entire posteri distraction time. Also, the external spring mechanisms in our rabbit model allows control and individual justification of the applied distraction forces at any time point of the experiment.

In our study, the operated rabbits tolerated the procedure and the distraction device well. The animals were not limited in their normal behavior. The current results suggest that dynamic mechanical distraction leads to reorganization of the lamellar architecture of the annulus. If sufficient cell death has occurred, the disc can recover once the leading has been returned in distraction as shown in the animals that were first loaded for 28 days than distracted for 28 days. This leads to a chronic state of decompression in the annulus, which appears to direct metaplasia of chondrocytes, reduction of the number of cell death, and the concomitant production of fibro-cartilage in the substance of the annulus. This results in increase of disc thickness. The biomechanical results in our experiment showed that the flexibility of loaded discs was higher than of distracted discs. Specimens of both study groups, however, showed significantly less flexibility than untreated control discs. These results suggest that moderate disc degeneration stimulated through 28 days of disc compression leads to decreased segment flexibility. Temporary disc distraction decreases the flexibility of compressed discs. This could be due to increased disc height. The segmental flexibility was significantly less with moderate disc degeneration, accompanied by a tendency to stabilization of the mo-
tion segment through temporary distraction. This suggests that changes through disc degeneration on histologic, cellular, and biomechanical levels occur early. Based on these findings, it seems to be important to intervene at an early time point when disc degeneration was initiated in order to maintain disc health or stimulate tissue repair.

It has also been shown in other studies that disc tissue has a very limited ability to regenerate.6,14,15 We have shown that not only cell viability is reduced under mechanical loading but also that cells that are still alive show an impaired metabolic activity and delayed cell growth. Therefore, once the degenerative process has reached a certain level, it is difficult to stop or reverse it with currently available techniques. One possible approach to the management of disc disease would be to apply dynamic distraction to the disc to a early time point to increase disc height stimulating disc diffusion resulting in increased water content and finally leading to increased intervertebral disc nutrition. In the current study, we stimulated disc regeneration to the degenerated rabbit intervertebral disc. Dynamic mechanical distraction may play an important part in revising the effect of disc degeneration, and further studies will have to demonstrate whether this is a suitable method to influence disc cell metabolism and disc regeneration.

■ Conclusion

The current study showed that disc degeneration creates a vicious circle with progressive deterioration in oxygen, nutrient, and waste transport leading to cell death and depletion of the matrix. The in vitro New Zealand white rabbit model allows, on a macroscopic and microscopic level, the investigation of intervertebral disc regeneration initiated through dynamic disc distraction.

The current results suggest that dynamic mechanical distraction leads to a chronic state of decompression in the anulus, which appears to direct metaplasia of chondrocytes, reduction of the number of cell death, and the concomitant production of fibro-cartilage in the substance of the anulus. This results in increase of disc thickness. Early intervention when disc degeneration was initiated seems to be important to maintain disc health or stimulate tissue repair.

■ Key Points

- Disc degeneration was induced by axial dynamic loading.
- The application of dynamic mechanical distraction leads to reorganization of the architecture of the disc.
- This article represents a basic in vitro model to study the effect of controlled dynamic disc distraction on degenerated intervertebral discs.

References