

Regeneration of Intervertebral Disc Tissue by Resorbable Cell-Free Polyglycolic Acid-Based Implants in a Rabbit Model of Disc Degeneration

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Study Design. Different biologic strategies exist to treat degenerative disc disease. Tissue engineering approaches favor autologous chondrocyte transplantation. In our one-step-approach, a resorbable cell-free polyglycolic acid (PGA)-based implant is immersed in serum from whole blood and implanted into the disc defect directly after discectomy.

Objectives. The aim of our study was to investigate the capacity of a cell-free implant composed of a PGA felt, hyaluronic acid, and serum to recruit disc cells and stimulate repair tissue formation *in vivo* after microdiscectomy in a rabbit model.

Summary of the Background Data. Disc tissue has a limited ability to regenerate after the degeneration process was once initiated. Therefore, we developed a cell-free resorbable implant that is able to attract local cells into the defect and induce proper repair tissue formation.

Methods. The cell-free implant consisting of PGA and hyaluronic acid was immersed in allogenic serum and implanted into the disc defect after discectomy in New Zealand white rabbits. One week and 6 months after the operation, the disc height index and the T2-weighted signal intensity index were determined using plane radiographs and magnetic resonance imaging. Finally, discs were explanted and investigated histologically. Animals with discectomy only served as controls.

Results. In our animal studies, we could demonstrate that the T2-weighted signal intensity of the operated discs decreased in both groups 1 week after surgery. However, after 6 months, the T2-weighted signal intensity index increased by 45% in the implanted group whereas the index decreased further by 11% in the sham group. This

corresponded to changes in the disc height index. Furthermore, the histologic examinations indicated cell migration into the defect and showed tissue regeneration.

Conclusion. The implantation of a cell-free PGA-hyaluronic acid implant immersed in serum after discectomy induces regeneration, resulting in improvement of the disc water content and preservation of the disc height 6 months after surgery.

Key words: degenerative disc disease, proteoglycan, discectomy, nucleus pulposus, matrix protein, polyglycolic acid, cell-free implant. **Spine 2008;33:1527–1532**

Back pain is one of the major causes of disability and thus has a major socioeconomic impact. The lifetime prevalence of low back pain is 75 to 80% of the population. Disc degeneration is the main cause of back pain.^{1,2}

Current conventional treatment of disc degeneration includes medication, steroid injection, physical therapy, and surgery. All these strategies are limited to treat the symptoms and not the underlying biologic alterations of the disc. However, there is now a growing interest in developing strategies to address the underlying biologic imbalances that lead to symptomatic degenerative disc disease.^{3–6}

The intervertebral disc (IVD) has a unique structure, composed of a tough outer ring (anulus fibrosus) and a gelatinous inner core (nucleus pulposus). Morphologically, the anulus fibrosus consists of concentric lamellas rich in collagen fibers, whereas the nucleus pulposus is a gelatinous cushion rich in proteoglycans. During aging, the nucleus pulposus is transformed from a turgid gel of proteoglycans into a more desiccated fibrocartilaginous structure that appears similar to the inner anulus. So, degeneration of the vertebral disc is associated with progressive changes in material properties, matrix composition, and morphology.^{2,3,7} Therefore, the biologic repair of an IVD can be achieved theoretically by stimulation of the synthesis of matrix molecules and/or the prevention of matrix degradation or cell death.

Current tissue engineering approaches for the biologic repair of IVD tissue focus on the transplantation of cultured autologous disc-derived cells. Recently, Meisel *et al* have shown that the implantation of autologous culture expanded disc-derived cells reduces back pain signifi-

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cantly and may prevent loss of disc heights.⁸ More advanced tissue engineering approaches favor the use of resorbable biomaterials combined with autologous cells. The use of biomaterials for cell transplantation ensures initial mechanical stability and surgical manageability, allows even distribution of cells, and may guide tissue formation and regeneration.⁹ In cartilage regeneration, autologous chondrocytes embedded in graft-stabilizing scaffolds of hyaluronan, collagen, or synthetic polymers have shown to be clinically effective for the treatment of focal articular cartilage defects.^{10–12} In IVD repair, hyaluronan-based scaffolds augmented with bone marrow derived stem cells could prevent fibrous tissue formation and endplate disruption after nucleotomy in a pig model.¹³ Furthermore, the implantation of collagen scaffolds could restore disc heights and biomechanical properties of the disc after nucleotomy in a bovine model.¹⁴

Taking advantage of the mechanical and regenerative properties of resorbable polymers and precursor cells, we developed a cell-free resorbable polymer-based implant that is composed of a polyglycolic acid (PGA)/hyaluronan scaffold and immersed in allogenic serum for the regeneration of IVD tissue. The cell-free implant may recruit residing disc cells and local precursor cells into the defect and induce the formation of a disc repair tissue.

In this study, we used an *in vitro* and a rabbit model of disc degeneration to investigate the following hypotheses:

- Can we show migration of IVD chondrocytes or mesenchymal stem cells *in vivo* using serum from whole blood?
- Can we reduce the loss of disc heights after nucleotomy by implanting the cell-free PGA-based implants *in vivo*?
- Can we induce the development of disc repair tissue by implanting the cell-free PGA-based implant *in vivo*?

■ Materials and Methods

In Vivo Animal Studies

Preparation of Cell-Free Disc Implants. Resorbable felt ($15 \times 20 \times 1 \text{ mm}^3$) of pure PGA (Alpha Research Deutschland GmbH, Germany) was immersed in 330 μL hyaluronic acid (10 mg/mL Ostenil, TRB Chemedica AG, Germany). The implants were freeze-dried for 16 hours using a lyophilisator (Leybold-Heraeus, Germany) and stored in a desiccator at room temperature.

Surgery. All experimental protocols were approved by the Animal Protection Board of the Senate of Berlin. Twelve male New Zealand white rabbits (Harlan Winkelmann, Borcheln, Germany) weighing in average 1.9 kg at the time of surgery were divided in 2 groups (6 animals in each group). Anesthesia was induced with 0.1 to 0.15 mL/kg ketamin (Ursotamin) intramuscular and 0.1 to 0.15 mL/kg medetomidin (Dormitor) and maintained using an isoflurane (0.5–1.0%/v/v), NO_2/O_2 (1.5 L/min) gas-mixture. Through a retroperitoneal approach, the spine was located and a microdiscectomy L5/S1 with partial resection of the annulus fibrosus was accomplished. The PGA-based implants (5 mm in diameter) were immersed in allogenic

serum (Kraeber, Germany) and implanted in 6 rabbits. Serving as controls, microdiscectomy L5/S1 without implantation of the cell-free implant was performed in 6 rabbits. Finally, the wound was closed in layers. After 6 months, animals were killed using pentobarbital (Trapanal) and a potassium overdose. Then, the operated discs were explanted and investigated histologically according to the study protocol.

MRI and Radiographic Analysis. Magnetic resonance images (MRI) and lateral plain radiographs were performed under general anesthesia (ketamin 0.1 mL/kg intramuscular and dormitor 0.1 mL/kg i.m) 1 week and 6 months after surgery. MR images were taken using a 1.5-T imager (GE Twin Speed 1.5T, General Electric, Milwaukee). The rabbits were positioned supine on a quadrature surface coil and sagittal T2-weighted spin echo images parallel to the lumbar spine were obtained (repetition time TR 3200 milliseconds; echo time 130 milliseconds; number of excitations 20; field of view 14 cm; slice thickness 3 mm; no phase wrap). The T2 signal intensity level of a normal and the operated disc were quantified using Osirix software (Osirix Medical Imaging Software, Version 2.7.5). Therefore, an oval region of interest of 0015 cm^2 was created and measurements were performed placing the region of interest into the center of the disc of T2 weighted sagittal images. The T2 signal intensity index was calculated by dividing the measured T2 signal intensity of the operated disc by the T2 signal intensity of a normal disc. For the radiographs, a high potential generator (Revolution XRD, General Electric) was used (voltage 54 kV, current 6 mAs). Vertebral body heights and disc heights were measured using Osirix software. The disc height index was calculated as described by Lu *et al* for comparisons between study groups.¹⁵ The measurements have been performed by 2 experienced physicians (radiologist and neurosurgeon) independently and the results have been averaged.

Histopathology. Explanted IVD segments were cut in 3 coronary slices of 5 mm thickness and decalcified in 0.05% ethylene-diamine tetra-acetic acid for 4 weeks and subsequently embedded in paraffin. The paraffin blocks were sectioned into 2 μm sections from ventral to dorsal using a microtome. The sections were stained with hematoxylin and eosin and safranin O, respectively, for evaluation.

Statistical Analysis. Data are presented as mean and as single plot values. Statistical analyzes included the Mann-Whitney *U* test for the comparison of 2 study groups and the Wilcoxon test for paired samples in follow up. $P < 5\%$ was considered significant.

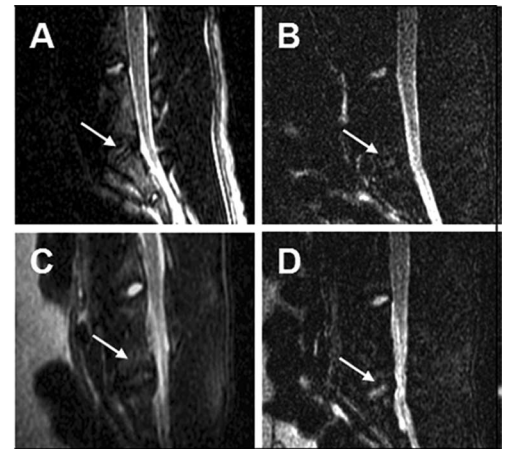
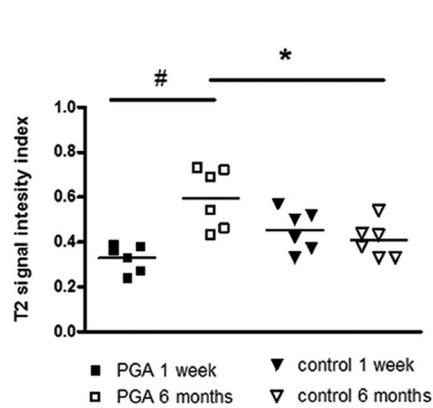
■ Results

Rabbit Model of Disc Degeneration

To our knowledge, we used for the first time an open surgery microdiscectomy model for the evaluation of biologic disc repair strategies. In contrast to previously used disc degeneration models, where a needle aspiration of the nucleus pulposus was performed, our approach is more closer to the real clinical situation. Performing microdiscectomy, this includes partial resection of the annulus fibrosus and a more destructive preparation of the nucleus pulposus. Therefore, regeneration is hindered.

Nevertheless, the MRI examination showed that the T2 signal intensity index decreased 1 week after surgery in both groups (sham and PGA implanted, 0.45 ± 0.04

Figure 1. **Left:** T2 signal intensity index of sham operated and PGA-implanted animals taken 1 week and 6 months after surgery. The index increased by 45% ($0.33 \pm 0.06 \rightarrow 60 \pm 0.06$) in the PGA implanted group ($\#P < 0.05$), whereas the index decreased by 11% ($0.45 \pm 0.04 \rightarrow 0.41 \pm 0.03$) in the sham group (n.s.). The intergroup comparison (PGA group = 0.60 ± 0.06 and sham group = 0.41 ± 0.03) after 6 months was also significant ($*P < 0.05$). **Right:** Representative images of sham **A, B** and PGA implanted **C, D** animals. Significant recovery of T2-weighted signal intensity after 6 months is seen in discs of PGA implanted animals (D) compared with very low signal intensity in sham (B). The arrows indicate operated disc.



and 0.33 ± 0.03 , respectively, n.s.). However, after 6 months, the T2 signal intensity index increased by 45% (0.60 ± 0.06) in the implanted group whereas the index decreased by 11% (0.41 ± 0.03) in the sham group ($P < 0.05$) (Figure 1).

Correspondingly, the radiographic analysis demonstrated disc space narrowing 6 months after surgery in the sham and PGA implanted group. However, the mean disc height index was significantly ($P < 0.05$) lower in the sham-implanted group compared with the PGA implanted group (0.075 ± 0.007 and 0.097 ± 0.005 , respectively) (Figure 2). Furthermore, the sham group showed osteophyte formation as sign for progressive degeneration (Figure 2B, black asterisk).

The histologic evaluation showed regenerative effects and formation of repair tissue in intervertebral discs after nucleotomy and implantation of the cell-free PGA-based implant (Figure 3). The discs, which were treated with cell-free implants after nucleotomy, appeared normal in height and showed considerable amounts of disc repair tissue (Figure 3A). Chondrocytes with normal cytomorphology were situated in small clusters (Figures 3B, C,

encircled), as it is typical for chondrogenesis. Also the intercellular matrix showed characteristics that are typical for a proteoglycan-rich cartilaginous tissue with a dense and homogenous yellowish safranin O staining of proteoglycans (Figure 3D). The repair tissue was of regular cellularity and there were no signs of inflammation or malignant transformation. In the sham-operated cases without implantation of the cell-free implant, no regeneration or repair tissue was detected (Figure 4). The intervertebral discs were flattened, and in the central region of the disc fibrotic, inhomogeneous tissue was evident (Figure 4A). A low cellularity, consisting only of fibroblasts (Figures 4B, C, arrows) and few inflammatory cells was detected, but chondrocytes were missing. Safranin O staining showed a reddish extracellular matrix indicating fibrosis (Figure 4D).

Discussion

This study evaluated whether resorbable cell-free PGA-based implants immersed in serum from whole blood can be a treatment option to repair disc damage and inhibit degeneration. We followed the hypothesis that in the

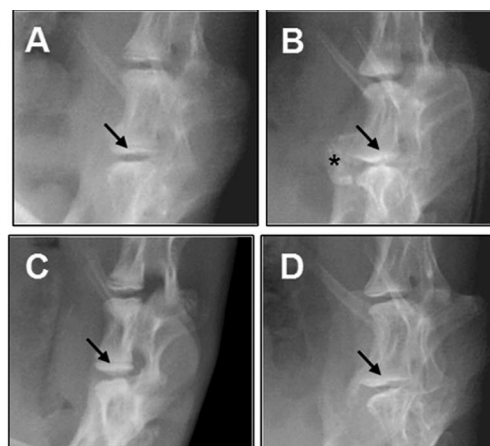
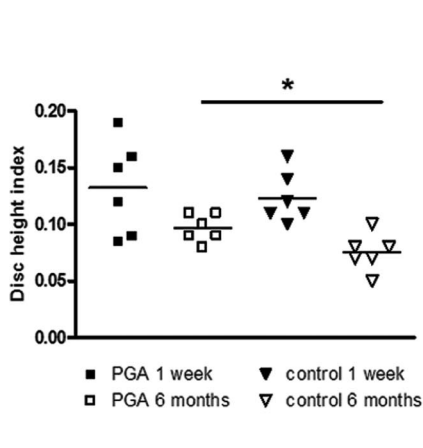


Figure 2. **Left:** The disc height index (DHI) of sham operated and PGA-implanted animals taken 1 week and 6 months after surgery. The index decreased in both groups after 6 months. However, the decrease was significantly lower 27% ($0.133 \pm 0.017 \rightarrow 0.097 \pm 0.005$) in the PGA implanted group compared with the sham group 39% ($0.123 \pm 0.009 \rightarrow 0.075 \pm 0.007$) ($*P < 0.05$). **Right:** Representative lateral radiograph image of sham **A, B** and PGA implanted **C, D** animals. PGA-implanted animals showed a lower decrease in DHI compared with sham animal (see arrows). Furthermore, sham animals showed ventral osteophyte formation (*) demonstrating progression of the degenerative process.

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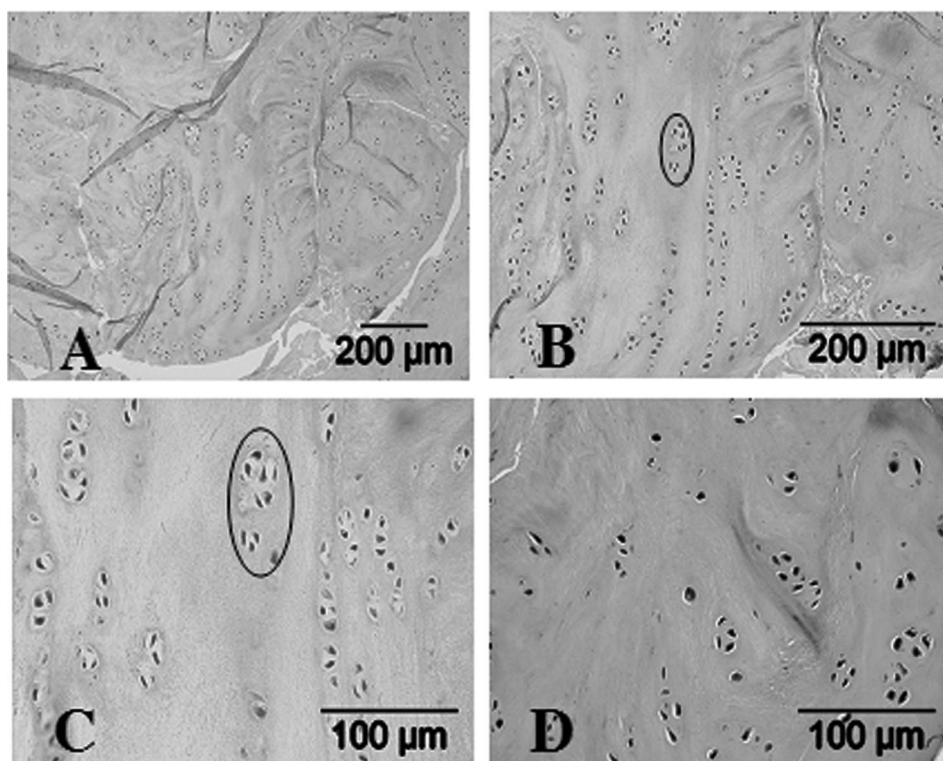


Figure 3. Hematoxylin and eosin (A–C) and safranin O (D) staining of the central area of decalcified intervertebral discs 6 month after nucleotomy and implantation of PGA-constructs. The encircled small clusters of chondrocytes within a dense and homogeneous proteoglycan-rich matrix indicate tissue regeneration and repair tissue formation.

case of disc injury, local nucleus pulposus, anulus fibrosus cells, and mesenchymal progenitors from adjacent vertebral segments migrate into the defect and form undifferentiated fibrous repair tissue. The capacity of local cells to migrate, proliferate, and to form repair tissue may be affected and lowered by the degree of degeneration of the disc. Therefore, we supposed that a “smart”

substrate that initially stabilizes the disc, attracts local cells into the defect, and initiates matrix protein production may induce proper repair tissue formation. Attraction of local cells has been shown by the migration of chondrocytic cells on stimulation with serum from whole blood. Implantation of the “smart” substrate (PGA felt, hyaluronic acid, and serum) formed disc repair tissue

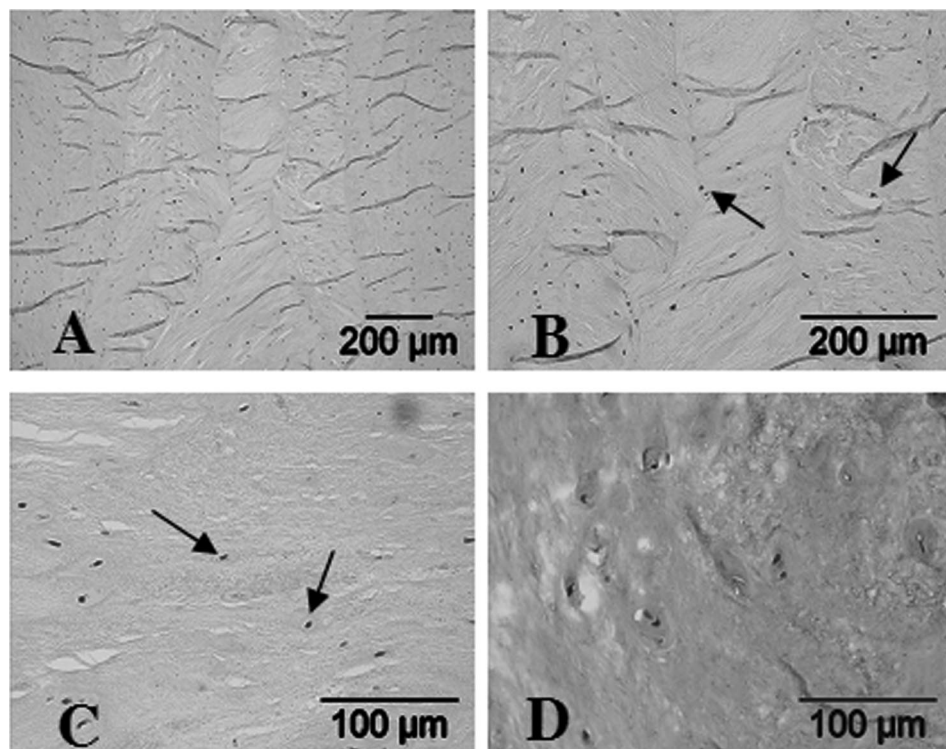


Figure 4. Hematoxylin and eosin (A–C) and safranin O (D) staining of the central area of decalcified intervertebral discs 6 month after nucleotomy only (sham). The arrows indicate fibroblasts within an homogenous matrix with low cellularity.

and prevented loss of disc height as assessed by histologic and radiologic analysis.

Prevention of disc height and restoration of T2-weighted signal intensity on MRI are 2 major parameters for evaluating disc degeneration in clinical settings. In the normal aging process, a decrease of disc height occurs with a decrease in water content associated and a reduction in proteoglycans in the nucleus.^{1–6,15–21} A high signal intensity of T2-weighted images in MRI is often used indirectly to evaluate water content in the IVD.^{18,20} Based on these parameters, regeneration of the disc using resorbable cell-free PGA-based implants was achieved successfully in our rabbit model with water and proteoglycan restoration. Disc chondrocytes of animals with PGA-implants produced an extracellular matrix that contained components similar to normal intervertebral disc tissue. Positive evidence of proteoglycan content was supported by accepted histochemical staining techniques such as Safranin O-Fast Green. Although a morphotypic nucleus pulposus was not generated, cells that could appropriately be considered disc chondrocytes were identified in the intervertebral discs of animals with PGA-based constructs. No evidence of necrotic changes was present, nor were there any active signs of tissue vascularization.

Our data are conform with results using chondrocyte transplantation or transplantation of mesenchymal stem cells to stimulate disc regeneration.^{4,6,17,21} However, the use of cell-free PGA-based constructs for disc regeneration has advantages compared with transplantation techniques. The use of cell-free resorbable implants have the advantage that only one surgical intervention is necessary and donor site morbidity as occurring in cell-based transplantation procedures is avoided. Cell-free implants are storable, have a considerably longer shelf life than cell-based grafts, and can be used on demand for the treatment of discs after nucleotomy. In addition, biomaterials ensure initial mechanical stability and may allow early loading of the disc. Especially, polymers have been shown to be mechanically stable. For instance, the PGA felt used in this study withstood a maximal tensile load of 38 N without rupture of the material. In contrast, a copolymer of PGA and polylactic acid failed at a maximum tensile load of 15 N and collagen ruptured when a load of approximately 10 N was applied.²²

Implantation of the cell-free implant is a one step procedure, which means implantation of the constructs can be performed directly after the microdiscectomy and enables immediate mechanical support. As shown in this study, the cell-free implant may enhance the healing response by stimulating cell migration of local intervertebral disc cells or stem cells from the vertebral bone marrow and by subsequent promoting tissue formation. Recently, it has been shown that stem cell migration can be stimulated by, for instance, synovial fluid and human serum derived from whole blood.^{23,24} Mesenchymal stem cells derived from bone marrow have the capacity to develop towards multiple mesenchymal tissues, in-

cluding cartilage, bone, and fat.^{25,26} In addition, the PGA-hyaluronan-based implant has been shown to induce articular cartilage formation after microfracture in a sheep model.²⁴ Therefore, it is most likely that the cell-free implant may enrich local cells at the defect site by cell attraction and may guide repair tissue formation.

In summary, our results show the efficiency of the PGA-based constructs for cell recruitment as well as for chondrogenic differentiation and matrix protein formation in our microdiscectomy model. Therefore, we believe that the combination of scaffolds with release systems and recruitment technology may be a promising and effective disc tissue repair approach after microdiscectomy.

■ Key Points

- Polyglycolic acid based implants regenerate intervertebral discs.
- Using a rabbit disc degeneration model, we show that cell-free polyglycolic acid based implants form disc repair tissue after nucleotomy.
- The proof of principle *in vivo* shows maintenance of disc height after implanting the polyglycolic acid based construct.

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