Is a collagen scaffold for a tissue engineered nucleus replacement capable of restoring disc height and stability in an animal model?

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Abstract The idea of a tissue engineered nucleus implant is to seed cells in a three-dimensional collagen matrix. This matrix may serve as a scaffold for a tissue engineered nucleus implant. The aim of this study was to investigate whether implantation of the collagen matrix into a spinal segment after nucleotomy is able to restore disc height and flexibility. The implant basically consists of condensed collagen type-I matrix. For clinical use, this matrix will be used for reinforcing and supporting the culturing of nucleus cells. In experiments, matrixes were concentrated with barium sulfate for X-ray purposes and cell seeding was disclaimed in order to evaluate the biomechanical performance of the collagen material. Six bovine lumbar functional spinal units, aging between 5 and 6 months, were used for the biomechanical in-vitro test. In each specimen, an oblique incision was performed, the nucleus was removed and replaced by a collagen-type-I matrix. Specimens were mounted in a custom-built spine tester, and subsequently exposed to pure moments of 7.5 Nm to move within the three anatomical planes. Each tested stage (intact, nucleotomy and implanted) was evaluated for range of motion, neutral zone and change in disc height. Removal of the nucleus significantly reduced disc height by 0.84 mm in respect to the intact stage and caused an instability in the segment. Through the implantation of the tissue engineered nucleus it was possible to restore this height and stability loss, and even to increase slightly the disc height

of 0.07 mm compared with the intact stage. There was no statistical difference between the stability provided by the implant and intact stage. Results of movements in lateral bending and axial rotation showed the same trend compared to flexion/extension. However, implant extrusions have been observed in three of six cases during the flexibility assessment. The results of this study directly reflect the efficacy of vital nucleus replacement to restore disc height and to provide stability to intervertebral discs. However, from a biomechanical point of view, the challenge is to employ an appropriate annulus fibrosus sealing method, which is capable to keep the nucleus implant in place over a long-time period. Securing the nucleus implant inside the disc is one of the most important biomechanical prerequisites if such a tissue engineered implant shall have a chance for clinical application.

Keywords Lumbar spine · Nucleus replacement · Disc implants · Collagen matrix · Tissue engineering · Biomechanical testing

Introduction

Intervertebral disc (IVD) prolapse has been reported to be most frequent in young adults [11]. Spinal fusion and discectomy are two common surgical procedures of treatment [3]. Rigid spinal fixation has several limitations due to the loss of segmental mobility and the potential progress of degeneration in adjacent segments. In contrast, discectomy benefits good short-term effects of pain relieve, but it reduces disc height and increases segmental instability. This can lead to more stress in the remaining annulus but also in the

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facet joints [3, 4, 14, 15], which may lead to an accelerated further degeneration.

Restoring disc height and mobility would require a replacement of the morbid nucleus pulposus by an artificial compound. Several artificial nucleus implants have been developed and some of them have already been clinically applied but the success was not optimal yet [2, 6, 10, 13, 19, 21, 24].

Therefore, more and more biological solutions are discussed. Recently, it has been shown that seeding or reinserting cells inside the intervertebral disc may preserve disc structures by slowing down the degeneration processes [17, 18]. It was also hypothesized the cell insertion might restore the annulus and nucleus tissue [8].

The future might also show trends to use tissue engineered nucleus implants, which is of great interest for orthopaedics and neurosurgeons for the last few years. Sato et al. [20] for example, were able to grow annulus cells in an atelocollagen scaffold which was then implanted in rabbits. He could show that cells were viable and that they were able to proliferate after implantation. Alini et al. [1], used Collagen-I/Hyaluronan scaffolds to culture nucleus/annulus cells. He showed that the cells proliferate but more proteoglycan was released into the culture medium and did not stay in the implant. Even stem cells could be a source of cells for tissue engineering, as they may differentiate into cells with an IVD-like phenotype as proven with a DNA copy of the mRNA (cDNA) [22].

Tissue engineered nucleus implants may provide more mechanical stiffness compared to pure biological solutions. The requirements therefore are to provide a scaffold with adequate mechanical properties, which allows to culture suitable cells in it, for example, *intervertebral disc cells or mesenchymal progenitor cells (MPCs)*. A recent study showed that a three-dimensional collagen matrix might suitably serve as scaffold for cell seeding, that could be used to serve as a nucleus replacement [16]. It could be shown that

these cells were able to proliferate and that cell viability was still 92% after 4 weeks. The cell morphology showed spindle-shaped and rounded cells and a tissue specific gene expression pattern.

The success of such an implant, however, is not only dependant on its biological performance but also on the biomechanical requirements and reliability. Further important requirements are, appropriate implantation techniques and adequate biomechanical performance after implantation. In this study, we investigated whether implantation of the new tissue engineered nucleus implant into a spinal segment after a nucleotomy is able to restore disc height and flexibility.

Methods

Implant

The implant basically consists of condensed collagen type-I matrix (Fig. 1). For clinical use, this matrix will be used for reinforcing and supporting the culturing of nucleus cells. In experiments, matrixes were concentrated with barium sulfate for X-ray purposes and cell seeding was disclaimed in order to evaluate the biomechanical performance of the collagen material. The appropriate ratio between barium sulfate and collagen material was estimated by means of radiographs from different ratios of the implant (3, 7.5, 15 and 60%). It was found that at least a ratio of 50% barium sulfate should be used to visualize small peaces of the implant due to the expected implant extrusion.

Specimen preparation

The in vitro testing was performed on six lumbar functional spinal units (FSU), which have been taken from 5 to 6 months-old calves. The specimens were carefully stripped off the soft tissue and muscle layers

Fig. 1 Condensed collagen matrix concentrated with barium sulfate for radiographic observations. The matrix has been used as a nucleus replacement in these experiments (*left*). This collagen matrix may serve as a three-dimensional scaffold to allowing cell seeding inside (*right*)

preserving all bony structures and ligaments. The cranial and caudal ends of the specimens were embedded into polymethylmethacrylate. In each specimen, a standardized oblique incision was performed from a right lateral approach. A minimal invasive access to the interior of the disc was achieved by a five to six mm long cut from the upper to the lower endplate using a scalpel blade (#11). The annulus fibers were cut parallel to the orientation of the outer annulus fibers, thus preserving the fibers in this direction. Through this fissure, an IVD defect was created by means of complete removal of the nucleus pulposus (Fig. 2). Subsequently, the rest of the cavity of the intervertebral disc was filled with the implant. Proper application of the implant was controlled by radiographic visualization (Fig. 3).

For the first experiment, a specimen with the implant was scanned with a micro CT (Fan Beam, Stratec, Pforzheim, Germany) in a resolution of 0.16×0.16 mm having a slice thickness of 0.32 mm. Implant fit and volume was subsequently controlled after three-dimensional reconstruction (Fig. 4).

Biomechanical testing

The caudal end of FSUs were fixed in a custom-built spine tester [23]. The cranial end of the upper vertebra was fixed to a gimbal system, which allowed the upper vertebra to rotate freely and translate in all directions. Each rotation axis of the gimbal system was driven separately. The motion response of specimen was recorded with rotational potentiometers attached to the gimbal system. Pure bending moments were applied continuously with a constant loading rate of $1.5^\circ/\text{s}$. The specimens were loaded according to the internationally accepted recommendations with pure bending

Fig. 4 Quantification of the implanted volume of the collagen nucleus replacement. Cranial caudal radiograph (*left*) and the reconstructed volume obtained from a microCT scan (*right*)

moments of ± 7.5 Nm to move towards lateral bending right/left (+/-), flexion/extension (+/-) and left/right (+/-) axial rotation. In each direction of this flexibility test, the specimens underwent 3.5 loading cycles whereas the first 2.5 served as precycles.

Data evaluation

Motion data were evaluated for range of motion (ROM) in terms of rotation, neutral zone (NZ) and angle at unloaded posture (kyphosis/lordosis angle). NZ describes the laxity of specimens, which was defined between the two intersection points at 0 Nm due to the hysteretic characteristics of FSU motion pattern. The maximal motion of FSUs reaching the bending moment applied was defined to be the ROM in one direction. The neutral position is the mid-point between the two intersection points defining the NZ. We defined the kyphosis/lordosis angle to be the change in the neutral position of a reduction stage with respect to the intact stage. Values of ROM and NZ of the defect situation were related to the initial

Fig. 2 Specimens depicted at the stage of intact, without nucleus and after implantations. Nucleotomy was performed with Rongeurs from a right lateral approach. Implantation of the nucleus replacement was achieved using a guidance tube for the implantation

Fig. 3 Radiographs which show disc height (h) in the intact state ($h=0$), after nucleotomy, and after implantation

starting posture of intact specimens by adding the kyphosis/lordosis angle.

The ROM was assumed to be non-normal distributed, therefore, a general non-parametric test for more than two conditions of one specimen's group, the Friedmann-test, was used to proof for tendencies of significance. If the Friedmann-test resulted in a $P < 0.05$ then a Wilcoxon's U -test (signed rank test) was used to show statistically significant differences between annulus closure methods. In all tests, P -values with less than 0.05 were considered to be statistically significant and P -values between 0.05 and 0.1 were interpreted as showing statistical tendencies. P -values were not adjusted for multiple comparisons.

Results

Removal of the nucleus induced a slight kyphosis of 0.47° in the segments, whereas the implantation could restore this offset to -0.1° (slight lordosis). Nucleotomy reduced the disc height by 0.84 mm (range 0.36–1.15) in respect of intact stage. Through the implantation of the tissue engineered nucleus it was possible to restore this height, and even led in some cases to a slight increase in disc height with a median of 0.07 mm (range 0.46 to 0.17) compared to the intact stage. Median flexibility of intact specimens was in order of 3.96° ROM and 0.88° NZ in flexion, $ROM_{Ext}=3.11^\circ$ and $NZ_{Ext}=0.91^\circ$ in extension (Fig. 5). Nucleotomy caused a significant loss of stability resulted in $ROM_{flex}=5.45^\circ$, $ROM_{ext}=3.59^\circ$ and $NZ_{flex}=1.59^\circ$ and $NZ_{ext}=1.57^\circ$. Application of the implant significantly stabilized FSUs to a $ROM_{flex/ext} = 4.28/3.36^\circ$ and NZ of 0.57° and 0.54° , respectively. There was no statistical difference between the stability provided by the implant and intact stage regarding flexion/extension movements. Results of right axial rotation and left lateral bending showed the same trend compared to flexion/extension. In lateral bending, the ROM resulted in a strong asymmetrical behavior due to the annulus access at the right side. However, during flexibility measurements, implant extrusions have been observed in three of six cases while loaded in right lateral bending (Fig. 6).

Discussion

The results of this study showed that a collagen scaffold for vital nucleus replacement is able to restore disc height and provide physiological stability to intervertebral discs. However, if the implant is inserted with the standard technique through a hole which is left

Fig. 5 Range of motion (ROM) and neutral zone (NZ) in degrees of the six specimens. Specimens were tested intact, after nucleotomy and after implantation of the nucleus replacement. NS Not significantly different; * significantly different with $P < 0.05$; + statistical tendencies of a difference with $0.05 < P < 0.1$

after a mechanical nucleotomy, it is very likely that the implant is extruded already after a few load cycles.

This is more or less the problem with all nucleus replacement implants, which are currently at different stages of pre clinical and clinical investigations. Therefore, different implant designs shall result in a lower risk of extrusion. Mostly, the goal is to increase the size of the implant after insertion. Hydrogel-derived implants for example, like the Aquarelle (Stryker Spine, Allendale, NJ, USA) increase in size because the implant soaks up water and swells to a size much larger than the hole. However, animal experiments showed a still high extrusion rate ranging from 20% with a posterolateral approach to 33% with an anterior approach [5, 12].

Another nucleus implant made of polycarbonate urethane (PCU) Newcucleus (Zimmer, Spine) curls itself up, during the implantation and forms a spiral within the cavity. But even this implant started to extrude with the end of the implant after a few months of implantation.

Fig. 6 Sequence of images from implant extrusion during the flexibility tests in the spine tester. Extrusion mostly occurred in the direction of lateral bending

The most extensively studied nucleus replacement device is the Prosthetic Disc Nucleus (Raymedica, Inc., Bloomington, MN, USA), which is composed of a structural polymeric hydrophilic hydrogel that is constrained in a woven polyethylene fabric jacket. The original version consisted of anterior and posterior implants, which are oriented in a transverse position within the disc cavity of the denucleated disc. Because first clinical trials showed an extrusion rate of more than 20%, these two cushions were sutured together after the implantation, which strongly reduced this risk. The current “solo”-version consists of only one implant with a larger size.

Due to the limited availability of human cadaver specimens, this feasibility test was performed on the lumbar region of calf specimens. It was found that lumbar calf discs may serve as model for this kind of experiments because the size and shape and structure of the disc is similar to the human lumbar disc [25]. Previous biomechanical investigations showed also similarities regarding spinal flexibility [25].

The nucleotomy was performed through a small incision from a right lateral approach to ensure a free view to the access. The annulus is known to consist of several concentric fiber layers with alternating fiber orientation directions. With this access, the trauma of the annulus was kept minimal in order to preserve the fibers of one direction while fibers of the other direction were cut. This minimal access will also enable us to test different sealing methods in further studies. The denucleation exhibited an unstable situation for specimens, which was in agreement with previous findings [7, 9, 24].

This experiment showed that such collagen implants in principle, are able to restore the biomechanical characteristic of an intervertebral disc, but however, suggests a high risk of extrusion. This implant was already extruded not after a few thousand cycles, but already during the standard flexibility test at ± 7.5 Nm. Therefore, it is important to search for the possibilities to seal the annulus or to prevent extrusion in another way. A solution to this problem is one of the most important biomechanical prerequisites if such a tissue engineered implant shall have a chance for clinical application.

References

1. Alini M, Li W, Markovic P, Aebi M, Spiro RC, and Roughley PJ (2003) The potential and limitations of a cell-seeded collagen/hyaluronan scaffold to engineer an intervertebral disc-like matrix. *Spine* 28(5):446–454; discussion 453
2. Allen MJ, Schoonmaker JE, Bauer TW, Williams PF, Hingham PA, Yuan HA (2004) Preclinical evaluation of a poly (vinyl alcohol) hydrogel implant as a replacement for the nucleus pulposus. *Spine* 29(5):515–523
3. Bao QB, Yuan HA (2002) New technologies in spine: nucleus replacement. *Spine* 27(11):1245–1247
4. Brinckmann P, Grootenboer H (1991) Change of disc height, radial disc bulge, and intradiscal pressure from discectomy. An in vitro investigation on human lumbar discs. *Spine* 16(6):641–646
5. Carl A, Ledet E, Yuan H, Sharan A (2004) New developments in nucleus pulposus replacement technology. *Spine J* 4(6 Suppl):325S–329S
6. Di Martino A, Vaccaro AR, Lee JY, Denaro V, Lim MR (2005) Nucleus pulposus replacement: basic science and indications for clinical use. *Spine* 30(16 Suppl):S16–S22
7. Frei H, Oxland TR, Rathonyi GC, Nolte LP (2001) The effect of nucleotomy on lumbar spine mechanics in compression and shear loading. *Spine* 26(19):2080–2089
8. Ganey TM, Meisel HJ (2002) A potential role for cell-based therapeutics in the treatment of intervertebral disc herniation. *Eur Spine J* 11(Suppl 2):S206–S214
9. Goel VK, Goyal S, Clark C, Nishiyama K, Nye T (1985) Kinematics of the whole lumbar spine. Effect of discectomy. *Spine* 10(6):543–554
10. Husson JL, Korge A, Polard JL, Nydegger T, Kneubuhler S, Mayer HM (2003) A memory coiling spiral as nucleus pulposus prosthesis: concept, specifications, bench testing, and first clinical results. *J Spinal Disord Tech* 16(4):405–411
11. Jonsson B, Stromqvist B (1995) Influence of age on symptoms and signs in lumbar disc herniation. *Eur Spine J* 4(4):202–205
12. Klara PM, Ray CD (2002) Artificial nucleus replacement: clinical experience. *Spine* 27(12):1374–1377
13. Korge A, Nydegger T, Polard JL, Mayer HM, Husson JL (2002) A spiral implant as nucleus prosthesis in the lumbar spine. *Eur Spine J* 11(Suppl 2):S149–S153
14. Meakin JR, Reid JE, Hukins DW (2001) Replacing the nucleus pulposus of the intervertebral disc. *Clin Biomech (Bristol, Avon)* 16(7):560–565
15. Natarajan RN, Andersson GB, Patwardhan AG, Verma S (2002) Effect of annular incision type on the change in biomechanical properties in a herniated lumbar intervertebral disc. *J Biomech Eng* 124(2):229–236
16. Neidlinger-Wilke C, Wurtz K, Liedert A, et al (2005) A three-dimensional collagen matrix as a suitable culture system for the comparison of cyclic strain and hydrostatic pressure effects on intervertebral disc cells. *J Neurosurg Spine* 2(4):457–465

17. Nishimura K, Mochida J (1998) Percutaneous reinsertion of the nucleus pulposus. An experimental study. *Spine* 23(14):1531–1538; discussion 1539
18. Okuma M, Mochida J, Nishimura K, Sakabe K, Seiki K (2000) Reinsertion of stimulated nucleus pulposus cells retards intervertebral disc degeneration: an in vitro and in vivo experimental study. *J Orthop Res* 18(6):988–997
19. Ray CD (2002) The PDN prosthetic disc-nucleus device. *Eur Spine J* 11(Suppl 2):S137–S142
20. Sato M, Asazuma T, Ishihara M, et al (2003) An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annulus fibrosus cells using a tissue-engineering method. *Spine* 28(6):548–553
21. Schoenmayr R, Buch C, Lotz C (1999) Prosthetic disc nucleus implants: the Wiesbaden feasibility study. 2 years follow up in ten patients. *Riv Neuroradiol* 12(suppl 1):163–170
22. Steck E, Bertram H, Abel R, Chen B, Winter A, Richter W (2005) Induction of intervertebral disc-like cells from adult mesenchymal stem cells. *Stem Cells* 23(3):403–411
23. Wilke HJ, Claes L, Schmitt H, Wolf S (1994) A universal spine tester for in vitro experiments with muscle force simulation. *Eur Spine J* 3(2):91–97
24. Wilke HJ, Kavanagh S, Neller S, Haid C, Claes LE (2001) Effect of a prosthetic disc nucleus on the mobility and disc height of the L4–5 intervertebral disc postnucleotomy. *J Neurosurg* 95(2 Suppl):208–214
25. Wilke HJ, Krischak ST, Wenger KH, Claes LE (1997) Load-displacement properties of the thoracolumbar calf spine: experimental results and comparison to known human data. *Eur Spine J* 6(2):129–137