Spinal Fusion: Techniques Results and Limitations

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Spinal fusion or vertebral arthrodesis has first been used around 1910 to treat deformity cases and spinal tuberculosis. Its aim was to abolish movement through bony healing of several spinal segments. Spinal fusion is used nowadays not only to treat infections and deformities but also trauma and degenerative conditions. One of the most common uses is in degenerative disc disease whereby fusion is used in order to stop painful or abnormal movement. Although initially fusion was performed posteriorly, later posterolateral, anterior and combined fusions have been performed. In order to achieve the best possible fusion mass surgeons have tried to obtain fusion simultaneously at the front and back of the spine. One such technique is the so called PLIF technique (posterior lumbar interbody fusion) where bone graft is inserted through the spinal canal into the intervertebral disc following retraction of its contents. A variation of this technique is the TLIF type of fusion (transforaminal lumbar interbody fusion) consisting of introducing the intervertebral graft through the foramen avoiding thus violating the spinal canal. Anterior fusion techniques in the lumbar spine involve approaching the front of the spine either in a transperitoneal or a retroperitoneal route. The advent of rigid fixation mainly using pedicle screws has improved fusion rates although this resulted in increased cost and morbidity. Anterior grafting techniques have evolved towards the use of hallow cages made out of carbon fiber, PEEK or titanium. Fusion cannot be achieved without the use of either bone graft or bone substitutes (save exceptional cases). More recently bone morphogenetic proteins have been commercialized and their clinical use is expanding. The aim of BMPs and other bone substitutes is to avoid donor site morbidity linked with autograft harvesting.

Complications of spinal fusion mainly include infection, nerve root injury related to misplaced pedicle screws and pseudarthrosis. The latter can be quite difficult to diagnose. Several papers in the past have pointed out the poor relation between imaging studies and clinical results. Long term complications include adjacent segment disease in which the level between the fused and unfused spine shows signs of degeneration instability or spinal canal stenosis.

Although spinal fusion in deformity and trauma cases has undoubtedly clear clinical benefits, this procedure is considered controversial in treating degenerative disc disease. Until 1999 there was little scientific evidence on the effectiveness of fusion in treating degenerative lumbar spondylosis (3). A good quality prospective trial published in 2001 showed evidence that fusion was superior to conservative treatment (2). A more recent study though showed no clinical difference between fusion and conservative management comprising exercises and cognitive intervention (1). Even though fusion might still have its place for deformity, trauma, infections and some tumors its use in degenerative disc disease might be overtaken in the future by newer more physiological solutions.

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Disc arthroplasty, Surgical approaches

Gunzburg R., Willocx P

address

A surgical approach requires knowledge of surface anatomy, radiological anatomy and finally surgical approach anatomy. Patients expect a cosmetically acceptable scar leading to minimally invasive approaches. Current disc replacements, however, require a strict anterior approach. The exact position of the iliocava junction and left iliac vein has to be known. It is essential to have a table which is adjustable during surgery, allowing for orthogonal fluoroscopy. The surgical approaches are different for a one level L5-S1 and for more than one level. The two surgical approaches are discussed. Special attention has to be given to the superior hypogastric plexus: cauterization has to be avoided, especially in males (retrograde ejaculation). Other potential complications comprise vascular injuries, urethral injuries, postoperative ileus, and abdominal hernias. Limitations to the indication of disc replacement surgery are not yet established according to ‘evidence based’ principles and are mostly flowing out of ‘common medical sense’: one needs facet joints devoid of advanced osteoarthritis, good quality abdominal vessels and therefore a relatively young patient, spondylolysis or listhesis are considered to be contra indications as are spinal stenosis, major radiculopathy and a history of anterior (retroperitoneal) surgery. Previous spinal surgery other than discectomy at the painful level is also thought to be a contra indication as are fracture, spinal tumor, general or local infection, evolving autoimmune disease, pregnancy, morbid obesity, psychiatric disturbances, and major bone disease.

A New Procedure for Total Nucleus Removal from the Posterior Approach

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INTRODUCTION: A traditional discectomy aims at removing primarily herniated nerve compressing tissue. Contrary, nuclear replacement devices require as much nucleus material removed as possible in order to optimize the positioning and size of the device as well as associated load transfer capabilities while minimizing endplate disruption [1, 2]. Therefore, total nucleus removal (TNR) is a requisite for a successful clinical outcome. However, until now, a method for TNR from the posterior approach has not been available. The purpose of the study was to document and develop a method for posterior monoportal TNR.

METHODS: Twelve human cadaveric discs were used in an iterative posterior monoportal approach to document the capability of a wide range of instruments in removing nucleus tissue from various anatomical zones. New instruments were developed if zones could not be reached using traditional surgical instruments. The thoroughness of each step was evaluated using an imaging balloon filled with contrast medium and taking fluoroscopic images from multiple directions. A templating technique, assessing the shape and volume of the nucleus cavity, was used to guide the TNR procedure. The implant used was a two-part in situ curable polyurethane, which is implanted through a 5.5mm annulotomy under controlled pressure while in liquid form and being contained within a polyurethane expandable balloon (Disc Dynamics, Inc., DASCOR™).

RESULTS: Performing a posterior monoportal TNR while maintaining a minimal annulotomy proved difficult with traditional nucleotomy instruments. Consequently a surgical map of the nucleus (Figure 1) was created, based on a given instruments ability to reach a particular zone. Furthermore visualization techniques for intraoperative assessment of the procedure were developed. With a specific sequence of fluoroscopic views and the measurement of calibrated pictures, important target criteria could be controlled. Measurement in the human cadaver studies demonstrated that TNR could be accomplished and the nucleus replacement device accurately conformed to the size and shape of the nucleotomy space, as predicted by intraoperative measurements and calculations.

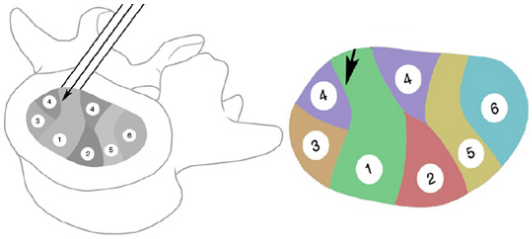

Fig. 1: Surgical map for TNR from a posterior approach

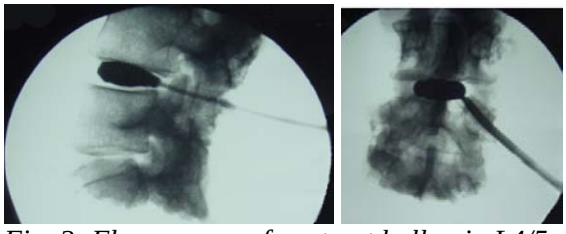

Fig. 2: Fluoroscopy of contrast ballon in L4/5 of a posterior TNR approach

DISCUSSION & CONCLUSIONS: A posterior TNR using a monoportal approach presents technical challenges due to anatomical limitations, particularly at the lower lumbar levels. By utilizing a mapping technique for nucleus removal combined with fluoroscopic imaging of a contrast-filled balloon, remaining nucleus material was identified and systematically removed. Experimental findings have demonstrated the technique's reliability which is currently being adopted in a prospective multi-center clinical study.

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Transient Reduced Mineral Density Associated with BMP-enhanced Spinal Fusion

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INTRODUCTION: BMP-2 has gained broad acceptance as an adjuvant to spinal fusion when used with an interbody fusion device. Given that BMP promotes regional changes in bone mineral density, bone mass, and bone geometry, the purpose of this study was to evaluate metabolic reactions that might affect both short-term and long-term stability of the interbody fusion.

METHODS: The clinical and surgical experience of 17 patients treated for degenerative lumbar spine disease has been compiled. In combination with dorsal fixation with pedicle screws each patient received two poly-ether-ether-ketone (PEEK) interbody cages with 6 mg of BMP-2 in each cage. Patient follow-up consisted of pre-operative radiographs and clinical evaluation, followed further at 3 months, at 6 months, and at 1 year after surgery. Seventeen patients, 8 males, 9 females, mean age 67 years, 14/17 at L4-5. Radiographs, Spiral CT reconstruction, and BMD measurements were used to interpret morphology, cage stability, and fusion at 3 months, at 6 months, and 1 year following surgery.

Data were collected in a retrospective analysis of all patients. Dallas Questionaire and Prolo Score and overall pain on Visual Analog Scale (VAS) were used to assess outcome. Particular attention was focused on vertebral bone tissue adjacent to the fusion mass above and below the fused level.

RESULTS: Clinical assessment demonstrated clear improvement in all patients over the course of the 1-year follow-up. An unexpected finding, apparent at 3-month's follow-up in every patient, was reduced mineral density at the vertebral endplate both above and below the level of fusion. This regional osteopenia was transient in all patients however, and at both the 6-month and at the 1-year evaluation had resolved.

DISCUSSION & CONCLUSIONS: Although confirmation by biopsy could not be obtained, apparent osteolysis adjacent to the BMP-stimulated fusion mass may be interpreted by two mechanisms. Interbody fusion, or surgery in general, creates an inflammatory stimulus to the vertebral bodies, and when coupled with BMP stimulation has the potential to enact a metabolic response where osteoid deposition overrides mineral supplementation. Despite the fact that trabecular bone may indeed be forming within the matrix, delayed mineralization interpreted by radiographs would suggest a lack of bone formation. A potential second mechanism might be accounted in considering regional, humoral hypercalcemia. While generally associated with myeloma and hormonal-laden systemic effects, a potential for high concentrations of anabolic growth factor to stimulate regional osteoclastic mechanisms can not be discounted. In conjunction with the PLIF techniques in this series of patients, sufficient stability was attained through the construct to delimit the transient finding as an observation rather than as an adverse event. With the advent of resorbable fixation systems for vertebral application, recognizing a transient potential for reduced mineral density following high doses of BMP underscores the importance of sustaining fixation sufficient for achieving therapeutic intent.

DISCLOSURES: Partial support for this project was provided by Sofamor Danek.

Total disc arthroplasty: consequences for sagittal balance and lumbar spine movement.

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Study design. This in vivo biomechanical study was undertaken to analyse the consequences for sagittal balance and lumbar spine movement in three different lumbar disc prostheses.

METHODS: A total of 105 patients underwent total disc replacemen in three different centers. The Maverick prosthesis was used in 46 patients, SB Charité device was used in 49 patients and Prodisc device was utilized in 10 patients. The analyse was computer assisted, using Spineview and Matlab softwares. The intra and interobserver reliability and measurement uncertainty was performed. The analysis of lateral X-ray films in flexion-extension, allowed to measure the prosthesis postioning, the range of motion, the localization of the mean center of rotation, the vertebral translation and the disc height, for each prosthesis device. The sagittal balance was analysed on full spine film. The parameters studied were described by Duval-Beaupère. The results were compared to the data found in literature, and compared to 18 asymptomatic patients, and 61 asymptomatic subjects, concerning the sagittal balance.

RESULTS: Prostheses allowed an improvement of the range of motion of less than 2°. The range of motion of L5-S1 prostheses ranged from 11.6 to 15.6% of the total lumbar motion during flexion-extension. At L4-L5 level, the range of motion decreased when there was an arthrodesis associated at L5-S1 level. There was no difference of range of motion between the three prostheses device. The mean center of rotation was linked to range of motion, but did not depend on the prosthesis offsetting. The disc height improved for any prosthesis, and decreased in flexion or in extension, when the prosthesis is off centered. Translation indicated a minor increase of range of motion at L4-L5 level for the Maverick *versus* the SB Charité. L5-S1 arthrodesis was linked with an increase of the Pelvic Tilt. The lumbar lordosis curvature increased between L4 and S1, even more when a prosthesis was placed at L3-L4 level.

DISCUSSION & CONCLUSIONS: Total disc arthroplasty is usefull in the surgical management of discogenic spinal pathology. The three prostheses studied allowed to restore the disc height, the range of motion, without disrupting the sagittal balance, but induced modification of the lumbar curvature. Lumbar prosthesis seems to be an alternative to lumbar fusion.

Enhanced osteointegration by biochemical surface modification: covalent linking of collagen I to intervertebral metal disk surface

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INTRODUCTION: In a recent paper[1] Cunningham suggested that, among the major areas on which the long-term successful performance of total disc arthroplasty is based, the most important and most challenging aspect is for the implanted device to encourage osseointegration at the bone–metal interface while preserving the biomechanical properties of motion. To enhance osteointegration, present models of intervertebral disks rely mostly on micro-mechanical, titanium-based approaches or ceramic coatings. Contemporary biomaterials surface science points to the immobilization of biological molecules that can direct events at the tissue/implant interface as a promising new approach to osteointegration[2]. In this communication, we present our result on *in vivo* evaluation of immobilization of collagen type I to metal surfaces and its application to an existing vertebral disk.

METHODS: Ti samples were modified as reported in[2] by a process involving deposition from propene plasma, followed by acrylic acid grafting and collagen type I (Kensey Nash, Lot. No. 24903) covalent linking. Six rabbits were used for bone implantation. The femur middiaphyses was exposed and samples were transversally implanted, up to a total of 12 implants for each material. Animals were sacrificed at 4 weeks. Evaluation was performed by hystomorphometry and measurement of push out force using a MTS apparatus. The same surface modification process was applied to a Charite intervertebral disk, to evaulate suitability to intervertebral disks coating.

RESULTS: Results of *in vivo* testing are reported in Table 1. Histomorphometry data show that both bone to implant contact and bone ingrowth are signficantly higher on the collagen coated sample. Also functional evaluation by the measurement of push out force shows a signficant improvement on the coated sample. Figure 1 shows the Charite disk after the coating process. Sample was toluidine blu stained to disclose the presence of the collagen layer.

Table 1. Results of in vivo testing at 4 weeks

	Control	Collagen coated	*p*
Bone to Implant contact (%)	62.7±23.4	77.7±17.8	<0.01
Bone ingrowth(%)	85.3±11.7	91.8±6.8	<0.01
Push out Force (Mpa)	13.6±4.0	20.7±6.8	0.003

Surface analysis, by X-ray Photolectron Spectroscopy, confirms the homogeneous coating by collagen molecule on the disk surface.

Fig. 1: Charite disk bearing a covalently linked layer of collagen.. Toluidine blue staining shows coating homogenety

DISCUSSION & CONCLUSIONS:

Collagen type I coating of titanium, obtained by specific surface functionalization and covalent linking[2], shows enhanced bone ingrowth and bone implant contact, together with enhanced mechanical performances, in a rabbit model. The same process can be applied to existing intervertebral disks, yielding a metal disk that presents a homogeneous collagen-rich surface layer. Moreover, the surface layer can entrap charged molecules like growth-factors, opening the way to a multifunctional type of surface, combining biochemical function and drug release. These findings show that emerging methods of biochemical surface modification[2] can contribute to the development and improvement of intervertebral disks.

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The myths and the facts of disc arthroplasty surgery

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The idea to replace the intervertebral disc by a mechanical prosthesis has long been around and many attemps have been made in the last century already to find a clinical viable solution.

It's only in the last good 10 years that disc replacement has become a clinical issue, first in Europe with the development of models, which seemed to yield acceptable clinical results. It's only in the last 5 years and with the fact of the outcomes in FDA studies in the United States, when disc replacement became a major issue in spine surgery. This is exemplified in the fact that there is a spine arthroplasty society, which within a few years grew up to a society with almost 1500 members, and with the occurence of a big number of companies developing and producing disc arthroplasty devices, aspiring to participate in a market, which is estimated between 1 and 3 billion US$ in the next few years worldwide.

Although the concept to maintain a mobile segment instead of fusing it, specifically in degenerative spine disease is very appealing, there are major mechanical as well as biological issues, which are still poorly understood, and scientifically not well supported. Furthermore prospective randomized studies do mostly not have a longer follow-up than of 2-4 years, most of them only 2 years, where disc arthroplasty is usually compared in the endpoint outcome with spinal intervertebral fusions. Even in the classical studies the disc replacement has been compared with a non-ideal anterior intervertebral fusion, after two years most of the parameters seemed to be approximately the same, independent whether there was a disc replacement or a fusion.

It remains to good clinical science with well-designed studies and to the basic scientists to support the enthusiasm for disc replacement with reliable data in order not to be driven by marketing issues but by well-documented science. The dream to have a maintenance of a mobile lumbar and cervical spine in spite of degenerative disease is very valid and the endeavors in science and clinical development are justified.

The ultimate solution may not be a mechanical disc replacement but much more a biological disc replacement with options to induce regeneration of a degenerated disc and intervertebral joints.

Allogeneic intervertebral disc transplantation: Results of pre-clinical and clinical studies

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INTRODUCTION: The concept of motion preservation after intervertebral disc excision is attractive and gaining popularity. Current methods of preservation have included nucleus replacement and prosthetic intervertebral disc replacement. While the latter is particularly gaining popularity, the long term results of an artificial disc are still not known.

The concept of intervertebral disc transplantation is borrowed from the success of other large organ transplantation. Since 1992, the authors have conducted a series of experiments to study the feasibility of such a procedure in a primate model using autografts, fresh allografts and fresh-frozen allografts[1,2].

METHODS: The surgical procedure involves a near complete excision of the intervertebral disc and its bony end-plate in the recipient, while from the donor the whole intervertebral disc is excised en-bloc with its bony end-plate. Size and height matching is carried out before insertion of the appropriate donor disc.

RESULTS: The transplanted discs were shown histologically to be viable, metabolically active, and biomechanically be able to maintain mobility and stability. However, with long term follow-up, the transplanted disc appears to undergo progressive degeneration[1,2].

DISCUSSION & CONCLUSIONS: This is the first study to demonstrate that allogeneic disc transplantation to be viable. With fresh frozen allografts, there appears to be no significant immunologic response. Healing of the end-plates and therefore stability of the transplanted disc occurs reliably. However, before healing of the bony end-plates and re-establishment of a blood supply, nutrition to the transplanted disc maybe lacking and hence cell necrosis may occur. Such that with a reduced cell population inside the nucleus, they are unable to maintain the extracellular matrix, and maybe responsible for the progressive degeneration after transplantation. Nevertheless, it was felt that as long as the segment was clinically asymptomatic, such transplantation could at least postpone a fusion of the segment which may have long-term effect on the juxtafusion levels.

Since 4 years ago, one of the coauthors has started a pilot series of 8 cases of fresh-frozen disc allograft in the human cervical spine. The medium-term follow up results to date have been very satisfactory. Neurologically the patients have all improved. There was no pain, radiologically there was no sign of instability and the segments were still mobile on flexion-extension.

Future directions include a detailed characterization of the cellular and metabolic events after transplantation, and use of autologous culture expanded stem cells or nucleus cells to repopulate the transplanted disc.

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Scaffold Characterisation for Nucleus Pulposus Regeneration

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INTRODUCTION: The overall goal of this project is to develop an in-situ cross-linkable, biodegradable cell-seeded scaffold for the recapitulation of degenerated intervertebral discs. In a previous study we have shown that microbial Transglutaminase (mTGase) is an efficacious enzymatic cross-linking agent for protein based substrates [1]. The specific aims of this study are to i.) investigate the efficacy of mTGase in cross-linking type II collagen and glycosaminoglycans (GAGs) and ii.) generate biomechanical control data from bovine nucleus pulposus (NP) explants under creep loading in confined compression. The hypotheses behind these objectives are that mTGase cross-linking will enhance the physicochemical properties of the scaffold and that compression modulus data obtained from NP explants will elucidate biomechanical properties necessary for tissue engineering of the NP.

METHODS: Scaffolds were prepared with collagen (C-9301, Sigma) to GAG (C-4384, Sigma) dry weight ratios of 2:2 and 2:5 respectively. Ca^{2+} independent mTGase (Activa®WM, Ajinomoto Co. Inc., Japan) was purified by cation-exchange chromatography (specific activity of 27000nmol putrescine incorporated/mg/hour). Collagen was cross-linked with 0-250µg/ml mTGase and the amount of cross-link formation was quantified by amino acid analysis.

A confined compression fixture made from polytetrafluoroethylene was designed for use with a Dynamic Mechanical Thermal Analyser (DMTA®2980, TA Instruments Inc., DE, USA). Creep loading (constant load of 18N) of bovine NP tissue was carried out over a three hour period at 37ºC.

RESULTS: The amount of ε(γ-glutamyl)lysine increased rapidly with increasing mTGase concentrations up to 50-100µg/ml, with a plateau at higher mTGase concentrations (Fig. 1). Preliminary data obtained from creep loading bovine lumbar NP tissue (Fig.2) showed a compression modulus of 0.475MPa after 180 minutes.

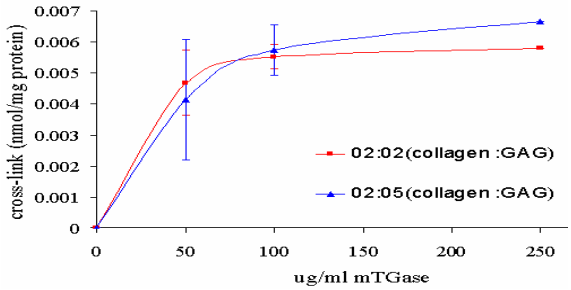

Fig. 1: Amount of cross-link formed in mTGase cross-linked collagen-GAG scaffolds (n=2).

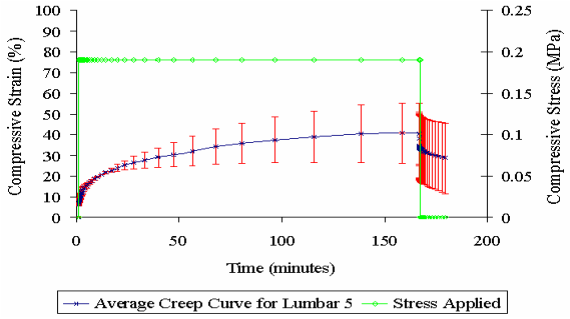

Fig. 2: Creep curve obtained from bovine lumbar (L4-L5) NP tissue. (n=3).

DISCUSSION & CONCLUSIONS: Optimal cross-link formation was found to occur at 50-100µg/ml of mTGase. Future biomechanical studies will compare the strength of mTGase cross-linked scaffolds to bovine NP tissue. Since the extracellular matrix protects the cells in the NP from stresses, recreating the biomechanical environment that the cells are exposed to *in vivo* could potentially induce more effective synthesis of disc tissue.

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Preclinical Evaluation of Intrinsically Radiopaque Hydrogels for Replacing the Nucleus Pulposus

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INTRODUCTION: Nucleus replacement is a promising new approach to treat mild cases of degenerative disc diseases. Current prosthesis designs [1-2] have several disadvantages: they are radiolucent, which makes exact positioning within the annulus difficult, and they do not fill the entire cavity, which alters the stress-distribution in the intervertebral disc. Here we describe a new type of prosthesis based on intrinsically radiopaque hydrogels that possess controllable swelling and mechanical properties. To serve as a nucleus replacement, the prosthesis should meet the following requirements: 1)Dry implantation through a small incision in the annulus with subsequent *in situ* swelling to fill the entire nucleus cavity; 2)Excellent visibility with X-ray fluoroscopy and MRI, without artifacts; 3)Adequate mechanical properties and fatigue resistance.
We designed and studied different hydrogels that fulfill these criteria. From one material, a prototype nucleus prosthesis was prepared, which was evaluated in a realistic model.

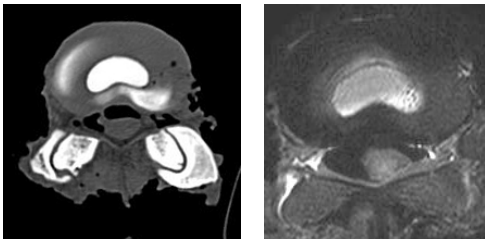
METHODS: Hydrogels were prepared by copolymerizing either N-vinyl pyrrolidinone (NVP) or hydroxyethyl methacrylate with the radiopaque monomer 4-iodobenzoyl-oxo-ethyl methacrylate (4IEMA) [3] in the molar ratio 94/6. The resulting copolymers are indicated by N94 and H94 respectively.
Swelling studies were conducted at room temperature in PBS. Their E-modulus was determined at 37 °C in a water bath. Biocompatibility was verified *in vitro*, using the MTT and Live/Dead assays, and *in vivo*, by subcutaneous implantation in mice. The mice were sacrificed after 1 week and 3 months. PMMA was also implanted as a reference material. A prototype nucleus prosthesis was made from N94 and was implanted into an explanted porcine lumbar spine. It was allowed to swell overnight and visualized using CT and MRI.

RESULTS: Swelling studies showed that N94 absorbs more water than H94, however their E-moduli are comparable (table 1) and within the desired range of 0.2 – 4 MPa [4]. The *in vitro* cytotoxicity assays showed that both materials are non-cytotoxic.

Table 1. Equilibrium water content (EWC) and E-modulus

	EWC	E-modulus
N94	74%	1.4 MPa
H94	23%	1.2 MPa

After one week of implantation in mice there was only a mild acute inflammation. After 3 months a thin fibrous capsule surrounded all implanted materials and macrophages were only sporadically detected. The prototype prosthesis was easily implanted through a small incision in the annulus, which was closed with suture. After overnight swelling, the hydrogel filled the entire cavity and clear CT and MRI scans could be obtained (fig. 1).
Fig. 1: CT scan (left) and MRI scan (right) of N94 prosthesis in a porcine model.



DISCUSSION & CONCLUSIONS: Both N94 and H94 appear to be biocompatible hydrogels. With its high swelling capacity, N94 will be easiest to implant through a minimal incision in the annulus. The prototype implantation proved the suitability of the new nucleus prosthesis and visualization was excellent with both, clinically important, visualization techniques, especially as compared to current designs. Future studies will include fatigue resistance tests and long-term implantation in living animals.

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Can the Biomechanical Disc Function Be Restored by a Collagen Matrix Nucleus Replacement

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INTRODUCTION: The goal of a nucleus replacement is to restore the origin disc height and motion. Recently, it has been shown that rebuilding the nucleus by seeding autologous cells inside the intervertebral disc (IVD) is capable to enhance anatomical integrity, which also preserves vital structures maintaining nutrition transport and metabolism[1]. 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: 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 order to evaluate the biomechanical performance of the collagen, the collagen matrix was concentrated with barium sulfate for x-ray purposes but no cells were seeded (Fig.1a). The in vitro testing was performed on six bovine lumbar functional spinal units (FSU), aging between 5 and 6 months. In each specimen an oblique incision was performed from a right lateral approach. Through this access the nucleus was removed and replaced by a collagen-type-I matrix (Fig.1b). Proper application of the implant was controlled by radiographic visualization.

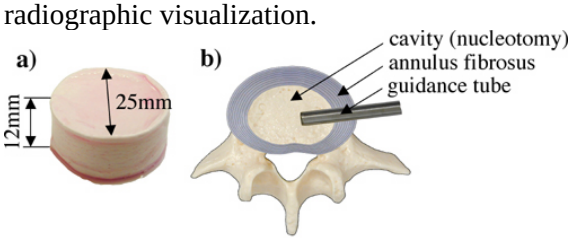

Fig.1: a) collagen matrix, b) implantation.

Spinal segment flexibility was assessed using a custom-built spine tester[2]. Unconstrained pure moments of ±7.5Nm were applied in axial rotation, flexion/ extension and lateral bending. For each tested stage (intact, nucleotomy, and with implant) flexibility and height measurements were performed. The 3rd load cycle was evaluated to determine the range of motion (ROM) and neutral zone (NZ) in each direction. Paired t-test was employed. In order not to loose significance the p-values were not adjusted for multiple testing.

RESULTS: Removal of the nucleus reduced disc height by 0.88±0.37mm in respect to intact stage. In contrast, implantation of the vital nucleus replacement increased the disc height by 0.15±0.4mm compared to intact stage. The average flexibility of the intact specimens was 7.9±4.3° ROM and 3.0±2.3° NZ in flexion/ extension. Nucleotomy caused a significant loss of stability and resulted in 10.0±4.7° ROM and 3.5±2.2° NZ ($p<0.05$). Application of the implant significantly stabilized FSUs to a ROM and NZ of 7.6±2.0° and 1.4±0.3°, respectively ($p<0.05$). There was no statistical difference between the stability provided by the implant and the intact stage. Similar trends were found in lateral bending and axial rotation compared to flexion/extension. However, implant extrusions have been observed in three of six cases.

DISCUSSION & CONCLUSIONS: Artificial and tissue engineered nucleus replacement has been of great interest for orthopaedic and neurosurgeons. Early treatment of disc degeneration or IVD prolapse are intended to be the main indications for tissue engineered nucleus replacement. The results of this study reflect the principal 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 IVD will be considered as an objective for future biomechanical studies.

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Re-Swelling Degenerate Intervertebral Discs by Percutaneous Injection: an *In-Vitro* Investigation

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INTRODUCTION: Healthy intervertebral discs (IVD) are swollen structures that support substantial static and dynamic loads through hydrostatic pressurization of the fluid-filled nucleus pulposus (NP). During the diurnal loading cycle, the disc is compressed and the fluid within it seeps out. The NP contains a high concentration of proteoglycans (PG), which effect a high osmotic potential, drawing water back into the disc during rest. Although the exact mechanism of disc degeneration remains unclear, it is widely known that degenerate discs generally contain less PG and hence less fluid [1]. Restoration of the natural swelling capacity of the disc would appear to be a critical requirement for the effective treatment of damaged or degenerate discs. The concept of artificially increasing the osmotic potential of PG-extracted intervertebral discs by direct injection of a biocompatible, osmotically active gel was investigated in this project as a means to recover their mechanical function.

METHODS: Porcine coccygeal discs were isolated with endplates intact. An apparatus was designed to measure the temporal changes in the swelling force of the discs. The discs were placed in a bath of 2M NaCl solution, and loaded to 4 N by a porous platen to which a load cell was connected. When the disc had reached equilibrium, the 2M solution was replaced by 0.15M NaCl. The transient (osmotic) swelling force was captured by the load cell and the maximum value recorded. The samples were then immersed in 4M GuHCl for six days to extract the PGs from the nucleus and were tested again. A viscous gel of high molecular weight polysaccharide alginate (1.5% alginate) was then injected into the NP, and tested again. The effectiveness of PG extraction comparing extracted (n=7) to control (n=10) discs was determined using the Alcian blue dye test [2]. The effects of mechanical damage (cutting and piercing the annulus) were also investigated using the same testing method.

RESULTS: The swelling force of cut discs (n=8) increased by 15% (±27), while that of the pierced discs (n=3) decreased by 11% (±52). The PG content of the discs decreased by an average of 40% after PG extraction (*Fig. 1(a)*). The intrinsic swelling force exerted by discs following PG extraction decreased, on average, by almost 85%. Injection of the alginate recovered 10% of the original swelling force (*Fig. 4(b)*).

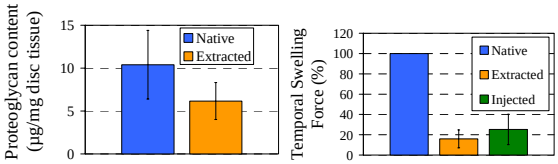

Fig. 1 (a) Proteoglycan content (left) (b) Swelling pressure (n=10) (right)

DISCUSSION & CONCLUSIONS: It was shown that the bulk swelling response of the disc can be reproducibly measured *in-vitro*. Loss of PG content had a disproportionate influence on whole disc swelling. A sensitivity study performed using a numerical model of the experiment showed that nucleus diameter has the greatest influence on swelling force, followed by swelling pressure and then annulus stiffness. Cutting the annulus had the effect of increasing the swelling force if more than ¾ of the annulus was sectioned. Annular piercing was found to reduce the total swelling force, and therefore could have negative implications if employed in potential therapy. Injection of alginate partially restored the intrinsic swelling behaviour of the 'degenerated' disc, but only a very limited amount of gel could be introduced into the dense tissue matrix. Additional stimulation designed to produce new disc matrix material is likely required for complete functional repair. Alginate could possibly be seeded with autologous NP cells for biological, minimally-invasive disc repair [3].

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Is the Disc a Source of Back Pain?

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The disc as a source of back pain is a controversial topic. This is primarily because disc degeneration occurs as part of normal aging and presents as often without associated back symptoms as with. It is also controversial because at the present time the only method to determine if a disc is painful is discography which is a diagnostic technique with low sensitivity and specificity. One reason why disc degeneration is considered a potential source of back is that it is sometimes the only identifiable abnormality. Further, it can easily be observed on magnetic resonance imaging and is known from in vitro studies to be biomechanically different from a healthy disc. There are also biochemical theories about the pain resulting from degenerative changes which are difficult to prove in vivo.

The disc as a secondary source of back pain is much less controversial. In fact disc degeneration is a necessary presence for herniations to occur. Also when the disc degenerates it loses height. This has implications on the relationship of the moving surfaces of the facet joints creating facet osteoarthritis and leads to stenosis because of the facet osteoarthritis and the bundling of the ligamentum flavum. Thus facet osteoarthritis and spinal stenosis can be seen as secondary to disc degeneration. Another secondary disease entity is degenerative spondylolisthesis where the biomechanically weaker disc allows slippage of one vertebra on its neighbor.

In summary, disc degeneration is a cause of secondary spinal conditions which are common and not infrequently lead to surgical intervention. If it is also a source of primary back pain, remains controversial and will be explored further in the presentation.

Cell-cell interactions and the cytoskeleton in organisation of the developing intervertebral disc

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INTRODUCTION. The intervertebral disc develops from two key components: the embryonic notochord and sclerotomally derived mesenchyme. Initially, the mesenchymal cells surround the notochord and, then under the influence of a variey of embryonic patterning systems, form regular repeating dense annular condensations of sclerotomal cells interspersed with more widely spaced cartilage precursor cells of the early developing vertebral bodies. As the vertebral body cartilage differentiates, the annular condensations shows evidence of differentiation into inner and outer regions. Shortly afterwards, the notochord rapidly bulges in the region of the developing disc, and thins in the vertebral bodies, from which it eventually disappears. The bulges form the foetal nucleus pulposus, and as they enlarge the annular condensations differentiate into the cartilaginous inner and fibrous outer annulus fibrosus. The ends of the vertebral bodies on either side form the cartilage endplates. The subsequent fate of the foetal nucleus pulposus is species dependent. Some species, eg mice and rats, retain notochordal cells as the predominant cell type in the nucleus pulposus throught life. Others, eg. horse, lose them before they are born. Many are intermediate between these extremes, including human beings, where notochordal cells are lost by about 8 years old, although a few may persist for longer. The nucleus pulposus becomes populated by a chondrocyte like cell population whose origin has not been conclusively demonstrated, but is likely to be from the surrounding cartilaginous inner annulus fibrosus and endplates. In the foetal annulus, cells have to organise the deposition of highly ordered arrays of collagenous lamellae to form the adult structure of the annulus fibrosus. The first stage of this process involves orietantion of cells into sheets of oriented fibroblasts, with cell orientations being organised at angles of around 50-60 degrees of the cells in the preceeding sheet. Work in our laboratories has examined how this orientation process ocurrs, and how the orientation of cells my be related to the oriented deposition of collagen.

METHODS. Rat intervertebral lumbar spines from foetuses, neonates and older animals, up to 2 years, were frozen, cryosectioned and labelled for a variety of molecules associated with the cytoskeleton, cell-cell and cell-matrix interactions and collagen production and examined by conventional and confocal microscopy.

RESULTS AND DISCUSSION. Early stages of cell orientation occur as cells assemble large actin stress fibres in their cytosol, coincident with cell elongation; within a cell layer all of the actin fibres are parallel, ensuring all of the cell have the same orientation. This organization is maintained for the remaining foetal period as the collagenous lamellae are deposited. In addition to orienting cells, the actin cytoskeleton may have a direct role in matrix orientation. Firstly, alpha5beta1 integrin at the cell surfaces is associated with stress fibres intracellularly and oriented fibronectin extracellularly, and secondly, examining another tissue with a high degree of collagen lamellar organization, the cornea, we have shown that intracellular vesicles containing type I procollagen localize along actin filaments, suggesting that collagen is trafficked along the stress fibres. At early stages of cell orientation, expression of gap junction proteins is prominent, suggesting coordination of cell behaviour in organizing the lamellae, along with cadherin expression showing cell-cell binding. Highly organized expression of vinculin and cadherins at slightly later stages suggests the maintenance and enhancement of cell-cell contacts using actin-associated adherens junctions, possibly retaining relative cell orientation of lamellae. Remarkably, the actin stress fibres are rapidly lost after birth, suggesting that cells now orient to the deposited extracellular matrix, rather than using their own intrinsic mechanisms.

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The notochordal cell in the postnatal intervertebral disc

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INTRODUCTION: During embryogenesis of the intervertebral disc, the cells of the notochord play a critical role in initiating tissue formation, and may be directly responsible for development of the nucleus pulposus. In some species, including humans, these notochordal cells may eventually be lost, perhaps through apoptosis or terminal differentiation, and are replaced by chondrocyte-like cells[1]. However, there is some evidence that the notochordal cells may persist in at least some humans.

The nucleus pulposus of the intervertebral disc undergoes substantial changes during aging and degeneration, which can compromise disc function and lead to chronic pain and debility. One of the first morphological changes is the loss of the notochord-derived cells[2]. However, the significance of loss of the notochordal cells and subsequent degeneration of the disc has not been thoroughly studied.

METHODS: We have used numerous tools, including confocal scanning microscopy, transmission electron microscopy, cryogenics, immunohistochemistry, and real-time PCR to investigate the functional behavior of adult-derived notochordal cells.

RESULTS: Both embryonic notochord and adult notochordal cells have been long described as "physaliferous," meaning they appear "foamy" in conventional histological sections[3]. We have found that while this term is accurate, it only begins to describe the structure of these unique cells. The cells contain massive vacuoles which take up as much as 80% of the cell volume (Fig. 1), surrounded by dense actin cortices and multilamellated membranes (Fig. 2)[4]. These vacuoles appear to function at least in part in osmoregulating the cells[5]. The cells are occasionally multinucleated, a phenomenon which can become more common *in vitro*. *In situ*, the cells exist in tightly joined clusters of up to 100 cells (Fig. 3). Within these clusters, the cells are connected via gap junctions, and maintenance of these connections seems to be important for *in vitro* survival[5].

In situ, the notochordal cells do not exist in isolation. We have begun to identify interactions between notochordal cells and other cell types, including chondrocytes, annulus fibrosus cells, and mesenchymal stem cells. Notochordal cells stimulate gene expression in these cell types, particularly genes for small proteoglycans.

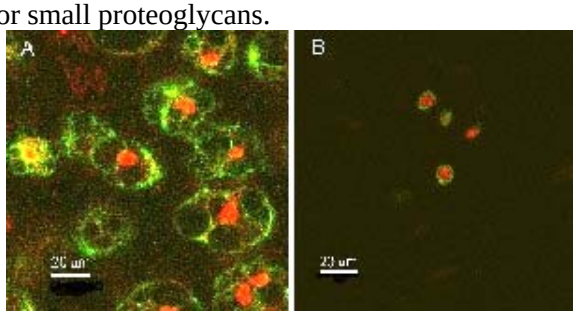

Figure 1. Actin structure of disc cells, (A) young disc (B) aged disc. Note the presence of large vacuoles in the young cells. Green: actin; red: cell nuclei. Bar: 20 μm.

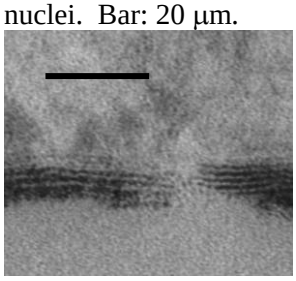

Figure 2. TEM of the multilamellated membrane around notochordal cell vacuoles. Bar: 500 nm.

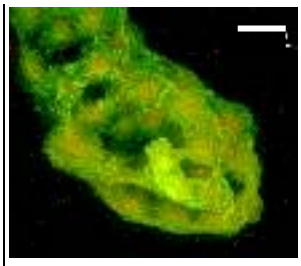

Figure 3. 3D reconstruction of a notochordal cell cluster containing over 30 cells. Bar: 20 μm.

DISCUSSION & CONCLUSIONS: While we have only begun to scratch the surface, the results thus far are quite intriguing. The fundamental questions which face us as a research community are (1) what function do the notochordal cells serve after formation of the axial skeleton? (2) why do the notochordal cells disappear during maturation? (3) do the notochordal cells and their gelatinous matrix serve a role in the pathogenesis of intervertebral disc degeneration?

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ACKNOWLEDGEMENTS: This work was supported by the Canadian Institutes of Health Research under grant number 64385.

Molecular Phenotypes of Notochordal Cells Purified from Nucleus Pulposus via Fluorescence–Activated Cell Sorting

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INTRODUCTION: The immature nucleus pulposus (NP) is populated by cells of notochordal-origin that are larger and contain more extensive cytoskeleton and vaculoes [1,2,4]. The disappearance of these cells with age is believed important in regulating metabolic shifts that occur in the aging intervertebral disc [1]. Work in our laboratory has shown that cells derived from the notochordal cell-containing NP do not respond to physical stimuli and soluble mediators to the same extent as fibrochondrocyte-like cells of the disc [3], suggesting a unique phenotype for these cells. In this study, we developed a new technique to purify notochordal-like cells from immature NP cells by fluorescence-activated cell sorting (FACS) and characterized their unique molecular phenotype by mRNA and integrin expression patterns.

METHODS: Primary cells were isolated from the anulus fibrosus (AF) and NP of skeletally-immature porcine and rat spines. NP cells were sorted by both fluorescence and size on the FACStarPLUS with smaller AF cells (10-15 μm cell size) as the reference population. Sorted cells were collected into two fractions: large NP cells (higher fluorescence and larger than AF cells) and small NP cells (lower fluorescence and smaller than AF cells). Real-time RT-PCR analysis was used to characterize gene expression in these two cell populations for key extracellular matrix related proteins (collagen types I and II, aggrecan, decorin, biglycan, lumican, MMP1, 2 and 3, TIMP1 and 2). Expression levels of integrin subunits (α1, α5, α6, β1) were analyzed by flow cytometry with appropriate antibodies.

RESULTS: FACS analysis showed that the NP contained a majority of cells that were larger than AF cells (Fig. 1), with auto- and blue fluorescence higher than AF cells (Fig. 2). Microscopic examination of the sorted large NP cells demonstrated the existence of vacuoles within many cells of this population, consistent with the appearance of notochordal cells identified in previous reports [1,2,4]. In comparison to the sorted small NP cells, large NP cells expressed lower mRNA levels of type I collagen, biglycan and TIMP1. A greater number of these large NP cells also expressed α6 and α1 integrin subunits and a higher expression level of β1 subunits as compared to small NP cells (Table). These differences point towards a potential difference in integrin-mediated interactions with collagens and laminin in the matrix of NP.

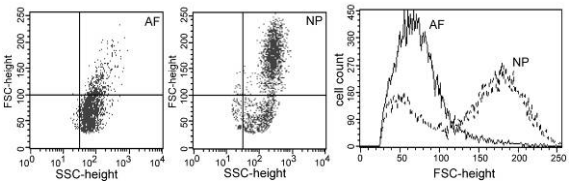

FIGURE 1: Flow cytometry analysis by light scatter only for cell size in rat IVD cells.

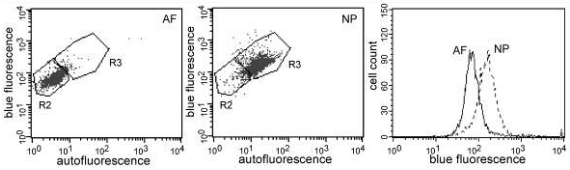

FIGURE 2: Flow cytometry analysis by auto and blue fluorescence in rat IVD cells

TABLE. % positive cells and mean fluorescence intensity (MFI) of integrins in porcine NP cells.

	Large NP		Small NP	
	% of (+)		% of (+)	
subunit	cells	MFI	cells	MFI
α1	**35**	**10**	**10**	**5**
α5	74	27	55	19
α6	**39**	**20**	**19**	**9**
β1	96	124	95	67

CONCLUSIONS: Notochordal-like cells of the NP have a molecular phenotype (i.e. mRNA and integrin expression) distinct from that of the small NP cells, suggesting their unique metabolic contributions and interactions in the intervertebral disc. This technique presents the possibility to identify unique gene expression profiles or metabolic markers for these notochordal-cell like population.

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The Structure, Degradation and Lifespan of Aggrecan in the Human Intervertebral Disc

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INTRODUCTION: Intervertebral discs consist of an outer annulus fibrosus (AF) and a central nucleus pulposus (NP). The ability of the discs to resist compression is aided by their high proteoglycan content, particularly in the NP. Intervertebral disc degeneration involves loss of proteoglycan in the NP, which to some extent is compensated by an increase in the inner AF. Disc proteoglycans exist in two populations – those that are aggregated with hyaluronan and those that are non-aggregated. The aggregated proteoglycans are derived from aggrecan, as in cartilage, though their structure has not been well characterized. At present it is not clear if the non-aggregated proteoglycans are also derived by proteolysis of aggrecan or whether they are distinct.

METHODS: AF and NP from lumbar discs was extracted with 4 M guanidinium chloride containing proteinase inhibitors. Proteoglycans were recovered by associative CsCl density gradient centrifugation, and fractionation through Sepharose CL-2B separated aggregated from non-aggregated proteoglycans. The aggregated proteoglycans were further subdivided by dissociative CsCl density gradient centrifugation. Proteoglycan structure was analyzed by gel electrophoresis in 1.2% agarose, following trypsin, chondroitinase ABC or keratanase II digestion. Products were identified directly by toluidine blue staining or immunologically following transfer to CPC-coated nitrocellulose. Antibodies to the CS1 core protein or to KS chains were used for immune detection. Proteoglycans were also analyzed for aspartic acid racemization to assess their residence time within the extracellular matrix.

RESULTS: Trypsin digestion cleaves the human aggrecan core protein in all structural regions, with the exception of the CS1 region, which remains intact and can be separated from other fragments by virtue of its larger size and slower electrophoretic mobility. Anti-KS analysis showed that CS1 fragment was devoid of KS, and treatment with keratanase prior to analysis did not alter its mobility. Treatment with chondroitinase did alter the mobility of the CS1 region, as expected, but did not alter that of the KS fragments, indicating that the majority of the CS2-derived fragments are devoid of KS chains. Analysis of the non-aggregated proteoglycans after trypsin treatment revealed that the CS1 region was present in the molecules of larger size but not in those of smaller size. KS-containing fragments of identical size to those present in the aggregated proteoglycans were present in both large and small non-aggregated proteoglycans. The proportion of D-aspartic acid in the proteoglycan increased with age, indicating long residence times within the extracellular matrix, with the proteoglycans having a mean half-life of 10-15 years.

DISCUSSION & CONCLUSIONS: The aggrecan core protein possesses three adjacent regions to which glycosaminoglycan (KS or CS) chains are attached - the KS-rich region, the CS1 region, and the CS2 region. Current models of aggrecan structure suggest that KS may be present in all regions. However, the present work indicates that the CS1 region is devoid of KS, and that if KS occurs in the CS2 region then it is only in areas devoid of CS. The present work also indicates that the majority of disc non-aggregated proteoglycans are derived from aggrecan by proteolysis, as their trypsin-generated fragments were identical to those derived from the CS1 region and the KS-rich region of aggrecan. The relatively long half-life of the disc proteoglycans accounts for the high proportion of non-aggregated molecules present in the disc matrix, as degradation products can accumulate. The reason for the retention of the non-aggregated proteoglycans in the disc is unclear, but it is probably related to the large size and avascular nature of the tissue. Finally, it was apparent that proteoglycan structure and turnover in the AF is analogous to that in the NP.

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Mechanical Influences in Disc Degeneration

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INTRODUCTION It is important to establish what intervertebral "disc degeneration" really is, and to distinguish it from normal ageing. We suggest that disc degeneration should be defined as a cell-mediated response to gross structural disruption. Such disruption is an easily-detected, unambiguous marker of impaired disc function, which does not occur inevitably with increasing age, and which is more closely related to pain than any other feature of ageing discs[1]. Structural disruption is irreversible, because adult discs are incapable of repairing gross defects. Furthermore, it naturally *progresses*, by physical and biological mechanisms, and so is a suitable marker for a degenerative process.

MECHANICAL INFLUENCES Certainly, this definition simplifies the issue of causality: excessive mechanical loading disrupts a disc's structure and precipitates a cascade of non-reversible cell-mediated responses which lead to further disruption. Cadaveric experiments and mathematical models have shown how various combinations of compression, bending, and torsion can cause all of the major structural features of disc degeneration, including endplate defects, radial fissures, radial bulging, disc prolapse, and internal collapse of the annulus[2]. Damage can be created by a single injury, or by wear-and-tear "fatigue" loading. Animal experiments confirm that structural disruption to disc or endplate *always* leads to cell-mediated degenerative changes[3].

OTHER INFLUENCES: Other features of degenerated discs can be considered as predisposing factors for, or consequences of, structural disruption. Genetic inheritance and impaired metabolite transport can make the disc matrix physically weaker and so vulnerable to injury. So too can age-related changes in collagen cross-linking, and loss of water and proteoglycans from the nucleus. Elevated levels of cytokines and MMP's may represent attempted repair[4], as in other connective tissues, and they could be triggered by the abnormal matrix stresses which follow structural disruption. Ingrowth of blood vessels and nerves doubtless represent a late consequence of altered mechanics and biochemistry in severely disrupted tissues. Defining disc degeneration in terms of structural disruption therefore leads to a simple conceptual framework which incorporates all known features of degenerated discs.

DISCUSSION It is important to realise that "excessive" loading does not mean high loading. The fact that disc degeneration is common even among sedentary people with no history of spinal injury suggests that an unfavourable inheritance, middle age, inadequate metabolite transport and accumulating fatigue damage can weaken some discs to such an extent that physical disruption occurs during the activities of daily living. This speculation is supported by the very wide range of tissue strengths reported in cadaveric experiments.

CONCLUSIONS Disc degeneration should be defined as a cell-mediated response to progressive structural disruption. The underlying cause is tissue weakening arising primarily from genetic inheritance, ageing, and nutritional compromise. The precipitating cause is structural disruption arising from injury or fatigue failure.

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Effects of mechanical loading on disc metabolism

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INTRODUCTION: Disorders of the intervertebral disc are commonly implicated in low back pain and often associated with mechanical overloading. The purpose of this paper is to review recent studies describing the effects of mechanical loading on intervertebral disc metabolism. This goal is to define hypothetical models that provide a quantitative relationship between mechanical loading and intervertebral disc cell metabolism with the eventual goal of obtaining a predictive model between mechanical loading and disc remodeling.

METHODS: The review will cover recent animal modeling and tissue culture studies. In our recent work, quantitative relationships between mechanical loading and disc cell metabolism are obtained using a rat tail model for in vivo studies and bovine caudal discs for whole disc organ culture studies.

Studies on the rat tail model utilized an external fixator that was surgically installed into the tail vertebrae of rats in vivo to allow precise mechanical control over the intervertebral joint loading conditions. These studies addressed the specific influences of loading mode (immobilization, compression & shear), as well as cyclic loading magnitude, frequency, and duration on the short-term intervertebral disc cell anabolic and catabolic gene expression responses.

Studies involving whole discs in organ culture utilized bovine caudal discs that were loaded with static and diurnal compression to evaluate the influence of mechanical loading on cell viability, biosynthetic activity, and disc structure.

DISCUSSION & CONCLUSIONS: We hypothesize that disc degeneration results from mechanical damage to the tissue combined with an imbalance between anabolic and catabolic biosynthetic activity. Damage may be quantified mechanically as well as biologically.

Recent results demonstrate a threshold of mechanical loading is required to promote a biosynthetic response. Mechanical loading below that threshold (e.g., immobilization) or above it (e.g., 1 Hz -- 1 MPa cyclic compression) can stimulate gene expression responses (e.g., Fig. 1).

Anabolic and catabolic responses of disc cells must both be considered. An imbalance between anabolic and catabolic gene expression may lead to protein synthesis or loss (e.g., Fig. 2).

Future studies evaluating the influence of mechanical loading on disc metabolism is necessary to develop a more quantitative and predictive relationship between mechanical loading and disc remodeling.

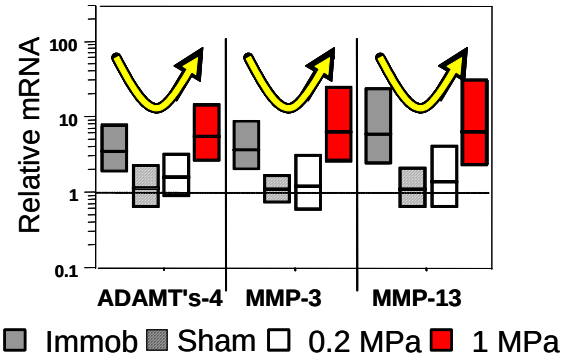

Fig. 1:Relative mRNA expression of annulus cells in response to mechanical loading regimes in vivo using a rat tail model. The amplitudes of 0.2 MPa and 1 MPa correspond to 1 Hz cyclic compression loading, Immob=immobilized motion segments.

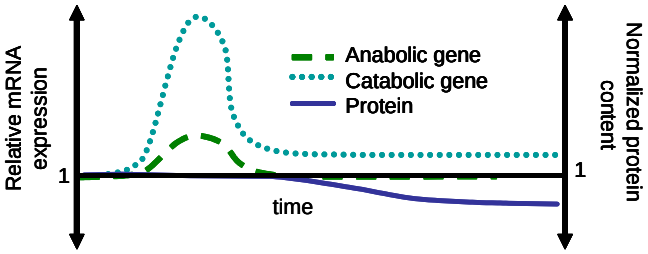

Fig. 2: This schematic of a catabolic remodeling response is one of several patterns describing relationships between changes in gene expression and protein level.

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Intercellular Communication via Gap Junctions in the Annulus Fibrosus

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INTRODUCTION: Annulus fibrosus cells have an elaborate morphology[1,2] and complex mechanical[3] interaction with the extracellular matrix. Evidence of the gap junction protein connexin-43 has been found in bovine and human annulus fibrosus cells[2,4]. To fully determine their mechanobiological behaviour it is essential to investigate possible intercellular communication. Fluorescence recovery after photobleaching (FRAP) has demonstrated functional gap junctions in the nucleus pulposus and cartilage[5,6]. The aim of this study was to determine the activity of gap junctions in the *intact* annulus fibrosus.

MATERIALS AND METHODS: Discs were obtained from 8 bovine tails within 4 hours of slaughter. Circumferential outer (OA) and inner (IA) annular specimens (~1 mm thick) were bluntly dissected from the posterior disc. All specimens were incubated in calcein-AM (5 μM, Molecular Probes) in Ca^{2+}-free PBS (1.5 h, 37°C) and then washed in 100 times volume PBS for 10 minutes (3x). Some specimens were treated with the gap junction blocker 1-octanol (1 mmol/L, Sigma-Aldrich). Cells were imaged on a Zeiss LSM 510 confocal microscope (63x 1.2 NA water immersion objective; 488 nm argon laser; 1.3 μm optical slice). An initial image was recorded (t_{ini}). An oval region of interest (ROI) was then fit around the cell body and visible processes of a cell. This ROI was then photobleached at 100% intensity for 10-15 seconds (until ~40% original fluorescence). Images were obtained immediately following photobleaching (t_0), after 10 min (t_{10}) and 20 min (t_{20}) of recovery. The mean pixel intensity within a cell of interest was determined and normalized to an uninvolved cell in the image field (Image J, NIH). Percent recovery was calculated from: $[(t_{20} \text{ or } t_{10}\text{-}t_0)/t_{ini}] \times 100$.

RESULTS: A clear return in fluorescence was observed in cells of the OA, which did not occur when treated with octanol (Fig. 1). In the OA, a significant difference was observed between the percent recovery of untreated (n=17) and octanol treated (n=15) cells at both t_{10} and t_{20} (Fig. 2, $p<0.05$). In the IA, only 50% of the untreated cells showed a greater then 10% recovery in fluorescence. No significant difference was observed between the percent recovery of untreated (n=14) and octanol treated IA cells (n=10) at t_{10} or t_{20} ($p>0.05$).

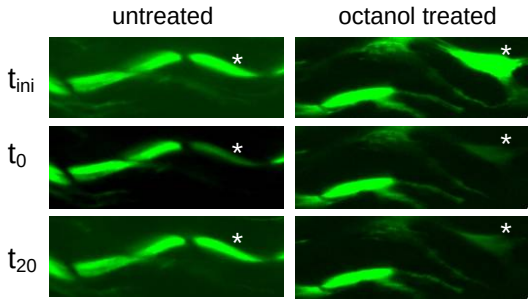

Fig. 1 Return of fluorescence in cells(*) of the OA and no return when treated with octanol.

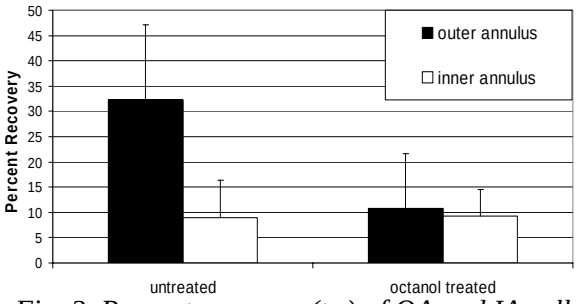

Fig. 2 Percent recovery (t_{20}) of OA and IA cells untreated and treated with octanol.

DISCUSSION: This study demonstrates functional gap junctions in the *intact* annulus fibrosus. The percent recovery of fluorescence in untreated outer annulus cells is similar to the 25-35% recorded in chondrocytes[6]. Functional gap junctions have been demonstrated in cultured human annulus fibrosus cells with decreased communication observed with aging[4]. Future research will explore the potential role of functional gap junctions in the mechanobiology of healthy and degenerated annulus fibrosus cells.

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Regulation of gene expression in intervertebral disc cells by low and high hydrostatic pressure

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INTRODUCTION: Intervertebral disc structures are exposed to wide ranges of intradiscal hydrostatic pressure during different loading excercises reaching a minimum during lying or relaxed sitting and a maximum during lifting weights with a round back[1]. We hypothesize that these different loading magnitudes influence intervertebral disc (IVD) cell metabolism, causing either anabolic effects or degenerative processes depending on their magnitudes. Therefore the aim of this study was to assess changes in gene expression of human nucleus and annulus cells after the application of low hydrostatic pressure (0.25 MPa) and high hydrostatic pressure (2.5 MPa).

METHODS: IVD cells isolated from human disc biopsies (n=23) were seeded into three-dimensional collagen-type-I matrices and exposed to the different loading magnitudes by specially developed pressure chambers. The lower pressure range (0.25 MPa, 30 min, 0.1 Hz), was applied with a recently published device by using an external compression cylinder[2]. For the application of higher loads (2.5 MPa, 30 min, 0.1 Hz) the cell-loaded collagen gels were sealed into sterile bags with culture medium and stimulated in a newly developed water-filled compression cylinder by using a loading frame. These methods allowed the comparison of loading effects in a wide physiological range under equal three-dimensional culture conditions. Cells were harvested 24h after end of stimulation and changes in the expression of genes known to influence IVD matrix turnover (collagen-I, collagen-II, aggrecan, MMP1, MMP2, MMP3, MMP13) were analyzed by real-time RT-PCR. A Wilcoxon signed-rank Test and a Wilcoxon 2-sample Test were performed to detect differences between stimulated samples and control samples and differences between low and high hydrostatic pressure. Multiple testing was considered by adjusting the p-value to 0.007.

RESULTS: Both regimes of hydrostatic pressure influenced gene expression in nucleus and annulus cells but differences in responses magnitude-to-magnitude and region-to-region were detectable. Low hydrostatic-pressure (0.25 MPa) tended to increase collagen-I expression of both annulus and nucleus cells ($p<0.05$) and aggrecan expression of nucleus cells ($p=0.031$) but significantly decreased nucleus cells MMP3 expression ($p=0.001$). The effects on all other catabolic target genes tended to decrease in both annulus and nucleus cells. High hydrostatic pressure (2.5 MPa) tended to decrease gene expression of all anabolic proteins with significant effects on aggrecan expression of nucleus cells ($p=0.004$) and a strong tendency of decreased collagen-I expression of both annulus and nucleus cells ($p=0.016$). MMP1, MMP3 and MMP13 expression tended to increase in both annulus and nucleus cells with strong tendencies for nucleus cells ($p=0.02$ for MMP13) and annulus cells ($p=0.016$ for MMP1).

DISCUSSION & CONCLUSIONS: These results demonstrate that hydrostatic pressure as one of the physiological stimuli of the intervertebral disc may regulate matrix turnover in a magnitude dependent way. Low hydrostatic pressure (0.25MPa) tends to result in anabolic effects, whereas high hydrostatic pressure (2.5MPa) tends to cause catabolic effects. Therefore, hydrostatic pressure may play an important role in the maintenance of intervertebral disc matrix but also in its degradation.

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Cell Mechanics and Mechanobiology in the Intervertebral Disc

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INTRODUCTION: Substantial biological remodeling of the intervertebral disc occurs in response to altered mechanical loading, changes that may contribute to the health or degeneration of the intervertebral disc. Work in our laboratory has focused on understanding the responses of isolated intervertebral disc cells to physical stimuli, and both the factors and mechanisms that play a role in regulating those responses *in vivo*.

CELL MICROMECHANICS: Modeling has predicted that cells within the intervertebral disc experience spatially-varying mechanical stimuli, including hydrostatic pressure, fluid-flow, deformations and osmotic pressure changes, that may vary depending on cell morphology, mechanical properties of both cell and extracellular matrix, and the means for physical interaction between cell and matrix. Work in our group has studied the biological responses of isolated cells when exposed to these representative stimuli *in vitro*. Cells of the anulus fibrosus are generally responsive to short-term periods of direct compression and osmotic pressure change, as quantified by altered gene expression profiles for cytoskeletal and matrix proteins and cytoskeletal organization. Cells of the immature nucleus pulposus exhibit fewer responses to these same stimuli *in vitro*, differences that may be partly attributed to the more extensive cytoskeletal network and stiffer properties of these cells.

CELL-MATRIX INTERACTIONS: To determine the mechanisms that govern these spatial differences in cell mechanobiology, our work has recently focused on studying how mechanical stimuli are transduced to cells within the disc. Integrins are known to be important mediators of cell-matrix interactions in other systems, with demonstrated roles in regulating cell survival, adhesion, cytokine responses, and mechano-biology. In the intervertebral disc, we observe a spatially varying pattern of integrin expression with integrins that interact with collagens, laminins and fibronectin for most cells. These integrins are shown to play a role in mediating intervertebral disc cell adhesion to particular matrix substrates that are similar for both anulus fibrosus and nucleus pulposus cells; however, the observations differ from findings for both fibroblasts and chondrocytes, pointing to a phenotypic difference for cells of the intervertebral disc.

COLLAGEN KNOCKOUT MODELS OF DISC DEGENERATION: Evidence for human disc disease points to a role for mutations in the genes encoding type IX collagen, a molecule that may mediate cell-matrix interactions In order to better understand

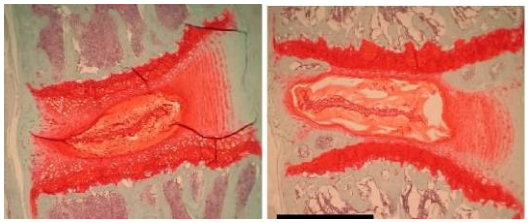

Figure: IVD from (left)wild-type and (right) Col9a1 -/- mouse aged 9 months

this interplay, our laboratory has begun to study mice with an inactivated *Col9a1* gene that express no type IX collagen. Discs exhibit more degenerative changes compared to wild-type littermates (Figure). Stiffness of the knee joint cartilage was measured to be significantly less in the mutant mouse; however, similar matrix changes have not yet been evaluated for the intervertebral discs. Our laboratory is investigating two potential hypotheses to explain the observed disc changes in the *Col9a1* -/- mice: (1) a mechanically compromised extracellular matrix is associated with elevated wear-and-tear of the disc, which leads to premature degenerative onset; or (2) altered cell-matrix interactions with the collagen deletion alter transduction of mechanical signals from matrix to cell, and hence alter cell mechanobiology.

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The Role of Extracellular Matrix and Matrix Molecular Fragments in Disc Degeneration.

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INTRODUCTION: The annulus fibrosus and nucleus pulposus have very different extracellular matrix organizations but have many matrix proteins in common. The remarkable lamellar organization of the type I and II collagen fibrils in the lamellae in the annulus provides an effective mechanism for controlling bending and twisting. While the fine type II collagen fibrillar network trapping abundant aggrecan helps maintain a fluid-like state, the nucleus pulposus with aging and degeneration matrix proteins are increased, lost, fragmented or covalently modified. Because it is postulated that there is a reciprocal feedback relationship between the matrix and the cell, these matrix modifications could accelerate degeneration.

METHODS: The thesis of matrix modification accelerating degeneration is explored in a review of the literature.

RESULTS: There is abundant evidence in the disc especially in the nucleus for the accumulation of many kinds of matrix molecular fragments. For example, aggrecan fragments accumulate after birth and increase in early degeneration. Fibronectin is an example of a protein that accumulates with age and degeneration. It is present at low levels in the low Thompson grade disc but the total fibronectin dramatically increases with grade and becomes a major non-collagenous protein during degeneration component of tissue[1]. In addition, multiple fibronectin fragments become dominant. It is well established that the fibronectin fragments produced in vitro with purified enzymes induce decreased anabolism and increases catabolism in many tissues. This may also happen in the disc *in vivo*.

The small leucine-rich proteoglycan biglycan increases in age and degeneration. Biglycan alters cell signalling via interactions with the EGF receptors[2].

With age, the proteins that have long life, such as collagen, become increasingly glycated. Glycated molecules frequently alter normal signaling pathways or can initiate alternative signaling via receptors such as RAGE. (Ref)

DISCUSSION & CONCLUSIONS: In both the aging and especially the degenerated disc there is an accumulation of non-collagenous matrix molecules. However, fragmentation and covalent modification of matrix molecules are also observed. All of these can modify cellular responses and frequently promote matrix catabolism and decreased matrix synthesis. These matrix changes must be considered as mechanisms for promoting disc degeneration.

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Proteinases in Degeneration of the Intervertebral Disc: Cause and Effect.

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Proteinases are necessary for the homeostasis of connective tissues, including the intervertebral disc. Increased proteinase activity, however, has been implicated in disorders of the disc, including disc degeneration, herniation and scoliosis. Proteinases reported in the disc are many and varied, including the serine proteinases, the matrix metalloproteinases (MMPs) 1,2,3,7,8,9,13 and the aggrecanases[1]. Between them, this array of enzymes is capable of degrading all the matrix components in the disc. There may be a chronological expression of enzymes, with different ones being produced at different stages of the disease[2], as is the case for MMPs in articular cartilage during osteoarthritis. Similarly different groups of enzymes may have a relevance to different stages of disc degeneration; aggrecanases to date appear to be less prominent in disc degeneration than MMPs, but this may reflect tissue being obtained for study predominantly in late stage disease.

There are many ways in which enzyme activity, the important factor physiologically, can be modulated. MMPs, for example, are produced as inactive precursors. In addition there are at least 4 endogenous inhibitors, TIMPs, which when bound to the MMPs render them inactive.

Immunohistochemistry has shown that these enzymes and their inhibitors can be produced not only by blood vessel cells, when present, in the disc, but also by the disc cells themselves[1]. Certainly disc cells *in vitro* can synthesise MMPs and TIMPs. MMP production and activity, both *in vitro* and *in vivo*, can be altered by loading, depending on the type, size and frequency of the load and the source of the cells, whether annulus or nucleus. For example, annulus cells up regulate MMPs 3, 13 and ADAMTS-4 differentially in response to the magnitude of load, irrespective of the frequency applied, whereas nucleus cells respond to different frequencies and loads[3].

MMP production is very sensitive to cytokines, with IL-1 or TNFα stimulating disc cells to produce more MMPs. Many cytokines and other potentially stimulatory molecules such as iNOS, thromboxane, monocyte chemoattractant protein (MCP-1) and TSG-6 (TNFa Stimulating Gene), are produced in degenerate discs, again by both disc and blood vessel cells, and are likely to influence proteinase production and activity. Disc cells closest to blood vessels are often more strongly immunopositive for MMPs than those further from them[1], suggesting that they may be produced in response to stimulation by cytokines within the blood vessel.

The well known effects of proteinases are the denaturation or degradation of the extracellular matrix, with some enzymes being more effective against certain molecules. For example, the collagenases, MMPs 1,8 and 13 are necessary to cleave the collagen triple helix *in vivo*, with MMP13 being particularly effective at denaturing type II collagen. Once the cycle of degradation has begun, it can self perpetuate in more ways than one. Fragments of matrix components created by enzyme activity can stimulate the production of more enzymes and some enzymes, eg serine proteinases and MMP2, can activate other MMPs. In addition to the matrix effects, MMPs may influence cell activity. MMP1 has been shown to confer resistance to apoptosis in some cell types[4]. It remains to be seen if any of the proteinases could influence disc cells in a similar manner.

It is important to understand the mechanism of disc degeneration, which factors are key players and what parameters they are dependent on. Only with this knowledge can there be correct targeting of future therapies such as pharmaceutical intervention or gene modification.

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The Course of Macroscopic Degeneration in The Human Lumbar Intervertebral Disc

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INTRODUCTION: Previous studies predominantly addressed macroscopic alterations of the intervertebral disc during aging and degeneration in a descriptive, qualitative manner[1-5]. So far, quantitative data on age-related macroscopic alteration is still sparse. The objective of this study therefore was: 1) to provide a semi-quantitative description of the temporal course of macroscopic features of age-related disc changes. 2) Explore the relation and sequence of different features such as clefts and tears. 3) To provide a conceptual morphological framework of disc aging and degeneration.

METHODS: A total of 248 mid-/parasagittal sections of lumbar motion segments originating from 41 routine autopsies (7mt-88y; asymptomatic back) were analyzed semi-quantitatively for macroscopic changes of intervertebral discs, endplates and adjacent vertebrae. An array of macroscopic markers based on Thompson's grading was graded for every motion segment and correlated with the respective age of the donor.

RESULTS: Nuclear fibrous transformation, anular disorganization as well as endplate and vertebral body alterations process predominantly in the first two and in the 5th to the 7th decades. In the 3rd and 4th decade only little progression of the alterations is apparent. Cleft formations in the nucleus pulposus and anulus tears show a delayed appearance mostly starting in the 2nd decade of life. In general, nuclear clefts precede anular tear formations. Within the anular tears, radial and concentric tears demonstrate a similar course over time while rim lesions mostly develop after the 6th decade and independently from the others. Significant differences are observed between the upper and lower lumbar spine with lower segments exhibiting more extensive alterations in several parameters.

DISCUSSION & CONCLUSIONS: This study semi-quantitatively demonstrates that nuclear fibrous transformation and anular disorganization in the motion segment precede the formation of tears and clefts in the intervertebral disc. This strongly indicates cleft formation as a consequence of these alterations during aging and/or degeneration. The temporal sequence suggests a strong correlation of cleft and tears formation starting with nuclear clefts. Rim lesions appear independently and substantially later in life. Our results support the concept that disc degeneration starts in the nucleus. Finally, it emphasizes that the extent of macroscopic alterations already being apparent in the second decade of life is a challenge to any tissue engineering and repair attempt.

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Accelerated intervertebral disc degeneration in scoliosis versus physiological ageing develops against a background of enhanced anabolic gene expression

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INTRODUCTION: Molecular consequences of long term deformation and altered mechanical loading of intervertebral disc tissue (IVD) in idiopathic scoliosis have yet to be elucidated. In order to get insight into such mechanisms we studied alterations in IVD tissue from human scoliotic spines on the histological and on the molecular level. We hypothesized that disc degeneration is accelerated in scoliosis compared to normal ageing and that this is reflected by an altered gene expression profile.

METHODS: Semiquantitive histological analysis of IVDs [1], was performed in scoliotic adolescents (surgery group, age 10-22, mean 14,2 years, n=16) and compared to non-scoliotic adolescents (autopsy group, age 8-17, mean 13,3 years, n=8), normal adults after traumatic injury of vertebrae (surgery group, age 28-55, mean 35,6 years, n=7) and mature normal adults (autopsy group, age 44-77, mean 63,2 years, n=10) which served as controls. Molecular analysis was performed by a custom made cDNA array [4] which harboured 48 genes for cartilage, bone, adipose tissue, mesenchymal progenitor cells, but also genes functioning as transcription factors or morphogens. The gene expression pattern of 16 scoliotic IVDs (age 10-22, mean 14,2 years) and of 7 normal IVDs (trauma surgery, age 28-55, mean 35,6 years) was analyzed and related to the histological scoring of the same sample.

RESULTS: Histological grading revealed significantly enhanced degeneration in the scoliosis (HDS 5,3) versus age-matched control IVDs (HDS 2,25; p=0.001). Degeneration phenomena were similar to those observed in normal aging discs of older age (n=7; mean 35,6 years HDS 5,6). This shows that IVD degeneration in the scoliotic group (HDS 2–10 points, mean 5.3 points) seems to occur about 20 years ahead of time. cDNA array analysis revealed higher mRNA levels for AGC1 (mean 15-fold; p=0.002), COL11A1 (mean 9-fold; p=0.007), COL12A1 (p=0.037), biglycan (p=0.027), decorin (p=0.016), lumican (p=0.023), chondromodulin1 (p=0.012), and MIA (p=0.037) in the scoliotic than in the trauma disc tissue, while COL1A1 levels were equal. On average COL2A1 mRNA was 5.7-fold higher (p=0.057) in scoliotic discs. No differences in mRNA levels were evident for molecules involved in matrix catabolism like MMP3, MMP13, MMP17, and TIMP1.

DISCUSSION & CONCLUSIONS: This is the first study correlating histological scoring and gene expression profiling in IVDs. In conclusion, alterations in the tissue over time graded by a morphologic degeneration score were not reflected by the actual gene expression profile of the cells. Morphologic disc degeneration was accelerated by about 2 decades in scoliosis versus physiological ageing and developed against a stronger anabolic matrix metabolism at younger age or in response to the altered mechanical environment of the tissue.

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Factors Influencing Nutrition of the Intervertebral Disc

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INTRODUCTION: The disc is the largest avascular tissue in the body with cells in the centre of an adult lumbar disc 6-8 mm from the nearest blood supply. In order to function and remain viable, the cells require an adequate nutrient supply, and also an efficient means of removing products of metabolism such as lactate. Nutrients reach the cells of the nucleus pulposus by diffusing from blood vessels of the vertebral body through the cartilage endplate into the disc matrix under concentration gradients set up by cellular metabolism[1]. Even in a normal healthy disc, oxygen and glucose levels in the centre of the nucleus. Lactic acid is produced by disc cells at high rates and accumulates, leading to a fall in tissue pH. Failure of nutrient transport is apparent in degenerate discs[2]; it can lead to further fall in pO_2 ,glucose concentrations and pH hence affecting cellular activity and even cell survival adversely and may thus be one route to disc degeneration. At present, there are no non-invasive methods for measuring levels of nutrients and metabolites in human discs. However as transport of small solutes such as glucose and oxygen is mainly by diffusion[3], gradients can be calculated provided values of relevant parameters viz. endplate 'permeability', solute diffusivity through the matrix, disc dimensions and rates of cellular consumption are known. Here we give some results from measurements of these parameters in bovine and human discs.

METHODS: Measurements were made on human discs removed at surgery for routine treatment of the scoliotic deformity or bovine caudal discs (18-24 months). Diffusivity of solutes of different molecular weights was determined using a concentration-gradient method[4]. Rates of nutrient consumption and lactate production were measured in a custom-built metabolism chamber with custom-built electrodes[5]. Endplate permeability in human discs was estimated by electrochemical N_2O measurement[6]. Concentration gradients were calculated from the diffusion equation using a finite element method[7].

RESULTS: Solute diffusivities fell as solute molecular weight increased and as hydration fell. Rates of metabolism showed a non-linear dependence on substrate concentrations and also varied with factors such as mechanical stress. Computation of nutrient profiles showed that with loss of endplate permeability, increase in rates of nutrient consumption or lactic acid production or a fall in diffusivity, nutrient concentrations could fall to levels unable to maintain cellular activity or viability

DISCUSSION & CONCLUSIONS: Calculations show that nutrient concentrations throughout the disc can be predicted provided accurate values of material constants are available. Much more data is required before effect of external factors on nutrient concentrations (e.g. various mechanical activities) can be reliably estimated.

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The Genetics of Intervertebral Disc Degeneration

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Introduction The molecular basis for degenerative disc disease (DDD) is poorly understood. This study aimed to test the contribution of genetics to DDD in the Southern Chinese population using a case-control approach.

Methods Since 2001, 804 volunteers between the ages of 18 and 55 were recruited from the general population. Symptoms and life-style were assessed by questionnaire, DNA was isolated from blood samples, and DDD was assessed by MRI for each level using the Schneiderman's classification and summated to provide a total DDD score. A DDD score of 0 was defined as no disc degeneration and these individuals were used as controls. Annular tears, disc and end-plate herniations were also objectively graded.

The frequencies of known predisposing genes such as Trp2 and Trp3 alleles in collagen IX; Taq I and Fok I alleles in Vitamin D receptor (VDR); the promoter polymorphism in metalloproteinase 3 (MMP3); as well as an additional 7 polymorphisms in 6 candidate genes (MMP3, MMP8, MMP13, aggrecasnase 1, aggrecan and interleukin 1) were determined and correlated with MRI findings.

Results The prevalence of lumbar disc degeneration was 67%, lumbar disc herniation was 30%, annular tears were 30%, Schmorl's nodes was 10% and ossified yellow ligament was 6%. When stratified by age, lumbar disc degeneration was present in 40% of the population between 18 and 30 years, while 85% had disc degeneration by 50 years. Lumbar disc herniations occurred most commonly in the 18 to 30 age group, while the incidence of annular tears increases with age and were present in 33% of individuals between 50 to 55 years. There is no correlation between annular tears or mild disc degeneration with back pain.

The Trp2 allele was present in 20% of the population and was associated with a 4-fold increase in the risk of developing annular tears at 30-39 years and a 2.4-fold increase in the risk of developing DDD and end-plate herniations at 40-49 years. Affected Trp2 individuals had more severe degeneration. The Trp3 allele was absent from the Southern Chinese population.

The t allele of Taq I in VDR gene was significantly associated with DDD, with a relative risk of 2.61. Further subgroup analysis showed that under 40 years of age, the relative risk was even higher at 5.97. Similarly, disc herniation was significantly associated with t allele, with a relative risk of 4.64.

No additional significant associations could be identified in either type IX collagen, VDR, promoter region of MMP3, or the 7 other new polymorphisms.

Discussion

This is the largest-scale population study to date using MRI to precisely define DDD. For the first time, we demonstrated that the Trp2 allele is a significant risk factor for the development and severity of degeneration. The contrasting Trp allele frequencies between Finns and Chinese is the first indication that the genetic risk factors for DDD varies between ethnic groups. In addition, we were also able to demonstrate an age-dependent association between the t allele of VDR Taq I and a higher risk of developing DDD and disc herniation.

Such studies will provide a new angle to understand the underlying mechanism of DDD. Future work will focus on identifying additional candidate genes to test in a case-association study approach, finding early onset DDD families for linkage studies, and to understand the mechanism of disc degeneration using a proteinomics approach *and* transgenic mouse models.

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Determinants of Disc Degeneration and Pathology: A Major Paradigm Shift

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INTRODUCTION: The traditional view over much of the last century was that disc degeneration was primarily a result of mechanical insults and injuries imposed on normal aging. However, in 2002, conducting a review of "degenerative disc disease", incorporating recent research, Ala-Kokko concluded, "Even through several environmental and constitutional risk factors have been implicated in this disease, their effects are relatively minor, and recent family and twin studies have suggested that sciatica, disc herniation and degeneration may be explained to a large degree by genetic factors." This represents a major shift in the understanding of determinants of disc degeneration. My research group's work which has contributed to this dramatic shift will be presented.

METHODS: Initially, we conducted a series of studies investigating the influences of commonly suspected environmental and behavioral risk factors on disc degeneration and back symptoms using monozygotic twins highly discordant for the factor of interest, selected from the population-based Finnish Twin Cohort. Exposures were verified and data on potentially confounding factors were gathered in an extensive structured interview. Disc degeneration was assessed both qualitatively and quantitatively from MR imaging using a 1.5 Tesla scanner. The data from these studies were aggregated to allow multivariable analysis to examine the relative effects of lifetime exposures and familial aggregation on disc degeneration. Familial aggregation represents the effects of genetic and other shared family influences (e.g., shared childhood environment). Analogous data were later collected of dizygotic twins to allow classic twin studies of genetic and environmental influences. We currently have data on 600 twins. DNA analysis using a candidate gene approach and haplotypes is being conducted in search of genotypes associated with disc degeneration and back pain.

RESULTS & DISCUSSION: Our findings indicate that while physical loading involving materials handling, bending, and twisting appear to influence disc degeneration, the effect size is modest, which helps explain the inconsistent results of previous studies of the effects of occupational loading. No effects of occupational driving on disc degeneration were found. Conversely, disc degeneration was explained to a great degree by familial aggregation. In the multivariable analysis, 43-61% of the variance in disc degeneration, depending on lumbar region, was explained by familial aggregation, whereas age and occupational physical loading together explained 11-16%. The identification of gene forms associated with disc degeneration, such as TaqI and FokI of the VDR gene, each explaining 6.5% of the variance in nuclear signal intensity, allow investigations of gene forms as modifiers of environmental effects.

The degenerative findings of outer annular tears and disc height narrowing were consistently associated with history of back-related symptoms, and thus are of particular interest. Preliminary results of a classic twin study bivariate analysis revealed that the variance in the lifetime back pain variables explained by genetic influences was accounted for by the genetic correlation with disc height, suggesting such disc findings as one possible pathway through which genes influence back symptoms.

CONCLUSIONS: Disc degeneration and pathology as currently seen in developed countries appears to be largely genetically determined. Environmental factors do appear to play a role, but the identification of these factors and the estimation of their effect sizes is much more complicated than once thought and likely involves complex interactions.

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Recent advances in MRI markers of disc degeneration

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INTRODUCTION: Magnetic resonance imaging (MRI) has also been used as a non-invasive measure of disc degeneration. In this paper the recent quantitative MR methods and their implications for characterizing degenerative disc disease will be discussed.

METHODS: Normal intervertebral discs show a bright clear signal from the nucleus pulposus, and there is no signal from the annulus fibrosus. Disc degeneration is shown by a change in the signal form the nucleus pulposus to give an irregular outline and a reduction in signal intensity. In the intervertebral disc, the uptake of Gd DTPA enhancement has been observed clinically often in normal appearing discs (1) and there is a high prevalence disc degeneration in asymptotic populations. Post-operatively contrast enhanced MR is used to distinguish scar tissue from recurrent herniation (2-4). However, it is still not possible to define the precise anatomic source of a patients symptoms on the basis of the MR. In an effort to improve the capability of MR techniques to quantitatively assess disc degeneration, surrogate MR measures of tissue hydration, such as relaxation times (T_1, $T_{1\rho}$ and T_2), water diffusion, and spectroscopic (HRMAS) methods are also proposed.

RESULTS: In vitro $1/T_1$, showed significant effects of hydration and collagen content(5); similarly $1/T_2$ and water content for disc tissue show significant correlations (5), (6). Boos et. al (7) using age and gender matched symptomatic and asymptomatic disc herniations, showed that symptomatic disc herniations showed significantly shorter $T_1$and T_2. Grading T_2 weighted images has also gained recent popularity (8). Relaxation time, $T_{1\rho}$ depends on the proteoglygan content and to some extent water content. Molecular diffusion shortens the spin-echo signal and has been used to study disc degeneration in vitro. In the spine, the addition of diffusion has been shown in multiple applications (9-13) to provide additional diagnostic information. Line Scan Diffusion Imaging (14), (15), gives high quality diffusion weighted images and apparent diffusion coefficient (ADC) and anisotropy maps in the human spine. HR-MAS data correlated well with Thompson grade and an increase was seen in the levels of unbound hydroxproline and glycine in annular tissue which is directly associated to collagen breakdown. HR-MAS also detected a decrease in N-acetyl concentration of nucleus pulposus associated with proteoglycans with degeneration.

DISCUSSION & CONCLUSIONS: Quantitative imaging appears promising may potentially provide information with regards to disc degeneration and biochemistry and further studies are warranted.

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Non-Invasive Determination of Nucleus Pulposus Proteoglycan Content Using $T_{1\rho}$ MRI

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INTRODUCTION: MR and radiographic imaging have successfully been used to detect late-stage degenerative changes in disc morphology, height, and hydration. However these imaging methods cannot reliably detect disc constituent changes thought to contribute to early degeneration, such as loss of proteoglycan (PG) in the nucleus pulposus (NP). $T_{1\rho}$-weighted MRI, defined as the spin-lattice relaxation in the rotating frame, is linearly correlated with proteoglycan content in articular cartilage [1] and may be able to detect PG content in the intervertebral disc. The objectives of this study were (1) to determine whether there is a relationship between $T_{1\rho}$ and PG content in human intervertebral disc tissue and (2) to demonstrate the feasibility of performing *in vivo* $T_{1\rho}$ imaging in the lumbar spine.

METHODS: Fresh-frozen cadaveric human lumbar spine sections (ages 15, 25, 45, 51, 67) were imaged on a 1.5T clinical MR scanner. A series of sagittal plane $T_{1\rho}$-weighted images were acquired (4mm slice, Tr/Te=3000/12 msec, spin lock time (TSL) 15 to 75 msec). $T_{1\rho}$ was calculated on a pixel-by-pixel basis by fitting intensity data to the following exponential function: $S(TSL) = S_0 e^{-(TSL/T1\rho)}$. Mean $T_{1\rho}$ was taken from a 5mm circular region of interest in the center of the NP (n = 19 discs). A series of T_2-weighted images (4mm slice, Tr = 2000, Te = 15 to 75) were used to generate quantitative T_2 maps. Site-matched NP samples were analyzed for water and glycosaminoglycan (s-GAG) content using the DMMB method. Linear regressions between $T_{1\rho}$, T_2, water content, and s-GAG were performed. A volunteer (age 29, no back symptoms) underwent $T_{1\rho}$ imaging (sagittal plane, Tr/Te = 3000/12, TSL = 15 to 70) and a $T_{1\rho}$ map of the *in vivo* lumbar spine was generated.

RESULTS: There was a strong correlation ($r=0.7$, $p<0.01$) between $T_{1\rho}$ and s-GAG content. T_2 was moderately correlated with s-GAG content ($r=0.5$, $p<0.05$). Neither $T_{1\rho}$ nor T_2 were significantly correlated with water content. The *in vivo* scan successfully generated a $T_{1\rho}$ map (Fig 1). $T_{1\rho}$ times (~100msec) from the *in vivo* scan were within the range of the cadaveric $T_{1\rho}$ values.

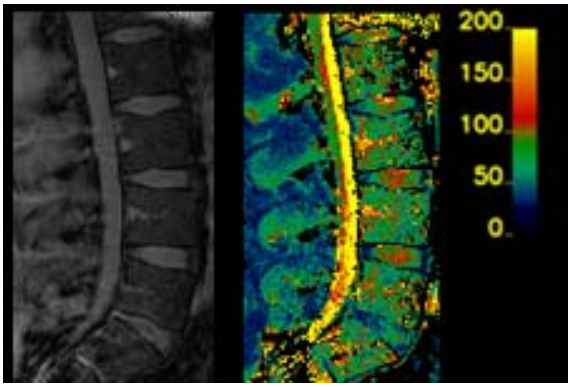

Fig.1: In vivo $T_{1\rho}$ image and map

DISCUSSION & CONCLUSIONS: In this preliminary study, $T_{1\rho}$ was found to vary in proportion to s-GAG content in the intervertebral disc. Thus, $T_{1\rho}$ MRI may provide a non-invasive technique to determine PG content in the intervertebral disc. $T_{1\rho}$ was better correlated with s-GAG than T_2. Use of $T_{1\rho}$ may be an improvement upon previous attempts to determine biochemical content using quantitative MR and spectroscopy [2-4] because it does not require a contrast agent, can be performed relatively quickly in a clinical scanner, and provides a spatial map of PG content. This technique could potentially be used to diagnose early degeneration and to assess the efficacy of new biologic disc treatment strategies.

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Micro Observations the Intervertebral Disc

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INTRODUCTION: The Intervertebral discs (IVD) function and dysfunction is governed by the structural architecture of collagen fibres in the annulus fibrosus (AF); at the macro scale for the overall performance of the disc and at the micro scale where it influences cell behaviour. To understand such mechanical behaviour the three-dimensional collagen fibre architecture must be quantified in intact IVDs. Conventional imaging modalities lack either the spatial resolution (e.g. x-ray diffraction [1]) or have limited penetration (e.g. optical, electron and confocal laser microscopy [2]) to yield mechanically important information. This study aims to validate the origin of the alternating layers of fibre texture observed within intact, hydrated and unfixed IVDs using Scanning Acoustic Microscopy (SAM) [3].

METHODS: The three-dimensional structure of intact, unfixed human lumbar IVDs was imaged using SAM at 50MHz in pulse-echo mode. The micro-structure within the AF was observed and compared to optical and electron micrographs of individual lamellae, peeled by micro-dissection.

RESULTS: SAM images of the sagittal section of the disc is characterised by alternate light and darks bands (see Figure 1a) representing the interface between adjacent lamellae and collagen bundles respectively. Within the dark bands a uniform, highly oriented fibre texture that reversed between adjacent layers was observed (shown in Figure 1b). Resolution of the texture was limited by the acoustic system to 30 µm.

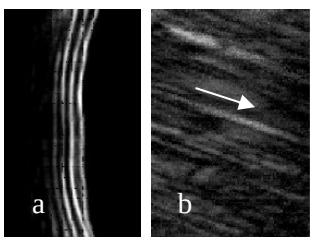

Figure 1

Optical microscopy (Figure 2) revealed that each lamella consisted of a highly organised fibrous structure with regularly spaced splits between fibres (spacing of 25 ± 5 µm).

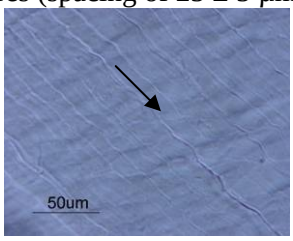

Figure 2

Electron microscopy demonstrated highly orientated collagen fibrils (diameter of 200 nm) identified by a banding structure (periodicity 60 nm) (see Figure 3b). Groups of collagen fibres of diameter 5 µm were observed (shown in Figure 3a).

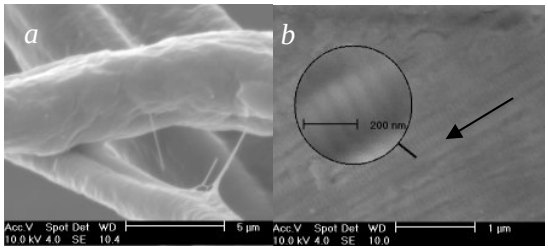

Figure 3

DISCUSSION & CONCLUSIONS: SAM operating at 50 MHz cannot resolve and therefore image individual collagen fibres on the micro scale. However, the regular defects in the fibre layers can be visualised as alternating layers of fibre texture and therefore provides an effective way of quantifying the three-dimensional fibrous structure of intact, hydrated, unfixed intervertebral discs.

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High Serum Level of Tnf-A Correlates with Chronic Low Back Pain but Not Acute Sciatica

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INTRODUCTION: Low back pain is the primary cause of disability in individuals younger than 50 years. Increasing evidence is available as to the importance of cytokines in acute and particular chronic pain. Cytokines can influence transduction, conduction, and transmission of the nociceptive signal, resulting in prolonged or permanent signalling to the brain's cognitive centres in the absence of a painful noxious or nonnoxius stimulus (Sommer 2001).
Several cytokines, including Interleukin 1 (IL-1), tumor necrosis factor (TNF), IL-6, and IL-10 are thought to influence nociception or pain. To date, there have been no studies of the production of inflammatory mediators in blood from patients with low back pain. We have therefore analysed levels of the proinflammatory mediators IL-1ß, IL-6, TNF-α in sera from patients with sciatica and low back pain, and their possible relationship to pain dimensions.

METHODS: In this prospective longitudinal study with a follow-up of six months, the course of serum concentration of interleukin 1 beta (IL-1ß), interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF-α) was measured by Bio-Plex cytokine assay in 31 patients with acute sciatica and 41 patients with chronic low back pain. Blood samples were taken at ten fixed times during follow-up, and cytokine values were adjusted to possible influential factors and correlated to the course of pain and clinical function to evaluate the predictive role of cytokine regarding therapy outcome.

RESULTS: At admission of the study and 10 days later, the proportion of TNF-α positive subjects (above 2pg/ml) was significant elevated among patients with low back pain compared to patients with acute sciatica. Median (SD) of serum TNF-α concentrations were significant higher in patients with chronic low back pain (n=41) than in patients with acute sciatica (n=31). In the whole period the pain of patients reduced from time to time. Elevated TNF-α serum levels are associated with a significantly improved pain in patients with chronic low back pain but not with acute sciatica. A close coherence exists between the cytokines IL-1ß, IL-6 and TNF-α together in blood of patients as with acute sciatica as with chronic low back pain. Neither age, sex, BMI, nicotine and alcohol consumption are related to the serum levels of cytokines.

DISCUSSION & CONCLUSIONS: As far as we know, this is the first analysis of parameters predicting a major clinical connection of cytokines in blood and low back pain. Our findings indicate that elevated serum levels of the proinflammatory cytokine TNF-α are associated with a significantly improved pain in patients with chronic low back pain but not with acute sciatica. We concluded that Detection of high level of TNF-α might be a marker for more pain in patients with chronic low back pain. and TNF-α probably play an important role in the chronic process of low back pain.

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Spine Surgery in a Large Animal Model: Experiences and Limitations

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INTRODUCTION: Sheep have become a convenient, economical and practical large animal model for orthopaedic research. For spine surgery, they are useful for investigating fusion devices, growth factors and their carriers, instrumentation methods, vertebroplasty-kyphoplasty methods, disc replacement and vertebral body corpectomy [1].

VENTRAL "ANTERIOR" LUMBAR INTERBODY FUSION (ALIF): In the breed we use, there are usually 7 sometimes 6, lumbar vertebrae. Using a left retroperitoneal approach, up to four lumbar discs can be readily accessed. Commonly, two fusion sites are used with a normal space in between. Cylindrical metal or ceramic cages (with or without growth factors and their carriers) can be evaluated [1]. If the interbody fusion site is unstable, unilateral instrumentation using pedicle screws will provide stability to the construct.

DORSAL "POSTERIOR" LUMBAR INTERBODY FUSION (PLIF) Using a dorsal approach, access to 3 or 4 levels are possible. Pedicle-screw instrumentation spanning several levels is possible. Access to the disc space using this approach is difficult.

TRANSVERSE LUMBAR INTERBODY FUSION (TLIF): Using a dorsal approach, single-level fusion by decortication of the transverse processes (with or without instrumentation) is used to compare bone-graft substitutes and growth factors and their carriers to autograft [2].

CERVICAL INTERBODY FUSION: With the sheep in dorsal recumbency, one or two fusion sites are preferred. Tri-cortical autograft or cancellous bone is available from the sternum, and avoids having to reposition the sheep following procurement from the iliac crest. Graft-containment implants can be evaluated [3] as well as interbody fusion devices or disc replacements. The cervical disc space is cup-shaped and smaller than the disc space of the lumbar region.

VERTEBROPLASTY: Three or 4 vertebral bodies can be drilled from the lateral side, cavitated and used to test bone-void fillers [1]. The vertebral body of sheep is too dense to drill a small hole in the outer cortical shell and inject a bone void filler under pressure or using kyphoplasty. CT scans are more useful than plain radiographs to document the extent of filling and monitor behavior of the filler over time. Mechanical testing of individual vertebral bodies and histology of surrounding bone, epaxial musculature and adjacent spinal cord, are useful endpoints.

LUMBAR CORPECTOMY: Removal of one vertebral body and replacement with an implant can be used to evaluate construct design, bone graft substitutes, and growth factors that are used to fill the prosthesis. Unilateral instrumentation using two pedicle screws in each vertebral body is essential for stability.

LIMITATIONS OF THIS MODEL: The shape and dimensions of quadrupedal vertebrae are very different than humans and implants and devices have to be adapted. Sheep vertebral body bone density is much higher than in elderly people which may limit some studies, although osteoporosis of the spine can be achieved in 6 months following ovariectomy and a dietary-induced metabolic acidosis [7]. Fusion of vertebral bodies in animals can be more rapid than in people so evaluation at early endpoints (6 mo. or less) is recommended in spine fusion studies. Skeletally mature (> 3.5 yrs. old) sheep are used in most studies except where growth disturbances such as scoliosis are being studied.

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Investigations using an Ovine Annular Lesion Model of Experimental Intervertebral Disc Degeneration

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INTRODUCTION

Intervertebral disc (IVD) lesions have a very poor healing capacity and are problematic to treat clinically. Controlled experimental annular lesions [1] also have a limited repair potential. The outer margins of such defects heal spontaneously within 3 mth, however their innermost regions do not heal even after a 2 yr recovery period and may propogate to form circumferential and radial tears affecting the nucleus pulposus (NP) leading to proteoglycan loss, NP degeneration and loss of IVD function. One of the problems inhibiting repair processes in the annulus fibrosus (AF) is that granulation tissue in the defect site is not effectively resorbed and replaced by new functional annular lamellae. Hyaluronan oligosaccharides (HA oligos) have been shown to stimulate a number of cell types to produce elevated levels of active matrix metalloproteases (MMPs) capable of ECM remodelling. Furthermore, HA oligos also stimulate articular chondrocytes to up-regulate a number of anabolic ECM genes (collagen-II, aggrecan, HAS-2) [2]. HA oligos therefore have the potential not only to stimulate matrix removal but also its replacement by de-novo synthesis. The aim of this study was to assess HA oligos for the promotion of ECM remodeling and matrix repair in an established ovine model [1, 3].

METHODS

Ovine AF and NP cells were established in monolayer and alginate bead culture in the absence and presence of HA oligos (10-16 mers, 0.05-1 g/ml). Conditioned media samples were collected for MMP analysis by gelatin zymography, cells were isolated and RNA extracted with TRIzol for RT-PCR to determine relative mRNA expression levels for MMP-2, 9; aggrecan and type I and type II collagen on days 2, 5 and 10 in bead culture. The HA oligos were also assessed in an ovine annular lesion model [1, 3]. The HA oligos (10 mg/ml) were administered to annular lesion sites via a gelatin sponge, no treatment and carrier plus sponge were also assessed for their abilities to promote repair of the annular lesion over a 3 mth recovery period. This was determined using a histological scoring scheme which evaluated lesion depth, degree of AF re-integration, proteoglycan loss, extent of blood vessel in-growth and macromolecular annular collagen re-organisation.

RESULTS

AF cells were poorly responsive to the HA oligos (0.05-1 g/ml) in monolayer culture with proMMP-2 levels only marginally elevated and MMP-9 unaffected. In contrast, proMMP-2 production by NP cells in monolayer culture displayed a strong dose dependant increase, MMP-9 was not affected. In alginate bead cultures the AF and NP cells were both responsive to the HA oligos which significantly elevated MMP-2 and MMP-9 activity in a dose dependant manner. Aggrecan, Type I and II collagen expression in the AF and NP cells however were differentially regulated by the HA oligos and were up-regulated in NP cells and down regulated in AF cells. The HA oligos did not significantly improve the healing response (as assessed by histological criteria) in the ovine AF lesion model [1, 3].

DISCUSSION & CONCLUSIONS

MMP-2 and 9 activity were both up regulated in AF and NP cells by the HA oligos however this was not evident at the mRNA level indicating that the HA oligos did not act at the transcriptional level at least with these MMP genes. In contrast, the anabolic matrix genes (aggrecan, type I/II collagen) were differentially regulated by the HA oligos in the AF and NP cells. The effect on NP cells was similar to the stimulatory properties displayed by HA oligos on articular chondrocytes [2] but clear differences were evident between the regulation of these genes in AF and NP cells.

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The Influence of Harvesting Methods For The Development Of An *In-vitro* Intervertebral Disc Model

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INTRODUCTION: There have been a large variety of animal models used to study the intervertebral disc. However, there has yet to be a live ex-vivo model that provides of a platform for long-term, dynamic loading, disc tissue studying and culturing. In this study the impact of harvesting method on the mechanical properties and nutrition diffusion of bovine coccygeal intervertebral discs under cyclic dynamic load was monitored. The objective was to determine the most suitable harvest method for the continued development of an in-vitro intervertebral disc model.

METHODS: Fresh bovine coccygeal intervertebral discs (n=21) were harvested with 3 different techniques: without endplates (NEP) (widely accepted method for IVD harvest[1]), with the cartilaginous endplates (CEP) (our newly developed method), with bony endplates (BEP) simultaneously from the same tail. The tissues were loaded in axial compression over 6 days under repeated dynamic 0.1-1.0 MPa for 12 hrs at 0.3 Hz and static load at 0.1MPa for 12 hrs (minimum physiological load to counter swelling[2]). The loading, in an environment mimicking physiological conditions, was facilitated by our novel bioreactor developed to incorporate computer controlled dynamic axial loading while collecting mechanical load-displacement data. To determine nutrient diffusion, the disc tissue was labeled with $^{35}SO_4$ on day 5 for 1 complete load cycle, 24 hrs. The stress-relaxation mechanical behavior of the tissue was fit using a Kelvin model[3] to determine time constants and permanent strain. Disc tissue was dissected into 6mm (superior-inferior) annular and nuclear plugs and further sliced horizontally into 1mm layers. Nutrient diffusion through the tissue was mapped by counting ^{35}S diffused into the 1mm segments.

RESULTS: The creep and recovery time constant, ranked from greatest to lowest, were CEP>BEP>NEP, indicating that the NEP method lost height and recovered height quickest. Permanent strain reached a steady state for both BEP and CEP methods but showed a tendency to further strain for NEP (Fig. 1) in the creep. Nutrition diffusion, ranked from greatest to lowest, in the nucleus, were NEP>CEP>BEP where BEP S35 count = 50% <CEP S35 counts. (Fig. 2)

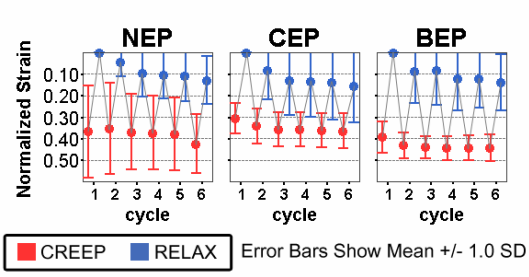

Fig. 1: Normalized maximum strain over the 6 loading cycles. Maximum strain during creep cycle is shown in red and relaxation shown in blue for each harvesting method.

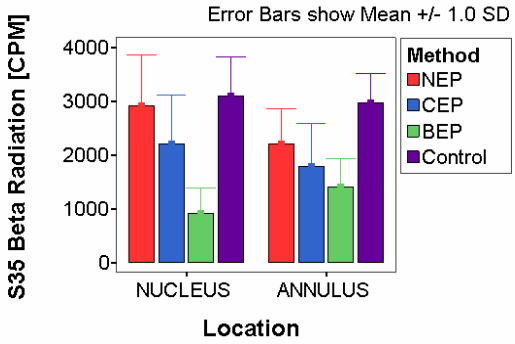

Fig. 2: CPM count for each harvest method and control. Mean nucleus counts shown first followed by annular counts.

DISCUSSION & CONCLUSIONS: The data demonstrated that the harvest method is critical in both mechanical stability and nutrient supply for long term tissue culturing. Although in nutrient diffusion the NEP method resulted with highest diffusion in both the nucleus and the annulus the CEP harvest method proved to be the best overall candidate for future in-vitro IVD model development.

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An *in vitro* tissue culture system for ovine caudal intervertebral discs with endplates

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INTRODUCTION: Arising from the avascular nature of intervertebral discs (IVDs), limited nutrient supply, in addition to mechanical loading, has become one of the main working hypotheses for induction of degenerative disc disease [1]. Because investigating these etiologies and their interaction *in vivo* is challenging, *in vitro* culturing systems are appealing. However, culturing disc explants has had limited success, e.g. culturing with adjacent endplates and static loads avoided 'disc swelling', but caused dramatic reduction of cell viability [2]. In this study, a new preparation and loading technique is evaluated for *in vitro* culturing of intact ovine caudal IVD explants with endplates.

METHODS: 12 Swiss alpine sheep (*Ovies aries*) were systemically anticoagulated and the caudal vasculature evacuated post-mortem as previously described [3]. The four most proximal of the caudal discs with adjacent endplates were then prepared with a precision histology band saw [3]. Discs were kept under standard culturing conditions (37° C, 5% CO_2) under diurnal loading (0.2 MPa for 8 h and 0.8 MPa for 16 h) and perfused with DMEM and 10% FBS. 2 discs were analyzed on day 0 (fresh) and 2 discs after culturing for 7 days.

Cell viability was assessed using the LIVE/DEAD® staining kit (Molecular Probes). Samples of disc tissue (~ 5 mm^3 of annulus or nucleus) were incubated under "free-swelling" conditions in serum-free DMEM for 3 hours [2]. Stained samples were visualized on an inverted confocal LSM (Zeiss). Total cell number and cell viability was assessed from 3 randomly chosen stacks of nucleus or annulus. Each stack represents a 100µm projection. The number of alive and dead cells was quantified with a custom image analysis macro (Zeiss).

RT-PCR was performed according to standard protocols in order to measure expression of 3 anabolic (*aggrecan*, *col I* and *col II*) and 2 catabolic genes (*ADAMTS-4*, *MMP-13*) [4]. Gene expression was quantified by ΔC_t values using the relative quantification method, which normalises C_t values relative to the gene expression of the ribosomal 18S gene. Relative mRNA values were then calculated:

$$Relative\ mRNA = 2^{-\Delta Ct} \quad (1)$$

Statistical significance was assessed by the Wilcoxon-Signed rank test pairing mean relative mRNA values (2 discs per sheep per group) for day 0 and day 7, per sheep.

RESULTS: LSM results showed a small but non-significant decrease in mean cell viability in both tissues (90% at day 0, 84% at day 7, *Figure 1*). In the nucleus, only *col II* was down-regulated and *MMP-13* (p = 0.04) was up-regulated after 7 days. In the annulus, both *aggrecan* and *col II* were down-regulated and *ADAMTS-4* was up-regulated after 7 days. All of these differences were of borderline significance, i.e. p = 0.05, except for where noted.

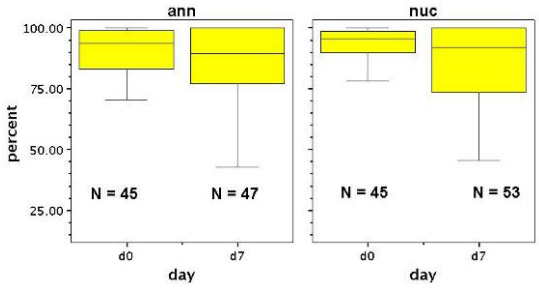

Fig. 1: Percentage of viable cells at day 0 and after 7 days of tissue culture in the annulus (ann) and nucleus (nuc).

DISCUSSION & CONCLUSIONS: Anti-coagulation and vascular evacuation enabled culturing of entire discs with intact endplates for up to 7 days without significant loss in cell viability. Although there was some borderline significant down and up regulation of anabolic and catabolic genes, respectively, over 7 days of culturing, this was not for all genes and less in the nucleus than annulus. These results may be further improved by application of higher frequency physiological loads.

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Biological Repair and a Preventive Approach for Disc Degeneration

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ANABOLIC PATHWAY: The effect of growth factors on the regulation of matrix metabolism and cell proliferation has been extensively studied in articular cartilage, whose cells are phenotypically closely related to some cells in the intervertebral disc (IVD). The clinical application of growth factors has been proposed as a method of enhancing cartilage repair in osteoarthritis and after traumatic cartilage injury. On the other hand, studies of growth factors using IVD cells are limited. Recently, the difficulties in culturing IVD cells *in vitro* were partially overcome by three-dimensional culture techniques using alginate gels or a pellet culture system. Studies on the effects of the bone morphogenetic protein (BMP) family , such as osteogenic protein-1 (OP-1, otherwise known as BMP-7) (1) and growth differentiation factor-5 (GDF-5, otherwise known as CDMP-1 or BMP-14) (2) in our laboratory and others (3) showed that it is possible to stimulate the biological activity of IVD cells.

For *in vivo* studies, the availability of recombinant protein and the use of gene transfer techniques accelerated progress in this field of research. The development of a reproducible animal model for quantitative studies in a reasonably sized animal, such as the rabbit, makes it possible to analyze the effects of growth factors *in vivo* (4). Recently, using the rabbit annular puncture model, our laboratory has shown that a single injection of the growth factor, OP-1, induced a significant restoration of disc height and an improvement in the histological and biochemical parameters of disc degeneration (5).

The results from these *in vitro* and *in vivo* studies clearly suggest the potential usefulness of recombinant growth factors as therapeutic drugs or as medical devices.

Catabolic Pathway: In addition to efforts to stimulate the anabolic pathway, further efforts have been made to inhibit the catabolic cascade, which causes disc degeneration, by the oral administration of a drug or an intradiscal signaling pathway modification technique.

In order to inhibit the degradation of the IVD by matrix degrading enzymes, such as metallo-proteinases and aggrecanase, the effects of the coumarin derivative, Esculetin, was assessed in both an *in vitro* and an *in vivo* setting. The oral administration of an esculetin prodrug showed a significant suppression of disc height loss and histological changes in the rabbit annular puncture model (6-8).

The nuclear factor, kappa B (NF-kB) transcription factor, plays an important role in the regulation of cytokines and metalloproteinases. An *in vitro* study, using the alginate culture system, showed a very high transfection rate with a naked decoy oligonucleotide (ODN) containing the NF-kB cis element with a resulting inhibition of the activity of IL-1 and TNF measured by several biological parameters (9). The intradiscal injection of the NF-kB ODN resulted in a significant recovery of the disc height loss induced by an annulus needle puncture (10).

An intradiscal injection therapy has a pharmacokinetic advantage over an intra-articular injection for a joint disease. This is because of the structural uniqueness of the IVD as an avascular and alymphatic structure, which is also isolated in a confined space where a single injection can be effective with a longer half-life as indicated by Thompson, Oegema et al. (11). In summary, therapies combining growth factors and anti-cytokine or enzyme agents will be optimal approaches to provide both structural and symptom modification for degenerative disc diseases.

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Disc Repair with Autologous Chondrocytes: A Pilot Clinical Study

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INTRODUCTION: This pilot study was designed to assess whether autologous disc chondrocyte transplantation will prevent disc degeneration in patients that have undergone discectomy. Cultured autologous cells were transplanted into the nucleus pulposus by a closed procedure. While it has been shown previously that disc cells sustain a phenotype in culture and that transplantation into canine disc demonstrates appropriate integration, the fate of transplanted cells in a human population has not been reported.

METHODS: Patients were actively recruited for this study from a population that was to be surgically treated for single level disc herniation. To be included in this study, patients could not have modic changes and must have failed previous conservative treatment. Patients were evaluated by VAS, Jenny neurological score, and an assessment of spine mobility was made. Intervertebral discs were also assessed qualitatively by MRI for disc height, degenerative changes (Modic), and fluid content. Disc material was removed by open microdiscectomy and intra-operative diagnosis of the disc (I-IV) was made. Cells were commercially expanded for transplantation under GMP conditions and maintained in culture with serum taken from individuals prior to their surgery. Transplantation occurred 3 months after the microdiscectomy procedure. Patients were hospitalized for 2 days following cell transplantation and required to wear a lumbar orthosis for the follwing 3 weeks. Follow-up occurred at 3, 6, 12, 24, and 36 months after the procedure.

RESULTS: MR images demonstrated changes at the surgical site that were characteristic of normal disc morphology. Introduction of cells had a positive affect on cell height, MRI signal, and matrix appearance. MRI changes were positive over time; showing enhanced central disc signal, and reduction in endplate effusion. All patients in the study showed improvement in the level of their low back pain and spine mobility was preserved or enhanced in 87.5% of the patients in this study. By 3 months, approximately 73% of the patients regained full motor sensation, and 82% of the patients achieved sensory recovery. Remaining symptoms were slight and in most cases not residual. Assessment on the VAS scale demonstrated a pre-operative mean of approximately 76mm that was reduced to 19mm in the final assessment. No secondary instability or degenerative change at adjacent levels was seen nor was progressive degenerative change at the treated intervertebral disc documented.

DISCUSSION & CONCLUSIONS: Autologous cells transplanted into a damaged intervertebral disc appear to retard degeneration. Evidence of matrix production and suppressed inflammation was evident by MRI, radiography, and by clinical assessment of pain. From these clinical results, autologous disc chondrocyte cell transplantation appears to offer the promise of retarding degeneration, maintaining intervertebral height, and stimulating matrix regeneration after micro-discectomy. Relief of pain, matrix production and integration, and no evidence of degeneration suggest autologous cell transplantation may be a valuable clinical tool for use in treating disc herniation.

Molecular Therapy of Intervertebral Disc

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INTRODUCTION: Currently there is no effective biological treatment for the Intervertebral disc. However, many different molecules of potential therapeutic benefit are being investigated. The purpose of this presentations is to review and categorize the molecules under investigation for potential therapy in preventing or reversing disc degeneration.

METHODS:
Review of literature on molecules that may be useful in the therapy of the intervertebral disc.

RESULTS: As the disc matrix undergoes turnover, small imbalances between synthesis and degradation can lead to significant changes in overall disc matrix content or long periods of time. One of the major goals of molecular therapy of the disc involves modulating this metabolic balance to the more favorable anabolic state (Figure 1). This can be accomplished by increasing synthesis or by decreasing catabolism. The list of molecules under investigation for potential benefit in biological therapy of the intervertebral disc repair continues to grow. The diversity of the molecules is such that these molecules no longer all fall into the classical terminology of "growth factor". Some of these molecules are not growth factors at all and some are not even cytokines. There are at least four different classes of molecules that may be effective in disc repair (Table 1). These include anticatabolics (e. g. TIMPs), non-chondrogenic mitogens (e.g. IGF-1, PDGF etc), chondrogenic morphogens (TGF-β1 and BMPs), and intracellular regulators (LMP-1 and Sox9). While there are some in vitro data on all of these molecules, few of these molecules have been tested in vivo with an animal model of disc degeneration.

DISCUSSION & CONCLUSIONS: As the current preliminary experiments are concluded, in vivo systems involving a more realistic model of disc degeneration will be necessary prior to attempting human studies.

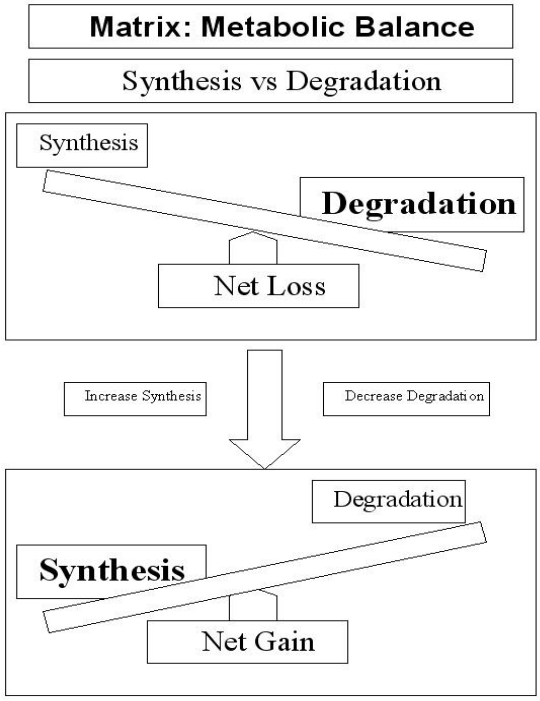

Fig. 1: Balance of disc matrix metabolism

Table 1. Potentially Therapeutic Molecules

Category	**Molecule**
Anticatabolic	TIMP-1
	TIMP-2
Mitogens	IGF-1
	PDGF
	EGF
	FGF
Morphogen	TGF-β
	BMP-2
	BMP-7 (OP-1)
	BMP-13 (GDF-6 or CDMP-2)
	GDF-5 aka CDMP-1)
	Link N
Intracellular Regulators	SMADs
	Sox9
	LMP-1

BMP-2 GENE TRANSFER ALTERS COURSE OF DISC DEGENERATION IN RABBIT MODEL

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INTRODUCTION: The concept of intradiscal gene therapy for the treatment of intervertebral disc degeneration has been extant since the late 1990s [1,2]. However, efficacy of gene transfer in altering the course of degeneration in a reproducible animal model of disc degeneration previously has not been established. In the current study, we injected degenerating rabbit discs with adenoviral vectors carrying the human BMP-2 gene to assess efficacy in terms of MRI and x-ray outcome measures.

METHODS: Intervertebral disc degeneration was induced in 13 skeletally mature female NZW rabbits by anterolateral stab of the L2-3, L3-4, and L4-5 lumbar discs by a 16-ga. needle to a depth of 5 mm. Discs L1-2 and L5-6 were maintained as intact controls. Three weeks post-stab, saline—with or without virus—was injected directly into the three stabbed lumbar discs of each rabbit with a 30-ga. needle. Group 1 (n=8) received the adenovirus construct Ad/hBMP-2 containing the therapeutic human BMP-2-encoding gene. Group 2 (n=5) received saline only. The rabbits were followed longitudinally with midsagittal T2-weighted MRIs preoperatively, and 3, 6, and 12 weeks post-stab. Degeneration was assessed at each time point by MRI Index (product of nucleus pulposus area and signal intensity). Plain x-rays were taken at week 12 to assess for osteophyte formation and possible fusion. Nucleus pulposus was harvested at 6 weeks post-stab from 5 animals (3 rabbits from Ad/hBMP-2 treatment group, and 2 rabbits from saline control group), cultured whole in serumless media for 48 hours, and supernatant analyzed by ELISA to measure hBMP-2 production (normalized to wet weight of harvested nucleus pulposus tissue).

RESULTS: MRI: Saline injection alone at 3 weeks failed to prevent significant decreases ($p<.05$) in MRI Index by 6 and 12 weeks post-stab. In contrast, discs injected with Ad/hBMP-2 at 3 weeks exhibited less decrease in MRI Index than the saline controls. By 12 weeks, the saline-injected discs had lost approx. 49% of their MRI Index, in contrast to only a 25% decrease in the Ad/hBMP-2 treated discs. Plain x-ray: Lateral and anterior-posterior plain films demonstrated no obvious bony intervertebral fusion in either the saline control or the Ad/hBMP-2 treated discs. There also was no discernable difference in osteophyte formation, disc height, or endplate sclerosis between the two groups. ELISA: BMP-2 levels detected by ELISA at 6 weeks post-stab were 72 ± 47 (pg/ml/mg) in the intact control discs, 34 ± 11 in the saline injected controls, and 217 ± 124 in the Ad/hBMP-2 injected discs. These data demonstrate that vigorous transgene expression was obtained in degenerating discs 3 weeks after their injection with Ad/hBMP-2.

DISCUSSION & CONCLUSIONS: The results of this study suggest that gene transfer of a growth factor encoding sequence, hBMP-2, can delay disease progression in an animal model of intervertebral disc degeneration, as assessed by clinically relevant T2-weighted MRIs. The mechanisms of therapeutic effect likely include sustained BMP-2 transgene expression as well as proteoglycan synthesis upregulation and increased disc water content. Direct adenovirus-mediated transfer of the hBMP-2 gene did not result in bony fusion in this rabbit model of intervertebral disc degeneration by 12 weeks. This study, to our knowledge, is the first demonstration of efficacy of gene transfer in favorably altering the course of degeneration in a reproducible animal model of disc degeneration.

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TOWARDS BIOENGINEERING A SCAFFOLD-FREE NUCLEUS PULPOSUS

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INTRODUCTION: Intervertebral disc (IVD) degeneration is a common problem and the treatment options for persistent back pain are limited. Recent studies have shown that reinsertion of nucleus pulposus can delay disc degeneration[1]. Our goal is to bioengineer an IVD *in vitro* that can be used to repair diseased discs. In this study we investigated whether it was possible to generate a nucleus pulposus-bone substitute construct *in vitro*.

METHODS: To generate the bone substitute material, porous cylindrical substrates (4mm diameter, 4mm height) were formed by sintering calcium polyphosphate powder (CPP)[2]. Nucleus pulposus (NP) was dissected from bovine lumbar or caudal spines or sheep lumbar spines. For selected experiments, articular cartilage was harvested from bovine metacarpal-phalangeal joints. The cells were harvested by sequential enzymatic digestion and placed on the upper surface of the CPP substrate[(2)]. To generate multi-tissue constructs, chondrocytes were placed on the top surface of the CPP and allowed to form cartilage in vitro for 2 weeks and then NP cells were placed on the top surface of the cartilage layer. These were grown in culture for up to 8 weeks. The constructs were evaluated histologically. For biochemical quantification the tissues were papain digested (40 μg/ml) for 48 hr at 65°C. The digest was assayed for DNA content using the Hoechst 33258 dye binding assay and fluorometry, glycosaminoglycan content using the dimethylmethylene blue dye binding assay and spectrophotometry, and collagen content using the chloramine-T/Ehlrich's reagent dye binding assay and spectrophotometry. Interfacial shear properties of the *in vitro*-formed tissues to CPP were assessed using a specially designed shearing jig held in an Instron universal testing machine.The data was analyzed using an unpaired t-test and significance assigned at $p<0.05$.

RESULTS: Histological evaluation of the bovine caudal cells placed on CPP showed that a continuous layer of tissue formed on the substrate surface by 2 weeks and attained a thickness of about 2mm by 6 weeks. The matrix contained sulfated proteoglycans and similar to the *in vivo* tissue, scattered individual cells had greater staining intensity suggestive of localized enhanced pericellular proteoglycan accumulation. Notochordal cells were present in the tissue formed by the bovine caudal cells. Scanning electron microscopy of the NP-cartilage-CPP (triphasic) constructs demonstrated that at 24 hours after the NP cells were placed on the cartilage layer the cells maintained their rounded morphology, similar to NP cells placed directly on CPP. At 8 weeks of culture histological examination of the triphasic constructs by light microscopy showed that a continuous layer of NP tissue had formed and was fused to the underlying cartilage tissue, which itself was integrated with the porous CPP. The incorporation of a cartilage layer stabilized the construct by improving tissue attachment to the CPP, as demonstrated by increased peak load and increased energy required for failure during shear loading.

DISCUSSION & CONCLUSIONS: *In vivo* the intervertebral disc is exposed to a range of mechanical stresses due to torsion, flexion, and extension of the spine. Since these forces are absorbed by all the tissues of the IVD and as they are all involved in degeneration, an implant should attempt to reconstruct the normal structural anatomy of the IVD including nucleus pulposus and cartilage endplate to ensure proper function. This study demonstrates that it is possible to generate a multi-component construct while maintaining the integrity of the different tissues.

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ACKNOWLEDGEMENTS: This work was supported by CIHR, The Arthritis Society and NSERC. CS and DH were supported by NSERC Fellowships. Jason Hong produced the CPP and Jian Wang helped with the mechanical testing.

STEM CELL APPLICATIONS IN CELL THERAPY FOR NUCLEUS REPAIR

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INTRODUCTION: There has been an increasing rise of interest in stem cell therapy, since it has provided new option in broad range of diseases. However, stem cell application to treat intervertebral discs has just begun. We have reported the regenerative effect of autologous mesenchymal stem cell (MSC) transplantation in treatment of disc degeneration. Despite the effectiveness of the procedure, its pathogenesis was unclear. In order to obtain evidence to clarify the mechanism in regenerative effect of MSC transplantation, we conducted an *in vitro* and *in vivo* study investigating differentiation and fate of the transplanted MSCs.

METHODS: *In vitro* induction study. Since differentiation ability of stem cells associates with microenvironment in which they are placed. Human NP cells or AF cell were cocultured with MSCs possessing GFP gene for 3 weeks in alginate beads. Using FACS Vantage cell sorter, only MSCs were retrieved and examined by flow-cytometry, immunocytochemistry, cDNA microarray and RT-PCR. In order to clarify cytokines related to induction, culture medium was analyzed by cytokine array. *In vivo* study. NZL white rabbits were divided into three groups (normal control; degenerative disc; and MSC transplantation models evaluated at 2, 4, 8, 16, 24 and 48 week). Autologous MSCs were isolated from marrow and were infected with retroviral vector expressing GFP. MSCs were embedded in atelocollagen gel and transplanted. MRI and radiographic evaluations, immunohistochemistry for C-S, K-S, type I, II, IV collagen, HIF-1 alpha and beta, HIF-2 alpha and beta, GLUT-1 and GLUT-3, and MMP-2 and RT-PCR for aggrecan, versican, type I, II collagen, IL-1 beta, IL-6, TNF-alpha, MMP-9 and MMP-13 were studied.

RESULTS: *In vitro* study. From flow-cytometric analysis, MSCs coculutured with NP cells in alginate expressed similar cell size and internal intensity with fairly large cells dominantly stained with keratin sulfate as observed in NP cells, whereas MSCs cocultured with AF cells showed that MSCs remained small in size and relatively low in intensity with no dominance in expression of proteoglycan epitopes. These findings were compatible with immunocytochemistry and RT-PCR results with turn over of type I and type II collagen expression. Several genes were found to be candidate for specific markers by microarray. Cytokine array results confirmed importance of TGF-beta, PDGF, IGF-1 and EGF during induction. *In vivo* study. MRI and radiograph results confirmed regenerative effects of the procedure. GFP-positive cell were detected in the nucleus throughout all periods with its percentage rising from 21±6% in 2 weeks to 55 ± 8% in 48 weeks, which proved survival and proliferation of MSCs. Immunohistochemistry showed positive staining of all proteoglycan epitopes and type II collagen in some of the GFP-positive cells. MSCs expressed HIF-1alpha, MMP2 and GLUT-3 expressing compatible phenotypic activity with nucleus pulposus cells. RT-PCR results demonstrated significant restoration of aggrecan, versican and type II collagen genes and significant suppression of TNF-alpha and IL-1beta genes in transplantation group.

DISCUSSION & CONCLUSIONS: Our data show that MSCs are capable of differentiating into cells expressing some of the major phenotypical characteristics of disc cells *in vitro*. Furthermore, MSCs transplanted to degenerative discs not only survive but also proliferate and differentiate into cells expressing phenotypes of nucleus pulposus cells with suppression of inflammatory genes *in vivo*. Results of the current study demonstrate some possible explanation for regenerative process of MSC transplantation to the degenerate disc.

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Topographical Guidance of Intervertebral Disc Cell Growth *In Vitro*: Towards the Development of Tissue Repair Strategies for The Anulus Fibrosus.

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INTRODUCTION: The anulus fibrosus of the intervertebral disc (IVD) consists of an aligned extracellular matrix, which is synthesised during development by similarly aligned IVD cells[1]. This highly organised structure may be severely disrupted during the onset or treatment of IVD prolapse. Of those prolapse cases that require surgical intervention, approximately 10% of patients re-herniate, most often at the original site of injury. Topographical guidance has been used previously to promote the alignment of tenocytes and of the extracellular matrix they synthesise in experimental models of tendon repair. Therefore, we have investigated the growth of IVD cells on micro-grooved polycaprolactone (PCL) *in vitro* to determine if topography can be used to promote IVD cell alignment and matrix synthesis.

METHODS: Cells isolated from the anulus fibrosus (AF) or nucleus pulposus (NP) of bovine IVD and expanded in monolayer to passage II were subsequently cultured on micro-grooved PCL for 1-4 weeks. Their growth pattern and the expression of extracellular matrix molecules were examined by scanning electron microscopy, video microscopy, immunohistology and RT-PCR (for type I collagen). IVD cells were cultured also on non-grooved PCL as a control.

RESULTS: AF and NP cells adhered to the PCL membranes, became fibroblast-like and proliferated to confluence (Figure 1).

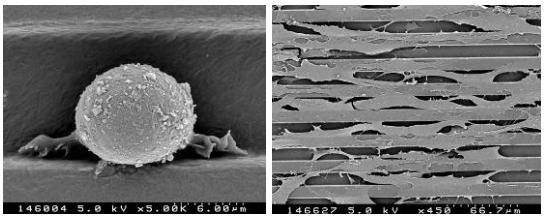

Fig. 1: An AF cell at 1 hour post-seeding (left panel), which has settled into the micro-groove of the PCL membranes and begun to extend filopodial and lamellipodial attachments onto the substratum. By day 14 (right panel) the majority of AF cells became elongated and aligned to the microgrooves/ridges.

Both cell types aligned to and migrated along the micro-grooves/ridges of the substrata, but AF cells were significantly more closely aligned than NP cells. In addition, AF cells were markedly more immunopositive for type I collagen, but less immunopositive for chondroitin-6-sulphated proteoglycans, than NP cells (Table I). There was no evidence of extracellular matrix deposition. Disc cells cultured on non-grooved PCL did not show any preferential alignment at sub-confluence and did not differ in their pattern of immunopositivity to those on grooved PCL.

Table 1. Immunoreactivity for Extracellular Matrix Molecules (ECM) on Disc Cell-Seeded Membranes of Polycaprolactone.

ECM Molecule	AF Cells	NP Cells
Collagen I	+++	+
Collagen II	-	-/+
Collagen III	-	-
KS PGs	++	++
C-4-S PGs	++	++
C-6-S PGs	+/-	++

DISCUSSION & CONCLUSIONS: Substrate topography was effective in aligning IVD cell growth. However, the differential response of AF and NP cells to topographical cues, and the fact that neither cell type deposited an extracellular matrix, suggests a need to optimise cell sources and / or environmental conditions further to promote the synthesis of an aligned repair AF tissue.

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ACKNOWLEDGEMENTS: Part-funded by the European Union. QLK6-CT-2002-02582.

The effect of severity of disc degeneration on mesenchymal stem cells' ability to regenerate the intervertebral disc: a rabbit model

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INTRODUCTION: Degenerative disc disease (DDD) is a consequence of alterations in the extracellular matrix composition of the intervertebral disc (IVD). Studies suggest that mesenchymal stem cells (MSCs) have the ability to arrest degeneration but not regenerate it. We hypothesize that this is related to the severity of degeneration. This study investigates the effect of different severity of degeneration on the ability of MSCs to halt or regenerate the IVD.

METHODS: Disc degeneration was induced in 9 New Zealand white rabbits at 4 consecutive lumbar levels by annular puncture[1]. The degeneration was allowed to progress for 1 month (early group, n=5) or 7 months (late group, n=4). Autologous MSCs were then isolated, expanded *ex vivo* and labeled with bromo-deoxyuridine (BrdU). $1x10^5$ MSCs were injected into 2 of 4 degenerated discs per rabbit. The other 2 levels were used as control levels that were either sham (induced but no further treatment) or injected with culture medium only. Serial radiographs of the spine were taken every 2 weeks. All rabbits were sacrificed at 16 weeks post-injection, the discs were retrieved for histological and immunohistochemical exam-ination. Disc heights on radiographs were measured[2]. A histological grading[3] was used to assess the severity of degeneration (Grade 0 = normal to Grade 5 = severe degeneration).

RESULTS: MSCs could be detected in all IVDs 16 weeks post-injection. For the early group, there were no significant differences in disc height between the sham discs, the medium-injected discs and the cell-injected discs. For the late group, all discs showed loss of height. The loss was significantly ($p<0.05$) greater for the cell-injected discs and the medium-injected discs than the sham discs (? because of double puncture). Histological grading of annular degeneration revealed no significant differences in the early group, whereas for the late group, the cell-injected discs were significantly ($p<0.05$) less degenerated than the medium-injected discs and sham discs (Table 1). This finding was further supported by histological confirmation of a near normal proteoglycan level within the posterior annulus fibrosus of the cell-injected discs (Figure 1).

DISCUSSION & CONCLUSIONS: Contrary to intuitive belief, MSCs injection has a more significant effect when introduced into more severely degenerated discs. Although evidence of halting progression of degeneration can be demonstrated, they do not have the ability to regenerate the disc fully or restore disc space height. It is not possible to conclude from the current study whether this failure to regenerate the disc is a problem of the model itself, an insufficient number of cells, a lack of a scaffold material, or that MSCs lack this ability. Further studies will be needed to ascertain the stage of disc degeneration that would benefit most from stem cell-based therapy.

Disc treatments	Early group	Late group
Sham	2.8	4.5
Medium-injected	2.5	3.8
Cell-injected	2.4	2.1

Table 1. Mean degenerative grades for IVDs subjected to different treatments.

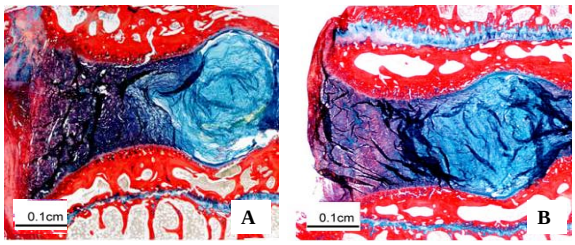

*Fig 1. Cells-injected discs (**A**) restored the proteoglycan level (stained blue)[4] when compared to sham discs (**B**) in the late group.*

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ACKNOWLEDGEMENTS: This study was supported by HKU Foundation Seed Grant and HKU Committee on Research and Conference Grants.

Nucleus Replacement with an *in situ* Curable Balloon Contained Polymer and Restoration of Segmental Kinematics

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INTRODUCTION: Disc height and annular pre-tension losses due to discectomy often impose altered segmental kinematics. These changes may further lead to segmental instability, which may not only accelerate the degenerative process and recurrence of pain, but may promote degeneration of adjacent levels as well. Ultimately, surgical treatment of such conditions often leads to fusion. The goal of nuclear replacement technologies is to restore physiological motion at the pathological level and prevent adjacent segment pathology while minimizing the annulotomy necessary for a total nucleus removal and effective implantation. The used Disc Arthroplasty System (DASCOR™) is a unique contained injectable nuclear replacement device. It is implanted into the nucleus cavity by injecting a custom formulated polymer under controlled pressure into a cavity conforming balloon through a small annular incision. The polymer cures in situ in a matter of minutes to a firm but pliable implant.

METHODS: To determine whether segmental kinematics of a lumbar motion segment having undergone a nucleotomy, can be restored with implantation of the device to those experienced by an intact motion segment we tested twelve human lumbar functional spine units (FSUs; age: 54±6yrs) in nondestructively compression (1200N), axial rotation (5.5Nm), flexion/extension (7.5Nm) and lateral bending (7.5Nm). Moments were combined with 500N compression. Each FSU was tested in three conditions: Intact, after nuclectomy and with an implanted device. Neutral zone (NZ), range of motion (ROM) and stiffness were obtained from load displacement curves and statistically compared between conditions using a repeated measures analysis of variance.

RESULTS: Compared to the intact state, nuclectomy significantly increased the NZ during all load modalities (Table 1, significant differences between conditions noted with an asterisk). Furthermore, nuclectomy increased ROM during all load modalities except lateral bending. Finally, nuclectomy increased stiffness only during flexion/extension and lateral bending. Implantation of the study device restored all kinematic parameters to near intact values.

Table 1. Segmental Kinematic Changes (%) with Respect to Intact

Load	*NZ*	*NZ*	*ROM*	*ROM*	*Stiff*	*Stiff*
	Nucl	*Impl*	*Nucl*	*Impl*	*Nucl*	*Impl*
Comp	*34*	*4*	*73**	*-8**	*-11**	*-2**
AR	*24*	*8*	*13**	*2**	*-4*	*-3*
F/E	*84**	*2**	*12**	*4**	*39**	*-8**
LB	*62**	*12**	*3*	*2*	*13**	*0**

DISCUSSION & CONCLUSIONS: The results of this study demonstrate that the study device is able to restore the segmental flexibility lost after a nucleotomy while still preserving segmental level biomechanics. The results of this study suggest that the device is biomechanically well suited to act as a long-term replacement for the degenerative nucleus pulposus.

ACKNOWLEDGEMENTS: Author (NO) Grant Research Support: A research grant was provided by Disc Dynamics INC to complete the study;

MRI and Biochemical Properties in Dynamic Loading Studies of Bovine Intervertebral Discs: Effect of Loading Conditions

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INTRODUCTION: Treatment modalities of intervertebral disc (IVD) degeneration currently focus on relieving the symptoms of spinal pain as opposed to intervening before disc degeneration has progressed too far. Quantitative MRI has the potential to become an accurate and non-invasive diagnostic tool of IVD degeneration. We have previously shown that targeting the collagen integrity of the nucleus pulposus (NP) of unloaded bovine coccygeal IVDs influences the MRI parameters [1]. Only upon introduction of 2h of dynamic loading was trypsin able to affect some MRI parameters [2]. The goal of the present study was to determine the effect of 16h of dynamic physiological loading in an open system on MRI and biochemical parameters.

METHODS: The NP of bovine 3-disc segments were injected with 40 µl of either Tris buffer or trypsin (5 mg) and placed in a plastic bag filled with 1L of saline. The disc segments were kept in solution at 37°C under dynamic loading (50N-300N-50N, 1Hz) or left unloaded for 16h. Sample sizes were as follows: n=4 for trypsin, loaded/ unloaded; n=2 for buffer, loaded/ unloaded. MRI and biochemical analyses of the NPs were performed.

RESULTS: Increasing the loading of the IVD segments from 2h to 16h led to a significant decrease in the apparent diffusion coefficients (ADCs) of the NP (Fig. 1A; [2]). Trypsin treatment of the NP in 16h-loaded discs significantly decreased the relaxation time T1 and the GAG content (Fig. 1B).

DISCUSSION & CONCLUSIONS: Results show that increasing the loading time with the use of an open system, i.e. disc segments in solution, caused a decrease in diffusion distances within the NP. Chiu *et al.* [3] reported that diffusion in human discs induced by loading is a function of the pattern of the load, with a step load leading to increased diffusion and a step displacement, as in this experiment, leading to decreased diffusion. Since the sample size is small, it remains to be determined if enzymatic digestion will play a role as expected on diffusion.

Loaded NPs that underwent trypsin treatment had lower T1 than buffer-treated NPs. As expected targeting the core protein of the proteoglycans decreased the water retention in the NP (though this was not significant due to the sample size) which in turn decreased T1. However, this is in contrast to our previous study [2] where 2h of dynamic loading in a closed system increased the GAG content and did not affect T1. Overall these results suggest that MRI and biochemical parameters are sensitive to loading conditions in the IVD.

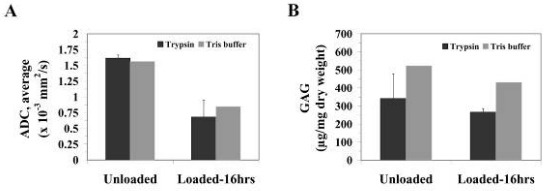

Fig. 1: The GAG content (A) and ADC (B) of bovine coccygeal NP injected with trypsin or Tris buffer and left unloaded or loaded for 16h.

REFERENCES: [1] J. Antoniou, F. Mwale, G. Beaudoin *et al.* (2003) Quantitative MR Imaging and Biochemical Quantification of Enzymatically Denatured Intervertebral Discs. *Orthopaedic Research Society*. [2] D. Périé, J.C. Iatridis, C.N. Demers *et al.* Assessment of compressive modulus, hydraulic permeability and matrix content of trypsin-treated nucleus pulposus using quantitative MRI. *J Biomech* (**In Press**). [3] E.J. Chiu, D.C. Newitt, M.R. Segal *et al.* (2000) *Spine* **26**(19):E437-44.

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In vivo effects of recombinant human growth and differentiation factor 5 on the intervertebral disc

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INTRODUCTION: Growth and differentiation factor-5 (GDF-5) is a member of the bone morphogenetic protein family. Although GDF-5 has been studied using the mouse tail compression model [1], there has been no quantitative data on the capacity of GDF-5 to repair the degenerative intervertebral disc (IVD). The purpose of this study is to determine the reparative capacity of recombinant human GDF-5 (rhGDF-5) in an *in vivo* setting using the rabbit annular needle puncture model [2].

METHODS: Twelve adolescent New Zealand white rabbits received an annulus puncture (18G) in two non-contiguous discs to induce disc degeneration. Four weeks later, PBS (10 µl) or GDF-5 (1 and 100 µg, in 10 µl) were injected into the center of the NP of previously punctured discs.

Radiological assessments: Disc height was radiographically monitored biweekly from the day of needle puncture injury to 12 weeks post-injection. IVD height was expressed as the disc height index (DHI)[3]. Percent DHI (%DHI = (postoperative DHI/preoperative DHI) x100) was subsequently calculated.

Histological Analysis: The experimental IVDs were excised from each vertebral body-disc-vertebral body unit, and each IVD were fixed in 10% formalin, decalcified, embedded in paraffin, sectioned and assessed by histology. Sagittal sections (5-8 mm) of each IVD were stained with hematoxylin and eosin, as well as Safranin-O.

RESULTS:

Changes in DHI: The intradiscal injection of GDF-5 significantly altered the time course of changes in disc height in degenerated IVDs ($p < 0.001$). At 4 weeks after injection, the %DHI in the GDF-5 group (both 1 µg and 100 µg) began to increase and return towards that of the non-punctured disc, whereas the PBS groups did not show any recovery of disc height during the 12 weeks after injection. At the 16-week time point, the DHI of GDF-5 injected discs remained significantly higher than that of the PBS-injected discs (PBS vs GDF-5: 1 µg and 100 µg, $p<0.001$).

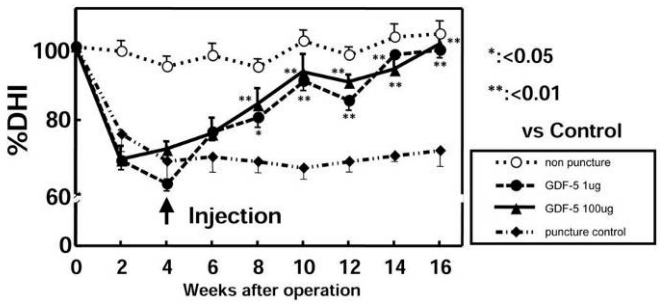

Fig. Change of percent DHI

Histological Analysis: rhGDF-5 treated discs showed a greater percentage of proliferating cells compared to PBS controls. However, the 100µg rhGDF-5 group demonstrated more cellularity over the 1µg group.

DISCUSSION & CONCLUSIONS: The preliminary results of this study showed that rhGDF-5 enhanced cell proliferation as well as matrix synthesis and accumulation in the rabbit IVD. The study provided encouraging preliminary evidence that a single injection of GDF-5 induced recovery of disc height in the IVDs of rabbits with degenerative changes previously induced by annular needle puncture. Stimulation of the anabolic cascade by rhGDF-5 could therefore prove useful as a therapeutic approach to delay the progression of disc degeneration or to promote the repair of the degenerating human IVD.

REFERENCES: [1] A. Walsh et al (2004) Spine **29**: 156-163. [2] K. Masuda et al (2004) Spine **29**: 1-9. [3] D. Lu et al (1997) Spine **22:** 1828-34

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Further characterisation of an ovine model of steroid-induced osteopaenia

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INTRODUCTION: Bone loss and increased bone fragility following long-term steroid therapy may result in painful vertebral fractures. A large animal model is needed to fully understand the pathogenesis of steroid-induced osteoporosis and to test potential implants. The sheep is a valid model since bone remodelling is similar to humans and the vertebral dimensions permit easy surgical manipulation. This study was undertaken to fully characterise the sheep as a model of steroid-induced vertebral osteoporosis.

METHODS: Osteoporosis was induced in ten 8-year-old lactating ewes by oophorectomy, weekly injection of 54 mg dexamethasone and a diet containing 0.2% calcium for up to 6 months. Iliac crest biopsies were taken at the start and end of treatment to measure a range of bone histomorphometric indices. Control animals were neither ovariectomised nor treated with steroids. Bone mineral density (BMD) in the lumbar spine (L2-L5) was measured by dual energy X-ray absorptiometry (DXA) after 0, 3 and 6 months of treatment. At each time interval sheep were killed and the entire lumbar spine (L1-L6) and distal and proximal femora were processed for histology, quantitative histomorphometry, mechanical testing, micro-CT (computed tomography) and for expression of a range of molecular markers using real-time PCR analysis.

RESULTS: After six months of treatment BMD in the lumbar spine decreased by 29.5% from baseline (Figure 1). Trabecular bone volume (BV/TV) of L2, L3 and L4 vertebrae (pooled) decreased by 31.4% and trabecular thickness (TbTh) decreased by 33.9%; $p<0.05$ (Figure 2). Cortical bone thickness decreased by 43.9%; $p<0.05$. BV/TV in the distal and proximal femora decreased by 48.6% and 42.5% respectively ($p<0.05$) and trabecular thickness decreased by 35.2% and 29.5 %. The average load at which L1 yielded decreased by 67.4%. Static measurements of bone formation decreased by 68.3% and bone resorption increased tenfold. Coll-1 gene expression was significantly down regulated in the vertebral bodies.

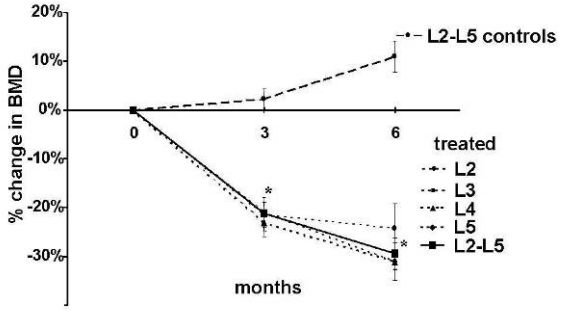

Figure 1: Percent change in lumbar spine BMD over 6 months of continuous steroid treatment

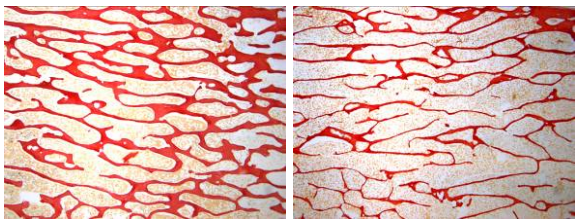

Figure 2: Vertebral trabecular architecture of control (left) and steroid-treated (right) sheep

DISCUSSION & CONCLUSIONS: Using DXA, cancellous bone histomorphometry and mechanical testing, this study has demonstrated significant trabecular and cortical bone loss in the sheep lumbar spine up to six months after ovariectomy and continuous steroid treatment. Significant loss of trabecular bone was also seen in the femora. These changes are the result of increased resorption and decreased formation of bone. This is supported by the molecular analyses. This ovine model is suitable for pharmacological trials and in-vivo assessment of vertebral augmentation procedures and orthopaedic devices.

Low Stiffness Biopolymer used for Mechanical Bone Augmentation in Osteoporotic Vertebrae

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INTRODUCTION: Bone cements generally used for vertebroplasty are PMMA or CaP based. Both materials are at least a magnitude stiffer (4-10 GPa) than vertebral trabecular bone (100-300 MPa). Adjacent segment fractures, a problem of vertebroplasty, is a likely result of this stiffening. A novel biopolymer, without this stiffening drawback, was biomechanically tested.

METHODS: 42 human vertebral bone cores were cut, characterized (BMD, porosity) and augmented with a three-part biopolymer, comprising a donor and acceptor with thiol and acrylic active groups, and a base reaction starter. A Teflon® mold held the bone core tightly while the polymer was injected, infiltrating the bone core completely. Augmented cores were kept in 37^{O} C saline. At seven time intervals (1h, 2h, 8h, 1d, 3d, 7d, 14d), the cylinders were compressed at 0.1% strain per second to failure. For controls, pure biopolymer samples at the same seven time intervals (n=21) and non-augmented bone cores (n=10) were tested. Failure strain, strength and stiffness were measured for the different time intervals.

RESULTS: Native bone shows a strength and stiffness strongly (r^2 > 0.9) linked to its porosity. Augmented cores, a composite of polymer and bone, showed a characteristic load-displacement curve with two linear elastic sections showing a distinct stiffness. The first leg was always stiffer, with a value comparable to the stiffness of native bone. At around 2% strain, the curve shows a knee, followed by a second leg with a lower stiffness. The composite's ultimate strength was 6.1 MPa after one hour, 12.7 MPa after eight hours and 15.5 MPa after two weeks (in comparison, native bone of average porosity is 2.7 MPa). Remarkable was the high failure strain at 9.1%, 16.1% and 20.3% (native bone, in comparison, is 2.2%). The stiffness of the augmented cores was 33.7 MPa, 67.3 MPa and 74.3 MPa, again for the same time intervals (native bone with average porosity is 145.1 MPa). Stiffness and failure strain curves were very similar for the bone-polymer composite and the polymer alone.

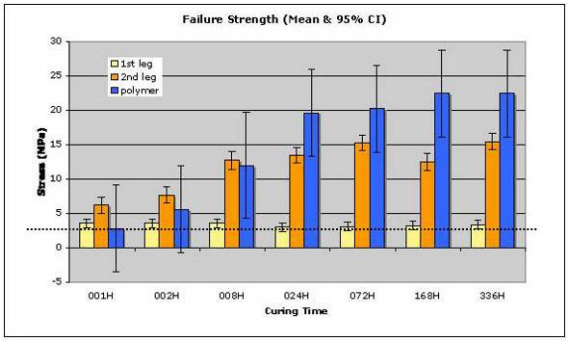

Fig 1: Ultimate strength of composite (1st and 2nd leg) and the polymer, plotted from left to right for increasing curing times, bars indicating group means ± 95% confidence intervals. The dashed line shows the average strength of native trabecular bone.

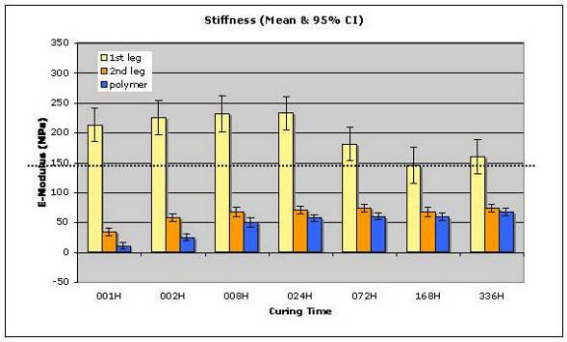

Fig 2: Stiffness of composite (1st and 2nd leg) and the polymer, plotted from left to right for increasing curing times, bars indicating group means ± 95% confidence intervals. The dashed line shows the average stiffness of native trabecular bone.

DISCUSSION & CONCLUSIONS: Nearly 80% of the fully cured augmented bone's ultimate strength and stiffness is reached after eight hours. Already after one hour, the composite's strength is double that of native trabecular bone (~ 2.7 MPa). Fully cured, the polymer's second leg stiffness is remarkably similar to that of very osteoporotic vertebral bone (~ 60-80 MPa). The novel biopolymer strengthens but does not stiffen osteoporotic bone. Whether cement with a lower stiffness will result in a lower incidence of adjacent segment fracture can only be shown clinically.

Osseogenetic collagen complex as an effective graft alternative in experimental posterolateral lumbar spine arthrodesis

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INTRODUCTION: Spinal fusion has been used since the late 19th century. Two frequentlyy applied techniques are anterior interbody fusion and posterolateral intertransverse process fusion. Posterolateral lumbar intertransverse process arthrodesis is the most common procedure performed. Autogenous bone is considered the golden standard for bone graft material used for spinal arthrodesis. Unfortunately, morbidity associated with autograft harvest may occur in as many as 25-30% of patients. Currently, there are several competitive strategies either to augment healing or to replace autogenous bone graft in the spine. These include biologically active bone void fillers with growth factors derived from animal bone.

METHODS: COLLOSS E by OSSACUR AG is a bone void filler extracted from the extra-cellular matrix of cortical diaphyseal equine bone. It is a lyophilisate in collagenous matrix form, consisting of Type I collagen chains with other insoluble proteins present. It is supplied in 20 mg vials. 12 New Zealand immune competent, white rabbits underwent single level, posterolateral intertransverse process arthrodesis bilaterally at L4-L5, using COLLOSS E alone or in combination with either a titanium mesh cage, a PEEK cage, a TCP block or a stent filled with COLLOSS E. The animals were divided in four group and were euthanized 6 weeks after surgery, the lumbar spines were excised, and the fusion assessed by manual palpation, radiographs, and CT.

RESULTS: Manual fusion status: In group A and B fusion success was achieved in 75% (3 out of 4 rabbits). In group C and D only the animal with COLLOSS E combined with a stent developed a spinal fusion, whereas the 2 rabbits with TCP plus stent failed to fuse.
Radiography and CT: Imaging of group A showed a very good bone formation in 75%, except one animal which completely failed to develop a spinal fusion. Using 6 or 12 vials of COLLOSS E did not influence the radiographic fusion mass.
Similar results were observed in group B, where in 50% of fusion sites a very good bone formation was seen. In group C and D, 2 of the 3 animals with stent implants and both rabbits with TCP blocks developed moderate bone masses (2+ and 3+).

DISCUSSION & CONCLUSION: Autograft bone is considered to be the most osteogenic graft material in spine fusion surgery.
These problems have prompted the search for bone graft substitutes to achieve spinal fusion [1]. DBM derived from human allograft is effective as graft extender and enhancer in the rabbit model of posterolateral lumbar spine fusion with fusion success rates of up to 100% [2,3]. Early studies with recombinant BMPs and purified BMP extracts were successful in a variety of rodent models, yet early clinical trials in humans resulted in disappointing outcomes.
Recombinant BMP-7 has demonstrated variable success in rodents, recombinant BMP-2 has demonstrated consistent success, and an bovine-derived mixture of BMPs has also led to spinal fusion in a rodent and monkey model, as well as in humans in 94% [4]. As promising as their results may be, dose relationships, carrier substrates, possible antibody formations, and unknown long-term outcome may provide continued challenges, and the high manufacturing cost may further preclude routine clinical application.
The data of the current study show favorable results of COLLOSS E in supporting spinal fusion in combination with the titanium mesh and the PEEK cage. The success rate of spinal fusion was 88% (7 of 8 animals) according to the radiological findings and 75% (6 of 8 animals) according to manual fusion criteria. These rates correspond to data when autografts or DBM are used in the rabbit model.
The presented data have to be seen as a pilot study with a relatively small sample size. Further studies may be conducted to investigate the use of COLLOSS E in conjunction with spinal implants.
COLLOSS E is effective in supporting new bone formation and in achieving a spinal fusion with comparable success rates as autografts. These results suggest the feasibility of COLLOSS E to support bone growth without the need for a second site bone graft harvest or internal fixation.

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ACKNOWLEDGEMENTS: OSSACUR AG, Obersteinfeld, Germany, provided support for this study.

Regeneration of nucleus pulposus after discectomy by autologous mesenchymal stem cells: a rabbit model

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INTRODUCTION: There is evidence to suggest that mesenchymal stem cells (MSC) introduced into the degenerated intervertebral disc have the ability to survive and halt the degeneration process[1, 2]. In clinical practice, one of the areas where this maybe useful, is using these cells combined with a scaffold for nucleus replacement after surgical excision of the disc. This study explores the ability of MSCs to survive and maintain disc height in a rabbit discectomy model.

METHODS: Autologous MSCs were isolated from 12 New Zealand white rabbits and expanded *ex vivo*. They were bromo-deoxyuridine (BrdU)–labeled, seeded onto gelatin scaffolds and re-implanted into the rabbit after either a posterior discectomy (n=8) or anterior discectomy (n=4). 4 lumbar disc levels were operated per rabbit: 1 control level with scaffold; 1 sham level (discectomy alone); and 2 levels implanted with BrdU-labeled MSCs. Immuno-histochemical and radiographic analyses were performed up to 12 weeks.

RESULTS: Immunohistochemical examination showed only 1 (12%) posteriorly operated rabbit but 3 (75%) anteriorly operated rabbits retained the MSCs. The BrdU-labeled cells could be detected within the nucleus pulposus, the annulus fibrosus and the cartilaginous end-plates up to 12 weeks post-operatively (Figure 1). There was evidence of new bone and osteophyte formation. Disc height measurements reviewed that both the scaffold group and the MSCs-implanted group were able to better maintain disc height than the sham operated group.

DISCUSSION & CONCLUSIONS: The IVD is a particularly challenging environment, as it is avascular and subjected to high repetitive mechanical loading. Moreover, a discectomy model, where the nucleus is evacuated, is a more demanding model for the stem cells to work than previous disc degeneration models. Nevertheless, this study demonstrates that MSCs in combination with a scaffold has the potential to act as a nucleus replacement in patients undergoing surgical treatment. However cells could not be easily retained in the posterior discectomy group probably because of the annular defect after posterior discectomy. Whereas in the anterior group, the annular flap may help to retain cells. For those that can retain cells we have demonstrated for the first time that these cells can remain inside the disc for up to 12 weeks. However, in none of the groups could disc height be maintained, and the scaffold alone group did just as well as the MSCs-implanted group. New bone formation and osteophyte formation could be an effect of the degenerative process or because of the transplanted MSCs transdifferentiating into osteoblasts. This study has showed that stem cell therapy has the potential to act as a nucleus replacement. Future work will need to assess the synthetic activity, the matrix composition and the fate of the retained MSCs. Work will also be needed to identify the best scaffold and cell source for this purpose.

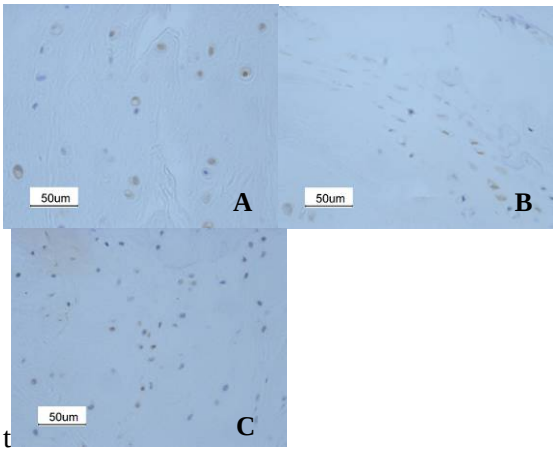

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*Fig. 1: The persistence of MSCs (brown cells) in all 3 disc compartments: the nucleus pulposus (**A**), the endplate (**B**) and the annulus fibrosus (**C**).*

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Impact of Trp2 allele mutation of α2 chain in collagen IX on the structural integrity of human annulus fibrosus

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INTRODUCTION: A preliminary study has suggested that Trp2 allele mutation in α2 chain of collagen IX (*COL9A2*) may attribute to early degenerative disc desease[2]. In intervertebral discs (IVD), collagen IX is found in close association with collagen II and it is hypothesized that collagen IX may play an important role in maintaining the structural integrity of the discs through its covalent cross-links with collagen II[2]. Collagen II is present in all three components of the IVD i.e. annulus fibrosus (AF), nucleus pulposus and end plates. This abstract focuses only on the AF. In the outer edge of the AF, the concentration of collagen II is very low[3]. The concentration of collagen II is known to increase from the outer edge towards the interior of AF in radial direction. Thus we have arrived at a hypothesis that, testing the mechanical properties of the inner AF region may enable us to find the influence of Trp2 on the mechanical properties of AF. Studies have been carried out in the past to determine the overall mechanical properties of the AF[1], but there is not much work done to find the microscopic mechanical properties of inner AF collagen II. Thus a study in the microscopic level is essential to figure out the fine differences in mechanical properties that are expected to be caused by mutations such as the Trp2 allele.

PROPOSED METHODS: We aim to test and compare 20 samples each from the Trp2 mutated non-degenerated and normal non-degenerated controls of human inner AF specimens. The samples will be collected from patients who undergo scoliosis correction procedure. The incidence of Trp2 among the south Chinese population is about 20%, which makes it easily possible for collecting both mutated and normal discs.

The annulus part of the disc will be carefully removed and genotyped before storing at -80° C. Thin sections of inner AF will be microtomed to a thickness of approximately 0.5mm using a cryostat. Individual fibers of length approximately 1.5mm will be dissected from the slice under a binocular microscope. The experimental setup and the tensile testing protocols from a previous study on rat muscle fibers will be adopted with some modifications[4]. The fibers will be held between the force and displacement transducers by gluing the ends. The movements will be controlled with a computer and servo controller. Based on our pilot study conducted on bovine AF specimens, a 2.5 N force transducer of frequency resonance 1 kHz was found to be appropriate for this purpose and the same will be used in the actual study. The force and displacement will be visualized in an oscilloscope and recorded into a computer for further analysis.

POSSIBLE OUTCOMES AND DISCUSSION: The outcome of this study can be of three types. 1) Trp2 affected AF fibers are weaker than non-Trp2 affected AF fibers. 2) Trp2 affected AF fibers are stronger than non-Trp2 affected AF fibers. 3) No significant difference between the tensile mechanical properties of Trp2 affected and non-Trp2 affected AF fibers. Outcome (1) will prove that, Trp2 plays a significant role in weakening the AF and thus leading to DDD. Outcome (2) will suggest that the role played by Trp2 is benign as far as the tensile mechanical properties of AF are concerned. Outcome (3) may mean one of the following two (a) Trp2 does not affect the mechanical properties of AF; (b) the testing method is not sensitive enough to discriminate the role played by Trp2. Outcome (3) will thus suggest for a nano-level testing involving individual collagen II fibrils.

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High Cement Viscosity Reduces Leakage Risk in Vertebroplasty

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INTRODUCTION: Extravasation of PMMA can lead to pulmonary cement and fat embolisms. To study the leakage phenomena in cadaveric experiments would require a large sample size due to the variability with living tissue. Consequently, the possibility of developing and implementing a physical model may be of interest.

METHODS: A physical leakage model of a vertebra was created to observe the leakage phenomenon due to a range of injection viscosities of bone cement. Open cell aluminum foam blocks with a porosity of 90% were used to model osteoporotic trabecular structure. A thin layer 1.8mm layer of PMMA served as the as a cortical shell. One 4mm diameter passage to the center of the model was used to locate the injection syringe while a second port (2mm diameter) located 10mm from the syringe port and orthogonal functioned as a perforating blood vessel. Four 2mm diameter ports allowed for the desired 3.45kPa internal pressure during injection. Each model was completely filled with a gelatin as a bone marrow stimulant. Blood expander acted as a blood stimulant by flowing through the model. Approximately 20cc of bone cement was injected into each model and a real time value of the cement viscosity was determined by extruding 20cc of cement from the same batch through an identical syringe and recording the extrusion force. The time required to leak was determined when the cement exiting the perforating vessel and cross the path of a photosensitive transducer.

RESULTS: A total of 33 tests were performed and results of interest were the time required to leak for a certain initial injection viscosity, the amount of cement leaked and the distribution of the cement spread within the model. Figure 1 shows cements with viscosities starting around 50Pa·s would leak early around 10 to 20 seconds after injection while higher viscosities around 325Pa·s would leak after 60 seconds. Most importantly, but not shown on either figure, is the fact that bone cements with viscosities greater than 350Pa·s did not leak.

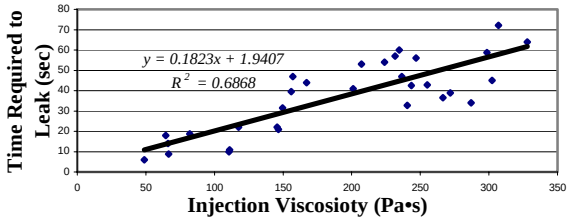

Fig. 1: Time Required to Leak as a Function of Injection Viscosity

Finally, in Figure 2, the distribution of the cement within the leakage model would tend to fall into three categories: high, medium, and low viscosity spreads.

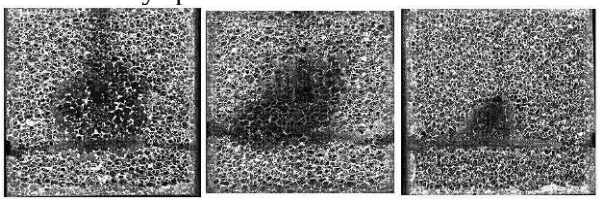

Fig.2: Section views of the leakage model exhibiting three different initial cement viscosities (from left to right: high, medium, low).

DISCUSSION & CONCLUSIONS: The model has shown a strong correlation between cement viscosity and leakage frequency and thus clearly identified bone cement viscosity as a key parameter influencing leakage. More importantly, our model suggests that a critical bone cement viscosity of 350Pa·s results in no leakage and by using this value clinically may reduce the risk of extravasation.

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Comparison of Pedicle Screw Spinal Fixation for Fractures: Bridge vs. Tension Band Construct

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INTRODUCTION: Posterior osteosynthesis of thoraco-lumbar fracture has been shown to settle in kyphosis if classic posterior Tension Band (T-B) type of fixation is utilized (Fig. 1a). This study compared the initial stability and pull out strength of parallel versus divergent posterior screw orientation within the vertebral body for posterior spinal fixation. It is believed that a divergent screw construct, creating a bridge-type (Bridge) fixation (Fig. 1b), is safe, significantly stronger, and will resist failure in kyphosis and screw pull-out significantly more then a parallel screw construct, which acts like a tension-band.

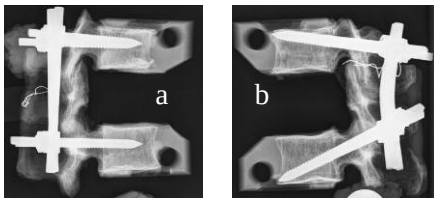

Fig. 1a,b:Radiographs of T-B (a) and Bridge (b) constructs. (levels T11-L1)

METHODS: We tested our hypothesis using: a finite element analysis (FEA); six synthetic models using the ASTM standard for vertebrectomy[1]; and six human cadaveric models quantifying construct stiffness and ultimate strength using an MTS machine.

The FEA (Finite Element Analysis) models were loaded at 100N, 300N, & 600N and the resulting displacements were generated for each load. With this data, stiffness curves for each construct were created.

For the ASTM corpectomy model, UHMWPE blocks with a tensile strength of 40 ± 3 MPa were fabricated to the standards dimensions with three models having T-B constructs. For the Bridge constructs, the pedicle screw orientation, with respect to the horizontal plane, were altered to 16.5º superiorly 26.4º inferiorly. The specimens were loaded using MTS Load under displacement control at a rate of 0.4mm/s. For the cadaveric models, six human fresh frozen spines were dissected and instrumented between T11-L1, and then had a corpectomy of at the T12 level. Three specimens of each construct were built. For the Bridge construct, a guide was designed to provide consistent placement of the schanz screw. The cadaveric model testing duplicated the ASTM model testing protocol.

RESULTS: All three modalities showed greater stiffness with the Bridge type fixation Fig. 2. The cadaveric model failure load was greater for the Bridge Construct compared to the T-B (Fig. 3).

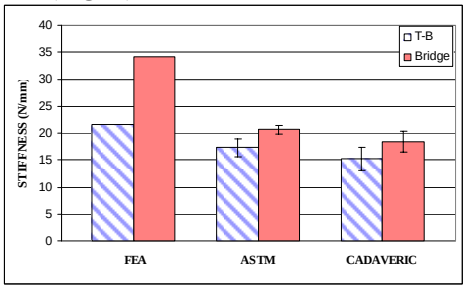

Fig. 2: Construct Stiffness comparison between FEA analysis, ASTM UHMWPE standard, and Cadaveric Model.

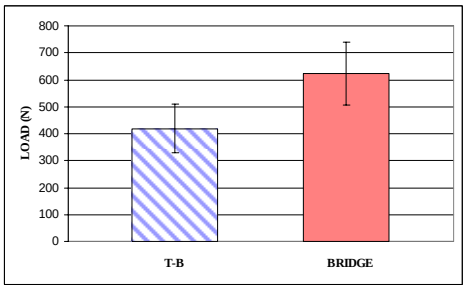

Fig.3: Cadeveric ultimate strength load for T-B and Bridge constructs.

DISCUSSION & CONCLUSIONS: If the added mechanical advantage of Bridge type fixation can off load the anterior column allowing it to heal before kyphosis occurs, then the added morbidity of anterior column reconstruction is not warranted in management of thoraco-lumbar fractures.

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Differential Functions of Cyclooxygenase 1 and 2 in Bone Repair

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INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for pain control after orthopaedic surgeries. They inhibit the enzyme cyclooxygenase (COX), of which two isoforms exist (COX-1 and 2). Non-selective COX inhibitors have been shown to delay bone healing, suggesting possible roles of COX-1 and COX-2 in bone repair. However the distinct functions of COX-1 and COX-2 during bone repair and formation are largely unknown. This study aims to investigate functions of the two enzymes by comparing the effects of different COX inhibition in fracture repair, using 2 well established mouse femoral fracture models with different mechanical environments. Further investigation using COX-1(-/-) and COX-2(-/-) mice is also carried out.

METHODS: To achieve different COX inhibition, mice were treated with ketorolac (non-selective COX inhibitor), valdecoxib (selective COX-2 inhibitor) or placebo (control). Fractures were created on both femora while one side was externally-fixed and the other side left unfixed. In another set of experiment, the same type of fracture surgeries were done in COX-1(-/-), COX-2(-/-) and wild-type mice (without any further treatment). For all the operated mice, radiographs were taken weekly. Three time points were chosen as 7, 14 and 21 days post-surgery. Histological analyses and mechanical testing were performed.

RESULTS: In the COX inhibition experiment, x-ray showed reduced callus formation in ketorolac treated mice, while mechanical testing showed significantly decreased stiffness of fractured femora from both NSAID groups. Histologically, ketorolac showed delayed healing in both fixed and unfixed fractures; while valdecoxib showed impaired healing in unfixed fractures only.

Interestingly, in COX-2(-/-) mice the impairment on bone healing was more severe. Radiographs showed reduced callus formation in COX-1(-/-) mice at 14 days, and in COX-2(-/-) mice at both 14 and 21 days. Mechanical testing at 21 day showed significantly decreased stiffness of fractured femora in COX-2(-/-) mice. Histology at 21 day showed severe delay in bone healing of unfixed fracture in COX-2(-/-) mice, and minor delay in COX-1(-/-) mice. The results of the two sets of experiments were summarized in table 1.

Table 1. The effect of COX inhibition (as compared to placebo), and the effect of COX gene knockout (as compared to wild-type) on bone repair

	Fixed fracture				Un-fixed fracture			
	K	**V**	**A**	**D**	**K**	**V**	**A**	**D**
x-ray	/	/	/	/	+	—	+	++
Histology	+	—	—	—	+	+	+	++
Mechanical testing	+	+	—	+	/	/	/	/

K: ketorolac (both COX-1 and COX-2 inhibition)
V: valdecoxib (COX-2 inhibition)
A: COX-1(-/-) mice
D: COX-2 (-/-) mice
+: inhibitory effect (reduced callus formation, delay in healing, or reduced mechanical stiffness)
++: severe inhibitory effect
—: no effect
/: data not available

DISCUSSION & CONCLUSIONS: This is the first study to fully investigate the effect of various COX inhibition, as well as the effect of COX-1 or COX-2 gene deficiency, in both fixed and unfixed fracture repair. These results suggest that COX-1 and COX-2 are both involved in bone healing, yet carrying differential functions. COX-1 has minimal function in fixed fracture healing and minor function in early stage of unfixed fracture healing. COX-2 has minor function in fixed fracture healing, but plays an essential role throughout unfixed fracture healing. As fixed fractures heal predominantly by direct bone formation, while unfixed via endochondral ossification, this study has significant implications on the mechanism of bone healing which is the subject of further research.

Establishment of an In-vitro whole Organ Intervertebral Disc / Endplate Culture Model

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INTRODUCTION: Primary or posttraumatic degeneration of the intervertebral disc and endplates are of major orthopaedic and socioeconomic importance. To study disc degeneration, generally, investigators use either animal models or *in-vitro* assays. However, it remains difficult to mimic the complex pattern of the disease *in vivo* and with current *in-vitro* systems. Moreover, cell or organ cultures exhibit often cell dedifferentiation due to profound changes of the intercellular matrix and the micro-environment.

Aim of the study was therefore to establish a whole organ disc / endplate culture system as a model for studying primary and posttraumatic disc and endplate degeneration

METHODS: Thoracolumbar and lumbar intervertebral discs including adjacent endplates were harvested from female 6 months old New Zealand White Rabbits and cultured in 6 well plates containing supplemented medium for up to 49 days. Concurrent changes of cell viability (Live/Dead®, Molecular Probes), total proteoglycan content (Alcian blue binding assay) as well as collagen I/II and aggrecan gene expression (RT-QPCR) were determined.

RESULTS: Whole organ disc / endplate cultures retained their viability over 49 days (from 81% ± 7% to 78% ± 2%, Fig. 1). Total proteoglycan content was stable over 28 days (23,8 ± 1,04 µg / mg disc to 20,83 ± 3,26 µg / mg, m±SD from two separate experiments). Quantitative PCR demonstrated a significant down-regulation of the aggrecan gene (decrease of 88% ± 10% for annulus (A) and of 44% ± 18% for nucleus pulposus (N) cells after 42 days, m±SD, from three separate PCR experiments, normalized to the expression of GAPDH) as well as of collagen type II mRNA (decrease of A: 96% ± 2%, N: 25% ± 1%). In contrast collagen type I gene expression was initially up-regulated until day 28 (A: 30,31 ± 12 fold, N: 193 ± 71 fold) and subsequently dropped by day 42 (A: 3,08 ± 1,3 fold, N: 56 ± 19 fold), still remaining significantly up-regulated compared to day 0.

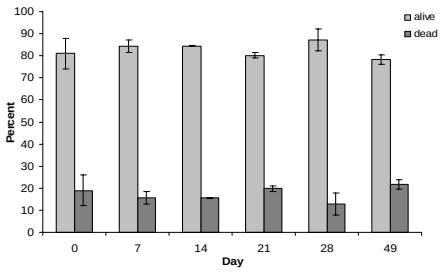

Fig. 1: Cell viability of disc cells after culture as a whole organ system. Live/Dead® viability assay. Mean ±SD of three separated experiments

DISCUSSION & CONCLUSIONS: We have demonstrated that constrained intervertebral disc / endplate cultures from rabbits remain viable for 49 days. This ongoing viability without administration of growth factors exceeds most current disc culture systems other then primary cultures[1]. Moreover, disc proteoglycan content did not change significantly over 28 days despite a marked down-regulation of aggrecan gene expression. In contrast, the collagen gene expression profile showed significant alteration, with up-regulation of type I and down-regulation of type II collagen. Diminished aggrecan gene expression and a switch in collagen type gene expression are commonly observed with degenerative disc disease[2].

The described disc / endplate culture system is a promising model to induce and analyse disc degeneration and study the interplay between intervertebral disc and vertebral endplates.

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ACKNOWLEDGEMENTS: AO Foundation, Davos, Switzerland.

Slowly Progressive Disc Degeneration in a Goat Model

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INTRODUCTION Factors compromising interspecies comparison of intervertebral disc (IVD) degeneration in currently used *in vivo* models include relatively small disc size, different notochordal cell status and/or the fast progression of induced degeneration[1].
The goat IVD has similar geometrical and mechanical properties compared to human IVDs.[2] Also, notochordal cells are absent in mature goats.[3] Therefore, the goat is a suitable animal for disc degeneration models and we investigated two methods to *slowly* induce disc degeneration: injection of 0.25% Chondroïtinase ABC (CABC) and vertebral endplate perforation with a 3.5 mm drill (EP).

METHODS In each of 17 skeletally mature Dutch milk goats, at random, two lumbar IVDs were induced with EP, two with CABC, two with PBS and two sham-treated (Control) IVDs. Follow-up periods were 4, 8, 12, 18 and 26 weeks (n≥3). Both before surgery and before sacrifice, X-rays were taken. Subsequently, the spinal column was harvested and directly scanned with MRI. Next, individual levels were cut into 3 mm sagittal slices and digitally photographed for macroscopic analysis
IVD height: For each goat, IVD space (IVDsp) and cranial vertebral body height (CVBh) were measured at three points and averaged, both in the pre-operative and the pre-autopsy X-ray. Subsequently, the disc height index (DHI; the IVDsp/CVBh ratio) was calculated for each time point, and used to determine the loss of disc height (expressed as % decrease).
MRI: All levels were scored using a modified Thompson classification for degeneration.[4]
Macroscopy: The digital photographs were scored using the Thompson grading system.[5]

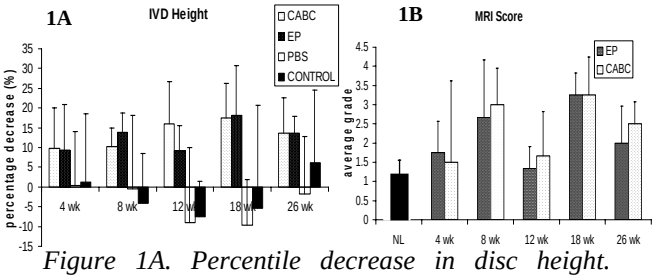

Figure 1A. Percentile decrease in disc height. Figure 1B. MRI grades scored according to the modified Thompson protocol. NL = reference goats.

RESULTS *IVD height:* No differences were found between control and PBS groups. The DHI was significantly decreased in EP treated levels at 18 weeks ($p<0.01$) and in CABC treated levels after 12 and 18 weeks ($p<0.05$) compared to PBS injected levels (Fig.1A.)
MRI: MRI data showed a trend towards degeneration over time but, due to low sample size, never reached significant levels over reference values (NL goats) (Fig. 1B).
Macroscopy: Control and PBS levels were not different. EP levels had significantly higher Thompson grades at 4 ($p<0.001$) and 18 ($p<0.001$) weeks and CABC treated levels at 8 ($p< 0.05$) and 18 weeks ($p<0.001$) compared to PBS injected levels. Also, scores increased significantly over time in CABC levels.

DISCUSSION Despite the limited number of animals, the data presented show a progressive trend of degenerative symptoms in time for both EP and CABC. At 26 weeks, these symptoms decrease slightly. Whether this is due to recovery or an artefact is now being addressed in a follow-up study. The non-significant increase of DHI, seen in figure 1A for the PBS/control levels, might be explained by different angles of the radiographs.
EP levels demonstrated gross morphologic destruction caused by the drill at all time-points, and EP was therefore considered to be less suitable for de- and regeneration research.
CABC levels demonstrated degeneration in approximately 2/3 of the treated levels. Because besides interspecies comparability, reliability and reproducibility of the degeneration are essential for a good degeneration model, this is also addressed in the follow-up study.
In conclusion: The goat disc degeneration model presented here is very promising, shows slowly progressive degeneration and mimics the human situation better than the currently used models.

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Notochordal Cells in Mature Caprine Intervertebral Discs

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INTRODUCTION: Currently used models studying degeneration *in vivo* vary considerably in the status of their notochordal cells. This status is relevant because the disappearance of these cells precedes the onset of disc degeneration in humans and it has been reported that they stimulate matrix production by the chondrocyte-like cells of the nucleus pulposus (NP) [1] Therefore they could influence the degeneration process. The small animals used for degeneration models also have relatively small discs and different mechanical loading in comparison with the human spine.[2]
To overcome these shortcomings we are developing a large animal disc degeneration model in the goat.

In this study we investigated whether notochordal cells are present in the intervertebral discs of mature Dutch milk goats.

METHODS: Six mature female Dutch milk goats over the age of three years were sacrificed. The intervertebral discs between T12-S1 were harvested and half of the intervertebral discs were selected for histologic preparation. Parasagittal slices were fixed in 10% neutral buffered formalin, decalcified in formic acid, paraffin-embedded and sectioned to 7 µm thickness slices. The sections were stained with hematoxylin and eosin (H&E) for cellular constituents. Four intervertebral discs of six goats (n=24) were carefully examined at different magnifications by two examiners.
Notochordal cells were defined as relatively large, vacuolated cells laying in clusters or in cord-like formations within the nucleus pulposus.[3] Chondrocyte-like cells were defined as relatively small, rounded cells lacking interconnections and vacuoles. [4]

RESULTS: Representative pictures of the histology specimens are shown in figure 1.
In none of the examined slices cells with the notochordal phenotype were observed. Chondrocyte-like cells were present throughout the entire NP in all of the specimens.

DISCUSSION Based on the observations of this study, we conclude that mature caprine intervertebral discs lack notochordal cells. This

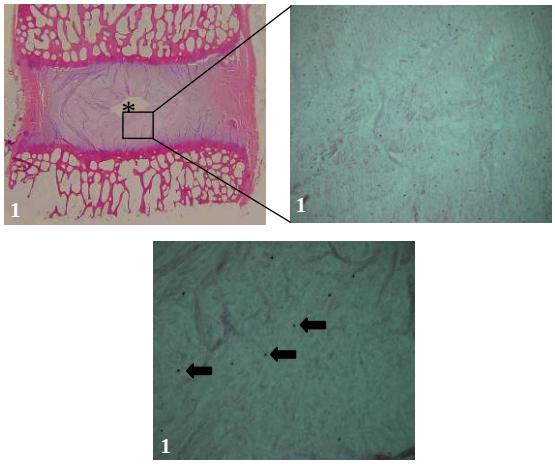

*Figure 1A to C: H&E staining. Figure 1A shows the entire intervertebral disc. 1B shows a representative detail of the NP (100X), 1C the same detail at a higher magnification (200X) Arrows show the chondrocyte-like cells. Cells with a notochordal phenotype were not observed. * = section artefact.*

was also observed in the (closely related) ovine model. [4] Additional ongoing studies address the recently described specific actin[5] and cytokeratin[6] stainings to verify the absence of notochordal cells. Also, fetal and juvenile goat intervertebral discs will be examined to study the presence of notochordal cells in these young nuclei pulposi.

The absence of notochordal cells in mature goats, and the presence of chondrocyte-like cells mimicking the human population, positively identifies the goat as a suitable animal for a disc degeneration model. This is corroborated by the more similar mechanical loading and geometry of the goat lumbar spine in comparison with the human spine.[7]

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Bioresorbable fibre reinforced composites for spinal fusion application: *In vitro* analysis

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INTRODUCTION: Poly(α-hydroxyl acids) have been studied as a material for bioabsorbable spinal fusion devices[1]. Their composites with osteoconductive component, hydroxyapatite, have also been studied for the same application[2]. In the current study a novel bioabsorbable composite composed of poly-L/DL-lactide 70/30 (PLA70) matrix containing osteoconductive component β-TCP, with or without poly-L/D-lactide 96/4 (PLA96) fibre reinforcement was studied.

METHODS: Laminar composites composed of 50 wt-% of PLA70 and 50 wt-% of β-TCP (50/50) with or without PLA96 fibre reinforcement were subjected to *in vitro* follow up in phosphate buffer saline (PBS) at 37°C. Compression strength was measured according to standard ISO 604:1993(E). Measurements were made in two directions, parallel and perpendicular to the laminar sheet structures to analyze the anisotropy of the material. Dimensions of test specimens were 10*10*4 mm. PBS was changed fortnightly and pH was checked shortly before change of PBS. Test specimens were gamma sterilized using 25kGy irradiation. Molecular weights were monitored by means of gel permeation chromatography.

RESULTS: Composites of this study demonstrated initial compression strength of 98-125 MPa after gamma-sterilization, depending on composition and direction of sample loading. When measured parallel to laminar structure, the composites retained ca. 50% of their initial compression strength for ca. 20 weeks, whereas in perpendicular direction composites retained ca. 50% of their initial compression strength after 40 weeks incubation in PBS (*Fig. 1*). No great differences on compression strengths between the fibre reinforced and non reinforced specimens could be measured in both directions analyzed. 38 week incubation decreased molecular weight (Mw) ca. 70% when compared to initial value of gamma-sterilized specimens which was ca. 49000 Da (*Fig. 1*). pH of the buffer saline remained near 7.4 during the whole follow-up and there were no great differences between specimens and control solutions (*Fig.2*).

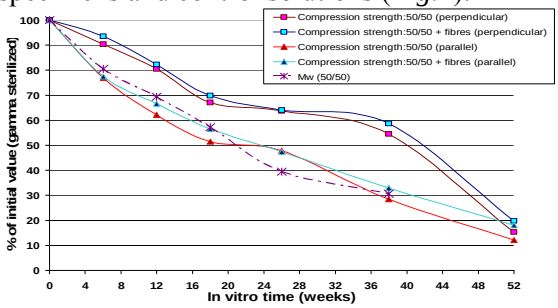

Fig. 1: Percentual compression strength retention and decrease of weight average molecular weight (Mw) in vitro (PBS).

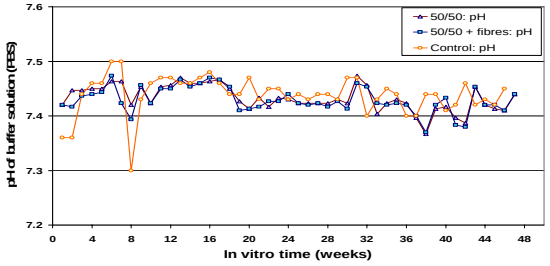

Fig. 1: The pH of the in vitro specimens and the control (PBS).

DISCUSSION & CONCLUSIONS: Compression strength retention *in vitro* followed the decrease of molecular weight and the pH of incubation media remained near 7.4 during the follow-up. Composites demonstrated anisotropic behaviour and they could retain ca. 50% of their initial compression strength for 20-40 weeks. Even after 52 week hydrolysis the weakest composites had compression strength of ca. 12 MPa which is comparable to that of cancellous bone (2-12 MPa[3]) and healthy intervertebral discs (11 MPa[4]). Therefore the studied composites may have applications as a material of spinal fusion devices.

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Potential Nuclear Replacement Materials: Developing a Screening Process

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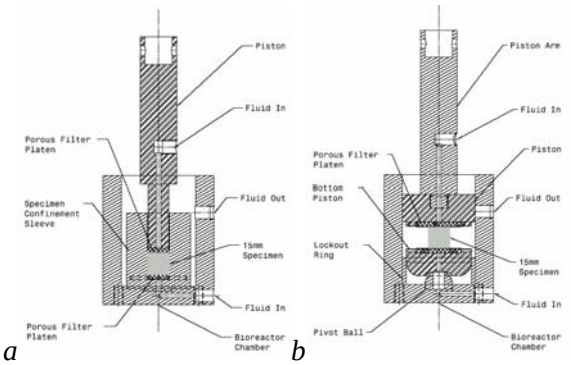
INTRODUCTION: Currently there is no established criterion for nucleus replacement materials. Initially non-cell-seeded materials are likely candidates for nuclear replacement in the near future. These suitable materials, then, need to restore under long-term nucleus swelling pressure, resist axial compression and must allow for considerable repetitive strain without mechanical failure. In this study a comparative on potential nuclear replacement materials and native nucleus tissue was conducted. The testing framework was derived from native nuclear material testing data, as well as, theoretical assumptions generated from general knowledge of spine biomechanics. The objective was to investigate potentially available biocompatible materials as nucleus replacements and to develop a material screening methodology for future available materials.

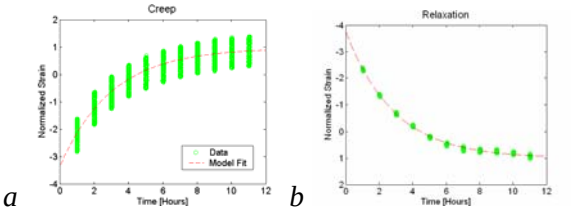
METHODS: A total of 100 specimen plugs (15 mm diameter, 15 mm height) were subjected to mechanical testing, with 5 repeated tests for each material. Screened materials include Polyvinylalcohol (PVA), Polyethyl- glycol (PEG), various Chitosan and bovine lumbar nucleus as the control. Two mechanical tests were conducted: confined and unconfined, to generate various mechanical properties of the material. Confined axial compression test (Fig.1a) alternating between 1 hour dynamic loading at 0.1-1.0MPa and 1 hour static loading at 0.1MPa (physiologic swelling pressure[1]) was used to determine creep and recovery rates. The loading, in an environment mimicking physiological conditions, was facilitated by our novel bioreactor developed to incorporate computer controlled dynamic axial loading while collecting mechanical load-displacement data. Permanent strain and time constants were calculated using a Kelvin model[2] curve fit (Fig. 2) on load-displacement data using the least-squares method. Unconfined axial loading tests (Fig.1b) at a 0.1%strain/sec rate was used to determine E-modulus, yield strength, elastic/plastic strain, and elastic recovery of the material.

RESULTS: Preliminary findings showed that native nucleus material behaved significantly different under confined and unconfined tests, and that, in comparison, most materials suffered large permanent strains under minimum physiological loads of 0.1Mpa.



a *b*

Fig. 1:Cross-sectional view of bioreactor chamber setup (a) for confined compression (b) for unconfined compression



a *b*

Fig. 2: Sample of normalized strain versus time data fit to the model equation of the (a) creep cycle and (b) relaxation cycle data using a least squares method. The data is shown by the green dots and the model fit is indicated by the dashed red line.

DISCUSSION & CONCLUSIONS: The material testing framework established through this study should be helpful for future material screening, but will also allow for cell-seeded materials, in a real tissue engineering setting, to be tested similarly.

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Role of endplates in contributing to compression behaviors of motion segments and intervertebral discs

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INTRODUCTION:
Intervertebral disc explants are commonly used to investigate the mechanics and mechano-biology of the disc. This study tested the hypotheses: 1) the mechanical behaviors of the intervertebral disc tested as explants are similar to those in the motion segments provided that endplate boundary conditions are similar, and 2) axial compression loading on motion segments will lead to non-recoverable damage in both the vertebral endplate and intervertebral disc. Rat caudal disc explants and motion segments were used as this is a common model for applying compression loading to the intervertebral discs in vivo, e.g., [1].

METHODS: Motion-segments (vertebra-disc-vertebra), disc explants, and single vertebrae were isolated from adjacent caudal levels of Sprague-Dawley rats (n=11). Motion-segments were harvested, potted in cyanoacrylate and tested with specially designed grips; disc explants were isolated using a scalpel and tested on rigid, porous platens; single vertebrae were potted and tested against rigid porous platens. All specimens underwent a force-controlled test protocol in PBS with protease-inhibitors and the following loading-stages: equilibration (0.5N-4hrs), 0.2MPa creep (4hrs), recovery 1(0.5N-6hrs), 1.0Mpa creep (4hrs), recovery 2(0.5N-6hrs). Elastic properties were determined from load, displacement and time data, while viscoelastic parameters were determined by curve-fitting to a stretched-exponential model.

A second series of experiments was performed to asses the influence of boundary conditions on transient behaviors of disc explants by modifying platen permeability from 3.2x10-10 to approximately zero while maintaining surface roughness (~20 um pores).

RESULTS: At 1.0MPa loading, deformations were similar in vertebrae and discs (Fig. 1). After accounting for vertebral deformations, disc deformations in the motion segments were not significantly different from explants ($p=0.4$). Time constants (as determined from stretched-exponential model) were significantly ($p<0.01$) larger for motion segments (τ=496±241s) than explants (τ=87±40s).

Permanent deformations in the motion segment and explant following the 1 MPa loading cycle and recovery were 0.6±0.6mm and 0.08±0.04 mm, respectively. Therefore, the largest permanent deformations were in the vertebrae, yet both measurements represented <10% permanent loss of height in the motion segment complex and explant, respectively.

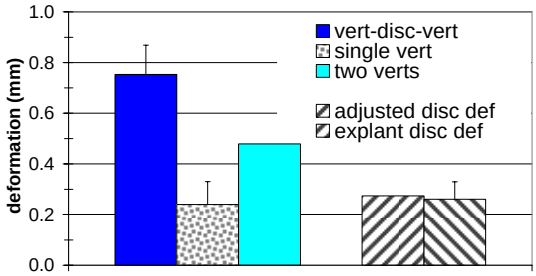

Fig. 1: Equilibrium deformations at 1 MPa for motion segment (vert-disc-vert), and 1 or 2 vertebrae (vert). Motion segment deformation was adjusted to arrive at disc deformation and compared with explant deformations.

DISCUSSION & CONCLUSIONS: Motion segment testing preserves in situ conditions most closely, however, disc explant testing provides maximum control over disc boundary conditions. Our results suggested disruption of the collagenous-network between the disc and vertebral endplate had minimal impact on disc equilibrium deformations compared to motion segments provided that vertebral deformations were accounted for. Differences in endplate boundary conditions between explants and motion segments had large impacts on transient behaviors with implications for pressurization and transport. Ongoing studies are investigating the influence of permeability and surface roughness on the equilibrium and transient behaviors of explants.

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The biomechanical effects of electrical stimulation on degenerative-like changes intervertebral disc

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INTRODUCTION: Low back pain (LBP) is associated with intervertebral disc (IVD) degeneration. Electrical stimulation (ES) has been found to be effective in relieving LBP, but the effects of the ES on degenerative changes of the IVD are not clear. Thus, the aim of this study is to investigate the biomechanical effects of ES on IVDs with degenerative-like changes induced via static compression.

METHODS: 27 male adult Sprague-Dawley rats (3-4 months old) were used. Two stainless steel pins were inserted into 8th and 9th caudal vertebrae of the rat. After three days rest, they were randomly divided into 3 groups (sham, control and ES groups). The sham group was allowed to rest without external loading while static compression of 11N was applied for one hour daily via the inserted pins to the caudal 8-9 discs in the control and ES groups [1] between days 4 and 17. ES was then applied to the rats in the ES group for 3 weeks between days 17 and 38 via two acupuncture needles inserted at the 8-9 disc level and an electro-stimulation device (IC-4107, Ito Co., Tokyo, Japan). The ES intensity was adjusted until visible muscle contraction was observed [2], and the ES frequency was 100Hz. Three 20-minute sessions of ES were applied weekly once every two days [3]. *In vivo* biomechanical properties of range of motion (ROM), angular laxity and compliance were measured before (Day 3) and after compression (Day 17) and after ES application (Day 38).

RESULTS: The changes in ROM and angular laxity from day 3 are shown in figures 1 and 2, respectively. Both ROM and laxity decreased significantly between days 3 and 17 in all groups, but the decreases between days 17 and 38 were only significant in the control group. No significant changes in ROM and laxity were found in ES group from day 17 to 38. The angular compliance decreased throughout the experiment in all groups and no significant difference between groups was observed.

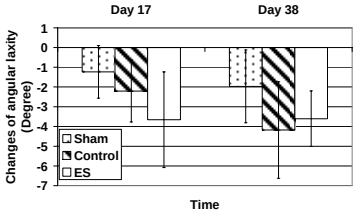

Fig. 1: The changes of angular laxity at days 17 and 38

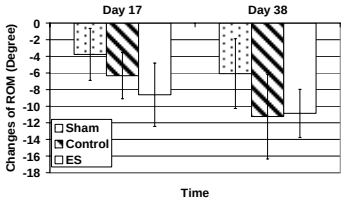

Fig. 2: The changes of ROM at days 17 and 38

DISCUSSION & CONCLUSIONS: Moderate daily static compression and ES application resulted in changes in the biomechanical properties of the IVD *in vivo*. The decrease in ROM is similar to the effect seen in human IVDs at the initial stages of disc degeneration. ES application has been found to induce biomechanical changes in the IVD, which can prevent further changes induced by the daily static compression.

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Lumbar Spine Fusion with a Novel Tantalum-Coated Carbon Fibre Cage Loaded with Colloss®

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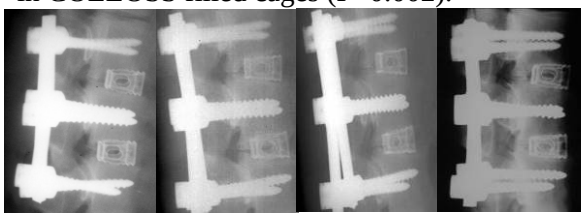
INTRODUCTION: Interbody spinal fusion has been performed increasingly in clinical practice, but failure to achieve a solid bony fusion occurs in 5% to 35% of the patients. Besides, implants of carbon fiber composite have been widely used in spinal applications. However, a series studies using carbon fiber reinforced cages showed a fibrous layer was constantly found interposing between the implant and the surrounding bone [1,2,3]. The aim of the present study was to tested whether the combination of a bovine bone protein extract-COLLOSS and a carbon fiber cage with a thin layer of biocompatible metal coating could improve the fusion results in a spinal fusion model

METHODS: 8 female Danish landrace pigs were operated, and lumbar spine interbody fusion of L3/4, L4/5 using tantalum coated C-C composite cages (Danfoss Bionics, Nordborg, Denmark) with pedicle screws fixation were performed on each pig. Cages packed with either autograft or a bovine bone collagen lyophylisate (COLLOSS®, OSSACUR AG, Oberstenfeld, Germany) were randomly assigned to the two levels anteriorly. Fusion was evaluated radiologically at 0, 4, 8, and 12 weeks post-op. All pigs were killed at 12 weeks and CT (after removal of pedicle screws), micro-CT and histology examinations were conducted

RESULTS: 7 pigs went through the observation without major complications.1 pig was excluded after 8 weeks due to implant related complications. Cages demonstrated good radio-transparency for serial evaluation of bone formation inside at follow-ups(Fig.1). With clinical CT evaluation, new bone formation could be clearly demonstrated inside the cage. Excellent biocompatibility was demonstrated by micro-CT images, in which bone in direct contact with the Ta-coated cages was abundant. Micro-CT evaluation showed that there were no differences of the bone volume fractions (BV/TV), surface densities (BS/BV) and trabecular thickness (Tb.Th) between the two graft materials. Only trabecular space (Tb.Sp) and trabecular number (Tb.N) had significant differences between them (P=0.02 and P=0.03 respectively). Histology sections demonstrated intimate contact of trabecular bone to the cage (Fig.2).Histomorphometrical comparison shown that only cartilage volume was slightly higher in COLLOSS filled cages (P=0.002).



0 week 4 weeks 8 weeks 12 weeks

Fig.1 Serial radiographic examinations of the same pig demonstrate the radiotransparent

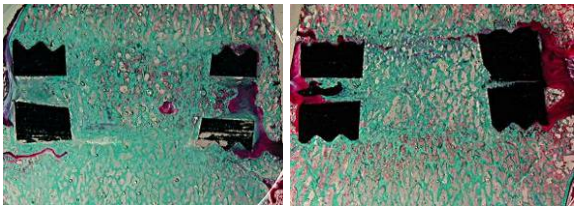


Fig. 2: Histological sections show the good bone-implant contacts and complete fusion with both Colloss(right) and autograft (left) .

DISCUSSION & CONCLUSIONS: The present study demonstrates when a thin layer of tantalum was coated on top of the carbon material; excellent interface binding was seen from both Micro-CT and histology. The bone formation can be followed with serial radiographs, while the thin Ta coating can serve as a marker and also an enhancement for bone anchorage. The bovine bone protein lyophilisate—COLLOSS achieved the same bone formation with that of autograft in this model after 12 weeks.

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ACKNOWLEDGEMENTS: This project was supported by the Danfoss Bionics A/S, Denmark; OSSACUR AG, Germany; and Nanoscience and Biocompatibility grant by Danish Research Agency

Design Considerations for a Multi-DOF Kinematic Spine Simulator

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INTRODUCTION: The kinematic analysis of the spine remains the subject of dialogue among researchers because of its complex biomechanics. As the industry for spinal devices and therapies continues to grow there is an increased need to accurately characterize the response of the spine.

A 6 Degree-of-Freedom, 8 controlled-axes Kinematic Spine Simulator has been designed to evaluate the functional kinematics of the spine under various simulated conditions. The simulator combines axial loading and rotation along with flexion/extension, lateral bending, and anterior-posterior and left-right translations. Typical applications for the Kinematic Spine Simulator include, but are not limited to, research of spinal fixation methods, interbody fusion, intervertebral disc research, and general spine biomechanics research.

METHODS: The Spine Simulator utilizes a servo-pneumatic axial/torsion testing system as its base platform and utilizes a combination of translational and rotational actuators with special restraint fixtures. Up to 8 actuators can be configured within the system and 24 channels of data are acquired. Each axis is independently controlled and incorporates independent measurement capabilities (including loads, moments, torques, and rotations). Table 1 describes the simulator forces and motions.

Table 1. 6 DOF, 8-Axes Kinematic Spine Simulator Forces and Motions

	Load	Motion
Axial	±5.6 kN	±50 mm
Rotation	±74 Nm	±50°
Flex./Ext.	±15 Nm	+120°/-60°
Lat. Bend	± 15 Nm	±60°
Ant./Post.	±1000 N	±50 mm
Left/Right	±1000 N	±50 mm

RESULTS: Figure 1 shows representative test data and includes the forces, torques, displacements, and rotations associated with axial loading and rotation, A-P and left-right translation, flexion-extension, and lateral bending. A unique feature of the simulator is its ability to provide pure bending in both flexion/extension as well as lateral bending. Figure 2 is actual testing performed under this pure bending mode.

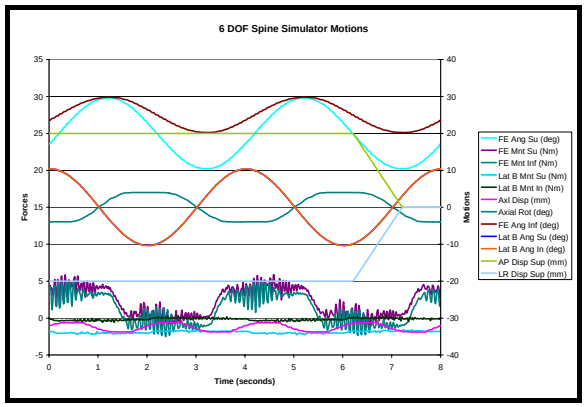

Fig. 1: Motion and Force plot from the Kinematic Spine Simulator.

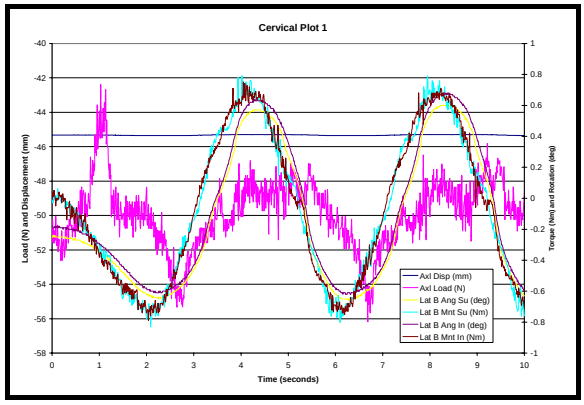

Fig. 2: 50 N Compression on a cervical spine segment with ±1.5 Nm of lateral bending.

DISCUSSION & CONCLUSIONS: The 6 DOF Full Spine simulator was designed to allow the researcher to perform a variety of tests related to spine kinematics. The development goal was to provide a system configuration that was suitable for quasi-dynamic simulation of typical spine kinematics and representative load bearing activities.

Various characteristics of the spinal components (single or multiple FSU) can be measured. These measurements can be useful for population of stiffness matrices indicating the spinal response to multiple loading conditions. This information can be useful in comparison between normal physiologic response and response after spine alteration, e.g. fusion and implants.

ACKNOWLEDGMENTS: We would like to acknowledge Mary Bilancini from the Institute for Spinal Disorders at the Cedars-Sinai Medical Center for her contributions.

Scaffolds for intervertebral disc tissue engineering created by flock technology

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INTRODUCTION: Flock technology is a method which has been used in textile technology for a long time but was not applied in the field of biomaterials until now. Flocking offers the possibility to create scaffolds with a high compressive strength despite of high porosity.
In this project we are creating a new type of scaffold for intervertebral disc tissue engineering using this technology[1]. Therefore the conventionally used materials have to be replaced by biocompatible and biodegradable ones which have to be electrically conductive to enable flocking.

METHODS: Principle of flock technology:
Flock technology is an old textile technology which is used to produce velvet like surfaces.
Flocking means to apply short fibres (0.3-5 mm) to a substrate which is covered with adhesive. The fibres are aligned in an electrostatical field and accelerated versus the adhesive covered substrate. Reaching the adhesive the fibres become stuck perpendicular to the substrate and give an uniform flock coating.

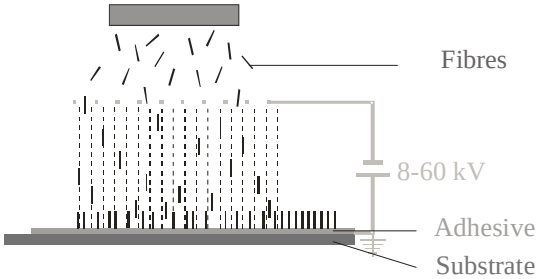

Fig. 1: Principle of the flock process

Materials: For flocking a Maag RF 400/500 Flocking Machine (Maag Flockmaschinen GmbH, Gomaringen, Germany) was used. Membranes made of crosslinked and mineralised collagen I were used[2] as substrate. Gelatin from porcine skin (BioChemika, Fluka, Chemie GmbH, Buchs, Switzerland) was used in a 10% solution as adhesive and as a model for Poly(3-hydroxybutyrate) (PHB)-fibres we used Polyamide (PA) -fibres with a diameter of ca. 30 µm. After flocking the scaffolds were chemically crosslinked with 1% EDC and freeze-dried.

RESULTS: The generation of new biodegradable scaffolds using flock technology was possible. The mineralised collagen I tapes are suitable as substrate and gelatin is a possible adhesive for the flocking process. After chemical crosslinking of the gelatin the scaffolds were stable under cell culture conditions.

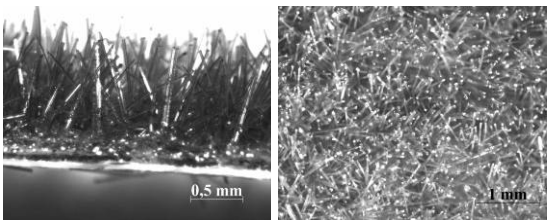

Fig. 2:Pictures of the scaffold (left: cross section; right: top view)

DISCUSSION & CONCLUSIONS: It could be shown that flock technology is a suitable method to create scaffolds for intervertebral disc tissue engineering. In further experiments the scaffolds will be characterized mechanically and seeded with chondrocytes and hMSCs for cell culture for several periods of time.
With this technology a variety of different scaffolds for tissue engineering can be produced using other substrates (like chitosan or PHB foils) adhesives (like chitosan, starch or hyaluronic acid) and fibres (like PLA or PGA), respectively. An optimisation of the scaffolds for application in different types of tissues should be possible.

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ACKNOWLEDGEMENTS: We thank the Deutsche Forschungsgemeinschaft for financial support (DFG-Paketantrag PO 392/27-1).

Influence of Cement Augmentation on Intradiscal Pressure: A Finite Element Study

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INTRODUCTION: Vertebroplasty and kyphoplasty are frequently used for internal stabilisation of a fractured vertebral body. Infiltration of bone cement (PMMA) into the vertebral body increases its stiffness significantly. Fractures in the adjacent vertebrae after vertebroplasty or kyphoplasty do occur occasionally. Thus cement augmentation is performed in some cases prophylactical.
The aim of the study was to determine the effects of volume and elastic modulus of PMMA and of outer loads on intradiscal pressure under physiological load that includes muscle forces.

METHODS: Using an osseoligamentous finite element model of the lumbar spine the volume and elastic modulus of PMMA inserted in the L3 vertebra were varied and the dependency on intradiscal pressure was determined. Two wedge-shaped fractures of the L3 vertebra were simulated. For the vertebroplasty (kyphoplasty) model an anterior height reduction of 35% (10%) related to the intact one was assumed. In order to simulate 'standing', the models were loaded with the upper body weight, a follower load [1] and a case-dependent force in the m. erector spinae [2]. The elastic modulus of PMMA was varied between 1000 MPa and 3000 MPa and the volume between 4 mL and 10 mL.

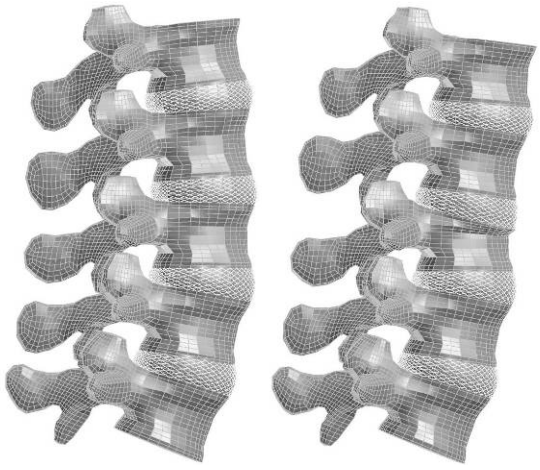

Fig. 1: Finite element model of the intact spine (left) and the fractured spine (right).

RESULTS: A wedge-shaped fracture of a vertebral body shifts the centre of gravity of the upper body anteriorly. This increases the flexion bending moment and thus the force in the m. erector spinae necessary for balancing the spine. Without compensation of the upper-body shift, intradiscal pressure in the discs adjacent to the fractured vertebra would increase by about 60% (20%) for vertebroplasty (kyphoplasty) compared to the intact lumbar spine. But even with shift compensation disc pressure is about 20% (7.5%) higher than normal.
Augmentation of the fractured vertebral body with bone cement has a much smaller effect on intradiscal pressure. The increase in that case is only about 2.4% for the intact vertebra as well as for the fractured. The effects of volume and elastic modulus of bone cement on intradiscal pressure are even smaller (1.3% and 0.2%, respectively) and thus negligible.

DISCUSSION & CONCLUSIONS: The effect of upper-body shift after a wedge-shaped vertebral-body fracture on intradiscal pressure and thus on spinal load is much more pronounced than that of stiffness increase due to cement injection. Our results do not suggest that vertebral-body fractures in the adjacent vertebrae after vertebroplasty or kyphoplasty are caused by the higher stiffness of the treated vertebra but by the anterior shift of the upper body. From the mechanical point of view a kyphoplasty is more advantageous than a vertebroplasty, especially for patients with osteoporotic vertebrae.

REFERENCES: [1] A.G. Patwardhan, R.M. Havey, K.P. Maede, et al (1999) Spine 24, 1003-1009. [2] T. Zander, A. Rohlmann, J. Calisse, et al (2001) Clin. Biomechanics 16, S73-S80.

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Effect of Disc Degeneration on the Mechanical Behavior of a Lumbar Functional Spinal Unit

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INTRODUCTION: Intervertebral discs provide flexibility of the spine and transmit and distribute large loads. In a degenerated disc the compressibility of the nucleus pulposus is increased and the disc height is reduced compared to a healthy disc. It is not fully understood how this affects the mechanical behavior of a functional spinal unit.
The aims of the study were to develop a finite element model of a lumbar motion segment which allows the simulation of different degeneration grades and to investigate the effect of disc degeneration on the mechanical behaviour of a functional spinal unit.

METHODS: A three-dimensional, nonlinear finite element model of the functional spinal unit L3/L4 was created (Fig. 1). Volume and rebar elements were used for the annulus fibrosus. The rebar elements represent the fibres. The nucleus was modelled as a fluid-filled cavity. All seven ligaments were integrated in the model and represented by tension-only spring elements with non-linear material properties. Besides a healthy disc, three different grades of disc degeneration (mild, moderate, and severe) were studied. Compared to a healthy disc their height was reduced 20%, 40% and 60%, respectively. With increasing disc degeneration the fibres and most ligaments buckle. The change in element length due to reduced disc height was compensated by offsetting their nonlinear stiffness curves. The compressibility of the nucleus was linearly increased from 0.0005 mm²/N (healthy disc, like water) to 0.15 mm²/N

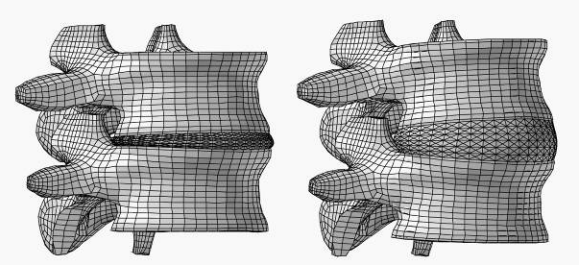

Fig. 1: Element meshes of a motion segment with a severely degenerated (left) and a healthy (right) disc.

(severely degenerated, like annulus fibrosus). Pure moments of 10 Nm were successively applied to simulate flexion, extension, lateral bending and axial rotation.

RESULTS: The finite element model predicts the same trends for intersegmental rotation and intradiscal pressure as obtained by others in *in vitro* studies [1, 2]. A mildly degenerated disc increases intersegmental rotation for all loading cases studied. With further increasing disc degeneration intersegmental rotation is decreased. There is a strongly non-linear relationship between intersegmental rotation and applied moment. With increasing disc degeneration the curves become more linear. The change of intradiscal pressure during loading is lower in a degenerated disc than in a healthy one. For axial rotation, the force in the facet joint increase with increasing disc degeneration. For extension and lateral bending, facet joint forces are higher for a mildly than for a moderately or severely degenerated disc. The maximum von Mises stress in the ground substance of the annulus increases with increasing disc degeneration for all loading cases studied.

DISCUSSION & CONCLUSIONS: The created finite element model simulates the global mechanical behavior of a degenerated disc very well. The predicted trends agree with in vitro measurements on cadaver specimens. Integration in a multisegmental finite element model of the spine will allow to study, for example, the effect of a degenerated disc on the adjacent segments.

REFERENCES: [1] D.S. McNally and M.A. Adams, (1992) *Spine* 17, 66-73. [2] M. Mimura, M.M. Panjabi, T.R. Oxland, et al (1994) *Spine* 19, 1371-1380.

ACKNOWLEDGEMENTS: Funding for this study was obtained from the Deutsche Forschungsgemeinschaft (Ro 581/16-1).

Notch 1 expression as a sign for proliferation in Anulus Fibrosus after trauma of the cervical spine? An histological, immunhistological and ultrastructural study.

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INTRODUCTION: We examined 90 samples from patients with lower cervical spine injury using histological, immunohistological and ultrastructural techniques to determine the effect of trauma on cell-signalling molecules.

METHODS: Anulus fibrosus (AF) from 30 patients (17y-78y) were removed during treatment for spinal trauma and fixed immediately. Fracture type according to Magerl`s classification was recorded. Samples were fixed for routine histology and immunocytochemistry in paraformaldehyde and embedded in Paraplast. Sections were stained with haematoxylin-eosin and immunolabelled with goat anti-human Notch 1(C-20), rabbit anti-human Notch 2 (25-255), rabbit anti-mouse Notch 3 (M-134), rabbit anti-human Notch 4 (H-225) (Santa Cruz Biotechnology), α-smooth muscle actin (Sigma) and PCNA (Sigma) in PBS for 1 hour and localised with appropriate fluorescent secondary antibodies. For TEM, tissue samples were fixed and processed using standard TEM procedures.

RESULTS: Histologically all samples contained cell clusters in the outer AF. Vessel invasion was apparent 26 days post trauma. Using TEM, damaged (necrotic) cells were prominent in the first week post trauma but after this time normal ultrastructure of disc cells were visible. No differences could be noted according to age or sex of the patients. Immunhistological labelling showed twenty patients to be Notch 1 positive whilst five samples were PCNA-positive. Most of the Notch 1 positive ells were found in cell clusters in the outer AF. Only five patients stained positive for Notch 2. α-smooth muscle actin positive cells were only present in blood vessels in the outer AF.

DISCUSSION & CONCLUSIONS: Our results show that after trauma, cell clusters were apparent in the outer AF, however these cells did not label for PCNA or alpha smooth muscle actin as has previously been observed (Johnson et al 2001; Hastreiter et al 2001). Our results suggest that the cell proliferation events leading to cluster formation occur very quickly after trauma (< 21 days). The expression of members of the Notch family of cell signalling molecules in these clusters would suggest that after proliferation the cells begin to differentiate along different pathways.

We do not know at the moment, if neovascularication of the disc tissue is based on migrating endothelia cells and/or proliferation of endothelia cells. In TEM sections, cells with processes, similar to migratory cells, were present near capillaries. This observation suggests that some cells might migrate in the damaged disc tissue. Proliferation of disc cells leading to cluster formations and vessel ingrowths leading to granulation tissue seem to be part of a repair strategy of the traumatised disc.

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Short term observations on Bonit® coated surfaces in self cutting implants

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INTRODUCTION: The stabilization of fractures in osteoporotic bone remains a challenging problem. A new type of implant for spinal fixation was developed to enlarge implant/bone interface and improve the osseointegration in osteoporotic bone. The implant is a perforated hollow cylinder, coated with a resorbable brushite coating (Bonit®, Calcium / Phosphor 1/1.1, DOT GmbH, Germany). In an osteoporotic sheep model these implants were used for the stabilization of a spinal fusion[1]. The implant showed bone ingrowth as expected, but a thin cell layer surrounding the cylinder surface was also visible after 16 weeks in situ. This observation led to the question whether the coating might have provoked these cellular reactions.

METHODS: Each implant was inserted after pre-drilling with a biopsy drill (7.35/6.45 outer /inner diameter) into the distal part of vertebra L3 or into the proximal part of vertebra L5[1]. This was done in two animals and one of them was sacrificed immediately after the insertion of the first implant, the other two weeks after operation. From each animal an implant was collected and the bone removed. The implants were carefully washed, cleaned in an ultrasound bath and dried. Finally they were coated with a 10 nanometer Carbon layer and investigated in a light microscope (Axiotech, Zeiss, Germany) and in a scanning electron microscope (SEM, Hitachi S4100, Japan). The SEM was equipped with a secondary electron (SE) and a backscattered electron (BSE) detector. An energy dispersive X-ray analysis (EDX, Isis300, Oxford, UK) was also performed.

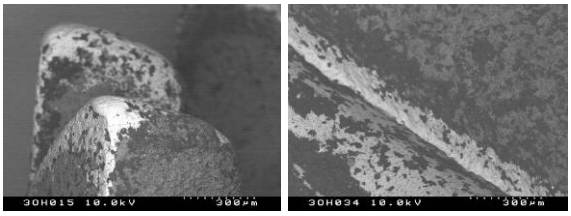

Fig. 1: BSE images of areas with worn off Bonit® coating on the outside of two teeth (left) and on a thread (right).

RESULTS: On both implants regions without Bonit® coating were found. The cutting edges of the implant (i.e. teeth, thread) exhibited region where the coating was almost completely worn off (Fig. 1) independently of the time in situ. In regions not involved in the cutting process (i.e. small cross-holes, Fig. 2) the resorption of the Bonit® was clearly detectable after two weeks. Here approximately 30% of the surface was devoid of any coating. The cylindrical part between the teeth and the thread had lost the coating only in a few places.
The EDX analysis confirmed Calcium and Phosphor in the coating and Titanium in the substrate.

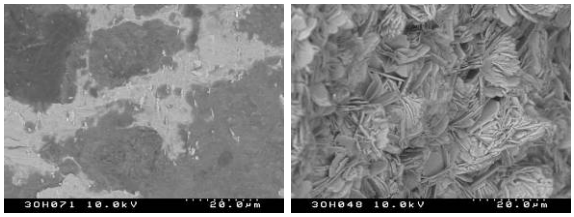

Fig. 2: Cross hole of an implant. After 2 weeks (left) most of the coating is resorbed (bright areas) compare to the original surface (right).

DISCUSSION & CONCLUSIONS: The present results suggest that the Bonit® coating is worn off in regions subjected to significant amounts of shear forces during implantation and possibly also during explantation. This especially includes the edges of the self cutting tread and the outer edges of the teeth. Here the implant obviously does not provide a design suitable for coating with Bonit®. According to Capello et al.[3] such critical parts of an implant need to be optimized for the type of coating used here.
The amount of resorption observed in regions shielded from shear forces is comparable to that reported by Szmuckler et al.[2] after 6 weeks in non osteoporotic pig jaw.

REFERENCES: [1]Kossmann T et al.(1999) *Orthopäde* 28:432-40. [2]Szmukler et al.(2000) *Proc. 13th Int. Symp. on Ceramics in Medicine*, Milan, 395-8. [3]Capello et al. (1998) *Clin. Orthop.* 355:200-11.

Comparison of experimental and numerical stress profiles in human disc show similar stress peaks why?

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INTRODUCTION: The disc is subjected to a combination of elastic, viscous and osmotic forces. The swelling tendency of the disc tissue and the tensile stresses in the collagen structure are highly interdependent [1].
Because experimental measurements of in vivo intradiscal stresses are difficult, different finite element approaches have been made. No published 3D finite element model of the disc includes osmotic prestressing. The purpose of this study is the comparison of experimental and numerical stress and pressure profiles in the human discs, which do not show a uniform pressure profile as assumed in a healthy human disc.

METHODS: In this study the fibril-reinforced poroviscoelastic swelling model of Wilson [2] was adapted. The disc model resembles one forth of a full disc consisting of 8952 3D 8-nodes elements and is described through a biphasic swelling model. The model distinguishes between an elastic non-fibrillar solid matrix, a 3D viscoelastic collagen fiber structure and osmotically pressurized fluid. For the non-fibrillar part a compressible neo-Hookean model was used. The swelling behavior of the disc was assumed to be solely due to osmotic swelling.
The stress in the disc is the sum of fiber stress, matrix stress and hydrostatic pressure. Simulations of the effects of changes in osmotic and axial mechanical load on hydrostatic pressure, osmolarity and disc shape were performed. Model predictions were compared to experimental results obtained from human cadaveric discs tested under similar loading conditions to those applied in these simulations [3].

RESULTS: Applying a linear increasing axial load of 500N raised the hydrostatic pressure to 0.33 MPa while an axial load of 1000N increased the pressure to almost 0.7 MPa. The model predicted the presence of stress peaks within the peripheral annulus that corresponded both in magnitude and width to those observed experimentally (fig 1). In the same way the centre of the disc (including some of the annulus elements) showed similar mechanical behaviour to the experimental results.

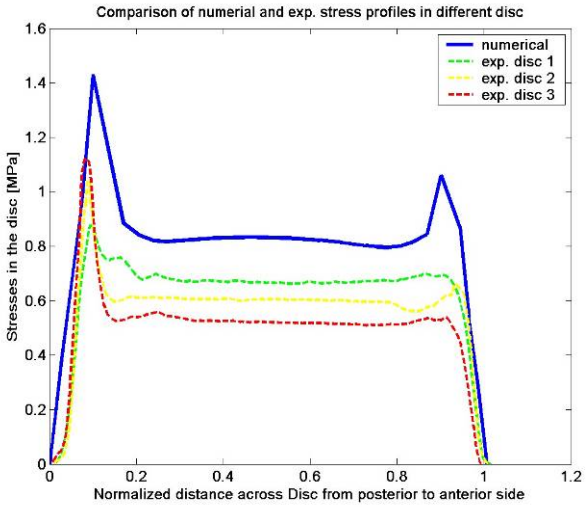

Fig. 1: *Comparison of experimental stress results with FEA predictions*

DISCUSSION & CONCLUSIONS: For the first time the intervertebral disc is modeled as a 3D osmotically pre-stressed fibril reinforced structure using finite deformation finite element analysis. The computed sum of the axial matrix stress and the hydrostatic pressure compares well with experimental results, as well as with intradiscal pressure measurements from the literature.

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ACKNOWLEDGEMENTS: This research is made possible through the support of the AOSpine and European Union (***EURODISC***, Project Number: QLRT-2001-02852).

TiO_2-coated Epoxy Replicas with Identical Surface Topography for Cell Culture Experiments

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[2]*AO Research Institute, Davos, Switzerland,* [3]*Institut Straumann AG, Basel, Switzerland*

INTRODUCTION: Surface topography has been shown to be one of the important surface characteristics affecting cell response[1]. In the past, diverse fabrication methods such as micro machining, plasma spraying, particle blasting and/or acid etching have been applied to fabricate stochastically rough micro- and nano-topographies[2]. However, there is a substantial need for cost-effective methods to produce large numbers of samples with identical surface topographies, without the need for specialized surface treatment such as blasting. Our concept of sample production is based on an epoxy replica technique using dental impression material[3]. The aim of this study was the investigation of surface roughness over several generations of epoxy replicas using standard surface characterization techniques.

METHODS: Masters of a rough (SLA; sand-blasted, large-grit, acid-etched; Institut Straumann AG) CP Ti disc were produced using dental impression material (vinyl polysiloxane). These samples served as negative replicas to cast epoxy resin. Cured epoxy substrates were coated with a 60 nanometer (nm) thick film of titanium oxide using reactive magnetron sputtering. Vinyl polysiloxane masters were cleaned and reused for the fabrication of further generations of epoxy replicas (up to a total of eight casts). Surface topography was characterized with White Light Confocal Microscopy and Atomic Force Microscopy (AFM). In the Scanning Electron Microscope (SEM), the same surface area was controlled over several generations. The chemical composition of the sputter-coated titanium oxide film was investigated with X-Ray Photoelectron Spectroscopy.

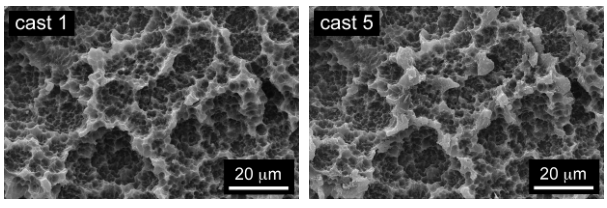

Fig. 1: Scanning electron micrographs of two different TiO_2-coated epoxy replicas made from SLA CP Ti disc. Left panel: first cast; right panel: fifth cast using the same vinyl polysiloxane master.

RESULTS: Roughness values R_a and R_t (measured with optical prolifometry) were the same within experimental uncertainty over all eight generations and in comparison to the original SLA CP Ti disc (Fig. 2).

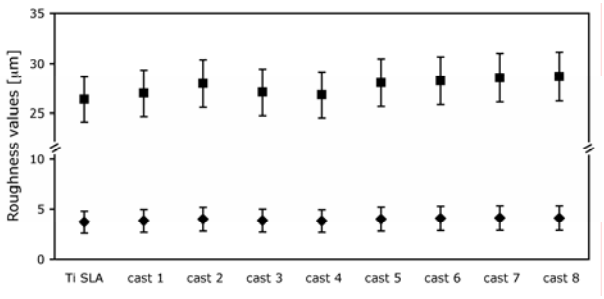

Fig. 2: Roughness values R_a (◆) and R_t (■) measured with White Light Confocal Microscopy (770 x 798 µm^2) of a CP Ti SLA disc and its eight generation replicas.

SEM investigations showed comparable topographies over all the eight consecutive casts. Only very rarely, some additional features were detected due to material transfer between casts. (Fig. 1, right panel). The chemical composition of the titanium oxide film showed pure TiO_2 (data not shown).

DISCUSSION & CONCLUSIONS: The epoxy replica technique is a simple and powerful tool to produce series of samples with essentially identical surface topographies. Such sample sets have the advantage of improving reproducibility and comparability in standard cell culture experiments. It is possible to use the same vinyl polysiloxane negative for the production of at least eight generations of epoxy replicas although the SLA surface used in this study is a highly complex 3-D topography with undercut features. The surface chemistry of epoxy substrates sputter-coated with TiO_2 is comparable to the native oxide film on the original SLA CP Ti surface.

REFERENCES: [1]Boyan *et al.*, in *Titanium in Medicine*, Springer-Verlag, p. 561-586, 2001. [2]Sykaras *et al.*, *Int J Oral Maxillofac Implants*, **15**: p. 675-690, 2000. [3]Wieland *et al.*, *J Biomed Mater Res*, **60**(3): p. 434-444, 2002.

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Effect of Osteoporosis on the Biomechanics of the Thoracolumbar Spine: Finite Element Study.

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INTRODUCTION: Osteoporosis is characterised by low bone mass and structural deterioration of bone tissue. This results in reduced bone strength and a high risk of fracture, especially in the aged population. Previous numerical investigations examined the biomechanical changes associated with osteoporosis but in many cases these analyses were limited to a single vertebral body or a single motion segment. [1, 2]. To date, there is still a lack of understanding of how osteoporosis impacts on the general spinal performance and the load transfer mechanism within. Therefore, the main goal of this study was to examine the effect of different bone densities on the biomechanical behaviour of an anatomically correct thoracolumbar spine. Using finite element analysis a range of spinal conditions including healthy bone (HB), osteopenic bone (OPN), moderate osteoporosis (OPR) and severe osteoporosis (SOPR) were modelled and compared.

METHODS: As shown in Fig. 1 a seven level (Th11-L5) spine model was created from a series of CT scans from a 63-year-old male cadaver and validated by the experimental methods [3].

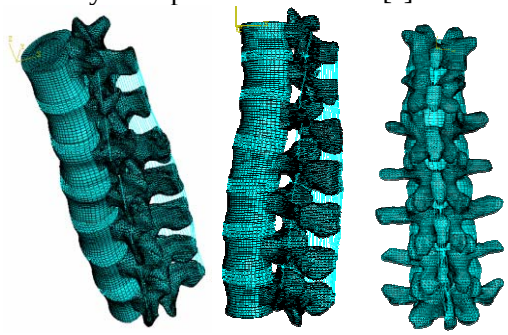

Fig. 1: 3D FE model of spine (Th11-L5).

Based upon data reported in the literature [4], different levels of bone tissue loss was simulated by changing the material properties of the cortical and trabecular bone in each segment. All models were subjected to a vertical compressive load of 750 N and a bending moment of 15 Nm to simulate flexion and extension. In this particular case the changes in the compressive stiffness and the displacement values in X, Y and Z-direction caused by the external load were examined.

RESULTS: As shown in Fig. 2, a drop in compressive stiffness was observed when osteoporosis and severe osteoporosis were simulated. By reducing the elastic properties of bone a decrease of 1, 9, and 12% was recorded for osteopenic, osteoporotic and severely osteoporotic spine, respectively.

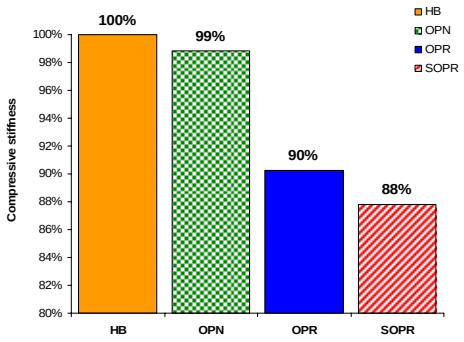

Fig. 2: Compressive stiffness calculated for all four FE models

In terms of displacement values, the behaviour of the healthy and the osteopenic spine were similar, however the displacement distribution changed significantly for the osteoporotic and severely osteoporotic spine. This suggests that the spine was less stable when the elastic properties of bone tissue were reduced, and additionally, unwanted motion occurred under a compressive load of 750 N.

DISCUSSION & CONCLUSIONS: Based on the FE model predictions, it can be seen that osteoporosis impacts significantly on the biomechanics of the thoracolumbar spine. The current FE analysis suggests that osteoporosis may lead to decreased compressive stiffness and reduced spinal stability, as a low bone density caused excessive movement between adjacent vertebrae. In the present case the "weakest link" in the spinal column was the Th12-L1 motion segment and this observation is in a good agreement with experimental data reported in the literature and clinical observations.

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Mechanical behavior of iv discs stabilizes in a loaded culture system

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INTRODUCTION: Fluid flow, into and from the intervertebral disc, plays a role in vivo in the mechanics of the disc. The regulating mechanism is the pursuit for balance between the external load on the spine and the osmotic pressure in the disc. During compression, fluid is pressed out of the disc whilst fluid flows back during rest. In vivo this leads to a stable pattern, in vitro however, earlier studies show no stabilization of mechanical behavior, even if the resting time exceeded the loading time [1,2]. In this study we investigate if the mechanical behavior of intervertebral discs moves towards a stable situation if the disc is exposed to a sustained cyclic loading in a culture system.

METHODS: four lumbar spines were harvested from 4-year-old goats. One disc (L3/L4) was tested within 2 hours after death and a second disc (L2/L3) was frozen (-20C) and tested 24 hours later. The disc was thawed before testing. Each disc was tested in a disc culture system for 24 hours. This period was divided into eight cycles: a loading phase (0.2-0.8Mpa, 0.5Hz sinusoidal, 2hours) and an unloading phase (0.2Mpa, static, 1hour). The samples were tested in a disc culture system flowed with medium (+10%FBS, antibiotics, 5%CO2). Load was applied through a hydraulic mechanical testing device (Instron 8872).

RESULTS: The loss of disc height during loading is compensated during rest after three full cycles (fig1 and fig2).
The increase of stiffness of the disc, due to fluid loss in the nucleus, stabilizes after four full cycles. The stiffness of the frozen disc is approximately 30% higher (fig3).

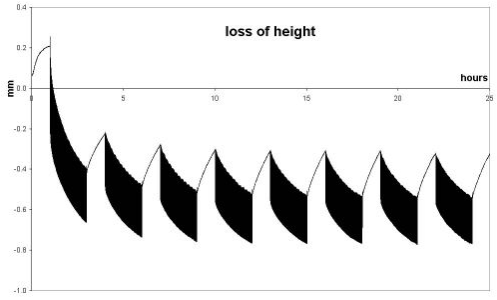

Fig. 1: Change of disc height (typical) in time.

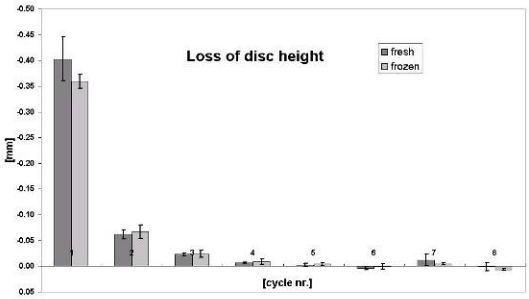

Fig. 2: Average loss of disc height per cycle.

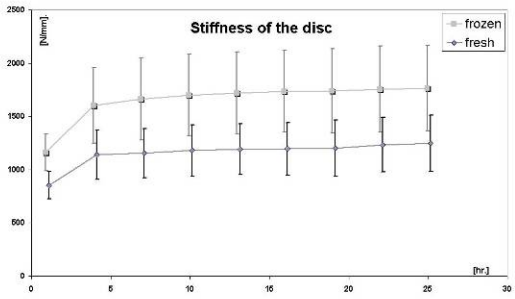

Fig.3: Change of stiffness in time.

DISCUSSION: The mechanical behavior of the disc stabilizes in four cycles but does not reach equilibrium during the loading or unloading phase. For a measurement of the mechanical properties, it is essential to cycle the system until this stable situation has been reached. The properties of the disc are then measured in its working range.

The shape of the displacement curve is not similar to curves known from the literature [3]. In vivo, the disc tends to equilibrium during the loading or unloading phase. This leads to a flattening of the displacement curve. We assume that in the present tests fluid flow in the endplate is hampered. This slows down fluid flow and equilibrium will not be reached.

Freezing of the samples appears to have influence on the stiffness of the disc but not on changes in stiffness or height. We presume that this is due to a changed stiffness of the annulus.

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An *in vivo* nutrient insufficiency induced ovine lumbar intervertebral disc degeneration model

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INTRODUCTION: Intervertebral disc degeneration is believed to play an important role in low back pain. The cells inside the disc rely on diffusion for nutrition and removal of waste products through the endplate[1]. Although correlation has been found between occlusion of endplate vascular openings and disc degeneration, causality has never been demonstrated in intact discs[2]. A model for nutrient insufficiency induced disc degeneration is being developed. In this study, we determine if the chosen method of blocking the major nutritional route results in inhibited perfusion and solute transport to the disc.

METHODS: Four sheep will be anaesthetized and the anterior lumbar spine exposed. A ~1cm wide thin slot will be sawn into the vertebrae parallel and adjacent to the endplates overlying the nuclear region of the L2-3 and L4-5 discs. After Ti-foils are inserted into the slots, the inhalation gas mixture will be changed to 70% N_2O and 30% O_2. At 5 min intervals, intranuclear concentrations of O_2 and N_2O will be measured amperometrically. Post-mortem, the vertebral vasculature will be infused with Procion red and thick sections examined to quantify the density of patent endplate capillary buds.

RESULTS: One pilot sheep has been completed. The blocks in the L2-3 disc were made without problems, in L4-L5 disc this was more difficult, and locations of the block were only partially overlying the nucleus. The N_2O diffusion measurements showed a clear inhibition of transport with the diffusion block (Fig. 1). However, O_2 concentrations only significantly differed in the cranial blocked discs (Fig. 2). Although perfusion inhibition has not yet been quantified, sections demonstrated clear decrease in the dye filled endplate capillary buds overlying regions of the blocked endplate.

DISCUSSION & CONCLUSIONS: In this study N_2O is used as a tracer for nutrient diffusion into the disc. It has been shown that it is possible to partially block the diffusion of N_2O into the disc. The defect was less efficient in blocking O_2. This could be because of differences in defects or a different diffusion rate of O_2. This model will be used to test causality between a diffusion block and disc degeneration in a longer term study.

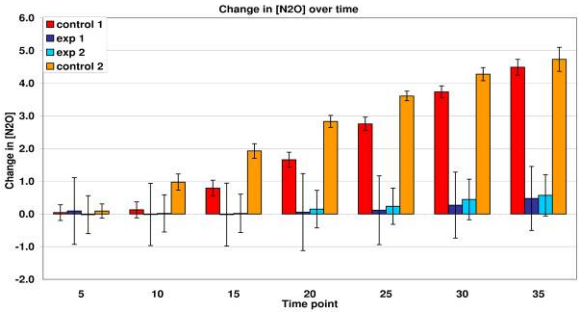

Figure 1. Increase in N_20 concentration over time relative to time 0.

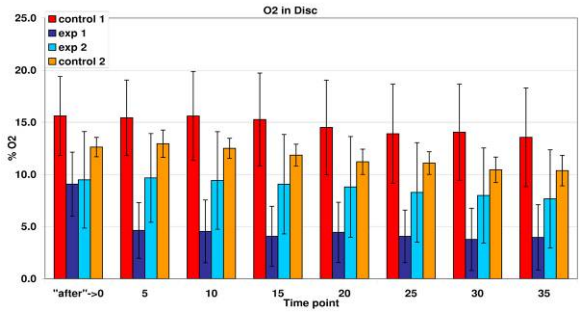

Figure 2. O_2 concentration in the discs.

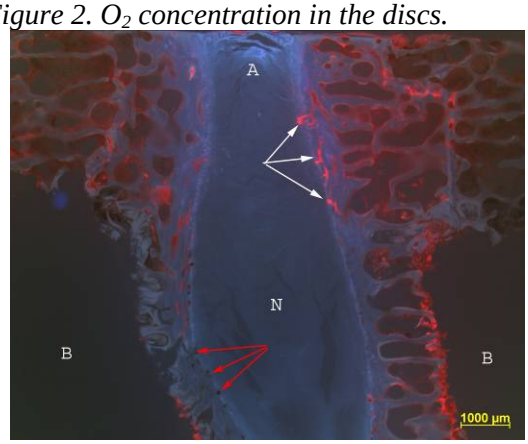

Figure 3. Procion red filled endplate capillary buds(white arrows) and non-filled bud (red arrows. (A=Annulus Fibrosus, N=Nucleus Pulposus, B=Block.)

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Mathematical modeling of the intervertebral disc nutritional transport

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INTRODUCTION: As there is no blood supply to the main body of the human intervertebral disc (IVD), the disc cells rely on diffusion and convection for the transport of nutrients and wastes. Difficulties associated with existing experimental techniques render mathematical modeling an attractive method for investigating the mechanical environment of the IVD and predicting nutrient transport. Commercial software package such as ABAQUS have been used to simulate nutrient transport under steady state [3] and dynamic [1] conditions. In this paper, we generalize the theoretical model [2] to include nutritional transport mechanism.

METHODS: We use the theory of mixture to develop a two phase mathematical model incorporating strain related osmotic swelling pressure and permeability, together with nutrients transport with convection and diffusion mechanism. The governing equations are:

Balance of mass

$$\frac{\partial \phi}{\partial t} + \nabla \cdot (\phi \frac{\partial U}{\partial t}) = 0 \qquad \text{Fluid phase}$$

$$\frac{\partial (1-\phi)}{\partial t} + \nabla \cdot ((1-\phi) \frac{\partial u}{\partial t}) = 0 \qquad \text{Solid phase}$$

Balance of momentum

$$\nabla \cdot (\phi \sigma_f) + F_f = 0 \qquad \text{Fluid phase}$$

$$\nabla \cdot ((1-\phi)\sigma_s) + F_s = 0 \qquad \text{Solid phase}$$

Constitutive laws

$$F_f = -F_s, F_f = P_f \nabla \cdot \phi + D1$$

$P_f \nabla \cdot \phi$ is interfacial force, $D1$ is a drag term:

Nutritional transport equation

$$\frac{\partial C}{\partial t} + \nabla \cdot \{C(\frac{\partial U}{\partial t} - \frac{\partial u}{\partial t})\} = \nabla \cdot (D \nabla C) - R(C)$$

Where ϕ, U, u, C denote the porosity, fluid displacement, solid displacement and nutrient concentration.

RESULTS: Numerical simulation has been employed to reduced one-d case. A sample disc was under 0.8MPa loading by 20 minutes and 40 minutes recovery. The disc height change and nutrient transport have been calculated.

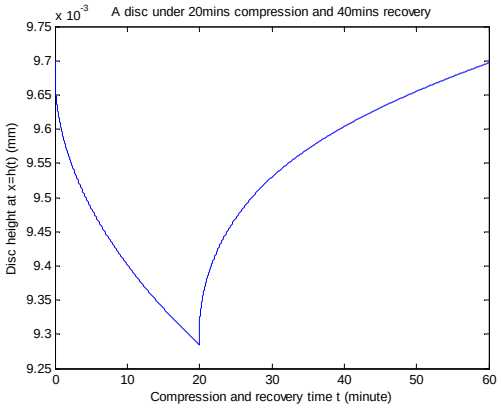

Fig. 1 The disc height change curve

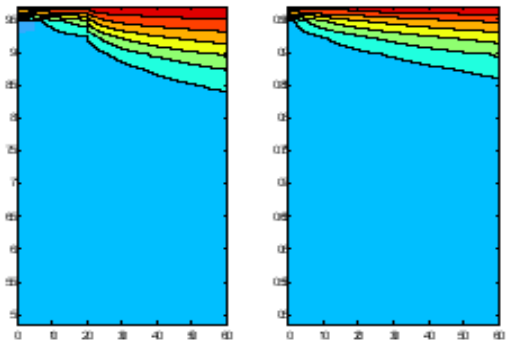

Fig. 2 Comparison of penetration between convection coupled nutrient diffusion and diffusion without fluid flow ($D=60\ \mu m^2/s$).

DISCUSSION & CONCLUSIONS: Our model agrees with the calculation in [1] that convection enhances the transport for large molecules significantly but has little effect for small molecules transport.

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Cells from the distinct regions of the intervertebral disc differ in terms of their mechanosensitivity

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INTRODUCTION: The intervertebral disc (IVD) as a spinal joint that shall allow movement and absorb impulsive load has to be regarded as a tissue of complex structure, expressed in its division into nucleus (N), transition zone (TZ) and annulus (A). These distinct regions differ not only in terms of their histological structure, constituent parts and cell types, but also in terms of their nutrient status, pH and representative mechanical load. As the three distinct zones of the IVD differ in so many respects, one may expect that the application of the two main mechanical loads, cyclic strain and hydrostatic pressure, will result in different cellular responses depending on the local origin of the cells.

METHODS: In order to determine if cellular responses to mechanical load are spatially-diverse, bovine disc cells (n=12) were isolated from the distinct regions (N, TZ, A) by collagenase-pronase digestion, expanded in monolayer and seeded in a three-dimensional collagen type-I gel. Cell-seeded gels were either stimulated by application of cyclic strain (1%, 2%, 4%, 8%; 1 Hz; 24 h) or by application of hydrostatic pressure (0.25 MPa; 0.1 Hz; 30 min) as previously described[1]. Load-induced changes in mRNA-expression of anabolic, catabolic and anti-catabolic genes relevant in the maintenance and degradation of the IVD-matrix were determined by real-time RT-PCR (collagen-I, collagen-II, aggrecan, MMP-2, MMP-3, TIMP-2). A Wilcoxon signed-rank Test was performed to check for differences between stimulated samples and control samples. Furthermore, a nonparametric Wilcoxon 2-sample Test was added to check for differences between the distinct regions of the IVD. Multiple testing was considered by adjusting the p-value to 0.008.

RESULTS:

Cyclic strain as well as hydrostatic pressure caused changes in gene expression in cells from each zone, but sign and extent differed. Nucleus and annulus as the gap-including zones showed deviant or even opposite responses in many cases. Effects of the transition zone mostly ranged in-between, confirming thus its intermediate position in the IVD. In nucleus cells, cyclic strain resulted in a significant increase of collagen-II ($p<0.001$), aggrecan ($p<0.001$) and MMP-2 ($p=0.002$) and there was a strong trend towards an increase of collagen-I ($p=0.009$). Additionally, cyclic strain tended to inhibit expression of MMP3 ($p=0.034$) and significantly inhibited expression of TIMP-2 ($p=0.001$) in nucleus cells. Effects tended to be contrariwise in annulus cells for collagen-I ($p=0.016$), collagen-II ($p=0.016$), MMP-2 ($p=0.031$) and TIMP-2 ($p=0.016$). Hydrostatic pressure tended to result in an inhibited expression of aggrecan, MMP-2, MMP-3 and TIMP-2 in nucleus cells but in an increased expression in annulus cells. Significant differences between N and A could be observed for collagen-I, collagen-II, MMP-2 and TIMP-2 (all $p<0.0007$) after the application of cyclic strain. Hydrostatic pressure tended to result in different effects between N and A for aggrecan, MMP-2 and MMP-3 (all $p<0.05$).

DISCUSSION & CONCLUSIONS: Findings indicate that cells from the three distinct regions of the IVD differ in their mechanosensitivity relating to cyclic strain and hydrostatic pressure. These regional differences concerning gene expression may be ascribed to intradiscal variations of the mechanical environment, cellular species, ECM and cellular morphology. Taking the results into account, subclassification of IVD cells is one of the primary preconditions for the better understanding of the biomechanical situation in the IVD.

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ACKNOWLEDGEMENTS: Supported by the European Union EURODISC (QLK6-CT-2002-02582)

Nickel Suppression in Ni-Ti Alloys by Plasma Immersion Ion Implantation Surface Treatment: New Materials For Orthopaedic Implantation

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INTRODUCTION: Nickel-titanium shape memory alloys (NiTi) are promising materials in orthopedic applications due to their unique properties. However, for prolonged use in a human body, deterioration of the corrosion resistance of the materials becomes a critical issue due to the increasing possibility of deleterious ions released from the substrate to living tissues. We have investigated the use of nitrogen, acetylene, and oxygen plasma immersion ion implantation (PIII) to improve the corrosion resistance and mechanical properties of the materials.

METHODS: Circular NiTi discs with 50.8% Ni were implanted with nitrogen, oxygen and carbon using plasma immersion ion (PIII) implantation technique. The elemental depth profile and surface chemical composition of the PIII treated samples were determined by X-ray photoelectron spectroscopy. The surface hardness and corrosion resistance properties of all samples were measured. The solutions taken from each sample after the corrosion test were analyzed for Ni and Ti concentrations using inductively coupled plasma mass spectroscopy. To investigate the cyto-compatibility of the samples, mouse osteoblasts expressed an enhanced green fluorescent protein (EGFP) were used in cell culturing. Cell proliferation was examined after examined after 2, 4, 6 and 8 days of culture.

RESULTS: After PIII treatment TiN, TiO and TiC are formed on the surface layer of each specimen after treatment. When compared with untreated NiTi, the corrosion resistance was five-fold higher, and the surface hardness and elastic modulus were 2-fold higher (Table 1). The concentration of Ni in the simulated body fluid for the untreated sample was 30ppm compared to undetectable levels in the PIII treated sample. There was no difference in the ability of cells to grow on either surface (Figure 1).

Table 1. Young's modulus and hardness of control and the treated samples surfaces

Sample	NiTi	Nitrogen implanted	Carbon implanted	Oxygen implanted
Young's modulus (GPa)	57	150 – 65	110 – 70	150 – 55
Hardness (GPa)	4.5	11 – 5	9.5 – 4.5	9 – 3.5

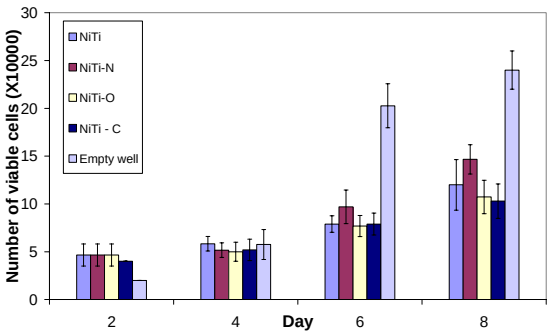

Figure 1. Cell proliferation versus number of days.

DISCUSSION & CONCLUSIONS: A number of surface modification schemes[1,2] have been studied to enhance the corrosion and wear resistance of NiTi alloys. However, PIII treatment is a method of nano-scale surface modification and not a coating, as such and unlike coatings, it does not de-laminate from the substrate. With enhanced corrosion and wear resistance, and negligible Ni release, PIII technology will allow NiTi alloys to be safely implanted in the human body. A new generation of "smart" orthopaedic implants will likely result.

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Corresponding author

Effect of TGF-β and BMP-2 on rat mesenchymal stem cell differentiation into disc-like cells *in vitro*

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INTRODUCTION: Current treatment for degenerated discs is limited and alternative methods have to be found. Mesenchymal stem cells (MSCs), differentiated into cartilage-like disc cells are a promising candidate for inducing intervertebral disc regeneration and need further development in animal models [1, 2]. *In vitro* protocols to induce differentiation into proteoglycan (PG) producing cells have been established for human MSCs using TGF-β [3], but TGF-β alone does not induce chondrogenesis in rat periosteal cells [4]. Therefore, we hypothesized that rat MSCs would differentiate into cartilage-like cells with a combination of TGF-β and BMP-2.

METHODS: MSCs were harvested from the bone marrow of 13 month old Wistar rats. Cells were expanded to the third passage and seeded at a density of 10 Mio/ml into a Fibrin carrier (45 mg Fibrinogen and 1 IU Thrombin/ ml; provided by Baxter). Carriers were disc shaped with a diameter of 5 mm and a height of 2 mm. They were cultured in the presence of either α-MEM and 1% ITS+ (control) or with additional dexamethasone, proline, ascorbic acid and TGF-β or BMP-2, or both (experimental groups). Samples were harvested at day 1, day 14 and day 21 and analysed for PG content spectrophotometrically (normalized to DNA content) and histologically (cryostat sections, Toluidine blue staining). RT PCR for collagen I, II and X, aggrecan, SOX-9 and osteocalcin was performed. All data was normalized to day 1. In case of a sufficiently high 18s value (housekeeping gene), but no detectable gene specific mRNA, the maximum number of cycles was taken for the analysis.

RESULTS: Spectrophotometrically undetectable to very low amounts of PG were found in the control groups. In all experimental groups similar amounts were found (0.039 to 0.070 µg PG per µg DNA). Histological sections showed faint metachromasia around cells (not shown). Collagen I was slightly upregulated on days 14 and 21 relative to day 1 in all groups (up to 21 fold). Collagen II and aggrecan were only slightly upregulated in the control and BMP-2 group (less than 15 fold), but both genes were clearly upregulated in experimental groups with TGF-β alone or in combination with BMP-2. In both groups values were highest at day 21. There was a synergistic effect of BMP-2 versus TGF-β alone (figure 1). Results from collagen X, SOX-9 and osteocalcin are pending.

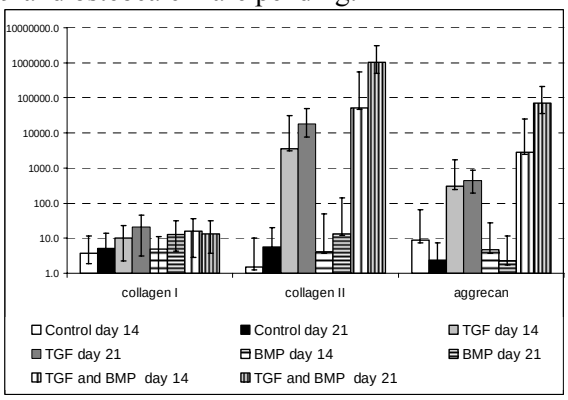

Fig. 1: Gene expression relative to control day 1. (Avg ± SD; n=4).

DISCUSSION: In contrast to the results of Hanada et al [4] TGF-β alone did induce the expression of collagen II in rat MSCs, but BMP-2 did not. Hence, rat MSCs behave more like human MSC than periosteal cells. Expression of collagen X and osteocalcin will determine, if the combination of both growth factor influences the amount and the kind of gene expression. Although we have a clear upregulation of the relevant genes our detection of PG was limited. In aggregates of human MSCs, 21 days have been shown to be sufficient to induce detectable amounts of PG [3]. However, this period may be too short for cells embedded within a fibrin carrier at the density we have used. We showed that TGF-β and BMP-2 have different effects upon gene expression of rat MSCs and that the combination of both is synergistic. Future studies will evaluate the interaction between these biological factors with mechanical ones.

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