Ex vivo evaluation of a 2nd generation Nucleus Pulposus Prosthesis (NPP II) and its ability to restore axial disc height in canine cadaveric specimens

Research Project Veterinary Medicine University Utrecht
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1. Abstract

The effect of a hydrogel nucleus pulposus prosthesis (NPP), made of N-vinyl-2-pyrrollidinone copolymerized with 2-(4'-iodobenzoyl)-oxo-ethyl methacrylate, on intervertebral disc (IVD) height was measured, as well as the assessment of the visibility on radiography, the extent of swelling and number of prostheses remaining intact and *in situ* after incubation. Both hydrophilic and hydrophobic NPPs were used, in a lumbar and lumbosacral size. The canine cadaveric specimens were radiographed in native state, after nucleotomy and after implantation and swelling of the NPP. The NPPs were weighted and measured before insertion as a xerogel and allowed to swell overnight at 37°C after insertion. After swelling the NPP was macroscopically evaluated *in situ* by dissection of the IVDs. Thereafter, the prostheses were weighted and measured again. Disc height was measured on radiography by two independent observers, using three different methods, where every method was measured twice. All methods were evaluated on inter- and intraobserver reliability, practical usefulness and restoration of disc height and compared with each other.

A restoration of disc height was found during measuring of the radiographs. Also, radiographs revealed intrinsic radiopacity of both hydrophilic and hydrophobic prostheses. Hydrophilic prostheses have a significant greater swelling ability compared to hydrophobic prostheses. Six out of eight (6/8) lumbar hydrophilic NPPs remained intact and *in situ* after overnight incubation, where 2 of them showed fragmentation. The fragmented NPPs were all migrated through the annulus fibrosus and no longer *in situ.* All (4/4) lumbosacral hydrophilic NPPs were extruded. Four out of five (4/5) lumbar hydrophobic NPPs remained intact and *in situ* after overnight incubation, while 1/1 lumbosacral hydrophobic prosthesis was extruded. No fragmentation of the hydrophobic prostheses was seen.
Future research should focus on the physical-mechanical properties of both hydrophilic and hydrophobic prostheses.

2. Introduction

Low back pain (LBP) is a common problem in both the human western society and in the canine population in general. LBP is often associated with intervertebral disc degeneration (IVDD)[23,36,67]. When a degenerated intervertebral disc is also painful the term degenerated disc disease (DDD) is used. DDD does not only affect human health but is also an economic burden on society. Medical costs, lost of production and disability benefits can lead to big expenses[23,40,65,67]. Veterinary research on IVDD could help to better understand IVDD and reveal new insights on etiology, pathofysiology and surgical possibilities. This may have a significant impact on both human and canine welfare. Development of new surgical strategies is needed, because current treatment is not always effective[36].

2.1 Anatomy of the intervertebral disc

The intervertebral disc (IVD) is incorporated into two vertebra[12]. The spine of the dog consists of 26 intervertebral discs in total[50]. The atlanto-axial joint is the only vertebral junction which does not have an interposing IVD[12]. In an axial, i.e. craniocaudal, view the cervical discs are almost circular in shape, the thoracic discs are more oval, while the lumbar discs are bean shaped[14]. The IVD is the biggest avascular structure in the body and is made of 3 anatomical structures: 1) an outer layer which is called the annulus fibrosus (AF), 2) a central structure which is called the nucleus pulposus (NP) and at the axial sides 3) the cartilagous endplates (CEP) [10,12,50]. These structures function to unite the spinal segments, protect the vertebral bodies and minimize shock and trauma in the spine, yet still permit flexibility[12,36,50].

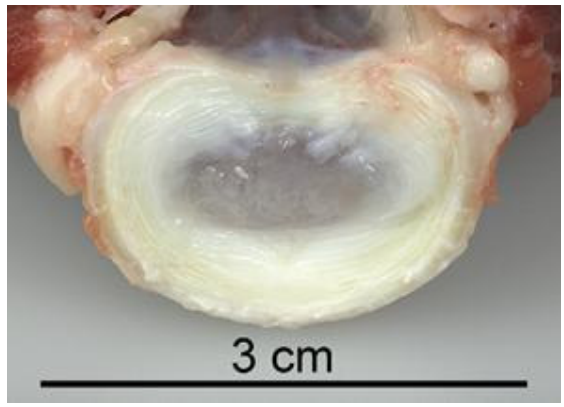


Figure 1. Craniocaudal view of a lumbosacral intervertebral disc of a healthy non-chondrodystrophic dog showing a clear demarcation between the AF with the concentric lamella and the NP. Also the concentric layers of the AF can be recognized[43].

In intervertebral discs, the AF consists of type I collagen lamellas which are arranged in concentric layers, with elastic fibers lying between these lamellae[21,47,50]. Type I collagen has an alternating pattern in which the fibers are orientated from one layer to the next[21]. Within one single lamellar layer the collagen fibers are arranged in the

same direction and have a 30 degree orientation towards the vertebral endplate[21]. The concentric construction of the lamellar layers has an important influence on the load distribution. The AF is important in shock absorption, withstands tension and provides mechanical strength and stability. The AF is ventrally two times thicker than dorsally, which is predisposing for herniation or extrusion dorsally[14,50]. It envelops the nucleus pulposus (Fig.1)[12,50,55].
The NP is eccentrically located in the dorsal third of the IVD and consists of randomly organized collagen fibers and radically organized elastin fibers. These elastin fibers are embedded in aggregating proteoglycans and type II collagen, the extracellular matrix (ECM). The ECM is produced by notochordal cells or chondrocyte-like cells in the NP[21,67,69]. The proteoglycan matrix attracts water, which leads to an osmotic swelling of the NP, resulting in a gelatinous structure with a high interstitial fluid content[47,50]. Chondrocyte-like cells within the NP also produce proteinases, which break down and remodel the ECM. This turnover is needed to maintain IVD properties (Table 1)[21].

Property	**Nucleus pulposus**
Protein content	13.6-21.9%
Water content	74-81%
pH	6.7-7.1
Complex shear modulus	7-21 kPa

Table 1. Human nucleus pulposus properties[32, 38].

As well as the AF, the NP is able to absorb shocks. It also spreads the compressive forces that reach the IVD and promotes fluid exchange between the vertebra and the IVD[50]. The NP lies in close contact with the CEP which connects the IVD to the vertebra (Fig.2)[12]. The CEP facilitates fluid and a nutrient exchange between these structures, and furthermore prevents the NP to protrude into the adjacent vertebral body[12,36,50]. The fluid exchange is needed to maintain a proper intra-discal pressure and appropriate disc height[1,52].

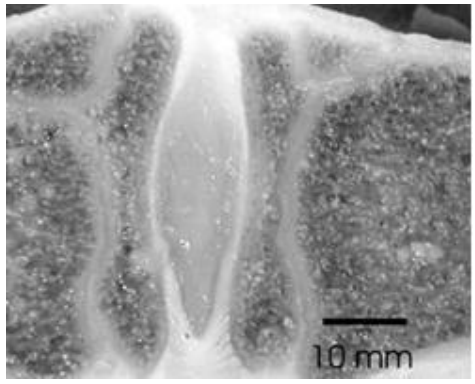


Figure 2. Macroscopical, sagittal view of the IVD. Grey mass is the central NP, surrounded with white AF. Both incorporated by the intact bony end-plates, indicated by an arrow[55].

2.2 Pathofysiology of canine intervertebral disc degeneration

IVDD occurs in all canine breeds, but DDD is mostly seen in chondrodystrophic breeds like the Dachshund, French Bulldog, Basset Hound, American Cocker Spaniel, Pekingese and Beagle[11,28,50]. Growth plates calcify earlier in development in chondrodystrophic dogs, resulting in shortened bones with a curved appearance[53]. Dachshunds develop disc herniation 12.6 times more than other breeds[25]. The overall prevalence of disc herniation in dogs had been reported as 2%[12]. DDD can be divided into 2 classifications, made by Hansen.

1) Hansen type I hernia nuclei pulposi (HNP) is described as a *chondroid* degenerative process, which occurs mostly in chondrodystrophic breeds but can also be found in non-chondrodystrophic breeds. This type of degeneration is characterized by a decrease in proteoglycans, by which the NP looses interstitial fluid. The loss in proteoglycans can already occur at an age of 2 months in Dachshunds[14,16,50]. This early decrease of proteoglycans is correlated to an early disappearance of notochordal cells within the NP, which produce proteoglycans[2]. In chondrodystrophic dogs, the notochordal cells already disappear during growth and will transform in or be replaced by chondrocyte-like cells, in contrast with non-chondrodrystophic dogs where the notochordal cells persist[19,31]. With a decrease in proteoglycans, the NP becomes more fibrotic and less gel-like and thereby looses its ability to absorb shock and spread forces[14,16,50]. The transparent gelatinous NP turns into a gray-white to yellow fibrocartilaginous tissue (Fig.3).

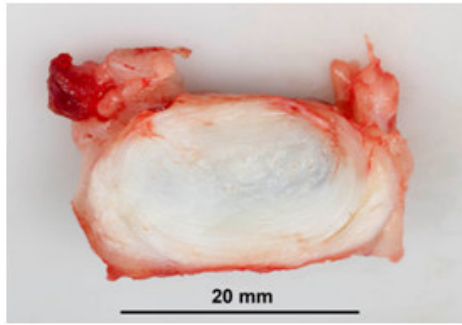


Figure 3. Craniocaudal view of the lumbosacral IVD from a chondrodystrophic dog. The transparent gelatinous NP had turned into a gray-white fibrocartilagous tissue [Personal collection H.C.Kranenburg].

At 1 year of age, chondrodystrophic dogs have 75-90% of there NP turned into a more fibrocartilaginous tissue[14]. When degeneration of the NP occurs, the AF will be loaded with more forces than usual (Fig.4), which can result in accelerated degeneration and rupture of the AF. As a result, a total rupture of the AF and a massive extrusion of the NP in the spinal canal can occur (Fig.5)[50]. The extrusion is explosive causing an acute compression of the spinal cord and thereby a severe inflammatory response and trauma to the spinal cord[12,13,50].

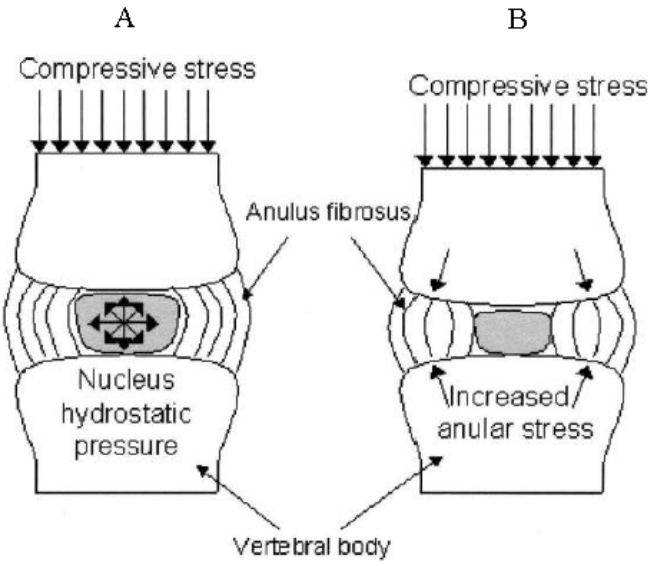


Figure 4. Distribution of the loads of the (A) healthy and (B) degenerated disc. Note the decrease in disc height[31].

2) Hansen type II HNP is described as a *fibroid* degenerative process, which occurs mostly in non chondrodystrophic breeds and is more related to aging. Most cases occur at an age of 6 to 8 years[14]. A described change in cell phenotype into a more fibrocytic phenotype results in shift from type II collagen to a less compliant type I collagen[21]. The degeneration is characterized by a fibrous collagenization and dehydration of the NP and degeneration of the AF[12,13,14,50]. Also, the vertebral endplates might become less permeable by which the nutrient supply and metabolism decreases. NP cells may die or become less viable leading to decreased proteoglycan production[21]. This process occurs with a partial rupture of the AF or bulging of the NP into the spinal canal (Fig.5). This causes a more chronic compression on the spinal cord[12,13,14,50].

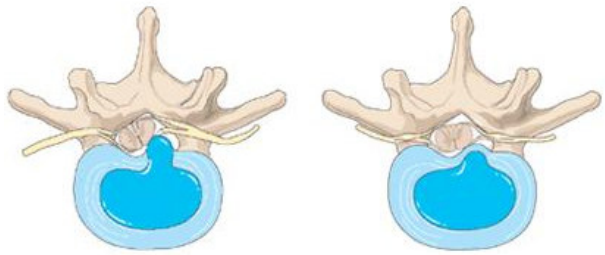


Figure 5. Craniocaudal/axial view of the IVD. On the left an extrusion (Hansen type I hernia nuclei pulposi) can be recognized. Whereas on the right a bulging (Hansen type II hernia nuclei pulposi) can be seen [www.theluklinskispineclinic.com].

This degeneration mostly due to wear-and-tear and ageing is the most common cause for IVD degeneration in humans[47]. Degenerative lumbosacral stenosis (DLS) is the most common cause of cauda equina compression in dogs and an example of Hansen type II HNP[61]. Clinical signs include caudal lumbar pain, pelvic limb lameness, reluctance to perform certain activities such as climbing stairs and jumping. It is most common in large to medium sized breeds of middle-age, with a predisposition for German Shepherd dogs[61].

The spinal cord can adapt to certain amounts of compression and mechanical displacement. When the spinal cord is no longer able to compensate, clinical signs will develop[50]. Clinical signs that can be seen in DDD are severe pain, ataxia and motor deficits like paresis or paralysis (Fig.6). When the lumbosacral disc in involved, bladder function, anal sphincter function and tail tone can be altered[50].

Figure 6. Dachshund with posterior paralysis as clinical sign of DDD [www.vin.com].

2.3 Diagnostics

An important step in the diagnostic process of IVD degeneration is the evaluation of information obtained from radiography, myelography, computed tomography (CT) or magnetic resonance imaging (MRI). Lateral and ventrodorsal radiographic views should ideally be obtained during general anaesthesia to ensure a good positioning and decrease movement[14]. Radiography can reveal a loss of disc height, sclerosis, spondylosis deformans and presence of a mineralized mass dorsal of the IVD space which is a highly significant finding of a herniated mineralized disc within the intervertebral canal[14,50,61]. Non herniated and calcified discs are a signs of IVD degeneration but not of IVD disease. Gas in the intervertebral space (Fig.7b), which is called 'vacuum phenomenon', is a rare but very accurate sign of IVD degeneration[39]. Loss in disc height (Fig.7a) is the most reported radiographic finding, but has a moderate sensitivity[14,39].

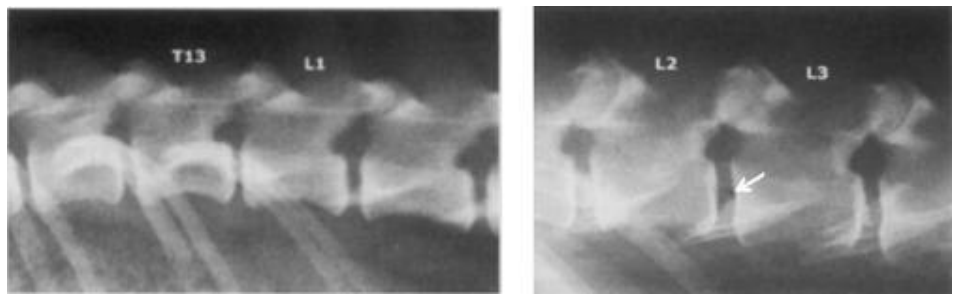


Figure 7a. On the left a Lateral radiograph of a 5-year-old Border Terrier. A loss in disc height can be Recognized between T13-L1. On the right a lateral radiograph of a Rottweiler dog with Hansen type I HNP, a gas lucency can be recognized between L2-L3[39].

Radiography alone is not enough for diagnosing IVD herniation, because it doesn't provide the degree of compression on the spinal cord and lateralization of the lesion[14].

Myelography is the method of choice to diagnose IVD herniation when MRI is not available. The accuracy for diagnosing IVDD is reported to be 72-97%[14].
Ventrodorsal and lateral projections should be obtained. Thinning or deviation of the contrast column is highly significant for diagnosing IVD herniation. Oblique projection is required to determine lateralization of the lesion[37,63].
CT generates a high-quality bone imaging and is a sensitive, noninvasive method used for diagnosing IVD herniation. It also provides information about lateralization of the lesion. CT and myelography have similar sensitivities in finding the location of the disc herniation. On the other hand, CT is more sensitive in finding chronic lesions due to the presence of a mineralized disc[14,34]. As stated before, mineralizing of an IVD is not necessary painful and can also be found in asymptomatic dogs. Contrast enhancement can visualize lesions that were not visible before contrast injection[14].

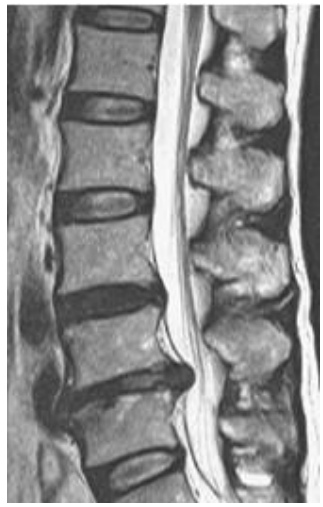


Figure 8. Sagittal T2-weighted MRI view of a 46 year old woman with chronic LBP. A disc herniation occurred between L4-L5, where also a decreased water content of the NP can be seen[59].

MRI is the best diagnostic tool for detecting IVD degeneration at an early stage. T2-weighted MRI reflects changes in water content of the nucleus and is thereby able to show degree of degeneration (Fig.8)[14,54]. Besalti et al. (2005) and Naude et al. (2008) reported that MRI and surgical findings had a complete agreement. Therefore, MRI is a good tool to guide surgical decisions. MRI is more accurate in finding the location and lateralization of the disc herniation and size of the extruded material[7,46].
Pfirrmann et al. (2001) developed a grading system (1-5) for human disc degeneration based on MRI, where grade 1 shows a clear distinction between annulus and nucleus and disc height is normal and grade 5 shows a lost distinction between annulus and fibrosus and a collapsed disc space. This is the most used grading system in human medicine. Bergknut et al. (2009) validated this grading system for the use in dogs and revealed that this system is highly reproducible for grading IVDD in dogs of various breeds and ages, at all spinal locations[4,54].

2.4 Treatment

Treatment of dogs showing clinical signs of IVDD, i.e. DDD, can be conservative as well as surgical. Conservative therapy includes corticosteroids, cage rest and muscle

relaxants. This treatment is solely indicated when pain is the only clinical sign[50]. When clinical signs reoccur again after 6 months of conservative treatment or conservative treatment is not effective enough, which is not uncommon, surgical therapy should be considered[50]. A surgical intervention will also be performed in more severe cases. Surgical strategies are fenestration, pediculotomy, dorsal laminectomy and (micro-) hemilaminectomy[22,60,61,62]. Fenestration can be performed lateral, dorsolateral or ventral and is mostly used at cervical level. Thereby the nucleus pulposus is removed, not necessarily facilitating decompression of the spinal cord[14].
Hemilaminectomy is the most popular approach for thoracolumbar IVDD, where a part of the dorsal arch is removed (Fig.9). This gives access to the lateral and ventral side of the vertebral canal, thereby also facilitating spinal cord decompression by removing herniated disc material[14].

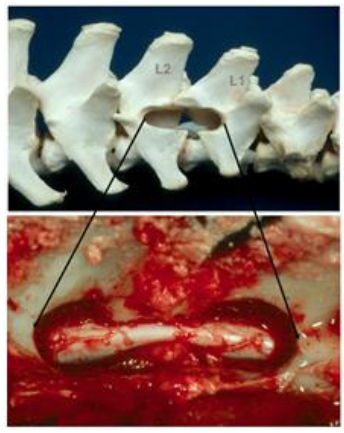


Figure 9. Hemilaminectomy of a L1-L2 segment [Personal collection B.P.Meij]

Dorsal laminectomy is usually performed at the lumbosacral level and provides decompression of the spinal cord by removing herniated disc material. The dorsal arch covering the spinal cord is totally removed in the midline using this strategy (Fig.10)[14,24]. It may however, also further destabilize the lumbosacral junction and cause more degenerative changes[42].

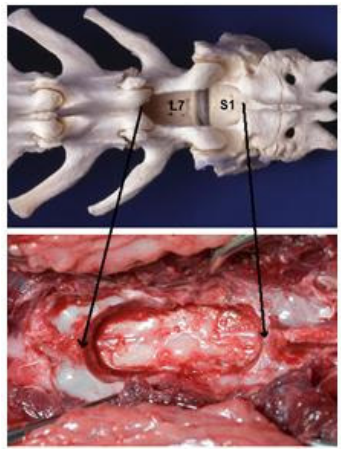


Figure 10. Dorsal laminectomy of a L7-S1 segment [Personal collection B.P.Meij].

Decompressive fusion of the vertebra, usually with the use of pedicle screws is the most used surgical method for DDD in humans. This is highly invasive, not always successful and leads to loss of function and flexibility of the concerning segments. Thereby, fusion can accelerate degeneration of adjacent discs and leads to adjacent segment disease (ASD).

All these surgical treatments are mostly focusing on elevating the clinical symptoms[36,47,57]. In order to recreate the natural function of the spine, a restoration of the NP is desirable[9,20]. This can be accomplished by gene therapy, molecular therapy such as growth factor injection, cell-based therapy or nucleus replacement[21,48,64]. Successful gene therapy not only needs efficient gene transfer to the cells but also a sufficiently long period of expression of these transgenes[21,48]. A number of vectors exist within gene-based therapies, such as viral-based vectors, gene guns and plasmids[49,58]. Previous research of Nishida et al. (1998) revealed that injection of a recombinant adenovirus transferred genes to cells within the intervertebral disc, resulting in manufacturing of desired proteins. Expression of the marker gene persisted in vivo for at least 12 weeks[49]. Duration of the gene expression can be limited by immunologic reaction to the viral proteins. Further investigation is needed to determine the maximum length of expression. Moreover, safety of possible vectors is of significant concern, because they have potential for an inflammatory reaction and spread of disease through viral infection[21,48]. In an *in vitro* study, Thompson et al. (1991) was the first to show that exogenous growth factors were able to stimulate proteoglycan synthesis in the NP[64]. Because of limited duration of this therapy and chronic character of the disease, the utility of this therapy may be limited[21]. Cell-based therapy such as reimplantation of NP cells or stem cells have shown good results[21]. A nucleus replacement device is another strategy for restoration of the NP. These devices can be divided into 2 classifications; the elastomeric and the mechanical (Fig.11). Elastomeric devices contain a hydrogel or a non-hydrogel replacement. A hydrogel consists of polymers which can absorb water and will swell when placed in the nuclear cavity[9]. This swelling is needed to restore biomechanical functionality after nucleotomy. Hydrogel-based nucleus pulposus prostheses (NPPs) have been in development for over 15 years[17]. Hydrogels can be made visible with radiographs, CT and MRI[9]. A non-hydrogel also consists of polymers, but these have shock-absorbing capabilities. Both gels can either be preformed or injectable[9,20]. Injectables only need a small incision or annulotomy, making the surgery less invasive[10].

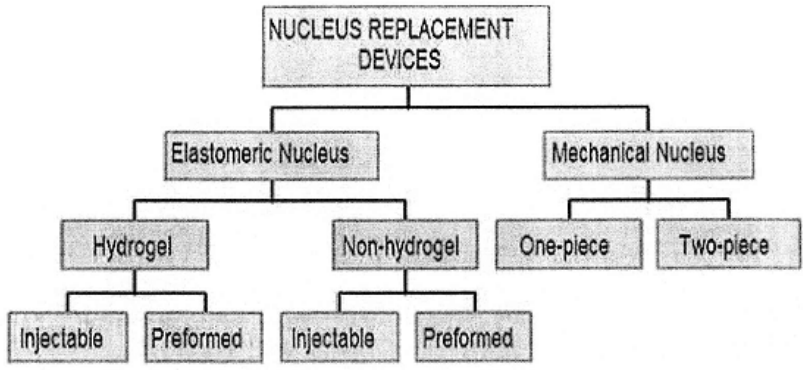


Figure 11. Classification of nucleus replacement devices[20].

Since the 1950s different nucleus pulposus replacement or reinforcement techniques have been investigated. Research shows promising results, still closure of the annulotomy points out to be a challenge. It has to be closed in a proper way to prevent the NPP to extrude through the annular incision, which is a common problem[3,17,20,35,59]. Migration of the prosthesis remains the primary source of complications[6]. Recurrence of extrusion by the prosthesis occurs within 4 to 6 weeks after surgery and is mostly related to the initial site of extrusion[15,22].
In human research, the prosthetic disc nucleus (PDN®) designed by Dr. Charles Ray has the most reported clinical history (Fig.12). A research of Shim et al. (2003) implanted a PDN® in 46 human patients and closed the annulotomy by suturing. Patients were followed for over 6 months, where four patients (8.7%) showed an extrusion of the PDN® device. Worldwide, the extrusion rate in humans is 12%[59].

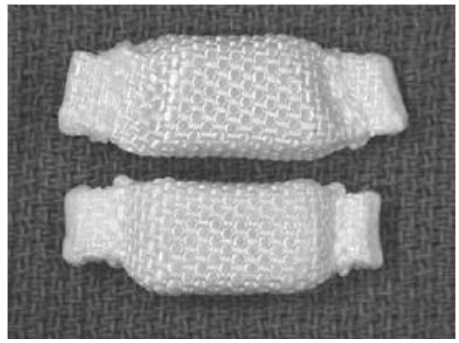


Figure 12. The prosthetic disc nucleus (PDN®)[17].

Previous veterinary research of Bergknut et al. (2010) revealed that a preformed NPP used in canine cadavers has promising qualities (Fig.13). This NPP was able to restore the disc height in 8 of 10 dogs. However, implant extrusion and fragmentation occurred in one of 10 dogs after closure of the annulus fibrosis with a mattress suture, tissue glue and a covering mesh[3].

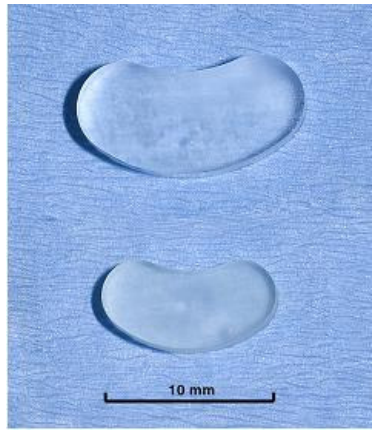


Figure 13. NPP I as xerogel on the bottom and in swollen state on top used in a research by Bergknut et al (2010)[3].

The extrusion rate not only depends on the closure technique, but also on the general state of the AF. An intact AF is the most suitable environment for NP replacement[17]. However, research of Berlemann et al. (2009) revealed that no implant extrusion occurred after implantation of a novel injectable protein hydrogel, Nucore®, in 14 patients with a herniated AF. Nucore® adheres to surrounding tissue when injected in the nuclear cavity and thereby seals the annular defect. No additional method was used to close the AF. Despite the bad condition of the AF, apparently no implant extrusion had been seen in the 2-year follow-up[5].

2.5 Radiographic analysis

Restoration of the natural disc height (Fig.14) after nucleotomy and replacement of the NP by the hydrogel is an important read-out parameter for testing the function of the NPP[10]. Decreased disc height can lead to increased load on the remaining AF and NP, the facet joints and the adjacent segments. Besides, it disturbs fluid exchange into the disc and can cause radicular pain resulting from a decrease in foraminal canal where the nerves run through[18,26,45,51]. The loss in disc height is proportional to the amount of nucleus removed. The disc height can be assessed by radiological imaging after nucleus replacement[3,47,50,61].

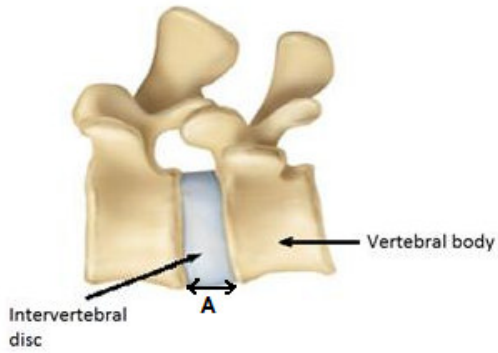


Figure 14. Lateral view of the intervertebral disc. Craniocaudal, axial measurement of the intervertebral disc is referred as 'disc height', indicated by the 'A' [www.smallincisionsbigresults.com].

The aims of this study were: 1) to radiographically investigate the effect of NPP II on IVD height in canine cadaveric specimen using three different measuring techniques, 2) to evaluate how many prostheses remained intact and *in situ* after incubation and 3) to investigate the extent of swelling of the prosthesis.

3. Materials and methods

Five lumbar spines were isolated from 5 healthy dogs and frozen at -20°. The spines are of dogs of approximately 15-20 kg and euthanized in an unrelated experiment. The segments included the spine from L1 to S3, with the pelvis attached. Prior to using, the spine was left to thaw for 24 hours at room temperature. Excess muscles were removed but ligamentous tissue was left intact.

3.1 Radiographic study of NPP II

Lateral and ventrodorsal radiographic views of spine 3, 4 and 5 (14 spinal segments) were obtained at three different conditions: 1) the native spine, to obtain the natural height of the IVD, 2) after performing nucleotomy, to confirm the possible loss in disc height and 3) after insertion of the NPP II and incubation of the specimen overnight at 37°C, to evaluate whether natural disc height is restored. Also it was important to radiographically determine the localization of the prosthesis in the nuclear cavity. The IVD height was measured on lateral radiographs by two independent observers and with three different methods, on standard computer screens using the software Image J®. All three methods were performed twice by each observer, using the same settings and magnification for the two observers. The data was collected in a Microsoft Excel® file. Only radiographs of IVDs in which the prostheses were still 'intact and *in situ'* after overnight incubation were used for measuring disc height. Prostheses which were fragmented and/or extruded from the IVD are considered not to be able to restore disc height.

Method 1
To measure the disc height, a method developed by N. Bergknut and L.A. Smolders based on an article of Hofstetter et al. (2009) was used[30]. The deepest point of the clefts needs to be determined. A single line running from the cleft of the cranial vertebra to the cleft of the caudal vertebra was measured (Fig.15).

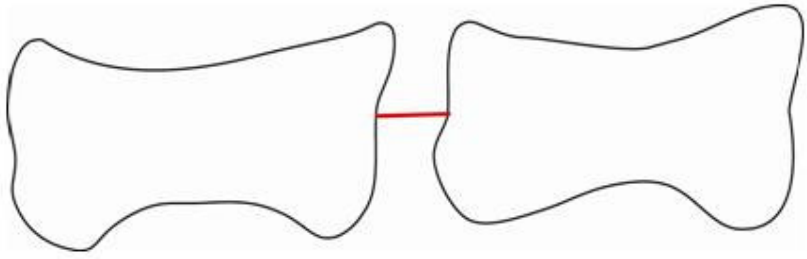


Figure 15. Method 1 for measuring disc height. A single midline is drawn.

Method 2
The second method was developed by H.C. Kranenburg based on an article of L. Twomey and J. Taylor (1985)[66]. Measuring disc height was performed by first drawing dorsal and ventral lines parallel to each other, connecting the most extreme dorsal and ventral corners of both adjacent vertebrae. In the arithmetical middle of these lines a third mid-line was drawn, which represents the disc height (Fig.16).

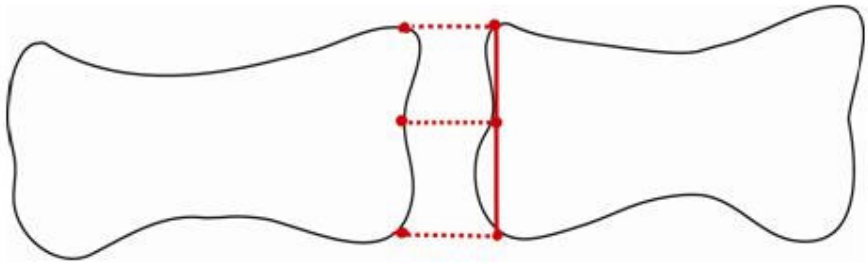


Figure 16. Method 2 for measuring disc height. A dorsal, ventral and mid-line disc height.

Method 3

To measure the disc height using a method developed by Masuda et al. (2004), the vertebras as well as the IVD were measured by using nine lines in total[41]. The dorsal and ventral lines A, G, C and I were drawn alongside the dorsal and ventral boundaries of the vertebra, in the same direction the vertebra runs. A vertical line can be used as a resource for determining the direction of the vertebra. Mid-lines B and H are drawn in the middle of the dorsal and ventral lines and also parallel to these lines. Lines D, E and F will be connecting the dorsal, ventral and mid-lines of the two adjacent vertebrae (Fig.17). The average IVD height was calculated following the formula: disc height index (DHI) = 2*(D+E+F)/(A+B+C+G+H+I).

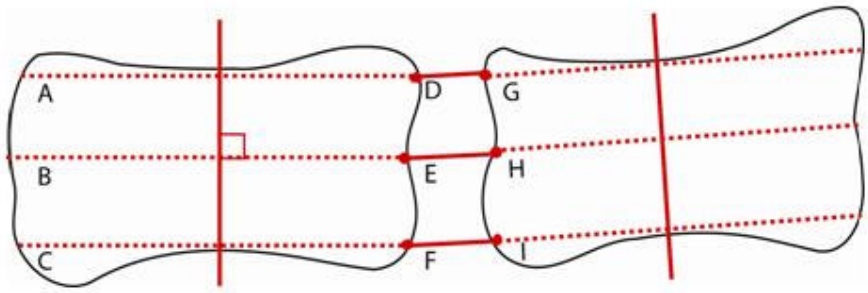


Figure 17. Method 3 for measuring disc height. The vertebra as well as the IVD are measured using 9 lines. The DHI is calculated using these lines.

3.2 Surgical implantation of the NPP II

Nucleotomy of lumbar IVDs was performed by making a stab incision via a left lateral approach. The incision was made in the middle of the IVD, parallel to the fibers of the outer annular ring using a surgical blade number 11. The NP was removed using a ball-tipped probe and grasping forceps. Nucleotomy of the lumbosacral segment was performed after performing a dorsal laminectomy. The annular incision was widened by using the grasping forceps, after which the non-hydrated NPP (xerogel) was manually inserted into the nuclear cavity. Hydrophilic prostheses were inserted in spine numbers 1, 2, 3 and 5, while hydrophobic prostheses were inserted in spine number 4 (Fig.18)

Figure 18. On the left a hydrophilic prosthesis after swelling with a greater blue core than the hydrophobic prosthesis after swelling with a red core on the right.

Two sizes of NPP II were used, a lumbar size and a larger lumbosacral size. The lumbar sized implants were inserted in the IVDs of L1-L2, L2-L3, L3-L4 and L4-L5, while lumbosacral sized implants were inserted in the IVDs of L5-L6 and L7-S1. Saline (0.9% NaCl) solution was injected into the nuclear cavity before closure of the annular defect to initiate hydration of the implant. During a pilot study the following method has showed to be the best for annular closure technique. A fascia transplantation which is placed in the annular canal followed by at least 2 simple sutures using Ethylon 4-0 with cutting needle and covering of the annular opening with a polypropylene mesh graft and tissue glue (Dermabond, Ethicon INC., Amersfoort, the Netherlands). Suturing of 3/5 lumbosacral IVDs could not be performed or failed due to lack of space and poor quality of the annular fibers. In these cases only the polypropylene mesh graft and tissue glue were used. Muscles were injected with amoxicillin clavulaanacid to prevent bacterial growth. Each specimen was wrapped in a plastic bag with PBS to prevent implant and specimen dehydration. The spines were incubated at a temperature of 37°C for 16-18 hours. Afterwards, the disc was carefully sectioned using a surgical blade 11 through the AF, closely to one of the CEPs to reveal the swollen implant.

3.2 Determining the size and mass of the implants

NPPs were measured using a Vernier caliper with an accuracy of 0.05mm. NPPs of spines 1 and 2 were only weighted and measured after swelling in the nuclear cavity and therefore not used in calculation of MSR (mass swelling ratio), EWC (equilibrium water content) and VSR (volume swelling ratio). After using both of these spines a new experimental design was developed, where NPPs of spines 3, 4 and 5 were weighted and measured before insertion into the nuclear cavity as well as after swelling in the nuclear cavity. Spine 3, 4 and 5 were used to calculate the MSR, EWC and VSR.

The MSR is the swollen mass divided by the dry mass. The EWC is defined as the weight percentage of water in the swollen hydrogel at equilibrium. The difference between the mass before and after swelling is divided by the mass after swelling followed by multiplication with 100.
Volume of the prostheses was calculated as length*width*height. The VSR is the swollen volume divided by the dry volume.

3.4 Data and statistical analyses

The software SPSS® was used for data and statistical analyses. *The effect of condition on disc height* (native, nucleotomy and after insertion of prosthesis) and intra- and interobserver reliability was analyzed for each method using a linear mixed model analysis. 'Observer' (2 levels: observer 1-2), 'condition' (3 levels: native, nucleotomy, inserted prosthesis), 'segment' (6 levels: segment 1-5 and 7), 'method' (3 levels: method 1-3) and 'number of measurement' (2 levels: measurement 1-2) were set as fixed effects, while spine (3 levels: spine 3-5) was set as random effect. A Bonferroni correction was applied to compensate for the multiple comparisons. The level for statistical significance was set to $P<0.05$. Each segment was selected separately by using 'select cases' within the linear mixed model to determine the effect on disc height on each single intervertebral disc.

The difference in mass and volume between hydrophilic and hydrophobic prostheses was also analyzed by using a linear mixed model. 'Size' (2 levels: size L2-L3 and L7-S1), 'intactness' (2 levels: not intact, intact), 'localization' (2 levels: *in situ*, extruded), 'swelling' (2 levels: swollen, not swollen), 'type of prosthesis' (2 levels: hydrophilic, hydrophobic) were set as fixed effects, while number of prosthesis (13 levels: prosthesis number 1-13) was set as random effect. Again, a Bonferroni correction was applied to compensate for the multiple comparisons and the level of statistical significance was set to $P<0.05$.

4. Results

4.1 Radiographic study of NPP II

4.4.1 Evaluation of radiographs and disc height measurements

Radiographs of IVDs in which the prostheses were 'intact and *in situ'* after overnight incubation were used for measuring disc height (9/14 segments). The NPP II was visualized after swelling by radiography. Both hydrophilic and hydrophobic prostheses were visible on radiography (Fig.19).

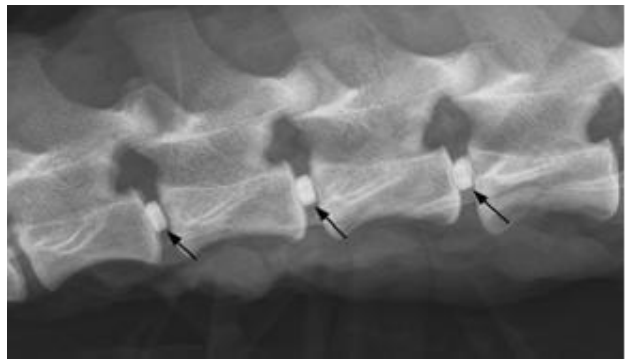
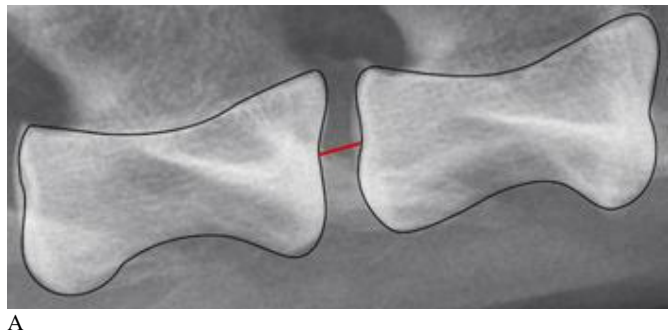
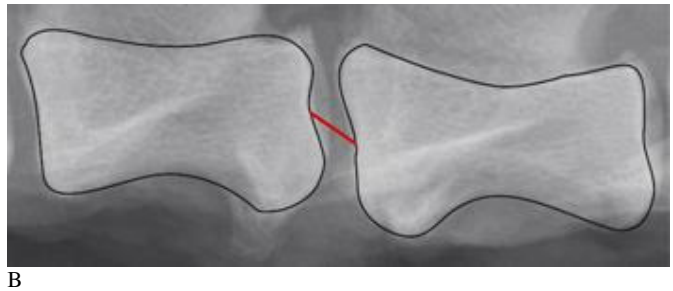


Figure 19. Lateral radiograph of lumbar segments of Spine 4 with swollen hydrophobic NPPs (arrows).

All three methods were applied to the radiographs of the native spine, after nucleotomy and after insertion and swelling of the prosthesis. Of all three methods good representations and less representative examples were encountered (Fig.20, 21 and 22).

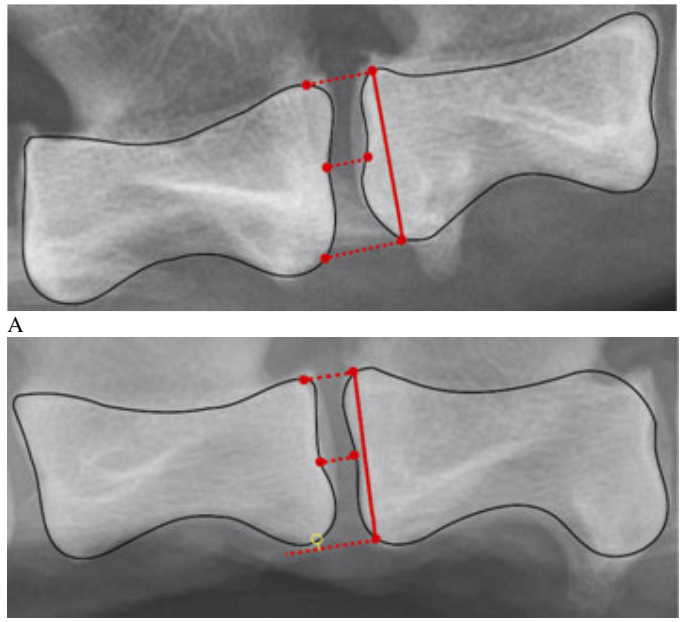


A



B

Figure 20. A) Good representation of method 1 and B) a less representative example can be seen, since the vertebrae did not lie straight opposite to each other.

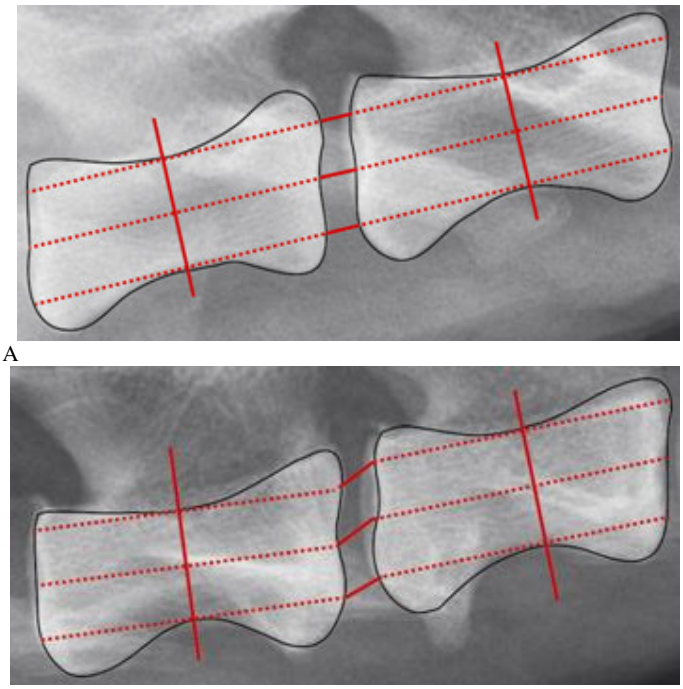


A

B

Figure 21. A) Good representation of method 2 on top and B) less representative example can be seen, since the ventral line did not connect the corners of the vertebra.

When the ventral line using method 2 did not connect the corners of the vertebra a lead line, indicated by the yellow line, was drawn from the corner of the vertebra to the ventral line to determine where this ventral line should end (Fig.21B).



A

B

Figure 22. A) Good representation of method 3 and B) a less representative example can be seen, since the vertebrae did not lie in a straight line.

4.4.2 Effect of condition on disc height

Method 1 and 2 revealed that disc height significantly decreased after nucleotomy and significantly increased after insertion of the NPP II into the nuclear cavity. Disc height in the native spine was not significantly different from disc height after NPP II insertion, which means the natural disc height was restored. Method 2 showed the greatest restoration of the disc height. Method 3 revealed that the disc height index significantly decreased after nucleotomy and increased significantly after insertion of the NPP II. Disc height index after NPP II insertion was significantly higher than disc height index in the native spine (Fig.23 and Table 2).

Condition	Method 1 (p-value)	Method 2 (p-value)	Method 3 (p-value)
Native - Nucleotomy	0.000*	0.000*	0.017*
Nucleotomy - Prosthesis	0.000*	0.000*	0.000*
Native - Prosthesis	0.841	1.000	0.006*

Table 2. Comparison of disc height per condition stratified by three methods used for disc height measuring. P-values were analyzed by the linear mixed model. * = statistical significant.

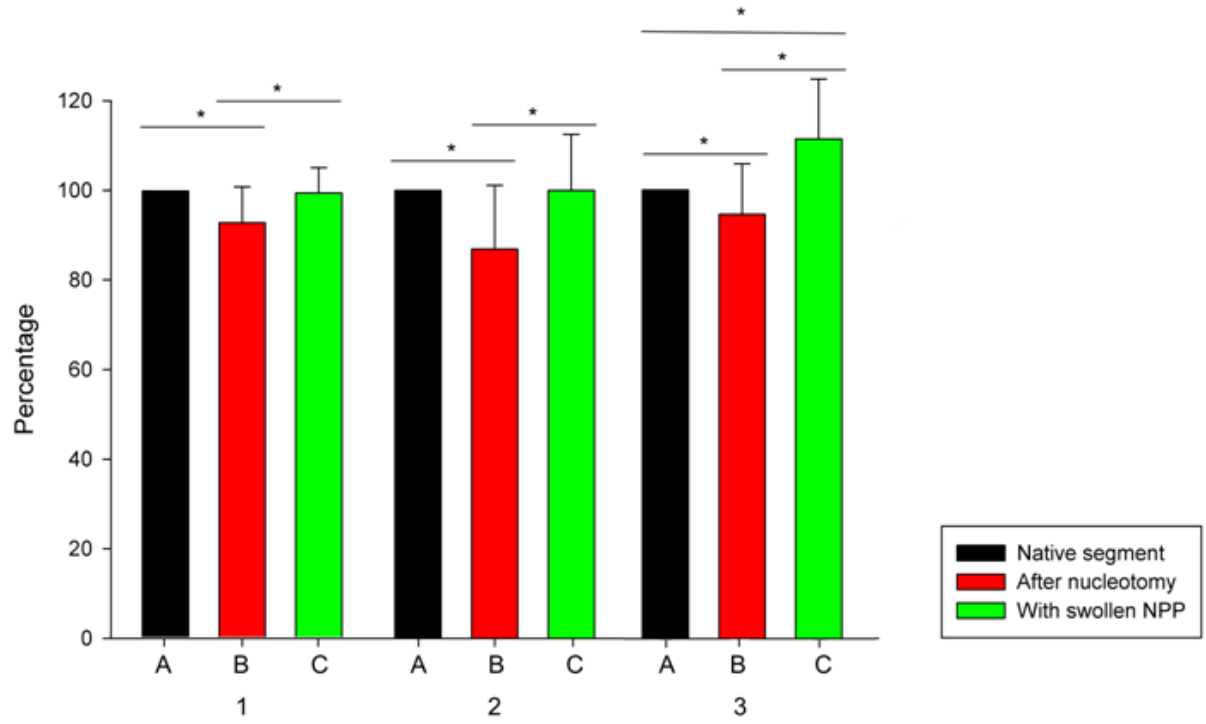


Figure 23. The effect on disc height measured by the three different methods. Native disc height is referred as 100%. * = statistically different.

Using method 1, in 7/9 segments disc height was restored. In both L1-L2 segments disc height was not restored. Using method 2, in all of the 9 segments disc height was

restored, so also in both L1-L2 segment. Using method 3 in only 5/9 segments disc height was restored.

4.4.3 Intra- and interobserver reliability

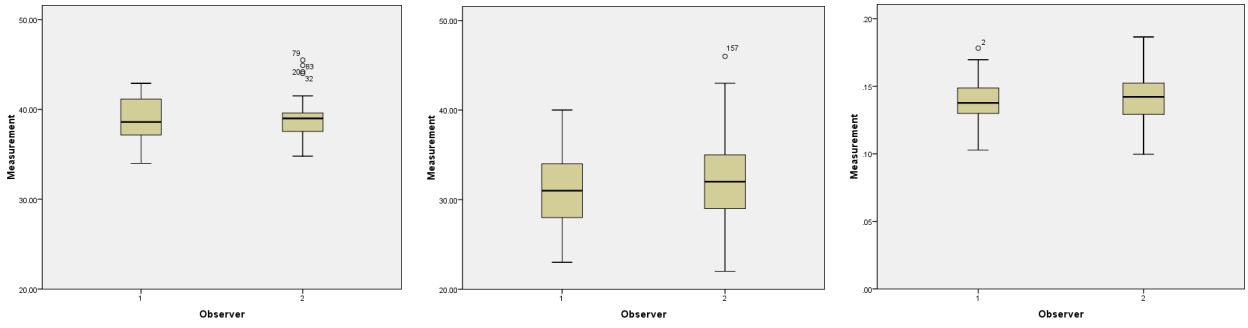
	Method 1 (p-value)	Method 2 (p-value)	Method 3 (p-value)
Interobserver (observer 1-2)	0.000*	0.113	0.335
Intraobserver (measurement 1-2)			
Observer 1	0.915	0.067	0.870
Observer 2	1.000	0.000*	0.183

Table 3. Inter- and intraoberserver reliability of the three used methods for disc height measuring.
* = statistical significant.

Method 1 had the best intraobserver reliability but was the only method showing a significant difference between both observers (Table 3). Outcome of method 1 revealed a structural difference in measurements between observer 1 and 2. During a consensus meeting method 1 was discussed. It appeared that one of the observers focused more on the middle of the cleft instead of the deepest point of the cleft, despite the description of the method. We made the decision to only select the deepest point of the cleft and performed a third round of measurements. The interobserver reliability of this third measurement had a p-value of 0.757, indicating no significant difference between the two observers any more (Table 4). A learning curve was seen, and the reliability of this method was improved. After the consensus meeting, method 2 had the lowest interobserver reliability (Table 4). Also method 2 had the lowest intraobserver reliability, also showing a significant difference between both measurements of observer 2 (Table 3). Interobserver and intra-observer reliability of method 3 lay in between method 1 and 2 (Table 3).

	Method 1 (p-value)
Interobserver (person 1-2)	0.757

Table 4. Interobserver reliability after consensus meeting. * = statistically significant.



A **B** **C**

Figure 24. Boxplots of measurements of observer 1 and 2 (after consensus meeting), of A) method 1 B) method 2 and C) method 3.

Boxplots reveal that within one specific method, both medians are equal if we compare the two observers.

4.2 Surgical implantation of the NPP II

Not all NPP II remained intact and *in situ* after incubation at 37°C (Table 5). Six of eight (6/8) lumbar hydrophilic NPPs remained intact and *in situ* after overnight incubation, where 2/8 showed fragmentation whilst being extruded through the annular canal and annular closing. Four of four (4/4) lumbosacral hydrophilic NPPs were extruded from the nuclear cavity. Four of five (4/5) lumbar hydrophobic NPPs remained intact and *in situ* after overnight incubation, while the one lumbosacral hydrophobic prosthesis was extruded. No fragmentation of the hydrophobic prostheses was seen. Four out of ten (4/10) prostheses (L1-L2, L4-L5, L5-L6 of spine 3, and L1-L2 of spine 4) that were rated as 'intact and *in situ*' were located very slightly eccentrically.

Location	Spine 1 (hydrophilic)	Spine 2 (hydrophilic)	Spine 3 (hydrophilic)	Spine 4 (hydrophobic)	Spine 5 (hydrophilic)
L1-L2					
L2-L3					
L3-L4					
L4-L5					
L5-L6					
L7-S1					

Intact and *in situ*
Intact but extruded from IVD
Not intact and not *in situ*
Not intact but *in situ*
Not used

Table 5. Number of prostheses rated as intact/not intact and in situ/extruded after incubation.

Both hydrophobic and hydrophilic NPPs filled the nuclear cavity perfectly which was seen after sectioning of the IVD at the end of the experiment (Fig.25).

Figure 25. Transverse view of a L2-L3 segment where a perfect fit of a hydrophilic NPP could be seen. The size of this NPP was designed for an L2-L3 segment.

4.3 Determining size and mass of the implants

The custom-made NPPs were weighted with accuracy of 4 decimals (Table 6).

NPP	Size	Implanted in spine; segment	Mass before insertion (g)	Mass after swelling (g)	MSR[I]	EWC[II] (%)
Hydrophilic	Lumbar	3; L1-L2	0.0777	0.1094	1.41	28.98
	Lumbar	3; L2-L3	0.0773	0.1130	1.46	31.59
	Lumbar	3; L3-L4	0.0763	0.1142	1.50	33.19
	Lumbar	3; L4-L5	0.0772	0.1149	1.49	32.81
	Lumbar	5; L2-L3	0.0769	0.1136	1.48	32.31
Hydrophilic	Lumbosacral	3; L5-L6	0.0927	0.1342	1.45	30.92
	Lumbosacral	3; L7-S1	0.0921	0.1542	1.67	40.27
	Lumbosacral	5; L7-S1	0.0929	0.1408	1.52	34.02
Hydrophobic	Lumbar	4; L1-L2	0.0764	0.0921	1.21	17.05
	Lumbar	4; L2-L3	0.0768	-	-	-
	Lumbar	4; L3-L4	0.0762	0.0905	1.19	15.80
	Lumbar	4; L4-L5	0.0761	0.1118	1.47	31.93
Hydrophobic	Lumbosacral	4; L5-L6	0.0922	0.1156	1.25	20.24
	Lumbosacral	4; L7-S1	0.0907	0.1191	1.31	23.85

Intact and *in situ*
Intact but extruded from IVD
Not intact and not *in situ*
Not intact but *in situ*

Table 6. Masses of the NPPs before and after insertion into the nuclear cavity.
[I]MSR: mass swelling ratio, [II]EWC: equilibrium water content.

The NPP inserted in IVD L2-L3 of spine 4 was damaged during excision and a piece of the prosthesis was lost and therefore not weighted after swelling. The NPP inserted in the IVD L3-L4 of spine 4 was not intact, but both pieces were present and could be weighted.
The average MSR of the 'intact and *in situ*' hydrophilic NPP II was 1.46, while the average MSR of the 'intact and *in situ*' hydrophobic NPP II was 1.22. The average EWC of the 'intact and *in situ*' hydrophilic NPP II was 31.32, while the average EWC of the 'intact and *in situ*' hydrophobic NPP II was 17.70.

The swelling ability of the 'intact and *in situ*' hydrophilic prostheses when looking at the MSR was 1.20 greater than the 'intact and *in situ*' hydrophobic prostheses (Table 7 and 10). Linear mixed model analysis revealed no significant difference in mass between 'intact and *in situ'* hydrophilic and 'intact and *in situ*' hydrophobic prostheses before insertion. After swelling, the difference in mass of the 'intact and *in situ'* hydrophilic and 'intact and *in situ'* hydrophobic prostheses was significant ($P<0.001$).

NPP	average MSR[I]	average EWC[II]
Hydrophilic; intact, in situ	1.46	31.32
Hydrophilic; not intact	1.56	35.83
Hydrophilic; extruded	1.60	37.15
Hydrophobic; intact	1.22	17.70
Hydrophobic; extruded	1.39	27.89

Table 7. Average MSR and EWC of the prostheses.
[I]MSR: mass swelling ratio, [II]EWC: equilibrium water content.

The hydrophilic prostheses which were rated as 'intact but extruded from IVD' had gained 1.09 times more weight than 'intact and *in situ*' hydrophilic prostheses, while the hydrophilic prostheses which were rated as 'not intact and not *in situ*' had gained 1.06 times more weight than 'intact and *in situ*' hydrophilic prostheses when looking at the average MSR (Table 5). The hydrophobic prostheses which were rated as 'intact but extruded from IVD' had gained 1.14 times more weight than 'intact and *in situ*' hydrophobic prostheses, when looking at the average MSR (Table 5). Statistical significant differences between 'intact but extruded from IVD' and 'intact and *in situ*' could not be obtained, since the amount of values was too low.

The custom-made hydrophilic NPPs measured (length x width x thickness) 9.05 x 3.86 x 2.40mm for the lumbar size and 9.15 x 4.48 x 2.44 mm for the lumbosacral size. The custom-made hydrophobic NPPs measured (length x width x thickness) 9.09 x 3.96 x 2.35 mm for the lumbar size and 9.15 x 4.46 x 2.45 mm for the lumbosacral size. Only NPPs inserted in spine 3, 4 and 5 were weighted before insertion as well as after swelling (Table 8).

NPP	Size NPP	Implanted in spine; segment	Size before insertion; length x width x height (mm)	Volume before insertion; length*width* height (mm^3)	Size after insertion; length x width x height (mm)	Volume after insertion; length*width* height (mm^3)	VSR[I]
Hydrophilic	Lumbar	3; L1-L2	9.05 x 3.86 x 2.4	83.84	10.94 x 4.33 x 2.95	139.74	1.67
	Lumbar	3; L2-L3	idem	83.84	11.24 x 4.51 x 2.74	138.90	1.66
	Lumbar	3; L3-L4	idem	83.84	-	-	-
	Lumbar	3; L4-L5	idem	83.84	11.84 x 4.46 x 2.63	138.88	1.66
	Lumbar	5; L2-L3	idem	83.84	11.17 x 4.39 x 2.67	130.93	1.56
Hydrophilic	Lumbosacral	3; L5-L6	9.15 x 4.48 x 2.44	100.02	11.78 x 4.81 x 2.89	163.75	1.63
	Lumbosacral	3; L7-S1	idem	100.02	11.08 x 5.35 x 3.15	186.73	1.87
	Lumbosacral	5; L7-S1	idem	100.02	11.01 x 5.18 x 3.16	180.22	1.80
Hydrophobic	Lumbar	4; L1-L2	9.09 x 3.96 x 2.35	84.59	9.65 x 4.23 x 2.49	101.64	1.20
	Lumbar	4; L2-L3	idem	84.59	-	-	-
	Lumbar	4; L3-L4	idem	84.59	9.76 x 4.20 x 2.48	101.66	1.20
	Lumbar	4; L4-L5	idem	84.59	10.38 x 4.41 x 2.70	123.59	1.46
Hydrophobic	Lumbosacral	4; L5-L6	9.15 x 4.46 x 2.45	99.98	10.25 x 4.79 x 2.55	125.20	1.25
	Lumbosacral	4; L7-S1	idem	99.98	9.97 x 5.00 x 2.58	128.61	1.29

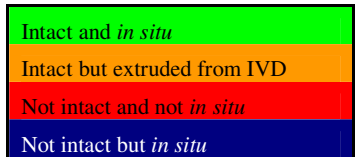


Table 8. Size and volume of the NPPs before and after insertion into the nuclear cavity.
[I]VSR: volume swelling ratio.

Again the NPP inserted in IVD L2-L3 of spine 4 was not measured after swelling, due to damaging during excision. The NPP inserted in IVD L3-L4 of spine 3 was not measured since it was not intact after incubation.
The average VSR factor of the 'intact and *in situ*' hydrophilic NPP II was 1.64, while the average VSR of the 'intact and *in situ*' hydrophobic prostheses was 1.22.
Again, statistical significant differences between 'intact but extruded from IVD' and 'intact and *in situ*' could not be obtained, since the amount of values was too low.

The swelling ability of the 'intact and *in situ*' hydrophilic prosthesis when looking at the VSR was 1.34 times more than the 'intact and *in situ*' hydrophilic prostheses (Table 9 and 10). Linear mixed model analysis revealed no significant difference in volume between 'intact and *in situ'* hydrophilic and 'intact and *in situ*' hydrophobic prostheses before insertion. After swelling, the difference in volume of the 'intact and *in situ'* hydrophilic and 'intact and *in situ'* hydrophobic prostheses was significant ($P<0.001$).

NPP	average VSR[I]
Hydrophilic; intact, in situ	1.64
Hydrophilic; extruded	1.84
Hydrophobic; intact, in situ	1.22
Hydrophobic; extruded	1.38

Table 9. Average VSR of the prostheses; intact vs. not intact.
[I]VSR: volume swelling ratio.

The hydrophilic prostheses which were rated as 'intact but extruded from IVD' increased 1.12 times more in volume than 'intact and *in situ*' hydrophilic prostheses.
The hydrophobic prostheses which were rated as 'intact but extruded from IVD' increased 1.13 times more in volume than 'intact and *in situ*' hydrophobic prostheses, when looking at the average VSR (Table 7).
Table 10 revealed an overall greater average swelling ability and a higher average EWC of intact hydrophilic prostheses, compared to intact hydrophobic prostheses. Hydrophilic prostheses had a significant greater swelling ability compared to the hydrophobic prostheses. Hydrophilic prostheses swell relatively more in volume than mass (Table 10).

NPP	average MSR[I]	average EWC[II]	average VSR[III]
Hydrophilic	1.46	31.32	1.64
Hydrophobic	1.22	17.70	1.22

Table 10. Comparison of measured properties of intact hydrophilic and hydrophobic prostheses.
[I]MSR: mass swelling ratio, [II]EWC: equilibrium water content, [III]VSR: volume swelling rate.

5. Discussion

5.1 Radiographic study of NPP II

The prostheses could easily be visualized by radiography, due to their intrinsic radiopacity. Radiographic examination, by means of fluoroscopy, can be of great importance during surgery to evaluate the location of the prosthesis after insertion.

Determination of the best method for measuring disc height is based on several criteria, which are practical usefulness and level of inter- and intraobserver reliability, but also probability of disc height restored was concerned.
Feasible en reliable measurement of lumbosacral disc height, with its specific anatomical characteristics, should also be one of the criteria to evaluate the three measuring techniques. But since all lumbosacral IVDs were excluded from disc measurement due to extrusion of all prostheses, no information was obtained on which methods would be best for measuring this particular segment. Unfortunately, these segments are of great importance since it is a common site for Hansen type II HNP in dogs.

Method 1 for measuring disc height was most practical, since clefts were relatively easy to be found and only one single line had to be drawn. Only when vertebrae were not situated straight opposite to each other the drawn line was not representative for disc height. Method 1 revealed to be the best method for measuring disc height in lumbar segments when inter- and intraobserver reliability was concerned. However, a consensus meeting and a third measurement was needed to obtain this inter- and intraobserver reliability. After the first two measurements the interobserver reliability of both observers showed a significant difference. It was important not to focus on the middle part of the cleft while drawing the midline, but focus on the deepest point of the clefts. Negative aspect regarding this method was that boxplots of this method are less symmetrical compared to method 2 and 3. Also, using method 1, in 7/9 segments natural disc height was restored, where in two L1-L2 segments (of spine 3 and 4) disc height was not restored. This finding is remarkable because the L1-L2 IVD is smaller than the segments L2-L3 – L4-L5 in which the same size NPP was inserted and disc height was measured to be restored. Although not significantly different, the prosthesis inserted in segment L1-L2 of spine 3 had less mass increase compared to other lumbar hydrophilic prostheses and the prosthesis inserted in segment L1-L2 of spine 4 had slightly less volume increase compared to one other lumbar hydrophobic prosthesis. More external pressure, since the IVD is small, could possibly result in incomplete swelling of the prostheses and thereby incomplete restoration of the disc height. Also, a measurement error could be an explanation for the finding that disc height is not restored in both L1-L2 segments.

Using method 2 disc height was restored in all of the 9 segments, so also in the two L1-L2 segments. This could imply it to be a better method compared to method 1, looking at the probability of disc height restoration. It was, however, often difficult to recognize and determine the exact corners of the adjacent vertebra. The most radiolucent boundary of the vertebra needed to be determined, while the less radiolucent boundary of the vertebra lies deeper and would give a distortion of the

positioned lines. Since both dorsal and ventral lines had to be drawn parallel to each other, it often occurred that one of these lines was not connecting the corners of the vertebra any more (Fig.21). While the midline position and direction was guided by the posterior and anterior lines, objectivity was excluded. The middle line is the representation of the disc height. This method had the lowest intra- and interobserver reliability of all three methods, also a significant difference in both measurements of observer 2 were found.

Method 3 turned out to be the most time-consuming method in practical usefulness, since nine different lines had to be drawn. Determining the direction of the vertebra and drawing the first line was difficult, while positioning the other lines was relative easy, due to strict instructions for use of this method. Only when vertebras were not situated straight opposite to each other line D, E and F will not be representative. Using method 3 only 5/9 segments disc height was restored. Also, disc height showed to be significantly larger after swelling of the prostheses when compared to the native disc height. It is unlikely that the IVD increased even more than native disc height, since the prosthesis is probably not able to produce such forces. These findings could imply the incorrectness of the measurements. Therefore, measurements obtained from radiograph of the native state and after nucleotomy should also be questioned looking at this findings.

To choose which of the measuring methods is best, more measurements, or probably more observers will be needed.

Position of the vertebra on the radiographs is of great importance, since it influences the measurement. When vertebras are not lying straight opposite to each other, the measurements will also be influenced and will be less representative for disc height. Consensus meeting appeared to be of great importance, while measurements could be done more accurate and a possible learning curve was seen. Possibly consensus meetings will be able to improve the other methods as well, but due to a lack of time it could not be performed regarding method 2 and 3.

5.2 Surgical implantation of the NPP II

The NPP could be inserted as xerogel, whereby the annular incision was kept as small as possible. Also, swelling of the implant is necessary to restore disc height. After swelling, the prosthesis incorporates itself between the annular rings. Implant migration occurred in 5/5 lumbosacral and 1/13 lumbar IVDs. The dorsal approach is possibly one of the factors that contribute to the high rate of extrusion at the lumbosacral segment. During insertion the prosthesis needed to be rotated 90 degrees to fit the nuclear cavity, due to this dorsal approach. On the other hand, previous research of Bergknut et al. (2010) revealed that prostheses inserted in the lumbosacral segments did not extrude from the IVD, also using a dorsal laminectomy and same insertion technique. Remarkable, all of the lumbosacral prostheses extruded in this research. A factor contributing to this different extrusion rate could be found in the altered shape of the prosthesis. The NPP used by Bergknut et al. (2010) was bean-shaped, where the NPP II used in this research was more oval and also more spherical in axial direction. The extrusion of the prosthesis in the L4-L5 segment of spine 4 was

probably due to the fact that the annular incision was larger in dorsal direction, resulting in a possible suboptimal closure of the annular defect.

All NPPs which stayed *in situ* also stayed intact during swelling in the confined IVD space. Only 2/12 hydrophilic NPPs and none of the hydrophobic prostheses were fragmented after overnight incubation at 37°C and swelling of the implant. The two fragmented NPPs were extruded and probably fragmented whilst being trapped in the annular closing and/or defect. The core of the hydrophilic prosthesis swells more and absorbs more water than the hydrophobic prosthesis core, due to its hydrophilic features. After insertion the prosthesis swells and tries to encounter minimal pressure, sometimes resulting in migration into the annular canal, which is created during the incision in the annulus fibrosus. When stuck in the annular canal the prosthesis suffers more forces, especially on the middle part of the prosthesis and can fragment.
Previous findings emphasize the importance of a small annular incision and a good annular closure technique. Closure of the annulotomy points out to be a challenge. It had to be closed in a proper way to prevent the NPP to extrude through the annular incision, which is a commonly reported problem[3,17,20,35,59].
We found the NPP to fill the nuclear cavity, which is important for the distribution of forces on annulus and secure natural functionality of this spinal segment.

Since only two sizes of prostheses (lumbar and lumbosacral) were used, there was not always a prosthesis available which exactly fitted the IVD. By this way, a clinical situation was imitated, where there is always an individual difference between the dimensions of the IVD. Except when the IVD is measured using MRI before performing an operation, it will be difficult to always achieve a perfect fit.

The hydrogels can be tested using biomechanical devices. Hydrogels should stay within the intervertebral disc space during biomechanical testing to maintain stability of the spine[47]. Physiological torsion and bending segmental properties are very important to provide normal movement and flexibility[47].
Biomechanical testing should reveal which of these prostheses has better physical-mechanical properties. Despite their swelling factor is less compared to the hydrophilic prostheses, the hydrophobic prostheses are capable of restoring disc height as well.

5.3 Determining size and mass of the implants

Hydrophilic prostheses had a significant greater swelling ability compared to the hydrophobic prostheses, probably due to more water uptake as a result of hydrophilic properties. Prostheses rated as ‘intact but extruded from the IVD’ had swollen more in volume and gained more weight in comparison to the ‘intact and *in situ’* prostheses. This is likely due to the fact that these prostheses are able to swell more freely without any external pressure. Hydrophilic prostheses had a relative greater volume swelling ability than mass swelling ability, while these swelling abilities were the same in hydrophobic prostheses. This is probably due to the difference in core, which swells more in volume in the hydrophilic prosthesis.

6. Conclusion

A NPP needs to possess several features before it will be suitable for *in vivo* use. The NPP should restore natural disc height, fill the entire nuclear cavity enclosed by the annulus ring perfectly. To be able to do so the NPP needs to be implanted in dry form (xerogel) and expand when present in the nuclear cavity. Prostheses should stay intact and remain *in situ* after insertion in the nuclear cavity and swelling during overnight incubation. Furthermore the prosthesis should be radiopaque and needs to have proper physical-mechanical properties and fatigue resistance.
Radiographs revealed intrinsic radiopacity of both hydrophilic and hydrophobic prosthesis. Method 1 and 2 for measuring disc height showed the best results, where method 1 had practical advantages and the best intra- and interobserver effect after a consensus meeting. Method 2 showed the most reliable measurements looking at disc height restoration and boxplots of interobserver reliability.
All NPPs which stayed *in situ* also stayed intact during swelling in the confined IVD space. Hydrophilic prostheses have a significant greater swelling factor compared to hydrophobic prosthesis. Proper annular closure technique is of great importance to facilitate the inserted prosthesis to remain intact and *in situ.*

This prosthesis meets up to most of the features a NPP needs to possess as discussed above. Future research should focus on the physical-mechanical properties of both hydrophilic and hydrophobic prostheses.

7. Acknowledgements

First I would like to thank Hendrik-Jan Kranenburg and Bjorn P. Meij from Utrecht University for their educational counseling and the opportunity to do my research at the Department of Clinical Sciences of Companion Animals. This was a good opportunity to see what research is really all about on a topic I was interested in. Thanks to them, I was also able to complete my research in three months, despite several misfortunes.

I would also like to thank David Onis for lots of his help and cooperation while performing this research and I enjoyed working together.

Furthermore I would like to thank Anneke Jansen as Designer Interactive Multimedia for her contribution to this report.

And last but not least, I would like to thank J.C.M Vernooij, Luc A. Smolders and Niklas Bergknut a lot for supporting me with data and statistical analyses, since this was one of the greatest challenges of this research.

8. References

1. Adams M.A., Hutton W.C (1983) The effects of posture on the fluid content of lumbar intervertebral discs. *Spine* **8**, pp 665-671

2. Aguiar D.J., Johnson S.L., Oegema T.R (1999) Notochordal cells interact with nucleus pulposus cells: regulation of proteoglycan synthesis. *Experimental Cell Research* **246**, pp 129-137

3. Bergknut N., Smolders L.A., Koole L.H., Voorhout G., Hagman R.E., Lagerstedt A., Saralidze K., Hazewinkel H.A.W., van der Veen A.J, Meij B.P (2010) The performance of a hydrogel nucleus pulposus prosthesis in an ex vivo canine model. *Biomaterials* **31**, pp 6782-6788

4. Bergknut N., Auriemma E., Wijsman S, et al. (2009) Pfirrmann grading of intervertebral disc degeneration in chondrodystrophic and non-chondrodystrophic dogs with low field magnetic resonance imaging. *Am J Vet Res* in pres

5. Berlemann U., Schwarzenbach O (2009) An injectable nucleus replacement as an adjunct to microdiscectomy: 2 year follow-up in a pilot clinical study. *European Spine Journal* **18**, pp 1706-1712

6. Bertagnoli R., Schonmayr R (2002) Surgical and clinical results with the PDN prosthetic disc-nucleus device. *European Spine Journal* **11**, pp 143-148

7. Besalti O., Ozak A., Pekcan Z., Tong S., Eminaga S., Tacal T (2005) The role of extruded disk material in thoracolumbar intervertebral disk disease. *Canadian Veterinary Journal* **46**, pp 814-820

8. Bibby S.R., Jones D.A., Ripley R.m., Urban J.P (2005) Metabolism of the intervertebral disc: effects of low levels of oxygen, glucose, and pH on rates of energy metabolism of bovine nucleus pulposus cells. *Spine* **30**, pp 487-496

9. Boelen E.J.H., Koole L.H., Rhijn L.W., Hooy-Corstjens C.S.J (2007) Towards a functional radiopaque hydrogel for nucleus pulposus replacement. *Journal of biomedical materials research* **83**, pp 440-450

10. Boyd L.M., Carter A.J (2006) Injectable biomaterials and vertebral endplate treatment for repair and regeneration of the intervertebral disc. *European Spine Journal* **15**, pp 414-421

11. Braund K.G., Ghosh P., Taylor T.K., Larsen L.H (1975) Morphological studies of the canine intervertebral disc. The assignment of the beagle to the achrondroplastic classification. *Research in Veterinary Science* **19**, pp 167-172

12. Bray J.P., Burbidge H.M (1998) The Canine Intervertebral Disk Part One: Structure and Function. *Journal of the American Hospital Association* **34**, pp 55–63

13. Bray J.P., Burbidge H.M (1998) The Canine Intervertebral Disk Part Two: Degenerative Changes – Non chondrodystrophoid versus Chondrodystrophoid Disks. *Journal of the American Hospital Association* **34**, pp 135–44

14. Brisson B.A (2010) Intervertebral disc disease in dogs. *Veterinary Clinics: Small Animal Practice* **40**, pp 829-858

15. Brisson B.A., Moffatt S.L., Swayne S.L., Parent J.M (2004) Recurrence of thoracolumbar intervertebral disk extrusion in chondrodystrophic digs after surgical decompression with or without prophylactic fenestration: 265 cases. *Journal of the American Veterinary Medical Association* **224**, pp 1808-1814

16. Buckwalter J.A (1995). Aging and degeneration of the human intervertebral disc. *Spine* **20**, pp 1307-1314

17. Carl A., Ledet E., Yuan H., Sharan A (2004) New developments in nucleus pulposus replacement technology. *The Spine Journal* **4**, pp 325-329

18. Chen J., Yan W., Setton L.A (2004) Static compression induces zonal-specific changes in gene expression fot extracellular matrix and cytoskeletal proteins in intervertebral disc cells in vitro. *Matrix Biology* **22**, pp 573-583

19. Cole T., Burkhardt D., Frost L., Ghosh P (1985) The proteoglycans of the intervertebral disc. *Biochimica et Biophysica Acta* **839,** pp 127-138

20. Coric D, Praveen M.D., Mummaneni M.D (2008) Nucleus replacement technologies. *Journal of neurosurgery: Spine* **8**, pp 115-120

21. Fasset D.R., Kurd M.F., Vaccaro A.R (2009) Biologic solutions for degenerative disk disease. *Journal of Spinal Disorders & Techniques* **22**, pp 297-308

22. Forterre F., Konar M., Spreng D., Jaggy A., Lang J (2008) Influence of intervertebral disc fenestration at the herniation site in association with hemilaminectomy on recurrence in chondrodystrophic dogs with thoracolumbar disc disease: a prospective MRI study. *Veterinary surgery* **37**, pp 399-405

23. Frymoyer J.W., Cats-Baril W.L (1991) An overview of the incidences and costs of low back pain. *Orthopedic Clinics of North America* **22**, pp 263-271

24. Gill P.J., Lippincott C.L., Anderson S.M (1996) Dorsal laminectomy in the treatment of cervical intervertebral disk disease in small dogs: a retrosprective study of 30 cases. *Journal of the American Animal Hospital Association* **32**, pp 77-80

25. Goggin J.E., Li A.S., Franti C.E (1970) Canine intervertebral disk disease: characterization by age, sex, breed, and anatomic site of involvement. *American Journal of Veterinary Research* **31**, pp 1687-1692

26. Gotfried Y., Bradford D.S., Oegena T.R (1986) Facet joint changes after chemonucleolysis-induced disc space narrowing. *Spine* **11**, pp 944-950

27. Grunhagen T., Wilde G., Soukane D.M., Shirazi-Adl S.A., Urban J.P (2006) Nutrient supply and intervertebral disc metabolism. *The Journal of Bone and Joint Surgery* **88**, pp 30-35

28. Hansen H.J (1951) A pathologic-anatomical interpretation of dis degeneration in dogs. *Acta Orthopaedica Scandinavica* **20**, pp 280-293

29. Hoerlein B.F (1987) Intervertebral disks. *Canine neurology* **3**, pp 470-560

30. Hofstetter M., Gedet P., Doherr M., Ferguson S., Forterre F (2009) Biomechanical analysis of the three-dimensional motion pattern of the canine spine segment C4-C5. *Veterinary Surgery* **20,** pp 49-58

31. Hunter C.J., Matyas J.R., Duncan N.A (2003) The notochordal cell in the nucleus pulposus: a review in the context of tissue engineering. *Tissue Engineering* **9,** pp 667-677

32. Iatridis J.C., Weidenbaum M., Setton L.A, Mow V.C (1996) Is the nucleus pulposus a solid or fluid? Mechanical behavior of the nucleus pulposus of the human intervertebral disc. *Spine* **21**, pp 1174-1184

33. Ishihara H., Urban J.P (1999) Effects of low oxygen concentrations and metabolic inhibitors on proteoglycans and protein synthesis rates in the intervertebral disc. *Journal of Orthopaedic Research* **17**, pp 829-835

34. Israel S.K., Levine J.M., Kerwin S.C., Levine G.J., Fosgate G.T (2009) The relative sensitivity of computed tomography and myelography for identification of thoracolumbar intervertebral disk herniation in dogs. *Veterinary Radiology & Ultrasound* **50**, pp 247-252

35. Jin D., Qu D., Zhao L., Chen J., Jiang J (2003) Prosthetic disc nucleus (PDN) replacement for lumbar disc herniation: preliminary report with six months'follow-up. *Journal of Spinal Disorders & Techniques* **16**, pp 331-337

36. Kandel R., Roberts S., Urban J.P (2008) Tissue engineering and the intervertebral disc: the challenges. *European Spine Journal* **17**, pp 480-491

37. Kirberger R.M., Roos C.J., Lubbe A.M (1992) The radiological diagnosis of thoracolumbas disc disease in the dachshund. *Veterinary Radiology & Ultrasound* **33**, pp 255-261

38. Kitano T., Zerwekh J, Usui Y., Edwards M, Flickere P, Mooney V (1993) Biomechanical changes associated with the symptomatic human intervertebral disc. *Clinical Orthopeadics and Related Research* **293**, pp 372-377

39. Lamb C.R., Nicholls A., Targett M., Mannion P (2001) Accuracy of survey radiographic diagnosis of intervertebral disc protrusion in dogs. *Veterinary radiology & Ultrasound* **43**, pp222-228

40. Maniadakis N., Gray A (2000) The economic burden of back pain in the UK. *Pain* **84**, pp 95-103

41. Masuda K., Aota Y., Muehleman C., Imai Y., Okume M., Thonar E.J., Andersson G.B., Howard S (2004). A novel rabbit model of mild, reproductible disc degeneration by an annulus needle puncture: correlation between the degree of disc injury and radiological and histological appearances of disc degeneration. *Spine* **30,** pp 5-14

42. Meij B.P., Suwankong N., Veen A.J., Hazewinkel H.A.W (2007) Biomechanical flexion-extension forces in normal canine lumbosacral cadaver specimen before and after laminectomy-discectomy and pedicle screw-rod fixation. *Veterinary surgery* **8**, pp 742-751

43. Meij B.P., Bergknut N (2010) Degenerative lumbosacral stenosis in dogs. *Veterinary Clinics of North America: Small Animal Practice* **40**, pp 983-1009

44. Nachemson A., Lewin T., Maroudas A., Freeman M.A (1970) In vitro diffusion of dye throught the end-plates and the annulus fibrosus of human lumbar inter-vertebral discs. *Acta Orthopeadica Scandinavica* **41**, pp 589-607

45. Natarajan R.N., Ke J.H., Andersson G.B.J (1994) A model to study disc degeneration process. *Spine* **19**, pp 259-265

46. Naude S.H., Lambrechts N.E., Wagner W.M (2008) Association of preoperative magnetic resonance imaging findings with surgical features in dachshunds with thoracolumbar intervertebral disk extrusion. *Journal of the American Veterinary Medical Association* **232**, pp 702-708

47. Nerurkar N.L., Elliot D.M., Mauck R.L (2010) Mechanical design criteria for intervertebral disc tissue engineering. *Journal of Biomechanics* **43**, pp 1017-1030

48. Nishida, K, Kang J.D., Suh J, Robbins P.D., Evans C.H., Gilbertson L.G (1998) Adenovirus-Mediated Gene Transfer to Nucleus Pulposus Cells: Implications for the Treatment of Intervertebral Disc Degeneration. *Spine* **23**, pp 2437-2442

49. Nishida K., Doita M., Takada T., Kakutani K., Miyamoto H., Shimomura T., Maeno K., Kurosaka M (2006) Sustained transgene expression in intervertebral disc cells in vivo mediated by microbubble-enhanced ultrasound gene therapy. *Spine* **31**, pp 1415-1419

50. Nunamaker D.M. (eds): Intervertebral disk disease, in Newton C.D. Textbook of Small Animal Orthopaedics. Ithaca, International veterinary Information Service, 1985

51. Ohshima H., Urban J.P.G., Bergel D.H (1995) Effect of static load on matrix synthesis ratesin the intervertebral disc measured in vitro by a new perfusion technique. *Journal of Orthopedic Research* **13**, pp 22-29

52. Paajanen H., Lehto I., Alanen A., Erkintalo M., Komu M (1994) Dirunal fluid changes of lumbar discs measured indirectly by magnetic resonance imaging. *Journal of Orthopedic Research* **21**, pp 509-514

53. Parker H.G., VonHoldt B.M., Quignon P et al. (2009) An expressed Fgf4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. *Science* **325,** pp 995-998

54. Pfirrmann C.W.A., Metzdorf A., Zanetti M., Hodler J., Boos N (2001) Magnetic resonance classification of lumbar intervertebral disc degeneration. *Spine* **26,** pp 1873-1878

55. Revell P.A., Damien E., Silvio L.D., Gurav N., Longinotti C., Ambrosio L (2007) Tissue engineering intervertebral disc repair in the pig using injectable polymers. *Journal of Materials Science* **18**, pp 303-308

56. Rossi F., Seiler G., Busato A., Habil., Wacker C., Lang J (2004) Magnetic resonance imaging of articular process joint geometry and intervertebral disk degeneration in the caudal lumbar spine (L5-S1) of dogs with clinical signs of cauda equine compression. *Veterinary radiology & Ultrasound* **45**, pp 381-287

57. Ruan D., He Q., Ding Y., Hou L., Li J., Luk K.D (2007) *Lancet* **369**, pp 993-999

58. Sabojima S., Kim J.S., Gilbertson L.G., Kang J.D (2004) Gene therapy for degenerative disc disease. *Gene Therapy* **11**, pp 390-401

59. Shim C.S., Lee S.H., Park C.W., Choi W.C., Choi G., Choi W.G et al. (2003) Partial disc replacement with the PDN prosthetic disc nucleus device: early clinical results. *Journal of Spinal Disorders & Techniques* **16**, pp 324-330

60. Stigen O., Ottesen N., Jaderlund K.H (2010) Early recurrence of thoracolumbar intervertrebral disc extrusion after surgical decompression: a report of three cases. *Acta Veterinaria Scandinavica* **52**, pp 10-17

61. Suwankong N., Voorhout G., Hazewinkel H.A.W., Meij B.P (2006) Agreement between computed tomography, magnetic resonance imaging, and surgical findings in dogs with degenerative lumbosacral stenosis. *Journal of the American Veterinary Medical Association* **229**, pp 1924-1929

62. Tanaka H., Nakayama M., Takase K (2005) Usefulness of hemilaminectomy for cervical intervertebral disk disease is small dogs. *Journal of Veterinary Medical Science* **67**, pp 679-683

63. Tanaka H., Nakayama M., Takase K (2004) Usefulness of myelography with multiple views in diagnosis of circumferential location of disc material in dogs with thoracolumbar intervertebral disc herniation. *Journal of Veterinary Medical Science* **66**, pp 827-833

64. Thompson J.P., Oegema T.R., Bradford D.S (1991) Stimulation of mature canine intervertebral disc by growth factors. *Spine* **16**, pp 253-260

65. Tulder M.W., Koes B.W., Bouter L.M (1995) A cost-of-illness study of back pain in the Netherlands. *Pain* **62**, pp 233-240

66. Twomey L., Taylor J (1985) Age changes in lumbar intervertebral discs. *Acta Orthopaedica Scandinavica* **56,** pp 496-499

67. Urban J.P., Roberts S (2003) Degeneration of the intervertebral disc. *Arthritis Research and Therapy* **5**, pp 120-130

68. Urban J.P., Smith S., Fairbank J.C (2004) Nutrition of the intervertebral disc. *Spine* **29**, pp 2700-2709

69. Yu J., Winlove C.P., Roberts S., Urban J.P (2002) Elastic fibre organization in the intervertebral discs of the bovine tail. *Journal of Anatomy* **201**, pp 465-475

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