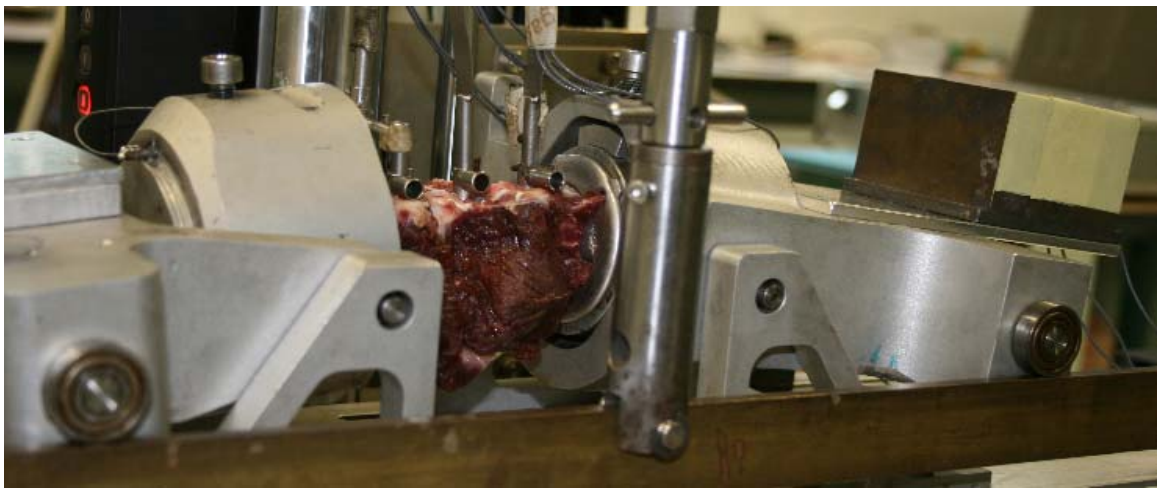
Ability of a 2nd generation Nucleus Pulposus Prosthesis (NPPV2.0) to restore axial disc height in canine cadaveric specimens before and after biomechanical testing



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Index

Abstract

A new treatment option to restore the natural function of the nucleus pulposus function and thereby preventing further clinical signs of intervertebral disc degeneration (IVDD) is the insertion of a nucleus pulposus prosthesis (NPP) in a nucleotomized disc. In humans, disc prostheses are already sparsely being used, but for dogs this option is currently not yet available.
The present study investigated whether after nuclectomy, insertion and swelling of second generation nucleus pulposus prosthesis (NPPv2.0) would restore the original axial disc height of an L2-L3 intervertebral disc.
Canine cadaveric specimens of 11 healthy Beagles were used and biomechanical tests (flexion/extension, lateral bending, and axial rotation) were applied to the spinal segments L2-L4 to gain information about the restoration of the biomechanical properties and the axial disc height. The biomechanical tests were carried out on 1) the native spine, 2) after nuclectomy, and 3) after insertion and swelling of the NPPv2.0. Lateral and dorsoventral radiographs were obtained and measured twice by two independent observers with a method developed by Hofstetter et al, and a method developed by Twomey and Taylor.
Compared to the intact spine, a significant ($p<0.05$) decrease in the mean disc height was found after biomechanical testing, using either measurement method. After insertion and swelling of the NPPv2.0, it was found that the mean disc height of an L2-L3 intervertebral disc can significantly be restored ($p<0.05$).
Before we can test the NPPv2.0 *in vivo* in dogs some additional tests of the physical en biomechanical properties of the NPPv2.0 needs to be performed but preliminary results are looking promising. In the future the NPPv2.0 may be a valuable addition to the surgical treatment options currently available for canine and human IVDD patients.

Keywords
Spine; Intervertebral disc; Intervertebral disc degeneration; NPPv2.0; Biomechanics; Measurement methods

Introduction

History

In dogs, thoracolumbar intervertebral disc degeneration is the most common cause of low back pain.[1, 2] In humans, intervertebral disc (IVD) herniation was first reported in 1824 and is also associated with low back pain (LBP). The most common cause of LBP in humans is also believed to be degeneration of the IVD.[3] This is a major health problem in the Western industrialized society.[4, 5] Nearly three quarters of the population on earth is affected with low back pain sometime in their life.[6] The high prevalence of low back pain also led to an explosion of costs and the economic impact in our Dutch society includes 1.7 % of the gross national product.[7, 5]

In dogs, IVD herniation was first described by Hansen in 1881 and during the last 60 years IVD herniation has been the focus of extensive research.[4, 8, 9] Intervertebral disc degeneration (IVDD) is categorized in Hansen type I and Hansen II, which refer respectively to extrusion of the nucleus pulposus and protrusion of the annulus fibrosus.[10]

Chondrodystrophoid dog breeds like the Dachshund, Pekinese, French Bulldog, and the Beagle, are predisposed for IVDD as described in several reports.[11, 9] The most commonly affected area of the back where disc extrusion or protrusion is present, is the thoracolumbar junction (vertebral bodies T11 through L2).[12] The clinical signs associated with IVD herniation in the thoracolumbar region range from spinal pain to paraplegia.[13]

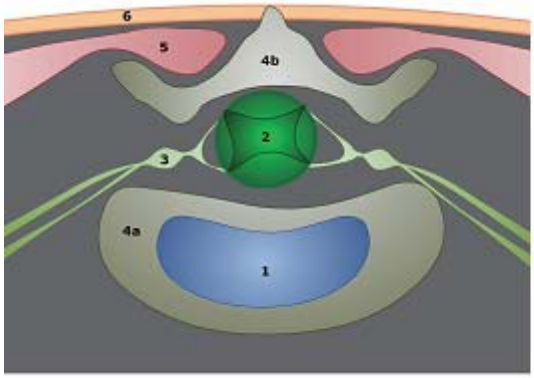
Several surgical treatments have been developed to treat the common cause of low back pain, but these treatments are still unable to offer an outcome that is prosthetic and at the same time physiologic.[14] A new treatment, insertion of nucleus pulposus prosthesis (NPP), has these properties and that makes the NPP suitable for clinical use to prevent further IVDD in early stages.[3]

Anatomy

An IVD exists between each vertebral body along almost the entire length of the spinal column. Only the first and second cervical vertebra does not have an interposing IVD. The spinal column collectively incorporates 26 intervertebral discs in the dog.[4, 8, 9]

The IVD is made up of three distinct anatomical regions (fig. 1).

1. The annulus fibrosus (AF), an outer fibrocartilaginous ring arranged in concentric layers which enevelops the nucleus pulposus.
2. The nucleus pulposus (NP), an ovoid central region of amorphous gelatinous material.
3. The hyaline cartilaginous vertebral endplates (EP), which cover the epiphyses of the vertebral bodies and are the cranial and caudal borders of the IVD.



	Key components
1	Nucleus pulposus
2	Spinal cord
3	Spinal nerves
4a	Annulus fibrosus and transition zone
4b	Vertebra
5	Epaxial musculature
6	Skin

Figure 1. Schematic image of a transverse cross-section through the dorsal part of a mature dog. (**Bergknut 2010**)

The AF is 1.5 to 2.8 times thinner dorsally than ventrally and this is why the NP is eccentrically located in the dorsal one third of a healthy non-chondrodystrophoid IVD (fig. 2).[8, 9] This location of the NP also increases the risk for extrusion or herniation of nuclear disc material dorsally towards the vertebral canal.[8]
The outer layer of the AF is composed of well-organized lamellar layers of type I collagen and the inner layer is composed of fibrocartilaginous material and are generally thicker than the outer layers. Seventy percent of the AF dry weight consists of collagen. The AF provides tensile strength and produces stability between the vertebrae.

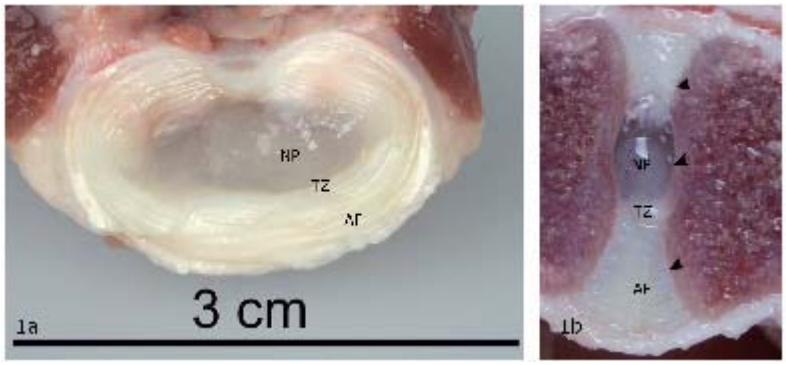


Fig. 2. Transverse (left) and sagittal (right) section through a L5-L6 IVD of a mature nonchondrodystrophic dog. The nucleus pulposus, annulus fibrosus, transition zone, and cartilaginous endplates are marked. (**Bergknut 2010**)

The central NP is a remnant of the notochord and contains 80% to 88% water and the remaining 18 % to 20% consists of disorganized type II collagen, proteoglycans, and glycoproteins. The NP acts as a shock absorber; equalizes the forces placed on the IVD and facilitates fluid exchange between the vertebra and the IVD.[8, 15, 9]
The hyaline cartilaginous vertebral endplates protect the vertebral bodies and functions as a semi permeable membrane which provides nutrients for the disc.[15, 9]
So, these three distinct structures, consisting of the NP, AF, and EP, function together to minimize shock and trauma to the spinal cord, protect the vertebral bodies, and unite the segments of the vertebral column.[9]

Pathophysiology

IVD herniation is initiated by degeneration of the disc and is associated with a loss of water from the NP. Loss of water is due to a decrease in the proteoglycan content and occurs with aging. This results in dehydration, further degeneration, and a loss of disc width. An increase in collagen content also occurs with age (fig. 3). As a result, the NP loses its ability to absorb shock and to dissipate the forces equally over the IVD.[2, 15, 9] In the AF, the proteoglycan content also decreases with age, and the ability to absorb shock decreases.[9]

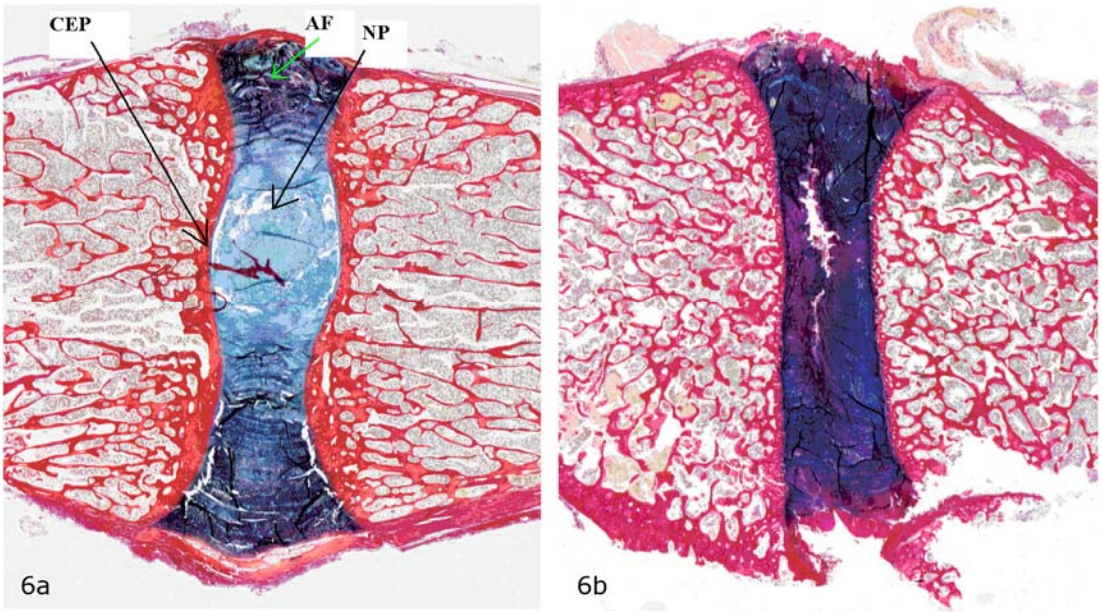


Fig. 3. Midsagittal histological sections of a) a healthy and b) a moderately degenerated canine IVD stained with picosirius red an alcian blue.
Proteoglycans are stained ligt blue (with Alcian blue) and collagen type I is colored red (with picosirius red). A clear distinction between the NP which largely consists of proteoglycans, and the AF which is composed of a mixture of proteoglycans and collagen type I can be made in section A. Unlike section B, where no clear distinction between the NP and AF can be made and an increase in collagen content can be seen throughout the IVD. A cleft transecting the NP can also be seen. (**Bergknut 2010**)

IVD Degeneration

Degeneration of the intervertebral disc can be classified in chondroid metaplasia and "fibrous" metaplasia. Chondroid metaplasia occurs in chondrodystrophoid dog breeds (Dachshund, Pekingese, French Bulldog, beagle, basset hound, American cocker spaniel, fig 4). These breeds are characterized by a disturbed enchondral ossification which finally results in short limbs.[16] The dachshund is predisposed to IVDD and develops disc herniation 12.6 times more often than other breeds.[11, 2, 8, 12] Fibrous metaplasia occurs in nonchondrodystrophoid dog breeds. Mixed breeds, German shepherd dogs, Greyhounds, Labrador retrievers, Rottweilers, Dalmatians, and Doberman pinschers are reported to develop type I IVDD. German Shepherd dogs are predisposed to develop Hansen type II IVDD, but can also develop acute Hansen type I IVD extrusions.[11, 8, 17]

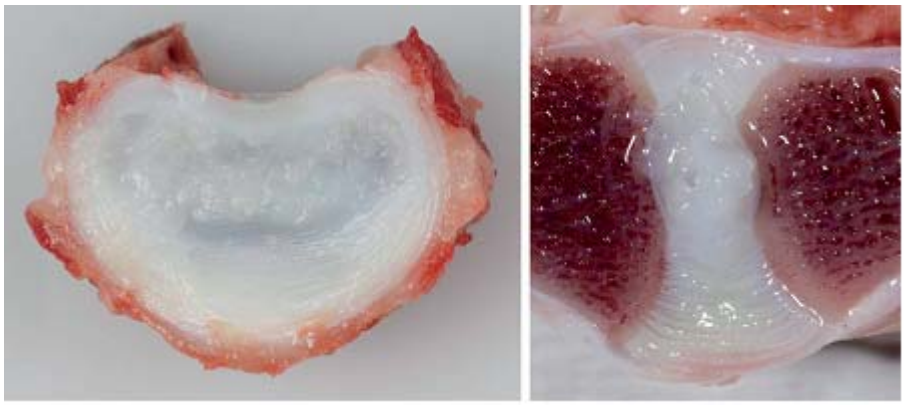


Fig. 4. Transverse (left) and sagittal (right) section through a L5-L6 IVD of a 2 year-old chondrodystrophic dog. The NP consists of fibrocartilaginous material and a widened transition zone. Unlike the AF which is normally structured (**Bergknut 2010**).

	Chondroid metaplasia	**"Fibrous" metaplasia**
Dog breed	Chondrodystrophoid	Non-chondrodystrophoid
Features	- loss of glycosaminglycans - increase in collagen content - decrease in water content	- degeneration NP and AF
Age	≥2 months of age	≥ 7 years according to literature, at an earlier age according to Ninke Gahrmann
Location	All discs along entire length of the vertebral column	A single disc along the vertebral column
Consequences	Herniation of NP material through AF into vertebral canal	Bulging of NP within AF → dorsal IVD protrusion
Calcification	Calcifications in several discs (mean of 2.3 calcified discs per dog)	Mineralization is infrequent
Gelatinous NP transformed to hyaline cartilaginous tissue	75% to 90% of the dogs by 1 year of age	High noncollagenous protein levels into old age

IVD Extrusion and Protrusion
Hansen has described two types of degenerative changes in the intervertebral discs of both nonchondrodystrophoid and chondrodystrophoid dog breeds. [2, 9]

I. Hansen type I disc degeneration is more associated with chondrodystrophoid dog breeds and occurs with subsequent extrusion of nuclear material through a total ruptured AF into the spinal canal (fig. 5). [8, 9, 18, 19] This Hansen type is characterized by replacement of mesenchymal cells of the NP with chondrocyte-type cells and this process starts in the transitional zone (separation of the NP from the AF). The gelatinous material in the NP will be

transformed to a more hyaline and fibrocartilaginous tissue. The massive extrusion of the nuclear material into the vertebral canal is also accompanied by a severe inflammatory response. Fibrinous adhesions between the extruded disc material and the dura mater occur in chronic extrusions.[8, 9] Chondrodystrophoid dog breeds can also develop Hansen type II protrusions, but to a lesser extent. IVD herniation most commonly occurs between 3 and 7 years of age in chondrodystrophoid dog breeds. IVD disease in these breeds occurs at an earlier age because of the accelarated degenerative changes in the intervertebral discs.[8, 20] IVD-related problems in these dog breeds occur generally in the cervical and thoracolumbar spine segments.[18]

II. Hansen type II disc degeneration is more associated with the nonchondrodystrophoid dog breeds and is characterized by bulging of the NP to a partial rupture and weakening of the AF, which results in annular protrusion into the spinal canal (fig. 5). This IVD protrusion is associated with fibroid degeneration by Hansen. [8, 18, 19] But in histological material Ninke Gahrmann used for her research project, not any fibrocyte was observed in NP tissue and evidence for the typical fibroid degeneration missed. Hansen type II IVD protrusion has a slower onset than the type I extrusion and occurs with aging. IVD herniations in these breeds occur at 6 to 8 years of age and are primarily recognized in large non-chondrodystrophoid dogs.[8, 21] In the study of Ninke Gahrmann, no significant difference between the mean ages of chondrodystrophoid dog breeds vs. nonchondrodystrophoid dog breeds was found, but in this research, this was the age at which patients were offered for surgical treatment at the clinic and not the age of onset of clinical symptoms.. In these dog breeds, the IVD-related problems mainly occur in the lower cervical or lumbar spine segments. [18]

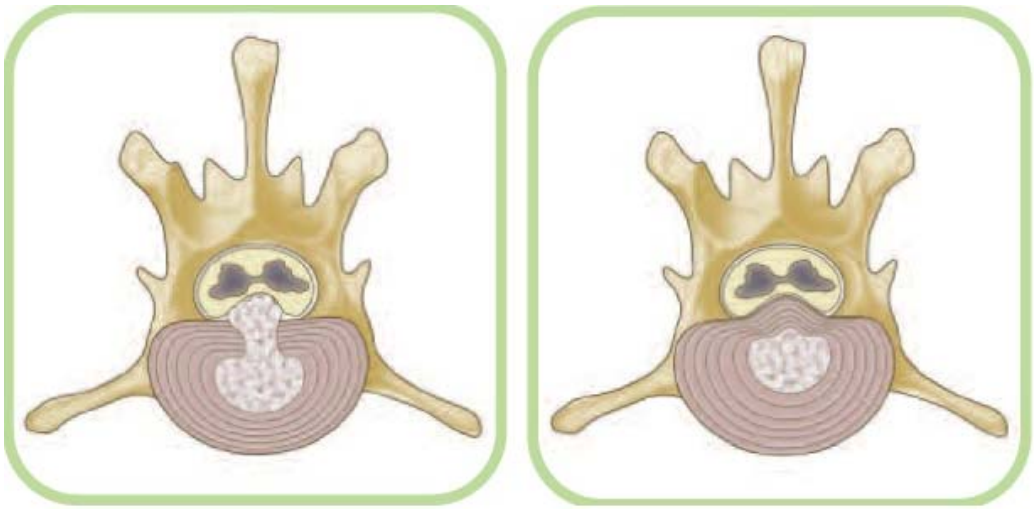


Fig. 5. Craniocaudal view of the IVD. On the left image, an extrusion of degenerate disc material through the dorsal annulus can be seen (Hansen type I IVDD). On the right image, a protrusion of intact dorsal annulus following collapse of the IVD space can be recognized (Hansen type II IVDD). (**www.vetgrad.co.uk**)

Degenerative lumbosacral stenosis (DLLS) can also be caused by Hansen type II IVD herniation and is generally seen in medium and large-breed dogs with a predisposition in German shepherd dogs. DLLS results in the cauda equine syndrome. [15, 22]

Clinical Signs

Clinical signs develop when the spinal cord is no longer able to compensate to compression and mechanical displacement. Location of the lesion, the size of the protrusion, and the dynamic force of the compression affect the severity of symptoms.[9]

Because of the relatively small ratio of spinal canal to spinal cord in the thoracolumbar region, the effects of spinal cord compression are most readily seen in this area. IVD herniation in the thoracolumbar region can result in severe pain, ataxia, and neurologic deficits that range from mild paraparesis to paraplegia with or without loss of deep pain perception (fig. 6). Sensory paralysis (absence of sensory function "deep pain") for over 24 hours guarantees a poor prognosis. When the lumbosacral area is damaged, urinary or fecal incontinence, and a low carriage of the tail may be reported. [8, 9, 15]

Fig. 6. Dachshund with paralysis posterior as a clinical sign of IVDD. (**www.vin.com by B.P.Meij).**

Diagnosis

Neurologic examination, radiography, myelography, and computed tomography (CT) or magnetic resonance imaging (MRI) are important tools to confirm the diagnosis "intervertebral disc degeneration". [8, 15]Also the medical history and the physical examination should not be left out.[9]

Information about duration of the problem, rapidity of the onset, status of the problem, history of spinal problems or trauma also are necessary to proceed with further diagnostic tests.[9]

I. Cervical IVD disease (CIVDD) is responsible for approximately 15 % of all canine IVDD cases.[23] Clinical signs in CIVDD are severe neck pain, guarding of the neck, ataxia, and muscle fasciculations without neurologic deficits. Also hemiparesis and tetraparesis or has been reported in several cases. Labrador Retrievers and Rottweilers are most commonly affected. [8, 2423]
II. IVD disease in the thoracolumbar region (TLIVDD) is associated with severe pain, ataxia, reluctance to move, aggressive behavior, varying degrees of back pain and neurologic deficits. Chondrodystrophic dog breeds are most commonly affected (with a predisposition for the Dachshund), but also the German shepherd dog is able to develop Hansen type I degeneration.[2, 8, 9, 17]

III. Clinical signs associated with DLLS are neurologic deficits (urinary or fecal incontinence), pelvic limb lameness, caudal lumbar pain, and a reluctance to perform activities. The German shepherd dogs and working dogs are predisposed to develop DLLS, and a common occurrence of DLLS has been reported in large breed dogs.[15]

The preliminary diagnosis of these IVD diseases are based on the information mentioned above, supplemented with the results of neurologic and orthopedic examinations. Imaging techniques should be used to confirm the diagnosis.[15]

Radiographic evaluation should be performed under general anesthesia to eliminate the problem of motion and facilitate proper positioning.[8, 9]
The "golden standard" for early recognition of disc degeneration is MRI, because of the clear images of the soft tissues of the spine, and precise distinction of pathologic changes. Disc calcification is supportive of disc degeneration, but IVD herniation cannot be demonstrated with IVD mineralization.[8, 19]
Radiographic changes of canine intervertebral disc herniation are characterized by:

- narrowing of the intervertebral disc space (fig. 7)
- narrowing of the articular facets
- small intervertebral foramen
- increased opacity of the intervertebral foramen (fig. 8)
- calcified material in the vertebral canal

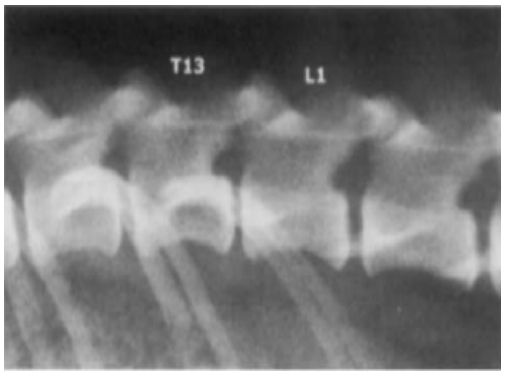


Fig. 7. Lateral radiograph of a 5-year-old Border terrier with narrowed intervertebral disc space (T13-L1) intervertebral as a potential sign of disc protrusion (**Lamb**).

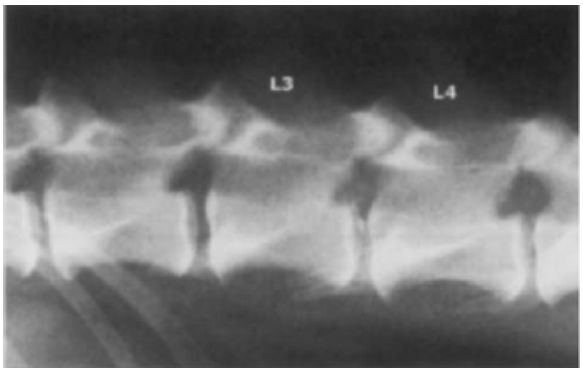


Fig. 8. Lateral radiograph of a 5-year-old mixed breed dog with increased opacity of the foramen as a sign of IVD protrusion (**Lamb**).

Imaging techniques used to confirm the diagnosis of CIVDD are radiography, myelography, computed tomography (CT) or magnetic resonance imaging (MRI). The diagnosis of TLIVV can also be confirmed with the same imaging techniques as CIVDD. MRI is superior to myelography for determining lesion localization and lateralization, and radiographs and myelopgraphs are more accurate than CT.[8]
To diagnose DLLS, convention radiography, stress radiography, epidurography, and discography have been used. But also CT and MRI are diagnostic tools to confirm the diagnosis of DLLS. [15]

Chondrodystrophoid dog breeds are susceptible to Hansen type I disc degeneration and release a large volume of mineralized nucleus material into the spinal canal,

which results in narrowing of the intervertebral disc space and increased opacity of the spinal canal.
In non-chondrodystrophoid dog breeds is it more difficult to recognize radiographically Hansen type II disc degeneration, because of the less extruded mineralized disc material within the vertebral canal.[25]

Treatment
The treatment for intervertebral disc degeneration in common can be classified in a conservative treatment, a surgical treatment, or a combination of both.
CIVDD can be treated with cage rest, anti-inflammatory drugs, and muscle relaxants, but when the dog does not respond to medical management, decompressive surgery is required. Decompressive surgery is also required when acute severe neurologic deficits are observed. Further, persistent pain, muscle spasm, and perceptible neurologic deficits are indications for a surgical treatment. Ventral slot decompression is most commonly used, but also dorsal laminectomy, and hemilaminectomy (fig. 9) can be considered. [9, 24]
Medical management of *TLIVDD* includes cage rest (prevention of nuclear extrusion through the ruptured AF), corticosteroid therapy, and muscle relaxants. Indications for medical therapy include ataxia, pain, and paresis.[8, 9] Surgical treatment is required when dogs do not respond to medical therapy, or in case of severe or progressive neurologic deficits, and for patients with minimal neurologic deficits or back pain alone.[8, 9] Surgical decompression of extruded disc material can be performed through lateral, dorsolateral, and ventral fenestrations, dorsal laminectomy, and hemilaminectomy.[8, 9, 26] The last treatment, hemilaminectomy, is usually obtained. And fenestration is often performed as a prophylaxis to prevent continued extruded material postoperatively.[9, 26]

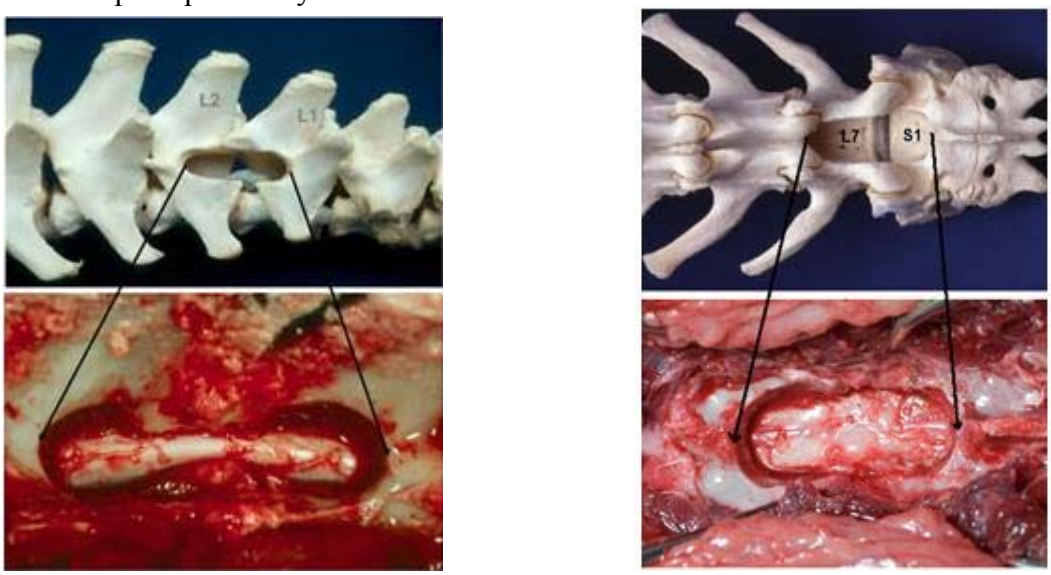


Fig. 9. Hemilaminectomy (left) of a L1-L2 segment and a dorsal laminectomy (right) of a L7-S1 segment (**B.P. Meij**).

Medical therapy of *DLLS* includes body weight reduction, a change in exercise pattern, and the use of NSAIDs. When dogs respond temporarily respond to this conservative treatment or when dogs show neurologic deficits, surgical treatment is recommended. Dorsal laminectomy is most commonly used.[22] This surgical

procedure may be extended with dorsal annulectomy and nucleotomy, unilateral or bilateral foramenectomy, and rarely, facetectomy.[15, 22]

Intervertebral disc degeneration can also be treated with regeneration of the damaged intervertebral disc. Disc degeneration results in a loss of proteoglycans and disc height and directly affects the biomechanical function of the IVDs. Treatments to repair the intervertebral disc tissue are:

- Exogenous growth factors which are able to stimulate the synthesis of proteoglycans in the NP. Biosynthesis of the nucleus increased fivefold by TGF-β, and the transition zone proliferation increased threefold by EGF.[27]
- Genetic modification of the disc cells through gene transfer so that the cells continuously manufacture the desired growth factor themselves.[28]
- Autologous chondrocyte transplantantion which is able to repair disc damage, retard disc degeneration, and restore disc height. The disc chondrocytes produces components similar to normal IVD tissue (proteoglycan content and both type I and type II collagen).[6]
- Direct injection of the protein (osteogenic protein-1, also known as BMP-7) into the NP or AF which is a new treatment for IVD degeneration, but proper nutrition must be available.[29]

Another treatment to prevent further degeneration and recreate the natural function is replacement of the nucleus pulposus. The NP can be replaced with hydrogel polymers or non-hydrogels polymers (fig. 10).[30, 31]
An example of a hydrogel polymer, which is implanted after nuclectomy, is the nucleus pulposus prosthesis (NPP) which is used in a research project of Bergknut et al (2010). A NPP consists of the hydrogel N-vinyl-2 pyrrolidinone copolymerized with 2-(4'-iodobenzoyl)-oxo-ethyl methacrylate. This NPP is able to swell *in situ* to fill the nucleus cavity, and significantly restore disc height (in 8 of 10 dogs). It also has intrinsic radiopacity and enables imaging by radiography or CT, and MRI. After implantation, disc height and mobility of the spinal segment will be restored; this may result in reduced pain and further degeneration of the spinal segment.[3]

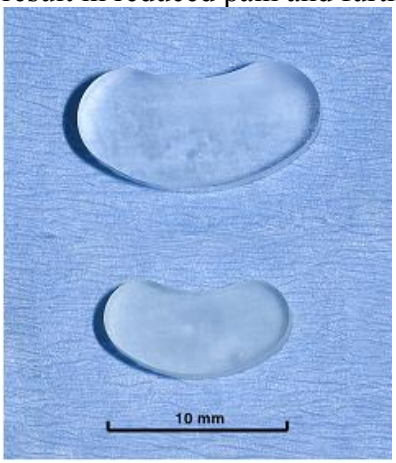


Fig.9. The nucleus pulposus prosthesis: comparison between a swollen/hydrated (above) and xerogel/dry state (beneath) prosthesis. (**Bergknut 2010**)

The NP can also be replaced by non hydrogel polymers which have some shock absorption capabilities. Hydrogels and non-hydrogels are classified as elastomeric and

are either preformed or injectable (fig. 11). Mechanical devices (nonelastomeric) could also be used to replace the nucleus pulposus and can be subdivided into 1-and 2-piece designs.[30, 31]

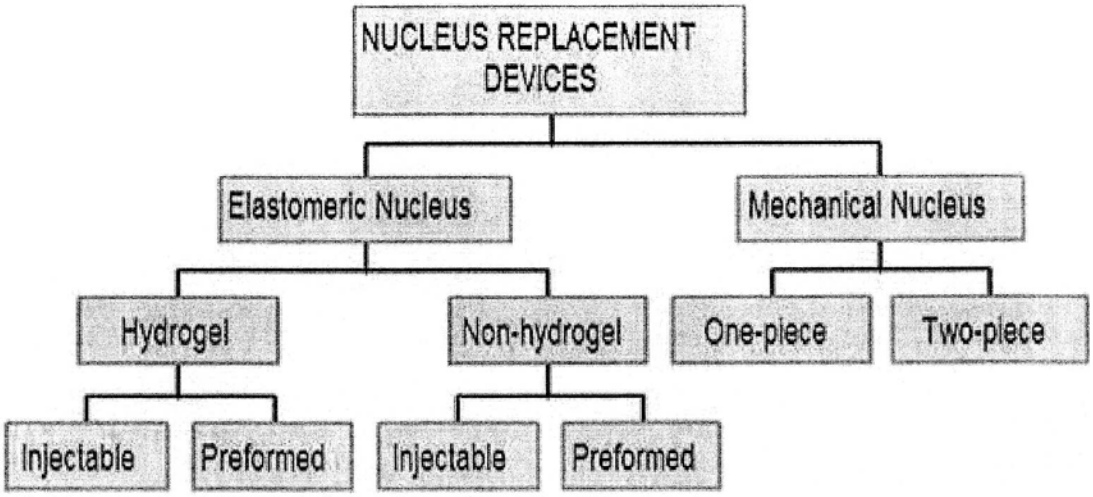


Fig. 10. Classification of elastomeric-and mechanical nucleus pulposus replacements. (**Coric 2008**)

Research Aims

In this study we used a second generation preformed nucleus pulposus prosthesis (NPPv2.0) to restore the axial disc height. This study contains three research aims:

1) A literature review on canine IVDD.
2) To answer the research question: "Will the axial disc height be restored, both before and after biomechanical testing*, when the NP is replaced with a preformed NPPv2.0?"
3) To evaluate two radiographic measurement methods for determining the restoration of axial disc height?

*
Biomechanical testing will be explained in the chapter "Materials & Methods".

Materials & Methods

Biomechanical cadaveric study

Second generation Nucleus Pulposus Prosthesis (NPPv2.0)
A NPPv2.0 consists of the hydrogel N-vinyl-2 pyrrolidinone copolymerized with 2-(4'-iodobenzoyl)-oxo-ethyl methacrylate. In this study, eleven prostheses were used and two of them were hydrophobic cored NPPs and nine of them were hydrophilic cored NPPs. Before the research project actually started, two of these nine hydrophilic cored NPPs were used in a pilot study. But both types of prostheses were hydrogel polymers and therefore by definition hydrophilic. Because of the intrinsic radiopacity of these prostheses, they could be visualized by radiography in this study.

Specimen preparation
Eleven lumbar spines were isolated from cadavers of healthy Beagles (weight range 11.0 – 17.0 kg; age approximately 2 years of age) and frozen at -20°. The spinal segments included the T13 to L6 spine. In preparation for biomechanical testing, the lumbar spine was thawed at room temperature for 24 hours.
The spinal segments were cut at T13-L1 and at L5, and cleaned of all residual musculature, with care taken to preserve all ligamentous tissue, so that the final specimen includes L1 to L5. Next, the transverse process of L2 was removed. Screws were inserted along the axis from T13 to L1 and in L5 for fixation, and the cranial (T13-L1) and caudal (L5) lumbar ends were embedded in two metal cups (fig. 11C). These cups were filled with heated (60°C) cerro-low147 (Cerro Metal Products Co., Bellefonte, PA).
Three L-shapes flags were attached to the ventral side of each vertebral body (L2, L3 and L4). Each flag contains three light emitting diodes (LEDs), used to record the movement of the spine during each loading cycle. The specimens were wrapped in moist towels before testing and were kept moist by regular spraying with saline (0.9 % NaCl) solution, during biomechanical testing.

Specimen testing
Three important devices were used to test the spinal specimens. First of all, a hydraulic materials testing machine (Instron Model 8872, Instron Corporation IST, Toronto, Ontaria, Canada, fig. 11B), which applies the load onto the spinal specimen. In this case, the load onto the segments was maximum 2 Nm (physiological moment). Subsequently, a 4-point bending device was used for bending the spine. A constant bending moment from L2 to L4 was subjected to the spinal segments by this device. Each loading cycle consists of one complete flexion-extension cycle, once complete lateral bending cycle, and one axial rotation cycle. These bending moments will be started and increased (at a constant rate) to the physiological moment of 2 Nm. This loading direction will also be reversed to a maximum of 2 Nm. In order to obtain reliable data, the loading cycle will be repeated 3 times in all three planes. These two devices were connected to each other.
To record the movement of the three L-shaped flags, an optoelectronic 3D movement registration system (Optotrak 3020, Northern Digital Inc, Waterloo ON, fig. 11A) was

used. Displacements and loads can be determined from angles and moments by this device.
A computer was used to get the recordings from the materials testing machine and these recordings were saved to the hard disc of the computer and will be used for analysis.

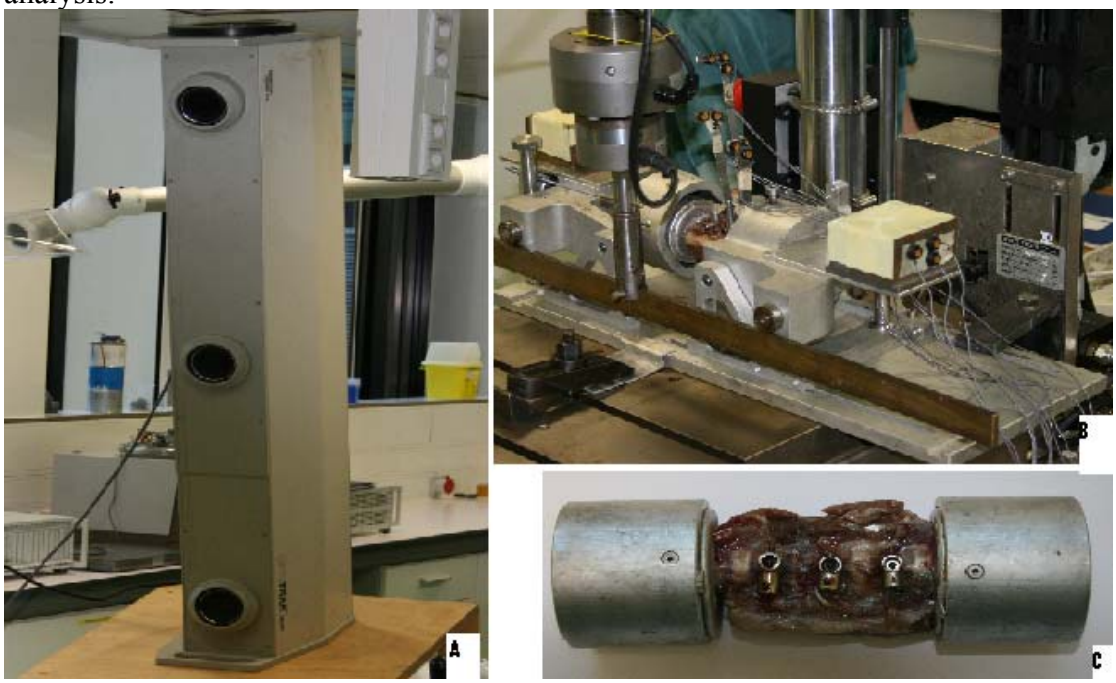


Fig. 11. Photograph of a cadaveric canine lumbar spine specimen fixed in two metal cups (A) for testing on a 4-point bending hydraulic testing machine (B) for load–displacement measurement during flexion (C) (**H.C. Kranenburg)**.

Testing steps
The spinal specimens were tested in three series of recordings:

1. Intact spine. This step will generate baseline values, which can be used in subsequent comparisons for each individual spine segment.
2. After surgical nuclectomy of the L2-L3 spinal segment.
3. After surgical implantation and swelling of the new preformed NPPv2.0 into the lumbar segment.

Each series consisted of three loading cycles and the sampling rate for both load and displacement measures were 50 samples/s. After each series, the spinal segment was removed from the testing machine to undergo the different surgical techniques.

Surgical implantation of the NPPv2.0
A nuclectomy of the L2-L3 disc has preformed in the second series. A surgical blade number 11 was used to make an incision in the middle of the IVD. Grasping forceps and a ball-tipped probed were used to remove the NP (fig. 12-2). After removal of the nucleus material, an aluminium test prosthesis was used to probe the nuclear cavity (fig. 12-1, in this photograph respectively, a NPPv2.0 without swelling, a swollen NPPv2.0 in the nuclear cavity and a freely swollen NPPv2.0 in a 50 mL tube with NaCl saline can be reported). In the third series, the NPPv2.0 was inserted into the nuclear cavity through a 5-mm transverse incision in the dorsal annulus fibrosus (fig. 12-3).

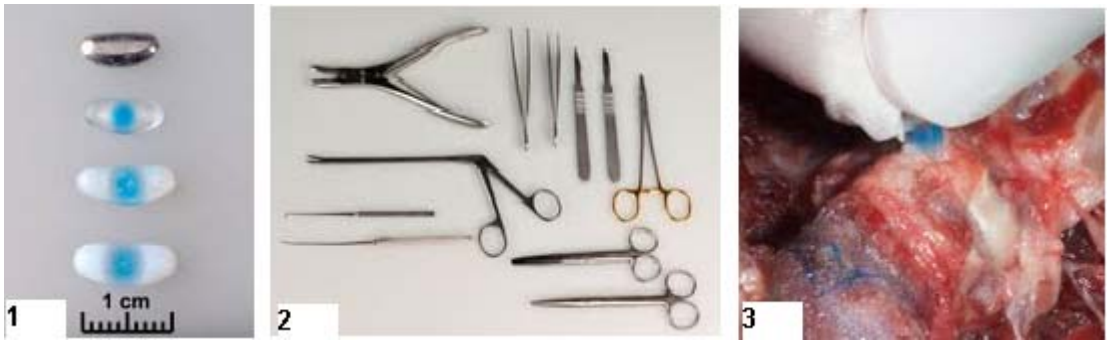


Fig. 12. Photograph of the NPPv2.0 (1), surgical instruments (2), and surgical implantation of the NPPv2.0 (3) (**H.C. Kranenburg).**

The next step was closure of the annular incision which was preceded by transplantation of fascia within the annular canal. Subsequently, the annular gap was closed with three stitches across the incision using PDS 3-0, and reinforced with tissue glue (Dermabond, Ethicon INC., Amersfoort, the Netherlands). Finally, a piece of polypropylene mesh was glued over the annular effect.
Before the spines were incubated at a temperature of 37°C for 18 hours (to achieve complete swelling of the implant), the remaining musculature was injected with amoxicillin clavulaanacid to prevent bacterial overgrowth. Thereafter, the spines were wrapped in a plastic bag with PBS to prevent dehydration of the spine segments and the NPPv2.0.
The day after, when biomechanical testing has occurred, the swollen NPPv2.0 was removed from the disc by an incision closely to the cartilaginous endplate. A surgical blade 11 was used to make this incision through the AF. The NPPv2.0 was removed to evaluate macroscopically (intact/*in situ*/shape) and thereafter, the prosthesis was weighted.

Radiographic study

Radiographic study of the NPPv2.0
Lateral radiographic views of spine 1 to 11 were obtained at six different conditions:

1. the native spine, before biomechanical testing, to confirm the natural disc height of the IVD
2. the native spine, after biomechanical testing, to determine whether the disc height changed by biomechanical testing,
3. after performing nuclectomy and before biomechanical testing, to obtain the loss in disc height,
4. after nuclectomy and biomechanical testing, to determine whether the loss in disc height changed by biomechanical testing,
5. after implantation of the NPPv2.0 and incubation of the specimen overnight at 37°C and before biomechanical testing, to evaluate whether natural disc height was restored,
6. after implantation and biomechanical testing of the NPPv2.0, to determine whether natural disc height was restored and the prosthesis provide physiological stability to the IVD.

The localization of the NPPv2.0 in the nuclear cavity was also important to be visualized. Therefore, dorsoventral radiographic views have also been taken in condition five.
The L2-L3 IVD height was measured by two independent observers on standard computer screens and two different measurement methods were used to measure the IVD height on lateral radiographs. The radiographs were also subsequently blinded and randomized. Both observers used the software program Image J® and both measurement methods were performed twice by each observer. Image J® calculates in pixels, but finally all values were converted to millimeters. All results were stored in a Microsoft Excel® file.

Method 1
The first method to measure the disc height was based on an article of Hofstetter et al. (2009) and has been further developed by N. Bergknut and L.A. Smolders.[34] First of all, the deepest points of the clefts in both cranial and caudal vertebra need to be determined. Subsequently, a single line from the cleft of the cranial vertebra to the caudal vertebra must be drawn (Fig. 13).

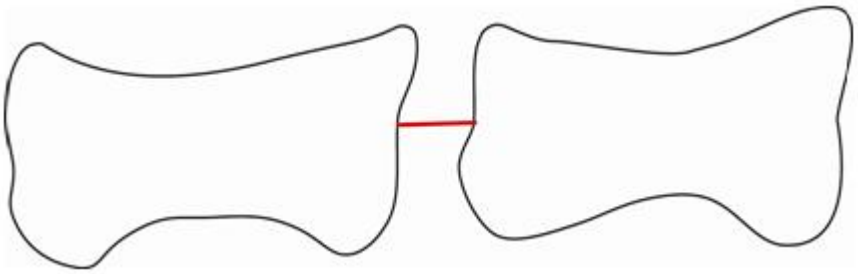


Figure 13. Method 1 for measuring disc height. A single midline is drawn. **(H.C. Kranenburg)**

Method 2
The second method to measure the IVD height was based on an article of Twomey and Taylor (1985) and has been further developed by H.C. Kranenburg.[35] Measuring disc height was performed by first drawing a ventral line from the corner of the cranial vertebra to the corner of the caudal vertebra. Next, a single line is running from the corner of the cranial ventral vertebra to the corner of the cranial dorsal vertebra and in the middle of this line a dot is drawn. Then a second line is drawn (near the point) parallel to the first ventral line, which represents the disc height and extends between the clefts of the cranial and caudal vertebrae (fig. 14).

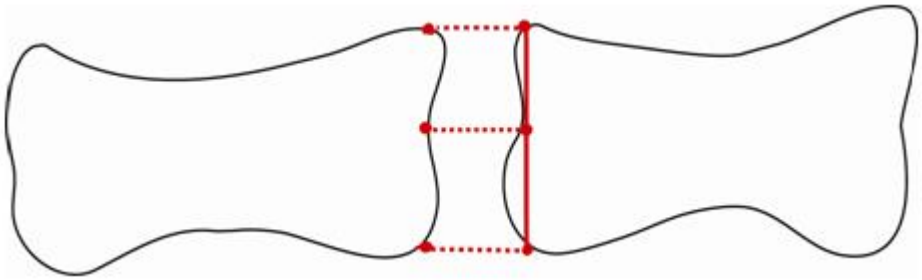


Figure 14. Method 2 for measuring disc height. A ventral line and midline is drawn. **(H.C. Kranenburg)**

Statistics

Data and statistical analyses

The software SPSS® was used for data and statistical analyses. Intra- and interobserver reliability was analyzed for each measurement method. The mean disc height ± standard deviation (SD) also was calculated for the effect of condition on disc height (native, nucleotomy and after insertion and swelling of NPPv2.0). A linear mixed model was used to compare them.

"Observer" (two levels: observer 1 and 2), "moment" (three levels: moment 1-3), "test moment" (two levels: before and after biomechanical testing), "method" (two levels: method 1-2) and "number of measurement" (two levels: measurement 1-2) were set as fixed effects, whereas "spine" (11 levels: spine 1-11) was assigned to a random effect. LSD (none) 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. Only the spinal segments where the preformed NPPv2.0 stayed intact and *in situ* were included in the statistical calculation.

Results

Biomechanical cadaveric study

Evaluation of the surgical insertion of the NPPv2.0
Insertion of the implant itselves was a relatively simple operation, depending on the preceding nuclectomy. Insertion of the fascia in annular canal and suturing the incision in IVD took most of the time.
The implant was visualized with lateral and dorsoventral radiographs, before and after overnight swelling. Totally 11 prostheses were used and 82 % (9/11) of the NPPv2.0 were hydrophilic cored prostheses and the other two prostheses were cored hydrophobic. All NPPs that stayed *in situ* also remained intact.
The two hydrophilic cored NPPs, used in the pilot study, did not remain *in situ* and were herniated through the annular incision and one of them was even fractured, probably because of its very eccentric position into the annular canal.
Of the hydrophilic implants, used in our actual study, all NPPs (7/7) remained *in situ* and intact. At the beginning of our study, the prostheses were located slightly eccentric, but during investigation they were located at more central position. Both hydrophobic prostheses stayed *in situ* and intact, but also slightly eccentric. Both types of prostheses (hydrophilic and hydrophobic) could be visualized by radiography (Fig. 15).

Fig. 15. Hydrophilic cored NPPv2.0 (left image, blue core) and hydrophobic cored NPPv2.0 (right image, red core), both after swelling. **(H.C. Kranenburg)**

Stability of the spinal segment
After nuclectomy, the range of motion (ROM) of the spinal segment (L2-L3) significantly increased, which results in a decreased stability of the spinal segment. After implantation and swelling of the NPPv2.0 overnight, the ROM significantly decreased, and this finally results in a restoration of the stability of the spinal segment. Further biomechanical results of this cadaveric study will be discussed by David Onis in his honor programme report.

Radiographic study

Lateral radiographic views were obtained at the six different conditions and measured with both measurement methods. Good representations and less representative examples were obtained with these two methods.

Loss of disc height after nuclectomy

After nuclectomy and using method 1 to measure the IVD height, there was a significant difference ($p<0.001$) between the mean disc height in the native spine and that after nuclectomy. The mean disc (± SD) height for the spinal segment in the native state was 3.663 ± 0.107 mm and 3.385 ± 0.106 mm after nuclectomy.
When compared with method 2, there was also a significant difference ($p=0.000$) in losing disc height after nuclectomy. The mean disc (± SD) height for the spinal segment in the native state was 3.546 ± 0.110 mm and 3.329 ± 0.110 mm after nuclectomy (fig. 16).

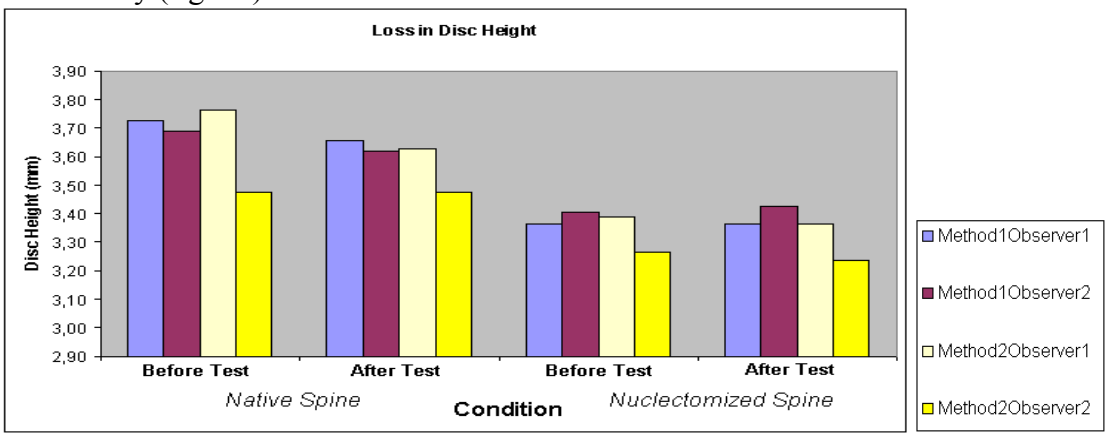


Fig. 16. Loss in disc height after nuclectomy, measured twice by two observers with two different measurement methods.

Disc height restoration after insertion and swelling of the NPPv2.0

I. After insertion and swelling of the NPPv2.0, and making use of measurement method 1 (developed by N. Bergknut and L.A. Smolders) to measure the IVD height, a significant difference ($p<0.001$) between the mean disc height after nuclectomy and that after implantation and swelling of the NPPv2.0 was found. The mean disc (± SD) height for the spinal segment after nuclectomy was 3.385 ± 0.100 mm and 3.706 ± 0.100 mm after insertion and swelling of the NPPv2.0 (fig. 13).
II. The same results were obtained after using measurement method 2 (developed by H.C. Kranenburg). A significant difference ($p<0.001$) was also found and the mean disc height after nuclectomy was 3.329 ± 0.105 mm and 3.647 ± 0.105 mm after insertion and swelling of the NPPv2.0 (fig. 17).

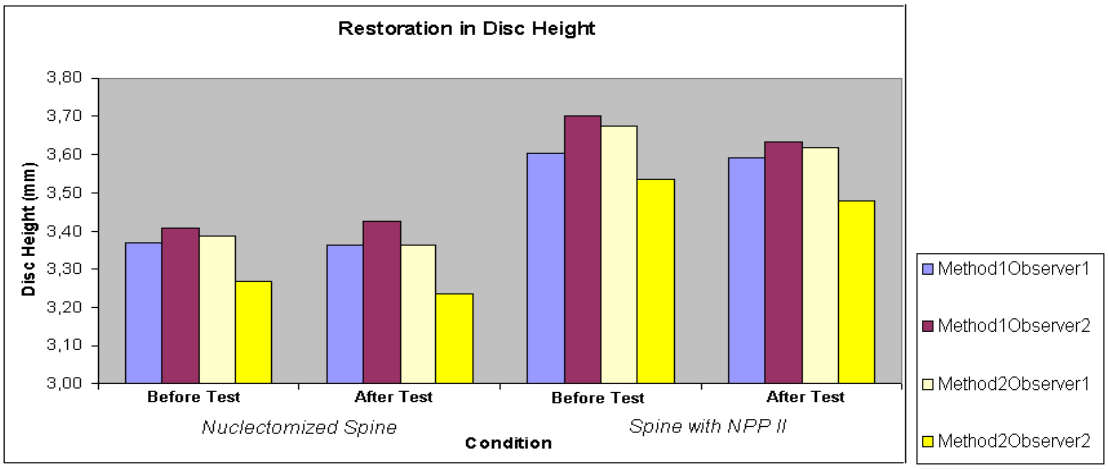


Fig. 17. Disc height restoration after insertion and swelling of a preformed NPPv2.0, measured twice by two observers with two different measurement methods.

Intra-and interobserver reliability

I. Method 1 had the best intraobserver reliability regarding to observer 1, but showed a significant difference between the two measurements of observer 2. The interobserver reliability between the two observers for measurement method 1 also showed a significant difference (table 1).
II. Unlike method 2, this had the best interobserver reliability with no significant difference between the two observers. On the other hand, the intraobserver reliability of observer 1 is lower compared to method 1, and observer 2 showed also a significant difference with method 2 (table 1).

Table 1. Inter- and intraobserver reliability of the two used methods for disc height measuring.
* = statistical significant.

	Method 1 (p-value)	Method 2 (p-value)
Interobserver (observer 1-2)	0.014*	0.455
Intraobserver (measurement 1-2)		
Observer 1	0.369	0.093
Observer 2	0.000*	0.033*

The biomechanical tests did not influence the restoration of the intervertebral disc height. No significant difference was found before and after biomechanical testing at any time (p>0, 05).

Discussion

Biomechanical cadaveric study

The intervertebral disc needs a proper disc height to function normal. Dorsoventral and lateral radiographs can be taken with digital radiography to measure the IVD height.[3]

Increased loading on the NP decreases matrix protein biosynthesis, which results in disc degeneration. An increase in intradiscal pressure can cause a further decrease in disc height, because of a reduced fluid transfer into the disc. A loss of disc height also results in instability of the spine, because of an increased range of motion and a decreased stiffness of the vertebral column. A decrease in volume of the foraminal canal, as a result in loss of disc height, may also result in radicular pain.[32]

All these things can result in back pain. Reduction in disc height can also lead to more stress in the remaining annulus and facet joints, which results in further degeneration.[33]

To restore disc height and provide physiological stability to intervertebral discs, the results of this study showed that nucleus pulposus prosthesis of the second generation for vital nucleus replacement is able to do this.

Surgical insertion of the NPPv2.0

The NPPv2.0 fills the nuclear cavity, and this is required to provide an even pressure over the endplates, whereby further degeneration of the IVD will be prevented.[36, 37] Migration of the NPPv2.0 will be prevented because the prosthesis absorbs water after one night incubation. This swelling is also important to restore the IVD height.

The position of the NPPv2.0 was not always perfectly in the middle, but slightly eccentric to the insertion of the pole (fig.18). The slightly eccentric position of the NPPv2.0 can probably be explained by an annular closure technique, which doesn't close the annular canal at the transitional zone. This allows the prosthesis to move throught the AF-NP transition zone and in the annular canal.

Another explanation could be that we, as inexperienced surgeons, in the beginning were not able to completely remove all the remaining NP tissue from the nuclear cavity and the NPPv2.0 came to be slightly eccentric.

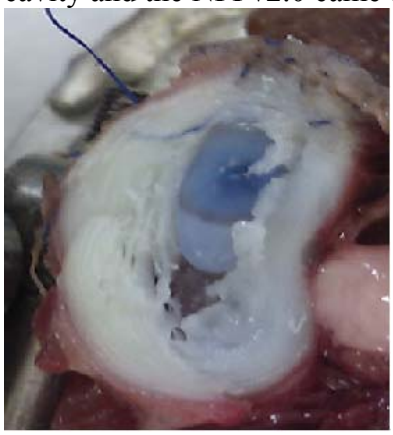


Fig. 18. Slightly eccentric position of the NPPv2.0

All NPPs that stayed *in situ* also remained intact. In the pilot study, two of the hydrophilic prostheses migrated and one of these was even fragmented. The two

hydrophobic prostheses stayed *in situ* and intact. One of the major limiting factors for successful IVD engineering after disc herniation is the lack of sufficient strategies to deal with the damaged AF.[38] Herniation can be prevented when the annular incision can be decreased and the annular-closing technique will be improved.
Cross stitches might be important, because in 7/7 spinal segments in which these stitches were applied, all prostheses stayed *in situ* and intact. Deep tightening of the fascia seems to work, although the technique still needs to be improved. Tightening reins on both ends would be a good way (fig. 19 and 20). When the problem with the annulus closure is solved, this NPPv2.0 might be a good alternative to the current surgical strategies for IVDD.

Ideally in a test situation all essential procedures, for instance AF suturing, should be executed by one single person to minimalize the number of variables. The spines should also be prepared in an exact way. In this study spines were prepared by three different researchers and each person cleaned the spinal segments of residual musculature and ligamentous tissue on his own way, which results in more or less musculature on the segments. This could have an effect on the stability of the spine and subsequently on the range of motion of the spine.

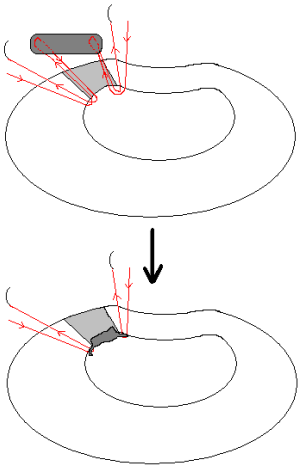


Fig. 19. Diagram of securing fascie with reins.

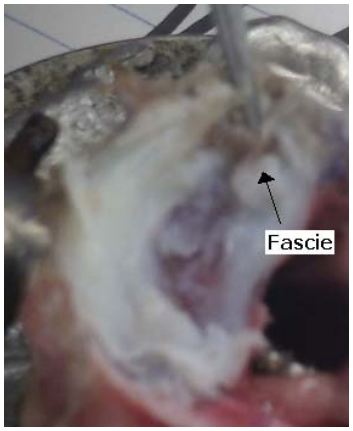


Fig. 20. Annulus closure with fascie.

Whether the NPPv2.0 was able to restore the natural biomechanical properties, I would like to refer you to honor program report of David Onis.

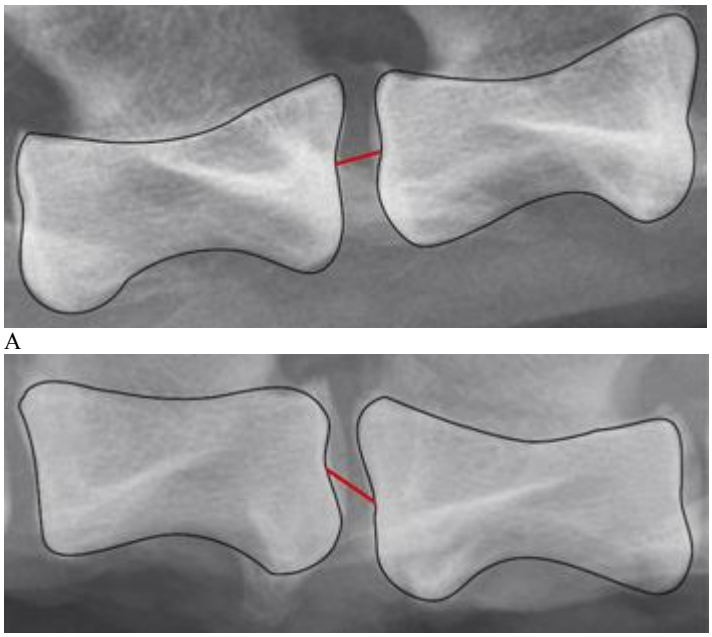
Radiographic study
Two measurement methods were used to obtain IVD height on lateral radiographic views. Dorsoventral radiographic views have also been taken to visualize the localization of the NPPv2.0.

To determine what the best method for measuring disc height, several factors have to be taken into account:

1. practical usefulness
2. interobserver reliability
3. intraobserver reliability

Method 1

This method needs only one single line to be drawn, in terms of practical usefulness this method is the best. It is less time-consuming than method 2 and the clefts are easy to recognize in most cases. Occasionally, the vertebras are not exactly opposite each other and the line which is drawn is not representative for the IVD height (fig.21).
In terms of interobserver reliability, method 1 is less useful. There is a significant difference between the two independent observers when making use of method 1, after the consensus meeting.
When intraobserver reliability of method 1 is compared between the two independent observers, observer 1 showed no significant difference between the two measurements, whereas observer two showed a significant difference. Unfortunately, I cannot think of an explanation for this remarkable difference.



A

B

Fig. 21. 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. **(H.C. Kranenburg)**

Method 2

When this method is compared to method 1, regarding to practical usefulness, then there could be reported some differences. First of all, method 2 is more time-consuming than method 1, because more lines have to be drawn before disc height can be determined. This does not mean that method 2 is less useful to determine the axial disc height. It is also difficult to determine the corner of the cranial dorsal vertebra, because the boundary of that angle is less clear. But this has nothing to do with the method itself. The quality of the radiographic views in this case leaves much to be desired.
And with this method, just like method 1, the vertebras are not always exactly opposite to each other and the middle line is not completely representative for the disc height. The middle of the line, drawn from the corner of the cranial ventral vertebra to the corner of the cranial dorsal vertebra, is not always exactly the deepest point of the

cleft. But if this principle is applied to all radiographic views, it gives a good representativeness of the restoration of the disc height.
The interobserver reliability for this method showed no significant difference, which means that method 2 was applied in a similar way by two independent observers. This method had the lowest intraobserver reliability for observer 1, but still no significant difference was reported. A significant difference is already observed between both measurements, regarding to observer 2.

Generally, a protocol must be drawn for both measurement methods and taking radiographic views. Subsequently, a control segment measurement should be included. The parameters in this study should also vary as little as possible, but this depends on several factors. For example, the cadaveric specimens released for this study; these specimens had a relative range of weight-and age and that might influence the outcome of the biomechanical tests and the ability of the disc height to be restored after insertion and swelling of the NPPv2.0.

Conclusion

The main research question in this study was; "Would the axial disc height be restored after implantation of a preformed second generation hydrogel NPPv2.0 in a nucleotomized disc, also after applying biomechanical tests?
Based on the results above, this study showed that NPPv2.0 was able to restore the axial disc height of the nuclectomized lumbar IVD after replacement of the nucleus pulposus by a preformed NPPv2.0 and application of biomechanical tests.
Another important outcome of this study was that all NPPs that remained *in situ* also remained intact.
The radiographic views were used to evaluate this restoration of axial disc height and were measured by using two different radiographic methods; method 1 described by Hofstetter et al. and method 2 described by Twomey and Taylor. Our results reveal that, in terms of practical usefulness, the method developed by Hofstetter et al. is most easily manageable to evaluate disc height restoration. The method developed by Twomey and Taylor revealed to be the best method for measuring disc height in lumbar segments when intra-and interobserver reliability is concerned. Choosing the best radiographic measurement method depends on where to focus is in veterinary practice.
Before we can test the NPPv2.0 *in vivo* in dogs some additional tests of the physical en biomechanical properties of the NPPv2.0 needs to be performed but results are looking promising. In the future the NPPv2.0 may be a valuable addition to the surgical treatment options currently available for canine and human IVDD patients.

Acknowledgements

First of all, I would like to thank Hendrik-Jan Kranenburg and Bjorn Meij, my supervisors for helping me with this research. They gave me the opportunity to perform this study about the restoration of axial disc height after insertion and swelling of the NPPv2.0. I have learned a lot about performing research, the pros and cons involved in this type of work.

I also would like to thank David Onis, because we did a lot of teamwork in the last few months and he helped me with the practical part of this study in Amsterdam and also with the statistical analyses required to explain the results. Subsequently, I would like to thank Albert van der Veen, for helping David and me with the biomechanical tests at VU medical centre.

And finally, I would like to thank Julie Huizinga, because this is a sequel of her study and she exactly explained the two measurement methods I made use of to measure the restoration of the axial disc height.

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