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The performance of a hydrogel nucleus pulposus prosthesis in an *ex vivo* canine model

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ABSTRACT

A nucleus pulposus prosthesis (NPP) made of the hydrogel N-vinyl-2-pyrrolidinone copolymerized with 2-(4′-iodobenzoyl)-oxo-ethyl methacrylate has recently been developed. The special features of this NPP, i.e. intrinsic radiopacity and its ability to swell *in situ* to fill the nucleus cavity and restore disc height, were investigated *ex vivo* in canine spinal specimens. L7-S1 intervertebral discs were isolated from three canine spinal specimens, and the dimensions of the nuclei pulposi were measured. Based on these averaged measurements, the NPP prototype was made and inserted in its dry form (xerogel) into a canine cadaveric spinal segment and allowed to swell overnight at 38 °C. The integrity of the NPP and the filling of the nucleus cavity were assessed before and after swelling, using radiography, computed tomography, and magnetic resonance imaging. The ability of the NPP to restore disc height was assessed on radiographs of 10 spinal specimens. Thereafter the NPP was macroscopically assessed *in situ* by dissection of the spinal specimen.

Both on imaging and macroscopically, 9/10 NPPs appeared to have a near perfect fit and disc height was restored in 8/10 spinal segments. The NPP may thus be an acceptable treatment option for low back patients meeting the requirements for NPP treatment.

1. Introduction

Low back pain (LBP) is a major health problem in the Western world, [1,2] and its incidence has increased dramatically over the last two decades [3]. The most common cause of back pain is believed to be intervertebral disc (IVD) degeneration [4,5]. IVD degeneration can apart from discogenic pain also lead to spinal instability and stenosis of the spinal canal causing severe clinical symptoms. Most patients suffering from IVD degeneration respond well to conservative and medical treatment. Surgical treatment is preserved for patients refractory to medical treatment. The most common surgical treatment of these patients is decompressive surgery combined with spinal fusion. The primary aim of this procedure is to reduce pain. It does however leave the patients with altered biomechanical properties of the spine which can lead to adjacent segment degeneration [6,7]. For patients with IVD degeneration at a single level, without spinal canal stenosis, total disc replacement can be a treatment option. Total disc replacement, contrary to spinal fusion, preserves near normal biomechanical functionality of the spinal segment. However, severe complications such as implant migration and failure may occur [8,9].

New treatments aim at being minimally invasive and at intervening earlier in the degenerative process by restoring the nucleus pulposus (NP) function and/or supporting regeneration of the IVD. Regenerative treatments have been investigated but in experimental settings only [10–12], and although minimally invasive surgical procedures coagulating the NP or the posterior annulus are currently used, they are considered controversial [13,14]. One drawback is that they reduce IVD height and do not restore the normal biomechanical function of the NP, leading to further

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degeneration of the spinal segment [15]. Another treatment option is implantation of a NP prosthesis (NPP) after nucleotomy of the degenerated disc, but this procedure requires that the annulus fibrosus is intact. The Prosthetic Disc Nucleus (PDN)[1], which is commercially available, has been implanted in over 5500 human patients worldwide [16,17]. Criteria for the use of any NPP are: 1) disc degeneration manifest by morphologic changes in the NP, 2) a competent annulus fibrosus, and 3) a minimal IVD height of 5 mm [18,19]. These requirements mean that the implant is suitable for only a small group of patients with low back pain, but in these patients implantation will restore disc height and mobility of the spinal segment, thereby relieving pain and may also prevent further degeneration of the spinal segment [20–23] as well as adjacent segment degeneration, which is a common problem after spinal fusion [6,7]. Although preclinical and preliminary clinical data show promising results [16,17,24], long term clinical studies are still lacking.

However, most commercial NPPs are not designed to completely fill the nuclear cavity. Complete filling of the nuclear cavity and even distribution of the load over the endplates can halt further degeneration of the annulus fibrosus, whereas incomplete filling is likely to lead to incorrect biomechanical loading and to progression of annular degeneration or even implant migration [20–22]. Boelen et al. proposed applying custom-made synthetic hydrogel NPP implants designed to precisely fill the nuclear cavity [25]. The NPP is made of a radiopaque synthetic hydrogel biomaterial in its water-free state, taking into account the increase in size due to three-dimensional swelling of the material *in situ*. The NPP is implanted in its dry state, so that only a small annular opening is needed, and then absorbs fluid from the surrounding tissue and swells to fill the nucleotomy cavity and thus restore disc height. The radiopacity of the implant enables imaging by radiography or computed tomography (CT), and the absorbed fluid makes magnetic resonance imaging (MRI) possible.

The aim of this study was to test this NPP *ex vivo* in canine lumbosacral segments (L7-S1). A clinically adapted mode of implantation of the NPP in the nuclear cavity of the L7-S1 intervertebral disc is reported. Swelling, fit, and restoration of disc height of the NPP *in situ* were monitored by radiography, CT, and MR imaging.

2. Materials and methods

2.1. Manufacturing of the nucleus pulpous prosthesis

The NPPs were made in a bean-shaped form, to copy the shape of the normal NP. The biomaterial is a chemically cross-linked hydrogel that is synthesized through a free-radical polymerization reaction from four reactive vinyl-type monomers: N-vinyl-2-pyrrolidinone (NVP), 2-hydroxyethyl methacrylate (HEMA), 2-(4′-iodobenzoyl)-oxo-ethyl methacrylate (4-IEMA), and allylmethacrylate (AMA). NVP and HEMA are responsible for the hydrophilic nature of the material, 4-IEMA provides intrinsic radiopacity to the NPPs as the material contains covalently linked iodine, and AMA results in chemical cross-linking [18]. The NPPs were manufactured in two steps: (i) polymerization and (ii) computer-controlled machining of the hydrogel in its dry state.

All chemicals were purchased from Acros[2]. NVP and HEMA were distilled under reduced pressure to remove inhibitory additives. The monomer 4-IEMA was synthesized from HEMA and 4-iodobenzoylchloride as described previously. AMA and 2,2′-Azobis-(isobutyronitrile) (AIBN) were used as received. NVP, HEMA, 4-IEMA, and AMA were weighed into a 500-mL round-bottom flask in the molar ratio 71.8:20.4:5.8:2. The total mass was about 200 g. AIBN (0.03 mol %) was added and completely dissolved in the monomer mixture. The reaction mixture was divided over a number of Teflon tubes (inner diameter 15 mm, wall thickness 1 mm, length 300 mm), which were closed with a stopper at one end. The tubes were filled to maximally 60%. The monomer-filled tubes were partially immersed in a thermostated oil bath and a computer-controlled time/temperature profile was run as follows: (i), constant temperature at 40 °C for 1 h; (ii) slow heating to 50 °C over 1 h; (iii), constant temperature at 50 °C for 6 h; (iv), slow heating to 60 °C over 1 h; (v) constant temperature at 60 °C for 6 h; (vi) slow heating to 80 °C over 1 h; (vii), constant temperature at 80 °C for 4 h; (viii), slow heating to 100 °C over 1 h; (ix), constant temperature at 100 °C for 4 h; (x) slow heating to 130 °C over 1 h; (xi), constant temperature at 130 °C for 2 h; (xii) slow cooling to ambient temperature (over 8 h). The procedure yielded transparent, glassy rods, which were removed from the Teflon tubes.

NPPs were machined from the polymer rods, using a five-axes computer-controlled lathe/milling system. As the material is a hydrogel, cooling with water had to be avoided, which meant that the machining process was performed slowly.

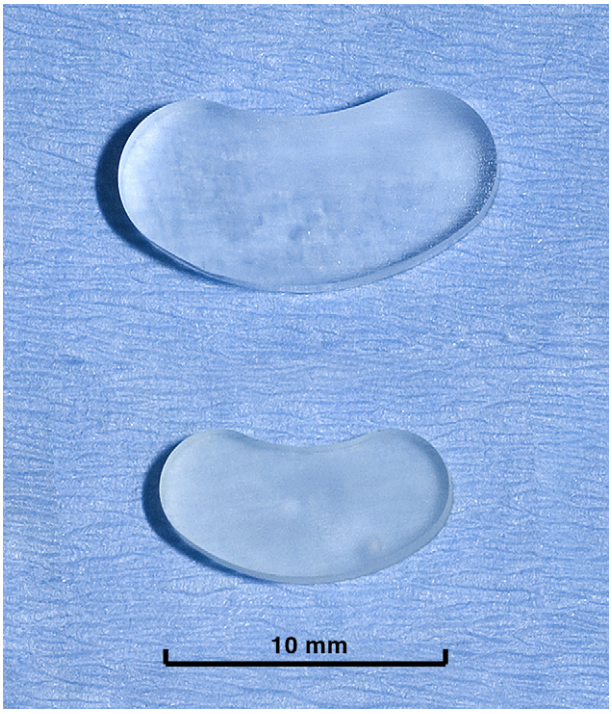

Fig. 1. The nucleus pulposus prosthesis: dry state/xerogel (bottom) and hydrated/swollen (top).

2.2. Determining the size of the implants

Four lumbosacral spines were isolated from the cadavers of healthy mixed-breed dogs (weight range 22.9–24.0 kg; age range 15–22 months) euthanized in an unrelated experiment. Three spines were used to determine the dimensions of the implants and the fourth spine was used for the implantation of the NPP (see below). MRI was performed using a 0.2 T open magnet[3]. For optimal resolution of the IVDs, a mixed signal sequence was used (DESS – Dual Echo Steady State) with a repetition time of 41 ms, an echo time of 12 ms, and a slice thickness of 1.194 mm. The MR images were obtained and assessed on standard computer screens using the software Web1000 5.1[4]. MRI showed the L7-S1 IVDs to be normal. The IVDs were then isolated by careful dissection for measurement of the width, height, and thickness of the NPs with a Vernier caliper (accuracy 0.05 mm). The NPPs were made on the basis of the mean dimensions of these three lumbosacral IVDs. Based upon our experience during pilot studies, the implants were made 20% smaller to compensate for residual NP material after nucleotomy. A swelling factor of 1.3 in each dimension was taken into account when making the implants, representing a volume swelling factor of 2.2 (1.3^3) [18,25]. The custom-made NPPs measured (width × height × thickness) 10.4 × 4.5 × 2.4 mm in the unswollen state, and 13.5 × 5.9 × 3.1 mm in the swollen state (Fig. 1).

2.3. Surgical implantation and imaging of the NPP in situ

Implantation of the custom-made NPP was studied using the spine from the fourth healthy mixed-breed dog. A dorsal (posterior approach) laminectomy was

[1] Raymedica, Inc., Bloomington, MN.
[2] Acros, Landsmeer, The Netherlands.
[3] Magnetom Open Viva, Siemens AG, Utrecht, the Netherlands.
[4] Clinical Review Station, Agfa Gevaert N V.