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Translation of an engineered nanofibrous disc-like angle-ply structure for intervertebral disc replacement in a small animal model

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ABSTRACT

Intervertebral disc degeneration has been implicated in the etiology of low back pain; however, the current surgical strategies for treating symptomatic disc disease are limited. A variety of materials have been developed to replace disc components, including the nucleus pulposus (NP), the annulus fibrosus (AF) and their combination into disc-like engineered constructs. We have previously shown that layers of electrospun poly(ε-caprolactone) scaffold, mimicking the hierarchical organization of the native AF, can achieve functional parity with native tissue. Likewise, we have combined these structures with cell-seeded hydrogels (as an NP replacement) to form disc-like angle-ply structures (DAPS). The objective of this study was to develop a model for the evaluation of DAPS in vivo. Through a series of studies, we developed a surgical approach to replace the rat caudal disc with an acellular DAPS and then stabilized the motion segment via external fixation. We then optimized cell infiltration into DAPS by including sacrificial poly(ethylene oxide) layers interspersed throughout the angle-ply structure. Our findings illustrate that DAPS are stable in the caudal spine, are infiltrated by cells from the peri-implant space and that infiltration is expedited by providing additional routes for cell migration. These findings establish a new in vivo platform in which to evaluate and optimize the design of functional disc replacements.

1. Introduction

Chronic low back pain affects over 50% of the population aged 65 or younger, and prevalence increases with age [1], resulting in more than US$100 billion in medical costs and lost wages in the USA [2]. Lumbar intervertebral disc degeneration has been implicated as a causative factor in low back pain, and deficiency in disc function is closely tied to the degeneration of its components [3–5]. Current surgical strategies for treating symptomatic disc degeneration, including spinal fusion and total disc arthroplasty, do not restore native joint mechanics and are associated with downstream complications. For example, fusion of a lumbar motion segment limits mobility and may accelerate adjacent segment degeneration [6], while prosthetic discs are subject to subsidence and migration, with limited benefit in comparison to fusion [7,8]. Thus, the role of these interventions in treating low back pain is controversial and continues to evolve.

A number of therapeutic strategies have been developed for each stage of the degenerative process to preserve or restore function of the intervertebral joint. Early in the degeneration process, interventions with cell, gene or pharmaceutical therapies may maintain disc function by reducing inflammation and preventing further matrix degradation [9–11]. A more substantial approach will likely be necessary for the treatment of end-stage disc disease, due to depletion of the endogenous cell population and irreversible deterioration of tissue structure. In such circumstances, a composite (or whole disc) approach would be required, where the entirety of disc structure and function is replicated. Towards that end, a number of studies have reported on co-cultured nucleus pulposus (NP) and annulus fibrosus (AF) components for tissue-engineered

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total disc replacement. These cell-seeded engineered discs have been evaluated in vitro [12,13], in the subcutaneous space [14–16], and have recently been placed in situ between rat lumbar and caudal vertebrae [17,18], illustrating the rapid advances in this regenerative approach to engineered disc replacement.

However, current engineered discs do not replicate the hierarchical AF organization required to support multiaxial spinal loads. The AF comprises lamellae, discrete fibrous sheets with specialized collagen alignment. Within each lamella, fibers run in a single direction, ranging from 20° to 50° with respect to the transverse plane, and adjacent lamellae have opposing fiber orientations, producing an angle-ply structure [19]. Multi-directional load-bearing during compression, torsion, flexion/extension, lateral bending and shear is supported by tensile reinforcement provided by fibers oriented in these directions [20]. An engineered disc may need to incorporate aspects of this native design for proper function of the regenerated tissue.

We have previously used electrospinning to generate engineered materials that recreate the organized fibrous architecture of the native AF [21–25]. Electrospun scaffolds with aligned nanofibers permit cell attachment and promote directed matrix production for reinforcement in principal loading directions [26–28]. Specifically, electrospun poly(ε-caprolactone) (PCL) seeded with either AF cells or mesenchymal stem cells increases in functional properties with in vitro culture, approaching native tissue properties at the single and multi-lamellar length scales [21,22,28]. Single strips of aligned scaffold can be arranged concentrically, precisely mimicking the alternating fiber alignment of native tissue, to form disc-like angle-ply structures (DAPS) [24]. Like their single layer counterparts, these constructs mature both compositionally and mechanically over time in culture, indicating their potential for use in total disc replacement.

The objective of this study was to develop a disc replacement model in which to evaluate DAPS in vivo. The murine caudal spine, used in many disc studies to investigate degenerative processes [5,29] and engineered disc replacements [30,31], is an ideal candidate for preclinical studies given the ease of surgical access and the ability to avoid critical structures (e.g. spinal cord and spinal nerves). In the context of disc tissue engineering, the rat tail model serves as a high-throughput system to screen engineered disc designs and inform large animal studies. Thus, we developed a rat tail disc replacement model in which native caudal discs were removed and replaced with the electrospun AF region of engineered DAPS. Here, our focus on the AF was to specifically assess the potential for colonization of and matrix deposition in electrospun scaffolds in the in vivo disc environment. Given early findings of graft displacement, we also developed an external fixation system to stabilize the disc space. Further, since early studies showed poor infiltration of the AF region of the DAPS by endogenous cells, we included sacrificial layers within the DAPS structure to provide additional routes for cell migration.

2. Materials and methods

2.1. Preparation of DAPS

DAPS were fabricated to reproduce the hierarchical structure of the native AF (Fig. 1A and B) [24]. Aligned nanofibrous sheets (thickness = 250 μm) were formed by electrospinning a 14.3% w/v solution of PCL (Shenzhen BrightChina Industrial Co., Hong Kong, China) dissolved in a 1:1 mixture of tetrahydrofuran (THF) and N,N-dimethylformamide (DMF) (Fisher Chemical, Fairlawn, NJ) (Fig. 1C). The polymer solution was extruded at a rate of 2.5 ml h^{-1} through a 13 kV-charged 18G needle. Fibers were drawn across a 15 cm air gap onto a grounded mandrel rotating with a surface velocity of 10 m s^{-1}. The resultant sheets of aligned nanofibers were cut into strips with fibers aligned at 30° relative to the strip long axis to mimic the native AF fiber architecture (Fig. 1D) [22,24]. Two strips with opposing fiber orientation were wrapped concentrically and fixed with a spot weld to form the AF region of the DAPS (Fig. 1E).

Preliminary characterization of DAPS fabricated in this manner included the measurement of DAPS geometry and compressive mechanical properties ($n = 7$). First, a non-contact laser device was used to measure height [32] and images of DAPS were taken with a digital camera and processed in Matlab to determine inner and outer diameters [33]. DAPS were then tested in unconfined compression on an electromechanical testing system (Instron 5542, Instron, Norwood, MA). First, a 0.5 N preload was applied and allowed to relax over 300 s. Next, three consecutive compressive strain ramps of 5% magnitude were applied with a 300 s relaxation period between each ramp. The compressive equilibrium modulus was defined as the slope of a line fit through points at equilibrium after 5%, 10% and 15% strain. The mean DAPS dimensions were: 5.1 ± 0.4 mm outer diameter, 1.0 ± 0.1 mm inner diameter and 1.9 ± 0.3 mm height. These dimensions allow a press-fit into the (caudal) C8/C9 disc space and are comparable to the native rat caudal disc geometry (4.15 mm outer diameter, 2 mm NP diameter, 0.94 mm height [34]). The compressive equilibrium modulus was 12.6 ± 4.3 kPa and was lower than that of the native rat caudal disc (238 kPa [17]), but was expected to increase after implantation as cells infiltrate and deposit a collagenous matrix.

2.2. Surgical implantation of DAPS into the rat caudal spine

In a first set of surgeries, DAPS consisting of only the AF region were implanted into the caudal spines of Sprague Dawley rats (male, 7–9 months, 478 ± 11 g) in accordance with local institutional regulations. Rats were first anesthetized and, using the sacrum as an anatomical landmark, the (caudal) C8 and C9 vertebral bodies were located via fluoroscopy (Orthoscan HD, Orthoscan, Inc., Scottsdale, AZ). Then, the dorsal skin spanning the vertebral bodies was incised, the dorsal tendons were partially separated from their bony insertions adjacent to the C8/C9 disc with a scalpel, and the native disc was removed. A double-armed non-absorbable suture was passed through the DAPS center, fed through the disc space and tied exterior to the ventral skin to anchor the implant in place (Fig. 2A). The incision was then closed with non-absorbable simple interrupted sutures. Post-surgical management included prophylactic treatment of infection (cefazolin, 15 mg kg^{-1} subcutaneous, 1 day pre-op and 2 days post-op), inflammation (meloxicam, 1 mg kg^{-1} subcutaneous, 1 day post-op), and pain (buprenorphine, 0.1 mg kg^{-1} subcutaneous, 3 days post-op). Rats were returned to normal cage activity and euthanized at either 14 ($n = 6$) or 28 days ($n = 9$). Additional rats were assigned to a discectomy-only control group, in which the native disc was removed in its entirety but no implant was placed. These rats were also euthanized at 14 ($n = 4$) or 28 days ($n = 4$).

2.3. Evaluation of disc height

To evaluate implant stability, caudal spines were imaged fluoroscopically pre-operatively, immediately post-operatively and at regular intervals through 28 days (Fig. 2B). Disc height index (DHI) [35], a standard technique used to normalize disc height to vertebral body length, was quantified from lateral fluoroscopic images using a custom Matlab program (Fig. 2C). The disc and adjacent vertebral bodies areas (A_{VB1}, A_{VB2}, A_D) and widths (W_{VB1}, W_{VB2}, W_D) were quantified digitally. The mean vertebral body lengths (L_{VB1}, L_{VB2}) were defined as $L_{VB} = A_{VB}/W_{VB}$, the disc height (H_D) as