Acta Biomaterialia 59 (2017) 192–199

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Initial investigation of individual and combined annulus fibrosus and nucleus pulposus repair *ex vivo*

Stephen R. Sloan Jr.[a], Devis Galesso[b], Cynthia Secchieri[b], Connor Berlin[c], Roger Hartl[c], Lawrence J. Bonassar[a,d,*]

[a] Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY, United States
[b] Fidia Farmaceutici S.p.A, Abano Terme, Italy
[c] Department of Neurological Surgery, Weill Cornell Brain and Spine Center, New York-Presbyterian Hospital, New York, NY, United States
[d] Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, United States

ARTICLE INFO

Article history:
Received 24 March 2017
Received in revised form 26 June 2017
Accepted 28 June 2017
Available online 29 June 2017

Keywords:
Annulus fibrosus
Nucleus pulposus
Intervertebral disc
Collagen
Hyaluronic acid

ABSTRACT

Novel tissue engineered and biomaterial approaches to treat intervertebral disc (IVD) degeneration focus on single aspects of the progressive disease and hence are insufficient repair strategies. In this study, annulus fibrosus (AF) and nucleus pulposus (NP) biomaterial repair strategies were used individually and combined to treat IVD degeneration modeled in *ex vivo* rat-tail motion segments by annulotomy and nucleotomy. An injectable riboflavin cross-linked high-density collagen gel patched defects in the AF, while NP repair consisted of injections of a modified hyaluronic acid (HA) hydrogel. Qualitative imaging showed the annulotomy and nucleotomy successfully herniated NP material, while the HA NP injections restored intact NP morphology and the collagen AF patches sealed AF defects. Assessed by quantitative T2 magnetic resonance imaging, combined repair treatments yielded disc hydration not significantly different than intact hydration, while AF and NP repairs alone only restored ~1/3 of intact hydration. Mechanical testing showed NP injections alone recovered on average ~35% and ~40% of the effective instantaneous and equilibrium moduli. The combined treatment comprising biomaterial AF and NP repair was effective at increasing NP hydration from NP repair alone, however HA injections alone are sufficient to improve mechanical properties.

Statement of Significance

Intervertebral disc degeneration affects an estimated 90% of individuals throughout their life, and is a candidate pathology for tissue engineered repair. The current standard of clinical care reduces spinal articulation and leads to further degeneration along the spine, hence great interest in a regenerative medicine therapy. Literature studies focused on biomaterial repair strategies for treating degenerated discs have partially restored native disc function, however no studies have reported the use of combined therapies to address multiple aspects of disc degeneration. This initial investigation screened injectable biomaterial repair strategies *ex vivo*, and through complementary outcome measures showed a combined therapy restores disc function better than individual approaches. This study is the first of its kind to address multiple aspects of disc degeneration, using clinically-oriented biomaterials in a well-established animal model.

© 2017 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Intervertebral discs (IVDs) are the cartilaginous structures between adjacent vertebrae critical to the mechanical stability and articulation of the spine. Each IVD is a composite tissue of a fibrous ring of annulus fibrosus (AF) that encompasses the proteoglycan-rich nucleus pulposus (NP). Progressive disc degeneration including IVD degradation, collapse, and herniation is estimated to affect 90% of the United States population throughout their lifetime, and chronic back pain is the leading cause of workplace absences [1]. Lesions in the AF result in herniation of the NP, leading to radicular pain from impingement of surrounding nerves.

* Corresponding author at: Department of Biomedical Engineering, 149 Weill Hall, Cornell University, Ithaca, NY 14853, United States.
E-mail address: lb244@cornell.edu (L.J. Bonassar).

http://dx.doi.org/10.1016/j.actbio.2017.06.045
1742-7061/© 2017 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

The innate healing ability of IVDs is poor as they lack a direct blood supply and have low cell density [2].

The interventional standard of care for degenerated discs involves partial or complete removal of the pathologic disc, with possible fusion of adjacent vertebrae through fixation, graft material, and/or device implant. Current interventions are inadequate as they impair the flexibility and mechanical environment of the spine, leading to further degeneration in adjacent discs [3]. Studies have applied mechanical repair techniques such as suturing to the AF, however these fail to improve healing in long-term studies [4–6]. Recent efforts focused on developing biological and tissue engineered solutions include annular repair, NP replacement/rehydration, and whole IVD replacement [2,7–17].

Biomaterials for annular repair must seal AF defects, prevent further herniation of the NP, restore biomechanical properties of native IVDs, and promote tissue healing [2,12,18,19]. Promising annular repair strategies have involved injectable biomaterials, able to fill irregular defects with favorable tissue adhesion and cell infiltration [18,20]. *In vivo* investigations have shown annular repair strategies maintain IVD morphology and proteoglycan content in damaged discs over time compared with untreated controls [20–25]. Collagen was the chosen biomaterial for annular repair in this study due to its past success *in vivo*, biocompatibility and mechanical properties [12,20,25].

NP replacement and rehydration techniques using injectable biomaterials have been shown to improve mechanical properties over untreated controls, restore NP hydration, and restore proteoglycan production, however the necessary needle puncture delivery through the AF can precipitate disc degeneration [13,26–38]. In native NP, hyaluronic acid (HA) attracts and retains water, which allows the NP to pressurize inside the disc and resist mechanical loads. In this study, a modified HA (HYADD4®) was chosen for NP repair to mimic native HA and restore NP hydration. HYADD4® and similar HA derivatives have excellent cytocompatibility and low toxicity both *in vitro* and *in vivo* [26,37,39,40]. HYADD3® has previously been used for *in vivo* investigations of NP replacement, where HA injections into injured IVDs supported host cell infiltration, ECM remodeling, and preserved disc height compared to injured, untreated controls [26].

Recent advancements in biological repair strategies have shown successful preclinical outcomes *ex vivo* and *in vivo*, however they only target one aspect of IVD degeneration. AF repair alone is not sufficient to rehydrate and pressurize the NP, while NP replacement leaves discs prone to further herniation without healing the AF [7,20,41]. The objective of this initial screening study is to assess the efficacy of individual and combined biomaterial AF and NP repairs to restore proper disc mechanical properties and NP hydration in an *ex vivo* rat-tail model.

The caudal rat-tail IVD is an appropriate model for this initial investigation of individual and combined repair strategies as it is an adequate size to study disc surgery and AF puncture has been robustly established as a method to provoke disc degeneration [42–46]. Repair strategies that improve mechanical properties in the rat-tail *ex vivo* model correlate well with improved mechanical and radiographic outcomes *in vivo* [20,25,41]. Many novel repair therapies were initially investigated in the rat-tail model, which yielded insightful screening results to guide biomaterial development and characterization [14,20,41,47–49].

2. Materials and methods

2.1. Collagen gel for AF repair

AF repair was performed using a high-density collagen gel, known to preserve mechanical properties and morphology of punctured IVDs *in vivo* and *ex vivo* [20,41]. Collagen fibers were harvested from rat-tail tendons as previously described [41,50,51]. The collagen injection used to patch AF defects was prepared at 15mg/mL by mixing 20mg/mL collagen in 0.1% acetic acid with a working solution of 10× Dulbecco's Phosphate Buffered Saline (DPBS), 1 N sodium hydroxide, and a riboflavin 1× DPBS solution. Riboflavin was prepared with 1X DPBS to create a final injection concentration of 0.06 mM. Upon injection of the final mixture into the defect location, the collagen gel was exposed to 468 nm blue light for 40 seconds to cross-link the gel into the shape of the defect *in situ*.

2.2. Modified hyaluronic acid for NP repair

NP repair was performed via injection of HYADD4® (Fidia Farmaceutici S.p.A., Abano Terme, Italy) as packaged by the manufacturer through an AF defect into the NP space. HYADD4® is non-crosslinked HA modified with C_{16} repeating side chains at a degree of substitution of ~3%, with similar charge density and swelling ability as natural HA [52]. The grafted alkyl side chains increase hydrophobic interactions within the polymer, resulting in a stable hydrogel at 10 times lower concentrations than natural HA, with enhanced resistance to degradation *in vivo*. The safety and efficacy of HYADD4® has already been proven for clinical use in joint therapies in the US and for spine therapies in Europe [39]. HYADD4® exhibits excellent cytocompatibility, and has been shown to promote chondrocyte proliferation and function *in vivo*. HYADD4® hydrogels were used as manufactured for clinical applications, where the polymer was dissolved inphosphate-buffered saline at a concentrationof 8 mg/mL [52]. The clear, homogenous hydrogel was stable at room temperature.

2.3. Mechanical testing

A total of 32 caudal rat-tail IVDs were used in this preclinical investigation for their well-established disc degeneration model and excellent screening ability for IVD repair strategies [14,20,42–49]. Frozen Sprague-Dawley rat-tails were thawed in room temperature DPBS for 15 min, dissected for their most proximal full motion segment (vertebra-IVD-vertebra) and loaded into an Enduratec ELF 3200 mechanical testing frame (Bose, Eden Prairie, MN) using custom grips (McMaster-Carr, Aurora, OH) as previously described [41]. Each motion segment was wrapped in a DPBS-soaked wipe to prevent sample dehydration, and the initial gauge length was recorded after 10 min of relaxation in the testing grips at 0 N load to equilibrate transient effects. Segments were subjected to a uniaxial stress-relaxation protocol comprising steps of 5% compressive strain to a total displacement of 20% initial disc height. An annulotomy and nucleotomy was then performed on each motion segment at the initial gauge length, where each IVD was incised with a #11 scalpel to create a ~1 mm^2 window in the AF, followed by 40% compression to herniate the NP. Smooth stress/strain curves during the application of the 40% strain and histological slices of compressed motion segments did not show signs of endplate damage or internal fracture (data not shown). After nucleotomy, an identical mechanical testing protocol was performed. Damaged motions segments were then treated with either **A**) collagen AF patch as described previously [20,41], **B**) HYADD4® injection into the NP or **C**) combined AF and NP repair strategies (Fig. 1). Motion segments were equilibrated at the initial recorded gauge length for 30 min following treatment, and then a third round of identical mechanical testing was performed on the treated segments. Time between dissection and the end of the three rounds of mechanical testing was ~2.5 h per sample.

The raw load data from mechanical testing was fit to a poroelastic model using a custom MATLAB script (Mathworks, Natick, MA)

Download English Version:

https://daneshyari.com/en/article/6449065

Download Persian Version:

https://daneshyari.com/article/6449065

Daneshyari.com