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Angle-ply biomaterial scaffold for annulus fibrosus repair replicates native tissue mechanical properties, restores spinal kinematics, and supports cell viability

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ABSTRACT

Annulus fibrosus (AF) damage commonly occurs due to intervertebral disc (IVD) degeneration/herniation. The dynamic mechanical role of the AF is essential for proper IVD function and thus it is imperative that biomaterials developed to repair the AF withstand the mechanical rigors of the native tissue. Furthermore, these biomaterials must resist accelerated degradation within the proteolytic environment of degenerate IVDs while supporting integration with host tissue. We have previously reported a novel approach for developing collagen-based, multi-laminate AF repair patches (AFRPs) that mimic the angle-ply architecture and basic tensile properties of the human AF. Herein, we further evaluate AFRPs for their: tensile fatigue and impact burst strength, IVD attachment strength, and contribution to functional spinal unit (FSU) kinematics following IVD repair. Additionally, AFRP resistance to collagenase degradation and cytocompatibility were assessed following chemical crosslinking. In summary, AFRPs demonstrated enhanced durability at high applied stress amplitudes compared to human AF and withstood radially-directed biaxial stresses commonly borne by the native tissue prior to failure/detachment from IVDs. Moreover, FSUs repaired with AFRPs and nucleus pulposus (NP) surrogates had their axial kinematic parameters restored to intact levels. Finally, carbodiimide crosslinked AFRPs resisted accelerated collagenase digestion without detrimentally effecting AFRP tensile properties or cytocompatibility. Taken together, AFRPs demonstrate the mechanical robustness and enzymatic stability required for implantation into the damaged/degenerate IVD while supporting AF cell infiltration and viability.

Statement of Significance

The quality of life for millions of individuals globally is detrimentally impacted by IVD degeneration and herniation. These pathologies often result in the structural demise of IVD tissue, particularly the annulus fibrosus (AF). Biomaterials developed for AF repair have yet to demonstrate the mechanical strength and durability required for utilization in the spine. Herein, we demonstrate the development of an angle-ply AF repair patch (AFRP) that can resist the application of physiologically relevant stresses without failure and which contributes to the restoration of functional spinal unit axial kinematics following repair. Furthermore, we show that this biomaterial can resist accelerated degradation in a simulated degenerate environment and supports AF cell viability.

1. Introduction

Intervertebral discs (IVD) support axial compressive loading of the spine while allowing for flexibility and a defined range of motion during activities of daily living. IVD's are comprised of two distinct regions: the central gelatinous core known as the nucleus pulposus (NP) which is circumferentially constrained by the annulus fibrosus (AF). The NP is a highly-hydrated tissue composed primarily of collagen type II and aggrecan, which provides load support due to its low permeability and the generation of intradiscal pressure (IDP). The AF is a highly organized lamellar structure consisting of 15–25 sheets of collagen type I with fibers

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aligned in alternating orientations of ±28–43° to the transverse axis of the spine yielding an 'angle-ply' architecture [1]. The AF functions to circumferentially confine the NP and resist tensile strains experienced during rotational and bending spinal motions [2,3].

Annually, over 5.7 million Americans are diagnosed with IVD disorders including IVD degeneration (IVDD) and herniation (IVDH) which ultimately compromise the structural integrity of the AF [4]. This results in a loss of IVD height, impaired IVD mechanical function, patient pain, and disability [5–11]. Accordingly, discogenic low back pain (LBP) affects approximately 80% of the adult population during their lifetime resulting in a diminished quality of life [4,12–14], and estimated healthcare expenditures exceeding $85.9 billion [15,16]. Surgical treatments for late-stage IVDD include spinal fusion and total disc replacement, however these methods suffer from significant drawbacks [17]. Newer technologies, including NP replacement (NPR) are being developed as interventional strategies to mitigate IVDD progression [18,19]. Such devices have not yet realized clinical utility, due in part to the lack of a mechanically robust AF repair method. Additionally, the nearly 500,000 patients undergoing discectomies annually to remove protruding/herniated NP tissue may benefit from a biomaterial that can restore AF integrity following the procedure. Studies demonstrate that conservative discectomies (i.e. those removing minimal NP material) often result in maintenance of IVD height, biomechanics and improved patient outcomes; however these patients are at increased risk for re-herniation, and incur significant reoperation costs [20–29]. These detrimental consequences may be moderated via utilization of an AF repair method.

Accordingly, there has been a recent increase in the development of AF repair strategies ranging from simple mechanical closures to biomaterial scaffolds. While many biomaterials developed for AF repair have been assessed for their ability to promote tissue regeneration *in vitro* [30–34], few have undergone thorough testing to evaluate their mechanical competency required for implantation into the spine [34–37]. Moreover, even fewer have been assessed for their contribution to restoring functional spinal unit (FSU) kinematics following injury and repair; arguably one of the most important functional outcomes of any motion preserving/sparing spinal implant. Finally, biomaterials to be implanted into a damaged IVD must demonstrate resistance to accelerated degradation as investigations have illustrated increased concentrations of destructive proteases which could jeopardize their mechanical integrity [38–40]. This is of particular importance for biomaterials composed of extracellular matrix (ECM) components, which often have to be chemically crosslinked to impart resistance to accelerated enzymatic degradation, yet should demonstrate cytocompatibility. Taken together, a critical need exists to create an effective AF repair biomaterial, which demonstrates the ability to survive in the mechanical, and biochemical environment of the damaged IVD and which will allow for eventual integration or regeneration of healthy AF tissue. The development of such a biomaterial may reduce the rate of IVD re-herniation, improve patient outcomes, and delay the need for spinal fusion procedures [41].

We have previously reported the development of a novel collagen sheet-based annulus fibrosus repair patch (AFRP) biomaterial derived from decellularized porcine pericardium, which has been assembled using a simple, scalable, and repeatable process. The resulting AFRPs have been shown to mimic the multi-laminate angle-ply (i.e. layered) architecture and basic tensile mechanical properties of the human AF [42]. Herein we aimed to further mechanically evaluate this biomaterial for its tensile fatigue strength, resistance to impact loading, attachment strength to IVDs, and its ability to assist in restoring axial kinematics following repair of injured FSUs. Additionally, we have assessed the ability of various crosslinking chemistries to render AFRPs resistant to accelerated protease degradation and evaluated their effects on AFRP tensile properties. Finally, considering the long-term goal of using this biomaterial in conjunction with autologous or allogenic cells to regenerate healthy AF tissue, we evaluated the ability of the AFRP to support AF cell viability and infiltration.

2. Materials & methods

2.1. Fabrication of annulus fibrosus repair patches (AFRPs)

Multi-laminate angle-ply AFRPs were developed and assembled from decellularized porcine pericardium as previously described by McGuire et al. [42]. AFRPs were maintained in a phosphate buffered saline storage solution containing protease inhibitor at 4 °C for up to two weeks prior to testing.

2.2. Preparation of functional spinal units

Bovine tails from 2 to 3-year-old calves were obtained from a local abattoir and transported on wet ice to the lab within an hour. Excess tissue surrounding the vertebral bodies and intervertebral discs were removed via dissection and functional spinal units (FSUs: vertebrae-IVD-vertebrae) were isolated via shears. Three FSUs were harvested from three caudal levels (cc1-2 to cc3-4: IVDs closest to the rear end) and were potted using wood screws and urethane potting resin to prevent slippage of the samples during testing. In general, bovine IVDs have been shown to have similar swelling pressure, geometry and resting stress compared to human lumbar IVDs [43]. Prior to testing, FSUs were wrapped in gauze saturated with storage solution and stored at −20 °C. Samples were thawed within the sealed zip-lock bag, which was submerged for four hours in PBS at ambient temperature thus not allowing tissue swelling.

2.3. Biomechanical evaluations of non-crosslinked multi-laminate AFRPs

2.3.1. Biaxial impact burst strength of AFRPS

Biaxial impact burst strength testing was modeled after ASTM D1709: "Standard Test Methods for Impact Resistance of Plastic Film by the Free-Falling Dart Method" with modification. Representative samples of AFRPs (2-, 3-, and 6-ply; n = 5/group; AFRP dimensions: 2-ply; 12 mm (L) × 12 mm (W) × 0.5 mm (T), 3-ply; 12 mm (L) × 12 mm (W) × 0.75 mm (T), 6-ply: 12 mm (L) × 12 mm (W) × 1.5 mm (T)) were tested using a custom designed free-fall impact testing drop-tower (Supplemental Fig. 1A). The base platform of the drop-tower consisted of a tissue holding clamp and four vertical rails, which guided a free-falling platform. The tissue holding apparatus consisted of two stacked blocks lined with course-grit sandpaper each having an aligned thru-hole of 6.25 mm diameter. AFRPs were sandwiched between the two blocks centered over the two thru-holes. Subsequently, a 6 mm steel ball attached to a 3-inch pushrod was placed in contact with the AFRP via the thru-hole in the superior block. Various weights ranging from 0.18 to 0.58 kg were stacked on the free-fall platform, which were then dropped from a constant height of 0.254 m. Impact energy (E) was calculated using the equation for kinetic energy, $E = \frac{1}{2} * m * v^2$, where m = mass and v = velocity. The resultant ball-burst pressure was calculated given the maximum force at rupture and its relationship with ball-burst pressure and geometric constraints according to established procedures [42,44,45]. AFRPs were kept moist throughout testing via saline spray.