Decellularized Pericardium as a Biologic Patch for Annulus Fibrosus Repair

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Introduction: Thirty-one million Americans experience low-back pain (LBP) at any given time.1 LBP is the single leading cause of disability worldwide and its prevalence in the United States is approximately 80%.2 One of the main causes of LBP is herniation and/or degeneration of the nucleus pulposus (NP); the central gelatinous core of the intervertebral disc (IVD). Surgical approaches used to ameliorate these conditions include removing herniated NP fragments, and if pain and disability progress, spinal fusion and total disc arthroplasty are last resort options. More recently, nucleus pulposus (NP) replacements developed from either inanimate synthetic or viable tissue engineered materials have been proposed as an early-stage intervention to treat NP herniation or IVD degeneration. However, in order for such techniques to be employed, it has become clear that the fibrous layers of the IVD (known as the annulus fibrosus - AF) that sequester the NP must be mechanically competent. The AF is composed of approximately 10-25 concentric sheets of type I collagen fibers interspersed with elastin and glycosaminoglycan. The fibers within these sheets are oriented at 40-70° to the horizontal axis of the spine and alternate in opposite directions with each consecutive layer allowing for resistance to the hydrostatic pressure generated by the confined NP as well as opposing tensile and torsional loads generated during bending. Currently, few AF repair ‘patches’ are commercially available and surgeons must rely on closing AF insufficiencies with sutures. Researchers continue to develop materials that attempt to mimic the biochemical and/or the mechanical properties of the native AF by employing natural and synthetic materials using various manufacturing techniques including electrospinning3. However, an ideal AF repair material has yet to be developed. We hypothesize that an ideal patch material for repair and regeneration the native AF can be developed from adjoined sheets of decellularized porcine pericardium due to its aligned type I collagen fiber architecture and mechanical strength. The objective of the research presented herein was to assess the histological and mechanical properties of decellularized porcine pericardium as a biomimetic AF repair material.

Methods: Methods: Fresh porcine pericardium was harvested and decellularized according to previously published methods with minor modification (i.e. without elastase digestion).4 Decellularized samples were evaluated histologically for tissue micro-architecture, extracellular matrix component identification and for the confirmation of porcine cell nuclei removal via hematoxylin and eosin (H&E) (n=6) and Movat’s Pentachrome (n=6) stains. Decellularized single sheet samples (n=8) were also mechanically evaluated using a Diaphragm Burst test according to ASTM D3786 to evaluate multi-axial burst strength. Briefly, 3”x3” samples were visually inspected for defects, carefully secured into the standardized test apparatus and tested at a diaphragm insufflation rate of 95 ± 5 mL/min until rupture. Burst pressure was recorded as the difference between the maximum indicated rupture pressure and the pressure required to inflate the diaphragm. A triple-ply sheet of decellularized pericardium was also tested for burst strength. Additionally, uniaxial tensile testing of decellularized pericardium was performed using an MTS test frame fitted with a 100N load cell according to methods outlined by Holzapfel et al., with modification.5
into rectangular specimens and were pre-warmed at 37°C for 20 min in PBS. The length, width and thickness of each sample were determined via calipers and confirmed visually using NIH Image-J using a visual calibrator and the software measurement tool. Course grit sandpaper was used between the tensile testing clamps and the ends of the samples to prevent slippage during testing. Samples (n=6) were tested to failure at 1 mm/min after a preconditioning regime of 5 loading/unloading cycles was performed at 10 mm/min to 10% strain (determined from the sample’s initial grip-to-grip gauge length following the application of a 0.010 N pre-load). Modulus was calculated according to $E = \sigma / \varepsilon$, where $\sigma$ and $\varepsilon$ is the calculated engineering stress and strain, respectively. The modulus was determined from the linear region of the stress-strain curves between 0.05-0.1 (mm/mm) as performed by others.6 Fresh porcine pericardium samples were used as comparative controls. Statistical analysis was performed using Student’s two-tailed t-test. Significance was defined as $p < 0.05$. Results are represented as a mean ± standard deviation.

**Results:** Results: The presence of an aligned, fiber preferred direction is macroscopically evident in the decellularized porcine pericardium sheets (Figure 1.A). Histological evaluation via H&E illustrated the complete absence of cell nuclei (Figure 1.B) from the extracellular matrix (ECM) as compared to fresh samples (Figure 1.C). Elastin and collagen fibers remained within the decellularized ECM as indicated by Movat’s pentachrome staining (Figure 1.D). The measured thickness of decellularized pericardium ranged between 0.3-0.7 mm. No significant differences were found for the average linear region modulus of decellularized and fresh pericardium (20.59 ± 12.04 MPa and 16.97 ± 9.57 MPa, respectively). The average multi-axial burst strength for single sheets of decellularized and fresh pericardium was 10.83 ± 3.76 psi and 18 ± 7.58 psi, respectively. Additional burst testing of a triple-ply (three stacked sheets) decellularized pericardium sample achieved a burst pressure of 85 psi.

**Discussion:** Discussion: Closure of defects within the AF following NP herniation or implantation of an NP replacement material is a requirement. An ideal repair material should mimic the native ECM architecture and mechanical properties of the native AF while containing the NP by withstanding the pressures it generates. We hypothesized that decellularized porcine pericardium could effectively function as an AF repair patch. Despite complete removal of porcine cells, the decellularization process did not detrimentally alter the mechanical properties of the tissue and the native ECM architecture remained. Moreover, the resultant linear region elastic modulus is within the range of reported values for a single layer of human AF (12-24 MPa).6 Pressures generated within the constrained NP are reported to be in the range of 0.5-2.5 MPa (~70-350psi);3 and while single layer sheets of decellularized pericardium would not be able to withstand this, extrapolation from our triple-ply experiments would indicate that 9 sheets (a 4.5 mm thick patch of pericardium) would be adequate, however further analysis will be completed. Ongoing studies include quantitatively evaluating decellularized pericardium for DNA and alpha gal content as well as seeding studies of AF cells on multilayered patches.

**Significance:** The initial results from the research contained herein illustrate that decellularized porcine pericardium may be a suitable material for use to repair insufficient annulus fibrosus in patients with intervertebral disc degeneration or herniation.
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