Introduction: The paradigmatic approach to tissue engineering includes the use of tissue specific cells or stem cells, physiologically relevant biochemical and/or mechanical factors, and cellular support scaffolds to aid in the regeneration or replacement of damaged or diseased tissue. The foundation of this approach lies in the ability of the cells seeded within scaffolds to produce an appropriate extracellular matrix (ECM) that resembles that of the native healthy tissue, ultimately aiding in the restoration of normal tissue structure and function. Studies within scaffold based scaffolds derived from the decellularization of healthy xenogenic or allogenic tissue has generated much interest showing great promise as reviewed recently [1]. Scaffold development utilizing such techniques may prove advantageous as biologically appropriate scaffolds tailored towards specific tissues can be formed at the outset. Recent publications indicate that this approach is suitable for the regeneration of numerous tissues including heart valves [2], vascular grafts [3], and corneal tissue [4]. We hypothesize that an ideal scaffold can be developed for tissue engineering the human nucleus pulposus (NP) to aid in ameliorating intervertebral disc (IVD) degeneration through the successful decellularization of porcine NP tissue. The development and utilization of such a process will result in a scaffold with biochemical and biomechanical characteristics comparable to healthy human NP ECM. The objective of the research presented herein was to develop a decellularization process by which all cellular components and immunogenic epitopes are removed from the porcine NP tissue while concurrently maintaining native ECM components resulting in a scaffold conducive to maintaining human cell viability and proliferation.

Materials & Methods: Fresh porcine NP tissue was harvested into ice-cold decellularization (decell) solution (0.6% Triton X-100, 1% deoxycholic acid, 0.1% EDTA, 0.02% NaN₃ in 50mM Tris buffer pH 7.5). Tissue underwent ultrasonication for 10 minutes and was subsequently agitated at room temperature for 72 hours with changes of decell solution and 10 minute ultrasonication periods occurring every 24 hours thereafter. Subsequently, NP tissue underwent washes in distilled water and 70% ethanol, prior to incubation for 48 hours in a mixture of DNase/RNase (720 mU/ml) at 37°C. Hematoxylin & Eosin (H&E) and DAPI staining was performed on fresh and decell NP tissue to assess ECM architecture and removal of cell nuclei. Immunohistochemistry (IHC) using mouse anti-collagen type II and anti-chondroitin-6-sulfate / aggrecan coupled with a biotinylated secondary anti-mouse IgG was performed to qualitatively assess the presence of these ECM components prior to and following decell. Dimethylmethylene blue (DMMB) assays were performed to determine changes in glycosaminoglycan (GAG) content following decell. Fresh and decell porcine NP tissue underwent DNA extraction and purification according to protocols provided with a QiaGen DNeasy® total DNA purification kit to quantitatively assess cell removal. Extracted DNA was visualized via agarose gel electrophoresis with ethidium bromide, and quantified via densitometry. IHC for the presence of alpha-gal, a cellular epitope primarily responsible for immune rejection of xenotransplants [5] was performed using a Griffonia simplicifolia derived biotinylated lectin. Scaffold cytotoxicity was assessed by seeding human adipose derived stem cells (hADSCs) – passage 5) into 12-well plates covered with decell NP tissue that had been treated with 100% ethanol and subsequent incubation in 50% fetal bovine serum / Dulbecco’s modified eagle medium solution. Viability and proliferation were assessed at day 3 & 7 time-points using MTS and Live/Dead (L/D) assays. Results are represented as a mean ± standard deviation. Statistical analysis was performed using Student’s two-tailed t-test. Significance was defined in all cases as p<0.05.

Results: IHC and routine histological evaluations of fresh porcine NP tissue indicated an ECM rich in collagen type II and aggrecan (brown staining) within and around islands of NP cells (Fig.1.a-c). Following decell, the NP ECM appeared mildly disrupted, but did not exhibit any indication of intact cell nuclei via H&E (Fig.2.a). These results were corroborated by densitometric quantification of extracted DNA which indicated a 97.74% reduction in average DNA content comparing Fresh and decell NP tissue, respectively (Fig.3.a). IHC staining of decell NP tissue for collagen type II and aggrecan illustrates that appreciable amounts of each ECM component remained (Fig.2.b&c, respectively). DMMB assays quantifying GAG content confirmed that decell NP contained nearly 50% the mean GAG content found in fresh NP tissue (201.62 ± 44.31 and 430.85 ± 172.22 μg/mg dry NP tissue, respectively). IHC staining for α-gal epitope was negative in all decell NP tissue samples (Fig.2.d) while positive (brown) staining was observed within and around NP cell islands in fresh NP tissue (Fig.1.d). Live/Dead assays for hADSCs seeded on decell NP tissue illustrated nearly 100% viability at day 3 & 7 (data not shown) concomitant with an approximate 2.5-fold increase in cell number between the two time-points (Fig.3.b).

Discussion: We have successfully demonstrated for the first time, the ability to decellularize porcine NP tissue and its utility as a scaffold source for tissue engineering the human NP. All cellular remnants and antigenic epitopes were removed thus allowing for implantation of the scaffold into human IVDs without soliciting a severe host immune reaction. Additionally, we showed that the primary ECM components of the decellularized porcine NP tissue are similar to those found in human NP, are non-cytotoxic, and are conducive to hADSC re-population and proliferation. Others have attempted construction of scaffolds that mimic the native NP ECM [6] or utilize NP allograft material [7], however cell proliferation and viability in these materials remain concerns. Moreover, some fail to include whole aggrecan molecules, which are deemed critical for proper NP mechanical function [8]. Ongoing work includes differentiation studies on hADSCs seeded onto decell NP scaffolds, characterization of scaffold material properties and implantation into animal models of disc degeneration.