Development of a Tissue Specific Acellular Extracellular Matrix for Intervertebral Disc Regeneration Using a Gentle Decellularization Process

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Statement of Purpose: Low back pain (LBP) is an epidemic that affects up to 35 million people each year in the United States alone.[1] The leading cause of LBP is disc degeneration.[2] One clinical marker of disc degeneration is a loss in intervertebral disc (IVD) height due to breakdown of extracellular matrix (ECM) of the nucleus pulposus.[3] The nucleus pulposus is a gelatinous matrix rich in collagen type II and chondroitin sulfate proteoglycans (CSPGs).[4, 5] These CSPGs have sulfated glycosominoglycans (sGAG) side chains that attract water, enabling the nucleus to withstand substantial compressive loads.[4, 5] Fragmentation or cleavage of these CSPGs to remove the sGAGs has been implicated in disease progression because it results in a loss of the nucleus to hold water and therefore bear compressive loads.[3] An ideal solution would consist of a proteoglycan rich matrix to restore the hydrostatic compressive resistance of the disc. Tissue specific decellularized ECM hold promise as ideal matrices for regeneration of the IVD. Recently, Mercuri *et al* has developed a protocol to decellularize the nucleus pulposus of porcine cervical and lumbar spine.[6, 7] This work showed promising results during *in vivo* subdermal testing in a rat model. However, one limitation of this study is that is uses Triton X-100 to decellularize the nucleus pulposus. The use of non-ionic Triton X-100 has been shown to significantly alter the basal lamina in nerves and has the potential to fragment proteoglycans leaving them unable to form aggregates and attract water.[8] Our lab has previously developed a gentle decellularization process for use on human nerves that does not use Triton X-100, but rather more gentle anionic Triton X-200.[8, 9] The purpose of this research was to adapt our gentle nerve decellularization method for use on the nucleus pulposus of the IVD with the goal to remove cells and DNA, but maintain intact proteoglycans.

Methods: Cervical and lumbar segments were removed en bloc from 4 month old female Yorkshire-Landrace porcine. Each motion segment was carefully dissected to expose the nucleus pulposus. Over the 5 day decellularization process, samples were first washed in distilled deionized water, followed by serial washes in SB-10, Triton X-200 and SB-16, SB-10, Triton X-200, and DNase/RNase. All washes were performed at room temperature with radial agitation. After decellularization, samples were fixed, with 4% paraformaldehyde, blocked and permeabilized with 0.5% Triton X-100 and goat BSA in PBS, and labeled with DAPI and an antibody against CSPGs. Confocal imaging was performed to assess degree of decellularization and maintenance of CSPGs. Additionally, some samples were tested for maintenance sGAGs using a standard dimethyl methylene blue (DMMB) assay.

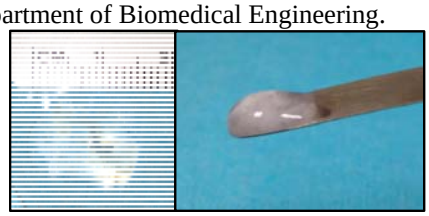

Figure 1 - Porcine nucleus pulposus after decellularization process.

Results: Nucleus pulposus samples were all macroscopically intact after decellularization (Figure 1). Confocal imaging revealed nearly complete removal of cell nuclei and nuclear debris in all samples tested throughout the entire thickness of the sample (Figure 2). In addition, intact CSPGs were found evenly distributed throughout both the untreated controls as well as the decellularized samples. Preliminary results of the DMMB assay indicate intact sGAG in the decellularized nucleus pulposus.

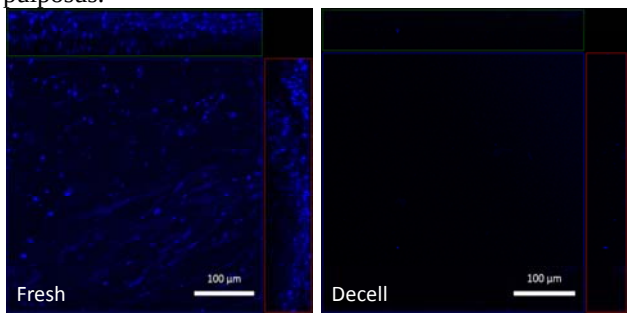

Figure 2 - DAPI staining showing presence of nuclei in fresh control tissue and removal in decellularized tissue.

C**onclusions:** These preliminary results indicate that we have successfully removed cellular components from the nucleus pulposus while maintaining an intact extracellular matrix with CSPGs. Further testing is needed to quantify the degree of DNA cellular removal. Future experiments will include mechanical characterization of the nucleus pulposus and quantification of the degree of aggrecan fragmentation by labeling against specific neoepitopes. Furthermore, *in vitro* and *in vivo* experiments will explore the efficacy of this tissue analog for regeneration and pain prevention.

References:

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