

Development of Solubilized Decellularized Nucleus Pulposus Tissue for Intervertebral Disc Regeneration

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INTRODUCTION: Intervertebral Disc Degeneration (IVDD) is a pathological condition initiating in the nucleus pulposus (NP), the gelatinous center of the intervertebral disc (IVD).¹ Healthy NP extracellular matrix (ECM) is characterized by high proteoglycan and glycosaminoglycan (GAG) content creating a hyper-hydrated tissue with hydrostatic pressure that resists compressive loads in the spine.² As the NP degenerates, it becomes more fibrotic due to a decrease in type II collagen and an increase in type I collagen. Some researchers have explored decellularized tissues as biomaterials for NP repair. In other fields, tissues have been decellularized and solubilized, resulting in ECM-based therapies with promising results demonstrating its ability to support *in vitro* tissue regeneration and mesenchymal stem cell (MSC) differentiation.^{4,5} Translation of solubilized ECM that is derived from NP has yet to be fully explored or evaluated. The objective of this work was to determine an optimal solubilization methodology for the NP and to characterize the resulting biomaterial. It was hypothesized that solubilized bovine NP (SBNP) can maintain high GAG and collagen content to make a cytocompatible biomaterial.

METHODS: NP tissue was extracted from bovine caudal IVDs, snap frozen, and pulverized. NP tissue underwent three different generalized solubilization methods: pepsin digestion (*pSBNP*), urea extraction (*uSBNP*), and urea extraction followed by DNase treatment (*udSBNP*). *pSBNP* underwent solubilization for 1-4 days (*pSBNP* 1-4) prior to neutralization, and urea solubilization was achieved using a modified protocol⁵ with a subset of samples undergoing a subsequent DNase treatment. All groups underwent biochemical analysis for GAG, collagen, and protein content using a dimethylmethylene blue assay (DMMB), hydroxyproline assay (HYP), and bicinchoninic acid (BCA) assay respectively. All samples were analyzed in comparison to fresh bNP (Fresh bNP) and pulverized fresh bNP (pFresh bNP) samples. Cytotoxicity of *pSBNP* 2, *uSBNP*, and *udSBNP* was assessed by seeding P4-P5 human adipose-derived mesenchymal stromal cells (hAdMSCs) and culturing them in growth media supplemented with concentrations of 5mg total protein/mL (Low) and 50mg total protein/mL (High) of each solubilized material. Cell viability, metabolic activity, and cell proliferation of each solubilization method was evaluated at 4 and 7 days. A sample size of n=4 was used for all outcomes and statistical comparisons were performed via a one-way or two-way ANOVA using GraphPad Prism software.

RESULTS: *pSBNP* showed no discernible difference between 1-4 days of digestion in residual DNA content (4.25 ± 1.5 , 4.25 ± 0.9574 , 3.75 ± 2.062 , and $3.5 \pm 0.5774 \mu\text{g}$ residual DNA content/mg dry weight), GAG content (56.1 ± 5.082 , 51.1 ± 11.13 , 45.98 ± 22.90 , $43.13 \pm 7.93 \mu\text{g}$ /mg dry weight), or collagen content (6.373 ± 1.750 , 7.453 ± 0.9047 , 8.313 ± 0.7796 , $8.693 \pm 0.8177 \mu\text{g}$ /mg dry weight). *udSBNP* showed statistically lower residual DNA content than *uSBNP* and bNP suggesting successful decellularization of the matrix ($p=0.245$ and $p=0.0009$, respectively). *udSBNP* and *uSBNP* groups showed higher levels of GAG content (724.2 ± 195.9 and $180.8 \pm 26.19 \mu\text{g}$ /mg dry weight, respectively) compared to *pSBNP*, but showed similar levels of collagen (4.093 ± 2.372 and $3.113 \pm 1.539 \mu\text{g}$ /mg dry weight, respectively). All study groups demonstrated cell viability above 90% at Day 7. At Day 7 the metabolic activity of Low *pSBNP* and Low *uSBNP* were not significantly different from that of the control group (containing only growth media) while all other groups showed significantly reduced metabolic activity.

DISCUSSION: This research sought to determine an optimal method for solubilization of the NP to develop a biomaterial that could be used for NP repair and regeneration. Previously, decellularized porcine NP had been solubilized using a pepsin-based procedure with some *in vitro* therapeutic effect on maintaining NPC phenotype,³ but solubilization had not been explored on bovine NP or with urea based solubilization methods. Our solubilization techniques were down selected into the most ideal groups based on their high GAG and collagen content. The methods that were considered the most desirable according to these criteria were *pSBNP* 2, *uSBNP*, and *udSBNP*. The solubilization methods were found to not be cytotoxic, as reflected by high cellular viability (>90%) across all groups after one week in culture, however some groups showed reduced metabolic activity. Future studies investigating gene expression changes in MSCs induced by these materials will be necessary. Urea solubilized bovine NP has the potential to serve as a biomaterial-based therapy for IVDD.

SIGNIFICANCE/CLINICAL REVELANCE: The degeneration of the NP can lead to IVDD which can lead to lower back pain. Since current treatments are limited in their ability to mimic the native ECM and natural environment of the NP, more *in vitro* treatments should be explored.

REFERENCES: 1. Newell N, et al. *J Mech Behav Biomed Mater*. 2017;69:420-434. 2. Schroeder GD, et al. *Semin Spine Surg*. 2016;28(1):2-7. 3. Wachs RA, et al. *Spine J*. 2017;17(3):435-444. 4. Wang B, et al. *J. Biomed Mater Res A*. 2018;106(3):829-838. 5. Rao Y, et al. *Stem Cell Res Ther*. 2022;13(1).

IMAGES AND TABLES:

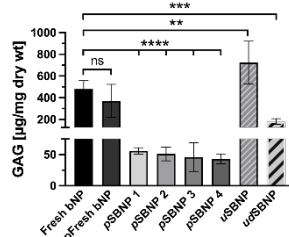


Figure 1. GAG content of SBNP measured through DMMB.

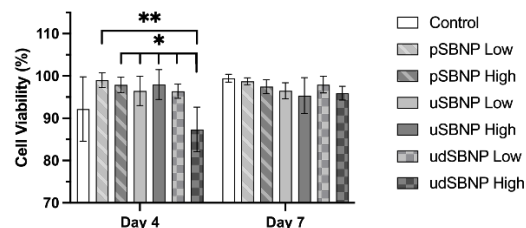


Figure 2. Cellular viability of SBNP cell lysates measured through Picogreen.