Cryopreserving Intact Intervertebral Discs for Increased Tissue Access

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INTRODUCTION: Low back pain is the leading cause of disability globally, affecting hundreds of millions of people worldwide. A major contributor to back pain is intervertebral disc (IVD) degeneration, which has treatment options limited to conservative pain management or invasive surgery. Efforts to develop regenerative therapies have been hindered by the limited availability of viable intact human IVDs for research. Studies often rely on animal models or in-vitro systems, which fail to replicate the complex mechanical and biochemical environment of the human spine. Creating a biobank of cryopreserved intact human IVDs with living cells would improve sample accessibility, accelerating research into new treatment strategies. Historically, cryopreserving intact IVDs has not been possible. To maintain cellular viability, whole tissue cryopreservation requires full saturation by chemical cryoprotectants (CPAs), which are toxic to cells within a short time window. In this work, we demonstrate a novel method using swelling-mediated transport to cryopreserve intact IVDs without compromising cellular viability, with potential for more efficient cryopreservation. A biobank of viable cryopreserved IVDs would enable researchers to uncover key pathways and pathologies, leading to new therapies for millions of people living with debilitating pain.

METHODS: IVDs were collected from freshly skinned Black Angus cows (24-30 months). Nucleus pulposus (NP) cells from three IVDs were isolated using enzymatic digestion in Dulbecco's Modified Eagle's Medium (DMEM) (0.2% Pronase E for 1 hour, followed by 0.025% Collagenase for 18 hours at 5% CO_2 and 37°C). NP cells were encapsulated in alginate beads and exposed to fresh media or one of two CPA solutions (10% dimethylsulfoxide [DMSO] and 10% propylene glycol [PG]/ethylene glycol [EG]) to determine the least cytotoxic solution. Live/dead assays measured cellular viability at multiple time points over 48 hours. Simultaneously, methods to improve CPA transport were explored. Nine IVDs were randomized into three groups (n=3): IVDs exposed to CPA without loading, IVDs pretreated with PrimeGrowth™ Isolation Medium then exposed to CPA without loading, and IVDs exposed to CPA following dynamic loading. DMSO saturation was tracked over 72 hours using Computed Tomography (CT). The full cryopreservation protocol was performed using the less cytotoxic CPA and more successful transport mechanisms and compared to negative and positive controls (n=4): fresh and unfrozen, no CPA and no compression, CPA without compression, and CPA with compression. The IVDs were cryopreserved at -80°C with a freezing rate of -1°C/minute for one week before being thawed and cultured of 24 hours.1mm samples were taken from each compartment of the IVDs and stained using a live/dead assay. Statistical analysis was performed using ANOVA followed by post-hoc t-tests. To further investigate cryopreservation methods, an additional nine IVDs were randomized into three groups (n=3): no compression, 0.25 MPa compression, and 0.5 MPa compression. IVDs were submerged in 70 mL of 79% DMEM, 1% P/S/A, 10% DMSO, and 10% EG for five days to ensure tissue saturation. IVDs were then placed in 100 mL of 1X PBS and compressed to remove CPAs. Osmolarity measurements were taken throughout the compression cycle to assess CPA expression over time.

RESULTS: Medium containing EG was less destructive than medium containing PG, bringing the window before cytotoxicity to 7.5 hours (Figure 1C). CT results showed non-loaded IVDs would not attain full saturation before cell death occurs, needing 72 hours to fully saturate the tissue (Figure 1B). PrimeGrowth did not show significant improvement of CPA transport versus the control ($p=0.85$); however, the use of swelling mediated transport produced significant improvement, reaching full saturation in 5 hours (Figure 1D). After cryopreserving IVDs for one week, the swelling mediated transport CPA group showed an average of 80-85% viable cells with no significant difference to the fresh, unfrozen controls whereas groups without swelling-mediated transport showed significantly less viability than the fresh, unfrozen controls ($p < 0.05$) (Figure 2B).The osmolarity expulsion showed the two compression groups released twice the CPA in the first minute of compression than the non-compressed, diffusion only controls (Figure 3).

DISCUSSION: This study presents a novel swelling-mediated method for cryopreserving whole IVDs. By using swelling-mediated transport, we were able to overcome CPA transport limitations and enable the cryopreservation of an intact IVD. This approach has resulted in maintaining cell viability across all regions of the bovine IVD soft tissue, showing no significant reduction compared to fresh, non-frozen controls. Additionally, the burst release seen in first minute of expelling suggests a potential direction to create a faster fluid flow gradient into and out of the IVD, leading to more efficient cryopreservation. A limitation of this work is that all experiments were conducted with bovine IVDs. Future directions of this project include applying this technology to human IVDs, optimized loading schemes, assessment of cellular phenotypes during preservation, and developing strategies to improve fluid flow.

SIGNIFICANCE/CLINICAL RELEVANCE: By using cryopreservation to store IVD tissue long-term, we lay the foundation for a tissue biobank that will improve access to appropriate samples for researchers.

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IMAGES AND TABLES:

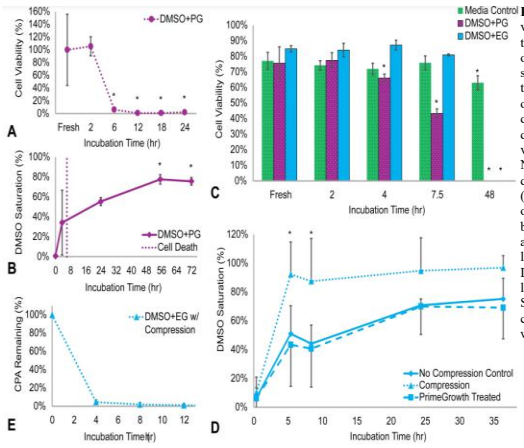

Figure 1: (A) Cellular viability of NP cells treated with DMSO+ PG over time. (B) DMSO saturation of whole IVDs treated with DMSO+ PG over 72 hours. Cellular death noted by the dashed line. (C) Cellular viability of encapsulated NP cells over time in different CPA medias. (D) DMSO saturation over 35 hours compared between PrimeGrowth and swelling-mediated loading. (E) The expel of DMSO+EG via dynamic loading over 12 hours. Significance from the control group is denoted with *.

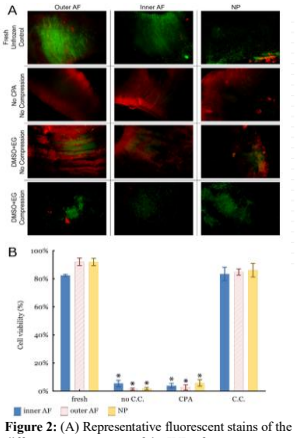

Figure 2: (A) Representative fluorescent stains of the different compartments of the IVD after cryopreservation. (B) Cellular viability quantified from the stained images. Significance from the control group is denoted with *.

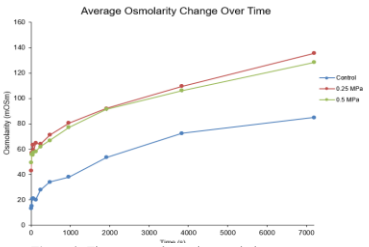

Figure 3: The average change in osmolarity throughout the expulsion cycle. Initial measurements were taken every fifteen seconds then used a logarithmic scale for the remaining measurements.