Comparative Viability, Potency and *In vivo* Efficacy of a Fresh or Cryopreserved Cell Therapy for the Treatment of Degenerative Disc Disease

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Introduction

- Cryopreserving cells provides many benefits for clinical use and commercialization, such as long-term storage, off-the-shelf usability, and the ability to complete safety and functional testing of the cells prior to human dosing.
- Our lab has identified a method to isolate progenitor cells directly from human disc tissue and create therapeutic cells known as *discogenic cells* to treat degenerative disc disease (**Figure 1**). We have optimized a formulation and method for cryopreserving these cells that is appropriate for clinical use.
- We compared the viability and potency of fresh and cryopreserved discogenic cells both *in vitro* (using a potency assay described at the 2013 ASGCT meeting) and *in vivo* (using a Gottingen minipig™ model).
- We hypothesized that the cryopreserved cells would be comparable to the fresh cells, which have been extensively tested previously.

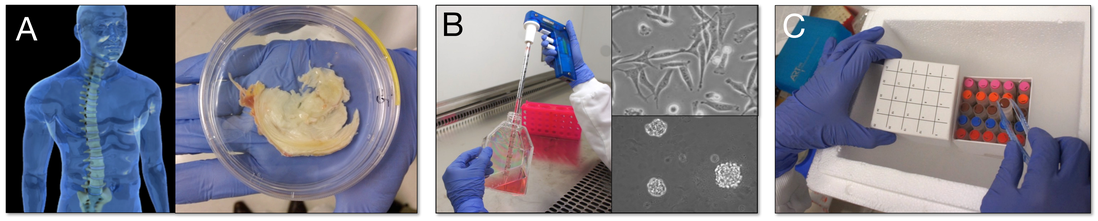


***Figure 1**: Process for creating discogenic cells. (A) Procure adult disc tissue. (B) Produce discogenic cells through proprietary multi-step process. (C) Create frozen bank of discogenic cells; release testing to ensure safety and consistency.*

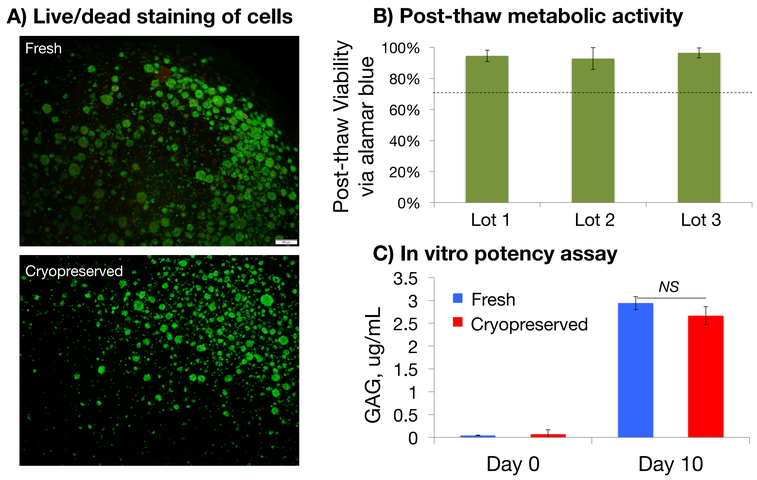
Methods

- Discogenic cells were combined with Profreeze™ and 7.5% DMSO. Cells were either tested prior to freezing (fresh), or after controlled rate freezing (cryopreservation) and storage at < - 150°C.
- Fresh and cryopreserved cells (post-thaw) were stained with live/dead fluorescent dye. Post-thaw, the metabolic activity of cells after 24 hours was assayed using Alamar Blue resazurin dye metabolic assay (n=6).
- Matrix formation (potency) was assessed by incubating a high concentration of cells in DMEM/F12 with 5% FBS, 10 ng/ml TGFb and 100 nM dex (n=6) for 10 days. The samples were digested with papain and the amount of GAG was quantified via DMMB assay.
- For the *in vivo* study, using a previously validated model, three lumbar discs of 4 Gottingen minipigs™ were injured (*note*: all animal work approved by private IACUC). After four weeks, each animal received either no treatment (injured control), gel control (1% w/v of high MW hyaluronic acid), 100,000 fresh cells with gel, or 100,000 frozen cells with gel (n=3/condition). Cryoprotectant was not removed prior to dosing. X-rays were performed every 4 weeks and disc height index calculated using 18 boney landmarks. Also, four weeks after injection, the discs were processed for paraffin histology and stained with H&E, Saf O and Masson's trichrome.

Results

- The cells remained highly viable after cryopreservation, as seen qualitatively via live/dead staining (**Figure 2, A**) and quantitatively using Alamar Blue across 3 lots (**Figure 2, B**).
- The *in vitro* potency assay for GAG production demonstrated significant and similar accumulation of GAG before and after cryopreservation (**Figure 2, C**).
- *In vivo,* the control discs continued to decrease in height after treatment, but both cell-treated discs showed static or improved disc height and were not significantly different from each other (**Figure 3, A**). Histology showed variable morphology across pig discs, with no unique differences between fresh and cryopreserved treatments (**Figure 3, B**).

***Figure 2: In vitro findings**. A) Live/dead staining shows cells viable after cryopreservation (green = alive; red = dead). B) Alamar blue assay showed that viability was above FDA required 70% threshold after cryopreservation for 3 lots (n=3). C) In vitro potency (GAG accumulation) is comparable between fresh and cryopreserved cells (n=5, NS = not significant via student t-test).*



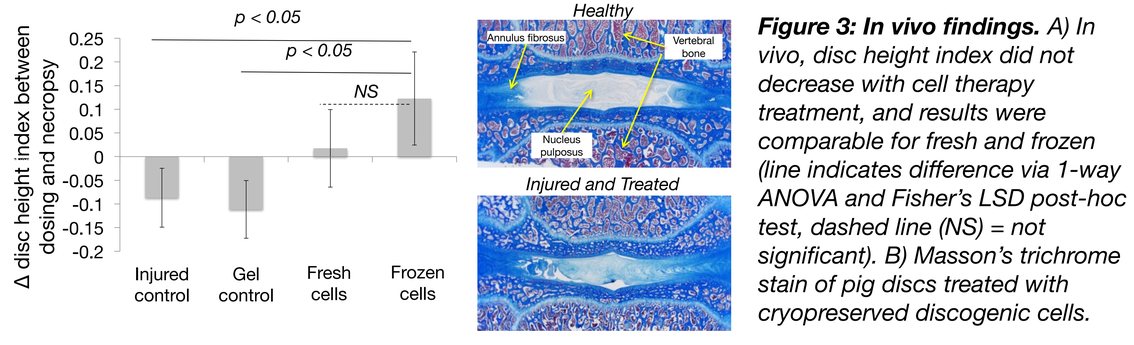


***Figure 3: In vivo findings.** A) In vivo, disc height index did not decrease with cell therapy treatment, and results were comparable for fresh and frozen (line indicates difference via 1-way ANOVA and Fisher's LSD post-hoc test, dashed line (NS) = not significant). B) Masson's trichrome stain of pig discs treated with cryopreserved discogenic cells.*

Summary/Conclusions

- Maintaining viability and potency after cryopreservation is a crucial step towards commercializing a cell therapy product.
- These studies demonstrate the cryopreservation protocols are appropriate and support the advancement of cryopreserved discogenic cells into clinical trials as a treatment for degenerative disc disease.