The Effect of Dosing and Cryopreservation on Efficacy and Safety of a Novel Cell Therapy for Degenerative Disc Disease Using a Porcine Model: Sub-Acute and Chronic Timepoints

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Introduction: Degenerative disc disease (DDD) is a primary cause of low back pain. Low back pain affects over 65 million people in the US and costs over $100B in care annually. There is a great need for novel treatments due to a current lack in options that regenerate the disc and provide long-term pain relief. Our lab has identified a method to isolate progenitor cells directly from human disc tissue and create a therapeutic cell population known as ‘discogenic cells’ through a multiple-step in vitro culture process. These cells are multipotent, express a unique profile of surface markers, and produce large amounts of endogenous proteoglycan, collagen and anti-inflammatory cytokines. At the 2014 ORS Annual Meeting, we presented in vivo safety and efficacy data for xenogenic injectable discogenic cell therapy (IDCT) delivered into 24 rabbits for 3 months. IDCT is a mixture of discogenic cells and viscous hyaluronic acid (600-800kDa, 1% concentration). The study demonstrated consistent improvement in disc height across a broad range of doses, repaired disc architecture via histology after application of IDCT, and consistent safety across multiple toxicological parameters. Since then, our lab has established a large animal model of DDD in Gottingen minipigs to continue testing the safety and efficacy of this potential spinal therapeutic. The objectives of the current study were to further optimize the large animal model, explore sub-acute (24 hour) inflammation and cell persistence, and compare cell concentrations and cryopreserved versus not cryopreserved (fresh) discogenic cells. Four weeks after injury, pigs were treated with various formulations of IDCT or control, and monitored for 1 day or 4 weeks. We hypothesized that the cells would persist for 24 hours with no signs of rejection or inflammation, despite the use of a xenogenic model. Further, we hypothesize that cell density would affect efficacy after 4 weeks and that both fresh and frozen discogenic cells would have similar efficacy in vivo. This study will help to identify the optimal dosing to be translated to human clinical trials, and also will help to finalize the formulation intended to use in human trials.

Methods: Using a previously validated model, three lumbar discs of 9 Gottingen minipigs were accessed surgically and the nucleus pulposus (NP) aspirated 3 times with a needle to induce degeneration (note: all animal work performed after approval of private IACUC). After four weeks, each animal received the same treatment in all injured discs (n=3), specifically either scaffold with 10,000 cryopreserved (CP) cells, 100,000 CP cells, 1,000,000 CP cells or 1,000,000 fresh cells, or scaffold alone, or a sham injection. The final two animals were either treated with 100,000 CP cells that were loaded with fluorescent calcein (to allow for traceability) or subjected to a sham injection. Needle placement was confirmed...
using multi-plane fluoroscopy (Fig 1D). Every 4 weeks for 8 weeks total, disc height was measured by 18 boney landmarks using x-ray images and normalized to week 0 values, resulting in a disc height index (DHI) that was then used to calculate the percent change in disc height from week 4 to week 8. Ex vivo MRI images were acquired at the end of the study using a Varian 7T DirectDrive MRI spectrometer. First, T2-weighted sagittal and transverse images were taken to identify the L1-L6 discs, and then transverse images taken for T2-mapping. For the two animals sacrificed after 1 day, one disc was dissected and the gross anatomy noted, and the NP imaged for the presence of fluorescent cells. The other discs were fixed and prepared for histology in paraffin. Sections were stained with H&E, safranin O, or Masson’s trichrome stain. For the 6 animals sacrificed after 4 weeks, all discs were prepared for histology as previously described. Finally, blood was collected from all animals prior to injury, 3-5 days after injury, and 3-5 days after dose delivery and analyzed for 22 hematological parameters and 19 clinical chemistry panel parameters. Clinical behavior and body weight was also noted throughout the study as measures of safety. Statistics: 1-way ANOVAs using Tukey’s post-hoc test was used for the DHI% data, with significance set at p < 0.05 and standard deviation graphed as error bars.

Results: By aspirating NP from the disc, significant changes occurred by the 4 week time point as seen via gross morphology (Fig 1A, B). Injury was confirmed via T2-weighted MRI by darkening of the disc compared to healthy adjacent discs (Fig 1C). One day after delivery, the human discogenic cells were found in high densities via calcein imaging (Fig 2A), showing that the xenograft were not rejected. Also, histology (H&E) did not reveal any inflammation at this sub-acute timepoint as demonstrated in the transition zone (Fig 2B) and in the NP (Fig 2C). Four weeks after delivery, safranin O and masson’s trichrome staining showed marked differences between healthy and injured discs, with some improvements noted after IDCT delivery as well as an absence of abnormal tissue after delivery of the progenitor cells (Fig 2D). H&E staining again showed a lack of inflammation (data not shown). Finally, disc height decreased to 64% of the original height 4 weeks after injury. After IDCT administration, the intermediate cell dose of 100,000 PF cells showed an improvement in disc height between 4 and 8 weeks compared to all other groups (p < 0.05) (Fig 3A). The disc height was comparable between CP and fresh cell formulations (data not shown). Finally, body weight was maintained throughout the course of the study (data not shown), with no concerning clinical behavior noted. Hematology and clinical chemistry panel results were consistent throughout the course of the study (three parameters are shown in Fig 3B).

Discussion: A large animal model of DDD was used to test the dosing and formulation of a novel cell therapy. A robust injury was formed after aspirating NP from the disc. One day after therapy administration, the cells persisted and no inflammation was noted. This may be due in part to the previously-characterized non-immunogenic properties of the cells, as well as to the fact that the disc is immune-privileged. Contrary to prior studies where only a 33X range of doses were tested and no dose-dependency was noted, in this study an optimized dose was found across a 100X range. The CP cells were equivalently efficacious to the fresh cells with respect to disc height; the ability to utilize CP cells allows for off-the-shelf usability in human patients and simplifies logistics. In this study, no major safety concerns were noted. A therapy containing discogenic cells continues to demonstrate efficacy and safety in animal models of DDD, suggesting that future human clinical trials of this therapy is warranted.
**Significance:** This work describes a broad range of safety and efficacy measures useful in evaluating novel therapies (cellular and non-cellular) for DDD and other intervertebral disc conditions. Further, this research supports the pre-clinical development of a novel therapy for the treatment of DDD in humans.

![Gross morphology of healthy and injured discs](image1)

![T2-weighted MRI image](image2)

![Dual-plane fluoroscopy](image3)

**Figure 1:** Gross morphology of A) healthy pig disc and B) injured disc after 1 month. C) T2-weighted MRI image shows darkening of disc after injury (blue circles). D) Dual-plane fluoroscopy used to ensure proper needle placement (blue circles) during dose delivery.
Figure 2: A) Calcein-labeled cells are found 24 hours after delivery in vivo (4X). H&E staining of B) transition zone (4X) and C) nucleus pulposus (20X) shows lack of inflammation 24 hours after delivery. After 1 month, D) safranin O stain and E) masson's trichrome stain shows healthy, injured, and IDCT-treated discs.

Figure 3: A) The 100,000 cell dose improved disc height compared to other cell doses and controls, as measured via x-ray (line indicates difference via 1-way ANOVA, p < 0.05). B) Representative safety parameters from hematology and clinical chemistry panels are normal throughout the course of the study (ALB = albumin, GLOB = globulin, Lym = lymphocytes)

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