

The Impact of Cryopreservation on In Vitro and In Vivo Potency and Safety of A Novel Cell Therapy in a Rabbit Model of Degenerative Disc Disease

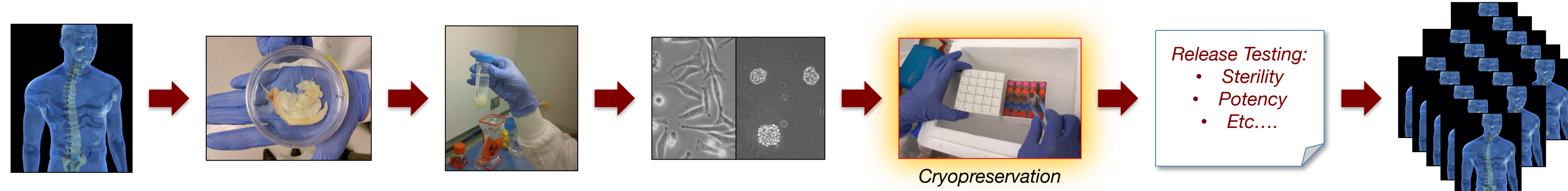
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INTRODUCTION

- We are developing a progenitor cell therapy called Injectable Discogenic Cell Therapy (IDCT) that treats degenerative disc disease.
- Cryopreservation is key to allow for commercialization and off-the-shelf usability. (Figure 1). However, it can result in loss of viability or changes to potency that could detract from the therapeutic potential of the treatment. Further, safety concerns may exist due to the presence of cryopreservatives, such as DMSO.
- The objective of the present study is to compare the *in vitro* and *in vivo* potency of fresh and previously cryopreserved discogenic cells. *In vitro*, the viability, recovery, and matrix-forming capacity was assessed. *In vivo*, a rabbit model was used to assess safety, disc height changes and histological appearance. We hypothesized that the *in vitro* potency assays would relate to the *in vivo* findings in the rabbit model, with cryopreserved discogenic cells behaving similarly to freshly isolated cells.

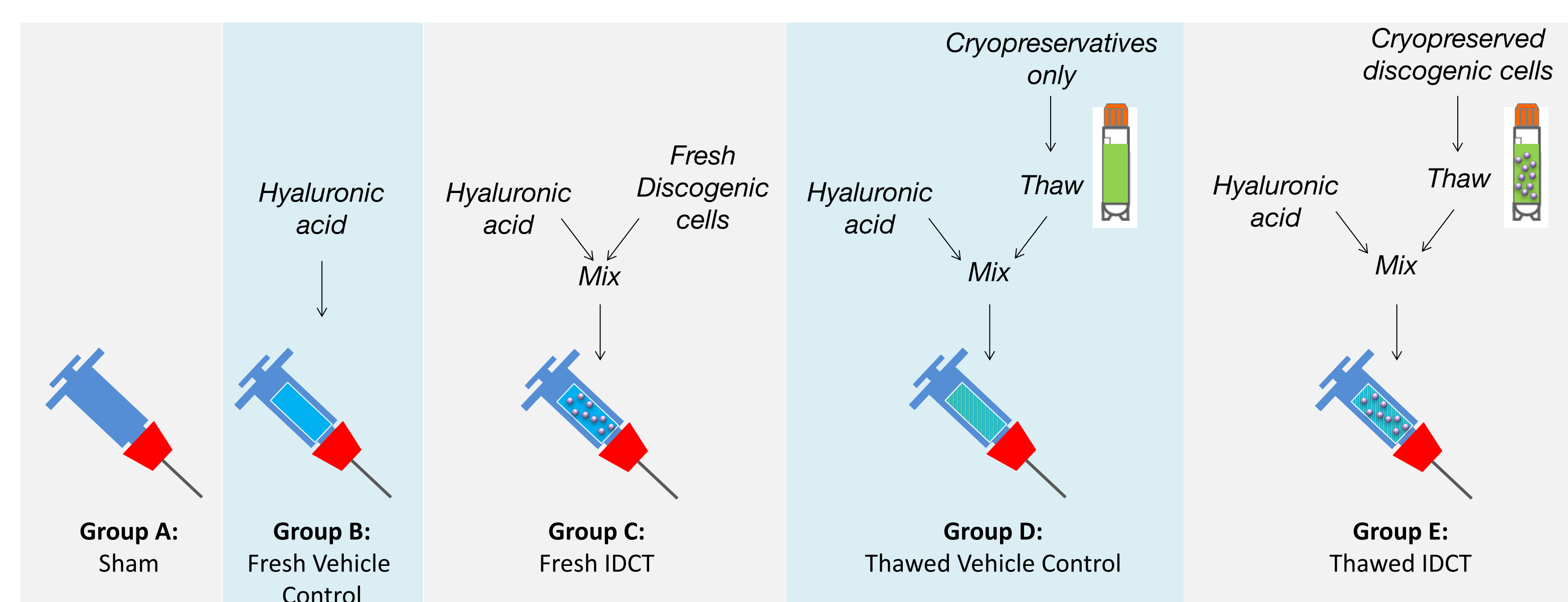
Figure 1: Process used to create discogenic cells.



METHODS

- Discogenic cells were produced from adult human disc tissue. Cells were either utilized immediately or cryopreserved in 7.5% DMSO and Profreeze™ using a controlled rate freezer.
- In vitro*: Viability was assessed via live/dead staining and trypan blue. Proteoglycan (potency) was quantified using DMMB assay after 10 days in culture (endogenous production) and after 14 days in micromass culture with chondrogenic medium.
- In vivo*: 10 female New Zealand White rabbits (5-6 months) were injured to induce degeneration using an annular stab model (L3/L4, L4/L5, and L5/L6). Four weeks later, discs were dosed as shown in Figure 2. Measurements included body weight, clinical observations, x-ray imaging (weeks 0, 4, 6, and 10 weeks) to assess disc height, and histology.

Figure 2: Design for rabbit study (n=6/group). Each injection contained 25 µl; dose was 30,000 cells.



RESULTS

- The cryopreservation protocol resulted in high viability and recovery of the cells after thaw (Figure 3A, B).
- Fresh and thawed cells produced comparable matrix in each of the potency assays that were utilized (Figure 3C, D).

RESULTS

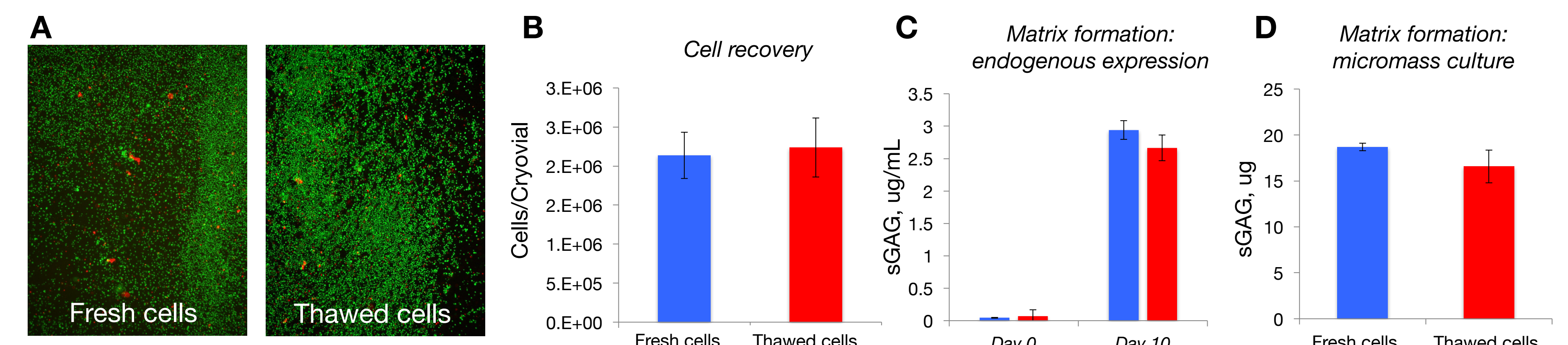


Figure 3: (A) Live/dead staining of cells. (B) Cell recovery (n=3 vials, with 4 counts per vial). (C) Endogenous proteoglycan production from 0 to 10 days as measured by sGAG (n=5/condition). (D) The micromass assay was performed and proteoglycan levels measured via sGAG (n=4/condition).

- In vivo*, no safety concerns were noted related to the cryopreservative agents, as seen by normal body weight (Figure 4A) and clinical observations as well as histology (Figure 4B).
- Six weeks after dosing of fresh and thawed IDCT, the nucleus pulposus contained collagen and proteoglycan, as shown via Masson's trichrome and Safranin O staining (Figure 4B).
- Disc height improved comparably for both fresh and thawed IDCT compared to all controls at 2 and 6 weeks after treatment ($p < 0.05$; Figure 4C); disc height improvement for a given treatment was consistent within animals, but varied across different animals ($p < 0.05$ via t-test).

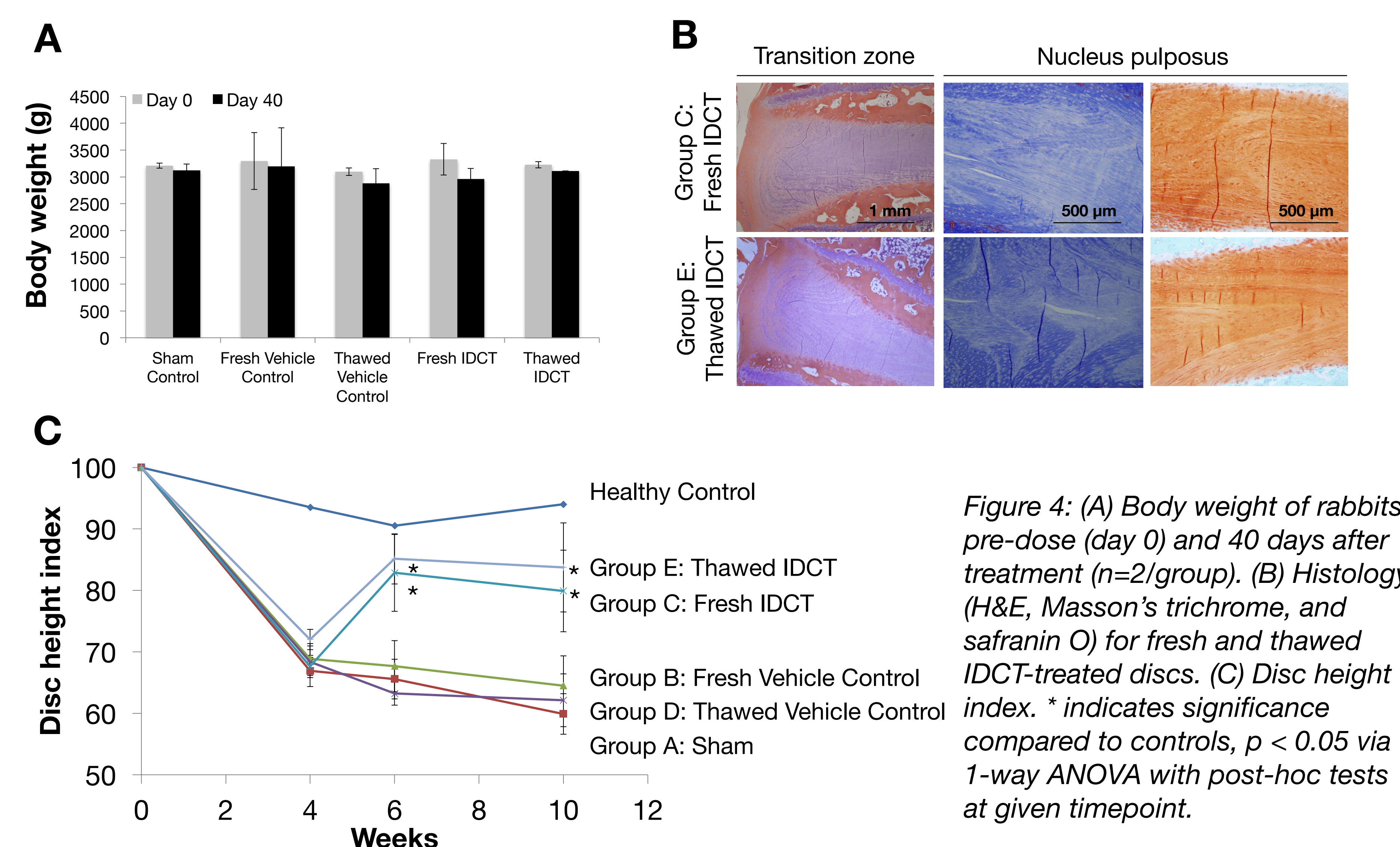


Figure 4: (A) Body weight of rabbits pre-dose (day 0) and 40 days after treatment (n=2/group). (B) Histology (H&E, Masson's trichrome, and safranin O) for fresh and thawed IDCT-treated discs. (C) Disc height index. * indicates significance compared to controls, $p < 0.05$ via 1-way ANOVA with post-hoc tests at given timepoint.

CONCLUSIONS

- While cryopreservation could have a negative effect on a cell therapy, this study demonstrates that IDCT remains viable and potent after thaw.
- In vitro* and *in vivo* findings were related, which supports the utility of *in vitro* assays as tools to limit the need for *in vivo* testing.
- No safety concerns were noted due to the use of DMSO or Profreeze™ cryopreservative, and disc height was improved as seen in previous animal studies.
- Human clinical trials are anticipated.