

# Assessment of Activity and Cell Persistence of a Novel Cell Therapy for the Treatment of Degenerative Disc Disease in a Gottingen Minipig™ Model: Xenogenic versus Allogeneic Considerations

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## INTRODUCTION

- Degenerative disc disease (DDD) is characterized by a breakdown of extracellular matrix (ECM) that results in decreased disc height, inflammation, changes to the endplate cartilage, and modulation of cellular phenotype.
- We have developed a method to isolate and expand progenitor cells from human disc tissue into a therapeutic cell population as a novel regenerative approach to disc repair.
- These cells are multipotent, express a unique profile of surface markers, and produce proteoglycan and collagen. Injectable discogenic cell therapy (IDCT) is the combination of these cells with a viscous hyaluronic acid carrier.
- The objectives of the present study were to assess 1) efficacy by disc height, 2) cell persistence after delivery, 3) differences between xenogenic and allogeneic cells, and 4) mechanism of action by gene expression analysis.
- We hypothesized that IDCT would be safe and result in improved disc height. Also, we hypothesized that neither the human nor pig cells would not persist to 2 weeks, and that gene expression for extracellular matrix molecules would not change for the native cell population.

## METHODS

- Degeneration was induced in L2-L3, L3-L4 and L4-L5 using stab/aspirate technique in 15 female Gottingen minipigs™ (9 months old). Four weeks later, animals were dosed with 150 µl of the groups in **Figure 1**. Cell dose was 100,000 cells.
- X-ray imaging of the spine was performed at weeks -4, 0, 2, and 6 weeks to determine the disc height index from 18 boney landmarks.
- At day 1, week 2, and week 6, the discs were isolated and processed for histology (Stem121, H&E), confocal imaging (for groups with Qdot® labels), and PCR. A Nikon A1 confocal microscope (205 nm excitation; 660-720 emission) was used to analyze the presence and location of Qdots® in 3 separate fields from 2 sections of each disc. Gene expression was quantified using internally validated TaqMan™ gene expression assays for aggrecan, collagen 2 and the housekeeping gene ACTB for pig species. Results were normalized to the housekeeping gene and then to the healthy control.
- At week 6, the spines from the remaining 6 animals were processed for histology (H&E, Safranin O, Masson's trichrome).

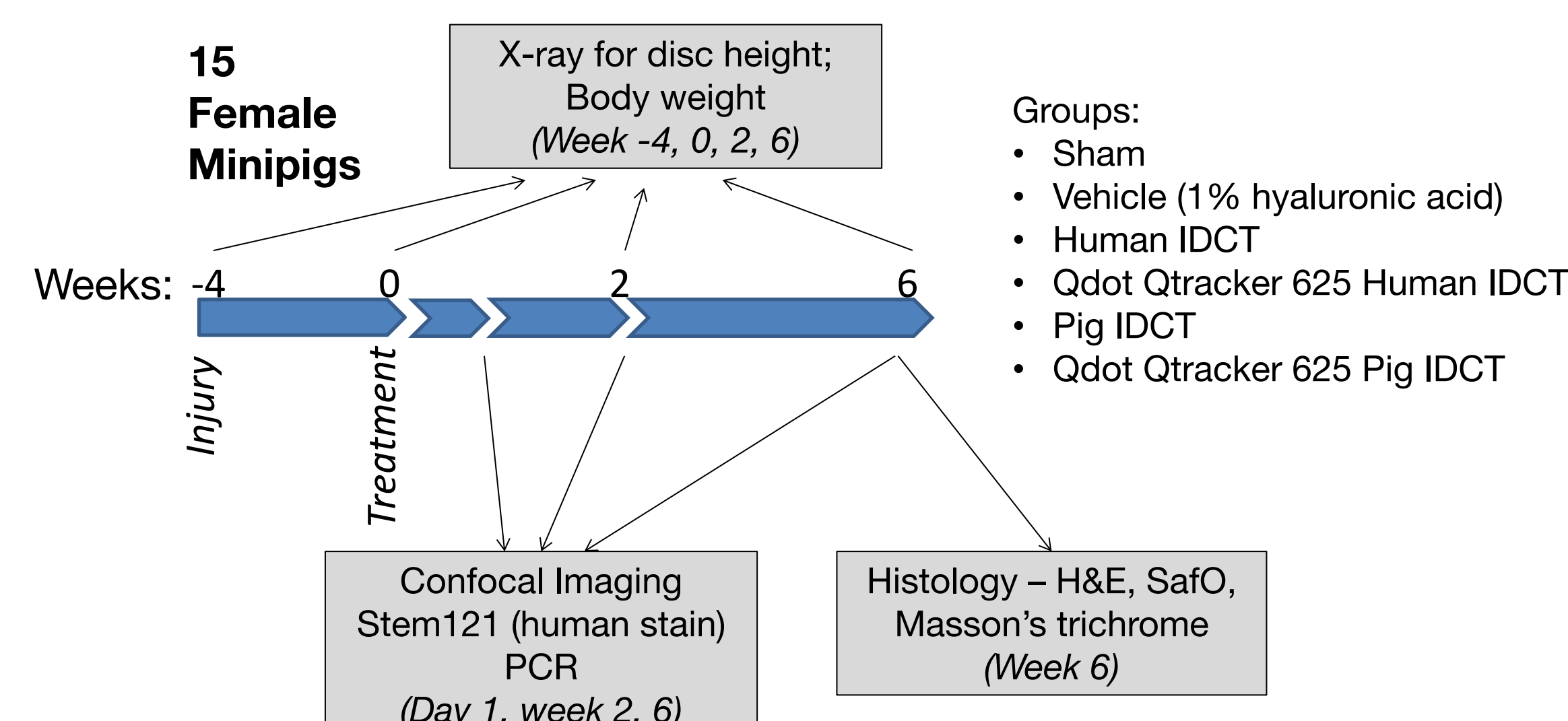


Figure 1: Schematic for animal study.

## RESULTS

- The injury method resulted in decreased disc height that was restored by application of IDCT but not sham or vehicle by 6 weeks after treatment ( $p < 0.05$  via 1-way ANOVA and post-hoc test). (**Figure 2B**).
- New ECM in IDCT-treated discs contained proteoglycan and collagen (**Figure 2C**), with no abnormal tissue noted as confirmed by gross appearance (**Figure 2A**).

## RESULTS

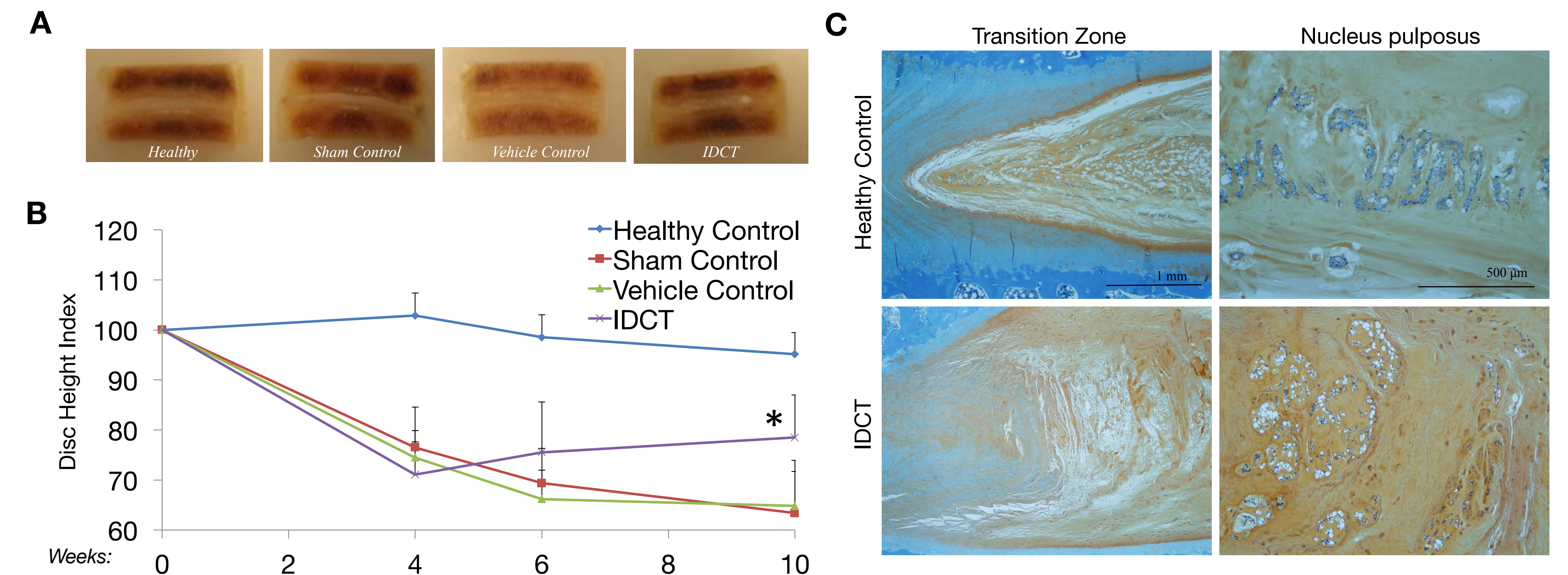


Figure 2: (A) Gross morphology of Gottingen minipig discs. (B) Disc height index (n=6/condition, \* Different than controls via 1-way ANOVA and post-hoc test,  $p < 0.05$ ). (C) Histology of transition zone and nucleus pulposus (safranin O stain).

- Qdot-labeled cells were identified through the NP and transition zone at 1 day, but not 2 or 6 weeks after treatment (**Figure 3A, B**). The results were similar between xenogenic and allogeneic models.
- Similarly, Stem121 staining was positive at day 1, but not later (**Figure 3C**).
- The improvement in disc height index was comparable between the allogeneic and xenogenic treatments, although the sample size was small (**Figure 3D**).
- PCR results showed that expression for aggrecan and collagen 2 by the native pig cells did not change significantly at day 1, week 2 or week 6 (*data not shown*).

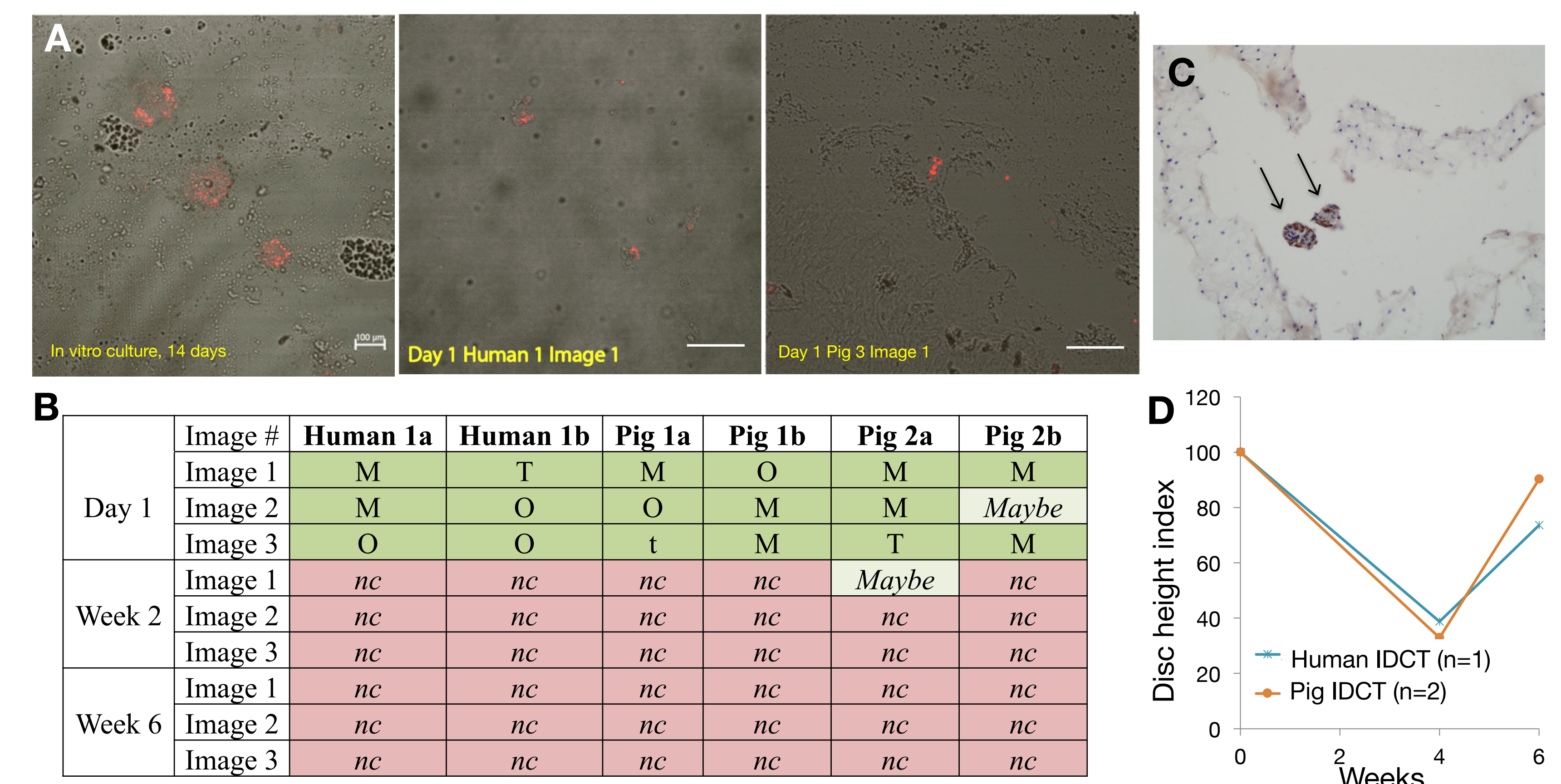


Figure 2: (A) Nanocrystal labeled cells. (B) Persistence of pig and human cells by timepoint (M = Middle; O = Outer region of NP; T = Transition zone; nc = No labeled cells found). (C) Stem121 stain for human cells at day 1. (D) Disc height index for human versus pig IDCT.

## CONCLUSIONS

- In a large animal model, IDCT is safe and results in improvement in disc height.
- The allogeneic and xenogenic treatments were comparable in terms of cell persistence (less than 2 weeks) and disc height improvement, supporting further use of the xenogenic model in future studies, which allows for better comparability to human clinical trial product.
- Gene expression for extracellular matrix molecule was not modulated after treatment with IDCT, suggesting that injected cells contribute directly to the new matrix formed.
- Human clinical trials are anticipated.