Human Tissue Procurement and Processing: Optimization of Tissue Handling

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Introduction

- Successful commercialization of an allogeneic cell therapy requires optimizing the processes used between tissue procurement and initial cell expansion in culture.
- We are developing a cell therapy to treat low back pain. The starting material is adult intervertebral disc tissue.
- In this study, we explored the stability of the tissue after procurement and prior to processing by incubating in storage specialized media at 4°C. Next, we explored the use of a vertical wheel vessel to expedite enzyme-driven tissue dissociation.

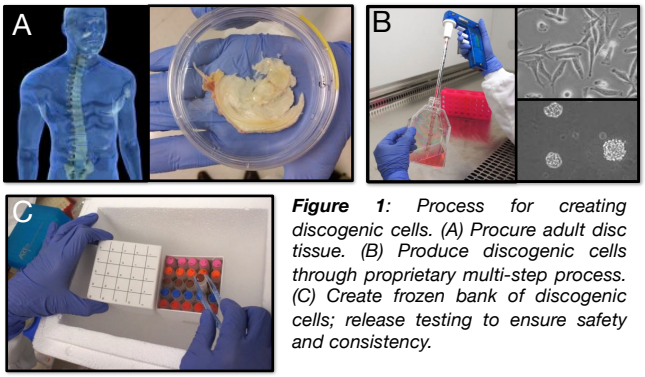
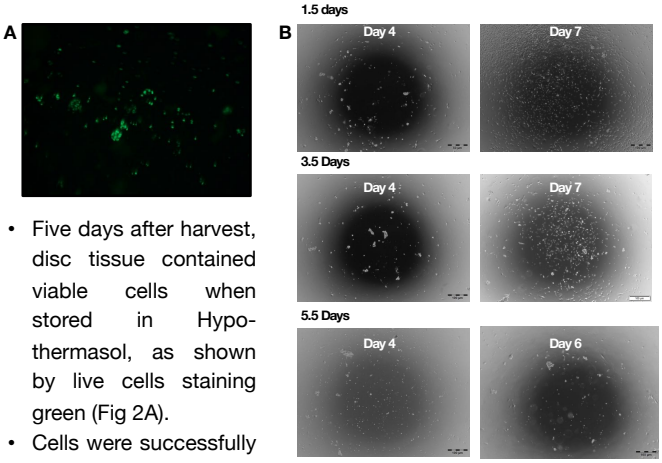


***Figure 1**: Process for creating discogenic cells. (A) Procure adult disc tissue. (B) Produce discogenic cells through proprietary multi-step process. (C) Create frozen bank of discogenic cells; release testing to ensure safety and consistency.*

Methods

- To evaluate tissue stability, minced human nucleus pulposus tissue was maintained in Hypothermasol (BioLife Solutions) for 1.5, 3.5 or 5.5 days at 4ºC. Live/dead fluorescent stain was used to visualize cells at each timepoint. Then, the cells were isolated enzymatically and plated onto flasks. Images of the flask were captured periodically to monitor cell adhesion and proliferation.
- To expedite enzymatic tissue dissociation, tissue was placed into a vertical-wheel vessel (28-40 RPM, PBSMini by PBS Biotech) that generates a rotating motion. Dissociation was monitored until chunks were no longer visible and resembled the end of normal, static dissociation.

Results



- Five days after harvest, disc tissue contained viable cells when stored in Hypo-thermasol, as shown by live cells staining green (Fig 2A).
- Cells were successfully grown from all conditions (Fig 2B).

***Figure 2**: (A) Live/dead stain of tissue after 5.5 days. (B) 10x phase images of cells on flasks.*

- Tissue dissociation was reduced from 14-20 hours to less than 5 hours in three independent experiments (Figure 3).

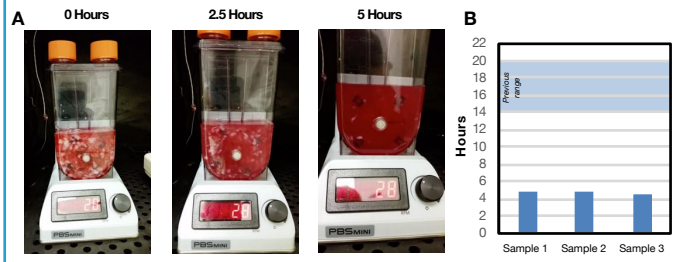


***Figure 3**: (A) Enzymatic dissociation of tissue in a vertical-wheel vessel. (B) In 3 samples, time to completion is faster than previous range.*

Results

- We have identified key enabling technologies to improve our manufacturing processing flexibility and efficiency. These new approaches still result in comparable cells at the end of the culture process.
- Additional optimization studies in other phases of the culture process are ongoing that can impact cost, consistency and quality.

This product is under preclinical investigation and has not been approved for use by the FDA.

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