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Optimization of AlphaLisa Assay for Aggrecan Detection During TMJ Fibrocartilage Stem Cell (FCSC) Differentiation

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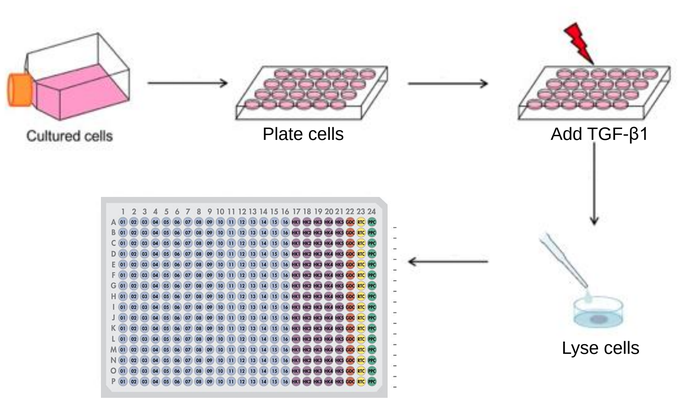
INTRODUCTION

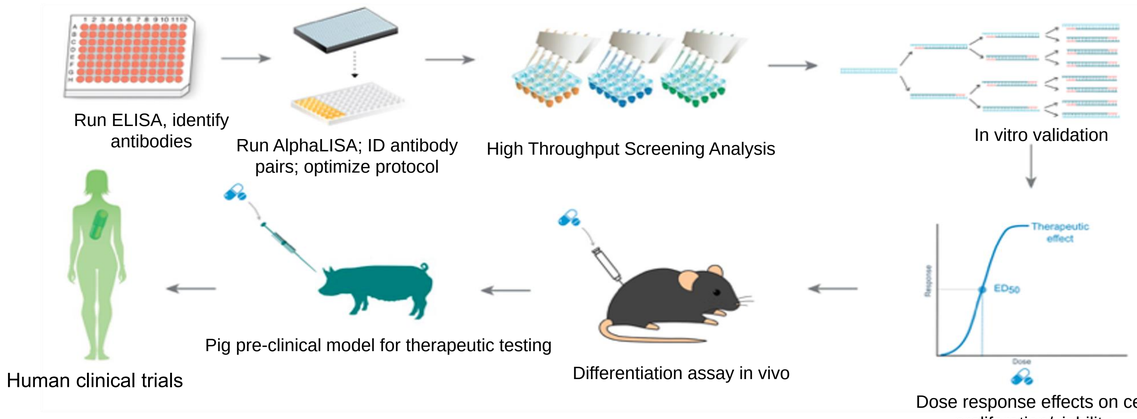
- TMJ is critical for movements of the jaw and activities such as speaking and eating
- In TMJ disorders, articular disc and/or joint undergoes degeneration or displacement, which significantly impairs normal functioning, causes pain, and decreases quality of life
- Despite the complex etiology and prevalence of TMJ disorders, it remains greatly understudied and poorly understood.
- No predictable form of treatment exists, with care primarily being palliative or surgical.
- Stem cell transplantation for regeneration comes with its own set of challenges such as immune rejection and engraftment
- Consequently, identifying a therapeutic compound that can induce differentiation of endogenous FCSCs into chondrocytes for cartilage formation is a promising approach for future treatment, that may overcome existing barriers with stem cell-based therapy in the area.

OBJECTIVES

- The Embree Lab has previously shown that intra-articular injections containing the Wnt inhibitor sclerostin induces endogenous FCSCs to regenerate cartilage in a rabbit TMJ-injury model.
- This project aims to further explore the therapeutic potential of this FCSC population by identifying approved drugs/small molecules that can be repurposed as TMJ fibrocartilage regenerative therapies.
- Immediate aims of this study are to 1) Develop an in-vitro phenotypic screen to quantify the differentiation of TMJ FCSCs using AlphaLISA for aggrecan; and 2) Submit protocol to the Columbia Genome Center for high throughput screening (HTS) analysis against clinical compound libraries to test potential candidates.

METHODS

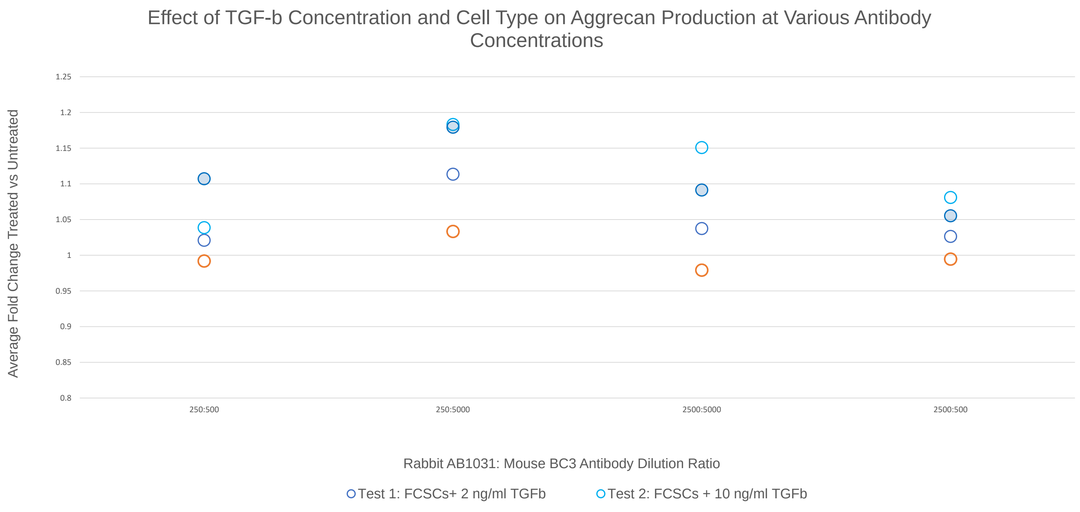


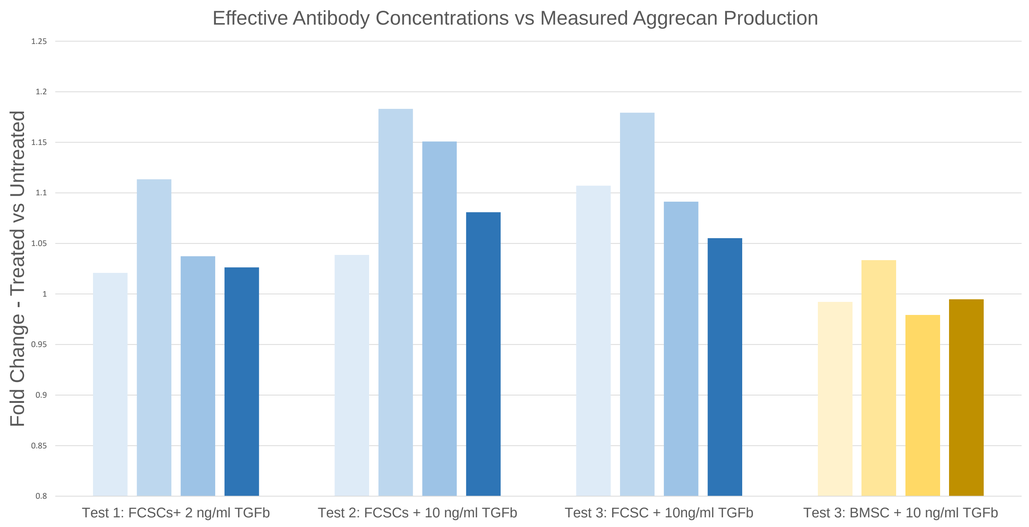


- Rat FCSCs and bone marrow stromal cells (BMSCs) previously isolated by Dr. Embree's lab were cultured and plated at a specified density in 384 well plates, incubated for the appropriate time in (20% FBS) media, and then serum starved overnight (in 2% FBS Media). To induce differentiation in positive controls, FCSCs were treated with the potent chondrogenic factor TGF-β1. After 2-3 days FCSCs were lysed. Then, the Ab pairs were formulated into various dilutions and added to the lysate. Lastly, acceptor beads were added, followed by the donor beads. Final plate sent for HTS analysis.

- When potential candidates are identified, they would require in vitro validation via RT-qPCR, dose response testing to examine effects on cell proliferation and viability, differentiation assays in vivo, pre-clinical model testing in a pig, and finally human clinical trials.

RESULTS & CONCLUSIONS





- The Ab's for aggrecan recognition and Ab pairs for donor and acceptor beads necessary for the AlphaLisa screen have been determined - BC3 (mouse aggrecan monoclonal Ab) for the donor beads and AB1031 (rabbit anti aggrecan polyclonal Ab) for the acceptor beads.
- Preliminary experiments have been run and analyzed by the HTS facility. The 250:5000 dilution exhibited the greatest fold change in signal-to-noise, and thus aggrecan expression/FCSC differentiation.

DISCUSSION

- Our initial findings indicate that AlphaLisa is a promising method for identifying drug candidates that can induce FCSC differentiation. However, further research is needed to increase the signal-to-background ratio of the screen.
- Once achieved, we can proceed with submitting the protocol to the HTS facility to test against clinical compound libraries.
- When potential candidates are identified, they would require in vitro validation via RT-qPCR, dose response testing to examine effects on cell proliferation and viability, differentiation assays in vivo, pre-clinical model testing in a pig, and finally human clinical trials.

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