## Project Title

**Developing a Novel Physiologically Relevant Model to Study Cartilage Regeneration**

### Abstract

Objectives

Osteoarthritis (OA) plagues a variety of mammalian species, including humans, dogs and horses. Many clinicians inject dextrose to stimulate mammalian immune systems to regenerate cartilage but some physicians raise doubts. Scientists have conducted few in vitro studies and not yet produced results with important translational implications. As a step towards elucidating mechanisms of dextrose-based cartilage regeneration, I aimed to develop an in vitro model more effectively representing cartilage growth in mammalian joint space and determine a dextrose dose that stimulates cartilage cell proliferation.

Methods

To create an in vitro model with higher translational potential, I extensively reviewed standards for culturing cells, selected a line better representing mammalian cartilage cells, formulated and custom-created a media within the normoglycemic range of most mammals (3.9-7.8 mM), and validated with Subject Matter Experts. To investigate proliferation, I empirically developed a dose schedule and physiologically relevant control. I experimentally optimized the colorimetric assay PrestoBlue (provides indirect measure of cell proliferation via metabolic activity). I conducted an experiment with 3 time points, 6 arms, and 4 replicates per arm/time point. To establish a preliminary therapeutic window that allows for a biphasic distribution, I tested a cubic regression model using the statistical significance criterion of p<0.05.

Results

Scientists and clinicians with expertise in dextrose injections for OA agreed in principle to a model with mouse cartilage cells in a DMEM/Ham’s F12 media mixture and a 4.24 mM glucose concentration. Several weeks of microscopic observation indicated the model’s ability to maintain cells. For the optimization experiment, I found incubating cells in the PrestoBlue assay for 20 minutes produced a highly linear correlation between cell number and absorbance. I anchored a dosing schedule at 25 and 400 mM hypertonic dextrose, with log defined intermediate points of 50, 100, and 200. A cubic regression model fit my dose-response experimental results (p<0.01) and enabled estimation of a therapeutic window centered around 250 mM.

Conclusions

Establishing a physiologically relevant in vitro model and effective dose for cartilage cell proliferation is a necessary first step towards designing informed molecular mechanisms and animal disease model research projects.

### Summary Statement

I establish a physiologically relevant in vitro model and hypertonic dextrose effective dose with the aim of advancing the study of regenerating cartilage in mouse disease models and subsequently a variety of mammalian species.

### Help Received

Professor Lin Chen provided lab space for me to conduct cell culture work and approved my research proposal. His lead post doc, Dr. Yi Kou, helped in troubleshooting laboratory challenges.