DRUG DELIVERY TO AVASCULAR TISSUE

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ABSTRACT

Osteoarthritis (OA) remains a debilitating disease affecting millions of people worldwide, characterized by cartilage which can delay or prevent the onset of OA. Cartilage is unable to regenerate due to a lack of blood vessel and nutrient supply. Additionally, drug delivery to cartilage presents a challenge as drugs fail to be retained within the knee joint space due to rapid clearance from the synovial fluid. *Bajpayee et al.* have successfully shown that positively charged proteins can penetrate through the full thickness of cartilage due to the tissue's negatively charged glycosaminoglycans (GAGs). Our three primary goals were to:

- Develop an osteoarthritic tissue culture model using cytokines and mechanical injury
- Quantify the biochemical properties of the cartilage tissue in both healthy and challenged cartilage
- Dye labeling of proteins and study of the transport properties of solutes in cartilage degeneration. There is an unmet need for the development of OA drugs

INTRODUCTION

Osteoarthritis (OA) is a common joint disorder in the United States and more than six hundred and fifty million people worldwide are affected by it. Osteoarthritis causes deterioration of the entire joint and affects multiple tissues within the joint space including cartilage, bone, meniscus, and synovial tissue. Cartilage is composed of an extracellular matrix, which consists of collagen (60%), aggrecans (35%), and chondrocytes (<5%). Cartilage is divided into the superficial zone, the intermediate zone, the deep zone, and the calcified cartilage.

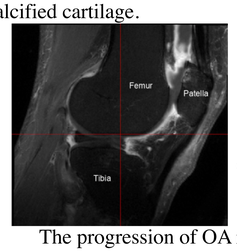

Figure 1: An x-ray image which demonstrates the degradation experienced by cartilage affected with osteoarthritis

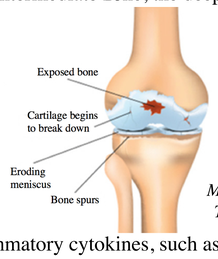

Figure 2: Osteoarthritic Knee

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The progression of OA results in pro-inflammatory cytokines, such as IL-1 and IL-6, to be released in tissue. Here, we develop an osteoarthritic tissue culture model using cytokine challenge by depleting the tissue of aggrecans. The model mimics tissue affected with OA and was used to quantify biochemical properties of the cartilage tissue. The mechanical stimulus from the dynamic mechanical analyzer (DMA) causes a direct impact to the extracellular matrix (ECM), and simulates the effects of a mechanical injury. After a mechanical injury, there is a higher amount of interleukin (IL-1), released, which causes inflammation and degradation in the tissue. If a mechanical injury is placed on a cartilage explant, more glycosaminoglycan (GAG) loss, cell death, and collagen loss will be expected. Due to cartilage being negatively charged, a positive peptide carrier, specifically avidin, was used to cause electrostatic attraction. The goal is to allow both uptake and penetration into the fullness of cartilage and retention of the drug in the knee joint for a long period of time.

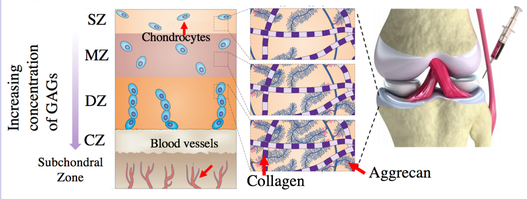

Figure 3: Cartilage Structure and Aggrecan Network

- 3mm x 1 mm cartilage disk
- 10 m or 1mm height
- Volume = lwh OR $\pi r^2 h$
- Volume = $\pi(1.5mm)^2(1mm)$
- Volume = $2.25\pi mm^3$
- Density = Mass/Volume
- 1 kg/m³ = Mass/2.25 πmm^3
- Mass = 2.25 x π
- **Mass = 7.07 mg**
- **Actual weight: 8.8 mg**

Figure 4: Calculation of Cartilage Explant

The research is scientifically significant because it contributes to the introduction of anti-inflammatory treatments of OA. Proper delivery and retention of drugs in the cartilage are necessary to achieve cartilage protection, to prevent cartilage matrix degradation, and to stimulate cartilage matrix biosynthesis.

EXPERIMENTAL METHODS

A. Developing an Osteoarthritic Culture Model

1. Harvested 3mm x 1mm bovine cartilage explants
2. *Developing a mechanically injured OA model:*
   1. Used DMA on cartilage explant by applying an unconfined compression of 50% strain to suppress the tissue from 1 mm to 0.5 mm thickness over a period of one second
   2. Cultured the cartilage explants in a serum free media for eight days
3. *Developing a cytokine challenged OA model:*
   1. Treated tissue in media with 5ng/uL of IL-1 for 4 days

Figure 5: Dynamic Mechanical Analyzer (DMA)
TA Instruments. 2018

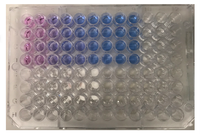

Figure 6: DMMB Assay using IL-1 and Control Samples

B. Measuring Biochemical Properties of Tissue

1. Created standard curve by taking a substance with a known concentration and absorbance in order to estimate an unknown concentration of the same substance
2. Performed Dimethylmethylene Blue Assay using control and osteoarthritic samples
3. Used standard curve to quantify and compare concentrations of GAGs between the two samples by reading the absorbance of each sample at 525 nm

C. Study Transport Properties of Solutes in Cartilage

1. *Conjugation of Protein and FITC Labeling:*
   1. Created 1mg/mL solution of FITC and 2mg/mL solution of BSA
   2. Added 100uL of FITC to BSA solution
   3. Dialyzed against Phosphate Buffer Solution (PBS) for 72 hours using 7kDa membrane to remove the excess dye
2. *Nonequilibrium Transports of Solutes in Cartilage:*
   1. Place a 6mm x 1mm cartilage explant in a custom designed transport chamber
   2. The upstream compartment was filled with 3uM Avidin and the downstream compartment was PBS
   3. The fluorescence of downstream solution was monitored over time using a spectrophotometer
3. *Equilibrium Intra-Cartilage Uptake of Solutes:*
   1. 3mm cartilage explants were equilibrated in 3uM bath of Avidin in a 96 well plate for 24 hours
   2. Changes in florescence were measured using the plate reader
   3. The amount of solutes uptaken by cartilage was calculated using standard curves

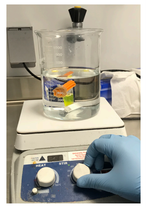

Figure 7: Attaching BSA to FITC experiment

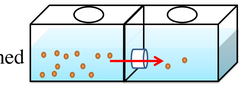

Figure 8: Transport chamber with solutes diffusing through cartilage explant

RESULTS

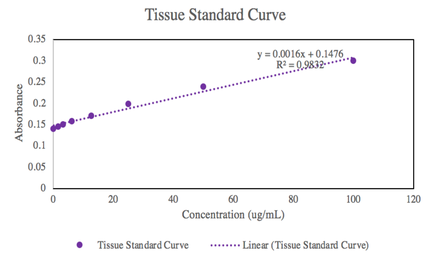

Figure 9: DMMB Assay Standard Curve made by taking cartilage explants with a known concentration, and measuring the absorbance. This will be used to find the unknown concentration of a explant by measuring its absorbance.

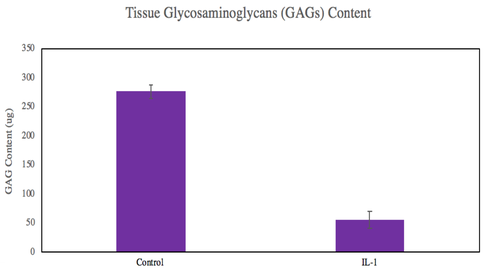

Figure 10: Concentration of Tissue GAGs Content in Control and IL-1 samples found by measuring the absorbance of a healthy cartilage explant and an explant treated with IL-1

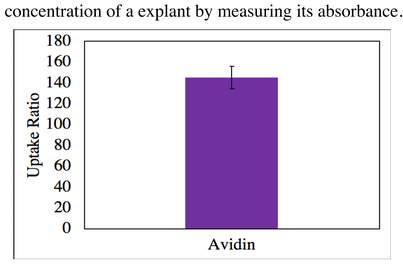

Figure 11: Uptake of Avidin into Cartilage Explants

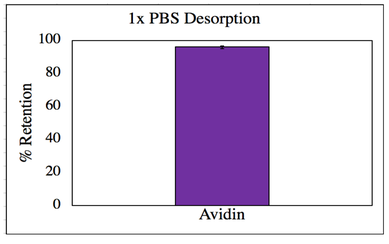

Figure 12: Retention in Cartilage Explants

RESULTS - Continued

$$\text{Molar F/P} = \frac{MW}{389} \times \frac{A_{495}/195}{[A_{280}-(0.35 \times A_{495})]/E^{0.1\%}}$$

Equation 1: FITC-protein conjugation equation used to calculate amount of FITC attached. The result was 6 FITC, which indicates 10% of the lysine amino acids in each protein had FITC.

$$\Gamma = \Phi K D_{SS} \frac{C_U - C_D}{\delta} \cong \Phi K D_{SS} \frac{C_U}{\delta}$$

Effective Diffusivity (D_{EFF}) = 3.8 x 10^{-7} cm^2/s
Steady State Diffusivity (D_{SS}) = 3.2 x 10^{-6} cm^2/s

Equation 2: Equation and Results to Measure the Rate of Effective Diffusivity and Steady State Diffusivity of Avidin

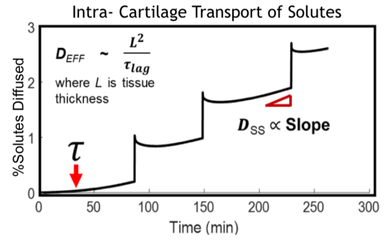

Figure 13: Graph of effective diffusivity of cartilage explants through protein chamber

CONCLUSIONS & FUTURE WORK

- Successfully created an osteoarthritic culture model. As seen in Figure 6, the tissue explants treated with IL-1 experienced dramatic losses in GAG content.
- Performed an injury using a DMA instrument put 50% strain on a cartilage explant and modeled the effects of a mechanical injury on cartilage
- With the help of various biochemical assays, such as DMMB dye assay, the effects of an injury were shown in both mechanical and cytokine challenge models
- A protocol for labeling the protein BSA with FITC dye was successfully created. Our calculations found that 10% of the lysine amino acids in the protein had FITC attached
- Transport properties of Avidin as a sample carrier was studied in cartilage. Avidin showed both high uptake and rapid diffusion in full thickness cartilage due to reversible charge interactions
- Proteins which are successfully able to penetrate cartilage will eventually be attached to pro-anabolic and anti-catabolic drugs. Such drugs include IL-1Ra, which counters the inflammation and degradation caused by IL-1. IGF-1, a growth factor to induce GAG production, or steroids such as dexamethasone, to relieve inflammation of OA

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