RESEARCH PLAN / PROJECT SUMMARY

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TITLE: Examining the Paracrine Effects of Adipose-Derived Mesenchymal Stem Cells in a Bovine Model of Osteoarthritis

Category: Biomedical and Health Sciences

A. RATIONALE

- **Background**

Osteoarthritis (OA) is a degenerative joint disease characterized by the breakdown of cartilage, which usually acts as a cushion between bones. This degradation causes joint pain and swelling as bones continually rub against one another. Moreover, the body's inflammatory responses result in the production of inflammatory markers and the generation of catabolic enzymes that can further degrade the articular extracellular matrix (ECM) (Mengshol et al., 2000). The production of inflammatory cytokines IL-1β, TNF, IL-6, IL-15, IL-17, and IL-18 are common in OA (Wojdasiewicz et al., 2014). These cytokines stimulate the production of matrix metalloproteinases like MMP 9 and MMP 13, which then degrade collagen and the ECM (Peter, 2006). Due to their ability to differentiate into various cell types, mesenchymal stem cells have provided insight for new treatments to an otherwise debilitating degenerative disease (Mei et al., 2017). Adipose-derived stem cells (ADSCs) have been shown to secrete exosomes, growth factors, cytokines, and other trophic factors that protect chondrocytes in osteoarthritic conditions and reduce the amount of matrix degrading enzymes (Platas et al., 2016).

- **Scientific Importance / Societal Impact**

The study will encourage the use of condition media over whole cell ADSCs in treatments for osteoarthritis because it will not induce a graft rejection and can be allogenic. It will also support the use of ADSCs cross-species, as pig ADSCs will be utilized to protect bovine cartilage.

B. RESEARCH QUESTION / HYPOTHESIS

- **Research Question**

Can the paracrine effects of adipose-derived stem cells exhibit protective effects on cartilage exposed to osteoarthritic conditions?

- **Hypothesis**

Previous studies have shown that ADSCs exhibit protective effects on cartilage (Platas et al., 2016), so it was hypothesized that the use of swine ADSCs condition media will result in an increase in survivability of bovine plug chondrocytes exposed to an in-vitro osteoarthritic condition.

C. RESEARCH METHODS

- **Procedures**:

Preparation of Stem Cells:
Adipose Derived Stem Cells will be obtained from the research facility and cultured in flasks containing 20ml of DMEM-F12 with 10% FBS and 1% P/S. They will then be transferred to Falcon Permeable Supports and allowed to seed in monolayer in their culture mediums.

Preparation of Groups:
Bovine cartilage plugs will be placed individually into 24 well plates. About 2.5 mL of media will be placed into each well. 4 total groups will be created: a regular control consisting of just the cartilage plugs, a positive control consisting of cartilage and ADSCs, a negative control consisting of cartilage and interleukin, and an experimental group consisting of cartilage, ADSCs, and interleukin. Well Plate 1 will hold the regular control and positive control. Well 2 will hold the negative control and experimental group. The permeable supports containing ADSCs will be placed over wells containing the Positive Control Group and Experimental Group. For each group, there will be four time points at which data will be collected: Day 1, Day 3, Day 7, and Day 14. Each time point will include 3 cartilage samples. The well plates will be kept in an incubator at 37^{o}C.

Addendum: Due to contamination, data from Day 14 was unable to be collected. Data from time points of Day 1, Day 3, and Day 7 were not affected by contamination and were successfully collected.

Cartilage Collection:
Once each time point is reached, the cartilage will be removed using sterile forceps and placed in small tubes labeled with the cartilage, its group, and its correlating time point. They will be stored in the freezer until all time points are collected to make data collection more streamlined and simple.

Histology:
Once all cartilage samples are collected, they will be embedded in paraffin and sectioned with a protocol similar to the one established by Schichnes et al., 1998. The samples will then be stained with Safranin O and plated in a protocol based on the one established by Kalscheur, 2001. The stained cartilage will finally be viewed under a compound light microscope with an attached camera where pictures will also be taken of the samples.

SOX 9 Immunohistochemistry Staining:
Immunohistochemistry staining of SOX 9 in the cartilage will also be done following a protocol established by Thomas, 2013. The results will be analyzed under a compound light microscope and using ImageJ.

Addendum: Immunohistochemistry staining of SOX 9 in the cartilage was done by another researcher in the lab, but I completed the analysis of the SOX 9 data.

- **Identify the Variables**
    - **Independent**: Timepoint (days), Presence of ADSCs and interleukin
    - **Dependent**: Presence of proteoglycans
    - **Constants**: Amount of condition media, Amount of cartilage, Incubation temperature

- **Identify the control of the experiment**

The regular control will be the cartilage alone without exposure to ADSCs or interleukin. The negative control will be the cartilage exposed IL-1β/6 without ADSCs. The positive control will be the cartilage exposed to ADSCs but not interleukin.

- **List *all* materials and supplies needed for the experiment**

- Bovine cartilage plugs provided by research facility
- 24 well plate
- Falcon Permeable Support for 24 well plate
- Culture media containing DMEM-F12 with 10% FBS and 1% P/S
- Micropipettes
- Adipose-Derived Stem Cells
- Microtome
- Paraffin
- Microscope slides
- Culture media containing interleukin 1β and 6
- Safranin O Stain
- Fast Green Stain
- Xylene
- 80%, 90%, 100% Ethanol
- Hematoxylin
- 1% Acetic Acid
- Distilled Water
- Small, capped tubes

- **Risk and Safety: Subject Specific Guidelines**

1. Human participants research: Not Applicable
2. Vertebrate animal research: Not Applicable

3. Potentially hazardous biological agents research:

a. Give source of the organism and describe BSL assessment process and BSL determination.
Adipose-Derived Mesenchymal Stem Cells: BSL 2 (~12mL), Bovine Cartilage: BSL 2 (24 plugs/~4mm) both supplied by the research institution.

b. Detail safety precautions and discuss methods of disposal.
Biological agents are stored in specific containers to be disposed of by research institute professionals. Lab coat, gloves, safety goggles, fume hood, and laminar flow hood will all be

used as safety precautions. Safety training will include: orientation to lab safety by Institute Director, lab disposal training and sterile technique training.

4. Hazardous chemicals, activities & devices:

- **Describe Risk Assessment process**

Chloroform - Harmful if swallowed, toxic if inhaled, causes skin and eye irritation, damage to organs if subjected to prolonged exposure; Xylene - Causes skin irritation, harmful if swallowed or inhaled; Safranin O - Skin irritation, eye damage; Eosin Y - Eye irritation; Trizol Reagent - Toxic if swallowed, respiratory irritation, skin burns, damage to organs through prolonged exposure

- **Detail supervision**

Research lab mentor/supervisor will be in the laboratory and monitoring all hazardous procedures.

- **Describe safety precautions and procedures to minimize risk**

Lab coat, gloves, and safety goggles will be worn in the lab. Any work done with xylene, Trizol, and chloroform will be done under a fume hood. Only a very small amount of chloroform that is necessary to the procedure will be used.

- **Discuss methods of disposal**

Chemicals and cells will be disposed in designated containers which are then properly disposed of by research institute professionals.

- **Data Analysis**

Safranin O stains red in the presence of proteoglycans, chondrocytes, and type II collagen, which are all present in healthy cartilage. When they are not detected, the cartilage will appear blue. Based on visual analysis of the stained cartilage sections, their level of degradation will be determined and recorded. Degeneration of cartilage will be shown to be blue, while healthy cartilage will appear red. The shades of the stain will also represent the degree to which cartilage is degraded or preserved. The SOX 9 protein should be present in healthy cartilage. An increase in levels of SOX 9 expression would represent cartilage that has not degraded.

D. BIBLIOGRAPHY

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