The Effect of Fibrin on the Differentiation of Dental Pulp Stem Cells - Research Plan

Rationale

In the field of regenerative medicine, pluripotent stem cells have many potential uses. A type of pluripotent stem cell, dental pulp stem cells have proven effective in regenerative endodontic therapy (Li, Parada, & Chai, 2017). Different factors, such as growth factors, transcriptional factors, and extracellular matrix proteins, can induce differentiation into cell types including odontoblasts, osteoblasts, chondrocytes, cardiomyocytes, neurons, and insulin secreting Beta cells (Potdar, 2015). We are choosing to focus on the effect of extracellular matrix proteins. As collagen is the natural scaffold for dental pulp stem cells, we will use collagen as the base of our hydrogel.

According to an experiment in revascularization of an immature tooth, fibrin may possibly be an ideal scaffold for the process because it enhances cell proliferation and differentiation and can act as a matrix for tissue ingrowth (Keswani & Pandey, 2013). Fibrin scaffolds have been found to be highly suitable for dental pulp regeneration because they promote a pulp-like tissue formation. Just some of the benefits of fibrin include its superior cytocompatibility and physiological degradation kinetics and nontoxic degradation products. Its formation of an extracellular matrix makes it ideal for culturing DPSC in vitro. Another benefit of fibrin usage is its relatively facile structural property manipulation by controlling concentration and ionic strength. This allows for optimization to mimic the viscoelastic properties of connective tissue ECM, fast nutrient diffusion, and homogenous cell encapsulation (Ducret et al, 2019). An injectable hydrogel of fibrin with polyethylene glycol (PEG) side chains has been used as a scaffold for dental pulp stem cells and resulted in high cell viability and

observable influence in odontogenic differentiation in a study designed specifically for applications in regenerative endodontics (Lu et al., 2015). The impact of fibrin scaffolds on dental pulp regeneration is leading us to study their potential impact on dental pulp stem cells.

If endodontic conditions can be nearly recreated, this opens up numerous possibilities for stem cell differentiation. Endodontic regenerative medicine is a major field of study in the broader field of regenerative medicine, as the stem cells from teeth can also be used in deriving other regenerative therapies, and the restoration of teeth can be widely studied due to the low risk and typically healthy subjects.

Research Questions

Does fibrinogen influence the odontogenic differentiation of human dental pulp stem cells?

Hypothesis

Human dental pulp stem cells cultured on collagen-fibrinogen hydrogels will show more evidence of odontogenic differentiation than those cultured on collagen-only hydrogels because fibrin has been proven to improve DPSC cell viability and proliferation.

Expected Outcome

We expect to see significantly more biomineralization and expression of genes indicative of DPSC osteo/odontogenic differentiation on the hydrogels containing fibrinogen.

Procedure

1. Culture DPSCs
    a. Warm flask of MEM Alpha, nutrient filled medium, to 37°C in hot water bath
    b. Transfer DPSCs from liquid nitrogen to flask

c. Free with DMSO, wash away with PBS

d. Transfer flask to biology incubator mimicking human internal conditions- 37°C, 5% CO_2

e. Change MEM Alpha medium every other day until transfer to hydrogels

f. Examine cell confluence with hemocytometer until approximately 80%

2. Prepare 15 2 mL wells of each hydrogel (collagen and collagen-fibrinogen)

   a. 6 for RT-qPCR (3 to be measured Day 8, 3 for Day 28), 2 for scanning electron microscopy (1 for Day 8, 1 for Day 28), 2 for EVOS microscopy (1 for Day 8, 1 for Day 28), 3 for rheology, and 2 without DPSCs

   b. Wash with MEM Alpha and store in incubator at 37°C, 5% CO_2

3. Plate DPSCs onto hydrogels

   a. Use trypsin to separate cells from flask

   b. Measure cell concentration to determine necessary volume of MEM Alpha to be added to result in concentration of 8000 cells per well

   c. Add necessary quantity of MEM Alpha

   d. Add cell solution to wells intended to contain cells

   e. Store in incubator

4. Maintain cell cultures

   a. Change MEM Alpha every other day

   b. Replace to incubator

5. EVOS Fluorescence Microscopy

a. Stain cells with 3.7% formaldehyde, 0.4% triton, and AF488 and DAPI stains on Days 8 and 28

b. Image the designated wells

6. Scanning Electron Microscopy

a. Dehydrate designated cells

b. Image those wells

7. Reverse Transcription Quantitative Polymerase Chain Reaction

a. Use QiAzol Lysis Reagent to aid in lysis of cells

b. Store in -80°C freezer

c. Isolate RNA with RNeasy Plus Universal Mini Ki

d. Perform RT-qPCR on Days 8 and 28

Data List

- Quantitative measure of expression of genes indicative of DPSC osteo/odontogenic differentiation
- SEM Images
- Fluorescence microscopy images
- Energy dispersive X-ray analysis from SEM imaging

Risk and Safety

The primary biological hazard of this experiment is infection or contamination from the DPSCs. The cells will be from a 13-year old's dental pulp, obtained from Stony Brook Dental

School. The BSL determination is BSL Level 2, due to the possibility of minor infection from the cultures. As there is no association with serious or lethal human disease with the experimentation with dental pulp stem cells, we deemed BSL Level 2 more appropriate than 3. To mitigate this risk, all cell work will be conducted inside the biosafety cabinet with appropriate precautions. Any cultured materials will be disposed in the biowaste bin, which will then be removed by Stony Brook University.

Several of the chemicals we will be using are also hazardous. Alexa Fluor 488 Phalloidin is harmful to the skin, liver, kidney, and heart. 200 uM L-ascorbic acid and 10 mM B-glycerol phosphate may be irritating to the mucous membranes and upper respiratory tract. Bleach is a severe irritant, presenting the risk of chemical burns. DAPI causes skin and eye irritation. DMSO causes mild skin and eye irritation as well, but long term exposure to it is carcinogenic. Ethanol, which we will be using primarily for sterilization for the biosafety cabinet, is a flammable CNS irritant that can cause heart and liver damage. FBS is associated with respiratory tract and skin irritation. Bovine fibrinogen is harmful by inhalation. Formaldehyde is a carcinogen. Penicillin streptomycin sol is an asthma inducing irritant. Propidium iodide and Triton X-100 can be irritants, and trypsin is linked to possible eye and skin irritation. The safety precautions we will use for these chemical hazards are use of lab coats, safety goggles, and gloves at all times in the lab. All work with these chemicals will be supervised by qualified scientists.

The glass pipettes we will use are risks if broken, and the high pressure and heat of the autoclave are potential hazards too, such as electric burns, shocks, or possible explosion. We will exercise caution in the handling of glass pipettes and the operation of the autoclave. All sharps will be disposed of in the appropriate container for safety.

As for all laboratory activity, caution and vigilance will be exercised at all times.

Data Analysis

The results of the EVOS microscopy and SEM will be qualitative, so the interpretation will be largely visual. The numerical data obtained from the RT-qCR will be tested for statistical significance of difference between gene expression for collagen and collagen-fibrinogen gels.

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Addendum

We decided to use gelatin gels in lieu of collagen gels due to gelatin's significantly lower price. Since gelatin is derived from collagen, we thought it would be the best substitution to create our desired properties in this model. The days chosen to take the measurements were changed to 12 and 31 due to scheduling of the laboratory.