Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Victoria Levy Grade: 11

   Email: victoria.levy2021@stanthonyshs.org Phone: (631) 806-0165

   b. Team Member: Vimala Alagappan c. Team Member: _____

2. Title of Project:

   The Effect of Fibrin on the DIfferentiation of Dental Pulp Stem Cells

3. School: Saint Anthony's High School School Phone: (631) 271-2020

   School Address: 275 Wolf Hill Road, South Huntington, NY 11747

4. Adult Sponsor: Paul Paino Phone/Email: ppaino@stanthonyshs.org

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☑ Yes ☐ No Tentative start date: _____

6. Is this a continuation/progression from a previous year? ☐ Yes ☑ No
   If Yes:
   a. Attach the previous year's ☐ Abstract and ☐ Research Plan/Project Summary
   b. Explain how this project is new and different from previous years on
   ☐☐ Continuation/Research Progression Form (7)

7. This year's laboratory experiment/data collection:

   06/28/19 (Actual Start Date: (mm/dd/yy))

   12/02/19 (End Date: (mm/dd/yy))

8. Where will you conduct your experimentation? (check all that apply)

   ☑ Research Institution ☐ School ☐ Field ☐ Home ☐ Other: _____

9. List name and address of all non-home and non-school work site(s):

   Name: Stony Brook University

   Address: 100 Nicolls Rd
   Stony Brook, NY 11794

   Phone/email: (516) 458-9011

10. **Complete a Research Plan/Project Summary following the Research Plan/Project Summary instructions and attach to this form.**

11. **An abstract is required for all projects after experimentation.**

Research Plan/Project Summary Instructions

A complete Research Plan/Project Summary is required for ALL projects and must accompany Student Checklist (1A).

1. All projects must have a Research Plan/Project Summary
   a. Written prior to experimentation following the instructions below to detail the rationale, research question(s), methodology, and risk assessment of the proposed research.
   b. If changes are made during the research, such changes can be added to the original research plan as an addendum, recognizing that some changes may require returning to the IRB or SRC for appropriate review and approvals. If no additional approvals are required, this addendum serves as a project summary to explain research that was conducted.
   c. If no changes are made from the original research plan, no project summary is required.

2. Some studies, such as an engineering design or mathematics projects, will be less detailed in the initial project plan and will change through the course of research. If such changes occur, a project summary that explains what was done is required and can be appended to the original research plan.

3. The Research Plan/Project Summary should include the following:
   a. **RATIONALE:** Include a brief synopsis of the background that supports your research problem and explain why this research is important and if applicable, explain any societal impact of your research.
   b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), ENGINEERING GOAL(S), EXPECTED OUTCOMES:** How is this based on the rationale described above?
   c. Describe the following in detail:
      - **Procedures:** Detail all procedures and experimental design including methods for data collection. Describe only your project. Do not include work done by mentor or others.
      - **Risk and Safety:** Identify any potential risks and safety precautions needed.
      - **Data Analysis:** Describe the procedures you will use to analyze the data/results.
   d. **BIBLIOGRAPHY:** List major references (e.g. science journal articles, books, internet sites) from your literature review. If you plan to use vertebrate animals, one of these references must be an animal care reference.

Items 1–4 below are subject-specific guidelines for additional items to be included in your research plan/project summary as applicable.

1. Human participants research:
   a. Participants: Describe age range, gender, racial/ethnic composition of participants. Identify vulnerable populations (minors, pregnant women, prisoners, mentally disabled or economically disadvantaged).
   b. Recruitment: Where will you find your participants? How will they be invited to participate?
   c. Methods: What will participants be asked to do? Will you use any surveys, questionnaires or tests? If yes and not your own, how did you obtain? Did it require permissions? If so, explain. What is the frequency and length of time involved for each subject?
   d. Risk Assessment: What are the risks or potential discomforts (physical, psychological, time involved, social, legal, etc.) to participants? How will you minimize risks? List any benefits to society or participants.
   e. Protection of Privacy: Will identifiable information (e.g., names, telephone numbers, birth dates, email addresses) be collected? Will data be confidential/anonymous? If anonymous, describe how the data will be collected. If not anonymous, what procedures are in place for safeguarding confidentiality? Where will data be stored? Who will have access to the data? What will you do with the data after the study?
   f. Informed Consent Process: Describe how you will inform participants about the purpose of the study, what they will be asked to do, that their participation is voluntary and they have the right to stop at any time.

2. Vertebrate animal research:
   a. Discuss potential ALTERNATIVES to vertebrate animal use and present justification for use of vertebrates.
   b. Explain potential impact or contribution of this research.
   c. Detail all procedures to be used, including methods used to minimize potential discomfort, distress, pain and injury to the animals and detailed chemical concentrations and drug dosages.
   d. Detail animal numbers, species, strain, sex, age, source, etc., include justification of the numbers planned.
   e. Describe housing and oversight of daily care
   f. Discuss disposition of the animals at the termination of the study.

3. Potentially hazardous biological agents research:
   a. Give source of the organism and describe BSL assessment process and BSL determination.
   b. Detail safety precautions and discuss methods of disposal.

4. Hazardous chemicals, activities & devices:
   - Describe Risk Assessment process, supervision, safety precautions and methods of disposal.
   - Material Safety Data Sheets are not necessary to submit with paperwork.

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.

References

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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.