Student Checklist (1A)

This form is required for ALL projects.

1. a. Student/Team Leader: Timothy Liu Grade: 12

   Email: tliu1@student.gn.k12.ny.us Phone: (347) 592-5367

   b. Team Member: _____ c. Team Member: _____

2. Title of Project:

   Synthesis and Analysis of a Novel Biodegradable Polyester Fiber Scaffold Derived from Poly(glycerol sebacate)

3. School: Great Neck South High School School Phone: (516) 441-4820

   School Address: 341 Lakeville Road, Great Neck, NY, 11020

4. Adult Sponsor: Carol Hersh Phone/Email: chersh@greatneck.k12.ny.us

5. Does this project need SRC/IRB/IACUC or other pre-approval? ☐ Yes ☒ No Tentative start date: 7/8/19

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:

   7/8/19 (Actual Start Date: (mm/dd/yy)) 8/9/19 (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: Rensselaer Polytechnic Institute

   Address: 110 8th St, Troy, NY 12180

   Phone/email: (518) 276-6000

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.

Rationale:

I. Tissue engineering is the growth of new tissue using cells, a scaffold, and growth factors
    A. The regeneration of tissue could treat millions of patients, whether by transplanting engineered tissue/organs (neo-tissue and neo-organs), or by endogenously aiding in regeneration (Furth & Atala, 2014)
    B. Engineered tissue can also be used in research (O'Brien, 2011)
        1. Engineered tissue can study tumor growth, emulating the microenvironment of a tumor better than cell culture
II. Scaffolds are 3D structures that mimic the ECM of cells and tissue, allowing for cell growth in engineered tissue (Stratton, Shelke, Hoshino, Rudraiah, & Kumbar, 2016)
    A. Must be biocompatible materials, often biodegradable polymers
    B. Provide physical support for cells and the structure of tissue
    C. Grant engineered tissue its mechanical properties
    D. Must be able to degrade in order for ECM to eventually take its place (O'Brien, 2011)
III. Poly(glycerol sebacate) is a biodegradable elastic polymer with potential for use as a scaffold in tissue engineering
    A. Its monomers, glycerol and sebacic acid, are both found naturally in the body
        1. Glycerol is a big component of triglycerides, and sebacic acid is a metabolic intermediate of fatty acids (Wang, Ameer, Sheppard, & Langer, 2002)
    B. Its elastic mechanical properties are suitable for soft tissue (eg skin, ligaments, nerves) engineering
    C. Glycerol's hydroxyl groups also allow for hydrogen bonding between backbones, biodegradation via hydrolysis, and crosslinking (Wang et al., 2002)
    D. PGS is unable to be electrospun
        1. The use of a high voltage to polarize a jet of dissolved(/melted) polymer and accelerate it towards a collector
            a) The solvent evaporates in the atmosphere before reaching the collector- solid fibers are collected
        2. Electrospinning creates micrometer-nanometer fiber mats with strong properties (Sill & von Recum, 2008)
IV. Modifications
    A. Octanediol was previously used to form a polyester elastomer (Yang, Webb, & Ameer, 2004)
        1. Poly(1,8-octanediol-*co*-citrate) (POC)
        2. Octanediol is the largest water soluble and nontoxic aliphatic diol
    B. Novozym 435 is an enzyme that helps catalyze esterification
        1. Would help increase molecular weight by aiding more in the reactions of primary hydroxyls of glycerol, not the secondary one (Ortiz et al., 2019)

Hypothesis/Engineering Goals: Increase molecular weight of PGS to allow for electrospinning. A greater molecular weight, achieved by the addition of 1,8-octanediol in a greater amount and Novozyme 435, will allow for PGS to be electrospun and to obtain improved mechanical and thermal properties.

Procedure:

I. Synthesis of PGOS
   A. 1:1:2, 1:3:4, and 1:4:5 molar ratios of glycerol:1,8-octanediol:sebacic acid
   B. Reaction procedure
      1. Under nitrogen, the mixture will be melted at 150° C, then brought down to 120°C for the first hour (hour including the melting process)
         a) Then, the temperature will stay at 120°C for the next 24 hours and continue under nitrogen
      2. After 25 hours, 0.5g of Novozyme 435 is added and the temperature will be brought down to 90°C for 2 hours, again under nitrogen
      3. Without nitrogen and at 90°C:
         a) The pressure will then be brought down to 100Torr for 4 hours
         b) The pressure will then be brought down to 75 Torr for 12 hours
         c) The pressure will then be brought down to 50 Torr for 12 hours
         d) The pressure will then be brought down to 25 Torr for 16 hours
   C. The polymer will be washed out of reaction tubes with chloroform in order to filter the enzyme out with filter paper
   D. The chloroform solution containing the polymer will be evaporated using a rotovap
      1. Chloroform will be further evaporated in a 40-50C oven under pressure for 3 days

II. Gel permeation chromatography assay
   A. 2mg of sample will be dissolved in 1mL of anhydrous tetrahydrofuran
   B. The solution will be transferred to GPC vials
   C. The GPC system will be calibrated with polystyrene and run

III. Differential scanning calorimetry assay
   A. 10mg of the required sample will be measured
   B. The sample will be heated to 250C, cooled to -60C, and again heated to 250C
      1. The sample is heated twice in order to ensure that the sample consists of amorphous regions, as the quick cooling prevents excess chain entanglement

IV. 1H Nuclear magnetic resonance assay
   A. Samples of 2-10mg will be dissolved in about 0.6mL deuterated tetrahydrofuran and run in a 500 mHz NMR spectrometer
   B. The program used will automatically shim and lock optimal parameters

V. Biodegradation assay
   A. 15mL of phosphate buffered saline (PBS) will be added to 20mg of electrospun and lyophilized fibers in 50mL centrifuge tubes

B. Tubes will be incubated at 37C and shaken gently at 100rpm for 3, 7, 11, 15, 19, 23, 27, and 31 days
C. For samples undergoing degradation for longer than 14 days, PBS buffer will be replaced every 17 days
    1. First, tubes will be washed with the following process
        a) Tubes will sit to allow all fiber to precipitate to the bottom
        b) Supernatant will be removed and deionized water will be added and removed
    2. 15mL PBS buffer will then be added
D. After each time period, tubes will be washed using the above process and freeze dried
    1. Then, fiber will be massed and undergo SEM imaging to visualize degradation activity

Risk and Safety:

- Tetrahydrofuran and chloroform, both possible carcinogens, must be used to dissolve PGS in solution or to clean glassware
    - Standard personal protective equipment will be worn and solvents will only be used under a BSL-2 fume hood
- Electrospinning of PGS into fibers requires a high voltage (17.5kV)
    - Interaction with electrospinning equipment when electric field is on will be done by a qualified scientist

Data Analysis:

- NMR data will be analyzed using the Mnova NMR program
    - Structure and the ratio of monomers in product will be obtained with chemical shift location and integration
- DSC data will be analyzed using the TA Universal Analysis program
- GPC data will be analyzed using Waters Breeze GPC software
    - DSC and GPC data will each be analyzed using a one-way ANOVA
    - T-tests will be performed between all ratios to find significant differences between specific groups
- Weight loss data is measured by comparing original mass to current mass
    - Data collected from the biodegradation assay will be graphed on a line graph to measure weight loss % by day

Bibliography:

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2. O'Brien, F. J. (2011). Biomaterials & scaffolds for tissue engineering. *Materials Today, 14*(3), 88-95.

3. Ortiz, C., Ferreira, M. L., Barbosa, O., dos Santos, J. C., Rodrigues, R. C., Berenguer-Murcia, Á., ... & Fernandez-Lafuente, R. (2019). Novozym 435: the “perfect” lipase immobilized biocatalyst? *Catalysis Science & Technology.*
4. Rai, R., Tallawi, M., Grigore, A., & Boccaccini, A. R. (2012). Synthesis, properties and biomedical applications of poly(glycerol sebacate) (PGS): a review. *Progress in Polymer Science, 37*(8), 1051-1078.
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6. Stratton, S., Shelke, N. B., Hoshino, K., Rudraiah, S., & Kumbar, S. G. (2016). Bioactive polymeric scaffolds for tissue engineering. Bioactive Materials, 1(2), 93-108.
7. Wang, Y., Ameer, G. A., Sheppard, B. J., & Langer, R. (2002). A tough biodegradable elastomer. *Nature Biotechnology, 20*(6), 602.
8. Yang, J., Webb, A. R., & Ameer, G. A. (2004). Novel citric acid-based biodegradable elastomers for tissue engineering. *Advanced Materials, 16*(6), 511-516.

Addendum:

The only change to the original plan was that the biodegradation assay was not performed, as there was trouble with getting an accurate mass measurement.