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

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

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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.