Appendix: Product Design Specifications

Title: Biomimetic Intestine for Traction Force Studies

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Problem Statement: The brush border of the human small intestine is comprised of absorptive enterocytes that undergo constant proliferation and bidirectional movement along the villous and crypt folds of the intestine. With a lifespan of twenty-four to forty-eight hours, enterocytes begin their life cycle as stem cells residing in the crypts of the small intestine; as these cells differentiate into mature absorptive enterocytes, they migrate from the crypt to the tip of the villus where they undergo cell death. The intestinal epithelium maintains a dynamic environment in which enterocyte migration from cell production until cell death is held in a stable, coordinated manner. In order study the cellular traction forces responsible for enterocyte migration, an *in vitro* model system mimicking the structure and dimensions of intestinal villi is needed. A biomimetic intestinal environment will be generated using a collagen scaffold mimicking the 3D topography of intestinal villi. Once seeded with epithelial cells, this scaffold will serve as a model to study the cell-cell forces responsible for the movement of epithelial cells in the intestine

Client: Professor Michael Murrell

Design Requirements:

1. Physical and Operational Characteristics
    a. Physical Requirements:
        i. Design must feature methods in generating rounded-tip 3D topographic, micro-needle array mold
        ii. Final mold must be able to generate a 3D collagen hydrogel scaffold
        iii. Cross sectional diameter of villi must be varied
            1. Range of 100µm-500µm
        iv. Height of villus: 600, 750, 900µm
        v. Spacing between villi: 250µm
    b. Safety
        i. Equipment required: UV/CO2 Laser Ablation System
            1. Safety concerns with improper equipment use
        ii. Materials required: TMPTA, methacrylated type-I collagen, phosphate buffer saline (PBS), and fluorescent beads
            1. TMPTA, methacrylated type-I collagen, and fluorescent beads have no safety concerns
    c. Accuracy and Reliability
        i. Mold must generate nearly similar collagen scaffolds having heights of 600, 750 and 900µm and varying in diameter
    d. Life in Service
        i. One semester
    e. Shelf Life

i. Mold used to create 3D collagen scaffold with villi architecture is expected to last several years
ii. Collagen scaffold generated using mold is expected to last several days if not weeks

f. Operating environment
    i. Room temperature (37° C)
    ii. Collagen to be contained in PBS Buffer: pH 7.4
g. Ergonomics
    i. Able to be held in human hand and operable my human hand
    ii. Mold must easily allow scaffold release
h. Size
    i. Scaffold
        1. Villi heights of 600, 750 and 900µm
        2. Villi diameter ranging from 100µm-500µm
    ii. Mold
        1. Millimeter scale: must hold several different micro-needle arrays of varying villi diameters and heights
        2. The mold will hold the following range of villi diameter: 100µm, 200µm, 300µm, 400µm, and 500µm
        3. The mold will hold the following range of villi heights: 600µm, 750µm, 900µm
        4. There will be 250µm spacing between the bases of neighboring villi
        5. The villi will be parabolic conical structures will rounded tips and bases
        6. The final mold will be rectangular with dimensions of 2x1cm and height of 1mm
i. Weight

    TMPTA density = 1.1g/cm^3

        1. Final mold = 1.1g/cm^3(2x1x0.1cm^3) = 0.22g

j. Materials
    i. TMPTA
    ii. Methacrylated type-I collagen
    iii. PBS
    iv. Fluorescent beads
k. Aesthetics, Appearance, and Finish
    i. Rectangular master mold (2x1x0.1cm^3)
    ii. Clear, plastic TMPTA mold.
    iii. Edges to be sanded and smoothed

2. Product Characteristics
    a. Quantity: 1 x Master Mold Prototype
    b. Target Product Cost: <$1000
        i. TMPTA: $45.40 per 100 g
        ii. Methacrylated type-I collagen: $169.50 per 1 liter

iii. PBS: $94.70 per 1 liter
iv. Fluorescent beads: $48.00 per 1 liter

3. Miscellaneous
   a. Standards and Specifications: No international standards or FDA requirements known.
   b. Customer: Professor Murrell would like to be able to create numerous collagen scaffolds based off of our mold. Therefore, he would like a mold that is both reusable and durable. He also would like to be able to view this under a microscope that has a 60x magnification. After our project is completed, Professor Murrell mentioned the possibility of creating his own mold, therefore he would like the process we use to be reproducible. It should be relatively easy and quick for him to follow our method, so we will take this into account when deciding which method to use.
   c. Patent-related concerns: Methodology of mold generation is not patented; therefore we have no patent related concerns.
   d. Competition: A group at Cornell has produced a microarray resembling intestinal villi using a laser-ablation method.