Biomimetic Intestine for Traction Force Studies

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Abstract

Epithelial cell migration and turnover on the villus structure in the intestine is a relatively unexplained phenomenon. It has been hypothesized that epithelial cells migrate from crypt to villus due to various traction forces produced by the topography of the small intestine. In order to quantify and study these traction forces, it is critical to construct an accurate model of intestinal villi. A 3D collagen scaffold featuring rounded, micro-needle arrayed topography is desired. Following seeding of epithelial cells on this scaffold, we hope to create a biomimetic model of the intestine. To generate uniform scaffolds, we investigated methods of creating a plastic, master mold used for the generation of a 3D collagen scaffold. Methods were judged based on their ability to generate villi structures of varying diameter (10-500μm), constant height (1mm), and rounded tops. We found three methods that fit these criteria: UV-LIGA, multi-photon excited photochemistry, and UV/CO_2 laser ablation. These three methods were evaluated based on ease of fabricating the master mold, access to equipment, reproducibility of methods, amount of training required, cost, and other factors. Overall, the UV/ CO_2 laser ablation method was determined to be the most effective, based on our design criteria, at generating the master mold with our desired features.

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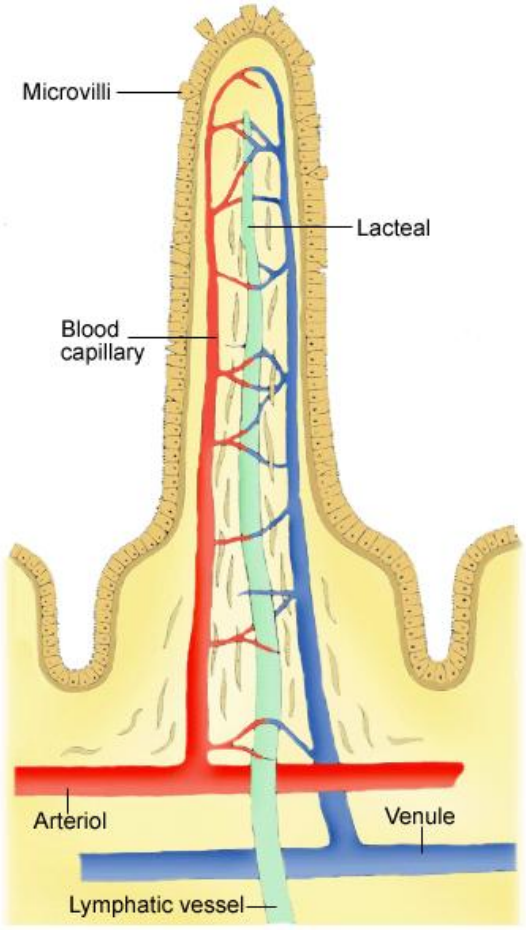
Background

Physiology of Intestinal Villi

The epithelial lining of the mammalian small intestine is structured into millions of tiny, finger-like projections called villi (Sherwood, 2011). Villi are comprised of a lacteal vessel, blood vessels, muscular tissue, and a mucosa layer all covered by a single cell layer of absorptive cells known as enterocytes (Sherwood, 2011). Nearly all nutrients, including amino acids and sugars, enter the systemic blood pathway by crossing the epithelium covered villi (Bowen, 2001). Then, nutrients are either diffused into the capillary network or carried by the lymphatic vessel (lacteal) located within each villus (Bowen, 2001). The purpose of the villus structure is to increase absorptive surface area in the small intestine (Sherwood, 2011). Villi typically project 1mm into the small intestine and feature a variety of cross-sectional diameters with an average diameter of 0.5mm (Sherwood, 2011; Caceci, 2013). Each villus is separated by deep invaginations known as Crypts of Lieberkuhn (Sherwood, 2011). In the crypts, enterocytes begin their life-cycle as proliferating stem-cells (Brunicardi et al., 2005).



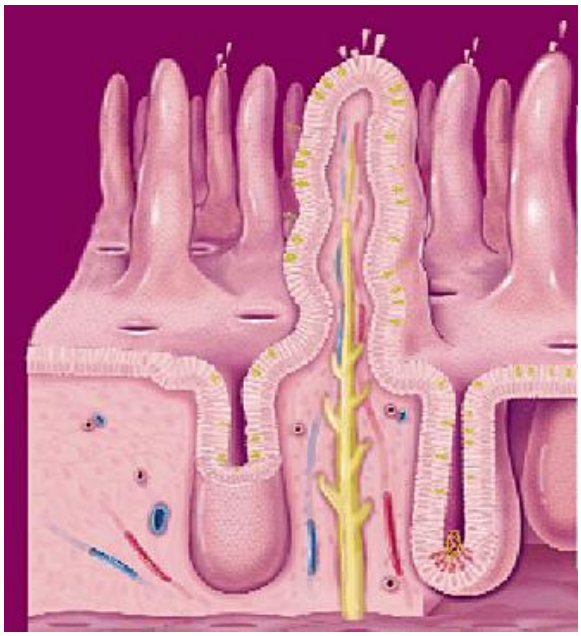


Figure 1: (left) Components of the intestinal villi. Enterocytes comprise microvilli depicted in this picture (Proffitt)

Figure 2: (right) Relationship between crypt and villi (Ganz, 2000)

With each stem cell division, one daughter cell remains in the crypt for subsequent division to ensure continuation of this process. The other differentiates into one of four cell types, the principal lineage being the enterocyte (Bowen, 2001). As enterocytes age, they divide and migrate up the sides of the villus (Bowen, 2001). At maturity, enterocytes are exceptional absorption cells, expressing numerous transport proteins and enzymatic characteristics, which are key to their function (Bowen, 2001). At the tip of the villus, they perish and shed off into the lumen for reabsorption (Brunicardi et al., 2005). The journey from the crypt to villus tip takes place between two to five days (Brunicardi et al., 2005). The small intestine lining undergoes continuous renewal due to the coordinated movement of enterocytes from crypt to villus tip during their life cycle of 24 - 48 hours (Brunicardi et al., 2005). The mechanisms underlying the rapid turnover of enterocytes in the small intestine are relatively unknown; however, it is hypothesized to be highly reliant on cell adhesion and traction forces (Basson, 2008).

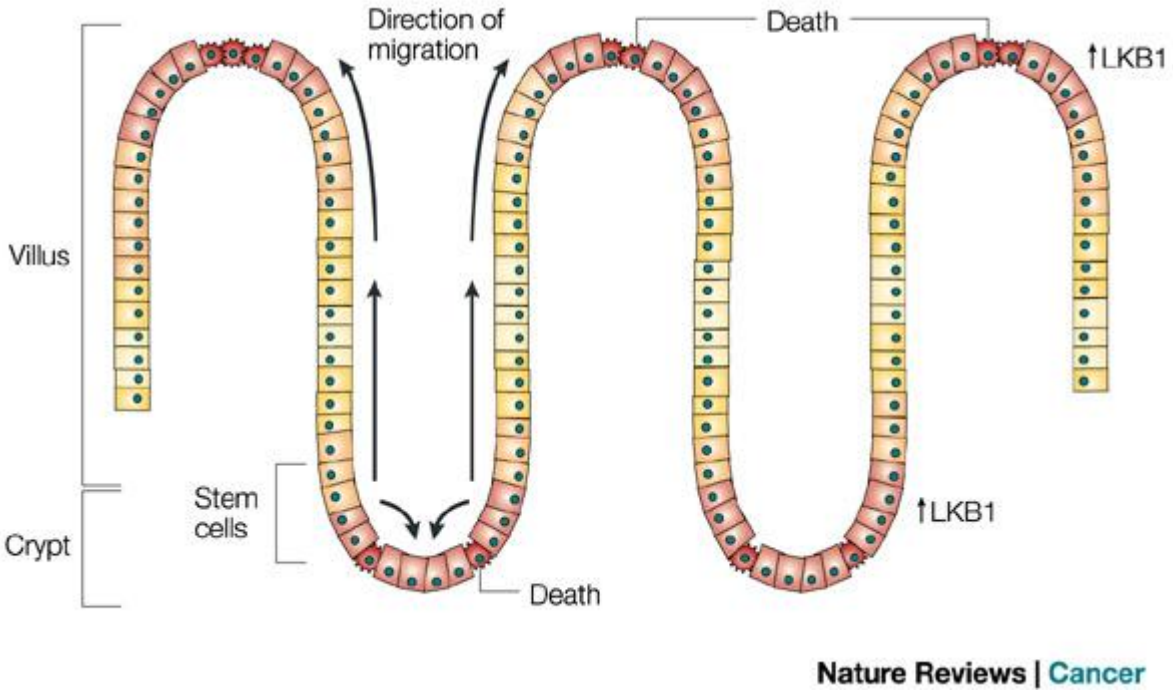


Figure 3: Migration of enterocytes from crypt to villus and subsequent cell life-cycle stages.

Client Information

Professor Michael Murrell, a faculty member of the Department of Biomedical Engineering at the University of Wisconsin-Madison, is interested in studying the factors and mechanisms underlying enterocyte migration in the small intestine. He is particularly interested in the traction forces caused by villi structure and their effects in the coordinated movement of enterocytes. In order to directly measure the traction forces involved in enterocyte migration, he would like to fabricate a realistic model of an intestine featuring three-dimensional (3D) villus topography and proliferating epithelial cells to be seeded on the villus structures. To better understand the effects of villus structure on enterocyte migration, he would like a model in which

villus diameter and height could be varied. Additionally, he would like an easily replicable method of generating this biomimetic intestine.

Client's Requirements & Design Constraints

The client has given several requirements for the final composition of the villi scaffold. First and foremost, the scaffold's microneedle array must maintain a certain specified topography. This topography must contain a tapered, conical tip on each cylindrical "villus" pillar. Another topographical constraint given to us is a minimum of 250μm spacing between the base perimeters of neighboring villus pillars. The spacing pattern will be maintained across the entire microarray scaffold to provide a uniform crypt bed between the villi projections, regardless of pillar diameter. In order for Professor Murrell to study the effects villi size plays on cell forces, we must create an array of pillar diameters with set increments. The diameters to be fabricated on the mold are 10 μm, 50μm, 100 μm, 200 μm, 300 μm, 400 μm and 500 μm. Each of these seven sizes must be solely contained within a 1 cm x 1 cm square section. The resulting scaffold will have a 7 cm x 1 cm dimension. Secondly, the final scaffold must be produced out of collagen type I material. Professor Murrell requires this specific protein-based biomaterial framework for seeding epithelial cells due to its common acceptance and use in cellular and tissue engineering. Collagen type I is an ideal material because of its natural origin and sites for cellular adhesion; it is therefore suitable for stem cell differentiation and oncogenesis. The potential for variability of the collagen requires purification to ensure homogeneity, however, collagen is routinely isolated from animal tissues including skin, tendon, or bone (Willerth et al., 2008). Additionally, our client's budget for the fabrication of the master mold and production of the collagen scaffold is $1000 USD.

Problem Statement

Mechanisms which underlie the coordinated migration of enterocytes from crypt to villus are unknown and have yet to be studied in an *in vitro* system. In order to observe and measure the cellular adhesion forces and traction forces involved in the rapid turnover of enterocytes within the small intestine, a precise model of the intestinal villi is needed. This model must feature realistic 3D topography of the small intestine, defined by round-tipped, conical structures to imitate villi. Ideally, this model would be fabricated using biologically compatible hydrogel scaffolding such as collagen; 3D structures on collagen scaffold would be seeded with intestinal epithelial cells in order to create a biomimetic model of the human small intestine. The creation of this biomimetic model would allow for the observation, direct measurement, and manipulation of variables involved in enterocyte migration.

Methods

A variety of methods exist for the creation of 3D topography on collagen scaffold, however, methods yielding highly reproducible, uniform scaffolds generally involve the fabrication of a master mold. This mold would then be used to create subsequent collagen molds featuring a negative volume which matches the desired 3D shape of the scaffold. For our

purposes, and per client specifications, the creation of a master mold followed by soft-lithography (use of polydimethylsiloxane (PDMS) to generate structures on collagen) will be the general methods used for the creation of collagen scaffold. Although we have defined a general method for the creation of 3D structures on collagen, methods for the creation of the master mold used in this process are still undefined.

Current Methods

Methods used for the generation of a plastic master mold used for soft lithography are constrictive. Most methods can only fabricate square pillar topography. These structures do no accurately represent the 3D rounded-tip, conical structure of villi. There are currently several methods of creating structures similar to the villi of the small intestine on a micron-scale sizing. Current methods include photopolymerization and micro-contact printing (3D printing). Photopolymerization is a process where a material is exposed to light radiation, usually UV radiation, and hardened in those areas. Then, the parts that were not irradiated are dissolved and eroded away, leaving the desired structures with a flat top. Another process is micro-contact or 3D printing. 3D printing builds a blueprint of a schematic block by block in a layer fashion. The Micro-contact printing currently available is unable to obtain the resolution required to produce villi of the size 5 μm in diameter accurately. Some 3D printers can get down to 10 μm in accuracy, however these structures are fairly blocky due to the fact that each part of a model in 3D printing is put down as a sphere and leaves small indentations between the intersections of each sphere which can throw off readings and make the structures unstable.

Methods for Collagen Scaffold Generation

In order to provide an optimal environment for cell proliferation and growth, an ideal collagen scaffold should be highly porous and permeable (Yao et al., 2005). These characteristics, however, cause structural instability in 3D microstructures fabricated from collagen; therefore, 3D topographic generated on scaffold typically break upon release from a PDMS mold (Golden et al., 2006). Sacrificial molding is a technique often used to prevent fracturing of the 3D structures generated on collagen and often involve the use of a dissolvable sacrifice mold (Golden et al., 2006; Sung et al., 2011; Yu et al., 2012). We plan to use a 2.5% calcium alginate sacrificial mold to prevent villus structures from breaking during mold release. Following the creation of a PDMS mold, a reverse mold using alginate will be created. This alginate mold will be used to fabricate the desired collagen scaffold. Following curing of the collagen scaffold, the alginate reverse mold will be dissolved using 60mM ethylenediaminetetraacetic acid (EDTA). Dissolving of the alginate mold prevents breaking of villi generated on collagen. Unlike conventional mold-peeling, sacrificial molding does not introduce shear forces when separating collagen scaffold from another mold.

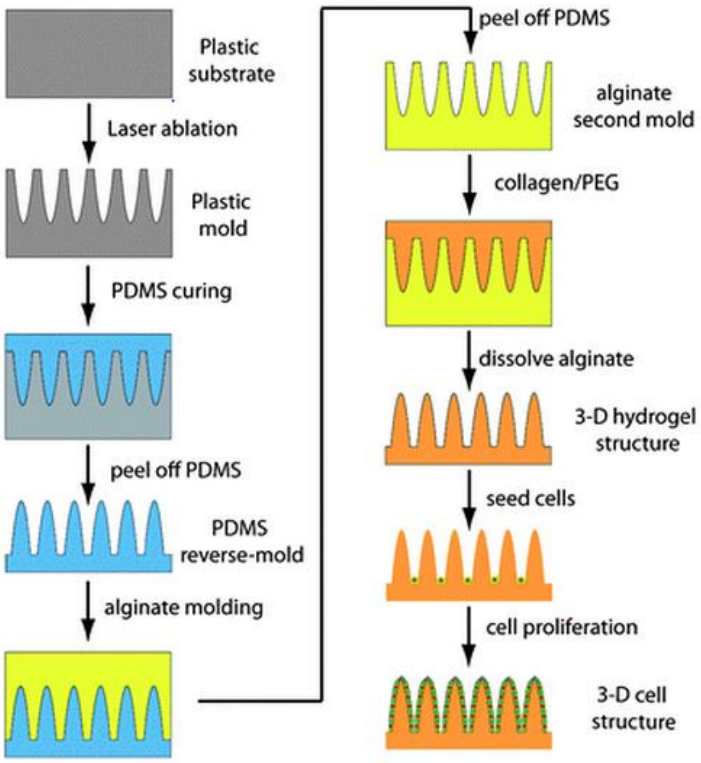


Figure 4: Overall fabrication process (Sung et al., 2011)

METHOD ALTERNATIVES:

Multi-photon Excited (MPE) Photochemistry

One possible method to make the master mold is through the use of multiphoton excited (MPE) photochemistry. This method is currently being used in Professor Campagnola's Lab; a grad student there, Visar Ajeti, is very enthusiastic about our project and offered to make the mold for us using this method. MPE photochemistry uses a laser to build 3D structures out of a variety of compounds, such as plastic or proteins (Ajeti). It focuses photons on the material and causes the molecules of the materials to become excited. This excitation forms cross-links which create 3D shapes in a 2D plane (Chen et al, 2012). This is done by using computer aided software. Different heights can be built by moving the field of the microscope down along the z-axis. This method is able to produce objects with heights of 1-1000μm and diameters of 1-500μm (Ajeti). These dimensions fall within the specifications of our project. The object could be formed with SU-8, which would allow us to use a mold made from this with the soft lithography method (Ajeti).

UV/CO2 Laser Ablation

The use of laser ablation for microfabrication has been steadily gaining momentum in the creation of three dimensional, microstructures (Waddell, 2006). Laser ablation systems can differ depending on the type of light used; UV laser ablation systems feature the use of ultraviolet radiation having wavelength of roughly 355nm whereas CO2 laser systems feature the use of

infrared radiation having a wavelength range of 9.3μm to 10.6μm (Lange, B., 2013). Both systems function in a similar manner, namely both utilize highly concentrated, powerful beams of radiation to break chemical bonds in metals and polymers in order to 'etch' materials (Lange, B., 2013; Waddell, 2006). Laser ablation systems feature the ability to freely manipulate in the x, y, and z direction by controlling laser intensity, length of exposure, and repeat exposure of material to the laser (Lange, B., 2013). Furthermore, most laser ablation systems are computer controlled, meaning program files featuring 2D or 3D design (such as CAD files or Corel Draw files) can be inputted into software that feeds into control of the laser (Yu et al., 2012).
Both systems offer precise resolution; CO2 laser ablation systems can etch diameters as small as 75μm (Lange, B., 2013). UV laser ablation systems are more precise and refined than CO2 laser ablation systems; they can etch diameters as small as 30μm featuring a high aspect ratio (Lange, B., 2013). The lack of materials required for fabrication, precision, and automation featured by laser ablation makes it an attractive method for the fabrication of 3D, microscale structures.

UV-LIGA

UV-LIGA stands for the German words meaning lithography, electroplating, and molding (Yang et al., 2012). This method features the creation of a photo-resistive mask, typically made from a chromium plate; etched using computer aided engraving (Ghodssi et al., 2011). For our purposes, this mask will resemble a plate with numerous circles of various diameters as negative area. SU-8 an epoxy based, negative photoresist will be used as the polymer for the master mold generating using this method (Lorenze et al., 1997). SU-8 starts out as a gel-like polymer, but it polymerizes into a hard polymer when exposed to heat or UV-light (Lorenz et al., 1997; Yang et al., 2012). In order to use SU-8 as our substrate, gel-like SU-8 will be put onto a glass plate coated with titanium and spun at 500 revolutions per minute for thirty seconds (Yang et al., 2012). Following spinning, the SU-8 will be baked at 90 degrees Celsius for three hours (Yang et al., 2012). The SU-8 is then removed from the glass and layered with a photo-resistive resin followed by the chromium mask. SU-8 is a compound that polymerizes through cross-linking as it is exposed to UV-light (Lorenze et al., 1997). Therefore, as UV-light contacts the area not covered by the photo-resistive mask, it will polymerize and form 3D structures. The methods used to generate microneedle arrays using UV-LIGA feature a backside exposure of the SU-8 to UV light (Yang et al., 2012). The height of the arrays depends solely on the length of exposure the SU-8 has to UV light, but typically has a maximum resist height of around 800um-1000um and a minimum lateral size of 0.2um (Ghodssi et al., 2011). Following these steps, a master mold of SU-8 would be created; however, prior to usage as a mold, SU-8 must be layered with a mixture of titanium and gold (Yang et al., 2012). A 30nm/100nm layer of Ti/Au can be added onto the SU-8 via sputtering (Yang et al., 2012). This metallic layer prevents subsequent molds made by PDMS, created using the SU-8 mold, from adhering to the SU-8 (Yang et al., 2012). The main attractive feature of this method is the ease of equipment use and access; without considering the engraving of the chromium mask, UV-LIGA requires only the use of a UV-light source (a mercury lamp), a spinerette, and a hot-plate or oven.

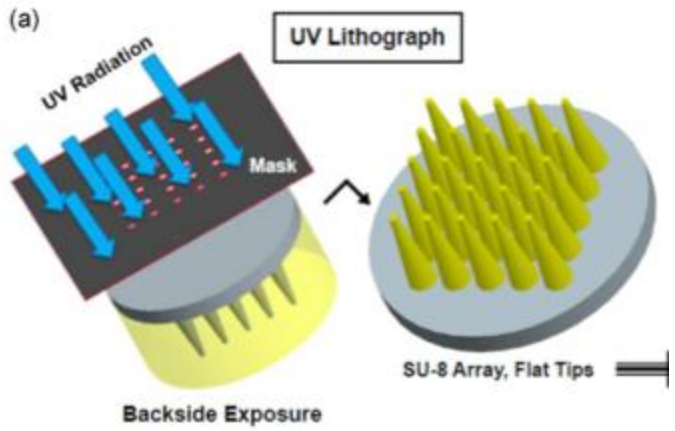


Figure 5: Backside exposure of UV radiation to SU-8 substrate to form microneedle array (Yang et al., 2012)

DESIGN MATRIX

After selecting our three method alternatives for the fabrication of the master mold, the methods were compared and contrasted using scaled criteria featured in a design matrix. Our design matrix is unconventional because methods, not products, are being compared.
The design of our product will be the same for each proposed method. We will be comparing three methods: MPE photochemistry, CO2 laser ablation, and UV-LIGA. Based off of client specifications and features we deemed important, the eight criteria in our design matrix were chosen. The criteria are listed in the order of their importance to the methodology: ease of fabrication, access to equipment, reproducibility, training, time, cost, durability, and safety.

Design Criteria (Weight)	Laser Ablation		MPE Photochemistry		UV-LIGA	
Ease of Fabrication (25)	4	20	3	15	2	10
Access to Equipment (15)	3	9	5	15	1	3
Reproduceability (15)	5	15	1	3	4	12
Training (15)	4	12	5	15	2	6
Time (10)	5	10	3	6	2	4
Cost (10)	5	10	3	6	2	4
Durability (5)	3	3	3	3	3	3
Safety (5)	3	3	4	4	3	3
Total (100)		**82**		**67**		**45**

Figure 6: Design Matrix

Ease of Fabrication

Ease of fabrication is our most important criteria, and therefore we gave it a weight of twenty five points. This is the most important factor in our design problem because of the incredibly small scale of our final design. With villi ranging from five to five hundred micrometers, our final product will be no longer than seven centimeters in total. Therefore, we need a method that makes creating this mold as precisely and easily as possible. Laser ablation won this category with a score of a four out of a five because it is almost entirely computer automated and very accurate. Unlike the other two designs, which must be made in labs and possibly result in error, laser ablation takes a computer aided design file and can accurately create it without introducing human error.

Access to Equipment

The next three categories are all equally weighted; however, access to equipment is slightly more important and was placed next in the list. Access to equipment is important to our project due to the size of our master mold. Going hand-in-hand with ease of fabrication, having easy access to equipment is important as there are not many methods capable of producing molds in microscale. MPE Photochemistry won this category with a score of five out of five because the equipment necessary for performing this method is located in the Engineering Centers Building, housed in Professor Campagnola's lab. Laser ablation received a score of three. Despite hearing numerous mentions of this laser ablation system, it remains elusive.

Reproducibility

Another important criterion is reproducibility which was also assigned fifteen points. Reproducibility would give Professor Murrell the ability to use our methods and make his own alterations. This criterion is important to Professor Murrell, because future studies may include the addition of different diameter cross sections, heights, or a combination of both features. Therefore, methods for generating the master mold must be easy to reproduce. Laser ablation received five out of five points for this category because it can be drawn up in a CAD file and created by a laser, resulting in minimal work for our client.

Training

The last criterion, worth fifteen points is the amount training required before fabrication can begin. Many of the methods we researched required the use of very expensive machinery and extensive training. Methods involving extensive training were weighted quite heavily in our design matrix. Professor Murrell believed that intense training hinders the time of fabrication and negatively affects reproducibility. This is true for MPE photochemistry; however, Professor Campagnola's lab is willing to create our mold for us. Therefore, we would require no training; as a result, this method received a score of a five out of five.

Time

Time is also an important factor and received a weight of ten points. We want a method that is accurate and quick to produce a master mold. Since we only have one semester to fabricate a biomimetic intestine, the method we choose cannot take months to create. Laser ablation won this category with a score of five out of five because it is a process that can be done in less than a week. Unlike MPE Photochemistry and UV-LIGA which involve chemicals and coatings of metals, laser ablation simply cuts into plastics. Clearly this process is much simpler and quicker, resulting in an obvious win in this category.

Cost

Along with time, cost is also an important criterion to our client. Professor Murrell supplied us with a budget of $1000; however, we would like to minimize our costs as much as possible. It was challenging to compare the costs of all three methods; however, we did our best to estimate. We were able to determine cost of all materials necessary for laser ablation; it came to a total of $350.40 without accounting for a possible laser operating fee. Operating fees may apply to the other methods as well. Unlike MPE photochemistry and UV-LIGA, CO2/UV laser ablation requires only one material, PMMA. MPE photochemistry and UV-LIGA both requires the purchase of SU-8, photo-resistive resin, Ti/Au layering substrate, and other additional chemicals. Furthermore, MPE photochemistry would require us to pay an outsourcing fee to Professor Campagnola's lab. In conclusion, laser ablation won this category with a score of five out of five based on our projections for the costs of all of the methods.

Durability

Durability and safety tied for the lowest weighted criteria, both having five points each. Durability is a criterion that Professor Murrell wanted us to include in our design matrix. He wants a mold that can withstand multiple usage. However, as a group, we determined that the mold created by any of these methods would all be made of equally durable material. Therefore, there was a tie between all of our designs in this category with a score of three out of five.

Safety

Our last criterion is safety with a weight of five points. All of our methods are fairly safe to use, and therefore safety was not rated highly. Our scores for this criterion were very similar, however, MPE photochemistry won this category with a score of four out of five because we would be outsourcing it to Professor Campagnola's lab and would not need to interact with the equipment nor the chemicals involved in the process.

Total Scores

Following score addition, we determine that laser ablation was the best method, having a score of 82. MPE Photochemistry placed second with a score of 67. Finally UV-LIGA placed last with a score of 45. All of these scores are out of a possible 100 points. The large discrepancies between the methods in our matrix clearly show laser ablation is the best method.

However, no matter which method we choose, all of them have inherent challenges that we will have to face when moving forward with our project.

Proposed Solution

Final Method Selection

Based on our design matrix, we have decided to use the laser ablation method for the creation of the master mold. Laser ablation not only had the highest total score, but also scored the highest in ease of fabrication, reproducibility, time, and cost. Professor Murrell agreed with our decision to pursue this method for the numerous reasons outlined in our design matrix. Professor Murrell also added that this is the method the team at Cornell used to create a nearly flawless microneedle array.

Final Method and Design Specifications

We will be using laser ablation to create the master mold. We will use a computer aided design program to create an accurate design for the laser to cut out. It will be a piece of polymethylmethachrylate that is 7cm x 1cm x 0.2cm. On the seven 1cm x 1cm square sections, we will have diameters of 10µm, 50µm, 100µm, 200µm, 300µm, 400µm, and 500µm. Each of the pillars in the square section will be spaced 250 um away from the next pillar measured from the base. These pillars will be etched to a depth of 1 mm. Once the master mold has been created, we will use the process of soft lithography as stated above (see section Methods for Collagen Scaffold Creation). Once we are left with a collagen scaffold, it will be ready for Professor Murrell to apply the epithelial cells.

Future Work

We have decided to use the method of laser ablation for creating the master mold. The next step in the design process is to locate the laser ablation machine. We have already began contact with Ian Linsmeier, a graduate student working in Professor Murrell's lab, who has used both the Epilog Laser Engraver 2005, located in the shared labs of the Department of Biomedical Engineering at UW-Madison, and the UV-laser ablation system, located in the Wisconsin Institute of Discovery, for microfabrication on plastic polymer.

Once we gain access to a laser ablation system, we will need to receive the proper training on equipment handling. At the same time, we will begin drawing a computer aided design file using a program such as AutoCAD or SolidWorks for our master mold design. Presently, however, we do not know the file type required by the laser ablation system in the WID, and cannot begin creation of a design file.

Following the completion of our master mold, we will begin the process of soft lithography leading up to the creation of a collagen scaffold. Depending upon time limitations, we may or may not aide Professor Murrell in the seeding of the collagen scaffold. However, we do plan to follow up with Professor Murrell throughout the time that he will be using the master mold and the subsequent collagen scaffolds generated from the mold.

Acknowledgements

Our team would like to thank Professor Murrell for presenting us the opportunity to assist him in his fascinating research. Through all the hardships and setbacks, Professor Murrell has supported us and pushed us on. It has been a wonderful experience creating the biomimetic intestine for him, and we wish him the best of luck in his future research. We would also like to thank Professor Ashton for his guidance during our project. Without him we never would have made the countless deadlines and noticed the important details. We would like to thank the countless labs we visited when trying to decide on the best method to create this mold. Finally, a special thanks to the University of Wisconsin-Madison's Department of Biomedical Engineering for their countless resources.

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Appendix: Product Design Specifications

Title: Biomimetic Intestine for Traction Force Studies

Team Members: Angela Beltrame, Kevin Knapp, Susanna Kwok, Shaun Pomerenke, Conor Sullivan

Date: September 28, 2013

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 10µm-500µm
        iv. Height of villus: ~1mm
        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: Polymethylmethacrylate (PMMA), PDMS, 2.5% Calcium Alginate, 60mM EDTA, 5mg/ml Type I Collagen, 0.3% glutaraldehyde, PBS buffer
            1. PMMA, PDMS, Calcium Alginate, EDTA, Type 1 Collagen have no safety concerns
            2. Glutaraldehyde is corrosive, harmful to the aquatic environment, and exhibits acute toxicity in high concentrations (however, since our concentration low, we need only be concerned direct skin contact)

3. 1,2-Dichlorobenzene is combustible, harmful if swallowed, and may cause skin irritation

c. Accuracy and Reliability
    i. Mold must generate nearly similar collagen scaffolds having height of ~1mm 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. EDTA: pH 8.5
    iii. 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 height of ~1mm
        2. Villi diameter ranging from 10um-500um
    ii. Mold
        1. Millimeter scale: must hold several different micro-needle arrays
        2. One mold will hold the following range of villi diameter: 10um, 50um, 100um, 200um, 300um, 400um, and 500um
        3. Each villi diameter will have a $1cm^2$ area where each villus is spaced 250um apart
        4. The final mold will be rectangular with dimensions of 7x1cm and height of 2mm (in order to generate villi with height of 1mm, additional height in mold needed – therefore, 2mm to be used for final mold)
i. Weight

    PMMS density = $1.18g/cm^3$

        1. Final mold = $1.18g/cm^3(7x1x0.2cm^3) = 1.400g$

j. Materials
    i. PMMA
    ii. 1,2-Dichlorobenzene (solvent for PMMA)
    iii. PDMS
    iv. 2.5% Calcium alginate
    v. 60mM EDTA
    vi. Type I Collagen
    vii. 0.3% glutaraldehyde (to cure collagen)
k. Aesthetics, Appearance, and Finish

i. Long rectangular master mold (7x1x0.2cm^3)
ii. Clear, plastic PMMS mold.
iii. Edges to be sanded and smoothed

2. Product Characteristics
    a. Quantity: 1 x Master Mold Prototype
    b. Target Product Cost: <$1000
        i. PMMA ($41.40/25,000 grams in bottle)
        ii. 1,2-Dichlorobenzene ($15.40/bottle)
        iii. PDMS ($36.50/50mL in bottle)
        iv. Calcium alginate salt ($22.70/bottle)
        v. 60mM EDTA ($19.30/bottle)
        vi. 8% Glutaraldehyde in H2O ($45.60/bottle)
        vii. Type I collagen ($169.50/bottle: may be provided by Prof. Murrell)
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.