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. In order to create such a scaffold, we plan to create a biomimetic scaffold mold to mimic the 3D topography of villi. A TMPTA master mold will be fabricated using multi-photon excitation photochemistry. This mold will feature villus diameters of 100, 200, 300, 400, and 500µm. Additionally, villus heights of 600, 750, and 900µm will be fabricated per each diameter. The goal of this project is to fabricate a master mold capable of generating collagen scaffolds to be used for traction force studies.

Client Information

Professor Michael Murrell
- Department of Biomedical Engineering at UW-Madison
- Molecular, cellular, and tissue mechanics
- Cellular Engineering
- Systems Biology
- Cell Motility and Tissue Dynamics
- Interested in manipulating mechanical properties of cells

Figure 1: Professor Murrell (Image taken from http://directory.engr.wisc.edu)

Background

Mammalian Small Intestinal Villi
- Tiny, finger-like projections
  - Humans: Height = 1000µm, Diameter = 350µm
- Increase absorptive surface area
- Covered with enterocytes
  - Life cycle
    - Begins in crypt
    - Ends at villus tip
      - 24-48 hour lifecycle
    - Coordinated throughout entire small intestine
    - Migration mechanism unknown
      - Likely due to traction forces

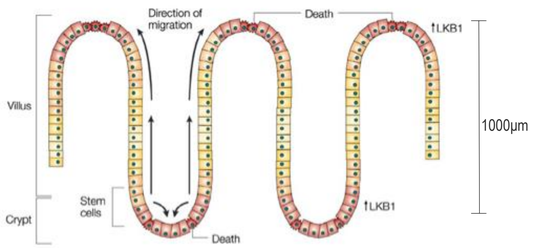

Figure 2: Direction of migration for enterocytes on small intestinal villi (Yoo et al., 2002)

Motivation

In order to directly measure the traction forces involved in enterocyte migration, a realistic model of the small intestinal lumen is required. This model must be a biocompatible scaffold featuring 3D villi topography and allow for cell seeding. Additionally, the generated scaffold must be easily replicable.

Client Specifications

- Parabolic, micro-needle array
- 250µm spacing between each pillar
- Diameter range: 100µm-500µm
- Height range: 500µm-1000µm
- Reusable master mold
- Master mold that easily creates hydrogel/collagen scaffolds
- Budget: $1000

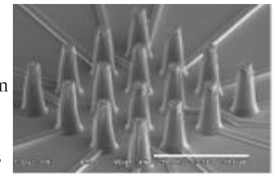

Figure 3: Microneedle array generated from UV-LIGA (Yang et al., 2012)

Design Matrix

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**

- According to the criteria listed in the design matrix above, the best method for our project is laser ablation. There were, however, unforeseen complications with this method.
  - Laser diameter: ~450µm
  - Height was uncontrollable
  - Parabolic curve generation not possible
  - Poor accuracy of dimensions and mold layout
  - Reflective surfaces causes machine error
- Final design utilizes MPE Photochemistry
  - 2nd option on design matrix
  - Campagnola Lab
    - Visar Ajeti

Client Specification	MPE Photochemistry Specifications	Met Client Requirements?
Max Villi Height 1000µm	Max Villi Height <1000µm Range of Heights Possible	✗
Diameter Range 100µm-500µm	Diameter Range 1µm-800µm (One time scan)	✓
Spacing 250µm	Spacing Computer controlled	✓

Multi-Photon Excitation Photochemistry

- Uses two photon beams to create structures in one plane at a time in a non-linear manner
- Freedom in all 3 dimensions
  - Control fabrication in x, y, and z direction
- Photopolymerizes/cross-links polymers to generate 3D structure
- 5 Watt lasers to galvanometer mirrors
- Automated fabrication
  - Limited to 800x800µm field of view
  - 1000µm height = maximum

Materials

- Trimethylolpropane-triacrylate (TMPTA)
  - High cross linking density
  - Hard products formed through photopolymerization
  - Good for use in radiation processing applications
- Methacrylated Type I- Collagen
  - Fundamental characteristics of type I collagen
  - Greater control over mechanical properties

Final Design: Progress

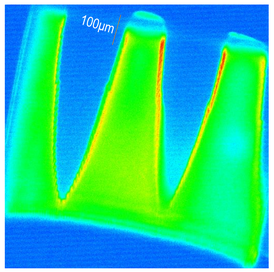

Figure 4: SEM image of prototype

Prototyping
- Initial prototype successful
- Parabolic/tapered cylinder
  - 500µm tall
  - 200µm diameter
  - TMPTA

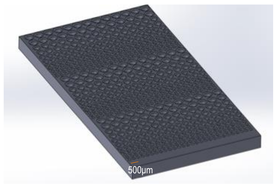

Figure 5: SolidWorks design of final mold featuring range of heights and diameters

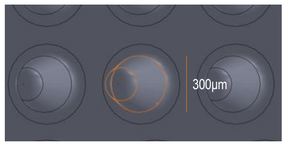

Figure 6: Zoom in of tapered topography of final mold design

- MPE Photochemistry software requires 3D images to be cut into:
  - 800x800µm sections or smaller (x and y-axis)
  - 3µm thick cross sections (z-axis)
- SolidWorks file conversion to BMP files
- Stitch sections to make overall mold

Expenses

Currently, we have spent $0. All materials and fabrication costs were generously donated by Campagnola's Lab.

Future Work

- Reverse molding to test creation of collagen/hydrogel scaffold
- Seeding cells on generated scaffold
- Variation of mold layout
- Increase range for diameter or height
- Fabricating crypts between each villus
- Changing mold layout
- Imaging collagen scaffold using confocal imaging and fluorescent beads

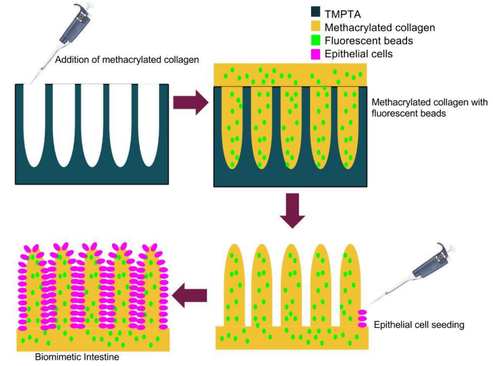

Figure 7: Methods for collagen mold generation (Image generated on Google Draw)

Citations

Basson, M.D. 1998. Role of integrins in enterocyte migration. Clinical and Experimental Pharmacology and Physiology 25(3-4):pg 280-285
Bowen, R. (2001, November 1). Villi, Crypts and the Life Cycle of Small Intestinal Enterocytes. arbl.cvmbs.colostate.edu. Retrieved October 9, 2013, from http://www.vivo.colostate.edu/hbooks/pathphys/ digestion/smallgut/life cycle.html
Chen, X., Su, Y., Ajeti, V., Chen, S.J., Campagnola, P.J., 2012. Cell Adhesion on Micro-Structured Fibronectin Gradients Fabricated by Multiphoton Excited Photochemistry. Cellular Molecular Bioengineering 5(3): p307319
Corporacion Mexicana de Polimeros, TMPTA Product Data Sheet. Retrieved December 3, 2013 from http://www.cmp.mx/docs/CMP-OM-033%20(TMPTA)%20-%20TDS.pdf
Cunningham, L.P., Veilleux, M.P., Campagnola, P.J. 2006. Freeform multiphoton excited microfabrication for biological applications using a rapid prototyping CAD- based approach. Optics Express14(19): p8613-8621
Proffitt, T. Small intestine: Structure and function of the small intestine. Retrieved on October 9th from http://www.elu.sgul.ac.uk /rehash/guest/scorm/109/package /content
Sherwood, L. 2011. The digestive system. In Fundamentals of Human Physiology 4th Edition. Cengage Learning, Stamford, CT. pg 436-476
Yang, W., Chen, Y., Huang, Y., Fu, Y., Tang, S., Fu, C. 2012. Engineering a biomimetic villus array for in vitro three-dimensional culture of intestinal epithelial cells. Journal of Microelectromechanical systems 21(6): p1418-1425
Yoo, L. I., Chung, D. C., Yuan, J. 2002. LKB1-A master tumor suppressor of the small intestine and beyond. Nature Reviews Cancer 2: p529-535

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