

Observation of Microvilli in Intestinal Epithelial Cells Utilizing a Scanning Electron Microscope

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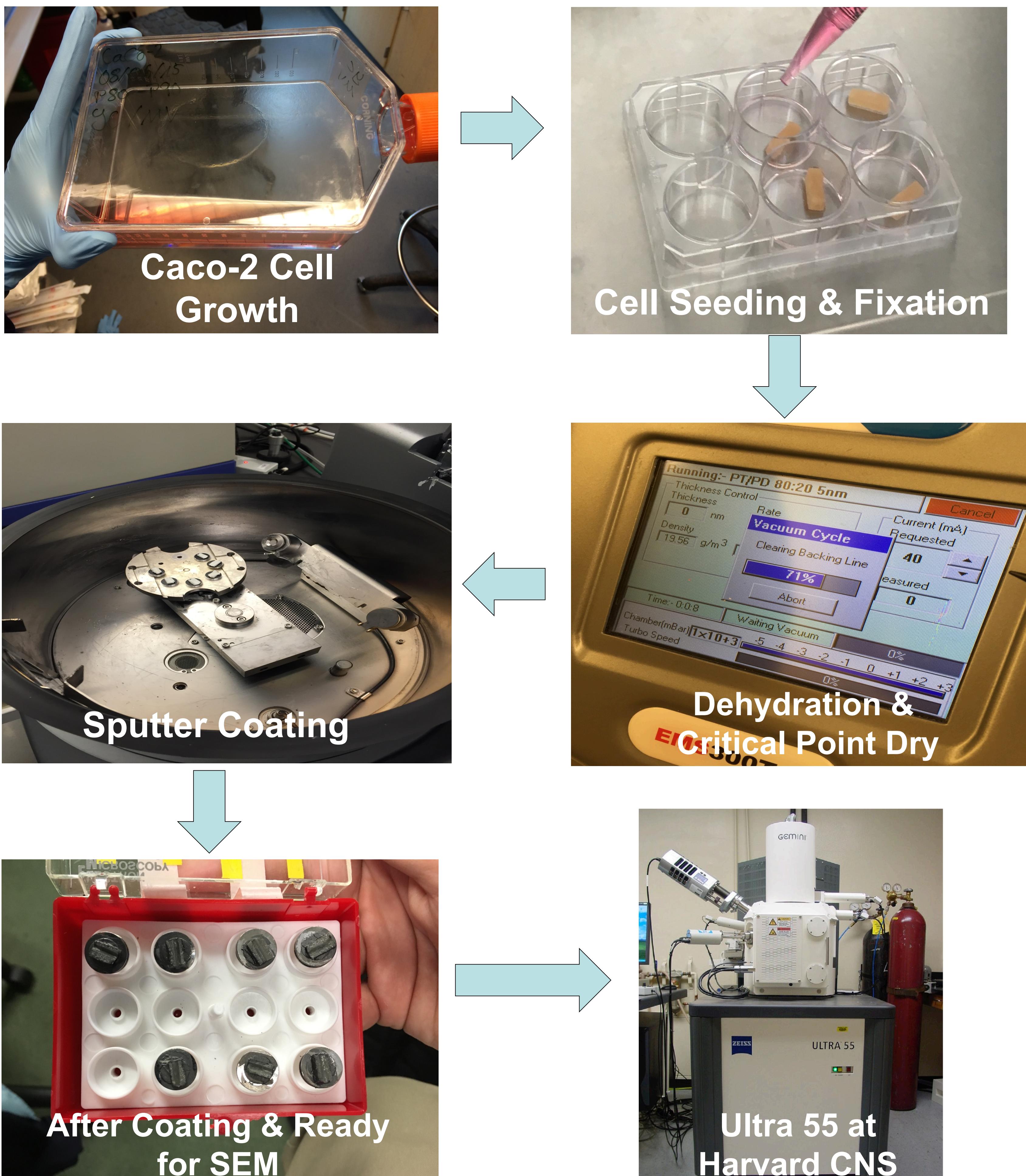
Background

A functional three-dimensional (3D) Intestinal System, made up of silk scaffold and human intestinal cells, has now been previously reported (1). This system has been used to test the effects of Titanium Dioxide Nanoparticles (TiO_2 NPs), in intestinal epithelium. TiO_2 NPs are widely found in food-related consumer products. Understanding the effects of TiO_2 on the intestinal barrier and absorption is essential for the safety assessment of these food-related products that will be in contact with the cells.

Motivation

The use of the Scanning Electron Microscope (SEM) is essential to assess the microstructure of cells, such as, the microvilli. SEM can also be used to observe the entire structure of nanoparticles. Thus far, SEM has been considered as a simple and efficient characterization method of the interaction between the nanoparticles and biological systems, TiO_2 NPs and intestinal epithelial cells. By SEM, we can clearly visualize where and when the TiO_2 NPs bind to the intestinal epithelium.

Sample Preparation for Scanning Electron Microscope (SEM)



Summary, Conclusion & References

Through the Scanning Electron Microscope, we have successfully captured a high density of microvilli in epithelial cells. Our next step is to apply this method to visualize the interaction between TiO_2 and intestinal epithelial cells.

References: (1) Chen Y. et al. Robust bioengineered 3D functional human intestinal epithelium. *Scientific Reports*. 2015 Sep 16; 5:13708. doi: 10.1038/srep13708.

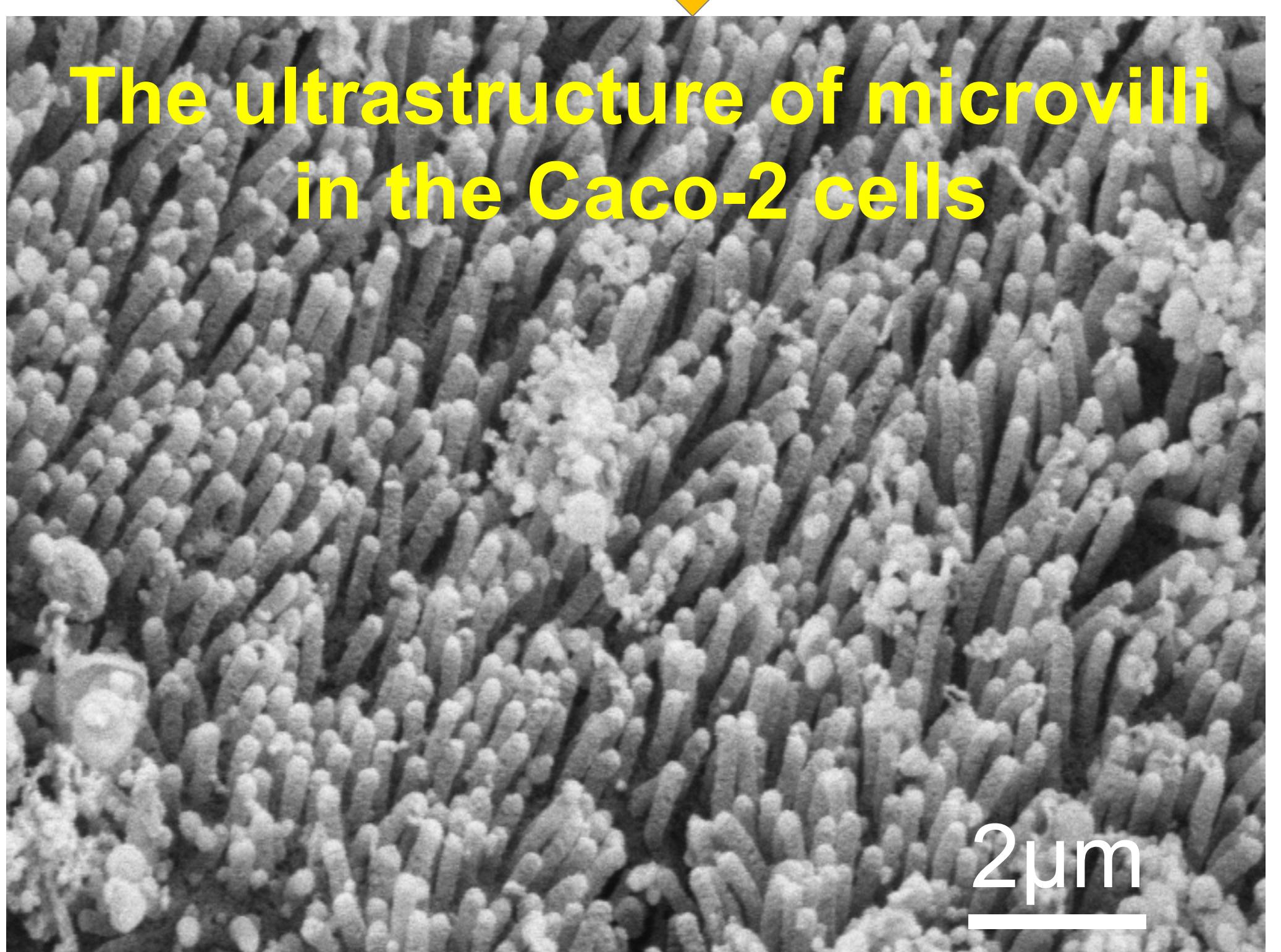
Silk Scaffold Preparation and Cell Seeding Methods & Materials

Silk fibroin was extracted from *Bombyx mori* silk worm cocoons and boiled in 2L of 0.02M Na_2CO_3 solution. The degummed fibers were then rinsed thoroughly with distilled water and air dried overnight. The dried silk fibers were dissolved in 9.3M LiBr and dialyzed to yield fibroin water solution. The supernatants are removed and set in scaffold molds. The Caco-2 cells were cultured on the membrane of transwell cell culture inserts at a density of $2 \times 10^5/cm^2$ and allowed to attach for 2 hours. The hollow Lumen of the 3D scaffolds was used to accommodate human intestinal epithelial cells. Collagen gels containing 2×10^5 H-InMyoFib/ml were prepared and delivered into the silk scaffolds with a Teflon-coated stainless steel wire to leave the hollow channel open for the seeding of Caco-2 and HT29-MTX. After, the hollow channels were loaded with the Caco-2/HT29-MTX cells at a density of 4×10^6 cells/mL.

Scanning Electron Microscopy (SEM) Methods & Materials

3D intestinal tissues were cross-linked with 2.5% GA, followed by progressive dehydration in a graded series of ethanols (30%, 50%, 75%, 95% and 100% for 30 min. each). The samples were dried by critical point drying with a liquid CO₂ dryer (AutoSamdri-815, Tousimis Research Corp.). Then, they were imaged using a scanning electron microscope (Zeiss UltraPlus SEM or Zeiss Supra 55 VP SEM, CaPrior rl Zeiss SMT Inc.) at a voltage of 2 ~ 3 kV. The samples were coated with a 10 nm. thick layer of Pt/Pd using a sputter coater (208HR, Cressington Scientific Instruments Inc., Cranberry Twp).

Acknowledgements



Dr. Ying Chen for guiding and inspiring me throughout the past 2 years, Professor Black for leading the Tufts University Biomedical Research HS Scholars Program, Dr. Kaplan for opening his laboratory to educating young scientists.

Co-culture of Intestinal Epithelial Cells on a 3D Silk Scaffold with Villi Structure

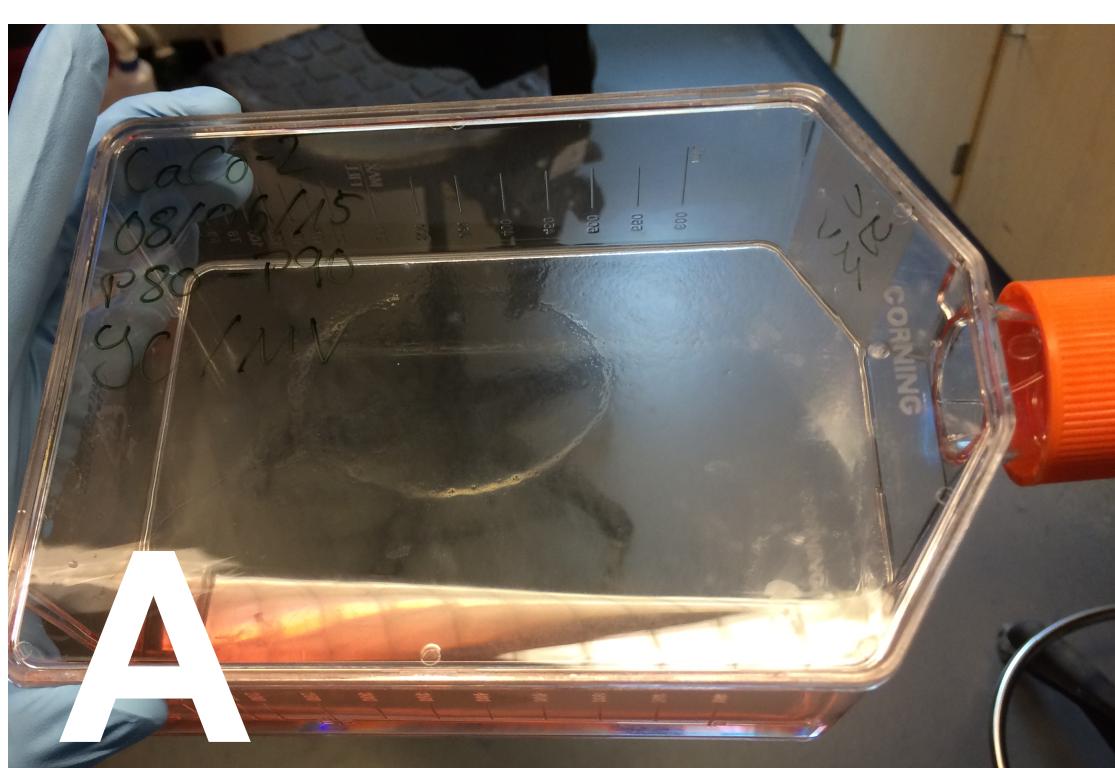
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Background

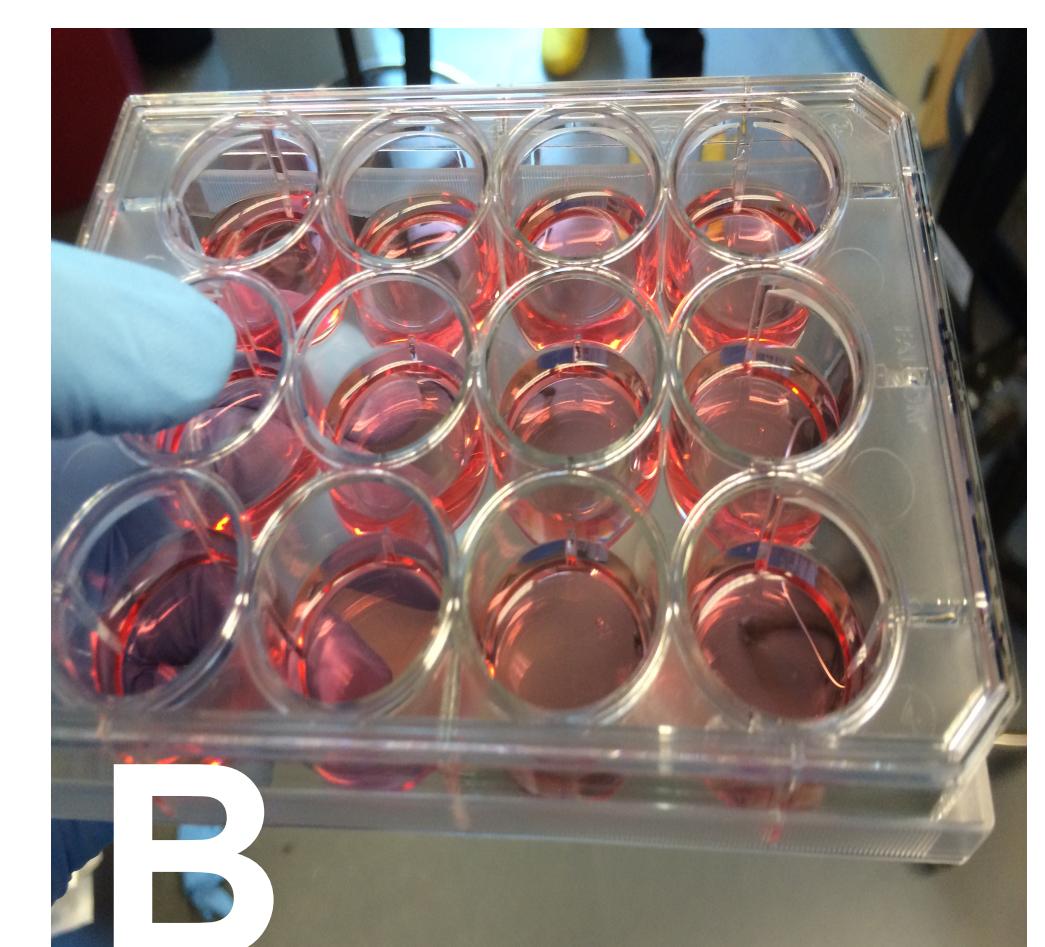
The small intestine displays a convoluted topography consisting of finger-like projections (villi), each villus approximately 0.5-1.6 mm in length. In this project, we are aiming to recreate the villi topography of the small intestine by fabricating a biodegradable and biocompatible villous scaffold using silk fibroin to enable the culture of Caco-2 and HT29-MTX with differentiation along the micro-projections (base-top axis) in a similar manner to native intestines.

Motivation

There are currently no robust in vitro human small intestines available. Silk can remodeled and represented as a tissue, capturing the biological complexity needed to develop a working, artificial small intestine. This artificial model will be a groundbreaking tool for studying pathogen interactions with the intestinal tissue.

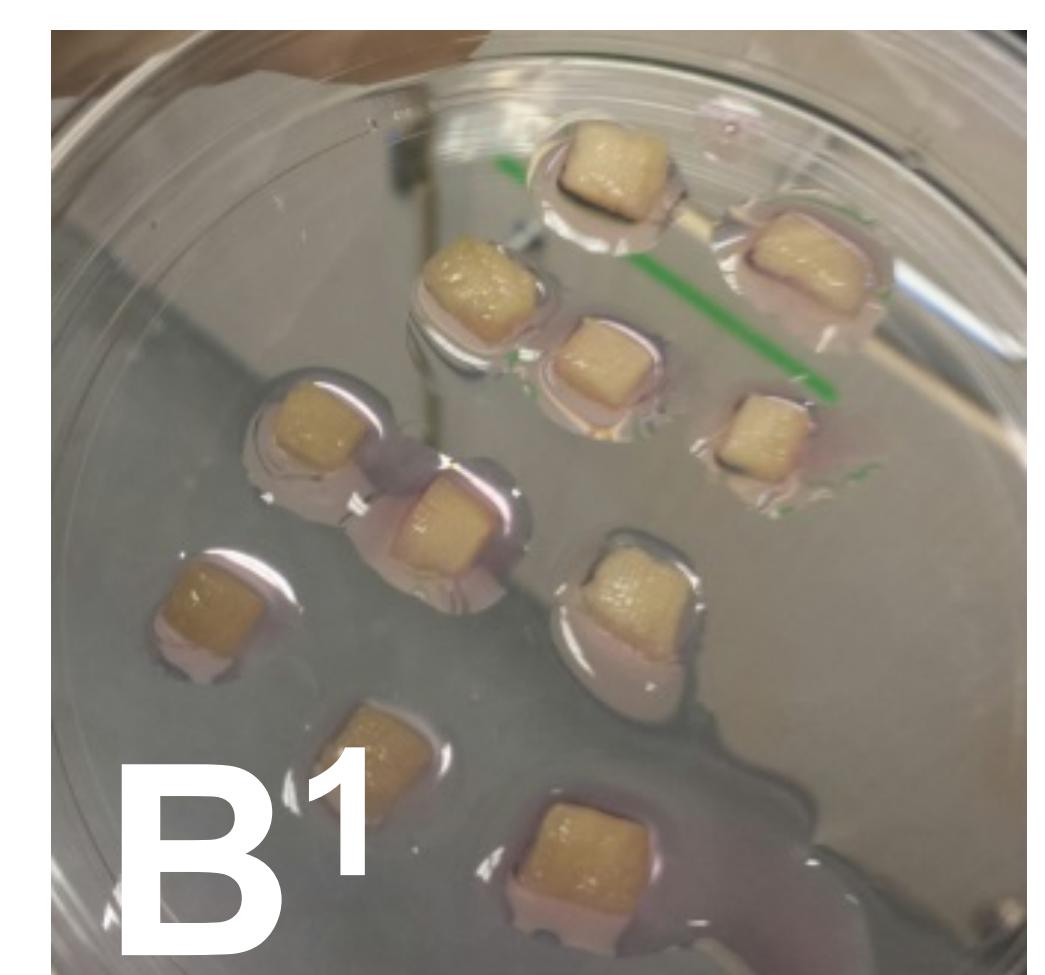


Cells seeded on 24 well tissue culture plate at a density of 2×10^5 cells/cm²



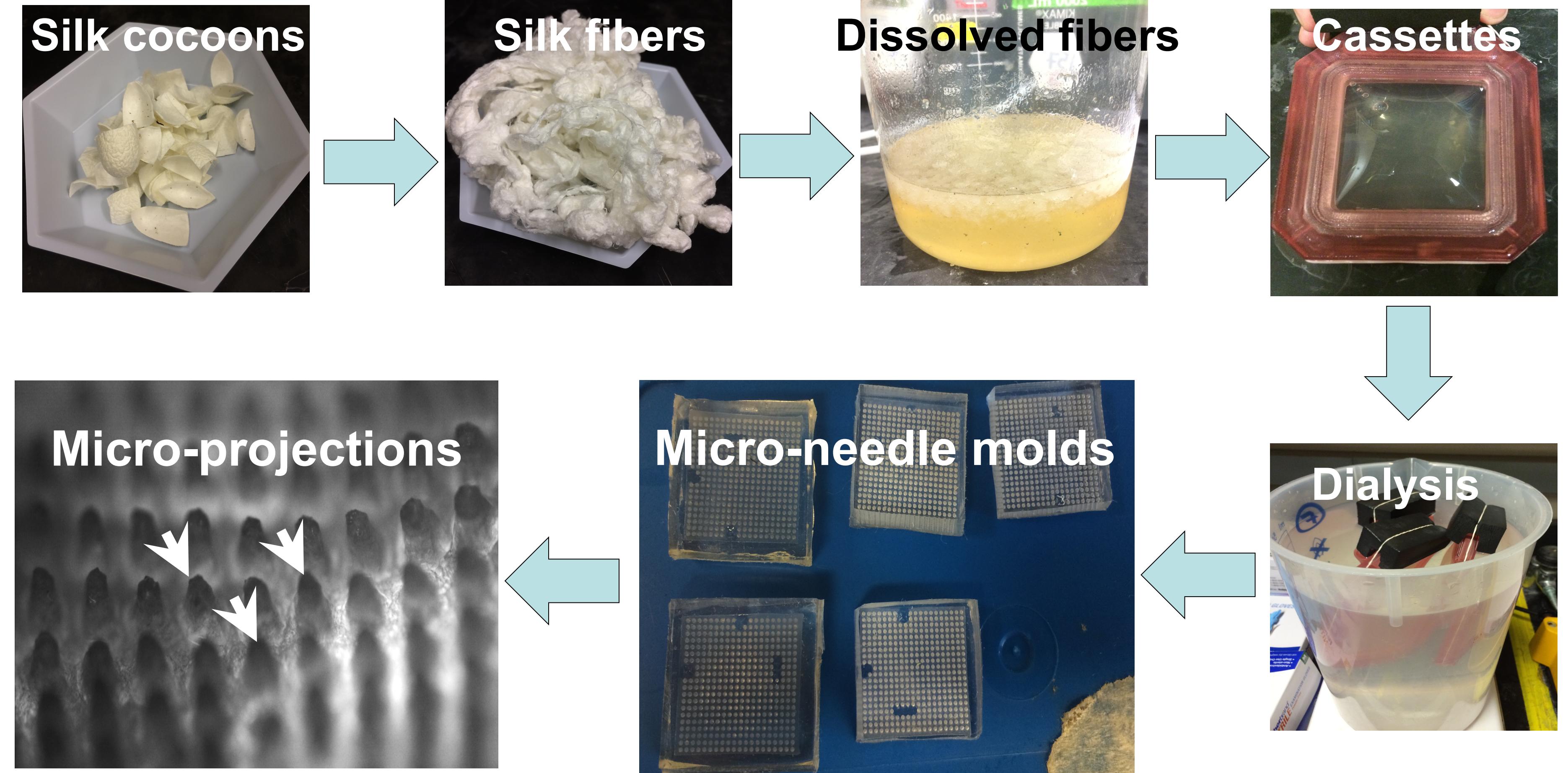
B

Cells seeded on scaffolds at a density of 5×10^5 cells/ml



B¹

Production of 3D silk scaffolds with micro-projections



Cell Culture Staining, Seeding and Imaging

The Caco-2 and HT-29MTX cells are cultured in a T-175 flask. After the cells are confluent, trypsin is added so that the cells detach off the bottom of the flask. The cells are resuspended, counted and seeded on 24 tissue culture plates and 3D silk scaffolds. Cells are maintained routinely for 21 days on the tissue culture plates and for up to 11 days on the scaffolds. Cells were fixed with paraformaldehyde for 15 min. The cells were stained with anti-human-E-cadherin (Abcam, 1:200) and DAPI (Invitrogen) overnight to visualize tight junction sites and nuclei. The cells were imaged using a Keyence fluorescence microscope or scanned using a Nikon A1R scanner and confocal 3D maximum projection images were assembled.

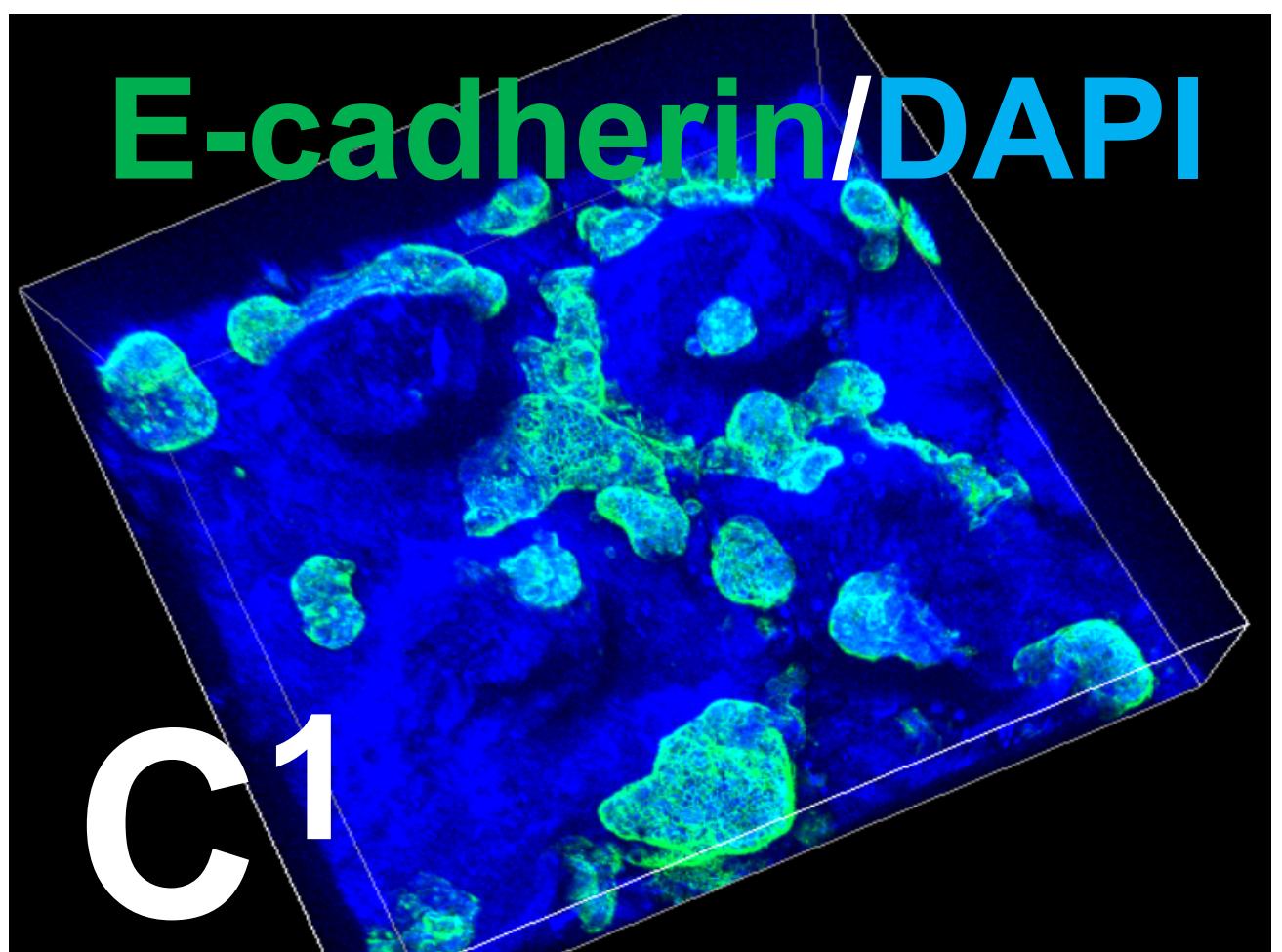
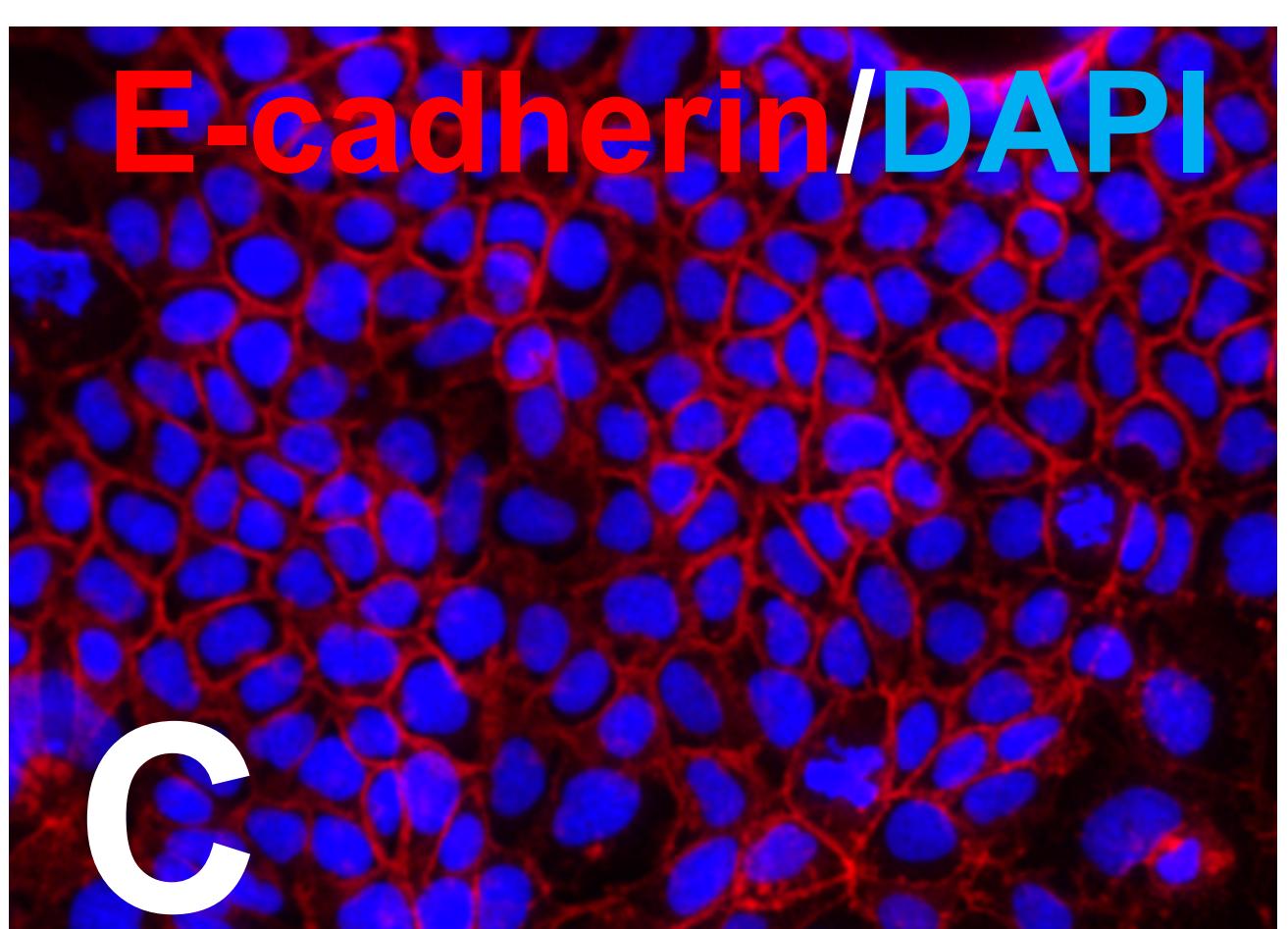
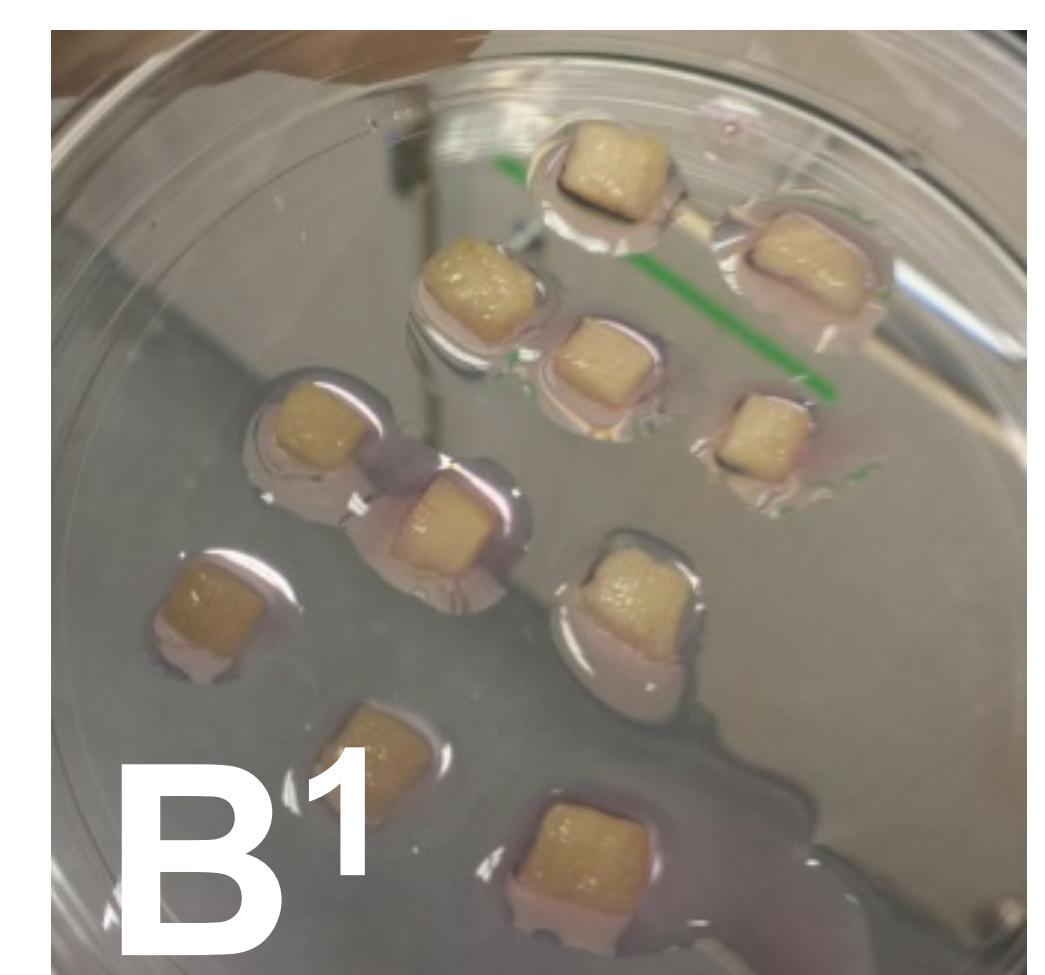
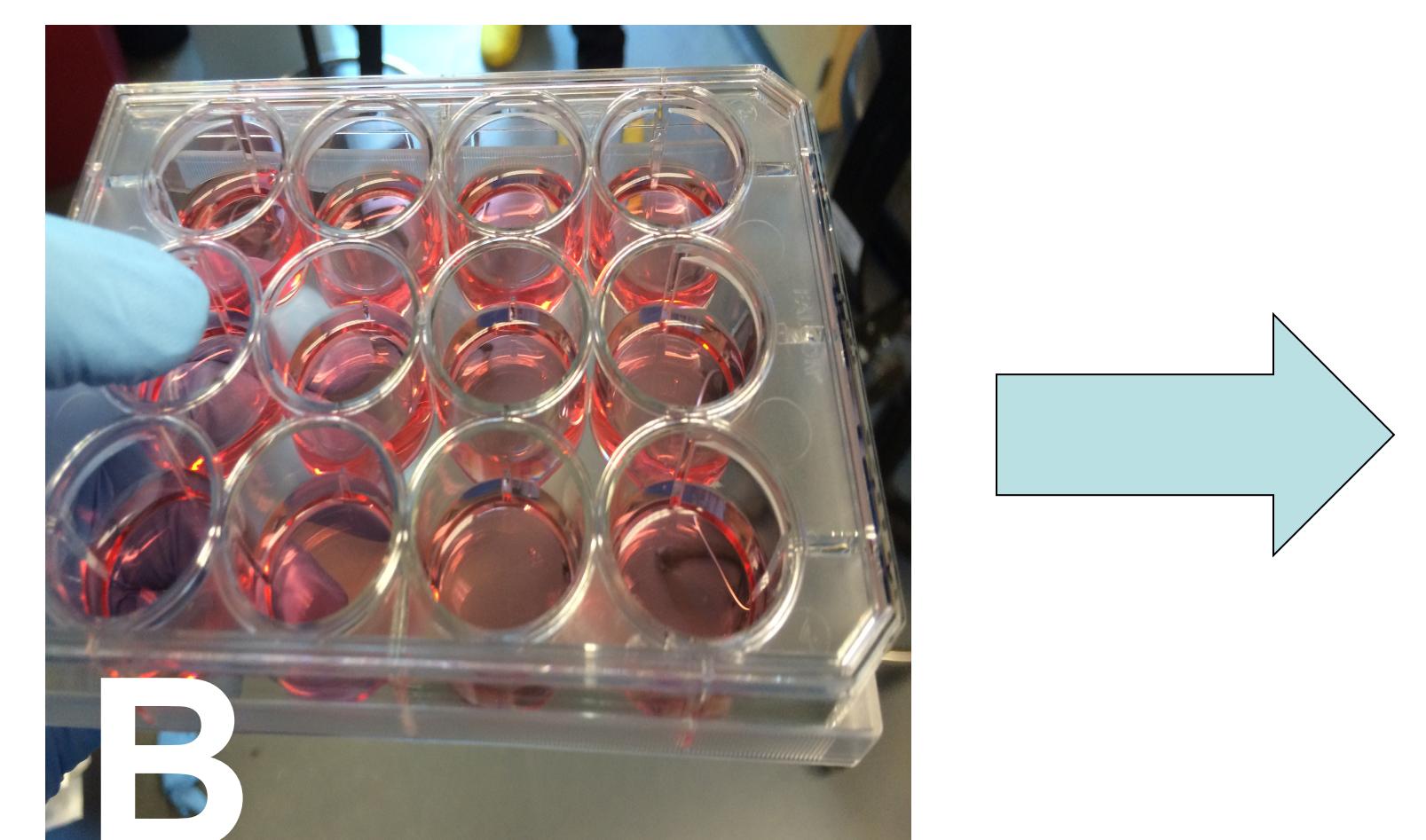


Fig 1. (A-C¹) Culture of cells on 2D tissue culture plates

A. T-175 flask of Caco-2 and HT-29MTX cells

B. Cells detach from flask using trypsin and seeded on 24 tissue culture plate

C. 2D image of Caco-2 and HT-29MTX cells (20x)

Fig 1. (A-C²) Culture of cells on 3D silk scaffolds

A. T-175 flask of Caco-2 and HT-29MTX cells

B¹. Cells detach from flask using trypsin and seeded on scaffolds

C¹. 3D image of Caco-2 and HT-29MTX cells (4x)

Silk Solution Methods & Materials

Silk fibroin was extracted from Bombyx mori silk worm cocoons. 5 g. of cocoons were cut into small pieces and boiled for 30 min in 2L of 0.02M Na₂CO₃ solution. The degummed fibers were then rinsed thoroughly with distilled water to remove residual Na₂CO₃ solution and air dried overnight. The dried silk fibers were dissolved in 9.3M LiBr at 60°C for 4 h. Then, the fibroin solution was dialyzed against distilled water for 3 days to yield fibroin water solution. The final concentration of the aqueous silk fibroin solution was about 5-6 wt%, which was determined by air drying a known volume of silk solution and massing the remaining solids. Silk solution was diluted to 4 wt% with distilled water for use and centrifuged twice for 20 min. each. The supernatants are removed and stored in the refrigerator until needed to make silk scaffolds.

Summary & Conclusion

Our Immunostaining of E-cadherin was a success on both 2D and 3D imaging. Unfortunately, in the 3D micro-projection scaffolds, we are unable to see the full coverage of cells on the micro-projections. We believe that single cells have migrated through the pores of the scaffold surface. Moving forward, we will optimize and decrease the scaffold pore size to improve the scaffold quality. We will also increase the cell seeding density on the scaffold in order to increase the cell attachment efficiency.

Acknowledgements

I would like to thank my mentor, Ying Chen for guiding me, Professor Black for leading the High School Intern Program, and Dr. Kaplan for allowing me to intern at his laboratory.