ILLINOIS INSTITUTE OF TECHNOLOGY

impact

Nayar Prize II, Phase I Quarterly Progress Report (*Q4*)
October 2017

Project: **Microfluidic Drug-Microbiota Interaction Platform**
Team: **Abhinav Bhushan, Genoveva Murillo, Rajendra Mehta**
Postdoctoral Scholars: **Sonali Karnik**
Students: **Chengyao Wang, Rongfei Wu, Kihwan Kim, Riddhi Dand, Tung Nguyen**

In the first year of our Nayar Prize II project, we have met and exceeded our Year One milestones (**Table 1**). We have made extraordinary progress to advance rapidly, including establishing collaborations with the leading groups on microbiome research. The support from the Nayar Prize was the catalyst that made this possible. Our overall project goals are to study the role microbiota play in influencing drug metabolism. We are developing a microfluidic platform to facilitate study of interactions between the drugs, intestines, and microbiota. The FDA recently allowed the first in vitro route to drug approval, which bodes extremely well for our technology.

As our Phase I milestones, we have 1) constructed biomembanes and 2) constructed microfluidic devices with biomembranes. Our work led to two poster presentations at national meetings, an award, the submission of a journal paper (attached with this report), an invention disclosure, and grant proposals. We also completed a market research study for commercialization of the microfluidic devices (attached). In addition, we have initiated studies to quantify the role colon-bacteria interactions play on drug metabolism related to the Phase II milestones. The accomplishments are summarized in **Table 1.**

Table 1: Summary of Milestones and Accomplishments		
	Milestone	**Status**
Phase I	Microfluidic platform	
	- Topological biomembranes	Completed
	- Microfluidic devices with biomembranes	Completed
	Commercialization	
	- Market research	Completed, pitch competition
	- Invention disclosures	Filed
	Training	Project supported one postdoctoral scholar, one Ph.D. student, two master's students, and one undergraduate student from Armour College of Engineering; one master's student from Stuart School of Business.
	- Collaborations - Grants submitted	Rush University and the University of Chicago
Phase II	Intestinal model in microfluidics	Part completed, part ongoing
	Intestine-drug interactions	Initiated (data included in report)

Phase I Summary:

1) **Construction of microfluidic device with biomembranes.** We have developed novel microfluidic devices that incorporate micrometer resolution-thick membranes, which are synthesized out of extracellular matrices. These extracellular matrices, which are supplied as a liquid, were coated on a glass slide and freeze-dried. The resulting sheet was peeled off and sandwiched between two fluidic layers to form a two-chamber microfluidic device using a novel fabrication process. The collagen fibrils can be seen clearly in the device. Cells on the microfluidic device with biomembranes have excellent viability for extended periods of time compared to the cells in a single layer device. These results are part of the manuscript that is attached (**Figure 1 in the attached manuscript**). We have further quantified mass transport of molecules through the membrane (**Figure 2 in the attached manuscript**).

2) **Market research.** In work led by a student from Stuart School of Business, we carried out market research on microfluidic devices. Based on primary and secondary research, we gained insights into market opportunity, customer pain points, and

pricing. A copy of the report is attached. A follow-up study was initiated to study the market opportunity for organs-on-chips for the drug and food industries. This work is ongoing. We have filed an invention disclosure with the university and have conducted preliminary investigation on forming an entity to commercialize the devices. We have also entered a startup pitch competition (results pending).

3) **Training.** The project has been instrumental in exciting students about research and has helped tremendously in recruiting students. The project has supported one postdoctoral scholar, one Ph.D. student, two master's students, and one undergraduate student from Armour College of Engineering, as well as one master's student from Stuart School of Business.

Preliminary Work Toward Phase II Milestones:

1) **Establishment of primary colon cells in the device.** We have now isolated and cultured primary colon and jejunum cells from animal (mouse) and human intestine (from a Crohn's patient) (**Figure 1A**). Primary cells are more representative of physiology than cell lines. These cells traditionally are grown as 3D organoids, which have the multicellular form of the organ but lack many features that are critical for organ function, such as vascular perfusion. We have been successful in growing the organoids in our devices and are now transitioning to the culture of layers of intestinal stem cells that are derived from these organoids.

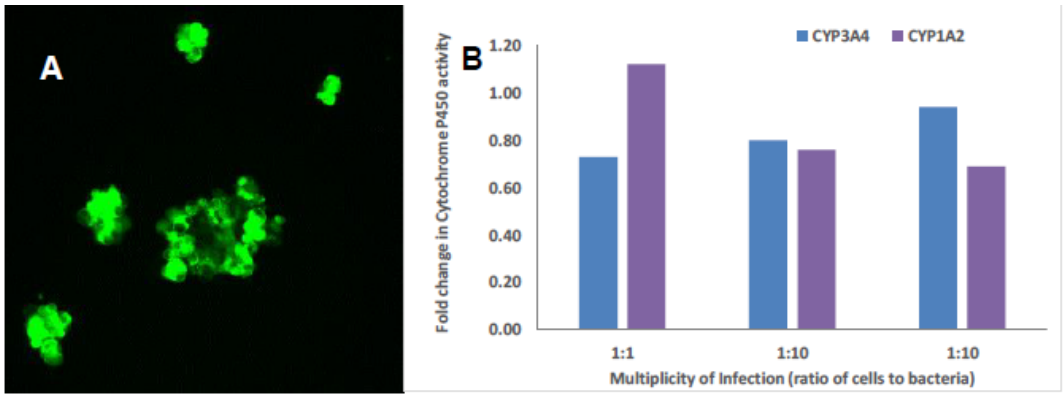

Figure 1. (A) Primary intestinal colon cells in the microfluidic device. The green color indicates live cells. **(B)** Modulation of activity of drug metabolizing enzymes by bacteria.

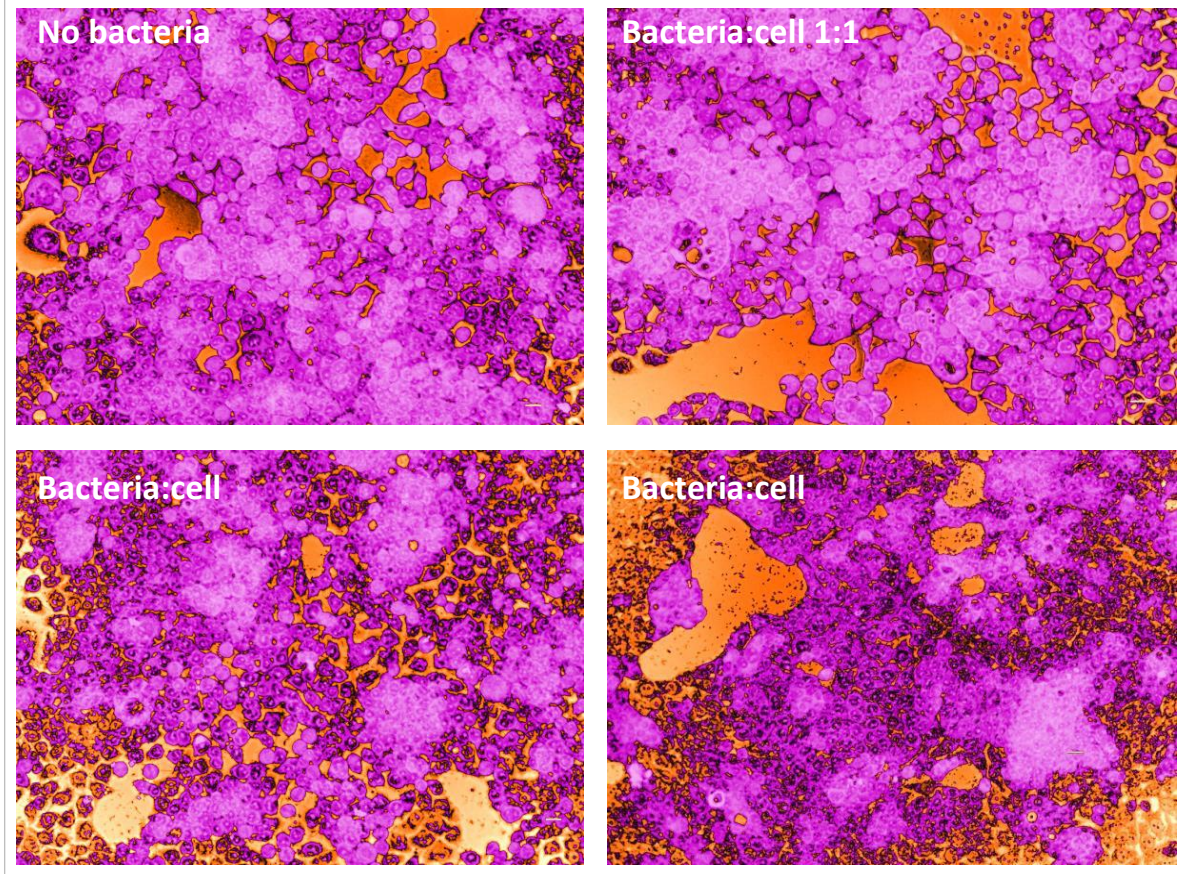

Figure 2. Invasion of cells by the bacteria is higher at higher bacteria:cell ratios. In these images, the black "dots" are the bacteria, the larger circular objects are the colon cells.

2) **Bacteria-colon interactions for drug metabolism.** We have initiated studies to investigate bacteria-colon interactions and their effect on the activity of Cytochrome P450, the enzymes that are responsible for metabolizing drugs. Our initial results indicate that the bacteria affect the activity of two of the most abundant Cytochrome P450s, namely CYP3A4 and CYP1A2. Interestingly, for CYP3A4, the activity increases with increasing number of bacteria, whereas for CYP1A2, the activity decreases (**Figure 1B**). The bacteria we tested in this case are VSL#3, a popular probiotic. The bacterial invasion of the cells can be clearly seen (**Figure 2**).

Poster presentations

Chengyao Wang, Nida Tanataweethum, Genoveva Murillo, Rajendra Mehta, Abhinav Bhushan. "A novel microfluidic device with an extracellular matrix-based membrane." *Experimental Biology*, 2017

Tung Nguyen, Abhinav Bhushan, "A simple, multilayer PET microfluidic device to reduce hydrophobic molecule absorption." Pittcon, 2017

- This work won First Prize at Klipatric Symposium 2017.

Journal paper

Chengyao Wang, Nida Tanataweethum, Sonali Karnik, Abhinav Bhushan. "A novel microfluidic intestine model with an extracellular matrix-based membrane." Under review.

Invited talks
Abhinav Bhushan, Rush University Medical Center. “Engineering microfluidic tissue models and cell-based sensors”, https://www.rushu.rush.edu/rush-medical-college/departments/immunity-emerging-pathogens/seminar-series

Collaborations
Each of our collaborators has been excited by the strengths of our group, Illinois Tech, and the support from the Nayar Prize (**Figure 3**). It is needless to say that without the Nayar Prize, venturing into any of these areas would have been impossible.
Ali Keshavarzian, Rush University – Role of microbiota along the gut-brain axis in the development of Parkinson’s disease
Eugene Chang, University of Chicago – Human intestinal model of Crohn’s disease

Grants submitted
Microfluidic gut platform to study mesenteric fat in Crohn’s. Rainin Foundation (finalist)

Microfluidic platform to discover interactions between intestine, liver, and bacteria. National Science Foundation CAREER (pending

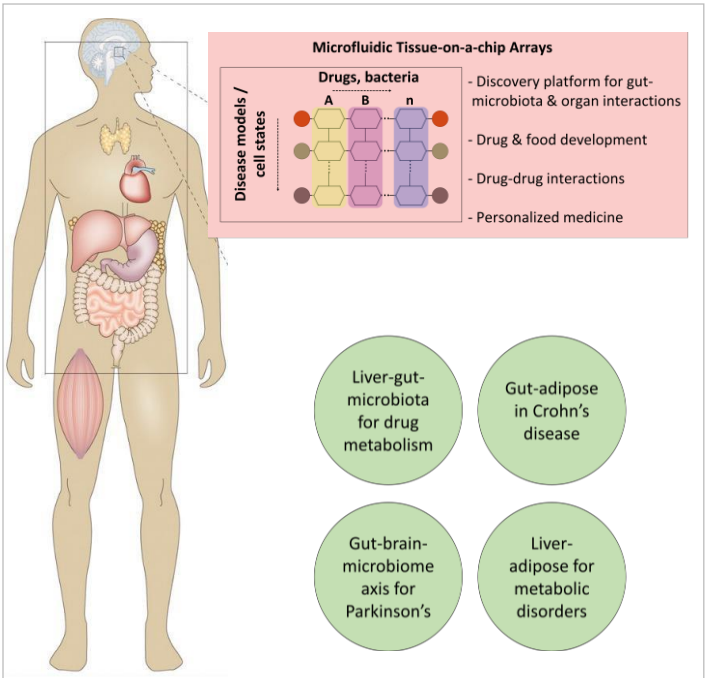

Figure 3. Sparked by the Nayar Prize.

Plans for Phase II and III:
We now have a much better understanding of the path toward translating this research. Very recent reports have been published of the critical role gut microbiota play on the efficacy of one of the most common drugs in the world, metformin. It turns knowledge has limited our ability to a priori test and validate bioactive molecules or drugs that can favorably utilize our own microbiota, pointing to the exciting possibility of our work here through the Nayar Prize. We propose to add an objective to the Phase II studies. Under this objective, we will study the role drugs play in regulating bacterial metabolites such as butyrate and propionate, which in turn affect Cytochrome P450 activity in the liver. In addition, due to the use of organoids and the intestinal stem cells, we no longer have to

incorporate the different cell types individually; therefore, we propose to replace Objective 2 of Phase II with establishing primary colon in the microfluidic device. Our revised objectives for Phase II are:

Phase II. Establish intestinal culture in the microfluidic device and characterize drug interactions. In Phase II, we will accelerate toward quantifying the role of bacteria on drug metabolism using colon in the microfluidic device.

Objective 1. Establish and characterize primary colon into the microfluidic device. We will isolate primary organoids and culture/expand them on conventional cell culture plates. Then, the cells will be seeded in microfluidic devices and cultured to confluency. After 24 hours of attachment, the cells will be cultured under perfusion at a flow rate of 5ul/hr. Culture media will also be flowed in the bottom chamber at the same flow rate. The media will be collected at periodic intervals to assess cell function. In Phase I, we had developed a model to establish a dual-oxygen-environment in the device. We will carry out experiments to establish this environment experimentally in order to mimic the intestinal microenvironment. We will characterize the cells using the functional assays (**Table 2**). We have expanded the list to include specific markers of intestinal cells.

Table 2: Assays to evaluate viability and function of cells and bacteria.

Viability	Functional assessment
- Morphology - Live / dead fluorescent assay	- Permeability with fluorescently labeled dextran - Mucus staining with alcian blue - Aminopeptidase activity - Cytochrome P450 activity - Cytokine secretion - qPCR on genes related to drug metabolism - Giesma differential stain - Cell markers Lgr5 – stem cell, SOX17 – definitive endoderm, VIL – villin, MUC2 – goblet cell marker, chromogranin A – endocrine cell marker, ZO-1 – tight junction, and lysozyme – Paneth cell marker.

Objective 2. Characterize role drugs play in regulating bacterial metabolites. t has been shown that metformin boosts the capacity of gut microbiota to produce certain shortchain fatty acids (butyric acid and propionic acid). Here, we will quantify the bacterial metabolites of the different strains of bacteria and their combinations in the presence of different drugs. The bacterium or the combination will be exposed to different concentrations and concentrations of drugs and the changes in the synthesis of the metabolites will be measured **(Table 3).**

Objective 3. Characterize role bacteria play in regulating activity of the colon drug metabolizing enzymes. We will continue our ongoing studies to quantify the role bacteria play in regulating activity of the drug metabolizing enzymes Cytochrome P450. It has been shown that metformin boosts the capacity of gut microbiota to produce certain types of short-chain fatty acids, such as butyric acid and propionic acid. In this objective, we will quantify the bacterial metabolites, especially short chain fatty acids of the different strains of bacteria and their combinations, in the presence of different drugs.

Table 3: Experimental matrix for Phase II study. A subset of the drug/bacteria combinations will be selected for the study.		
Intestine	**Drugs**	**Microbiota**
Caco-2 cells Primary organoids Intestinal epithelial and stem cells	5-Fluorouracil Digoxin Acetaminophen Genistein Sulfasalazine Prontosil Methotrexate SN-38G Metformin	- *E. coli* K-12, *E. coli* O157:H7 - Actinobacterium *Eggerthella lenta, Prevotella copri* - *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron* - VSL#3, which contains eight facultative, anaerobic, probiotic strains *L. acidophilus*, *L. plantarum*, *L. aracasei*, *Lactobacillus delbrueckii bulgaricus*, *B. breve*, *B. longum*, *B. infantis*, *Streptococcus thermophiles* - Enteroinvasive *E. coli* (serotype O124:NM)

The bacterium or the combination will be exposed to different concentrations and concentrations of drugs and the changes in the synthesis of the metabolites will be measured (**Table 3**).

We are on track to accomplish the objectives and milestones. (**Figure 4).**

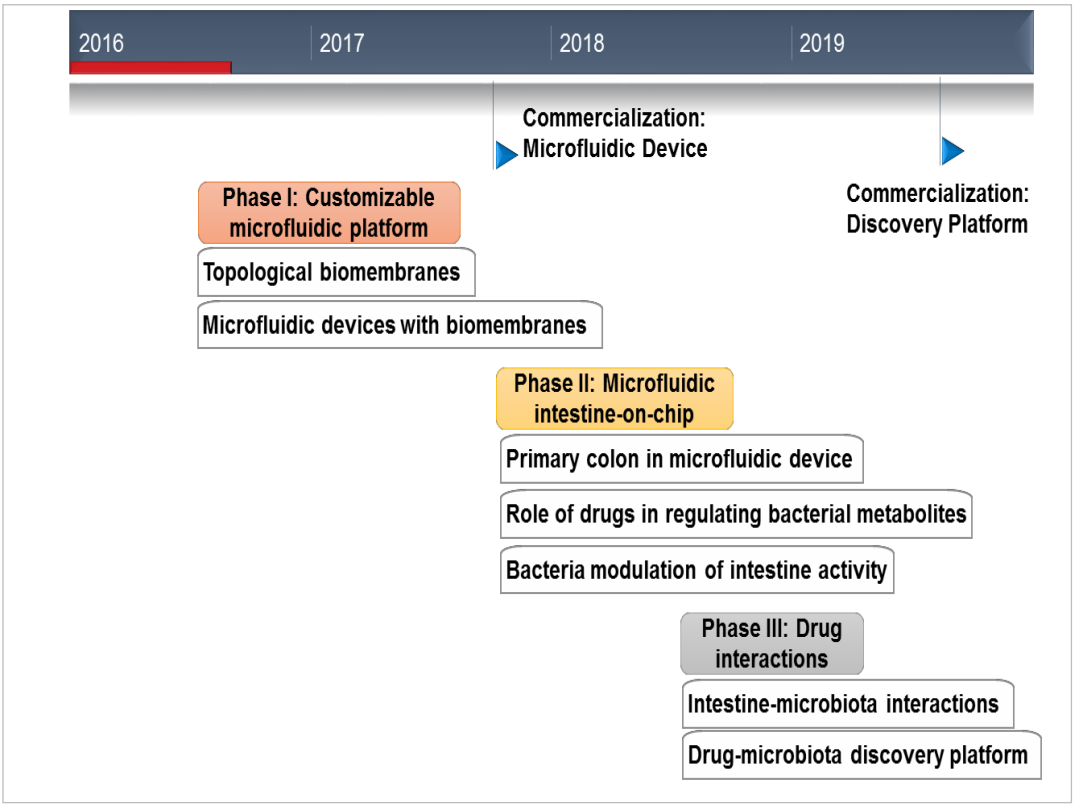

Figure 4. Timeline for the proposed project.