Development of a Continuous Sampling System for *In Situ* Monitoring of Anaerobic Coculture

By

Christopher Warburton, Undergraduate Department of Biomedical Engineering

Advisors

Nathan Swami, PhD, Department of Electrical and Computer Engineering
John Moore, PhD, Department of Electrical and Computer Engineering
Walter Varhue, PhD, Department of Electrical and Computer Engineering
Carlos Honrado, PhD, Department of Electrical and Computer Engineering

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Nathan Swami, Department of Electrical and Computer Engineering

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Christopher Warburton[a,1]

[a] Biomedical Engineering Undergraduate at the University of Virginia
[1] Email: csw9rh@virginia.edu

Abstract

Aside from surgical site infections, *Clostridiodes difficile* (*C. difficile*) infection (CDI) is the most common hospital acquired infection in the U.S., with over half a million incidences and 29,000 fatalities annually. A rapid point-of-care CDI susceptibility diagnostic tool would be an effective device to screen at-risk patients and better inform clinical management decisions. Impedance cytometry is a technology that has the potential for rapid CDI screening, as the device can detect the electrophysiological differences between *C. difficile* spores and vegetative *C. difficile.* With the Swami laboratory's current impedance sampling set-up, *C. difficile* germination cannot be fully understood because the sample is exposed to room air. *C. difficile* is an obligate anaerobe, quickly dying in the presence of oxygen; thus, in order to sample cultures at multiple time points, this capstone project aims to develop a continuous anaerobic coculture system for *in situ* monitoring of *C. difficile* germination via impedance cytometry. After developing a prototype to accomplish all required functions, the system was assembled and integrated into the impedance cytometry system. Pressure tests were conducted, and it was found that 110 mbar of pressure is required to produce the target sample flow rate of 10 μl/min. Additionally, the threshold pressure at which flow first begins is 60 mbar. Lastly, validation of sampling capabilities was confirmed through successful data collection on a solution of 7- and 2-micron beads. Utilizing the anaerobic system, the impedance cytometer was able to detect the size difference between the beads. This project is the first step in validating the device's capability to culture and sample anaerobic bacteria. Immediate next steps include confirming both the device's ability to maintain an anaerobic environment, and the possibility that *C. difficile* can grow in the system in a manner consistent with known growth trends.

Keywords: Microbiota, Impedance cytometry, Microfluidics, *Clostridiodes difficile*

Introduction

The human gut is home to over 100 trillion different microorganisms, collectively known as the gut microbiome (Ley et al, 2006). This diverse array of microbes is critical for many functions, including nutrient metabolism and immune system aid (Jandhyala et al, 2015). The microbiome community is a dynamic system, with the microbiota profile constantly changing to local environmental cues such as diet, the host immune system, and antibiotic disruption. These microbes modulate the composition of small molecules, or metabolites, by utilizing primary metabolites to survive, consequentially releasing secondary metabolites (Levy et al, n.d.). Antibiotic disruption of a "healthy" microbiome reduces overall microbial diversity and increases susceptibility of the human host to colonization and infection by pathogenic bacteria such as *C. difficile*. Aside from surgical site infections, CDI is the number one hospital-acquired infection (Chopra et al., 2019), with almost half a million incidences annually and 29,000 fatalities in the US (Lessa et al, 2015). Additionally, more than one billion dollars is spent each year in combatting CDI (Shah et al., 2016**).** Thus, there is a substantial need to develop tools that can rapidly quantify the patients' susceptibility to pathogenic bacteria following antibiotic treatment in the clinical setting. A rapid point-of-care testing method would improve patient outcomes both medically and financially, as current microbiota testing can only be performed in specialized laboratories (Miezeiewski et al, 2014). In prior research, absorbance assays have been used to quantify *C.*

difficile growth; however, due to the low sensitivity of assays, significant data can only be produced after around 15 hours of growth. At this time point, the bacteria culture is the past the germination phase and undergoing exponential growth ("Bacterial growth curve," 2018).

Single-cell impedance cytometry is a rapidly evolving technology that has the potential to quantify the susceptibility of host gut microbiota to pathogenic bacterial infections (Moore et al, 2020). Impedance cytometry is a tool used to assess the dielectric properties of individual cells. An alternating electric field at a specific frequency is applied to the cells, and information about the size and conductive properties of the cells is determined the cells' response to the field (Holmes, n.d.). In the Swami laboratory, we have utilized *C. difficile* as a model bacterium to test the ability of impedance cytometry to measure how alterations in microbiota derived metabolites affect the germination of *C. difficile* spores, which leads to CDI of the host. As seen in Figure 1**,** when *C. difficile* germinates, there are major changes in morphology, cellular structure, and machinery. In our most recent work in the Swami laboratory, with the use of impedance cytometry, we were able to successfully identify the presence of germinated *C. difficile* in its vegetative state. Figure 2 provides impedance cytometry data that we have collected on *C. difficile* cultures grown in various taurocholate concentrations. Taurocholate is a primary bile salt that can be found in the intestines and is known to induce

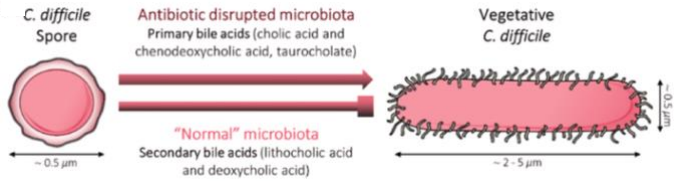

Fig. 1. *C. difficile* germination. When the gut microbiota is disrupted with antibiotics, *C. difficile* spores are able to germinate into the vegetative form. Vegetative *C. difficile* are have significantly different features and properties than the spore form.

germination of *C. difficile* spores (Howerton et al., 2011**)**. By culturing spores with no growth media, a threshold gate was established. Vegetative *C. difficile* appears to the right of the gate, and spore aggregates, along with other particulate matter in the media, appear on the left side of the gate. Between Figures 2b-2d and 2g-2i, it is evident that as taurocholate concentration increases, more germination occurs at the same incubation time. Additionally, at the same taurocholate levels, between 4 hours incubation and 5 hours incubation, more vegetative *C. difficile* was detected at 5 hours.

With the current sampling system, we are unable to obtain a complete picture of the growth kinetics and germination rates of *C. difficile* in various metabolite conditions. *C. difficile*, like most gut microbiota, is an

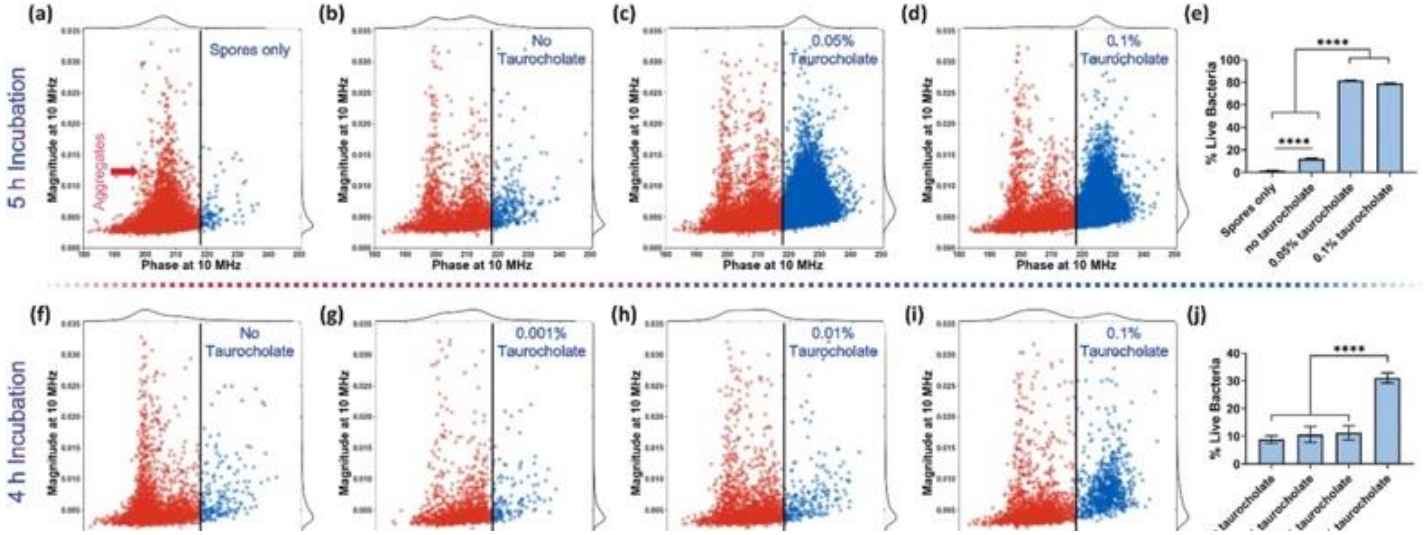

Fig. 2. Quantifying *C. difficile* spore germination after 5 h (a-e) and 4 h (f-j) of incubation under the indicated conditions: (**a**) in absence of growth media versus in presence of growth media contain taurocholate at indicated levels: (**b**) 0%; (**c**) 0.05%; (**d**) 0.1%. (**e**) Summary plot with variation of percentage of detected vegetative *C. difficile* for each condition. The analogous experiment conducted after 4 h of spore incubation in growth media containing taurocholate levels at the indicated levels: (**f**) 0%; (**g**) 0.001%; (**h**) 0.01%; (**i**) 0.1%; and (**j**) summary plot.

obligate anaerobe, quickly dying in the presence of oxygen ("Clostridium difficile-associated disease," n.d.). With our current sampling system, each culture being sampled is exposed to the atmosphere during sampling. Therefore, we only obtain data on a culture of interest at a single time point. Thus, each concentration level tested in Figure 2 was conducted on a completely different *C. difficile* culture. In order to understand *C. difficile* growth and germination kinetics using impedance cytometry, data needs to be able to be collected at multiple time points. Thus, this project aims to develop an anaerobic coculture system integrated with impedance cytometry to take continuous data on *C. difficile* cultures and learn about early germination and growth kinetics in various conditions. This project has the potential to lead to development of a point-of-care testing technology for rapid assessment of patient susceptibility to CDI following antibiotic treatment.

Materials and Methods

Materials

Materials for the Anaerobic System

Table S1 provides a list of the individual components of the anaerobic coculture system. The impedance cytometer used for sampling is the Ampha Z32 by Amphasys.

Materials for Preliminary Pressure Testing

To perform preliminary resistance testing, a Cetoni Nemesys low pressure syringe pump was controlled using QMixElements software, which produces user-defined, pulsation-free fluid flow. Pressure was measured using a 70 mbar Elveflow microfluidic pressure sensor which was attached to the Elveflow OB1 MK3+ and corresponding software.

Materials for Impedance Data Collection Validation

To test the function of the anaerobic coculture system, .1 µl of 7-micron polystyrene beads were added to 2 ml of 1x PBS. Following data collection on this solution, .1 µl of 2-micron polystyrene beads were then added to the solution l and impedance data was collected.

Methods

Design Constraints and Prototype Development

The first step of prototyping was to identify the desired functions of the system. It was determined that the system must perform two base functions. It has to be able to produce sample flow when desired by the user and has to provide an environment suitable for anaerobic bacteria to grow. The ideal environment mimicks the conditions of the gut. Thus, the device needs maintain the culture at physiological temperature and needs to provide anaerobic gas to the culture at all times. To satisfy multiple necessary conditions, positive pressure from an anaerobic gas source was utilized to produce sample flow while providing an environment suitable for anaerobic bacteria. An external heating source was decided to be the best method for maintaining the proper temperature of the culture.

Additionally, there were budget constraints on the design. The system had to be designed with a budget of approximately $6000, limiting the options for costly components such as regulators and heating mechanisms.

There were also constraints on the size of the system due to the location of the impedance cytometer in the laboratory. There is about 4 square feet of room available for the system in front of the impedance cytometer, which was necessary to consider while deciding which components to purchase. The components selected were chosen with the goal of feasibly fitting the device into the available lab space.

Another aspect that was considered was ease of implementation into the impedance cytometer. The Swami laboratory utilizes the impedance cytometer frequently for various experiments. Therefore, it was important that the anaerobic coculture system could be attached and detached from the impedance cytometer easily and quickly. This was accomplished by selecting the same microfluidic fitting sizes as the fittings on the impedance cytometer.

Safety features of the device were integrated into the design process as well. This involved the consideration of scenarios in which the device could potentially fail. One of the biggest risks is over pressurization of the system that could create a small explosion and lead to spillage of the anaerobic culture. This is a safety hazard because CDI can result from ingestion of as little as 100 spores (Moore et al., 2020**)**. To mitigate this risk, a backpressure regulator was incorporated to release gas when the pressure of the system exceeds a value defined by the user. The final prototype design can be seen in Figure 3. There are two modes that the system will be in during experiments. The first is when

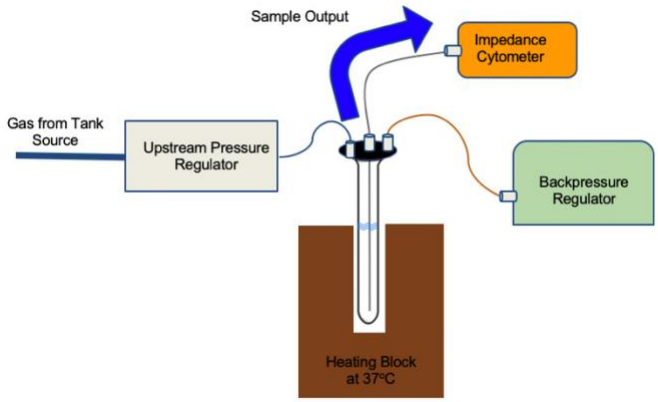

Fig. 3. Prototype design. Considering the necessary functions and constraints, this final prototype design was developed to be small, within the budget, and able to both culture bacteria and produce sample flow.

sampling is desired, in which the upstream regulator will produce a positive pressure great enough to produce sample flow at a rate of 10 µl/min. The other mode is when no sampling is needed and the culture is growing. The upstream regulator can be set to a low pressure such that the culture can receive anaerobic gas without production of sample flow.

Preliminary Resistance Testing

Following prototype development, the resistance of the impedance cytometer system had to be determined. The Fluigent Lineup Flow EZ upstream regulator comes in a variety of pressure ranges, ranging from 0-25 mbar up to 0-7000 mbar. According to the regulator's manufacturer, larger ranges of the regulator are less accurate at lower pressures, making it difficult to produce consistent flow at exactly 10 µl/min. This made identifying the pressure necessary to produce flow crucial.

To identify the resistance of the impedance cytometer system, the Nemesys syringe pump and Elveflow pressure system were connected to the impedance cytometer chip as shown in Figure 4. Various flow rates were inputted to the Nemesys, and the resulting pressure drop across the microfluidic chip was recorded using the pressure sensor. It was assumed that the entire pressure drop of this set-up occurred across the chip. It was also assumed that the majority of the resistance of the impedance cytometer comes from the chip, since the channel width is magnitudes smaller than the tubing width. Equation 1 was

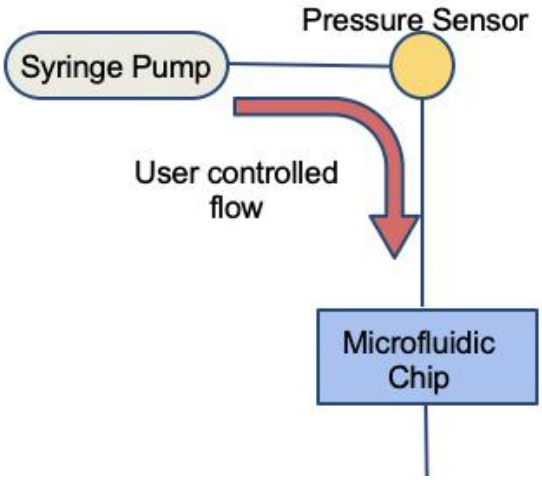

Fig. 4. Preliminary resistance testing design. A Nemesys syringe pump and Elveflow pressure sensor were used to control flow and record pressure drop across the microfluidics chip respectively. The relationship between pressure and flow rate was used to determine the theoretical resistance of the impedance cytometer in order to purchase the optimal upstream pressure regulator.

utilized, where ΔP is the pressure drop (mbar), Q is the flow rate (µl/min), and R is the chip resistance (mbar*min/µl).

$$\Delta P = Q*R \qquad \text{(Equation 1)}$$

A graph of pressure vs flow rate was created, and the slope of the line was recorded to be the theoretical resistance of the impedance cytometer. This value was then used to identify the theoretical pressure necessary to produce 10 µl/min sample flow in order to purchase the optimal upstream pressure regulator for the system.

Testing the Upstream Regulator Function

After the components of the system were ordered and shipped, it was necessary to determine the capabilities of the Fluigent Flow EZ Regulator. We needed to know how quickly the regulator could respond to a user defined increase or decrease in pressure. It was also important to understand the mechanism in which the regulator decreases pressure. Using the closed-system setup in Figure S2, it was determined that the regulator could increase and decrease pressure almost instantaneously. This is ideal for the user; however, the regulator's mechanism to decrease pressure is through a release valve that empties gas to the atmosphere, which could be problematic for the anaerobic cultures, as culture growth and germination kinetics would be altered by the presence of oxygen.

Air Leak Testing

Once the functionalities of the upstream regulator were understood, all the components were assembled to create the anaerobic coculture system. All fittings were initially tightened to a finger-tight level, and no additional tools were required for the assembly. An air leak test was then performed to ensure that the system is truly closed from the atmosphere. While connected to a house gas source, the system was pressurized to the level in which fluid flow was first apparent. Then, a solution of soap and water was applied to each of the fittings and observed for approximately four minutes. Bubbles forming around the fittings indicated that there was an active leak. Bubbles appeared on all three peristaltic fittings. To fix the leaks, the fittings were tightened half a rotation past finger-tight using an adjustable wrench.

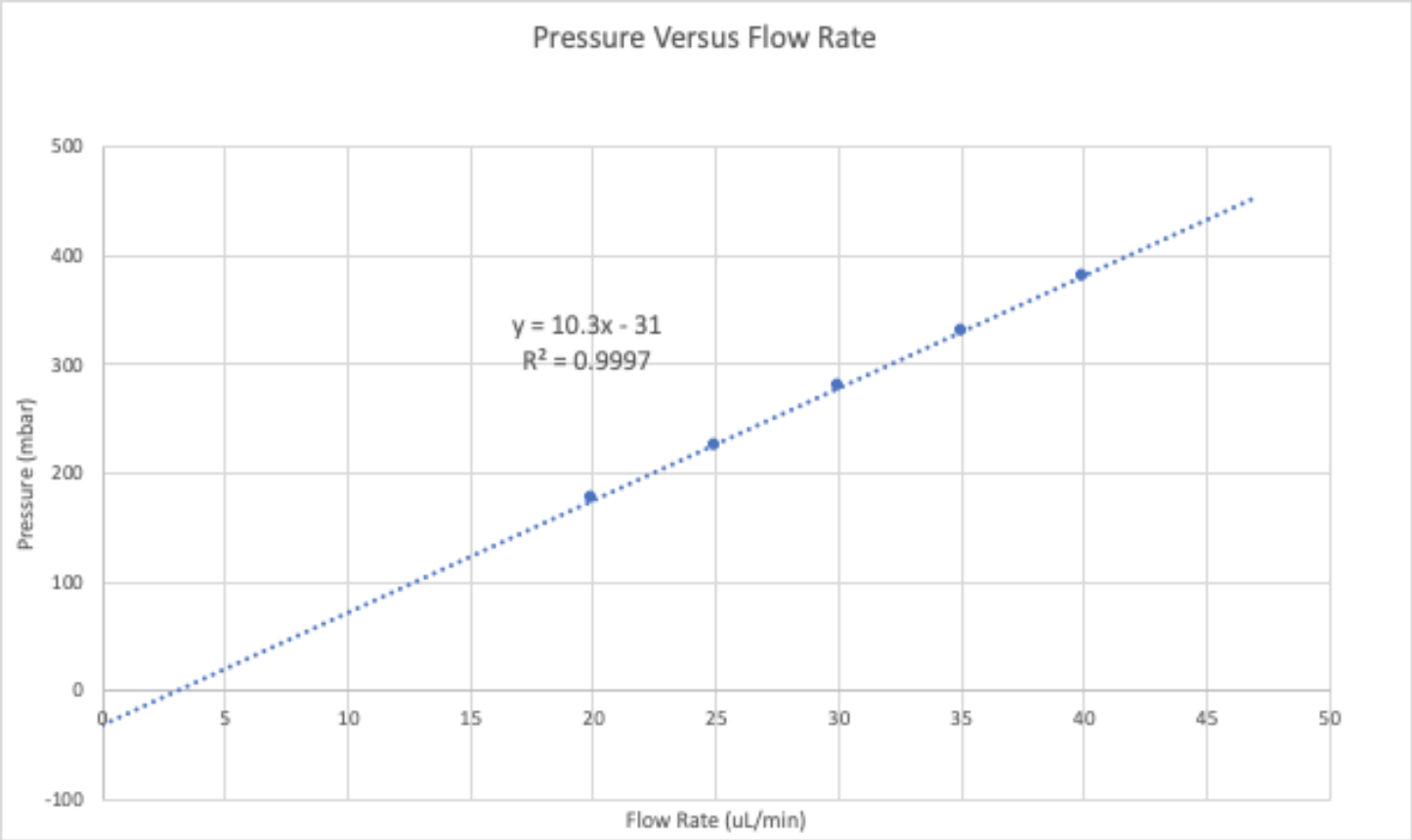

Fig. 5. Preliminary Resistance Testing Results. Five different flow rates were generated with the Nemesys syringe pump, and the corresponding pressure drops were recorded. The theoretical resistance was determined to be 10.3 mbar*min/µl. Using Equation 1, the theoretical pressure to generate 10 µl/min flow was 103 mbar. With an R^2 value of .997, the trendline was considered to be a good fit for the data.

Pressure Tests with the Impedance Cytometer

After the leaks were fixed, the anaerobic coculture system was ready to be connected to the impedance cytometer. The first step that needed to be completed was identifying the actual pressure necessary to produce 10 µl/min sample flow. Starting at 0 mbar, the upstream regulator was incremented 10 mbar at a time, and the resulting flow rate was detected using the flow sensor integrated within the impedance cytometer. Pressure was incremented at this rate until the flow rate reached 18 µl/min. Then, a flow rate versus pressure graph was generated, and the resistance of the system was identified from the reciprocal of the slope using the relationship from Equation 1. While performing this experiment, the threshold pressure at which flow begins was recorded as well.

Validation of Proper Impedance Cytometer Sample Collection with Anaerobic System

Once the pressure testing was complete, the next step was to confirm that the impedance cytometer could properly collect basic sample data using the anaerobic system. The 7-micron bead solution was placed into the anaerobic system and impedance cytometry data was collected for 5 minutes. Following this data collection, 2-micron beads were then added to the solution, and impedance cytometry data was collected for another 5 minutes. The purpose of these data collections was to ensure that the sample could effectively run through the system without being damaged from shear forces.

Results

Prototype Design

Considering the constraint and design requirements discussed in Methods, the final design prototype was created and can be seen in Figure 3.

Preliminary Resistance Testing Results

The pressure versus flow rate graph generated from the syringe pump and pressure sensor is shown in Figure 5. The theoretical resistance, or slope of the trendline, is 10.3 mbar*min/µl. Utilizing Equation 1, the theoretical pressure necessary to produce 10 µl/min flow was 103 mbar. Thus, the 0-345 mbar range Flow EZ regulator was purchased. Since the resistance was assumed from only the microfluidic chip, this regulator range leaves room for a potentially higher resistance. This results in a higher pressure required, as additional resistance could come from the microfluidic tubing.

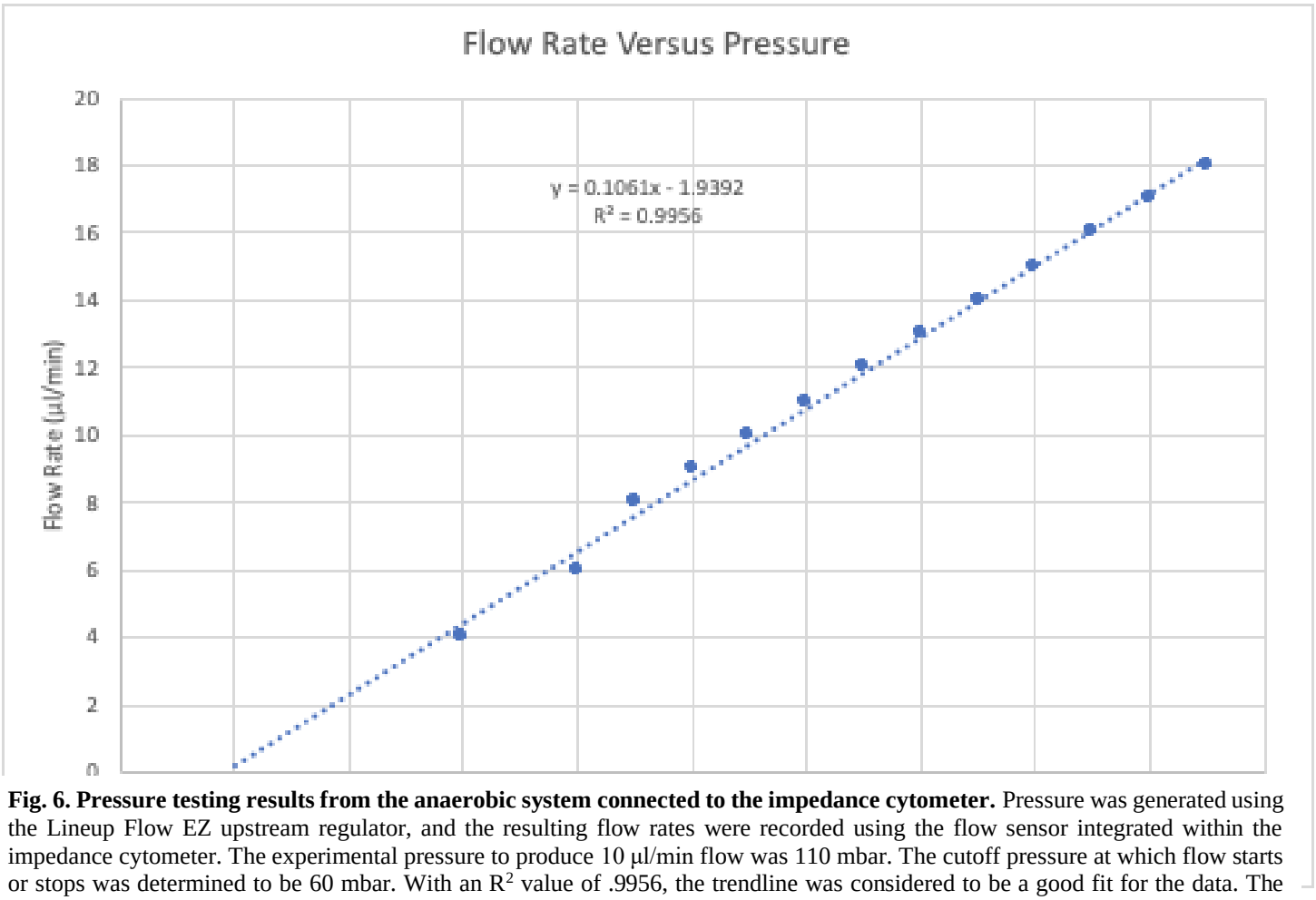

Fig. 6. Pressure testing results from the anaerobic system connected to the impedance cytometer. Pressure was generated using the Lineup Flow EZ upstream regulator, and the resulting flow rates were recorded using the flow sensor integrated within the impedance cytometer. The experimental pressure to produce 10 µl/min flow was 110 mbar. The cutoff pressure at which flow starts or stops was determined to be 60 mbar. With an R^2 value of .9956, the trendline was considered to be a good fit for the data. The trendline equation can be utilized by the user to quickly reach a desired sample flow rate.

Pressure Testing With Impedance Cytometer Results

The flow rate versus pressure data collected from the anaerobic system connected to the impedance cytometer is shown in Figure 6. 110 mbar of pressure was required to produce sample flow at 10 µl/min. The threshold pressure at which flow was produced was 60 mbar. Additionally, the reciprocal slope of the trendline was 9.43 mbar*min/µl.

Impedance Cytometry Validation Results

The impedance cytometry data collected on the 7-micron beads using the anaerobic system can be seen in Figure 7a. As evident in Figure 7a, there is a clear subpopulation of data present. This subpopulation is consistent with the 7-micron bead data previously collected using the standard impedance cytometry sampling set-up. The data previously collected can be seen in Figure S3.

The impedance cytometry data collected on the solution of 7-micron and 2-micron beads is shown in Figure 7b. In comparison to Figure 7a, an additional subpopulation appears near the bottom of the graph. Since the amplitude corresponds the physical size of the sample, the data appears to be valid, as the 2-micron beads are much lower on the graph than the 7-micron beads. Additionally, phase corresponds to the conductive properties of the cells. Since both beads are composed of the same material, they should generate similar phase shifts. This was confirmed with the data in Figure 7b.

Discussion

Overall, this capstone project shows promising results for a feasible anaerobic coculture system to be integrated with impedance cytometry. The anaerobic system was able to generate a pressure of 110 mbar in order to produce sample flow output to the impedance cytometer. The anaerobic sampling system was able to generate valid data on 7- and 2-micron beads. This is evident by the similarities between the 7-micron data collected using the standard impedance sampling set-up and the data collected with the anaerobic coculture system. Additionally, the anaerobic system was used to successfully distinguish between 7- and 2-micron beads in the same solution.

One of the challenges encountered during the project was the leaks in the peristaltic fittings. Initially, we thought that the fittings needed to be cleaned and reinserted to correct the leaks; however, this technique did not fix the

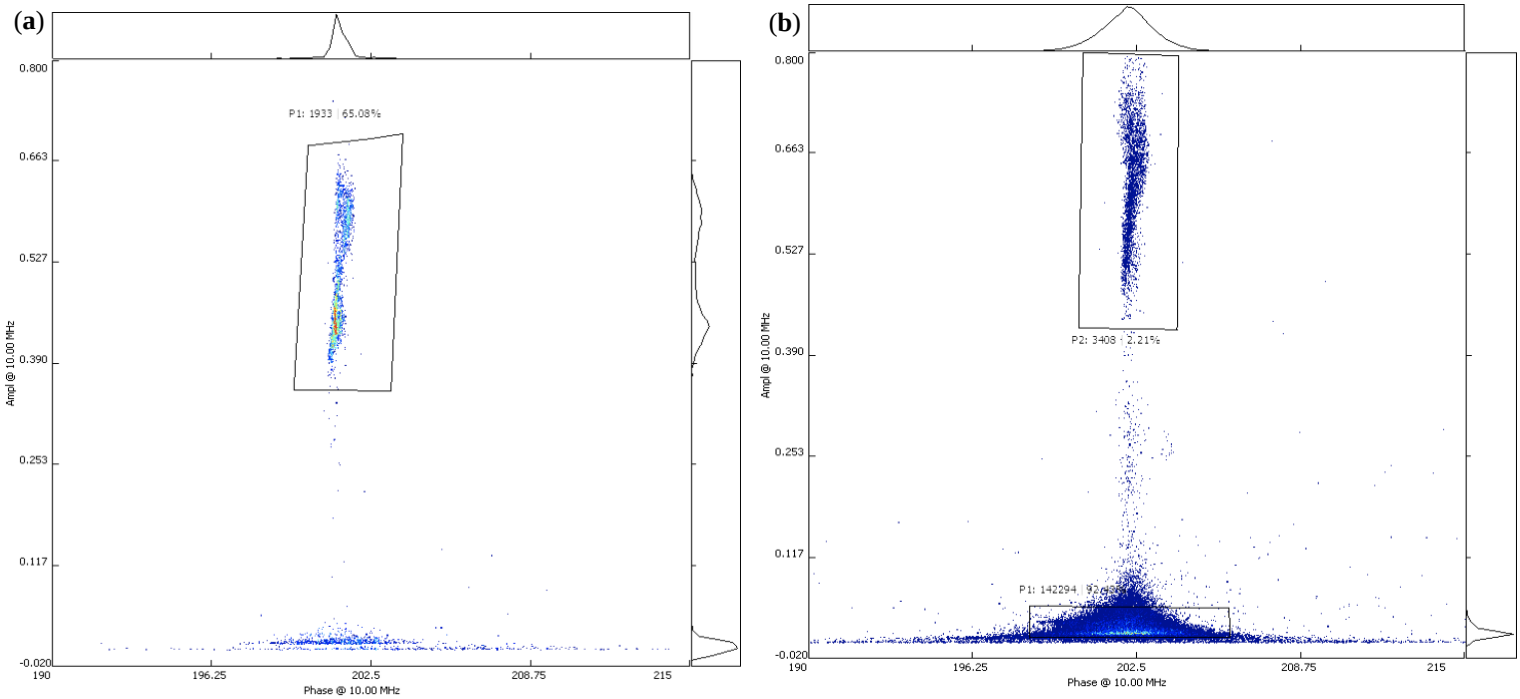

Fig. 7. Impedance cytometry data on polystyrene beads using the anaerobic coculture system. (**a**) shows the impedance cytometry data collected on the 7-micron bead sample. There is a clear population of data in the center of the graph, corresponding to the beads. (**b**) shows the impedance cytometry data collected on the solution of 7-micron and 2-micron beads. With the addition of the 2-micron beads, there is a new subpopulation that formed that is lower in magnitude than the 7-micron bead subpopulation.

issue, and the system was not able to reach pressures over 15 mbar. Most microfluidic fittings are only supposed to be screwed to finger-tight levels, and further rotation can potentially break the fitting. Tightening past finger-tight levels with a wrench was not our initial attempt.

With the COVID-19 pandemic, we lost approximately 3 months of laboratory time. This greatly impacted the expected timeline of the project. One of the immediate next steps of the project is to test the anaerobic coculture system's ability to maintain a truly anaerobic environment. To test this, an oxygen indicating tablet will be secured inside of the reservoir. The system should then be connected to the impedance cytometer and an anaerobic gas source, and samples should be taken every hour for 8 hours to mimics the conditions that the system will face with anaerobic bacteria samples. The oxygen sensing tablet should be recorded on video. Following the experiment, the video will indicate if the tablet ever senses the presence of oxygen.

If successful results of the oxygen testing experiments are recorded, *C. difficile* should then be cocultured in 0%, .001%, .01%, and .1% taurocholate concentrations. Each culture should then be sampled every 30 minutes for 12 hours, and the recorded growth trends should be compared to known growth patterns for *C. difficile* in increasing taurocholate concentrations in order to validate that the anaerobic bacteria can grow properly in the anaerobic system.

Next, *C. difficile* growth will be analyzed in various antibiotic treated mice fecal supernatant samples in order to understand how various antibiotic regimes affect *C. difficile* germination.

This capstone project has made impedance cytometry closer to being able to be used as a point-of-care rapid diagnostic technology for CDI. This technology has the potentially to save tens of thousands of lives, prevent hundreds of thousands of hospital-borne infections, and save billions of healthcare dollars annually. The anaerobic coculture system can additionally be utilized for other applications, such as the effects of various drugs on anaerobic bacteria cultures.

End Matter

Author Contributions and Notes

C.W., designed research, performed research, analyzed data, and wrote the paper with the help of the advisors. The author declares no conflict of interest.

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References

1. Ley, R.E., Peterson, D.A., Gordon, J.I., 2006. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124 (4), 837–848.
2. Jandhyala,S., Talukdar, R., Subramanyam, C., Vuyyuru, H., Sasikala, M., & Nageshwar Reddy, D. (2015, August 7). Role of the normal gut microbiota. Retrieved October 02, 2020, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4528021/
3. Levy, M., & Thaiss, C. (n.d.). Metabolites: Messengers between the microbiota and the immune system. Retrieved October 02, 2020, from http://genesdev.cshlp.org/content/30/14/1589.full.html
4. Chopra, T. & Navalkele, B. Clostridium difficile in the Hospital: Infection Prevention Considerations. *Infectious Disease Advisor* (2019). Retrieved May 6, 2021 from https://www.infectiousdiseaseadvisor.com/home/decision-support-in-medicine/hospital-infection-control/clostridium-difficile-in-the-hospital-infection-prevention-considerations/
5. Lessa FC, Mu Y, Bamberg WM, Beldavs ZG, Dumyati GK, Dunn JR, Farley MM, Holzbauer SM, Meek JI, Phipps EC, Wilson LE, Winston LG, Cohen JA, Limbago BM, Fridkin SK, Gerding DN, McDonald LC. Burden of Clostridium difficile infection in the United States. N Engl J Med. 2015 Feb 26;372(9):825-34. doi: 10.1056/NEJMoa1408913. PMID: 25714160.
6. Shah, D., Aitken, S., Barragan, L., Bozorgui, S., Goddu, S., Navarro, M., Xie, Y., DuPont, H., Garey, K., 2016. Economic burden of primary compared with recurrent Clostridium difficile infection in hospitalized patients: a prospective cohort study. J. Hosp. Infect. 93 (3), 286–289.
7. Miezeiewski, M., Schnaufer, T., Muravsky, M., Wang, S., Caro-Aguilar, I., Secore, S., Heinrichs, J. (2014, July 18). An in vitro culture model to study the dynamics of colonic microbiota in Syrian golden hamsters and their susceptibility to

infection with Clostridium difficile. Retrieved October 02, 2020, from https://www.nature.com/articles/ismej2014127

8. “Bacterial Growth Curve.” *Orbit Biotech*, Zodiac Brand Space Pvt. Ltd., (16 Apr. 2018). Retrieved May 1, 2021 from orbitbiotech.com/bacterial-growth-curve-generation-time-lag-phase-log-phase-exponential-phase-decline-phase/.
9. Moore, J. H., Salahi, A., Honrado, C., Warburton, C., Warren, C. A., & Swami, N. S. (2020, July 21). Quantifying bacterial spore germination by single-cell impedance cytometry for assessment of host microbiota susceptibility to Clostridioides difficile infection. Biosensors and Bioelectronics. Retrieved May 6, 2021 from https://www.sciencedirect.com/science/article/abs/pii/S0956566320304346?via=ihub.
10. Holmes, D., & Webb, B. (n.d.). Electrical Impedance Cytometry. Retrieved October 02, 2020, from https://link.springer.com/referenceworkentry/10.1007/978-90-481-9751-4_122
11. Howerton, A., Ramirez, N., Abel-Santos, E., 2011. Mapping interactions between germinants and Clostridium difficile spores. J. Bacteriol. 193 (1), 274–282.
12. Clostridium difficile-associated disease. (n.d.). Retrieved October 02, 2020, from https://microbewiki.kenyon.edu/index.php/Clostridium_difficile-associated_disease

Supplemental Materials and Figures

Table S1. Components of the Anaerobic Coculture System. A table of the materials used to create the anaerobic coculture system.

Part	Quantity
4 Port Elveflow Reservoir Size Small	1
0 - 345 mbar Fluigent Flow EZ Regular	1
Power Supply Kit for Flow EZ	1
Mechanical Regulator and Air Filter for Flow EZ	1
Thermo Scientific Heating Block	1
Heating Block 15 mL Tube Holder	1
2 MPa Zaiput Back Pressure Regulator	1
1/16" OD Microfluidic Tubing	1
3/32" ID Peristaltic Tubing	1
1/4 - 28 to 3/32" Barb Fitting	3
1/4 - 28 for 1/16" Fitting	2
Plug for 1/4 - 28 Port	1
Anaerobic Gas Tank Refills	1
Gas Tank Regulator	1

Figure S2. Set-up for Testing the Function of the Upstream Pressure Regulator. A closed system was utilized to understand the functioning of the upstream pressure regulator.

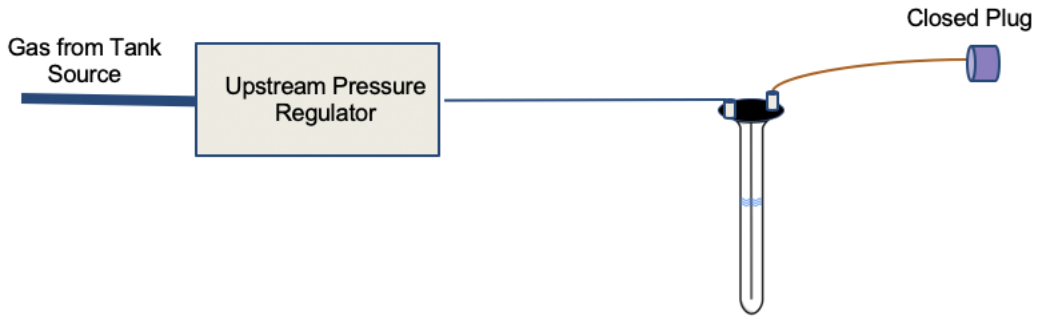

Figure S3. 7 Micron Impedance Cytometry Data Collected Using the Standard Sampling Set-Up. Previous experiments collecting data on 7 micron beads are consistent with the 7 micron bead data collected with the anaerobic system sampling set-up.