Measuring the Growth of a Single Bacterium Encapsulated within a Droplet of Growth Media Suspended in Oil in a 3D Printed Microfluidic Devices

Brigham Young University

Department of Chemical Engineering

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Project Purpose:

The purpose of my Honor's thesis project is to monitor the growth rate of a single bacterial cell in a droplet of growth media in oil within a droplet-forming microfluidic device.

Project Importance:

Today, antibiotic-resistant bacteria are a growing concern for public health.[1] Individuals who contract bacterial blood infections need immediate identification of the antibiotic resistance of the bacteria so the appropriate antibiotic regimen can be administered. Unfortunately, it can take up to 72 hours to produce the needed results.[2] Due to the need for rapid treatment, but lack of rapid identification, doctors often can only guess what type of bacteria is causing the infection, which can lead to inaccurate prescriptions, higher morbidity and mortality rates, and the development of bacteria with greater antibiotic resistance. These issues have motivated scientists to consider other methods for quickly identifying the antibiotic resistance of bacteria, one of them being the use of microfluidic devices as assays for antibiotic susceptibility tests. Microfluidics is a promising solution due to its low cost and material consumption, reliability, and potential ability to analyze the antibiotic resistance of bacteria at low concentrations (as is

typical with human blood samples). This could decrease the waiting time for test results through rapid and accurate tests, and thus save lives.

Recent studies at Brigham Young University (BYU) have focused on developing a microfluidic device that is 3D printed using poly(ethylene glycol) diacrylate (PEGDA) resin. The device encapsulates bacteria within aqueous droplets floating in oil as an assay for bacterial growth analysis. For these tests to be successful, bacterial growth must occur inside the droplets, since it is the key feature being measured. Yet in previous studies in our lab, bacterial growth in droplets was difficult to reproducibly achieve.[3] In this work, I aim to understand the requirements for growing *Escherichia coli* bacteria in an aqueous nutrient droplet in oil within a 3D printed PEGDA microfluidic device and develop procedures for accurately measuring that growth.

Project Overview:

Introduction and Background:

By 2050, it is estimated that antibiotic resistant bacteria could cause up to 10 million deaths annually if no action is taken to combat them.[4] There are many factors which contribute to the rise of antibiotic resistant bacteria, such as the use broad-spectrum antibiotics.[1, 5] Broad-spectrum antibiotics are those often prescribed while physicians are waiting for results from an antibiotic susceptibility test (AST), which can currently take anywhere from 24 to 72 hours.[2] To reduce the use of broad-spectrum antibiotics and the increasing mortality rates of bacterial infections, scientists are looking for ways to perform rapid ASTs that can help determine the correct prescription for treating a bacterial infection in a matter of hours rather than days. A promising technology for creating new rapid ASTs is the use of microfluidic devices (MFDs).

There are many kinds of MFDs that have been designed for performing ASTs, including MFDs that can form droplets of one fluid in a second immiscible fluid, such as water droplets in oil. For example, Warr et al. developed a device that could autonomously detect and encapsulate a single bacteria cell in an aqueous droplet surrounded by oil that was formed using pneumatically controlled digital microvalves. They reported growth within 4 hours.[3] Akuoko et al. developed a polydimethylsiloxane (PDMS) device that captured an average of 2 bacteria cells per droplet and reported a growth detection time of 30 minutes using fluorescence.[6] Ito et al. reported the development of a system that sustained bacterial growth in a single "culture" droplet by repeatedly merging and separating new droplets to and from the culture droplet.[7] Kaushik et al. detected bacterial growth in 20 pL droplets after one hour using a resazurin-based fluorescent growth assay.[8] In 2022, Hsieh et al. developed an AST assay that traps single bacterium in picoliter-scale droplets, and was able to detect growth from urine samples in 30 min using fluorogenic peptide nucleic acid probes.[9] Jakiela et al. demonstrated a microfluidic platform capable of forming droplets that serve as chemostats for long-term bacterial studies.[10] Lei et al. developed a droplet-microarray system for the growth analysis of *Pseudomonas aeruginosa* in nanoliter-sized droplets using a novel colorimetric readout method based on fluorescence.[11] In 2021, Qiu, W. and Nagl, S. created a PDMS MFD with a built-in optical oxygen sensor film to monitor bacterial growth based on the consumption of extracellular dissolved oxygen by *Escherichia coli* (*E. coli*) in droplets as an AST.[12] While these works represent great progress in improving droplet-forming microfluidic assays for ASTs, more work can still be done to enhance the flexibility, reproducibility, and simplicity of these devices.

Recent work at BYU has been focused on developing a droplet-forming microfluidic system that can obtain AST results in less than 4 hours. This is a novel and complex system that

can autonomously detect a single bacteria cell from a stock solution and encapsulate it in an aqueous droplet in oil. The system (called the Droplet-on-Demand, or DoD system) is based on a complex MFD which is viewed with a camera to provide computer image recognition and feedback to a series of pneumatically controlled microvalves to direct the flow of an aqueous stream and an oil stream and form aqueous droplets within the oil stream. The device is 3D printed using a custom 3D printer that was built by the Nordin lab group from the BYU Electrical and Computer Engineering Department.[3] Fabricating MFDs with 3D printing allows for much more complex designs that can have a wider range of features and thus applications, such as microvalves and vertical channels. The Nordin lab group has done extensive work to advance this technology.[13-25] In the BYU Pitt lab, we were able to use this technology to 3D print the complex MFD used in the DoD system. The device is made of a photosensitive resin containing 98.62% poly(ethylene glycol) diacrylate (PEGDA) monomer, 1% Irgacure 819 photoinitiator, which has an absorbance spectrum of 360-400 nm, and 0.38% avobenzone UV absorber, which absorbs light at wavelengths less than or equal to 360 nm.[15, 26] This resin composition is unique compared to typical droplet forming MFDs that are only 2D (all features are contained in a single plane) and made of glass, PDMS resin, or other polyurethane-based resins.[7-9, 11, 27, 28] I will use this DoD system during my study for measuring bacterial growth in aqueous nutrient droplets suspended in oil.

A critical feature of the DoD system is the measurement of bacterial growth in the aqueous droplets formed within the MFD. Bacterial growth demands very specific environments that include the correct oxygen concentration, temperature, and pH, along with a substantial food source and the absence of toxins. The DoD system was designed for use in rapid ASTs, but it was found during testing that bacteria don't grow well in droplets formed in the complex MFD: the

growth was inconsistent and insufficient for producing a strong, easily measurable fluorescent signal.[3] My research hypotheses are, based on our knowledge of the growth behavior and requirements of *E. coli*, that 1) a harmful substance is diffusing from the MFD into the aqueous droplets and killing the bacteria, and/or 2) there is not enough oxygen inside the oil surrounding the aqueous droplets to sustain bacterial growth. This study will investigate what is preventing the bacteria from growing at normal rates and will then develop a procedure that enables the growth of *E. coli* in aqueous nutrient droplets in oil in an MFD made with PEGDA resin.

Project Objectives:

The main objective of this project is to measure the growth of bacteria in an aqueous droplet in oil in a complex MFD (See Figure 1). To reach this objective, my two supporting objectives are 1) to reduce the cytotoxicity of my MFD, and 2) to use the complex MFD to form aqueous droplets in a perfluorocarbon oil containing sufficient oxygen to sustain bacterial growth.

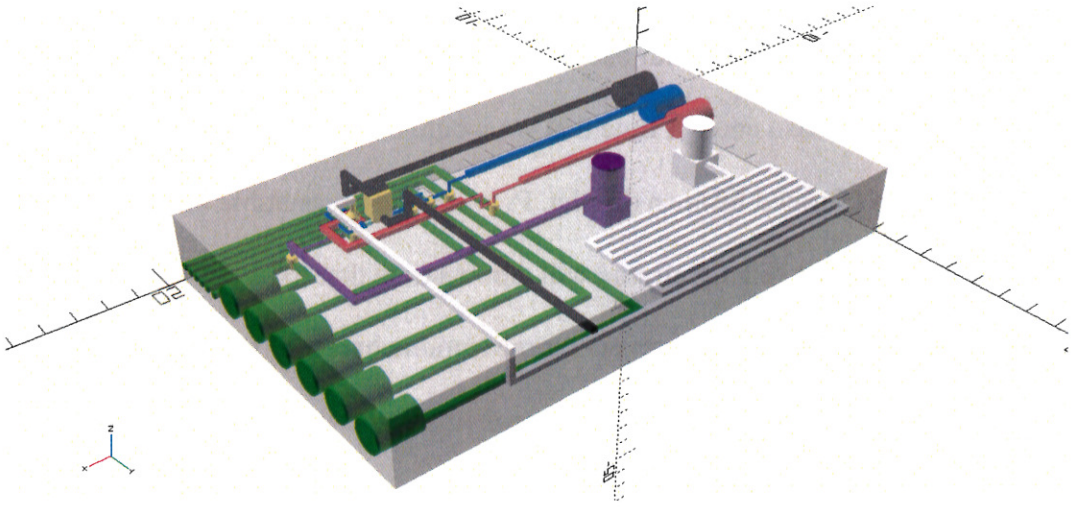


Figure 1: 3D view of the complex MFD designed for the DoD system that is operated via digital pneumatic valves to generate aqueous droplets in oil. Each green line is a pneumatic line, while the red line is the oil line, the blue line is the aqueous line, the black line is a second oil line to increase optical clarity of bacterial cell imaging, the purple line is a waste fluid line, and the white line is a serpentine channel designed to hold the aqueous droplets encapsulating the bacteria during growth measurements. The small yellow cylinders represent the digital pneumatic valves used for fluid control and droplet generation.

Research Plan:

To confirm my hypothesis, I first plan to determine the cytotoxicity of each component in the resin used to print the device. For all my experiments throughout this project, I will use *E. coli* which have a plasmid coding for green fluorescent protein. For this experiment, the bacteria will be grown in nutrient broth (NB) solutions containing the saturation concentration of each resin component. Their growth will be measured in a 96-well plate using a BioTek Synergy H1 Hybrid Reader (plate reader) over a 12-hour period, and the cultures with the most growth will be the least cytotoxic. By comparing the growth between cultures, I will determine which resin component is the most cytotoxic, which will inform me as I investigate potential methods for reducing the cytotoxicity of a printed MFD.

For most growth experiments performed during this project, I expect to measure bacterial growth over a 12-hour period. I chose 12 hours because of the design of the DoD system. The DoD system encapsulates the bacteria in droplets and then monitors those droplets inside the complex MFD for a period that may be as long as 12 hours, which means this is the maximum length of time during which droplets with bacteria in them could be exposed to cytotoxic chemicals. Twelve hours is also enough time that we would expect to see growth in a normal bacteria culture that isn't formed inside a droplet. Proving that measurable bacterial growth occurs inside a droplet during this timeframe will demonstrate that the techniques I will develop overcome the current theorized issues preventing measurable bacterial growth in the droplets made inside the complex MFD, and so for all experiments involving bacterial growth I will monitor the growth over a period of 12 hours.

Once the cytotoxicity of the resin components is generally known, I will investigate how to reduce the concentration of those compounds that diffuse into the channels within the PEGDA

device after it is printed. I plan to experiment with how treating the MFD with heat, rinsing it with isopropyl alcohol (IPA), and exposing it to ultraviolet (UV) light reduces its cytotoxicity, and then optimize the treatment that proves most effective at removing cytotoxic compounds. I hypothesize that the heat or UV treatment can reduce the amount of toxins in a PEGDA-MFD by further polymerizing any raw resin components remaining in the MFD, thus removing cytotoxic compounds by converting them to another product. The rationale behind the IPA treatment is that a continuous flow of IPA through the MFD's channels over time will elute out any cytotoxic chemicals in the MFD that could diffuse into the channel during device operation. As the IPA flows through the channels of the MFD, the chemicals will diffuse into the IPA and then be dissolved (due to their solubility in IPA). The IPA then carries the dissolved chemicals out of the MFD, effectively removing the cytotoxic substances from the device so they do not diffuse from the channel walls into the water droplets and kill the bacteria.

To prove the effectiveness of these treatments, I will use an MFD that consists of a single two-layer serpentine channel (not the complex droplet-forming MFD; see Figure 2). Several devices will each be subjected to a different treatment and then filled with NB for 12 hours, which will then be removed from the MFDs after the 12 hours and mixed into a suspension of *E. coli* containing resazurin (a growth indicator) in a 96-well plate. The plate will then be loaded into the plate reader, which will monitor the *E. coli growth* for 12 hours. I posit that any cytotoxic compounds remaining in the MFD after the various treatments will diffuse into the NB, and if they are present in the NB at a high enough concentration, they will prevent the bacteria from growing. Thus, the culture with the most bacterial growth indicates which treatment is most effective at removing toxins.

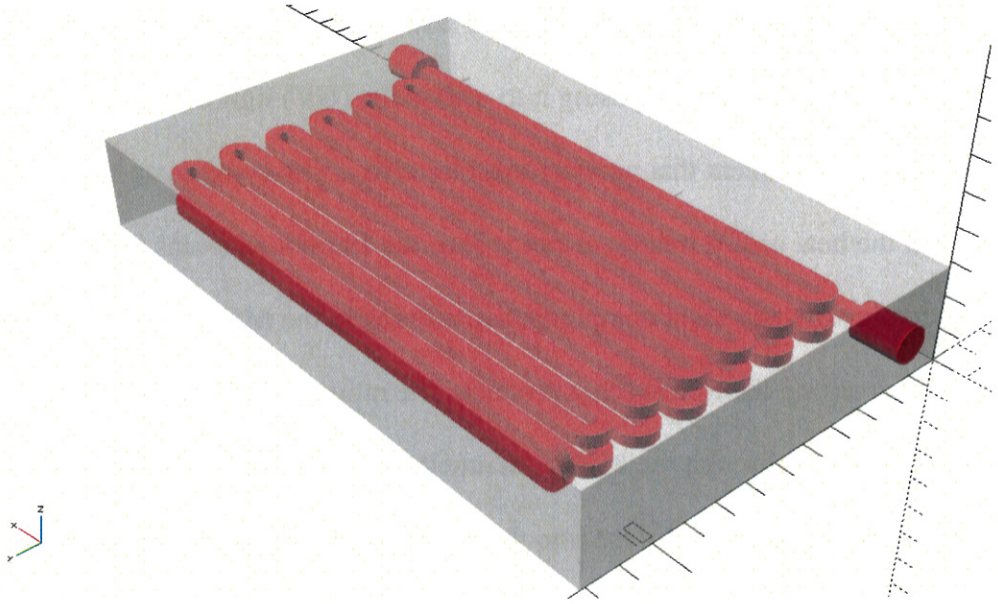


Figure 2: 3D view of an MFD consisting of a two-layer serpentine channel.

During the 12 hours of growth the plate reader will measure the fluorescent signal of the *E. coli*'s green fluorescent protein (GFP) and the fluorescence from resorufin in each well, giving me two different ways to measure cell growth. The GFP in each *E. coli* cell emits a green, fluorescent light when excited by a 485 nm wavelength of light. This allows us to count the number of bacteria at set time intervals and compare this number to a previous time to determine how much the bacteria have grown. Additionally, I will use resazurin, which is a metabolic fluorophore that slightly fluoresces until it is reduced into resorufin within a bacterium by unknown methods, although a current theory is that NADH (produced by bacteria during cellular respiration) is involved. Resorufin is highly fluorescent, and this signal is proportional to the metabolic activity and the number of living bacteria in a culture, which allows us to measure the growth by comparing how bright or intense the fluorescent signal is at various time points. The excitation and emission wavelengths of resorufin are about 570 and 580 nm, respectively.[3] This is outside the absorbance spectra of the photoinitiator and UV absorber used in the resin, so they should not affect the fluorescent signal produced by the resorufin. Resorufin is also nontoxic to

cells, making it suitable for use in long-term cultures.[29, 30] Thus, I will be able to deduce that the treatments resulting in the cultures with the highest GFP concentration and/or the strongest resorufin signal are the most effective treatments at reducing cytotoxicity.

After determining the best post-print treatment for bacterial growth, I will measure the bacterial growth in droplets formed in a T-junction MFD (See Figure 3) that has received the post-print treatment. A novel feature of the DoD system is its capability to reliably encapsulate 1 bacterium in a droplet with a diameter of 125 μm and a volume of 1 nanoliter (nL). To replicate this capture during my experiments, I will use the T-junction MFD to make aqueous droplets of a bacteria culture in HFE-7500 oil. The bacteria culture will be suspended in NB with a concentration of 10^6 CFU/mL, which equates to one bacterium per nL droplet. I will use HFE-7500 oil since bacterial growth in droplets surrounded by this oil has been reported previously.[6, 31] The results from this experiment will show whether the new treatment improves bacterial growth in droplets. The growth will be measured by taking fluorescence images of the droplets at different times during the growth cycle of the *E. coli* using a Nikon TE300 inverted microscope with a FLIR Blackfly S USB3 monochrome camera mounted on the microscope. The images will then be scanned using a computer system previously described to measure the grayscale pixel value.[3] A higher pixel value indicates greater fluorescence (resorufin production) and bacterial growth, and so I will be looking for each droplet in the post-print treated T-junction to have a higher pixel value than droplets in a T-junction with no post-print treatment. Using this method, I will complete my first primary objective and elucidate how much a post-print treatment can improve the growth of *E. coli* bacteria in droplets in PEGDA-MFDs by reducing the devices' cytotoxicity.

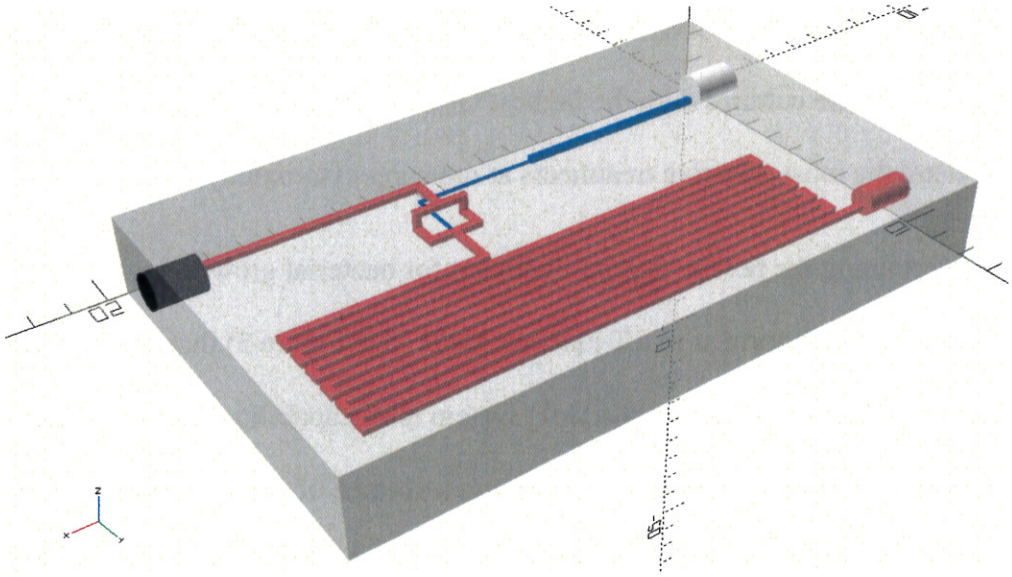


Figure 3: 3D view of a T-junction MFD. The blue line represents the aqueous line, and the red line represents the oil line until it is intersected by the blue aqueous line, which is where the aqueous droplets are formed in the oil. After this point, the red line represents the area which contains the aqueous droplets surrounded by oil.

My second primary objective is to grow bacteria in aqueous droplets suspended in perfluorocarbon (PFC) oil. Currently, a heavy mineral oil (a type of hydrocarbon oil with about 16 carbons in its molecular structure) is used as the oil phase when making droplets in the DoD system. When the DoD system was being developed, the heavy mineral oil was used, and so the DoD system is designed to operate with viscous oils. I theorize that this oil cannot hold enough oxygen to sustain bacterial growth in droplets and have found evidence of similar theories in the scientific literature.[12] The widely accepted practice in microfluidics is to use a PFC oil as the continuous phase (phase which constantly flows and surrounds the droplets formed of the second immiscible fluid) when encapsulating bacteria in aqueous droplets.[6, 8, 10, 28, 32-34] Hence, I plan to study the effect that different oils have on bacterial growth when bacteria are encapsulated in aqueous nutrient droplets suspended in oil, focusing especially on the effects of using hydrocarbon vs. perfluorocarbon oils. Different fluids can dissolve different concentrations of

oxygen, and so the ideal is to find an oil that has a high enough oxygen saturation value so that it will provide enough oxygen to permit bacterial growth in droplets.

There have been several studies concerned with measuring the oxygen concentration in different types of oils. For instance, a few novel methods for measuring the oxygen concentration in oils have been developed.[35-38] Besides this, Battino et al. reported the mole fraction of oxygen (O_2) in saturated hydrocarbons with 6-10 carbon atoms in their molecular chain at temperatures ranging from 243.15 K to 323.15 K. They measured the highest mole fraction for O_2 in n-decane to be 21.91×10^{-4} at 283.15 K.[39] Cuvelier et al. reported the oxygen concentration in dodecane and sunflower oil measured at the partial pressure equilibrium for different temperatures in the range of 5-50 ºC. The highest value found for dodecane was 130.3 mg/kg at 5 ºC.[40] Dias et al. reported the mole fraction solubilities of oxygen in three different fluoroalkanes at atmospheric pressure over a range of temperatures. They indicated that the end groups of the perfluorocarbons primarily dictate the solvation of oxygen.[41] Ding et al. reported the values for oxygen solubility in soybean oil and sunflower oil.[42] Jager et al. reported the oxygen solubility of water, perfluorodecalin, perfluorooctylbromide, and dodecafluoropentane (DDFP) as 6.3, 403, 527, and 29,421 mL_{O2}/L_{fluid}, respectively at 25 ºC (the value for DDFP was measured at 37 ºC).[43] Kubie measured the solubility in medical mineral oil at 24 ºC to be 0.134 ± 0.004 (units undeclared).[44] Ryu et al. showed that HFE-7500 is more oxygen soluble than tetradecane or other hydrocarbons.[45] Sklodowska and Jakiela measured the oxygen solubility in hexadecane, FC-40, FC-70, HFE-7200, and HFE-7500 to be 49.5, 51.9, 76.8, 64.4, 128.4, and >100 mL_{O2}/L_{fluid}, respectively at 1 bar pressure and various temperatures for each compound.[31] Vanhoutte et al. investigated the oxygen solubility of fluorine-functionalized ionic liquids, and reported that increased fluorination of liquids results in a higher oxygen solubility.[46] Wesseler et al. reported

the oxygen solubility of FC-75 and FC-47 to be 52.2 and 38.4 $mL_{O2}/100\ mL_{fluid}$, respectively at 25 °C. They also measured the oxygen solubility in many other fluorocarbon oils.[47] To view a graph comparing the before-mentioned data, see Table 1 below.

From Table 1, we can see that oxygen can have a mole fraction of around 25×10^4 in hydrocarbons, and around 45×10^4 in fluorocarbons. While there is a difference in the oxygen solubility between the different types of oils, it is only by a factor of about two. As this is not a significant difference, it appears that the lack of oxygen in the heavy mineral oil is not the only cause for the lack of growth previously observed in the DoD system.[3] This is why I will also be studying how to reduce the cytotoxicity of my devices, so that I can be sure that all factors affecting bacterial growth are addressed and optimized through my experiments. I expect that these experiments will help me know how much the bacterial growth is affected by using different oils when forming droplets.

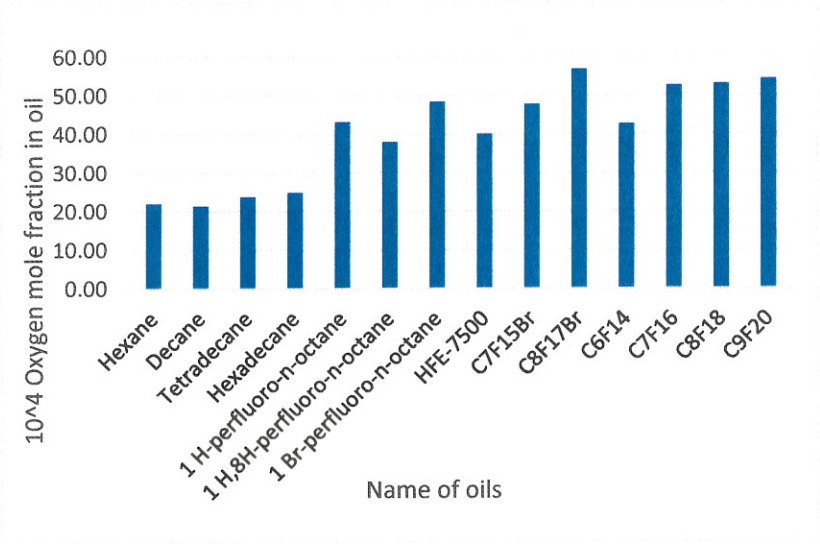


Table 1: Bar Chart comparing the mole fractions of O_2 found in different oils at 298.15 ± 1 K. The values shown were either reported by the sources previously mentioned in this proposal or in the Solubility Data Series published by the IUPAC-NIST.[48]

To remove external variables while measuring the bacterial growth in droplets in different oils, I will apply the post-print treatment method that I will have optimized to remove the

cytotoxicity from a PEGDA T-junction device (see Figure 3, pg. 10). After making droplets in the treated device, I will offload the droplets into a Teflon tube for measuring growth. This will prevent sustained exposure to any cytotoxic compounds remaining in the PEGDA devices after the post-print treatment and will enable me to measure the effect of different oils and their oxygen saturation on bacterial growth in droplets. If this strategy fails to produce quantifiable growth data, I will also consider making the droplets in a PDMS T-junction device, as this will completely remove all risk of cytotoxicity, but will introduce new variables such as the difference in oxygen permeability of PDMS compared to PEGDA. Despite this, I hope that I will still be able to compare the bacterial growth rate when using different oils based on the oxygen solubility in each oil. Some oils I initially hope to investigate are vegetable oil, heavy mineral oil, TMC FC-3283, Fomblin Y L-VAC 14/6, Galden HS 260, Gladen LS 200, and Novec 7500 PFC oil. I will also consider studying other oils if the results obtained from investigating these oils are unsatisfactory.

It would be helpful to know the oxygen concentration in each oil to be studied so that we could quantitively determine how much oxygen must be dissolved in an oil to sustain growth of *E. coli* bacteria in aqueous nutrient droplets. This would also be useful for future studies when determining whether an oil is or can be made suitable for use in droplet-analysis of growth of *E. coli*. Unfortunately, oxygen saturation nor oxygen concentration data are not available for all the oils intended for investigation within this study. If time permits, I will further investigate how to quantify these values, but in view of my main objective to measure bacterial growth it does not seem essential. The bacterial growth trends should be sufficient indication of whether there is adequate oxygen or not dissolved in the oils. To validate my anticipated experimental results, I will use the solubility of oxygen data available for some oils as found in the before-mentioned

literature to see if those data correlate with my anticipated experimental data when using the same or chemically similar oils.

Once I have determined a suitable oil for bacterial growth in aqueous NB droplets in oil, I will apply what I have learned to my main objective of measuring bacterial growth in droplets made in a complex MFD. I will first verify that the selected oil combined with the post-print treatment of the MFD does promote bacterial growth in droplets. This can be demonstrated using the T-junction MFD (see Figure 3, pg. 10). Depending on the oil selected, this may require that PEGDA-MFDs undergo some additional treatment to maintain good droplet formation, such as treating the device with fluoroacrylate when using a PFC oil.[49] This will be considered when evaluating the bacterial growth in droplets and the quality of droplet formation with the new conditions. The droplet quality will be measured by consistency of droplet-size (1 nL volume, ~125 μm diameter) and droplet stability over an extended time. After achieving bacterial growth in droplets under the new conditions, I will be ready to transition to experiments with droplet formation and bacterial growth in the complex MFD.

The complex MFD has a series of pneumatic valves that are essential for the proper functioning of this device (see Figure 1, pg. 5). Unfortunately, they are also very fragile and subject to frequent tearing or malfunction. This creates a concern of whether the new post-print treatment will negatively impact the functionality of the MFD's valves and prevent proper droplet formation. Hence before testing bacterial growth in droplets in the complex MFD, I will first have to test the droplet formation quality in the device after it has undergone the post-print treatment. This may require that some modifications are made both to the design of the complex MFD and to the post-print treatment process, but the goal will be to do so within an acceptable window of valve operation and measurable bacterial growth, if this is possible. Once I have

made any necessary modifications to the complex MFD to maintain droplet formation capabilities, I will be prepared to measure the bacterial growth in droplets in the complex MFD.

This final experiment will be conducted over a period of 12 hours. Using a bacteria-NB solution with a concentration of 10^6 CFU/mL, I will form droplets containing 1 bacterium per nL droplet in the complex MFD after it has received the developed post-print treatment and while using the newly selected oil. I will then monitor the droplets every 2 hours using the camera and microscope to take fluorescent images of the droplets and then measure the fluorescent intensity. Appropriate controls will be included as well. If the data indicates that over a 12-hour period there is an exponential increase in fluorescence, this will indicate that the bacteria is growing and that I have quantitatively measured it. It is possible that the method I will be using to measure the droplet fluorescence could have limited accuracy or sensitivity to the fluorescent signals.[3] If this is the case, I will have to investigate different methods for optically detecting bacterial growth using fluorescence. My main objective though is to indicate growth through measurement of the expected fluorescent signal, and so I propose that being able to measure an exponential increase in droplet fluorescence in the complex MFD by the end of 12 hours (or earlier) will be deterministic of successful bacterial growth.

Summary of Research Plan:

I propose that the herein contained research plan is an effective and time-efficient method to determine the factors affecting bacterial growth in aqueous NB droplets in oil in a complex MFD. I will first investigate the cytotoxicity of PEGDA-MFDs and if any interventional procedures are required to remove the cytotoxic chemicals. I will also investigate the suitability of different oils for use in such a system as the DoD system by measuring the bacterial growth in aqueous NB droplets in several oils, on the basis that different oils carry different oxygen

concentrations. I will then combine this new knowledge and use it to quantitatively measure the growth of bacteria in droplets in a complex MFD. Upon completing this project, this technology could advance the field of rapid AST diagnostics test so that patients can receive accurate prescriptions within hours of first entering the hospital rather than days, thereby reducing the mortality rate of antibiotic resistant bacterial infections and slowing the proliferation of these dangerous bacteria. After the completion of my project, further research could be done to enhance this AST technology such that it can be used against multiple types of bacteria to test several antibiotics at once. Further study could also be done to improve the measurement system for detecting bacterial growth and perfect the fabrication methods and design of the complex MFD so that it is more user-friendly.

I thank my thesis committee and the BYU Honors Department for their support and for considering my proposal, and I look forward to the opportunity to collaborate and contribute to this important work of getting more accurate antibiotic prescriptions to patients sooner.

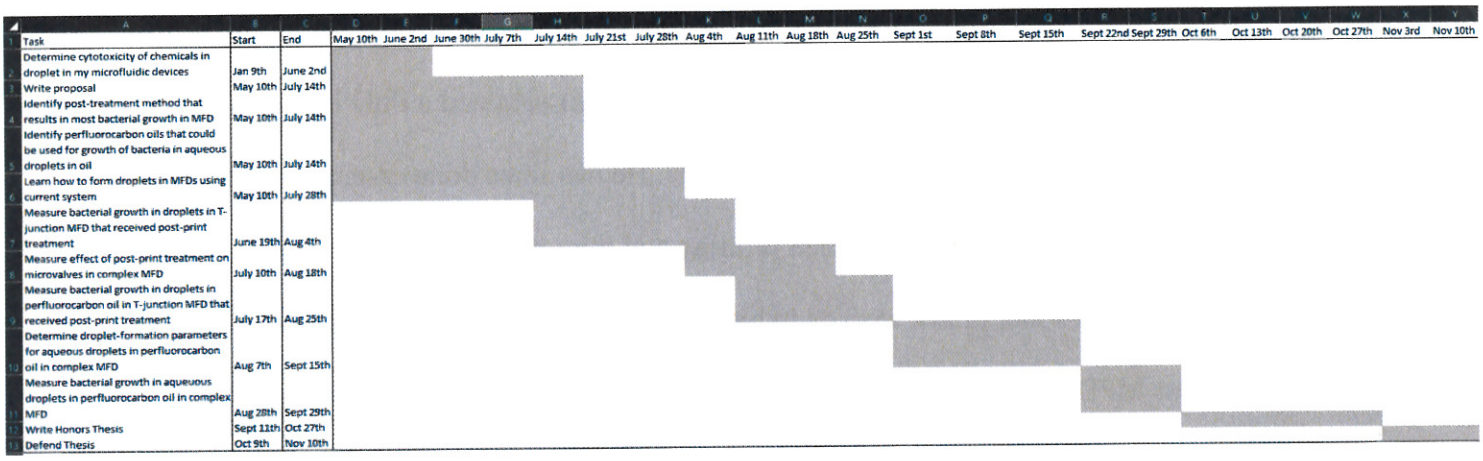
Qualifications of Thesis Committee:

My Faculty Advisor for this project is Dr. William Pitt, who is also the Chemical Engineering Honors Coordinator. Dr. Pitt earned his PhD at the University of Wisconsin. He joined the BYU Chemical Engineering Faculty in 1987 and has published over 184 peer-reviewed journal articles and obtained 9 patents. Lately Dr. Pitt has been conducting research to develop rapid diagnostic assays for testing antibiotics against bacteria. For the past year and a half, I have had the privilege to work in Dr. Pitt's lab and participate in several projects that were part of a graduate student's PhD dissertation. Dr. Pitt's previous research in material science, microfluidics, and diagnostics makes him very qualified to advise me on this project.

As Dr. Pitt is filling the role of both Honors Coordinator and Faculty Advisor, I have selected two Faculty Readers to serve on my thesis committee. The first will be Dr. Gregory Nordin from the Electrical Engineering Department. Dr. Nordin obtained a PhD in Electrical Engineering from the University of Southern California. He has since done extensive research to develop the technologies involved in 3D-resin printing and microfluidics. While I am not employed in Dr. Nordin's lab, I have worked in his lab frequently over the past one and a half years to make our MFDs using the custom 3D-resin printers he developed. I have also participated in his weekly lab group meetings from time to time to share the progress of my previous projects and to learn of the other developments happening within the lab from his students. Dr. Nordin's expertise will be valuable throughout the course of my project.

My second Faculty Reader is Dr. Ken Christensen from the Chemistry Department. Dr. Christensen obtained a PhD in chemistry from the University of Michigan in 1997, and then participated in two postdoctoral fellowships, one at the University of Michigan Medical School (1998-2002) and the other at the Harvard Medical School (2002-2004). His current research focuses on biochemistry and bioanalytical chemistry, for which he uses MFDs to grow and analyze mammalian cells. His background in microfluidics and cell viability in MFDs shows that he will be able to provide expert feedback on my thesis. I was recommended to request that Dr. Christensen be on my committee by Dr. Pitt because of his knowledge of cell growth in MFDs. I look forward to working with him and the rest of my committee throughout the duration of my research and am grateful for their willingness to serve on my Thesis Committee.

Project Timeline (Gantt Chart):



IRB Approval: N/A

Funding:

Item	Link	Cost
Teflon tubing (100 ft)	https://scicominc.com/ptfe-products/ptfe-tubing/	$36.00
Petri dishes	https://www.sigmaaldrich.com/US/en/product/sigma/p5856	$146.00
BD Difco NB	https://www.fishersci.com/shop/products/difco-nutrient-broth-general-purpose-medium/DF0003178?searchHijack=true&searchTerm=BD+234000&searchType=RAPID&matchedCatNo=BD+234000	$360.00
New mercury fluorescent lamp for Nikon TE300 inverted microscope	MicroscopeCentral	$145
PEGDA (500 mL)	https://www.sigmaaldrich.com/US/en/product/aldrich/475629	$157.00
Irgacure 819 (50 g)	https://www.sigmaaldrich.com/US/en/product/aldrich/511447	$218.00
Avobenzone (100 g)	https://www.tcichemicals.com/US/en/p/B3382	$138.00
Total		$1200.00

Culminating Experience:

After defending my thesis, I hope to share what I have learned with the scientific community. I plan to present my work at the 2024 BYU Biomedical Research Conference and the 2024 Annual Honors Conference. I also hope to present at the 2024 Rocky Mountain AIChE Regional Conference, whose date is to be announced. Finally, I plan to submit my work to be published in the *Micromachines* scientific journal.

References

1. CDC, *Antibiotic Resistance Threats in the United States, 2019*. 2019, U.S. Department of Health and Human Services: Atlanta, GA.
2. WebMD. *What is a Blood Culture Test?* 2022 [cited 2023 June 13th]; Available from: www.webmd.com/a-to-z-guides/what-is-a-blood-culture-test.
3. Warr, C.A., *Development of a 3D-Printed Microfluidic Droplet-On-Demand System for the Deterministic Encapsulation and Processing of Biological Materials*, in *Chemical Engineering*. 2022, Brigham Young University: Provo, UT. p. 136.
4. IACG, *No Time to Wait: Securing the Future From Drug-Resistant Infections*. World Health Organization.
5. WHO. *Antimicrobial resistance*. 2021 [cited 2023 June 13th]; Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
6. Akuoko, Y., et al., *Improving droplet microfluidic systems for studying single bacteria growth.* Analytical and Bioanalytical Chemistry, 2023. **415**(4): p. 695-701.
7. Ito, M., et al., *A Bacterial Continuous Culture System Based on a Microfluidic Droplet Open Reactor.* Analytical Sciences, 2016. **32**(1): p. 61-66.
8. Kaushik, A.M., et al., *Accelerating bacterial growth detection and antimicrobial susceptibility assessment in integrated picoliter droplet platform.* Biosensors & Bioelectronics, 2017. **97**: p. 260-266.
9. Hsieh, K., et al., *Combating Antimicrobial Resistance via Single-Cell Diagnostic Technologies Powered by Droplet Microfluidics.* Acc Chem Res, 2022. **55**(2): p. 123-133.
10. Jakiela, S., et al., *Bacterial Growth and Adaptation in Microdroplet Chemostats.* Angewandte Chemie-International Edition, 2013. **52**(34): p. 8908-8911.
11. Lei, W.X., et al., *Droplet-Microarray: Miniaturized Platform for High-Throughput Screening of Antimicrobial Compounds.* Advanced Biosystems, 2020. **4**(10).
12. Qiu, W.T. and S. Nagl, *Automated Miniaturized Digital Microfluidic Antimicrobial Susceptibility Test Using a Chip-Integrated Optical Oxygen Sensor.* Acs Sensors, 2021. **6**(3): p. 1147-1156.
13. Beauchamp, M., et al., *3D Printed Microfluidic Features Using Dose Control in X, Y, and Z Dimensions.* Micromachines, 2018. **9**(7): p. 326.
14. Esene, J.E., et al., *3D printed microfluidic device for automated, pressure-driven, valve-injected microchip electrophoresis of preterm birth biomarkers.* Microchimica Acta, 2022. **189**(5).
15. Gong, H., et al., *Custom 3D printer and resin for 18 μm × 20 μm microfluidic flow channels.* Lab on a Chip, 2017. **17**(17): p. 2899-2909.
16. Gong, H., A.T. Woolley, and G.P. Nordin, *High density 3D printed microfluidic valves, pumps, and multiplexers.* Lab on a Chip, 2016. **16**(13): p. 2450-2458.
17. Gong, H., A.T. Woolley, and G.P. Nordin, *3D printed high density, reversible, chip-to-chip microfluidic interconnects.* Lab on a Chip, 2018. **18**(4): p. 639-647.
18. Gong, H., A.T. Woolley, and G.P. Nordin, *3D printed selectable dilution mixer pumps.* Biomicrofluidics, 2019. **13**(1): p. 014106.
19. Haslam, B., et al., *Microfluidic reflow pumps.* Biomicrofluidics, 2015. **9**(4): p. 044104.
20. Nielsen, A.V., et al., *3D Printed Microfluidics.* Annual Review of Analytical Chemistry, Vol 13, 2020. **13**: p. 45-65.

21. Nordin, G.P., et al. *3D printing for lab-on-a-chip devices with 20 μm channels*. in *SPIE OPTO*. 2019. San Francisco, California, United States.
22. Rogers, C.I., et al., *Microfluidic valves made from polymerized polyethylene glycol diacrylate.* Sensors and Actuators B: Chemical, 2014. **191**: p. 438-444.
23. Rogers, C.I., et al., *Single-Monomer Formulation of Polymerized Polyethylene Glycol Diacrylate as a Nonadsorptive Material for Microfluidics.* Analytical Chemistry, 2011. **83**(16): p. 6418-6425.
24. Rogers, C.I., et al., *3D printed microfluidic devices with integrated valves.* Biomicrofluidics, 2015. **9**(1): p. 016501.
25. Sanchez Noriega, J.L., et al., *Spatially and optically tailored 3D printing for highly miniaturized and integrated microfluidics.* Nature Communications, 2021. **12**(1).
26. Warr, C., et al., *Biocompatible PEGDA Resin for 3D Printing.* Acs Applied Bio Materials, 2020. **3**(4): p. 2239-2244.
27. Cai, L.F., et al., *Droplet-based microfluidic flow injection system with large-scale concentration gradient by a single nanoliter-scale injection for enzyme inhibition assay.* Anal Chem, 2012. **84**(1): p. 446-52.
28. Christopher, G.F. and S.L. Anna, *Microfluidic methods for generating continuous droplet streams.* Journal of Physics D-Applied Physics, 2007. **40**(19): p. R319-R336.
29. Chen, J.L., T.W.J. Steele, and D.C. Stuckey, *Metabolic reduction of resazurin; location within the cell for cytotoxicity assays.* Biotechnology and Bioengineering, 2018. **115**(2): p. 351-358.
30. O'Brien, J., et al., *Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity.* European Journal of Biochemistry, 2000. **267**(17): p. 5421-5426.
31. Sklodowska, K. and S. Jakiela, *Enhancement of bacterial growth with the help of immiscible oxygenated oils.* Rsc Advances, 2017. **7**(65): p. 40990-40995.
32. Kao, Y.-T., et al., *Gravity-driven microfluidic assay for digital enumeration of bacteria and for antibiotic susceptibility testing.* Lab on a Chip, 2020. **20**(1): p. 54-63.
33. Kaushik, A.M., et al., *Droplet-Based Single-Cell Measurements of 16S rRNA Enable Integrated Bacteria Identification and Pheno-Molecular Antimicrobial Susceptibility Testing from Clinical Samples in 30 min.* Advanced Science, 2021. **8**(6): p. 2003419.
34. Zhang, P., et al., *A Cascaded Droplet Microfluidic Platform Enables High-Throughput Single Cell Antibiotic Susceptibility Testing at Scale.* Small Methods, 2022. **6**(1): p. 2101254.
35. Fakhri, A.B., et al., *Development of a Measure Cell to assess by Amperometry Dissolved Oxygen in Vegetable Oils.* European Journal of Lipid Science and Technology, 2023. **125**(4).
36. Feng, A., W. Cheng, and R.G. Compton, *Measuring the oxygen content of a single oil droplet.* Chem Sci, 2016. **7**(10): p. 6458-6462.
37. Newley, R. and P.B. Macpherson, *An oxygen electrode used to measure the oxygen content of oils.* Wear, 1977. **45**(3): p. 395-401.
38. Nunes Da Silva, I., et al., *Intelligent System for the Estimation of Gases Dissolved in Insulating Mineral Oil from Physicochemical Tests*. 2020, IntechOpen.
39. Battino, R., T.R. Rettich, and T. Tominaga, *The Solubility of Oxygen and Ozone in Liquids.* Journal of Physical and Chemical Reference Data, 1983. **12**(2): p. 163-178.

40. Cuvelier, M.E., et al., *Oxygen solubility measured in aqueous or oily media by a method using a non-invasive sensor.* Food Control, 2017. **73**: p. 1466-1473.
41. Dias, A.M.A., et al., *Solubility of oxygen in substituted perfluorocarbons.* Fluid Phase Equilibria, 2005. **238**(1): p. 7-12.
42. Ding, C., et al., *Experimental Study on the Solubility of O2 and N2 in the Liquid Oil at Low Pressure.* Advanced Materials Research, 2014. **908**: p. 145-152.
43. Jagers, J., A. Wrobeln, and K.B. Ferenz, *Perfluorocarbon-based oxygen carriers: from physics to physiology.* Pflugers Archiv-European Journal of Physiology, 2021. **473**(2): p. 139-150.
44. Kubie, L.S., *The Solubility of O_2, CO_2, and N_2 in Mineral Oil and the Transfer of Carbon Dioxide from Oil to Air.* Journal of Biological Chemistry, 1927. **72**(2): p. 545-548.
45. Ryu, G., K. Park, and H. Kim, *Interfacial properties of liquid metal immersed in various liquids.* Journal of Colloid and Interface Science, 2022. **621**: p. 285-294.
46. Vanhoutte, G., et al., *Fluorine-functionalized ionic liquids with high oxygen solubility.* RSC Advances, 2018. **8**(9): p. 4525-4530.
47. Wesseler, E.P., R. Iltis, and L.C. Clark Jr., *The Solubility of Oxygen in Highly Fluorinated Liquids.* Jounral of Fluorine Chemistry, 1977. **9**(2): p. 137-146.
48. Miyamoto, H., Y. Yampolski, and C.L. Young, *IUPAC-NIST Solubility Data Series. 103. Oxygen and Ozone in Water, Aqueous Solutions, and Organic Liquids (Supplement to Solubility Data Series Volume 7).* Journal of Physical and Chemical Reference Data, 2014. **43**(3).
49. Warr, C.A., et al., *Surface Modification of 3D Printed Microfluidic Devices for Controlled Wetting in Two-Phase Flow.* Micromachines, 2023. **14**(1).