

Rapid Separation of Bacteria from Blood Using
Surface-Modified Magnetic Nanoparticles for Sepsis Diagnosis

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A senior thesis submitted to the faculty of
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Bachelor of Science

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ABSTRACT

Rapid Separation of Bacteria from Blood Using Surface-Modified Magnetic Nanoparticles for Sepsis Diagnosis

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Bacterial septicemia is one of the leading causes of death in the United States. According to the CDC, over 26,000 patients died from septicemia in 2014 alone. Current diagnostic methods require anywhere up to 72 hours to identify the specific bacteria causing the full-body septic response. We are developing a method to reduce that critical time period to 2 hours by separating bacteria from blood using surface-modified magnetic nanoparticles (MNPs). The MNPs are modified with bis-Zn-DPA, a synthetic ligand that binds to both gram-positive and gram-negative bacteria. Two strains of gram-negative and two strains of gram-positive bacteria are used to prove that this method can be used to isolate any bacteria present in blood. The research is still in progress, but high bacteria capture percentages have been achieved.

Keywords: sepsis, nanoparticles, bacteria, E. coli, blood, bis-Zn-DPA

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Contents

Table of Contents	iv
List of Figures	v
1 Introduction	1
1.1 Sepsis	1
1.2 Surface-Modified Magnetic Nanoparticles	3
1.3 Microfluidic Devices	4
1.4 Work at BYU	5
1.5 Overview	5
2 Procedural Methods	7
2.1 Blood Acquisition and Preparation	7
2.2 MNP Surface Modification Characterization	8
2.3 Bacteria Culture to Quantify Capture	9
2.4 General Low Concentration Bacteria Capture Process	10
2.5 Magnetic Field Measurements	15
2.6 Filtration and Backwash Experiments	16
3 Results and Conclusions	17
3.1 Results and Analysis	17
3.1.1 Low Concentration Bacterial Capture in Blood	17
3.1.2 Parameters of Bacterial Release off Filter	21
3.2 Conclusions	22
3.3 Directions for Further Work	23
Bibliography	24

List of Figures

1.1	Sepsis process	2
1.2	Average sizes of bacteria, red blood cells, and magnetic nanoparticles	2
1.3	<i>Bis</i> -DPA-COOH used by J.J. Lee to capture bacteria from whole blood	4
2.1	SEM images of MNPs	8
2.2	In-house design of a steel magnetic rack	13
2.3	Magnetic field strength	15
3.1	Initial bacterial capture data	18
3.2	Bacteria capture with varying hematocrit by MNP amount used	19
3.3	MNP concentration effect on bacteria capture	20
3.4	Bacteria capture in buffer and plasma	20
3.5	Bacteria release by type of agitation	21
3.6	Bacteria release by flow rate	22

Chapter 1

Introduction

1.1 Sepsis

Bacteremia is a bloodstream infection caused by any bacteria present in the blood, which can quickly progress into sepsis—the body’s extreme response to the bacteria in the blood. Figure 1.1 details the sepsis process. Bacteremia can lead to a drop in blood pressure which restricts blood flow to essential organs. This can result in organ failure or death. Depending on the infectious organism, mortality rates are between 13-77% [1] and typically increases every hour the patient remains untreated.

Rapid identification of the bacteria causing the septic response is critical to effectively treating the patient. Current diagnostic methods of blood culture require up to 72 hours. The goal of this research aims to cut that time down to 2 hours.

The challenge in separating bacteria from blood comes from the relative sizes, as shown in Figure 1.2. There are 4-6 million red blood cells (RBCs) per milliliter of blood. The bacteria are 4 times smaller than the RBCs. Fewer than 10 colony forming units (CFU) per milliliter of blood can cause bacteremia [2], so searching for the bacteria is like searching for a needle in a haystack.

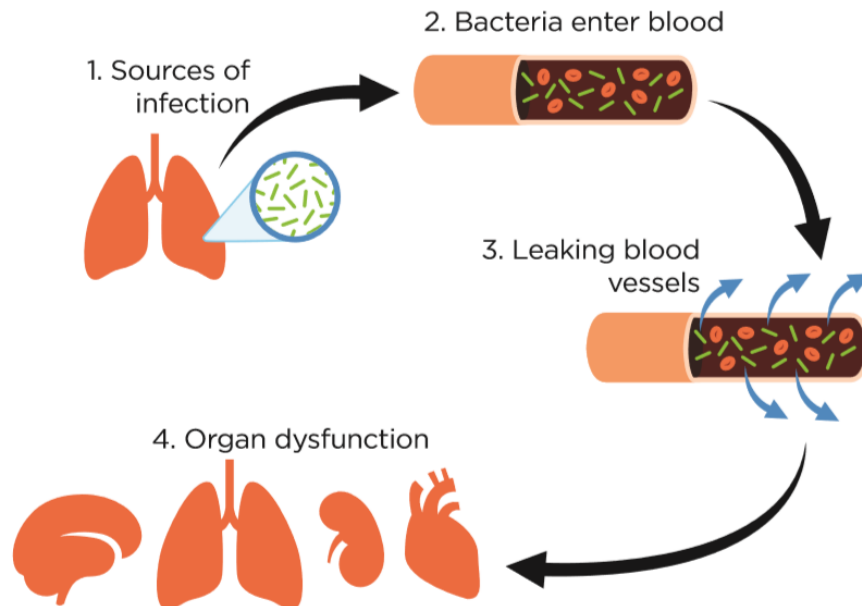


Figure 1.1 Diagram of how bacteremia leads to sepsis.

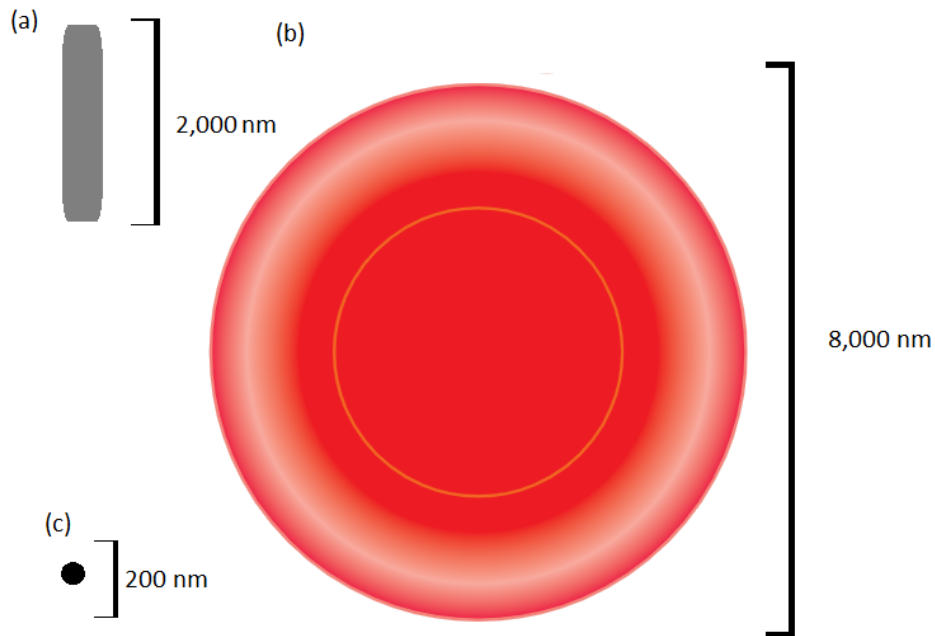


Figure 1.2 A diagram of the (a) average size of bacteria as compared to the average sizes of (b) a red blood cell and (c) a single biz-Zn-DPA coated magnetic nanoparticle. Image not to scale. "File:Diagram of a red blood cell CRUK 467.svg" by Cancer Research UK uploader is licensed under CC BY-SA 4.0.

1.2 Surface-Modified Magnetic Nanoparticles

For affinity capture of bacteria, we chose to modify iron oxide magnetic nanoparticles (MNPs) for a few reasons. First, we needed something that binds specifically to bacteria over all the other cells present in blood. Second, the method we use has to be general enough to capture both gram-negative and gram-positive types of bacteria. In this second case, both types of bacteria have anionic phospholipids on their surfaces that the MNPs bind to. We obtained 4 species of bacteria from Dr. Richard Robison at BYU to test this method on: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*. These types of bacteria make up a large percentage of sepsis cases [1].

To ensure that our MNPs can stick to bacteria, we first modify their surfaces with the process described by Lee *et al.* [3]. *Bis*-dipicolylamine (DPA), shown in Figure 1.3, is a synthetic ligand that has a high affinity for anionic phospholipids. After obtaining the DPA from J.J. Lee, we attach the ligand to the MNPs by a polyethylene glycol (PEG) linker, which requires a 2-step synthesis. By attaching this ligand to our MNPs, we are able to ensure the coupling of bacteria and MNPs and pull the bacteria out of blood by using a magnet. Christena Bentley focused on the MNP synthesis for the group.

The majority of the data presented uses the *bis*-DPA coated MNPs, but we tested two other kinds of surface-modifications: PSVue® attached to streptavidin-coated MNPs on both 1 μm and 100 nm sized MNPs, as well as Apolipoprotein-H (ApoH) on 200 nm MNPs. The PSVue® was purchased from Molecular Targeting Technologies, Inc. and came unbound to the streptavidin-coated MNPs. The ApoH-coated MNPs were purchased through ApoH Technologies, Inc. The results from the ApoH and PSVue® capture experiments indicated that they did not perform as well as the *bis*-DPA coated MNPs.

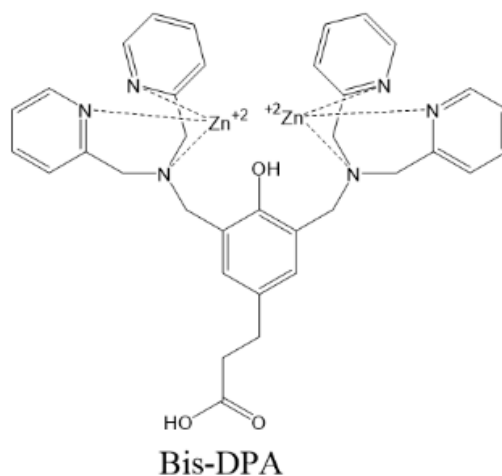


Figure 1.3 A diagram of the *Bis-DPA-COOH* used by J.J. Lee to capture bacteria from whole blood.

1.3 Microfluidic Devices

To capture spiked bacteria and MNPs from blood, Lee *et al.* used a microfluidic device and a magnet [3]. We wanted to expand on this idea and create a surface-modified microfluidic device that could selectively capture bacteria. We briefly explored the possibility of using a 3D printed filter structure to efficiently capture bacteria from blood.

Although not my area of research, the 3D printed filter project is relevant. The goal of that project was to chemically modify the surface of flat crosslinked poly(ethylene glycol) diacrylate (PEGDA) with a functional group that selectively binds to bacteria (e.g. polymyxin B (PMB) or DPA). Bacteria was captured with a 3D printed functionalized surface of PMB. The device was designed to have a grid structure of $18\ \mu\text{m} \times 20\ \mu\text{m}$ microfluidic flow channels. The inner volume was 0.0048 mL. After performing capture experiments with this device, Fischer Summers and Dr. Elisabeth Gates found problems with quantifying the large amounts of bacteria they were capturing.

This need to quantify bacteria capture leads to our current work and my thesis project. Section

1.4 details our planned improvements on the microfluidic device as well as the overall scope of my project.

1.4 Work at BYU

Instead of using the microfluidic device to only capture bacteria from blood, the goal is to 3D print a separate device to release the bacteria from the filter and remove the modified MNPs. My current research has focused primarily on releasing bacteria efficiently from a filter. The releasing protocol should also concentrate the bacteria into a small volume to be compatible with current molecular identification techniques. The target volume is in the μL range, specified as a single 'droplet' that contains a single bacterium and no modified MNPs. Chandler Warr, a graduate student in Dr. William Pitt's research group, has been working on a third microfluidic device to achieve this.

1.5 Overview

Before I joined the research group, Maren Skidmore, Wessley Call, Fischer Summers, Natalie Pugmire, and Dr. Gates started with a size-based separation of bacteria from blood by filtration with a fluorescence method to quantify how much bacteria they had captured from blood.

I joined the team in Fall 2017 when the group decided to move to a magnetic nanoparticle separation with a high concentration of bacteria and using a culture plating method to quantify how much bacteria they had captured from spiked blood.

In 2018, we wanted to see if we could get the MNP separation method to work with low concentrations, which were more realistic. Sepsis was found to have occurred when as few as 10 CFU/mL bacteria were present in blood [2]. To plate a low concentration of about 100 CFU/mL, we turned to vacuum filtration plating and a colony counting program to speed up the counting process. Ally Okolowitz, Christena Bentley, Kennedy Wandelt, Wessley Call, Fischer Summers, Dr. Gates

and I participated in these experiments.

Finally, in 2019, the group started collaboration with Dr. William Pitt and Dr. Richard Robison and changed focus from selectively capturing bacteria in blood to concentrating bacteria by efficient release from a filter into a small volume. Christena Bentley, Fischer Summers, Hope Callister, Robbie Bowers, Chandler Warr, and I have been working on different parts of the project.

The two goals of my research are to efficiently separate bacteria from blood using MNPs and effectively release bacteria off a filter. My thesis covers the methods used for the experiments in Chapter 2 and reports data and results in Chapter 3.

Chapter 2

Procedural Methods

This chapter includes the details of the acquisition and preparation of blood, MNPs, and bacteria, as well as experiment processes. Low concentration experiments of about 100-300 CFU/mL were performed with the goal of obtaining near 100% capture of bacteria from blood. The goal of the filtration and backwash experiments was to release bacteria off a filter with high efficiency.

2.1 Blood Acquisition and Preparation

Blood was obtained through healthy adult volunteers recruited on Brigham Young University campus. The only identification the researchers were given was the time the blood was drawn and the gender of the individual donating. Both descriptors were included as they were thought to impact bacteria binding. The time the blood was drawn was recorded in case clotting of the blood over time affected the results. The gender was included due to the red blood cell (RBC) count being higher in males than in females, thus resulting in a higher hematocrit in males. Blood from males has an average hematocrit of 37.0–49.6% and blood from females has an average of 31.3–44.3% [4]. Because of this difference, it was hypothesized that bacteria capture in male blood would be lower due to the generated steric hindrance.

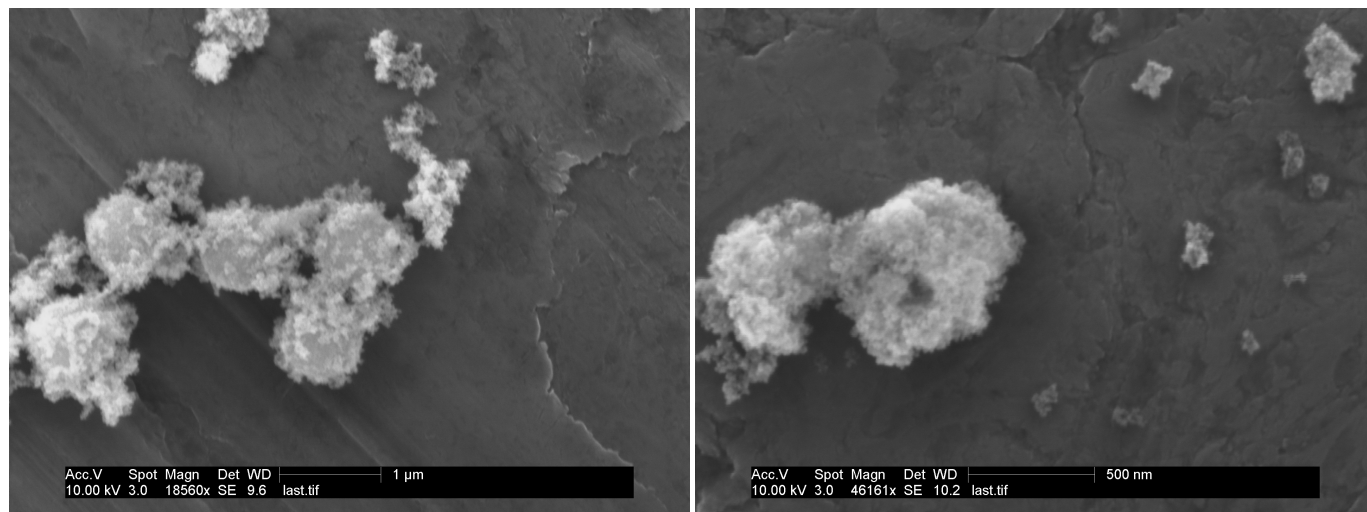


Figure 2.1 SEM images of (a) 1 μm magnetic particles coated with streptavidin and (b) 200 nm MNPs coated with ApoH. MNPs were diluted with water, the solution was dropped on a cleaned (with acetone and methanol) SEM stub, and dried.

Similarly, our early experiments using whole blood resulted in lower bacterial capture than diluted blood. Experiments were eventually standardized to use a dilution of 10% hematocrit, which was diluted manually with plasma after measuring the original hematocrit. More on this process can be found in Section 2.4.

2.2 MNP Surface Modification Characterization

To ensure that we had correctly modified our MNPs and to obtain approximate sizes, scanning electron microscope (SEM) images were used to image the modified MNPs. Figure 2.1 shows examples of images we obtained of MNPs coated with streptavidin and ApoH. The sizes of the MNPs we obtained were consistent with the sizes reported by the manufacturers.

The size of all three MNP types were measured with the Zetasizer Nano. The Zetasizer Nano was used to perform both hydrodynamic size and Zeta potential measurements of batches of MNPs. Hydrodynamic size measurements included a polydispersity index (PDI), which was used to describe

the degree of non-uniformity of the particles. Zeta potential is defined as the potential difference between the dispersion medium and the stationary layer of fluid attached to the particle and is related to the colloidal stability of each batch of MNPs.

The problem with size and Zeta potential measurements with the Zetasizer was that repeated measurements were not consistent with each other. The sizes obtained for the MNPs were often many times higher than the supposed actual size. We knew the MNPs tended to clump and stick to each other but didn't know if our measurements were accurate. We continue to take Zetasizer measurements but still have to evaluate if this is an effective way of characterizing MNPs.

2.3 Bacteria Culture to Quantify Capture

To quantify the amount of bacteria that was recovered from blood, the group tested if the MNPs affected the growth of the bacteria. This allowed us to know if we were able to compare a control without MNPs to samples containing MNPs. No difference in formed colonies of green fluorescent protein expressing *E. coli* (GFP) and the BL21 strain of *E. coli* was observed when comparing bacteria grown without ApoH or PSVue® MNPs to bacteria grown in the presence of ApoH or PSVue® MNPs. We concluded that bacteria did not grow differently in the presence of our surface-modified MNPs and that we would use culture plating as an acceptable bacteria quantification method. It is known in the microbiology world that the best statistical range for colony counting is 30-300 CFU per poured plate [5]. We generally only included data that fell within this acceptable range. The process to prepare the culture plates for the experiment is as follows:

1. Prepare the culture plates by following the instructions on the bottle of agar to make a total of 1 L of solution split into 3 separate Fleakers.
2. Measure 7.66 g of agar powder and 333 mL of water and combine into clean Fleakers.
3. Autoclave the Fleakers containing the solution using the liquid cycle setting.

4. Cool the Fleakers by leaving them on the counter for 10-15 minutes.
5. Pour the solution into sterile petri dishes, only pouring enough to cover the bottom of each dish. After about 20 minutes, the solution will solidified. Flip each plate over so that it sat out overnight upside down. Flipping the plates prevents condensation from forming and dripping down onto the agar, potentially contaminating them. If the plates are not used the next day for an experiment, they can be stored in the fridge for up to 2 weeks.

2.4 General Low Concentration Bacteria Capture Process

This section describes the typical bacterial capture experiment process. More information is on the group Box account under the Nanogroup > Data and Weekly Reports > Bacterial Capture > SOPs folder.

Experiment Preparation

The day before the experiment, someone in the group would follow these steps to prepare for the experiment.

1. Inoculate bacteria around 5:00 p.m. using 1 mL nutrient broth.
2. Let the bacteria grow overnight inside an incubator set to 37°C.
3. Prepare and autoclave 1X phosphate-buffered saline (PBS) buffer, deionized water, the filtration manifold and filtration supplies, pipette tips, and glass test tubes.
4. Set out culture plates upside down.
5. The next morning at 7:00 a.m., reinoculate the bacteria with 3 mL nutrient broth.

Blood Preparation

Two hours after reinoculating the bacteria, these steps were followed to prepare the blood for the experiment.

1. Relocate blood samples from the fridge to a table and allow the blood to equilibrate to room temperature.
2. Set calculated amount of whole blood aside for blood dilution steps.
3. Use the capillary tubes to take hematocrit measurements.
4. Centrifuge the remainder of the blood and capillary tubes for 10 minutes in Dr. William Pitt's centrifuge.
5. Separate the RBCs from the plasma using the 1 mL pipettor.
6. Dilute the whole blood with plasma to obtain the desired hematocrit. The spreadsheet titled Hematocrit Dilution Calculations found in the Google Drive Bacteria Data > Data folder will give the amounts of blood and plasma needed for the dilution.

Estimating Bacteria Concentration using Spectroscopy

The next step in the experiment is to prepare the bacteria for the samples by estimating the concentration of the bacteria through the absorbance of the bacteria at 600 nm. Bacteria preparation was typically performed while the blood was in the centrifuge.

1. Prepare a UV-Vis cuvette with 3 mL nutrient broth for the blank measurement.
2. Remove 1 mL nutrient broth and replace it with 1 mL bacteria.
3. Mix the solution 10 times with the micropipettor before measuring the absorbance.

After measuring the absorbance, we diluted the bacteria to the target concentration 1×10^7 CFU/mL through a conversion factor that depends on the type of bacteria used. Each conversion factor was found by making an absorbance-concentration curve which was taken during the growth period of the bacteria. The dilution amount was calculated by the target concentration divided by the average absorbance times the bacterial constant in mL. Because the total volume was chosen to be 1 mL, 1 minus the amount of bacteria gives the volume of nutrient broth needed. The amounts of measured bacteria and nutrient broth were combined to dilute to a total volume of 1 mL. After the first dilution was created, serial dilutions were made down to 1×10^4 CFU/mL, which was the dilution used to spike into the samples.

Sample Preparation

After diluting the blood and bacteria, the next step is to create the samples. Christena Bentley or Dr. Gates would prepare the MNPs beforehand, so all we had to do was remove the MNPs from the fridge and allow the solution to equilibrate to room temperature before starting sample preparation. Sample preparation was performed as follows.

1. Add 0.5 mL of blood to a polypropylene test tube for each blood sample.
2. Add 0.5 mL of plasma to a polypropylene test tube for each plasma control.
3. Add 10 μ L of diluted bacteria into each sample and control to spike each with bacteria.
4. Vortex each sample and control for 5 seconds.
5. Add 0.5 mL PBS buffer to each sample and control.
6. Add 20 μ L of MNPs to each sample and control.
7. Vortex each sample and control for 5 seconds.
8. After all samples and controls are finished, place them on the shaker in the incubator for 30 minutes at 37°C at 600 RPM.

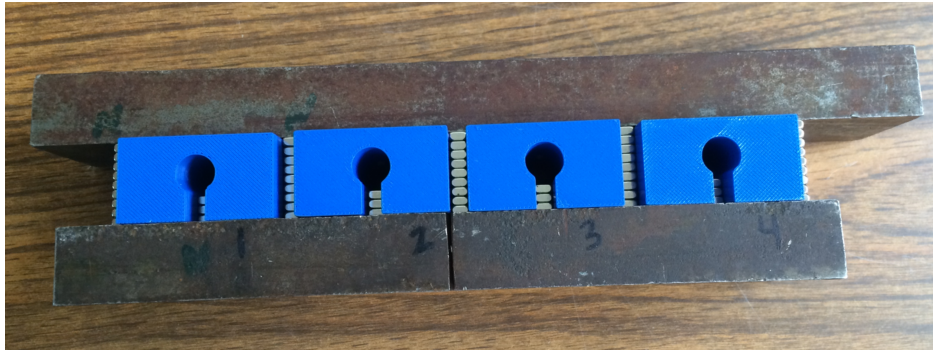


Figure 2.2 A picture of our first version of the steel magnetic rack with 3D printed separation inserts. The polypropylene test tubes sit snugly inside the circular opening where they are in close contact with the magnets.

Magnetization and Resuspension

After incubation, the next step is removing the samples and controls from the incubator and placing the samples in our custom magnetic racks with the caps loosened for 15 minutes. Figure 2.2 shows the in-house designed magnetic rack with four slots for the polypropylene test tubes to sit next to the magnet. The magnetic field strength measurements are described in Section 2.5. The controls were placed on the counter for 15 minutes. After the 15 minutes, the procedure to resuspend the samples was followed as described below.

1. While the tube is still on the magnetic rack, use a thin 2 mL serological pipette to remove the supernatant from the samples without disturbing the MNP pellet that formed on the side of the test tube.
2. Remove the tube from the magnetic rack and resuspend the pellet in 1 mL 1X PBS buffer.
3. Vortex each sample for 5 seconds.
4. Add 3 mL water to all samples and controls.

Vacuum Filtration

After magnetization and resuspension were performed, the next step is typically vacuum filtration. This section of the process was performed in the hood in the EB - B111 lab.

1. Use sterile tweezers to place 0.4 μm filters on the vacuum manifold.
2. Place a cup over the top of the filter and vacuum device.
3. Rinse the filter with 6 mL autoclaved water, then turned the knob on the manifold to allow suction.
4. After turning the suction off, pour a single sample or control onto each filter.
5. Rinse the container with 2 mL water and poured it onto the filter. Repeat this step.
6. Turn the knob to allow suction, and leave it on while rinsing the inside of the cup with 10 mL autoclaved water.
7. Remove the cup off the manifold and turn the suction off.
8. Using a different set of tweezers, pull the filter off the manifold and plate it onto an agar plate, making sure to not leave air bubbles in between the filter and agar.
9. Repeat the vacuum filtration steps until all samples and controls are filtered.
10. Place all plates in the incubator and leave overnight to grow.

Data Collection and Analysis

After the bacteria grew overnight, we took the plates out of the incubator and took pictures using an in-house designed culture picture box and uploaded the pictures to Box in the Nanogroup > Data and Weekly Reports > Bacterial Capture > 2020 Data > Culture Plate Pictures folder.

To count the colonies, Nick Morrill created a Python program called PyCFU that could identify and count the bacteria colonies. We had to manually go through and check that each 'colony' identified was actually a colony of the correct bacteria. The program is eventually supposed to learn what is a colony and what is not. However, we have yet to implement that feature yet as Nick is no longer working on the project. The results of these experiments are described in Section 3.1.1.

2.5 Magnetic Field Measurements

Magnetic field measurements were taken of the in-house magnetic rack referred to in Sec.2.4 because the force on the paramagnetic MNPs is dependent on the field gradient. Wessley Call and I used the Gauss meter available in Dr. Davis' lab to measure the magnetic field in intervals over a 1.5 cm distance of each slot of the magnetic rack, as shown in Figure 2.3. This was measured to know the variability in field strength between the different slots. The magnetic field levels decay exponentially for each slot. During the experiments, all magnetic interactions were performed as close as possible to the magnets as the field strength quickly dropped off as a function of distance.

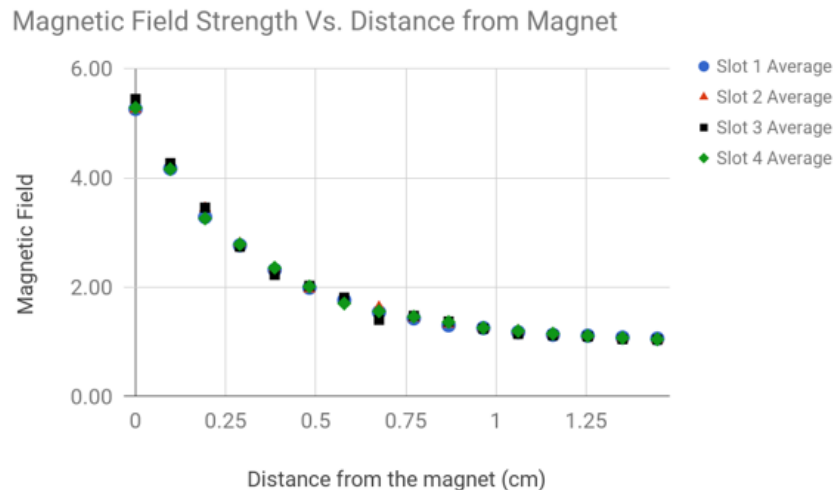


Figure 2.3 Magnetic field strength vs distance from magnets of each of the four slots of the in-house designed steel magnetic rack.

2.6 Filtration and Backwash Experiments

My current research is involved with these filtration and backwash experiments. These experiments were conducted to find out how to effectively release captured bacteria and attached MNPs off of a small filter in a manner that could be integrated with the process of releasing it into a small volume.

Fischer Summers and I performed the general bacteria capture experiment as described in Section 2.4 with a different filtration method. The capture medium was PBS buffer instead of blood, and the bacteria was diluted to 1×10^5 CFU/mL. After the PBS resuspension of each sample, instead of performing vacuum filtration, we filtered the solution of captured bacteria and attached MNPs through a polycarbonate filter with a pore size of $0.4 \mu\text{m}$ using a syringe pump. The filter was held in place by a 3D printed filter holder device made by one of Dr. William Pitt's students. The device had lure lock connections that enabled 1 mL syringes to attach to it on both ends. The filter was secured in place using an O-ring and the top and bottom of the device were secured together by an outer piece that screwed over the top. The size of the filter's pores ensured that free MNPs unattached to bacteria could get through the filter, but any bacteria or bacteria attached to MNPs could not get through and would be stuck on the surface. This procedure was the new filtration step.

The backwash step consisted of removing the attached syringes from the device and flipping the device around to prepare it for bacterial release. We then tried to release bacteria off the filter by using different methods: agitation of the liquid inside of the device by vibration or oscillation and using different flow rates to help push bacteria off the filter. Currently, we are testing if a magnet can pull captured bacteria and MNPs off the filter into PBS. The bacteria coming off the filter was captured in aliquots of $50 \mu\text{L}$, plated on culture plates, and left overnight to grow. The colonies were counted the next day. The results are described in Section 3.1.2.

Chapter 3

Results and Conclusions

After completing the experiments to determine if we could separate bacteria from blood, we obtained the Low Concentration Bacterial Capture in Blood data. The filter device experiments resulted in the Parameters of Bacterial Release off Filter data.

3.1 Results and Analysis

3.1.1 Low Concentration Bacterial Capture in Blood

For the low concentration bacteria capture experiments, the basic capture process was first tested. Our initial data is shown in Figure 3.1. Our capture in plasma (green dots) was higher than in female blood (purple dots), which was higher than our capture in male blood (red dots). These levels of capture were expected due to the nature of the higher hematocrit of male blood.

The next step was to determine exactly what variables contribute to the variation in the capture, or if it was just random, day to day variation. We began a series of experiments to isolate factors such as hematocrit, concentration of MNPs used, batch of MNPs used, incubation times, PDI, and MNP size.

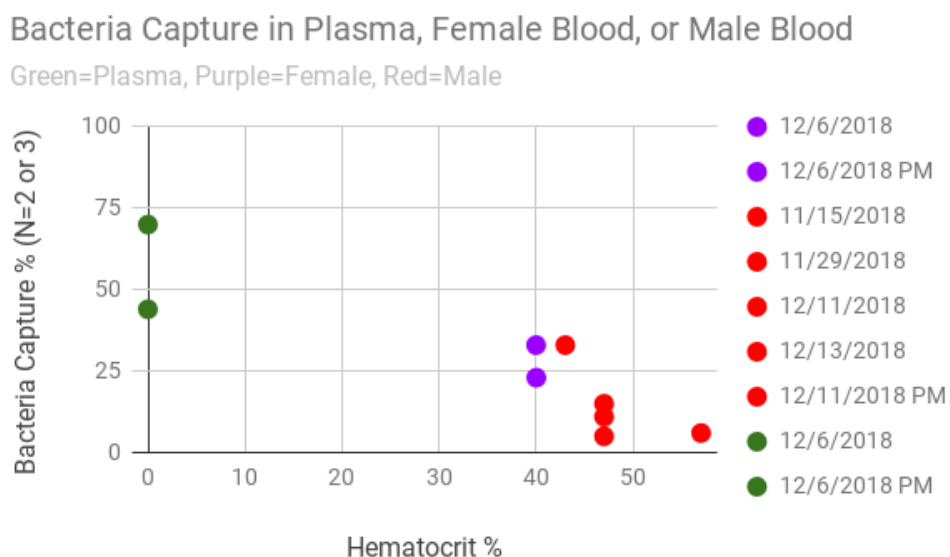


Figure 3.1 Initial bacterial capture percentage as a function of hematocrit percentage. N is the number of samples run on the given experiment date.

Hematocrit was experimentally found to have a large effect on bacteria capture. Figure 3.2 shows bacteria capture with varying hematocrit from 0 to 25% by batch of MNPs used. A large decrease in capture efficiency was observed as hematocrit percentage increased. These experiments were performed with DPA-coated MNPs. A capture percentage of 100% means that all the bacteria that we expected to spike into the sample was captured. Capture percentages higher than 100% were attributed to the nature of the variability of bacterial growth.

MNP concentration was also found to affect bacteria capture results. Figure 3.3 shows an increased percentage in bacterial capture at 40% hematocrit as MNP concentration increases. These experiments were performed with DPA-coated MNPs.

Capture might also vary by MNP batch. Figure 3.4 shows percentage bacterial capture from the different MNP batches. Blue dots represent experiments performed in a PBS buffer medium, red dots represent experiments performed in female plasma, and yellow dots represent experiments performed in male plasma. Capture percentages higher than 100% were caused by the nature of

the variability of the bacterial growth and were treated as full capture. No trend was found across this data, but looking at specific batches could help identify which particles performed badly and why they did so to avoid lower capture in the future. For example, though they had fewer data points, batches DA218 and DA219 had low capture rates in buffer (about 15%) compared to batches DA214 and DA215, indicating there might be a problem with the way batches DA218 and DA219 were made.

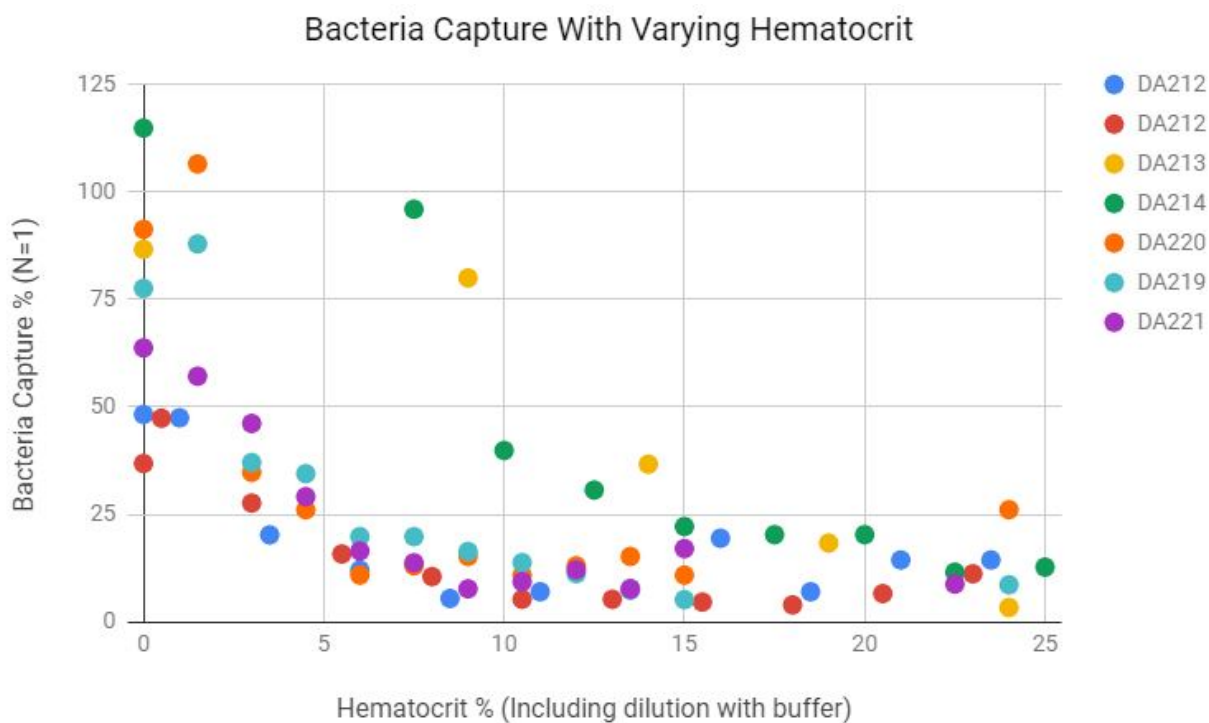


Figure 3.2 Bacteria capture percentage as a function of hematocrit percentage with color representing the batch number of MNPs used.

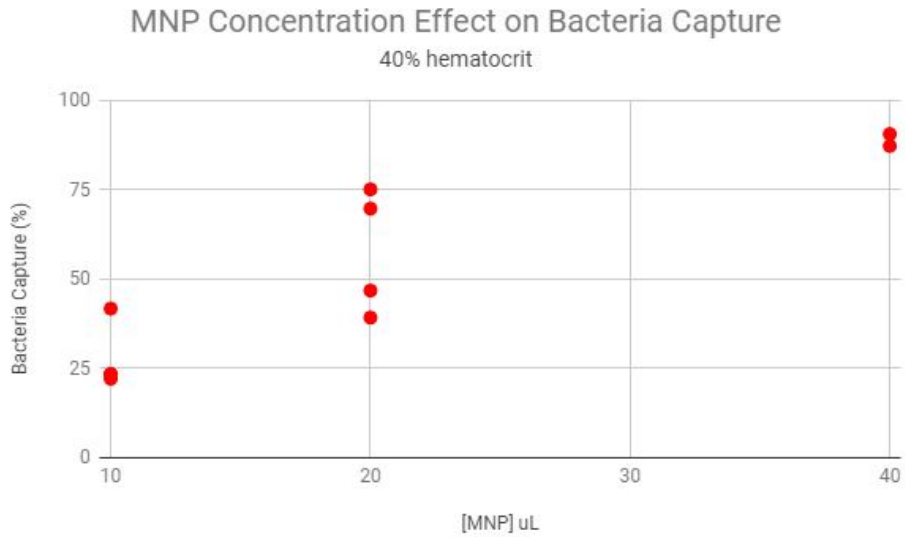


Figure 3.3 Bacteria capture percentage as a function of MNP concentration for samples of 40% hematocrit.

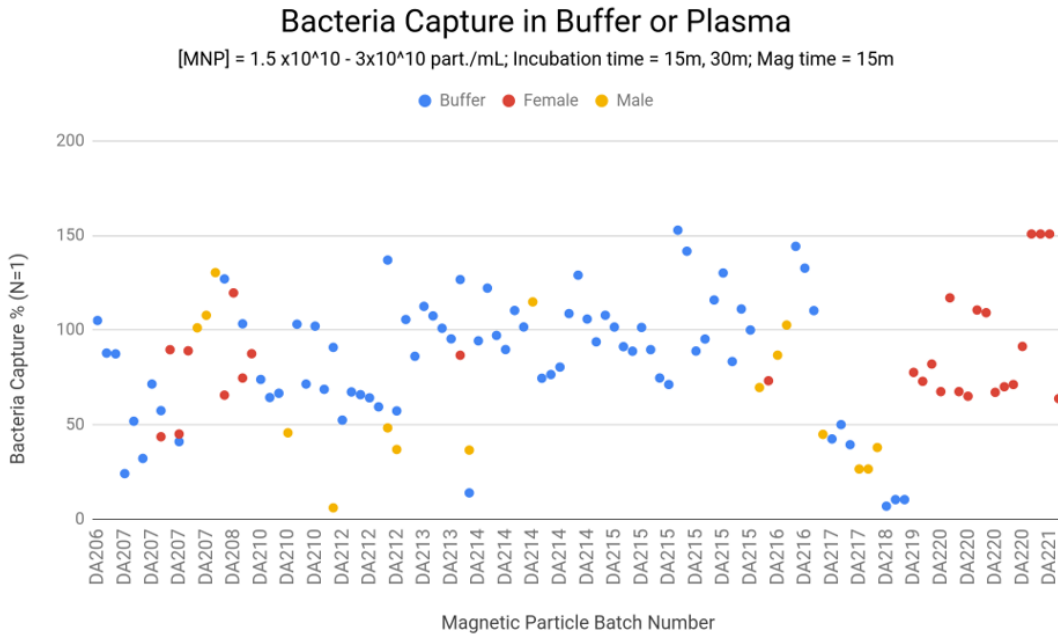


Figure 3.4 Bacteria capture in buffer and plasma by MNP batch number.

3.1.2 Parameters of Bacterial Release off Filter

Parameters that determined bacterial release from a filter included types of agitation and varying the flow rates. These parameters were tested by Fischer Summers and me with GFP-expressing *E. coli*. Figure 3.5 shows bacteria release by type of agitation. Agitation was hypothesized to prevent bacteria from sticking to the filter membrane or to more easily allow the bacteria to come off the membrane. Types of agitation tested were vibration, oscillation, and no agitation. Vibration was achieved by attaching cell-phone vibration motors to the housing of the filter holder device. Constant vibration applied vibration during both the filtration and backwash steps. All other types of agitation were applied during the backwash step only. The data shows that no significant difference in agitation on bacterial release was found.

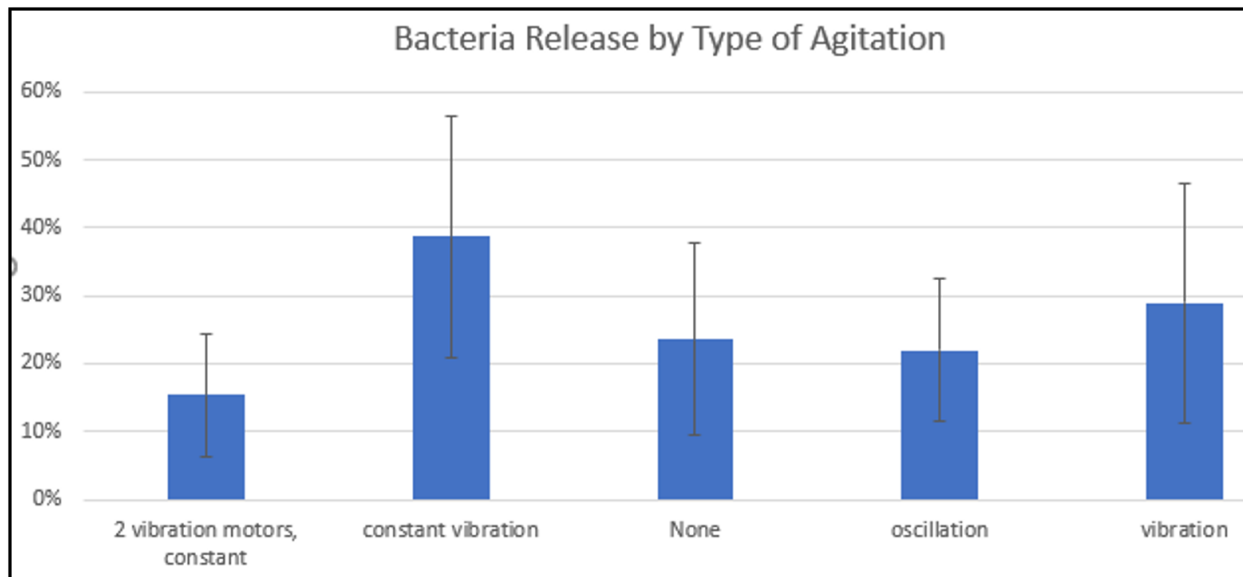


Figure 3.5 Agitation test results showing mean and standard deviation. The percentage of bacteria released for the five different methods of agitation.

Fischer and I also tested if a higher flow rate during the backwash step corresponded to a higher release percentage of bacteria off the filter. Figure 3.6 shows bacteria release by flow rate. These flow rates were achieved by changing the settings on the filter pump. The first five entries have

the same flow rate on the filtration and backwash steps. The variable 1.5-15 mL/min flow rate was 1.5 mL/min on the filtration step and 15 mL/min on the backwash step. It was hypothesized that a slower flow rate would allow the bacteria to gently stay on the membrane and that the faster backwash flow rate would then push the bacteria off the filter. The data shows no statistically significant difference between different flow rates. Bacterial release seems to be independent of both agitation and flow rate.

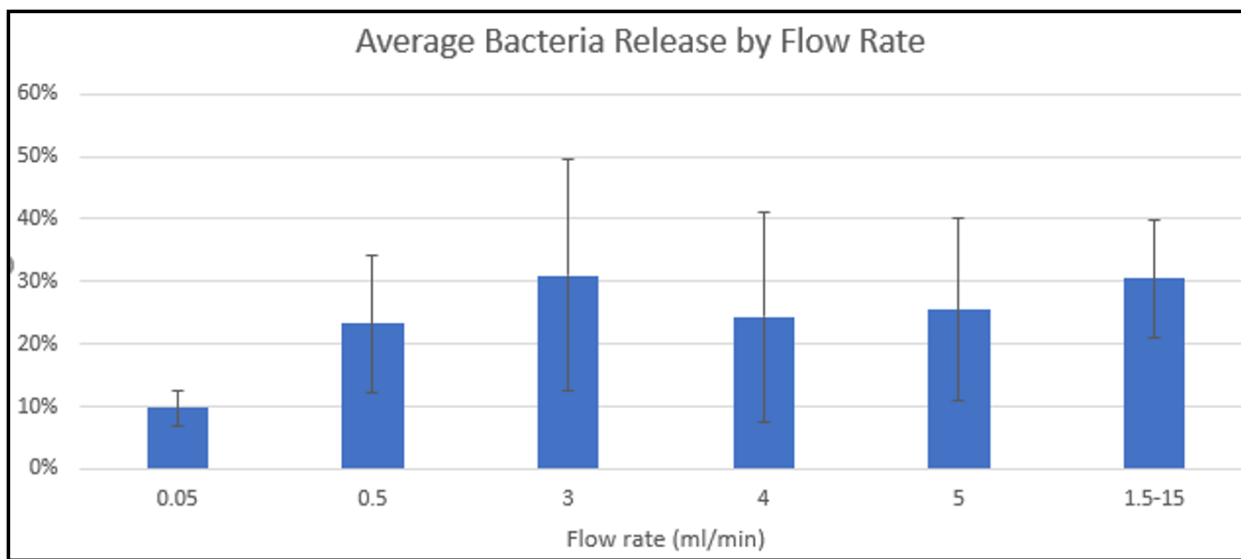


Figure 3.6 Flow rate tests showing percentage of bacteria by mean and standard deviation for five constant flow rates and one variable flow rate.

3.2 Conclusions

The main goals of the research project were to achieve a high bacterial capture percentage near 100% in blood and to achieve a high bacterial release percentage off a filter. This section will outline the findings of the research completed as well as the directions for future work.

We had aimed to achieve a bacterial capture rate near 100% in blood but did not achieve that consistently. Instead, we began focusing on what was limiting high capture, and found the factors

that affect the capture percentage. We started to test ways to improve our capture based on these findings, such as lysing the RBCs in the blood prior to performing the experiment, and standardizing the hematocrit of the blood to 10%. However, the project quickly switched focus from achieving high capture in blood to releasing bacteria off the filter.

We concluded that bacteria release off the filter was independent of agitation and flow rate, and that we needed to test a new method to release the bacteria. We designed an experiment to achieve this using our magnets to pull the bacteria and MNP complexes off the filter but have yet to complete it.

3.3 Directions for Further Work

Once the method to release bacteria off a filter is confirmed, we want to design and print a microfluidic device that we can release bacteria from a filter, detach any modified-MNPs from the bacteria, then release the bacteria into droplets containing 1 CFU per droplet. Those droplets can then be sent to a third party for rapid identification.

We will also have to go back to optimizing the bacteria capture from blood. Once we achieve near 100% capture with concentrations as low as 10 CFU/mL, we will be able to design a process that can separate bacteria from blood, release the bacteria and modified MNPs from a filter, detach the MNPs from the bacteria, and isolate the bacteria into small volumes for rapid identification for sepsis treatment.

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