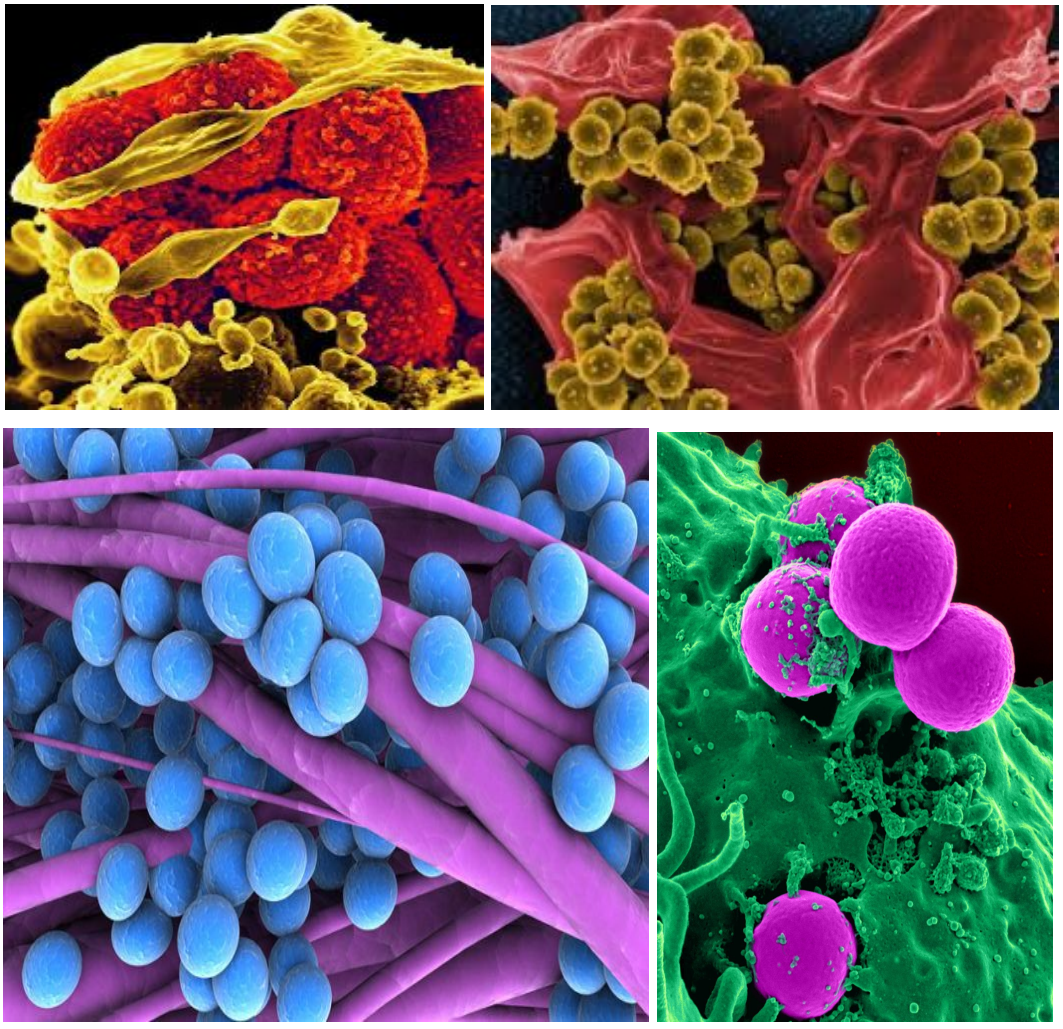
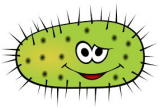
Preventing Nosocomial Central-Vein Catheter Infections: Effectiveness of Titania-Based Nanofiber Mats on MRSA

iCons 1 - Independent Case Study



Team I - Phoebe Lasic-Ellis, Hayley McIsaac, Simran Jeet, Sarah Kaunfer



I. Project Summary

Nosocomial infections are diseases that are acquired in hospitals. Among the most well-known -- and virulent -- infections is MRSA, or methicillin-resistant *Staphylococcus aureus*. This strain of *S. aureus* is capable of evading antibiotic treatment and is therefore extremely problematic for medical personnel. This bacteria is found on medical equipment such as polyethylene central vein catheters, which presents a unique threat since they act as an easy entry point for MRSA to be introduced into a patient's bloodstream. Antibiotic-resistant bacteria are inherently challenging to combat and have a high risk of becoming fatal to the patient, who is typically already weakened by other health issues. Therefore, it is crucial to find methods of treating MRSA that do not involve antibiotics to which *S. aureus* will inevitably become resistant. One promising method is the use of an antimicrobial coating that will, in theory, mechanically kill MRSA and other pathogens as they come into contact with medical equipment. Currently, polyethylene central vein catheters do not offer any protection against pathogens, meaning that central-line infections are only addressed after the patient has already become infected. An antimicrobial coating would ideally kill pathogens before they have the opportunity to enter a patient's body. This possibility begs the question: is it possible to lower the rate of MRSA incidence on polyethylene central-vein catheters by coating the surface in an antimicrobial material? There is research suggesting that titania-based nanofiber mats may be an effective antimicrobial coating for medical apparatus.

If an antimicrobial coating effectively functions to mechanically kill microbes because of its surface structure, then coating a polyethylene surface (to mimic a central vein catheter) with a polyurethane titania-based nanofiber mat will reduce the rate of MRSA incidence on a polyethylene surface.

By coating catheters with these titania-based polyurethane nanofiber mats, it is possible to prevent MRSA from colonizing that region and causing subsequent infection in humans.

II. Background

Most surgeries and medical procedures involve risk, but one of the most common is hospital-acquired infection. Hospital-acquired infections (HAIs) are a relatively well-known problem and are recognized by the Center for Disease Control and Prevention. HAI has been acknowledged by the CDC through its proposing state-by-state laws for prevention, as well as "gameplans" for prevention nationally. Despite these efforts, hospital-acquired infection still

affects many patients and requires more research to effectively combat[1]. Currently, certain tools and procedures, such as ventilators, needles, etc., are known for harboring antibiotic-resistant pathogens in hospitals. These pathogens, such as MRSA, exist almost exclusively in hospitals and are constantly exposed to powerful antibiotics, which leads to their rapid enhanced antibiotic resistance.

The focus of the research is on the niche area of nosocomial infections that occur as a result of central-vein catheter placement. Central-vein catheters provide doctors with a convenient way to deliver large volumes of necessary fluids, draw blood, and administer drugs simultaneously. The catheter itself is a highly trafficked area, for doctors and microbes alike, and can serve as an easy access point for microbes to enter into the bloodstream, causing bacteremia[2]. In 2011, catheter infections alone were responsible for 41,000 cases of bacteremia in the United States[3].

A new area of chemical engineering studies antimicrobial materials, which includes materials that may be able to prevent infections from catheters. Antimicrobial materials can have many mechanisms to eliminate microorganisms, including having the ability to release biocides, killing on contact, or immobilizing with cations[4]. The most promising potential application to MRSA is the titania-based polyurethane nanofiber mats, which were successful in eliminating wildtype *S. aureus*[5]. Currently, common hospital catheters are composed of silicon or polyurethane, neither of which possess inherent antimicrobial or bactericidal qualities[67]. These materials are essentially a blind spot within the field of disease prevention and are in desperate need of improvement. Therefore, the question remains: Is it possible to lower the rate of MRSA incidence on polyethylene central-vein catheters by coating the surface in an antimicrobial material? We believe that by layering catheters with these titania-based polyurethane nanofiber mats, it is possible to prevent MRSA from colonizing that region and causing subsequent infection in humans, without having to alter the current way central-vein catheters are made.

III. Methods

To study this theory, a laboratory study will be conducted to examine the nanofiber mats' ability to eliminate MRSA. A MRSA culture will be introduced to the mat and cell growth will be

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[1] "National HAI Targets & Metrics."
[2] Central Line-Associated Bloodstream Infections: Resources for Patients and Healthcare Providers (7 February 2011)
[3] *Infographic*. (2011)
[4] Kurtz, I.S.; Schiffman, J.D. (2018)
[5] Ibid.
[6] Marino, Paul L.
[7] Liu, L. C., and C. A. Siedlecki. (2016)

monitored. Currently, there is existing research on nanofiber mats with strains of wildtype *S. aureus* and other model organisms, however, none with MRSA[8]. Polyurethane will be used as a control substance, as it is the material a typical catheter is made of. The rate of cell growth will serve as a variable to be measured.

To measure the effectiveness of the titania-based polyurethane nanofiber mats in limiting the growth of MRSA, direct observation of the growth of cells over time under a microscope will work most effectively. To ensure the experiment is reliable and to reduce the probability of the data being skewed, three replicates of both the experimental (nanofiber mat layered over polyurethane) and control groups (polyurethane layer only) will be used. Additionally, a controlled environment will be provided to reduce the factors other than the materials that the catheters are coated with that affect the growth of MRSA. To ensure that the primary variable in cell growth is exposure to the titania-based nanofiber mats, the *S. aureus* will be grown at 37℃ (mammal internal body temperature) and will be given constant access to nutrients. To guarantee that the cells have appropriate nutrients to grow as necessary, MRSA cell solution will be inoculated onto a piece of nutrient agar, and the agar will be placed face-down onto the experimental and control surfaces[9]. Timepoints of both the control and the experimental groups will be taken every twenty-four hours for one week, recording the total initial number and final number of cells[10].

In the experimental group, it can be assumed that the cause of cell death is due to exposure to the nanofiber mat.

[8] Wahab, J.A. and Al Mamun, S. (27 January 2020)
[9] Yamada, Hiroyuki, et al. (15 August 2010)
[10] Ibid.

IV. Anticipated Results

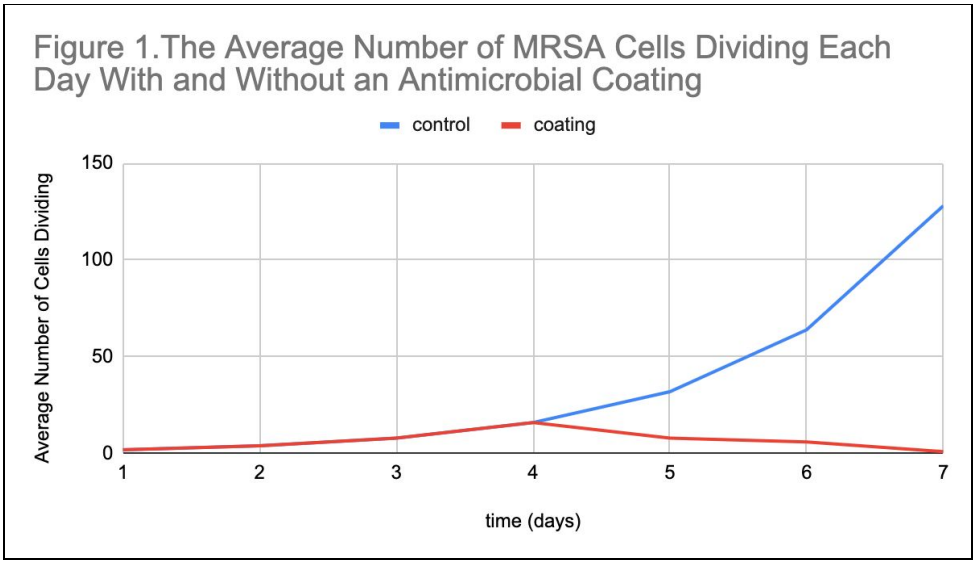


Figure 1: This graph shows the expected number of bacteria that are dividing (alive) over the course of seven days in conditions with and without the antimicrobial coating. To determine if the coating inhibits MRSA development, the team will analyze the Petri dishes once every twenty-four hours under a microscope and count the number of cells that are dividing (this is one of the most effective methods for determining how many cells are alive without disturbing the cells). The average doubling time for S. aureus at 37℃ with available nutrients is twenty-seven minutes[11]. A theoretical total number of viable cells can be calculated and compared to the experimental total to determine if the mats are impacting cell division. Further, the number of cell divisions over time will be graphed and the rate of cell division will be calculated (found by taking the derivative of the number of cell divisions). Counting how many cells are dividing once every twenty-four hours is a sufficient frequency to use to calculate the rate of cell division. If the coating is inhibiting the development of MRSA, then the derivative should eventually be negative, meaning that the graph has a downward slope to indicate a decrease in the number of living MRSA cells. It is expected that the population of bacteria in the control group will increase exponentially as there is nothing to inhibit their division. Finally, it is expected that the population of bacteria in the condition with the coating will grow initially, but will eventually succumb to the antimicrobial coating and decline until no living bacteria cells remain.

[11] Missiakas, D. M., & Schneewind, O. (February 2013)

Additionally, on day seven, the cells will be converted to liquid culture, dyed using a LIVE/DEAD assay, and analyzed using flow cytometry to estimate how many MRSA cells are alive after in each condition. This process involves washing the cells to eliminate residual agar, resuspending the cells in solution, and introducing a mixed dye. Only dead cells will have punctured membranes and will be permeable to the red dye. Cells with intact cell membranes will be tagged with a green dye. Next, a flow cytometer will fluoresce the cells with light and analyze the wavelength absorbance. It will quickly count a high volume of cells and report their viability. This process can only be applied to the samples once, so we intend to use it when the experiment is completed as a way of verifying our method of counting the number of cells that are dividing to determine how many cells are alive.

Figure 2. Hypothetical MRSA LIVE/DEAD Assay Results[12]

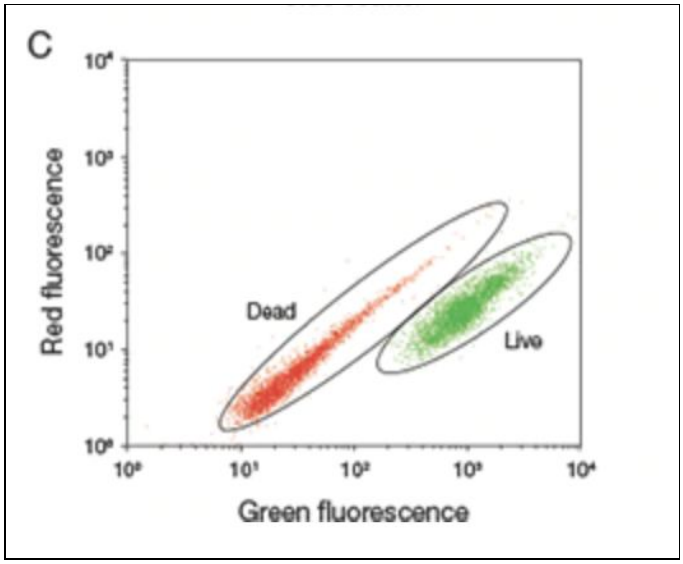


Figure 2: This figure displays ThermoFisher's hypothetical data gathered from flow cytometry. In this experiment, it is predicted that there would be a much higher concentration of dead cells than the data set pictured in the figure.

Figure 3. Flow Cytometry Cartoon[13]

[12] LIVE/DEAD Cell Viability Assays.
[13]Retrieved from https://www.abcam.com/protocols/introduction-to-flow-cytometry

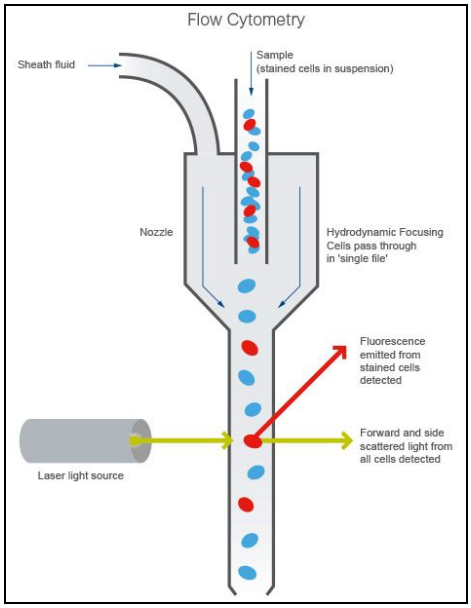


Figure 3: This illustration details how flow cytometry analyzes and sorts cells based on their reaction to dye and therefore their viability.

V. Timeline

To begin, a liquid *S. aureus* cell culture will be created using MRSA cells and standardized nutrient agar blocks. Then, two types of Petri dishes would be set up; one coated in polyurethane (to mimic a standard catheter) and the other coated in a layer of polyurethane as well as a layer of a titania-based nanofiber mat. The cell culture would be spread on the nutrient agar and added to each type of Petri dish. This process would then be replicated three times. All of the dishes would be incubated at 37℃ and videotaped throughout.

The Petri dishes will be checked once every twenty-four hours for a week. This observation would include analyzing each Petri dish under a light microscope and individually counting the number of cells.

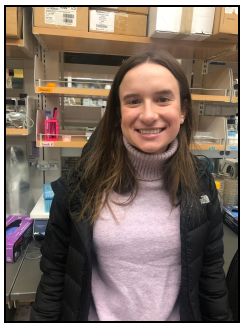
At the end of the week on day seven, calculate the average number of cells that are dividing each day for the control Petri dish and the experimental Petri dish. The average data would then be plotted as a function and the derivative would be calculated. Finally, the data would be analyzed for if/when the derivative is negative for each condition to determine if cell growth is inhibited in either condition.

VI. Materials and Budget

Material	Brand	Details	Cost	Running Total
BSL-2 Lab space[14]	Lab Central Facilities, Cambridge, MA	Includes basic materials and lab bench; cost per one week	$4,600.00/month	$1,150.00
500 mL Prepared nutrient agar	Carolina, Item #776366	None	$25.25	$1175.25
Oversized Petri dishes (12 count)	Carolina, Item #199279	None	$54.70	$1229.95
Microscope camera	AmScope, Item #MU1803-NI05	18MP USB 3.0 Color CMOS C-Mount Microscope Camera with 0.55X Adapter for Nikon Microscopes	$654.99	$1884.94
Medical-grade polyurethane TPU	Shenzhen Yi Yun Eco-Technologies Co., Ltd. Item #YN-M5803T	None	$7.60/kilogram	$1892.54
5 mL *Staphylococcus aureus* culture	Carolina, Item #155554A	Consulting with Dr. Margaret Riley of UMass Amherst	$19.20	$1911.74
LIVE/DEAD BacLight Bacterial Viability Kit	ThermoFisher Scientific, Item #L7007	Compatible with flow cytometry or microscopy	$638.00	2,549.74
Titania-based nanofiber mats	Made in-house	Consulting with Dr. Jessica Schiffman of UMass Amherst	“Low cost”	$2549.74+

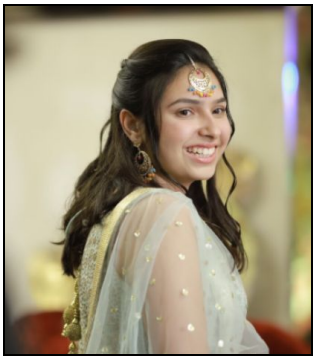
[14] MRSA Agent Information Sheet.

VII. Key Personnel



Sarah Kaunfer is a microbiology major and works as an undergraduate research assistant studying mycobacterial pathogenesis. She has developed knowledge of bacterial cultures and related lab procedures, as well as bactericidal materials. Throughout this project, Sarah would be responsible for assembling, monitoring, and measuring cell growth exposed to the nanofiber mats.

Phoebe Lasic-Ellis has basic pathophysiology and medical knowledge having been in the EMT academy this past year at UMass as well as knowledge on PPE and disease prevention. Additionally, she is aware of how to operate PCR and gel electrophoresis as well as micropipetting. In terms of roles and responsibilities, Phoebe would be responsible for leading proper protective guidelines and disease prevention as we will be working with a dangerous bacteria. She would also take responsibility for some procedural techniques as well as ultimately communicating findings in an understandable and user-friendly way.



Simran Jeet is a biology major, who has knowledge of making process maps, shadowing and working with the PIT team as a 4-year volunteer for CHA hospital. She has a lot of experience with gel electrophoresis as part of working with the Biogen Lab. Additionally, she is aware of and has performed basic lab techniques and procedures for both chemistry and biology. Simran will be responsible for performing some of the procedures along with recording and analyzing data from the procedures.

Hayley McIsaac is a biochemistry major, which lends itself to knowledge of antibiotic resistance and evolution. She has taken up through calculus 2 and has an understanding of the mathematical aspects of data analysis. Hayley will be responsible for quantifying and analyzing the data.

VIII. Relevance of Proposed Study and Broader Impacts

After exploring the issue of nosocomial infection, it is evident how much more research is required to eliminate this massive liability. Coating central-line catheters, as opposed to using antibiotics to treat MRSA, will ideally prevent the pathogen from developing further antibiotic resistance. Coating catheters is a more proactive approach to treating MRSA than waiting until the infection has already entered the body and resorting to antibiotics.

Research on catheter infection and preventative strategies would greatly benefit both treatment centers, such as hospitals as well as other healthcare organizations. Regardless, the outcome of this research will shed light onto a possible alternative to standard polyurethane central-vein catheters, which are known to be a major point of entry for MRSA and other major infectious pathogens. If research suggests that the titania-based nanofiber mats eliminate bacterial growth, then this coating could be incorporated into the state HAI plans for hospitals developed by the Center for Disease Control and Prevention[15]. If the research indicates that this coating is not helpful, or that another alternative method proves more efficient, then there will also be tremendous amounts to learn from those findings. It will be impossible to make headway on preventing hospital-acquired infection through catheterization without anticipating some error.

This research is also highly relevant to the general public. Sanitation and recovery are two integral parts of hospitalization. When a patient needs any treatment, such as central-line catheterization, for example, it is incredibly important that they do not fear further infection from receiving treatment. Funding for structured research could lead to the implementation of said results into current policy and healthcare plans for hospitals which would further alleviate concerns from the general public regarding the sanitation of the hospitals where they seek treatment.

IX. References

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Jared's Final Commentary:

Overall nice job! It's been great to see the evolution in your thinking and the refinement of your initial ideas into this final proposal! Ha, cool graphics on the cover :) Project Summary and Background are great. Footnote citation works well. There are two citations that are missing dates, which you should include. But this is otherwise well cited. You really clearly lay out the current situation, your research question, and hypothesis. Excellently done! Methods are clear and seem appropriate (Although you also have some Methods in your Anticipated Results - see

my comments in that section). One area you may want to specify is what kind of lab this work would be done in. I'm not positive, but I assume there are only certain labs certified to handle MRSA. If you specify the type of lab, or exact lab location where you would do this work, it would strengthen the methods just a little bit. (I see you do get into this level of detail in your Materials and Budget Section, but it is good to mention it in your Methods too). You also don't get into sources of uncertainty or error. Anticipated Results look good. If you found the results shown on your graph I'd be able to clearly see the difference between the two coatings. Because this is an average you should include error bars to show the standard deviation. There is obviously a clear difference by day 6 and 7, but reporting the standard deviation (by showing error bars) gives the reader a critical second piece of information, beyond just the average. This shows variability and if the error bars don't overlap it give the reader confidence there is a significant difference. If error bars were here, I could make a better judgment on whether day 5 is showing significantly different - it probably is, but without standard deviation, I'm less sure. Hmm I'm intrigued by your last sentence on page 5. You say you expect MRSA will grow initially, but eventually be inhibited and die from the coating. In real world conditions, do you think MRSA could survive long enough on the coating to successfully enter the human body? Your first paragraph on page 6 (in your Anticipated Results) is great, but it belongs in the methods section. What you are describing here is a method. It's ok to have a reminder about the method here before presenting a graph, but this is the first time you mention dyeing using the LIVE/DEAD assay. You also don't specify which sample the Figure 2 graph represents, is it the polyurethane control or the titania exposed experimental culture. It's probably the latter, but you should specify. Figure 3 belongs in your Methods section, since this isn't a result, but a graphic of your method. Timeline is ok, but is a bit Method's heavy. Timeline should be more of a quick overview, that's why a graphic like a calendar can be helpful. Here you get into a little too much Method's detail. Materials and Budget are excellent, very detailed and well thought out. Great job! Key Personnel looks really sharp with your photos and bios. You also do an excellent job connecting your experience with the work you propose to do for this study. Really nicely done! Relevance and Broader Impacts are well done. If your results show what you anticipate, it feels like the cost savings for hospitals (from having less MRSA infections and less lawsuits) would far outweigh whatever additional cost the titania coating would be. In other words, it seems like you have identified a real niche product that would be very popular. Really great job! I'd fund this research...and probably invest in your company if you found promising results and wanted to turn this research into a medical product line! Really, this seems worth pursuing, if you want to continue to develop this idea there are on campus grant funding opportunities for student research. See:
https://www.isenberg.umass.edu/centers/berthiaume-center-for-entrepreneurship/innovation-challenge. Feel free to talk to me if you want any guidance around this. It's been a pleasure having you all in class and to see your scientific thinking develop over the semester. Have a great summer :) Cheers, Jared