The Effects of Superparamagnetic Iron Oxide Nanoparticles on Biofilms

(4417 words)

Abstract

With the increase in implantable devices and surgical procedures, comes with an increase of infections such as chronic wounds, and pneumonia in cystic fibrosis patients. The cause for these infections stems from bacterial biofilms that are present on implantable devices. The use of nanoparticles has risen due to its relatively low toxicity in the human body and its relatively small size that increases the effectiveness of the nanoparticles penetrating the surface of the biofilm. The superparamagnetic iron oxide nanoparticles (SPION) and S. Epidermidis were grown with differing concentrations of nanoparticles in microcentrifuge tubes for 72 hours. The samples were then plated and incubated at 37 ^{o}C for 24 hours. Bacterial colonies that were larger than 1 mm were counted. Complete inhibition of the bacterial colonies occurred at 0.15 mg/mL. With this knowledge of iron oxide nanoparticles inhibiting growth of the bacteria, the present results provide us with much promise in utilizing Iron Oxide based nanoparticles for antibacterial and anti-infection properties.

Introduction

Metals have been used to as antibacterial agents before, but modern antibiotics have surpassed their efficiency, thus causing their use to slowly decline. However, metals in the form of nanoparticles have risen as a possible solution in inhibiting the growth and the formation of biofilms. The nanoparticles' efficiency may be due to their high surface to volume ratio which means an increased production of oxygen species. This allows for the nanoparticles to interact with the microbial membranes, which causes inactivation of the bacteria.

Biomaterial implants and devices, although successful in restoring function of certain body parts, pose a serious problem: microbial infections. Several biomaterial surface modifications have been developed to reduce biofilm formation but the microbial adhesion can not be fully eliminated. This causes long term treatment of biofilms to be a challenge.

S. epidermidis is the most common organism isolated from foreign material related infections such as infected prosthetic joints, intracardiac devices, artificial heart valves, and vascular grafts (Mack, D., & Rohde, H. 2015). A normally harmless bacterium, *S. epidermidis*, often becomes an opportunistic pathogen closely linked to the biofilm forming capability of the species. Although the bacteria is highly abundant on the human skin, it can quickly switch from a colonizing, to an invasive lifestyle. *S. Epidermidis* is also responsible for the large majority of nosocomial catheter related bloodstream infections. Such infections can cost up to $50,000 per episode and mortality can be as high as 2.5 percent (Loo, C., Young, P. M., Cavaliere, R., Whitchurch, C. B., Lee, W., & Rohanizadeh, R. 2013). There is also a lack of non-invasive treatments for the implantable infections and replacement surgery is also necessary.

Biofilms are the main contributor to fatal infections, such as chronic wounds, pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis media and catheter associated infections (Bjarnsholt, T. 2013). The growth of bacteria is categorized into two different phenotype groups : planktonic or sessile aggregates. However, the growth of biofilms primarily stems from sessile aggregates. Sessile aggregates, such as biofilm, are attached to surfaces and are made up of a large number of bacteria in order to form a microbial consortium (Parmar, S. 2013). The formation of the biofilms occurs in 5 steps: attachment, expansion, maturation, resistance, and detachment. Attachment occurs when the bacterial cells latch onto the surface of the implantable device through tail like structures. The cells then divide by communicating with chemical signals to their neighboring cells. When there are a sufficient amount of bacterial cells, the extracellular polymeric substance (EPS) is formed around the cells.

The EPS protects the cells from the immune system, toxins and antibiotics which makes the EPS one of the main causes for antibiotic and antimicrobial therapy resistance. Bacteria grown as a biofilm develops resistance to environmental stresses such as: antibiotics, disinfectants and host defenses (Thukkaram, M., Sitaram, S., Kannaiyan, S. K., & Subbiahdoss, G. 2014). Due to this, biofilms show extreme resistance to most usual antibiotic therapies. The EPS contains extracellular polysaccharides that minimize the penetration of antibiotics through the biofilm via neutralization of antimicrobial agents (Thukkaram, M., Sitaram, S., Kannaiyan, S. K., & Subbiahdoss, G. 2014). Neutralization is a process that occurs when an antibody blocks off a virus by binding to its cell surface receptors (Lebeaux, D., Ghigo, J., & Beloin, C. 2014). In order to increase the effectiveness in cell death within biofilms, new methods involving nanoparticles must be taken into account.

One way to prevent the formation of biofilm on medical devices, is to coat surfaces with biocompatible material. The discovery of nonbiological antibiofilm molecules would prevent bacteria from developing resistance to nonbiological agents. Using nanoparticles to coat the medical devices would be of great benefit due to the size of the nanoparticles. They are small enough to penetrate the biofilm but still large enough to have a long plasma half-life. Nanoparticles also offer a large surface to volume ratio ideal for mass loading of targeting drugs and antibiotics (Lebeaux, D., Ghigo, J., & Beloin, C. 2014).

Superparamagnetic iron oxide nanoparticles (SPION), for example, have received special attention due to the variety of applications present in the biotechnological field. Biosensors, antimicrobial activity, food preservation and hyperthermic cancer treatments are the few uses of SPION. Due to its biocompatibility and magnetic properties, it has also been used in biomedical research. Nanoparticles are less than 100 nm in diameter. Due to their small size and superparamagnetic properties, they can also be targeted to the infection site using an external magnetic field, causing deep penetration in a biofilm (Mack, D., & Rohde, H. 2015). This allows the nanoparticles to alter properties such as surface area, chemical reactivity, and biological activity.

In preventing the formation of biofilms on implantable devices, the use of iron-oxide based compounds has continued to rise due to its far lower inclination to induce bacterial resistance compared to current antibiotic therapies (Mack, D., & Rohde, H. 2015). It has been known that the probability of bacteria gaining resistance towards the iron oxide is remote because iron oxide simultaneously acts on multiple sites within bacterial cells, increasing chances in bacterial cell death . Superparamagnetic nanoparticles have been considered as a use

in preventing orthopaedic infections, such as septic arthritis, prosthetic joint infections, and osteomyelitis which is a condition that causes inflammation of bone. Bone is typically resistant to bacterial colonization, however certain instances that involve implantable devices can disrupt bone function and cause an infection.

The surface functions of the superparamagnetic nanoparticles are important in that their interaction with the bacterial cell membranes is crucial for cell death. The plasma membrane is a selectively permeable membrane that separates and maintains the needed intracellular components of the cell (Thukkaram, M., Sitaram, S., Kannaiyan, S. K., & Subbiahdoss, G. 2014). Nonpolar molecules can diffuse across the bilayer, however nanomaterials are not able to penetrate the bilayer on their own. In order to have the most effective cellular uptake, nanoparticles must be 50 nm or less in size. Iron oxide nanoparticles also contain an endocytic fate, which involves the nanoparticles being taken into macrophage cells, which is a large white blood cell, and then they are found in lysosomal bodies (Mack, D., & Rohde, H. 2015). Lysosomal bodies are organelles that contain enzymes that break down a large variety of biological polymers (Bjarnsholt, T. 2013, May).

Extensive research has been done to test the effects of both Gold and Silver nanoparticles on different bacterial biofilms, such as *Escherichia coli* and *Pseudomonas aeruginosa*. Previous tests have also tested the bacterial adhesion on a variety of different surfaces: GS, PS, PMMA, polymer brush coated PMMA, TCPS, and polymer brush coated TCPS. This gives insight on the range of materials the nanoparticles are most effective in preventing bacterial growth (H. 2014, September 23).

The main method used in previous experiments to test the effectiveness of the Iron Oxide nanoparticles in inhibiting growth is through calculating optical density, which utilizes the spectrophotometer (EN Taylor, TJ Webster. 2009). The nanoparticles and bacteria were grown in microcentrifuge tubes and then put through the spectrophotometer in order to calculate optical density, which in turn determined the growth of the bacteria and how effective the Iron Oxide Nanoparticles were in inhibiting bacterial cell growth. Although such methods were popular among the majority of the peer reviewed papers, there were no methods that discussed the use of the plating method. Utilizing the plating method after growth in the microcentrifuge tubes allows us to physically see and count the bacterial colonies.

Testing to see if nanoparticles inhibit the growth for S. Epidermidis is the first step in discovering new methods to completely prevent all future biofilm related infections.

Purpose

The use of implantable devices will increase with time, leading to an increased risk of infection. Infected implants require follow up routine care, increased pharmacy cost, and intensive care costs accounted for the majority of the incremental cost associated with infection. Although nanoparticles of different substances, such as silver, gold and zinc, have been found to decrease bacterial growth, none have become successful in inhibiting growth on metallic surfaces. This is because there have been issues in having the nanoparticles attach to the surfaces for an extended amount of time. The effectiveness of the nanoparticles on the surfaces of metallic surfaces for an extended period of time are still unknown. However, testing a variety of nanoparticles could increase the chances of finding the most effective nanoparticle to inhibit the

growth of the bacteria, along with offering additional benefits. Iron Oxide nanoparticles offer a new insight into the issue. Testing to see if the nanoparticles inhibit growth of bacterial colonies, could provide hope in testing to see if coating metallic surfaces with the Iron Oxide inhibit bacterial biofilms.

Testing new methods to prevent such infections allow us to become closer to finding effective strategies that will allow patients to be healthy, and healthy patients means saving the hospital time and money.

Hypothesis

The main question addressed in my research is as follows

Will Iron Oxide Nanoparticles at concentrations of 0.01 mg of nanoparticles / 1 mL of S. Epidermidis, .05 mg/mL, 0.1 mg/mL, 0.15 mg/mL and .25 mg/mL inhibit the growth of the S. Epidermidis?

Testing the effects of the iron oxide nanoparticles on *S. epidermidis* will include bacterial colony death due to the nanoparticle binding to the cell membrane proteins through electrostatic interactions, disrupting the bacteria function. An increase in the concentration of the Iron Oxide Nanoparticles per mL of bacteria, will decrease the number of bacterial colonies found when the bacteria is plate.

Null Hypothesis

The increase in the concentration of Iron Nanoparticles per mL of bacteria, will either increase, or cause no change to the number of bacterial colonies when compared to the control groups which contain only .5 mL of *S. Epidermidis.*

Experimental Variables

Independent Variable

Concentration of Iron Oxide Nanoparticles per 1 mL of S. Epidermidis.

Dependent Variable

The number of bacterial colonies per plate.

Safety Concerns

When working in the lab with the given substances, personal protective equipment will be used: safety goggles, closed toed shoes, and lab coat. Safe lab practices will also be instilled. *S. epidermidis,* although part of the human flora, may cause risk of developing infection to those with compromised immune systems. Ferrous Chloride is corrosive and an irritant when coming in contact with skin. It is also toxic to lungs, and mucous membranes and work with the substance will be done in the fume hood. Iron Oxide may cause respiratory tract irritation, mechanical eye and skin irritation and inhalation of fumes may cause metal-fume fever (Fisher Error Encountered. (n.d.). Retrieved 2017). Sodium Hydroxide, is a corrosive, irritant, and permeator when coming in contact with skin. If eye contact occurs, corneal damage or blindness may result. Ingestion and inhalation may cause tissue damage depending on length of contact.

Skin contact can produce inflammation and blistering. Inhalation of dust will produce “irritation to gastrointestinal or respiratory tract, characterized by burning, sneezing and coughing” (Fisher Error Encountered. (n.d.). Retrieved 2017).

Materials and Methods

Experiment done at CSUCI

1. 1 gram Iron (II) Chloride in 10 mL of H2O
2. 5 gram Iron (III) Chloride in 10 mL of H2O
3. 1 mg Sodium Hydroxide in 100 mL of H2O
4. *S. Epidermidis* freeze dried form
5. 6 mL Lysogeny broth obtained from Sigma Aldrich
6. 30 1.5 mL microcentrifuge tubes
7. Salt mannitol agar for 30 plates
8. Deionized H2O
9. P-1000 micropipettes
10. Analytical Balance
11. Serological Pipettes
12. LW Scientific 20 Liter Digital Incubator
13. Floor shaker for LB broth and S. Epidermidis
14. 30 inoculation loops
15. Four 45 mL falcon tubes
16. 100 mL graduated cylinder

17. 10 mL graduated cylinder
18. Fisher Scientific 6” stainless steel Scoopula Spatula scoops
19. Vortex Genie 2
20. 30 petri dishes

Synthesis of Iron Oxide Nanoparticles (Co Precipitation Method)

Stock Solution of Ferrous Chloride ($FeCl_2$) and Ferric Chloride ($FeCl_3$) and Sodium Hydroxide (NaOH)

a. 1 gram of $FeCl_2$ obtained from Sigma Aldrich in hexahydrate form, was measured using an analytical balance. The substance was then slowly transferred from a weighing boat dish into a 45 mL falcon tube. Distilled water was then added into a graduated cylinder until the meniscus reached 10 mL.
b. 5 grams of $FeCl_3$ obtained from Sigma Aldrich in hexahydrate form were measured using an analytical balance. Substance was then transferred into a 45 mL falcon tube. Distilled water was then added slowly into a graduated cylinder until the meniscus reached 10 mL.
c. 1 mg of NaOH was measured using an analytical balance. The NaOH substance was then transferred into a 100 mL beaker. Distilled water was measured into a graduated cylinder and then transferred into the beaker until the meniscus reached 100 mL.

4 mL of the $FeCl_2$ stock solution, and 1 mL of the $FeCl_3$ stock solution were then added into a falcon tube along with the 1 mL of the NaOH in order to synthesize the nanoparticles. The precipitate of the reaction were the nanoparticles. The falcon tube was then vortexed using the Vortex Genie 2. This process achieved the nanoparticle stock solution concentration of 2 mg / 10 mL.

Culturing the S. Epidermidis

The *S. epidermidis* was received in a freeze dried form, and was rehydrated in 6 mL of lysogeny broth (LB), which consists of : 10 grams tryptone, 5 grams yeast extract and 5 grams NaCl. The solution was then incubated for 24 hours or until the bacteria reached a late stationary phase.

a. 25 grams of Lysogeny broth was added into a 1 Liter volumetric flask. 1000 mL of deionized water was then added.
b. The solution was autoclaved for 2 hours. After the 2 hours, the broth was left to cool and after, the *S. Epidermidis* was then rehydrated.

Growing Nanoparticles and S. Epidermidis in Microcentrifuge Tubes

30 microcentrifuge tubes were then prepared

a. 15 microcentrifuge tubes were labeled as 'Control group', which contained .5 mL of the S. Epidermidis, and no nanoparticles.
b. The rest of the 15 microcentrifuge tubes were split into groups of 5. There were a total of 5 concentrations, and 5 control groups, one for each concentration.
c. The concentrations included were based on number of mg of nanoparticles per 1 mL of *S. Epidermidis* in order to achieve the appropriate concentration: 0.01 mg/mL, 0.05 mg/mL, 0.1 mg/mL 0.15 mg/mL, and 0.25 mg/mL.

For each concentration and control group, the tests were done in triplicates in order to achieve accurate results. P-1000 pipettes were used in order to obtain the correct amount of the nanoparticles. Serological pipettes were used in order to pipette 0.5 mL of S. Epidermidis into

the microcentrifuge tubes. Each microcentrifuge tube contained 0.5 mL of *S. Epidermidis* for all concentrations.

a. In the 0.01 mg/mL concentration, 25 μl of the the Iron Oxide nanoparticles were added using a micropipette.
b. The 0.05 mg/mL concentration contained 125 μl of the Iron Oxide nanoparticles
c. The 0.1 mg/mL contained 250 μl which were pipetted into the microcentrifuge tubes using a P-1000 pipette.
d. The 0.15 mg/mL contained 375 μl which were pipetted into the microcentrifuge tubes using a P-1000 pipette.
e. 0.25 mg/mL contained 625 μl which were pipetted into the microcentrifuge tubes using a P-1000 pipette.

After the nanoparticles were dispersed into the 0.5 mL of *S. Epidermidis*, each tube was immediately capped in order to prevent contamination.

The nanoparticles and bacteria were grown for 72 hours at 37 ^{o}C in order to imitate the body temperature if the nanoparticles were to be coated on an implantable device.

After 72 hours, the bacteria was taken out of the incubator, and each tube was vortexed due to substances collecting at the bottom of the tube.

Plating the Nanoparticles and S. Epidermidis (Streaking Method)

Next, the salt mannitol agar was prepared for 30 plates.

a. The Agar was used as a selective media for the the growth of the *S. Epidermidis*; 11.02 grams of the agar was added into a 1000 mL flask. Deionized water was added into the flask until the meniscus reached the 1000 mL line. The solution was heated to boil in

order to dissolve the medium completely. Agar was then sterilized by autoclaving at 121°C for 15 minutes. About 20 mL of agar was then poured into each of the petri dishes. Each plate was labeled with the appropriate concentration and kept in sterile bags to prevent contamination, and kept in a refrigerator until used.

b. The nanoparticles and bacteria were then plated using the streaking method with a loop, in order to count the bacterial colonies after 24 hours of incubation. There were sterile loops for each plate in order to reduce the chance of contamination. When plating, the lids of the plates were opened at an angle (clam shell) in order to prevent contamination of the samples.

c. After all 30 plates were streaked, the plates were then turned upside down, taped at the sides and then placed back onto a tray and placed into the incubator for 24 hours. After the 24 hour period, the plates were examined and counted for bacterial colonies. Bacterial colonies larger than, or equal to 1 mm were counted. Having this range allowed for the collection of data to be more accurate. Smaller colonies were taken into account, but not included in final data in order to increase consistency and accuracy of the data.

Results

Plates were analyzed after 24 hours of plating. For concentration .01 mg/mL which contained 25 microliters of Iron Oxide nanoparticles, the average number of bacteria colonies (1 mm >) was 5. .05 mg/mL which contained 125 microliters of nanoparticles, had an average of 4 colonies per plate; .1 mg/mL which contained 250 microliters of nanoparticles had an average of 5 bacteria colonies per plate; .15 mg/mL which contained 375 microliters of nanoparticles had average of 0 bacteria colonies (no growth); .25 mg/mL which contained 625 mg/mL of nanoparticles had an average of 0 bacteria colonies (no growth).

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1270.137	1	1270.137	10.175	.005[b]
	Residual	2246.863	18	124.826		
	Total	3517.000	19			

a. Dependent Variable: Bacterial colonies

b. Predictors: (Constant), Concentration mg/mL

Figure 1: Data output by ANOVA Excel analyzing the inhibition of bacterial cells

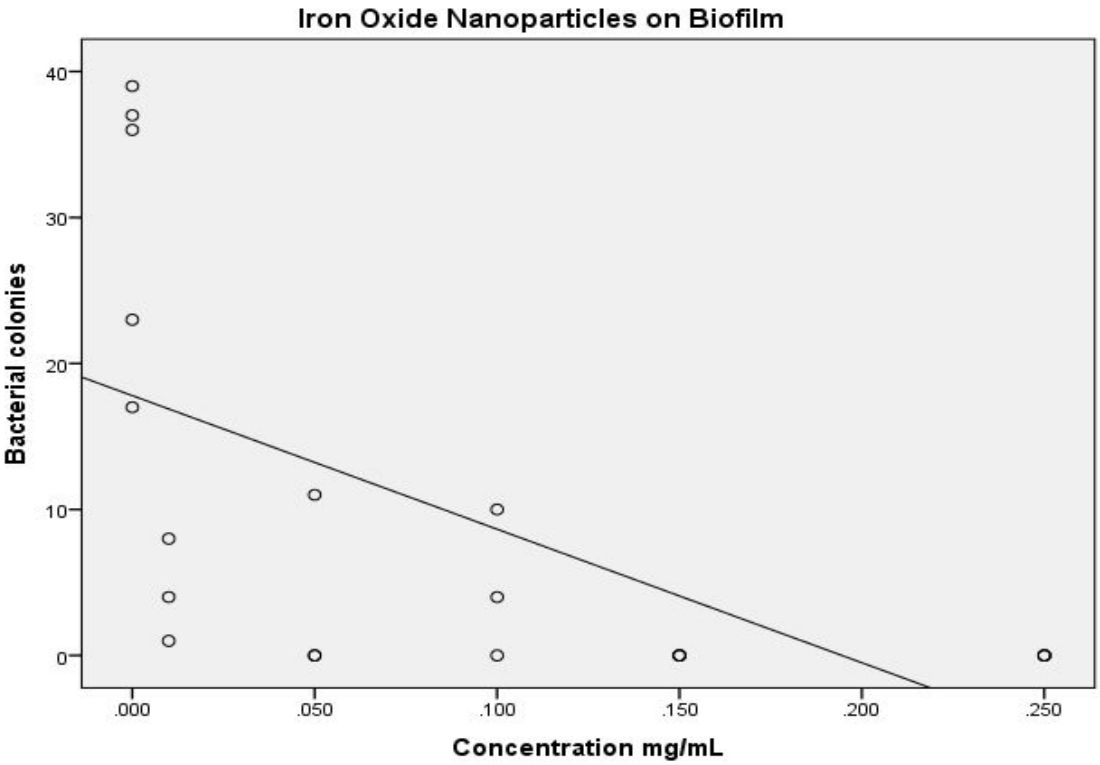

Figure 2: This chart shows that the increase in Iron Oxide Nanoparticles steadily decreases the number of bacterial colonies per plate. The bacterial colonies shown are a result of the average taken of all three plates with that specific concentration.

[SPION] mg/mL	Colonies
0.010	1
0.010	4
0.010	8
0.050	11
0.050	0
0.050	0
0.100	0
0.100	4
0.100	10
0.150	0
0.150	0
0.150	0
0.250	0
0.250	0
0.250	0
0.000	37
0.000	36
0.000	17
0.000	23

Data Table 3: Number of bacterial colonies per plate, including the control groups. This table does include the averages of 3 control groups. All bacterial colonies from all of the plates were taken into account for this table. As the concentration of the nanoparticles increased, the number of the bacterial colonies decreases.

Figure 4: Salt Mannitol Agar plates of the control group containing just .5 mL of S. Epidermidis after 24 hours of incubation at 37 degrees Celsius.

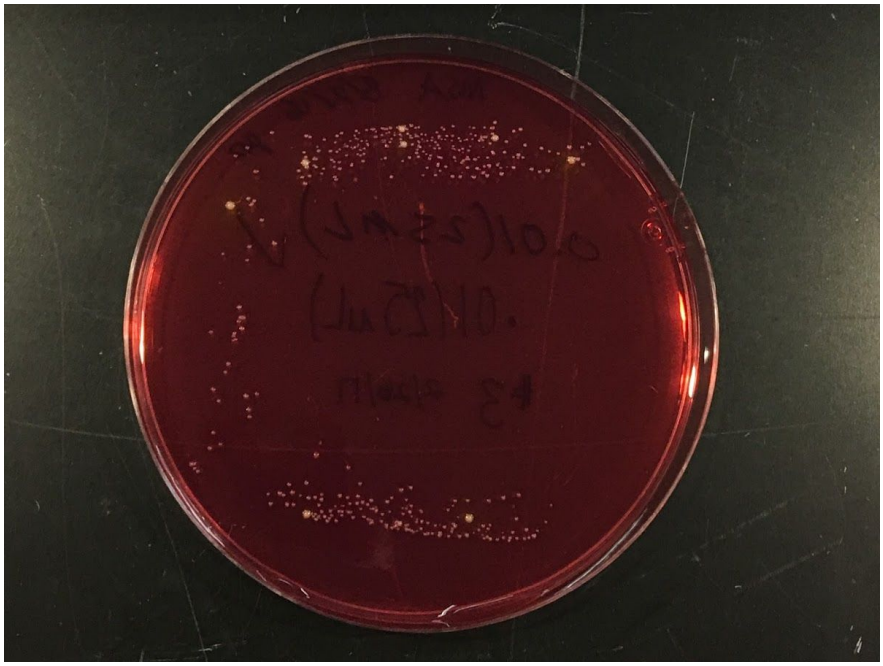

Figure 5: Salt Mannitol Agar plates of the lowest concentration of Iron Oxide Nanoparticles: .01 mg of nanoparticles per 1 mL of S. Epidermidis. The lowest concentration of the nanoparticles exhibited a dramatic decrease in the number of bacterial colonies.

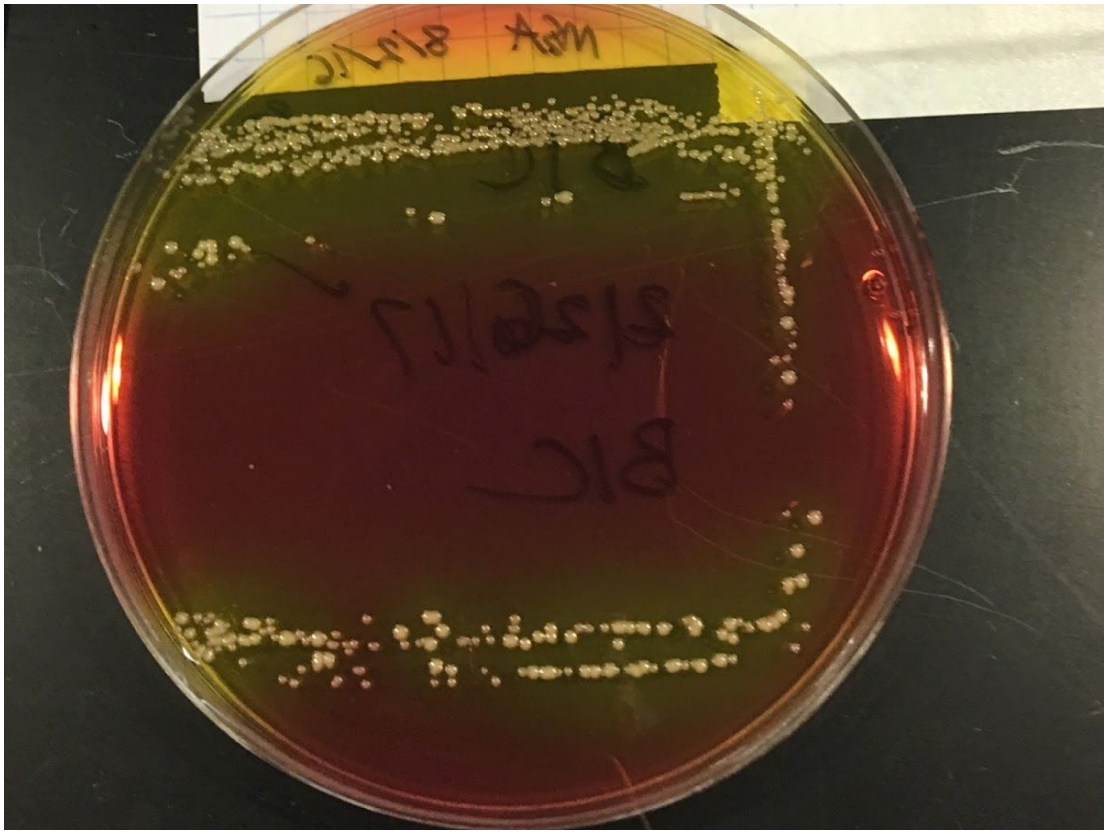

Figure 6: A control plate where only .5 mL of S. Epidermidis was streaked.

Statistical Analysis

To investigate the effect of Iron Oxide Nanoparticles on *S. Epidermidis biofilms,* a simple linear regression was conducted. The predictor was the concentration of the nanoparticles, and the outcome was the number of bacterial colonies. The predictor variable was found to be statistically significant [B = -.915, 95% C.I. (-1.518, -.312), $p < .05$], indicating that for every 0.01 ml unit increase in nanoparticle concentration, the bacterial colonies decreased by .915 units. The model explained approximately (36.1%) of the variability [R-squared = .361]. Therefore, the null hypothesis is rejected and the alternative hypothesis is retained.

Discussion

Nanoparticles offer a new and innovative insight into the complete inhibition of biofilms. They offer a high surface to volume ratio which increases effectiveness in mass loading of drugs and antibiotics. A high surface to volume ratio also generally includes the production of reactive oxygen species which can attack a large range of targets in order to exert antimicrobial activity. Previous studies have utilized gold and iron oxide nanoparticles in order to determine its effects on bacterial biofilm growth. *In vitro* experiments carried out on tissue culture polystyrene wells, were measured using optical density and showed that at concentrations of .01 mg/mL, gold nanoparticles showed a 13 % reduction in bacterial growth. However, biofilm growth increased when both gold and iron oxide nanoparticles were introduced, when compared to the control group [6]. In order to obtain the highest percent of inhibition of bacterial biofilms when using iron oxide nanoparticles, adhesion to nonmetallic surfaces is preferred [3]. Iron Oxide nanoparticles offer a new insight into the use of nanoparticles in the inhibition and inactivation of bacterial biofilms, such as *S. Epidermidis*.

This study showed that the complete inhibition of *S. Epidermidis* through iron oxide nanoparticles occurs highest at concentration of 0.15 mg/mL. The nanoparticles did not completely inhibit the growth of the *S. Epidermidis* at the lowest concentration of 0.01 mg/mL (25 μl of nanoparticles). Due to the iron oxide nanoparticles not immediately inhibiting 100% of the bacterial growth at the lowest concentration introduces, it demonstrates the relatively low toxicity. Since the main implication of the nanoparticles is to be coated on implantable devices, this low toxicity is favored. Also, due to a linear decrease in bacterial colonies, it indicates that the inhibition of the bacteria occurs gradually. At concentrations 0.01 mg/mL, 0.05 mg/mL and 0.1 mg/mL, half of the plates had no growth and the other half of the plates showed growth of about 4-5 colonies per plate. The control groups had an average of 34 colonies per plate which is drastically larger than number of colonies per plate with 0.01 mg/mL.

As the nanoparticle concentration in the bacteria culture increased, the concentration of the bacterial colonies decreased. Plates 0.15 mg/mL and 0.25 mg/mL had no growth present on all plates.

Previous studies have also indicated that this antibacterial activity exerted by the iron oxide nanoparticles may be due to the oxidative stress caused by ROS. The radicals in the ROS have been known to cause chemical damage to the DNA and proteins in bacterial cells. The electrostatic interactions between the nanoparticles and bacterial cell membranes causes physical damage, which in turn leads to cell death.

Sources of Error

Sources of error were present in both growing the bacteria and nanoparticles in microcentrifuge tubes and the plating of the samples. For the concentration of 0.1 mg/mL, one of the plates had no growth present however, the two other plates had 4 colonies and 10 colonies. Despite it having a higher concentration of nanoparticles when compared to the 0.01 mg/mL and .05 mg/mL samples, the two plates that did have growth had more bacterial colonies present when compared to the colonies found on the plates of the two lowest concentrations. Contamination may have occurred when the nanoparticles were pipetted into the microcentrifuge tubes containing *S. Epidermidis*. It may have also occurred when the samples were plated onto salt mannitol agar plates. Although sterile loops were used for each individual microcentrifuge tube, the plates may have not been opened using the "clam shell" technique that prevents outside contamination. The lids of a small portion of the plates were lying face up on the work bench which also increases the likelihood of contamination.

Further Work

Further testing could include growing the bacteria on a metallic surface that has been coated with the nanoparticles. Testing the effects of the nanoparticles on human health, to see if the nanoparticles pose any health hazard. However, the sample size should be increased also enable us to see if concentrations higher than .25 mg/mL decreased inhibition, instead of increasing inhibition. Increasing the sample size could also provide more accurate results. Testing could also include in vitro, and then in vivo testing. Testing different nanoparticles, such as silver nanoparticles and gold nanoparticles and comparing the number of bacterial colonies could also help with determining which nanoparticles offer the most benefits.

It was thought through common knowledge that negatively charged bacteria, which are more resistant to antibiotics than gram positive bacteria, would be more likely to adhere onto metallic oxides due to the positively charged metal surfaces.

However, despite previous assumptions, the adhesion between bacterial cells and iron oxide nanoparticles increases and is more adequate when the nanoparticles and bacterial cells are introduced on nonmetal surfaces due to an increased surface area in certain protein absorption. Utilizing different surfaces to grow the bacteria could further test this hypothesis and give insight into the limits of nanoparticles.

Conclusion

Nanoparticles are a nonbiological antibiofilm molecules that have been shown to inhibit the growth of S. Epidermidis. Utilizing nanoparticles could decrease the number of implantable devices related infections. Increasing the concentration of nanoparticles in *the S. Epidermidis*, decreased the growth of the bacterial growth. Through electrostatic interactions between the nanoparticles and bacterial cells, the Iron Oxide nanoparticles were able to penetrate the cell membrane and induce cell death by disrupting bacteria functions. Further work on testing the nanoparticles efficiency in inhibiting bacterial growth on different surfaces increases our knowledge in order to completely inhibit the formation of biofilms.

Acknowledgments

Thank You to Dr. Nikki Malhotra for her support and guidance, Dr. Zin Htway for assisting me in finalizing my methods and supervising my lab work done at CSUCI, along with Katherine Hutchinson for her guidance and supervision, along with CSUCI for funding and hosting my lab experimentations.

References

1. Bjarnsholt, T. (2013, May). The role of bacterial biofilms in chronic infections. Retrieved December 18, 2017, from https://www.ncbi.nlm.nih.gov/pubmed/23635385

2. Erik Unosson (2015) Antibacterial Strategies for Biomaterials https://www.researchgate.net/figure/277833092_fig2_Figure-2-The-five-stages-of-biofilm-development-Stage-1-Planktonic-free-floating

3. EN Taylor, TJ Webster - International journal of nanomedicine, 2009 The use of superparamagnetic **n**anoparticles for prosthetic biofilm prevention

4. Fisher Error Encountered. (n.d.). Retrieved March 23, 2017, from https://www.fishersci.ca/viewmsds.do%3FcatNo%3DI1163

5. Ion Anghel et. al 2014 https://www.sciencelab.com/msds.php?msdsId=9924050

6. Lebeaux, D., Ghigo, J., & Beloin, C. (2014). Biofilm-Related Infections: Bridging the Gap between Clinical Management and Fundamental Aspects of Recalcitrance toward Antibiotics.*Microbiology and Molecular Biology Reviews, 78*(3), 510-543. doi:10.1128/mmbr.00013-14

7. Loo, C., Young, P. M., Cavaliere, R., Whitchurch, C. B., Lee, W., & Rohanizadeh, R. (2013). Silver nanoparticles enhance Pseudomonas aeruginosa PAO1 biofilm detachment. *Drug Development and Industrial Pharmacy, 40*(6), 719-729. doi:10.3109/03639045.2013.780182

8. Mack, D., & Rohde, H. (2015). Structural basis of Staphylococcus epidermidis biofilm formation: Mechanisms and molecular interactions. *Front. Cell. Infect. Microbiol. Frontiers in Cellular and Infection Microbiology, 5*. doi:10.3389/fmicb.2015.00014

9. Parmar, S. (2013, August 5). Biofilms and Human Implants - MicrobeWiki - Kenyon College.

10. Sathyanarayanan, M. B., Balachandra Nath, R., Srinivasulu, Y. G., Kannaiyan, S. K., & Subbiahdoss, G. (2013). The Effect of Gold and Iron-Oxide Nanoparticles on Biofilm-Forming Pathogens. *ISRN Microbiology, 2013*, 1-5. doi:10.1155/2013/272086

11. Shunmuga Perumal, T. (2010). Biofilm Eradication and Prevention. doi:10.1002/9780470640463

12. Subbiahdoss, G., Sharifi, S., Grijpma, D. W., Laurent, S., Mei, H. C., Mahmoudi, M., & Busscher, H. J. (2012). Magnetic targeting of surface-modified superparamagnetic iron

oxide nanoparticles yields antibacterial efficacy against biofilms of gentamicin-resistant staphylococci. *Acta Biomaterialia, 8*(6), 2047-2055. doi:10.1016/j.actbio.2012.03.002

13. Thukkaram, M., Sitaram, S., Kannaiyan, S. K., & Subbiahdoss, G. (2014). Antibacterial Efficacy of Iron-Oxide Nanoparticles against Biofilms on Different Biomaterial Surfaces. *International Journal of Biomaterials, 2014*, 1-6. doi:10.1155/2014/716080

Katie Dang Project Timeline

June-August
Annotated 5 articles on the effects of Gemcitabine and Curcumin on Pancreatic Cancer cells.
Project Inquiry
Science Fair Chart

September 9
Practice writing e-mails to mentors
Rewrite 2 page project inquiry/proposal

September 15
Contacted Dr. Zin Htway for mentorship

September 17
CSUCI field trip and looked into the effects of nanoparticles on biofilm \
Also looked into cyanobacteria and phosphate removal

September 25
Focused primarily on biofilms on implantable medical devices
Worked on proposal
Looked into Iron Oxide as a possible substance to test on S. Epidermidis

October 1
Project Proposal Draft 1 Due

October 9
Revising draft 1
Reading and annotating more peer reviewed articles

October 10
Timeline due

October 10-16
Have at least 10 annotated articles (Read and annotate 2 each day)

October 17
Have project and methods finalized
Contact mentor for finalization

October 30
Have project approved by the IRB

November 15
Finish obtaining all materials for the project
Look over methods

December 1-10
Work on finalizing methods and looking over gap of knowledge

December 15
First draft of Academic Paper due

Before Winter Break
Discuss lab methods with mentor and start culturing S. Epidermidis

After Winter Break
Start testing
Test 3-5 times to finalize results

February 3
Registration deadline and paperwork due

February 15
Have all results finalized

March 15
Final paper due