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Effectiveness of AgNp Coatings on a PyC Surface in a Staph. Epidermidis-Infused Fluid Flow Model

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ABSTRACT

There is a medical need for a more effective heart valve coating that reduces both thrombotic potential and biofilm formation. The antimicrobial properties of silver nanoparticles have placed them under consideration for this application. The purpose of this study is to test the effectiveness of varying concentrations of silver nanoparticle (AgNP) coatings on a pyrolytic surface in a Staphylococcus epidermidis-infused fluid flow model. I hypothesized that as the concentration of the AgNP coating is increased, then there will be a decrease in the Staphylococcus epidermidis growth on the PyC surface because of the hydrophobic and antibiotic properties of silver nanoparticles. I produced a fluid flow model and tested the practicality of different concentrations of AgNps while running the fluid flow model by swabbing the plate for bacteria and incubating the bacteria for seven days. I found that the highest concentration of silver nanoparticles created the highest level of bacterial resistance and the lowest level of fluid retention when inside the model. Through these experiments, the unique properties of AgNp have been utilized to effectively create a more effective heart valve coating that both reduces the need for further medication after implantation, as well as the risk of biofilm formation on the prosthetic valve.

Introduction

Statement of Problem

Infectious Endocarditis is an infection of the heart's lining by microbial pathogens. Infectious Endocarditis (IE) is especially common in older patients and patients with preexisting heart conditions, drug users, and patients with poor gum health due to an increased amount of pathogens in the mouth that can reach the bloodstream (Mayo Clinic 2020). The tricuspid valve is most commonly affected by endocarditis and is the area for an estimated 50% of endocarditis valve infections. Yet, the largest risk factor for IE is the placement of an artificial valve, specifically mechanical valves. The usage of mechanical valves is seen as the best option for many patients, especially younger patients, due to their durability and longevity, as they can last up to 70 years (DrCiuffo 2021). The most common material used for mechanical heart valves is Pyrolytic Carbon (PyC) due to its low cost, easy manufacturing, and its biocompatibility. Mechanical valves last longer than 20 years whereas alternative valve options often need replacement in just 15 years. Yet, mechanical valves provide their drawbacks as well. The main downside of mechanical valves is the need for lifelong blood thinners. Heart valve implantation drastically increases turbulence, thus increasing thrombotic potential, or the potential for blood to clot, thus requiring long-term use of blood thinners, such as Warfarin (DrCiuffo 2021). All artificial heart valves also pose the heightened risk of IE. This risk comes from the increased chance of microorganisms forming biofilms on prosthetic valves. Biofilms are a slimy extracellular matrix that can form on synthetic heart valves due to their adhesive properties. These biofilms can develop on components of PyC heart valves and cause Prosthetic Valve Endocarditis, which can cause a vast range of cardiac issues. One of the most common organisms that

cause biofilms to form on PyC heart valves is Staphylococcus epidermidis. It can produce a multilayered biofilm on the outer surface of the synthetic valve, which could cause a wide range of cardiac issues, such as murmurs, heart failure, heart valve damage, and many other complications. Thousands of patients are affected by infectious endocarditis, and AgNp (silver nanoparticle) coatings may be a way to reduce this steady number (Mayo Clinic 2020).

Purpose

The purpose of this study is to test the effectiveness of different concentrations of silver nanoparticle (AgNP) coatings on a Pyrolytic Carbon (PyC) surface acting as a model heart valve, working against a Staphylococcus epidermidis-infected fluid flow model. The study aims to find an effective and affordable solution to prevent Prosthetic Valve Endocarditis. New studies show that superhydrophobic properties, where water droplets are repelled from the surface and roll off, would greatly reduce cell adhesion of droplets to the surface of the valve. This would greatly reduce the thrombotic potential or potential for blood clotting to occur, caused by the implanting of the synthetic valve. Silver nanoparticles (AgNP) have also been shown to have hydrophobic properties. This study aims to test a silver nanoparticle coating that is both hydrophobic and can be used to combat biofilm formation, as shown in my previous research, as well as recent studies concluded. (Cattaliotti 2009). I hypothesize that as the concentration of the AgNP coating is increased, then there will be a decrease in the Staphylococcus epidermidis growth on the PyC surface because of the hydrophobic and antibiotic properties of silver nanoparticles. Specifically, the highest concentration of AgNP would reduce the development of bacterial growth on the PyC surface at the greatest rate because it has the strongest antimicrobial ability, and the hydrophobic properties of the silver nanoparticles would help reduce cell adhesion at the greatest rate with the highest concentration. These tests may indicate that AgNp coatings should be used in medical practice in heart valve implantation.

Methods

Flow Model Construction

To produce a flow model that allows for visual comparison to the movement of blood through vessels, I developed an apparatus that simulates the flow of controlled, and bacterially-infused distilled water through a system of pipes with a PyC plate placed within the pipes allowing for bacterial collection. I placed a pump at the bottom of the piping system that pumped the fluid through the piping intended to represent that of a heart chamber, while the pipes carried the fluid throughout, similar to the actions of a blood vessel. The PyC plate resembled a model heart valve allowing fluid to flow through it into separate vessels, or in the model's case, the pipes.

Experimentation

After constructing the model, three plates were dip-coated in one of four AgNp concentrations, for a total of 12 dip-coated plates, making three trials for each concentration. The bacteria were then infused with a swab of agar cultured Staph. Epidermis bacteria, and mixed in an 8 mL solution of distilled water for each of the 12 trials. The fluid was then connected to the pump and the model was ready for valve implantation. The dip-coated plates were then placed into the model's tube, the bacterially infused fluid was connected to the pump, and the model was run using the pump for one unit of 8mL water with bacteria for each trial. The model was drained and then after the model dried (30-60s), the PyC plate was swabbed for bacterial collection. The bacteria were then swabbed onto a separate agar plate labeled with the plate's concentration as well as the trial number.

The cotton-tipped applicator was swabbed back and forth at 60-degree angles repeatedly to cover the entire plate and maintain a homogenous level of bacterial relocation for each plate. After placing the bacteria onto its agar plate, the plates were incubated for 7 days and growth/agar levels were recorded.

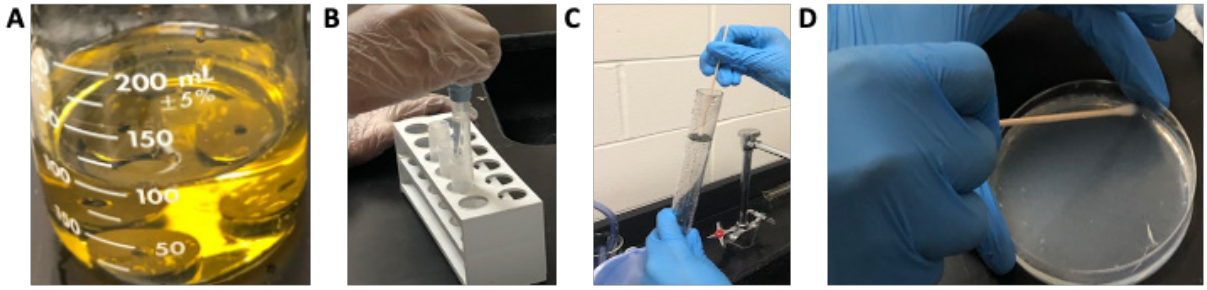

Figure 1. Images of Metholoogy. Dip coating process (A), bacteria mixing (B), swabbing after trial (C), and streaking onto agar plate (D).

Methods of Observation

Each day for 7 days of incubation, agar levels, as well as the colonial growth levels, were visualized, recorded, and photographed. The amount of fluid left on the plate after draining was noted during experimentation to show the strength of the AgNp's superhydrophobic properties at each concentration. This was noted by draining the model and viewing the amount of fluid left cohesively paired with the plate and its particles.

Results

Key:
[S1 - 50% colloidal silver(AgNp) concentration]
[S2 - 25% colloidal silver(AgNp) concentration]
[S3 - 5% colloidal silver(AgNp) concentration]
[S4 - 0% colloidal silver(AgNp) concentration (Control group)]

Table 1. Visual depictions of bacterial growth per day per concentration

Days after incubation	Bacterial growth level	Agar level remaining
Day 1	S1 - some bacterial growth (pre-colonial growth) S2 - some colonies forming S3 - vast colonial growth S4 - vast colonial growth	S1 - majority of agar remaining S2 - majority of agar remaining S3 - majority of agar remaining S4 - majority of agar remaining
Day 2	S1 - early forms of colonial growth S2 - complete colonial creation S3 - extensive colonial growth S4 - extensive colonial growth	S1 - majority of agar remaining S2 - slightly less agar remaining S3 - slightly less agar remaining S4 - slightly less agar remaining
Day 3	S1 - extensive colonial growth S2 - complete colonial growth S3 - complete colonial growth	S1 - slightly less agar remaining S2 - declining amount of agar remaining

	S4 - complete colonial growth	S3 - very little amount of agar remaining S4 - very little amount of agar remaining
Day 4	S1 - complete colonial growth S2 - complete colonial growth S3 - complete colonial growth S4 - complete colonial growth	S1 - increasingly declining agar levels S2 - almost no agar left in the plate S3 - nearly no agar remaining S4 - nearly no agar remaining
Day 5	S1 - complete colonial growth S2 - complete colonial growth S3 - complete colonial growth S4 - complete colonial growth	S1 - very little amount of agar remaining S2 - not much agar remaining S3 - even less agar remaining S4 - even less agar remaining
Day 6	S1 - complete colonial growth S2 - complete colonial growth S3 - complete colonial growth S4 - complete colonial growth	S1 - very little agar remaining S2 - very thin sheet of agar remaining S3 - very thin sheet of agar remaining S4 - very thin sheet of agar remaining
Day 7	S1 - complete colonial growth S2 - complete colonial growth S3 - complete colonial growth S4 - complete colonial growth	S1 - very thin sheet of agar remaining S2 - very thin sheet of agar remaining S3 - very thin sheet of agar remaining S4 - very thin sheet of agar remaining

Incubation day 1. A day after plating, the total bacterial growth was minimal. Bacterial growth increased as the silver concentration of each PyC plate decreased. The S1 concentration showed some bacterial growth. Vast colonies had not yet formed as they did for the S3 and S4 concentrations. The S2 concentration had some colonies forming, yet colonies were not as large or apparent as they were in the lower concentration groups (S3 and S4). The majority of the agar in the Petri dishes remained after the first day of incubation. It is also important to note that since the Staphylococcus Epidermis bacteria have an optimal incubation time of 24-48 hours, the first 2 days after incubation provide the most reliable information on the effects of AgNp coatings on Staph. Epidermis growth. As bacterial population size is influenced by fluctuations in growth rate, the next 5 days of incubation demonstrate the total difference in population size across the conditions.

Incubation day 2. The S3 and S4 concentrations experienced extensive colonial growth and the amount of agar left remaining in the S3 and S4 Petri dishes had significantly declined. The S1 plates showed early forms of colonial creation as bacteria was apparent throughout the plates, yet the majority of the agar remained in the

plates. The S2 plates showed colonial creation and the amount of agar remaining in the plates decreased since day 0.

Incubation day 3. Extensive colonial growth was present on the S1 plates, yet the majority of the agar remained on the plates. The S2 plates had little agar remaining, demonstrating that the bacteria had nearly used all of the agar for bacterial growth. The S3 and S4 concentrations had very little agar remaining as well and large visible colonies had formed throughout the plates.

Incubation day 4. The S3 and S4 concentrations once again showed the same trends in bacterial growth and agar availability, and the S2 plates had almost no agar left in the plate and the number of colonies left began to deteriorate due to a lack of agar remaining in the plate, similar to that of the S3 and S4 plates, yet still containing some colonial growth. The S1 plates had a majority of agar remaining in the plates. They also contain vast colonial growth, yet the colonies had not reached the size that they did on the other plates.

Incubation day 5. The S1 plates had a lower amount of agar left remaining in the plates than in previous days and large colonies began to form throughout the plates indicating larger bacterial growth. The S3 and S4 concentrations had nearly no agar left remaining in the plates and appeared as a thin sheet of agar and the number of colonies severely declined due to a lack of agar remaining in the plate. The S2 plates were very similar and did not have much agar remaining, yet had a greater portion of agar remaining than the S3 and S4 plates.

Incubation day 6. The S1 plates had a lower amount of bacterial colonies forming due to a declining amount of agar, signaling almost complete colonial decline, similar to that of all the other plates, yet taking much longer to reach this point. Each of the other plates had a very thin sheet of agar left in them, showing a near-complete colonial decline.

Incubation day 7. All plates had a very thin sheet of agar left in them, showing near-complete colonial decline at this point.

An important observation noted during the running of the flow model was the difference in fluid retention on the PyC plates across conditions. The S3 and S4 groups showed greater fluid retention than the S1 concentrations, thus indicating that the superhydrophobic properties of AgNps took place in reducing fluid retention on the PyC surface.

Table 2. Number of days for colonial growth and agar decline by concentration.

Concentration	# of days for colonial growth	# of days for noticeable agar decline
S1 (50%)	3	5
S2 (25%)	1	3
S3 (5%)	1	2
S4 (0%)	1	2

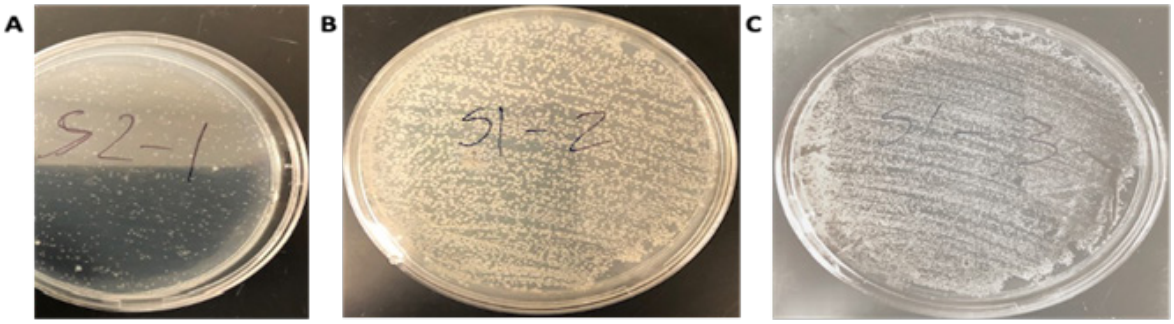

Figure 2. Agar plate growth on a day 2. Bacterial growth of condition S1 (50%) (A). bacterial growth of condition S2 (25%) (B). Bacterial growth of condition S3 (5%) (C). (Note labeling convention where "S#" indicates trial number and "-#" indicates condition number).

Discussion

Bacterial infection of medical devices remains an important issue. AgNps have superhydrophobic and antimicrobial properties. The experiments of this study support the hypothesis that increased concentration of AgNp coating results in a decrease in Staph. Epidermidis growth on the PyC surface. Specifically, the highest concentration of AgNP reduced the development of bacterial growth on the PyC surface at the greatest rate, indicating the strongest antimicrobial ability. The hydrophobic properties of the silver nanoparticles help reduce cell adhesion at the greatest rate with the highest concentration.

Overall, the S1 (50%) concentration showed the highest potential efficiency as a heart valve coating as it not only supported the practicality of using a silver nanoparticle heart valve coating but also showed the greatest resistance to bacterial growth in comparison to the three other concentrations. More testing should be done to determine the optimal concentration of AgNp coating on medical devices, acknowledging that this concentration may vary depending on the medical device and its use.

Another major result depicted by this experiment is how the superhydrophobic properties of the silver nanoparticles acted in response to the flow of the bacterial-infused model bloodstream, as indicated by the difference in fluid left on the PyC surfaces. AgNps have been known to have superhydrophobic properties yet real applications of this property have yet to be utilized in a variety of fields, such as cardiology. This property is specifically of interest to cardiology because it provides a way of reducing the thrombotic potential caused by the implanting of the synthetic valve. As valves are implanted, the thrombotic potential of the blood flowing through it greatly increases due to the synthetic components of the valve as well as the invasiveness of valve implantation and turbulence created by the edges and structure of the valve. These experiments indicate that the superhydrophobic and antibacterial properties of AgNp coatings can be effectively utilized to design a implantable heart valve device with a reduced risk of biofilm formation and reduced need for further medication after implantation such as anticoagulants.

One important limitation of this study is that toxicity of AgNps was not tested as no translational model was used. Previous studies have indicated silver nanoparticles can be toxic or have negative effects in humans when used as a coating (Tozzi 2001). However, there are a limited number of these studies and more extensive testing in translational models is needed. Another limitation is that these experiments were conducted with a basic model of circulatory and valve function. An important next step in determining the appropriateness and effectiveness of AgNp coatings on medical devices is to assess both toxicity and efficacy in advanced translational models of circulation. Further testing and optimization may reduce other patient medications as well as the risk of valve complications such as Infectious Endocarditis, heart attack, stroke, and others.

Conclusion

As demonstrated by this study and others, silver nanoparticle coatings on medical devices reduce thrombotic potential and biofilm formation. This study has shown that the highest concentration of AgNP reduced the development of bacterial growth on the PyC surface at the greatest rate because it has the strongest antimicrobial ability. This study also shows that the hydrophobic properties of the silver nanoparticles helped reduce cell adhesion at the greatest rate with the highest concentration. The superhydrophobic and antimicrobial properties of silver nanoparticles position them to be effective deterrents to thrombosis and biofilm formation in implanted medical devices.

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