May 5th, 12:00 AM

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Safety and Efficacy of Silver-Coated Biomaterials *in vivo*

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Abstract

Overtreatment and overuse of antibiotics in healthcare and agricultural settings have contributed to the selective pressure on bacterial strains to develop resistance. Resistance can develop as a result of mutations and subsequent resistance genes that allow bacteria to survive against antibiotics. Novel silver-oxide coatings were developed and were previously demonstrated to prevent adhesion of gram-negative bacteria (*Escherichia Coli* and *Pseudomonas Aeruginosa)* to the disc, but did not prevent gram-positive bacterial adherence (*Streptococcus Aureus*). In order to determine whether the silver-oxide coatings are bacterial static and may be preventing progression to biofilm formation, *in vivo* analysis of *S. Aureus* attached to discs was performed. Results show that the stages of biofilm formation and infiltrating immune cells were identifiable by scanning electron microscopy (SEM). These data will be used as the foundation to compare to *S. Aureus* and other gram-positive bacteria attachment to coated discs.

Background

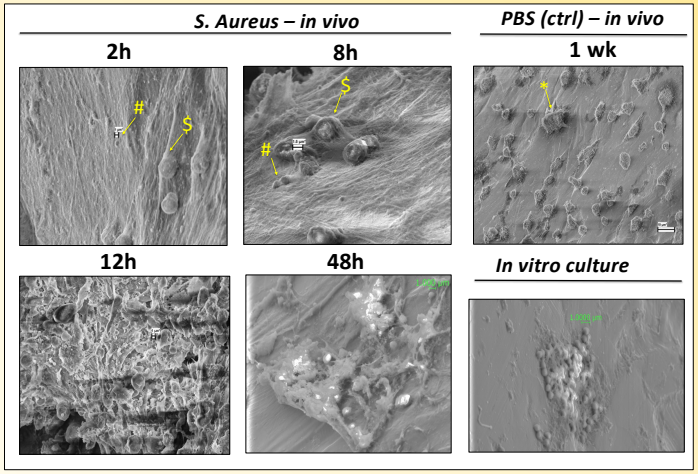
The use of antibiotics to treat microbial infections has been a mainstay of clinical practice since the mid 1900s.[1] Overtreatment and overuse of antibiotics in healthcare and agricultural settings have contributed to the selective pressure on bacterial strains to develop resistance. Resistance can develop as a result of mutations and subsequent resistance genes that allow bacteria to survive against antibiotics.[2] Prevention of infections is of paramount importance in the healthcare community. A prominent source of infection is from biomedical devices that have the potential to provide a surface for bacterial growth and adherence.[2] Studies estimate that prosthetic joint infections following knee and hip arthroplasties may occur in 1.5%-2.5% of patients. Further, the mortality rate of such infections may be up to 2.5%.[3]

The Demarest Lab's previous study demonstrated that unique silver-oxide coatings prevent adhesion of gram-negative bacteria (*E. Coli*, *P. aeruginosa*), but not adhesion of gram-positive bacteria (*S. Aureus*) to titanium discs *in vivo*. However, it is possible that the coatings are bacteriostatic against *S. Aureus* and could still be efficacious. Therefore, the goal of my project was to evaluate the adhesion of *S. Aureus* to uncoated titanium discs *in vivo* in order to confirm the different stages of biofilm formation are occurring in our model, as well as identify immune cells. These results will be used as the foundation to determine whether silver-oxide coatings are efficacious against *S. Aureus in vivo.*

Results

It was previously shown that *E. Coli*, *S. Aureus*, and *P. Aeruginosa* growth could be prevented *in vitro* by silver-oxide coated discs. It was also shown that after titanium discs were inserted into mice and bacteria injected, the bacteria would "home' and attach to the disc. Discs could be recovered from the animal and bacteria cultured in vitro and quantified; S. Aureus cultures were still able to attach to the discs while E. Coli and Pseudomonas culture were incapable (data not shown). The goal of this project was to identify the cells attached to the titanium disc when mice were infected with *S. Aureus* and determine the stages of biofilm formation in our model system.

We were also able to detect immune cells (neutrophils) attached to the disc in control samples[*]. We were able to detect individual *S. Aureus*[#], as well as ultrastructure[$] and biofilm formation. Of note, in vivo structures are similar to ultrastructure and biofilm formation detected on the discs growing with *S. Aureus in vitro*.



Conclusions

Our *in vivo* system is appropriate for developing *S. Aureus* biofilms. We were able to identify and distinguish between immune cells and *S. Aureus* in various stages of biofilm formation. These results will be used as the foundation to compare to *S. Aureus* attachment to coated discs and help the Demarest Lab to determine whether silver-oxide coatings are functioning in a bacterial static manner towards *S. Aureus,* or whether they are not efficacious against *S. Aureus* and/or other gram-positive bacteria. If the coatings function in a bacterial static manner, then they still may be efficacious in treatment. If they are not bacterial static, then the coatings will need to be reformulated. However, these preliminary data give the lab the tools needed in order to determine what is occurring with *S. Aureus* and other gram-positive bacteria directly on the surface of the biomaterial.

Materials & Methods

Disc Insertion and *S. Aureus* Injection

All animal protocols were approved by the RowanSOM IACUC. Mice wer ear tagged and temperature and weight recorded. Mice were the sedated using isoflurane and a small portion of their lower back flan was shaved using an electric razor. Mice were maintained unde anesthesia and injected with a dose of buprenorphine. A small incisio was made subcutaneously on the bank flank and uncoated titanium disc were inserted. The incision was closed using surgical staples an luminescent *S. Aureus* was injected subcutaneously into the surgica pocket. Mice were carefully monitored for two days.

Disc Recovery

Mice were euthanized and titanium discs were removed. Two of the disc were placed in LB media and allowed to grow overnight at 37C to confirr the presence of luminescent *S. Aureus.* The remaining 5 discs wer treated with various fixation solutions.

Fixation and Imaging

Discs were placed in 25% glutaraldehyde, 4% paraformaldehyde, 10% buffered formalin, and glutaraldehyde/paraformaldehyde overnight. Th next day samples were dehydrated in a series of ethanol washes fror 50% - 100% ethanol, dipped in HDMS solution, and then allowed to dr Samples were then imaged using scanning electron microscopy.

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Acknowledgements

This work was supported by the New Jersey Health Foundation, RowanSOM SMRF and PREP programs and RowanGSBS.