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Safety of Silver-Oxide Coated Biomaterials in Mice

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Abstract

It has been demonstrated that silver-oxide coatings designed by our collaborators are able to prevent *E. Coli* and *P. Aeruginosa* attachment to biomaterials *in vivo.* These findings demonstrate that such coatings show promise in preventing the development of biofilm on biodevices. However, it is unknown if the use of silver-oxide in this fashion is toxic *in vivo*. The goal of this project was to determine whether our silver-oxide coatings are safe to use *in vivo.* To assess the toxicity of our silver-oxide formula, mice were implanted with either silver-oxide coated titanium discs or uncoated titanium discs. Blood samples were drawn at pre-determined time points in order to determine AST and ALT levels via ELISA assay. Preliminary results demonstrate no acute liver injury after 3 months with the discs. However, it appears as if silver is accumulating in tissues over time. Histological analysis at one year shows evidence of simple steatosis in livers. While our group is continuing to investigate the safety and efficacy of these silver-oxides coatings *in vivo*, preliminary data shows that they may have some toxicity and the silver-oxide formula may need to be altered and retested.

Background

Silver is a material that has been used in medicine for hundreds of years to promote wound healing. Silver oxidizes when exposed to fluid and its ions act as antimicrobial agents. Because of the recent increase in healthcare acquired antibiotic resistant bacterial strains[1,2], the use of silver coatings for medical devices is being explored extensively. Because of its low solubility, certain components in the body can act as "sponges" for silver ions, with ionization in the local microenvironment being ideal[3]. Biofilm formation on implanted materials or biodevices is a common issue causing severe health consequences in patients including revision surgery[1]. This biofilm accumulation may be able to be combated through silver antimicrobial capabilities.

Dr. Demarest and her colleagues have developed a sputtering technique that can coat most solid materials with silver-oxide and control the elution rate by altering the sputtering formula[4]. These synthesized coatings also have three-orders-of-magnitude greater silver ion elution rates when compared to high-surface-area silver nanoparticles. Our collaborators have developed silver-oxide coatings that can prevent *E. coli*, *S. Aureus*, and *P. Aeruginosa* growth *in vitro*[4]. This silver oxide-coating is also effective against *E. Coli* and *P. Aeruginosa* attachment *in vivo*[5]. These findings demonstrate that silver-oxide coatings show initial promise in preventing the development of biofilm on biodevices. However, it is unknown if use of silver oxide in this fashion is toxic *in vivo*. Thus, the focus of this project was to determine if the silver oxide coatings are safe to use *in vivo.*

Results

In order to determine whether liver damage is occurring when mice are implanted with silver-oxide coated discs, AST and ALT Elisa assays were performed on serum. As a positive control for the assay, acetaminophen was administered to control mice in order to increase AST and ALT levels (Fig. 1). After 1 wk and 3 months post-insertion of discs the AST and ALT levels were not significantly increased in mice with silver-oxide coated discs compared to mice with uncoated discs (Fig 2). ICP-MS analysis of tissue at 1 wk post-insertion shows significant silver accumulation in some tissues and an increasing trend in many other tissues (Fig 3). Histological analysis demonstrates simple steatosis in the livers of mice with silver-oxide coated discs inserted compared to control mice with uncoated discs inserted (Fig 4).

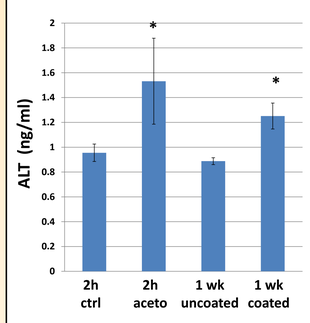

Fig. 1. Trial ALT assay. A trial ALT was performed at 1 wk using an acetominophen treated mice as a control in triplicate. Average values of the triplicate samples were graphed. ANOVA analysis was performed in order to determine significance. *p-value < 0.05

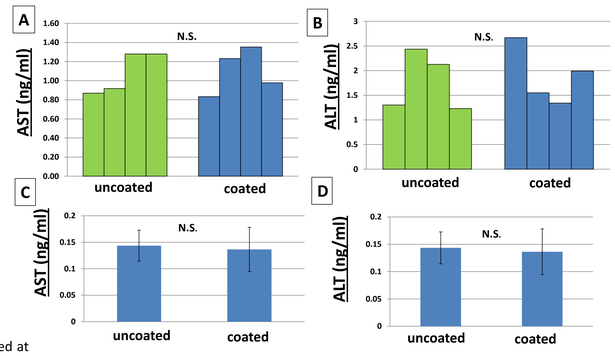

Fig. 2. ALT levels do not increase in mice 1-wk after disc insertion. AST and ALT were performed on serum for mice inserted with an uncoated or silver-oxide coated disc 1-wk post-insertion. ANOVA analysis was performed in order to determine significance. *p-value < 0.05; N.S. = no significant change

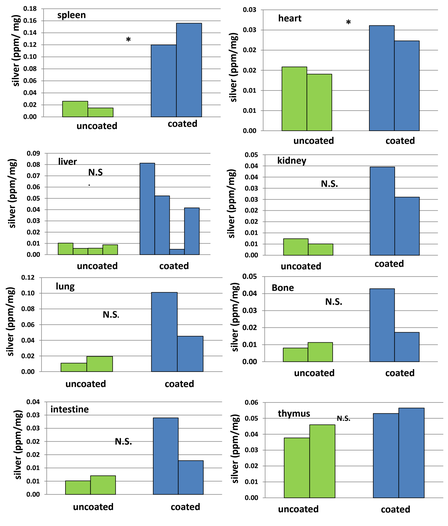

Fig. 3. ICP-MS analysis to determine silver accumulation in tissue. Mice were euthanized the indicated tissue was harvested 1-wk after insertion of coated or uncoated discs. Tissue was boiled in nitric acid and analyzed by ICP-MS to determine silver ppm/mg.

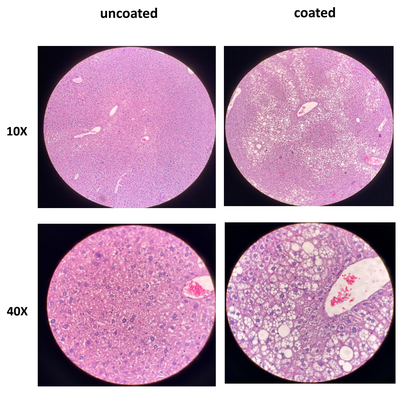

Fig. 4. Histological analysis to determine evidence of tissue injury. Mice were euthanized the indicated tissue was harvested 1-yr after insertion of coated or uncoated discs. Tissue was fixed in 10% neutral buffered formalin, processed, embedded in paraffin and sectioned at 5 microns. Sections were mounted on slides and H&E staining performed. Images were acquired using 10X and 40X objectives.

Materials & Methods

Disc Implantation
All protocols were approved by RowanSOM IACUC. The mice were placed in the induction chamber under anesthesia (isoflurane) and then hair was removed from the bank flank of the mouse with an electric razor. The mice were then re-anesthetized and placed into a nose cone and injected with buprenorphine in the cephalad region of the surgical site. An incision was created caudally forming a surgical pocket and the titanium disk (coated or uncoated) was inserted. The incision was then closed using surgical staples and mice were monitored until they were conscious.

Histological Analysis
Mice were euthanized using CO_2 inhalation and tissue was harvested (spleen, kidney, liver, intestine, thymus, heart, lung and bone). Tissue samples were fixed, processed, embedded, sectioned (5 microns) and stained with Hematoxylin and Eosin.

Silver Content Analysis
Mice were euthanized using CO_2 inhalation and tissue was harvested (spleen, kidney, liver, intestine, thymus, heart, lung and bone). Each tissue was weighed and 0.5-1 mg of tissue was added to a test tube containing 12 ml of 70% nitric acid. Samples were boiled at 150°C until tissue was liquified and the reaction equalized. Samples were diluted by adding 13 ml of ddH_20 and analyzed by ICP-MS to determine silver content.

AST/ALT Assay
Blood was collected (400 ul) from the facial vein of mice and allowed to coagulated for 2 hours at room temperature. Serum as then used to perform the AST and ALT Elisa assay according to the manufacturer's instructions.

Conclusion

Preliminary data suggests that although there is some evidence of silver accumulation in organs, however, there is no evidence that liver function is compromised. In contrast, histological analysis at later time points shows simple steatosis of the liver at the 1-year time point. Currently, ICP-MS analysis of 1-year tissue is being performed and AST/ALT assays are being repeated for short and long time points. Further histological analysis using autometallography is also being performed to determine the presence of silver in tissue and to complement the ICP-MS analysis.

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Acknowledgements

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