Characterization of the Antimicrobial Capabilities of Silver Carboxylate Against Persister Cells and Biofilms

Josue Marquez[1], Carolyn Lai[2], Jorge Garavito[1], Anna Rezk[1], Makenna Mette[2], Drew Clippert[2], Sai Allu[2], Liam Connolly[1], Christopher Born[1], Valentin Antoci[2], Dioscaris Garcia[1]
[1]Brown University, Warren Alpert Medical School, Providence, RI; [2]University Orthopedics, East Providence, RI
Presenting Author: Josue Marquez

Disclosures: Christopher Born owns stock in Biointraface, Inc.; Owns equity in BI Medical, LLC. ; Dioscaris Garcia owns equity in BI Medical, LLC.

INTRODUCTION:
Due to poor antibiotic stewardship and misuse, hospital acquired infections (HAIs) and surgical site infections (SSIs) by antimicrobial-resistant (AMR) pathogens are an accelerating threat to the US healthcare system. In 2009, a comprehensive study reported that approximately 2 million patients are affected by HAIs annually, costing the US healthcare system over $28 billion, and over 70% of the culprit bacteria were drug resistant. Furthermore, the stagnant discovery of new antibiotics has made our battle against AMR pathogens increasingly challenging and calls for the development of novel approaches to combat these infections. Organometallics with antimicrobial capabilities have emerged as a possible solution, specifically silver due to its multimodal bactericidal properties. To harness silver's capabilities, we have developed a silver carboxylate (AgCar) compound released via a titanium dioxide-PDMS (TiO_2-PDMS) matrix. Previous experiments in the Weiss lab have demonstrated AgCar/TiO_2-PDMS capability to penetrate the outer membrane of gram-negative bacteria.[1,2] Thus, in this study, we assess AgCar's ability to disperse clinically isolated Methicillin-Resistant *S. aureus* (MRSA) strains MW2 and VRS1 biofilms and negatively affect the viability of their persister cells.

METHODS:
Persister cells
To generate persister cells, MRSA MW2 strain was grown to stationary phase overnight, then exposed to 20X antibiotic minimal inhibitory concentration (MIC) of gentamicin and incubated for 24 hours to ensure the generation of persister cells. To generate a dose-response, 1X and 10X silver carboxylate delivered by the TiO_2:PDMS matrix were added into wells containing the persister cells and incubated for 24, 48, and 72 hours. Untreated cells served as the negative control, and 100% silver carboxylate without the matrix served as the positive control. Aliquots were removed at 24, 48, and 72 hours, serially diluted, and plated on tryptic soy agar for 24 hours to determine the number of cells at each condition.

Biofilm Inoculation, Staining, Imaging and Analysis
Single MW2 and VRS1 colonies were cultured overnight, and then put into 12-well plates at a concentration of $2x10^8$ CFU/mL. Sterile filter discs were placed in each well to provide texture for bacterial adherence and biofilm formation. After ensuring biofilm growth, bacteria were treated with experimental conditions (1X, 10X, 30X, 300X AgCar), positive control of 100% AgCar, and negative controls of untreated biofilms and TiO_2-PDMS matrix-only. Visualization of the biofilm components through confocal light scanning microscopy (CLSM) required staining with various dyes. Data was transferred to the software Image J (NIH) which was used to determine the pixel intensity of each of the dyes, construct a three-dimensional representation of the fluorescence pixel plot, and calculate the percent coverage of each of the biofilm components on the filter disc samples.

RESULTS:
Persister Cells Killing Assay
The lower the number of CFUs, the more successful the condition was at preventing the repopulation of bacterial cells by persister cells. In 1X AgCar, there was a significant reduction in CFUs at 24 hours of exposure when compared to the negative control (1X: $60x10^8$ CFU/mL, neg. control: $120x10^8$ CFU/mL). However, there was no significant difference in reduction at 48 and 72 hours of exposure between 1X and negative control. On the other hand, 10X AgCar demonstrated significant efficacy at 24, 48, and 72 hours of exposure, with a log6 reduction in CFUs at 72 hours. Finally, 100% AgCar almost completely eradicated MW2 persister cells.

Biofilms
Images were rendered depicting 3D fluorescence pixel plot of MW2 and VRS1 biofilms stained with SYPRO (red) for proteins, TOTO-1 (green) for eDNA, and Concanavalin A (blue) for EPS. Negative controls of untreated cells (A and H) and matrix only treatments (G and N) are consistent with high levels of purple and yellow fluorescence due to the overlapping presence of proteins, EPS and eDNA, signifying an untreated biofilm as it contains all three main components. Positive control of 100% AgCar (F and M), exhibited a general decrease in all fluorescence, with lingering blue and red fluorescence indicated remnant biofilm proteins and EPS, but without green eDNA signal. Experimental conditions B-D and I-K exhibited a dose-dependent decrease in fluorescent signals of all pigments, with 30X (D and K) having the greatest loss of blue fluorescence, indicating the most effective disruption of biofilm EPS.

DISCUSSION:
The assay involving persister cells strongly suggests that silver carboxylate can successfully kill persister cells, with 10X AgCar almost eradicating the repopulation of bacteria. By successfully killing all the remaining persister cells, reinfection after the removal of antibiotics could be prevented. The biofilm confocal images demonstrated a trend of increasing biofilm dispersal with increasing concentrations of AgCar treatment for both MW2 and VRS1 bacterial strains, which was expected as a higher concentration of AgCar contained within the matrix would lead to a greater release of silver ions and elicit a more prominent effect on the biofilms. There was also a noticeable impact on the amount of DNA and proteins, suggesting that the silver-based organometallic formulation was capable of damaging both the replicative ability and the functional structure of the biofilms and effectively reducing the presence of existing biofilms. The inconsistent results in the 300X fluorescence condition could be due to sample limitations that could be remediated by having a larger sample number from different areas of filter disc and/or multiple discs that were treated.

SIGNIFICANCE:
With the ongoing antibiotic resistance crisis, silver carboxylate is a potential and emerging solution with the ability to decrease rates of infection and mortality, combat both resistance and tolerance, and improve treatment of prevailing multidrug- and pan-resistant bacterial strains.

REFERENCES
1. Garcia D, Gardezi M, Suliman Y, Glasser J, Spake CS, Barrett C, Berns E, Jenkins D, Evans A, Cohen E, Eberson C, Hayda R, Daniels A, Green A, Owens B, Antoci V, Born CT. Fluorescent-conjugated antibodies as rapid ex vivo markers for bacterial presence on orthopedic surgical explants and synovium: A pilot study. J Orthop Res. 2021 Feb;39(2):299-307. doi: 10.1002/jor.24924. Epub 2020 Dec 2. PMID: 33225467.
2. Garcia D, Gilmore A, Berns E, Spake C, Dockery DM, Vishwanath N, Glasser J, Antoci V, Daniels A, Born CT. Silver carboxylate and titanium dioxide-polydimethylsiloxane coating decreases adherence of multi-drug resistant Serratia marcescens on spinal implant materials. Spine Deform. 2021 Nov;9(6):1493-1500. doi: 10.1007/s43390-021-00380-w. Epub 2021 Jun 25. PMID: 34173223.