Characterization of Silver Carboxylate's Microbicidal Mechanisms in Multi-Drug Resistant *Serratia marcescens*

Anna Rezk[1,2], BA; Jorge Garavito[1,2], BS & BA; Josue Marquez[1,2], BS; Carolyn Lai[1], BS; Alyssa Steinbaum[1], BS; Blaire Williams[1]; Makena Mette[1,2], BS; Christopher Born[1,2], MD; Dioscaris Garcia[1,2], PhD
[1]Diane Weiss Center for Orthopaedic Trauma Research, Rhode Island Hospital, RI, USA; [2]Warren Alpert Medical School of Brown University, RI, USA;
Presenting Author: anna_rezk@brown.edu

INTRODUCTION: There is an increased emergence of multi-drug and pan-resistant bacteria due to the poor stewardship and overuse of antimicrobials which is a paramount threat to the US healthcare system. As a result of these drug resistant bacteria, there are increased rates of hospital acquired infections (HAIs) and surgical site infections (SSIs) leading to millions of people becoming ill with antibiotic-resistant bacteria each year and billions of dollars in healthcare costs. The static progression and discovery of new antibiotics prompts an investigation into the development of novel antibiotic-independent antimicrobials. Much research has therefore pivoted to metallic with antimicrobial properties like silver due to its multi-modal microbicidal properties. However, despite silver's recorded multi-modal microbicidal properties, all studied formulations are subject to uncontrolled and unpredictable release of silver ions which can trigger human cell cytotoxicity. Nonetheless, silver carboxylate (AgCar) released via a titanium dioxide/polydimethylsiloxane (TiO_2:PDMS) matrix has emerged as a promising antimicrobial silver formulation due to its matrix chemistry which releases silver carboxylate in a controlled and predictable manner to decrease human cell cytotoxicity. However, the exact multi-modal microbicidal mechanisms of silver carboxylate in a TiO_2-PDMS matrix are still not characterized. Thus far, we have demonstrated that silver carboxylate is able to be a very potent and safe antimicrobial when compared to last-resort antibiotics in primary cell lines involved in wound healing, can penetrate the outer membrane of the Gram negative bacteria, disperse biofilms, and kill persister cells. This study continues that work to characterize silver carboxylate in a TiO_2-PDMS matrix's microbicidal mechanisms in multi-drug resistant, gram negative *Serratia marcescens*. This study establishes silver carboxylate's ability to generate reactive oxygen species (ROS) and decrease catalase production in multi-drug resistant, Gram negative *Serratia marcescens.*

METHODS:
Serratia marcescens was grown overnight to $1x10^6$ CFU/mL at 37℃. Bacteria were plated in 96 well plates and exposed to a gradient of AgCar-TiO_2-PDMS ranging from 1x to 150x for 6 hours. 10nm and 30nm nanoparticle silver as well as 100% silver carboxylate with no TiO_2:PDMS served as positive controls. 1% triton X and titanium dioxide/PDMS vehicle-only served as negative controls. To detect the release of ROS, bacteria were lysed and read via colorimetry for levels of hydroxylamine oxidation using the Cellular ROS Assay Kit (Abcam, Cambridge, MA). To detect the production of catalase, bacteria were administered hydrogen peroxide and read via colorimetry for levels of water and oxygen using the Catalase Activity Assay Kit (Abcam, Cambridge, MA).

RESULTS:
ROS: In *Serratia marcescens*, a 1x-30x concentration of AgCar produced a fold change of 1, or double the amount of ROS produced when compared to the cell blank ($p< 0.05$). AgCar 100x produced a 2 fold change ($p<0.05$) and AgCar 150x produced a 3 fold change ($p<0.05$) in ROS release, when compared to the cell blank. In the Gram positive organism, Methicillin-sensitive *Staphylococcus aureus*, AgCar 1x-150x produced a fold change of 3, or a 300% increase in ROS production, when compared to the cell blank.

Catalase: In *Serratia marcescens*, there were 0.08x, 0.07x, 0.98x, 0.08x, and 0.07x fold changes for 1x, 10x, 30x, 100x, and 150x AgCar TiO_2-PDMS matrices, respectively ($p<0.05$ for all). Therefore, there was an overall decrease in catalase production when compared to cell blank.

DISCUSSION:
AgCar TiO_2-PDMS matrices induced statistically significantly more ROS and concurrently less catalase as compared to nanosilver in *S. marcescens*, providing evidence for its bactericidal activity. The release of ROS, indicating bacterial oxidative stress and the decreased production of catalase (an antioxidant enzyme) in the Gram negative bacteria, *S. marcescens*, further supports the hypothesis that AgCar can penetrate the outer membrane of Gram negative bacteria and provides evidence of its mechanism of action.

SIGNIFICANCE/CLINICAL RELEVANCE:
Given the ROS generating and catalase inhibiting microbicidal mechanism of action of AgCar TiO_2-PDMS, AgCar TiO_2-PDMS has the potential to be utilized as an antimicrobial, especially synergistically with other antimicrobials.