The Development of a Broad Spectrum Antimicrobial Device Coating for the Prevention of Resistant Strain Bacterial Infections

INTRODUCTION
Advancements in medical technology and patient care have resulted in the longer lifespan and more active lifestyles of the aging population. Over the past two decades, the number of total joint replacements performed in the United States has risen drastically. It is projected that by the year 2030 more than 3.5 million total hip and knee replacements will be performed annually in the United States. Total joint replacement (TJR) technology has improved the quality of life of many Americans; however, these procedures are not without risk. Periprosthetic joint infections pose a serious procedural complication. These infections currently occur at a rate of 1-2 percent in primary procedures. Without improved treatments, it is estimated that perioperative related joint infections would exceed 35,000 per year in the United States of America by 2030.

Antibiotic resistant pathogens create a constant challenge for orthopaedic surgeons. Methicillin-resistant Staphylococcus aureus (MRSA) is responsible for an increasing number of surgical site infections. Due to these pathogens possessing the ability to change their resistance patterns to counteract antibiotics, it is imperative that an alternative method of treatment, one that circumvents bacterial resistance, is developed.

One solution to antibiotic resistance may lie with ceragenins, a class of compounds with antibacterial activities equipotent to those of antimicrobial peptides. Their broad-spectrum bactericidal capabilities make them especially useful against MRSA and other multidrug resistant pathogens. Cationic Steroidal Antimicrobial-13 (CSA-13), a ceragenin, will be used in this experiment to determine if it is effective in eliminating planktonic bacterial infections in vitro.

The goal of this work was to develop a polymer released antimicrobial device coating for the prevention of perioperative related TJR infections. It was hypothesized that the CSA-13 antimicrobial would exhibit potent bactericidal activity against MRSA in vitro. To test the hypothesis, a polymer coating loaded with the broad-spectrum CSA-13 was developed to challenge 5x10^8 colony forming units (CFU/mL) of MRSA in an in vitro model.

METHODS
Combination coatings of 0, 16, 18, and 20% w/w were fabricated by homogenizing CSA-13 with 1 mL of naphtha and 2 mL of a one part-room temperature vulcanizing (1-rtv) silicone polymer for 30 minutes. Specially designed titanium plugs were dip coated in the polymer to uniformly coat regions A and C (Figure 1). The device was rotated on an electric wheel at 13 rpm to ensure uniform coating thickness and was allowed to cure for seven days under ambient conditions.

A clinical strain of MRSA (ARUP 1709) was subcultured and grown in 5 mL of Brain Heart Infusion (BHI) broth for 2 hours at 39°C in a 200 rpm shaker/incubator to stimulate bacterial growth. A 1 mL sample was transferred to a beaker containing 100 mL of BHI and allowed to grow under agitation in the incubator for another 4 hours. The cells were washed, suspended in 10 mL of Phosphate Buffered Saline (PBS), and the bacterial concentration was determined through serial dilution. The bacterial suspension was diluted to 5 x 10^8 CFU/mL.

A control centrifuge tube was created by adding 1.2 mL of the bacterial suspension to 28.8 mL of BHI. Bare titanium and the 0, 16, 18, and 20% w/w coated bone plugs were placed in centrifuge tubes with an inoculum of 5x10^8 CFU MRSA. Experimental samples were then placed on Tryptic Soy Agar plates, incubated at 39°C overnight, and shaken at 200 rpm. The samples were collected at 0, 1, 2, 4, 8, and 24 hours and Dey-Engley Neutralizing broth (D/E broth) was used in serial dilution to deactivate CSA-13.

RESULTS
Scanning electron microscopy with EDX revealed an apparent uniform distribution of CSA-13 within the polymer coating. In vitro experimentation with 16, 18, and 20% CSA-13 demonstrated greater than a seven-log reduction in bacterial colonies within two hours. No bactericidal potential was demonstrated with the bare titanium plug or the pure silicone polymer coating (Figure 2).

DISCUSSION
The goal of this study was to establish a combination device coating capable of eliminating infections originating from MRSA. It was hypothesized that the polymer released CSA-13 would demonstrate potent bactericidal activity against MRSA in vitro. The results of this study supported the hypothesis, as it was observed that the concentrations of 16, 18, and 20% all possessed bactericidal activity. The 18% concentration possessed the most consistent bactericidal activity at the lowest concentration, exhibiting greater than 99% bacterial elimination in 8 hours. These promising results suggest that this system may have the potential to prevent perioperative orthopaedic device related infections when used as a combination coating.

Future work will focus on the characterization of the elution rate of CSA-13 from the polymer coating. In vivo studies will be conducted to evaluate the efficacy of the plug coated with 18% w/w of CSA-13 when used as an orthopaedic device coating on a TJR device.

SIGNIFICANCE
The novel broad-spectrum polymer released CSA-13 antimicrobial has demonstrated the ability to eliminate a heavy bacterial burden when used as an orthopaedic device coating in vitro. This combination device coating shows promise for preventing perioperative device related infections in vivo.

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