INTRODUCTION: Treating recurrent orthopaedic infections is a daunting challenge due to the possible presence of intracellular bacteria. A novel approach to eliminate intracellular pathogens is via cationic antimicrobial peptides (CAMPs). Unlike conventional antibiotics, amphiphilic CAMPs including cathelicidin LL-37 (Fig. 1) are comprised of hydrophobic and hydrophilic residues aligned on opposite sides of the peptide, facilitating their easy penetration through cell membranes and killing of intracellular pathogens. We hypothesized that cathelicidin LL-37 can be effective in eliminating intracellular bacteria. The objective of this study was to determine the efficacy of LL-37 against conventional antibiotics on *Staphylococcus aureus* (*S. aureus*), the main cause of orthopaedic infections, and to identify potent CAMPs for infection prevention.

METHODS: A clinical strain of *S. aureus* (SA1004) was obtained from a patient’s chronic wound and was studied in its log phase (exponential bacterial growth). Experiments were conducted using a 12-well plate. 4×10³ osteoblast cells (UMR106) were seeded in each well and the plate was incubated at 37°C for 36 hours. For bacterial culture, five *S. aureus* colonies were added to 5 mL of tryptic soy broth (TSB) and incubated at 37°C for 16 hours. 1 mL of overnight culture was added to 20 mL fresh TSB and was placed in a 37°C water-bath rocker for 2.5 hours for bacteria to enter log phase. Optical density of the inoculum was measured to determine bacterial concentration. Osteoblast cells were infected with bacteria for 2 hours at 500:1 *S. aureus* to osteoblast ratio. 50 µg of lysostaphin was added to each well and incubated for 2 hours to eliminate extracellular bacteria. LL-37, cefazolin, clindamycin, or plain media (control) was then added to the respective wells. After 2 hours, osteoblasts were lysed with 0.1% triton and intracellular *S. aureus* was plated on blood agar plates. Dilutions 10⁻¹, 10⁻², and 10⁻³ were made. The plates were inverted and incubated at 37°C for 18 hours. Colonies were counted using an Acolyte colony counter made by Symbiosis USA. In addition, the efficacy of LL-37 against extracellular *S. aureus* was examined by treating *S. aureus* with LL-37, lactoferrin-B, doxycycline, and cefazolin, for 30 min at various molar concentrations ranging from 10 nm to 100 mM under the same experimental conditions.

RESULTS & DISCUSSION: We found that LL-37 was effective in eliminating intracellular *S. aureus*. More bacteria were killed with increasing peptide concentration and LL-37 of 100 µM completely eliminated intracellular bacteria (Fig. 2). Compared to conventional antibiotics such as cefazolin and clindamycin, LL-37 was more potent in eliminating intracellular *S. aureus* (Fig. 3). Note that, in the literature, clindamycin has very good intracellular killing efficacy against a variety of bacteria including *S. aureus*. Moreover, our kinetic studies showed that LL-37 was effective in killing *S. aureus* within 2 hours and maintained its antibacterial activity for at least 24 hours (Fig. 4). In addition, we found that LL-37 was more potent than conventional antibiotics in eliminating extracellular *S. aureus* and it was potent in killing bacteria even at nano-molar concentrations (Fig. 5).

CONCLUSIONS: In the current study, *S. aureus* and *S. aureus* internalized within osteoblasts were treated with LL-37 and conventional antibiotics. Our results indicate that 100 µM concentration of LL-37 could completely eliminate intracellular bacteria within just 2 hours, and LL-37 was more potent in killing both intra- and extracellular *S. aureus*. This study is clinically significant because LL-37 could be a novel treatment to prevent infection recurrence and could be potentially the answer to bacterial resistance to conventional antibiotics.

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