

## Poly (d-amino Acid) Nanoparticles Loaded with Antibiotic effectively Target And Destroy Staphylococcal Biofilm.

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**INTRODUCTION:** Orthopedic device-related infections (ODRI) are characterized in a large part by antibiotic-tolerant biofilms forming on implants. D-Amino Acids can interact with *Staphylococcus aureus* biofilms through integration into the peptidoglycan of the bacterial cell resulting in disaggregation of the biofilm. In this study, we have assembled nanoparticles (NPs) that display D-Amino acids on their surface and load these NPs with the antibiotics to provide dual acting NPs.

**METHODS:** D-Amino acid NPs were synthesized by dissolving 10 mg of poly( $\alpha$ -N-acryloylphenylalanine)-block-poly( $\beta$ -N-acryloyl-D-aminoalanine) polymers into 1 mL of Dimethyl sulfoxide (DMSO) under stirring conditions. The final volume of 10 mL was achieved by adding distilled water, and the resulting mixture was dialyzed against distilled water for 48 hrs. Loaded NPs were prepared by adding 1 mg antibiotic (gentamicin, vancomycin and sitafloxacin) to the polymers. The dispersive and killing activity of NPs and loaded NPs was evaluated with colony-forming units (CFU) against *S. aureus* Mu12 and *S. epidermidis* 12 biofilms, confocal laser scanning microscopy (CLSM) and Transmission Electron Microscopy (TEM) against *S. aureus* biofilms grown on titanium disks in Tryptic Soy Broth (TSB) medium for 48 hrs, with media changes every 24 hrs. The cytotoxicity of the nanoparticles was also assessed against human embryonic kidney 293T cells (HEK293 cells) and human monocytic-leukemia cells (THP-1 cells) after 24 hrs of co-incubation.

**RESULTS SECTION:** TEM revealed that the NPs had an approximate diameter of 100nm. When a concentration of 250  $\mu$ g/ml of unloaded NPs was applied on staphylococcal biofilm for 24 hrs, a 1log reduction in CFU was observed ( $p = 0.0018$ , for *S. aureus* Mu12;  $p = 0.0013$ , for *S. epidermidis* 12). This was confirmed by scanning electron microscopy (SEM) and confocal evaluation, which showed that the NPs dispersed and created more gaps in the biofilm and reduced its thickness. The use of antibiotic loaded NPs resulted in a further reduction in CFU ( $p = 0.0014$  for *S. aureus* Mu12;  $p = 0.0011$  for *S. epidermidis* 12), but the most effective outcome with a 4log reduction ( $p = 0.0014$  for *S. aureus* Mu12;  $p = 0.001$  for *S. epidermidis* 12) was achieved when sitafloxacin solution was combined with sitafloxacin loaded NPs. Cell viability assay revealed that viability greater than 80% was observed at a concentration of 250  $\mu$ g/ml of NPs.

**DISCUSSION:** The application of these nanoparticles on Staphylococcal biofilm has shown promising results in reducing the CFU of the bacteria. Antibiotic loaded NPs proved to be the most effective approach. These findings indicate that D amino acid-based NPs have great potential for future antimicrobial therapies.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Nanotechnology offers great promise for improved diagnosis and therapy of many diseases, including bone and joint infection. In this study, D-Amino acids show efficacy in disrupting bacterial biofilm. As these amino acids are inherently safe, there is no toxicity concern. The application of these D-amino acids into nanoparticles, promises an effective, and safe means of reducing antibiotic tolerant biofilms, which are the main reason for recurrent bacterial infections.