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# Soluble Antimicrobial Peptide (AMP) Screening to Rationally Design AMP-Hydrogels that Selectively Prevent Biofilm Formation

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# Soluble Antimicrobial Peptide (AMP) Screening to Rationally Design AMP-Hydrogels that Selectively Prevent Biofilm Formation

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## INTRODUCTION

- Prosthetic implants and indwelling medical devices are used to replace, reinforce, or support tissues and organs, and their use has increased drastically over the past 50 years.[1,2]
- Such devices can become contaminated during the surgical procedure, or from bacterial colonization of the skin or other body sites of the patient or healthcare workers.[5]
- Bacteria in a biofilm secrete an extracellular polymeric substance (EPS), which is made of polysaccharides, proteins, and extracellular DNA, rendering bacteria less susceptible to antibiotics.
- This results in delayed detection of the biofilm-based infections and the best treatment may be to prevent the formation of biofilms initially.
- Antibacterial coating of surgical implants have been utilized but the promise has been hindered via the emergence of antibiotic and mutli-drug resistant infections thus an alternative method is antimicrobial peptides (AMPs).
- AMPs are part of the innate immune system and can eliminate pathogens including bacteria, fungi, and viruses, and are a promising alternative to antibiotics.
- P10 is derivative of LL-37 which prevents biofilm formation by inhibiting the initial attachment step.[28–30] Indolicidin exhibits strong anti-biofilm activity against MRSA via disruption of the membrane potential. $DD_{13}$ is a dermaseptin-derivative with enhanced cytotoxic activity that effectively incorporates into bacterial membranes and results in cell lysis.[36]

## METHODS

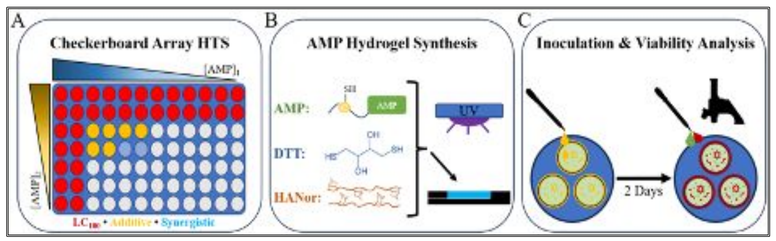


Figure 1: Schematic of experimental process. (A) Diagram of 96 well plate array for screening of AMPs at different concentrations (B) Chemical components used to synthesize AMP hydrogels. (C) MRSA process diagram.

*Peptide Synthesis*

- P10, $DD_{13}$RIP, , and and indolicidin were prepared using solid-state peptide synthesizer and confirmation via MALDI-TOF.

*Bacterial strains, media, and growth conditions*

- Deidentified patient isolates of MRSA and MSSA strains were obtained from Cooper University Hospital.
- Bacterial strains were grown overnight 37°C, with aeration in lysogeny broth (LB) or Mueller-Hinton broth (MHB) and diluted to a starting $OD_{600}$ to 0.05 in MHB for MIC determination and checkerboard assays.
- For biofilm growth, 1 mL of the diluted bacterial culture was added to each well of a 24-well plate containing a hydrogel mounted on a glass coverslip, incubated for 2 days at 37°C, and prepared for confocal.

*Checkerboard assays*

- Combination of AMPs were tested against MSSA and MRSA strains via a 96-well plate and the fractional inhibitory concentration index (FICI) was calculated.

*AMP hydrogel synthesis*

- HANor monomers were mixed with DTT, photinitatior, and different concentrations of AMPs dissolves in PBS and injected into 0.88 mm tall molds. The hydrogels were irradiated with UV and washed twice with PBS.

*Confocal microscopy*

- The live/dead biofilm viability assay was performed with a working staining solution SYTO 9 (green, live cells) and propidium iodide (red, dead cells). Twelve cross sections were taken from the three gels of each sample group.

*Mammalian cell viability*

- Viability was determined by using the number of live and dead cells to replace green and red % area, respectively.

*Stastical Analysis*

- Results were analyzed via a one-way analysis of variance (ANOVA) and tested versus the control hydrogels using Dunnett's multiple comparisons test.

## RESULTS

**AMP screens used to determine MICs of soluble AMPs against MSSA and MRSA**

- MICS for MSSA were 13.5 μM, 18.8 μM, and 87.5 μM for DD13-RIP, indolicidin, and P10, respectively. The MICs for MRSA were over 2-fold higher for DD13-RIP, 2.5-fold higher for indolicidin, and 1.4-fold higher for P10 in comparison to MSSA.

**Checkerboard arrays identify additive and synergistic AMP combinations against *S. aureus* isolates**

- MSSA: Indolicidin and P10 combination yielded 8 additive combinations and 5 synergistic combinations. $DD_{13}$RIP and P10 combination yielded 7 additive combinations and 0 synergistic combinations. No combination effect for indolicidin and $DD_{13}$RIP.
- MRSA: Indolicidin and P10 combination yielded 7 additive combination and 1 synergistic combination. $DD_{13}$RIP,and P10 or indolicidin combination yielded 7 additive effects against MRSA but not MSSA.
- Single AMP-loaded hydrogels at the MIC retain antimicrobial properties against MRSA (Figure 2) and combinatorial AMP hydrogels are effective against MRSA (Figure 3). These hydrogels also reduce bacterial bioburden on and within hydrogels.

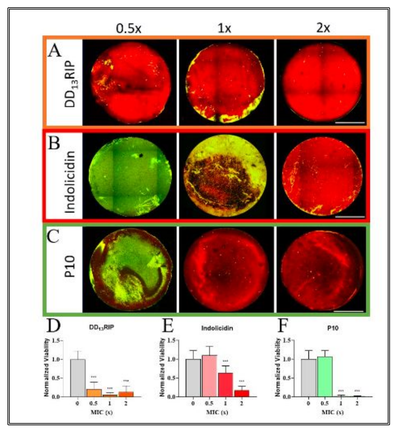


Figure 2. Examination of MRSA biofilm formation on different AMP-loaded hydrogels.

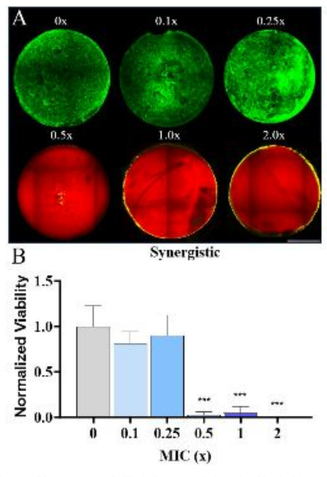


Figure 3. Synergistic effects of P10 and indolicidin on viability of MRSA atop different AMP-loaded hydrogels.

## CONCLUSION

- In this study, checkerboard arrays were used to identify soluble concentrations of DD13-RIP, indolicidin, and P10 that yielded combinatorial effects against MSSA and MRSA strains.
- The soluble screens identified a synergistic combination of P10 and indolicidin at a concentration 10- and 14- times lower than their MICs, respectively. This soluble AMP screening data was then used to design hydrogels with individual AMPs at their MIC, and we found that these hydrogels successfully prevented MRSA biofilm formation.
- While these AMP concentrations are already significantly lower than many other AMP-hydrogel platforms, we also found that AMP hydrogels with indolicidin and P10 at half of their synergistic concentration were also highly effective in preventing MRSA adhesion and biofilm formation.
- By using soluble screening AMP data, we rationally designed hydrogels with AMP dosing tailored to the pathogen of interest.
- Mammalian cells also adhere and are viable on these AMP-laden hydrogels, demonstrating that the AMPs selectively thwart bacterial colonization while maintaining high biocompatibility.
- This new paradigm for AMP-hydrogel design has potential for designing biocompatible, antimicrobial materials with applications as wound dressings and medical device coatings that prevent biofilm formation of opportunistic pathogens.

## REFERENCES

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REFERENCES