Antimicrobial Blue Light Therapy Resensitizes Methicillin-Resistant *Staphylococcus aureus (MRSA)* to Antimicrobial Peptides

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BACKGROUND

Antimicrobial blue light therapy (**ABL**) in the range of 400-470 nm is currently not routinely used in the clinic for the treatment of bacterial skin infections and wounds. However, bacterial catalases contain heme cofactors that can be photo-inactivated and leads to a toxic buildup of reactive oxygen species (ROS).

ROS can affect efflux pumps and ion gradients across the membrane and effectively lower the MIC of a drug.

Catalase is secreted by many human pathogenic bacteria to protect the bacteria from peroxide produced by neutrophils during the innate immune response. Additionally, all ESKAPE pathogens except for *Enterococcus faecium* secrete catalase, the latter is the least susceptible to blue light therapy. Bacterial catalase activity can be easily detected by adding peroxide to the bacteria and observing the generation of bubbles. Finally, Gram positive and negative bacteria, mycobacteria, molds, yeasts and dermatophytes are susceptible to blue light. Blue light is bactericidal.

SPECIFIC AIMS

Prototyped dressing on Tegaderm

The overarching goal of the Photonic Antimicrobial Wound Surface (PAWS) dressing program is to develop a wound dressing compatible with ABL (*i.e.* highly transparent, drug- or peptide-loaded, absorbent).

- **Aim 1:** Determine the synergistic efficacy of ABL with adjuvants (drugs and peptides)
  - Synthesis of antimicrobial peptides (AMP)
  - Combinatorial testing of antibiotics or peptides + ABL
- **Aim 2:** Incorporate adjuvant release functionality into the PAWS dressing
  - adjuvant release within 4h, minimal photodegradation
- **Aim 3:** Optimize the PAWS dressing design to improve manufacturability and optimize usability.
  - light weight, clear, drug-loaded, absorbent
  - minimal LED panel heat generation

METHODS

To determine if ABL could be used to re-sensitize methicillin-resistant *Staphylococcus aureus* (USA300) to antimicrobial peptides we synthesized the HHC-10 (KRWWKWIRW-NH_2) peptide using solid phase peptide synthesis (SPPS). The peptide was added to transparent peptide-loaded polyvinylpyrrolidone (PVP): acetic acid hydrogels designed for ABL therapy and tested in 96-well *in vitro* biofilm assays.

- Clear, transparent, drug- or peptide- loaded microneedle patches were developed for ABL + adjuvant delivery. Gels were adhered to Tegaderm.
- Microneedles were crystallized at room temperature and cast in silicone molds with ciprofloxacin or peptides

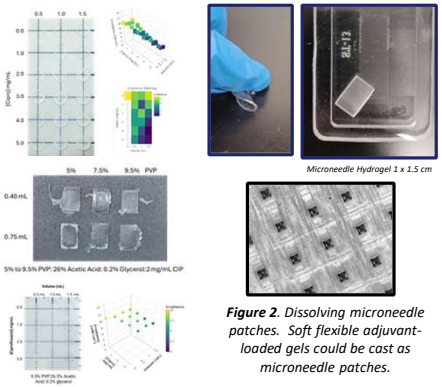


***Figure 1**. Hydrogel formulation for maximum loading of ciprofloxacin or peptides without loss of transparency*

Microneedle Hydrogel 1 x 1.5 cm

***Figure 2**. Dissolving microneedle patches. Soft flexible adjuvant-loaded gels could be cast as microneedle patches.*

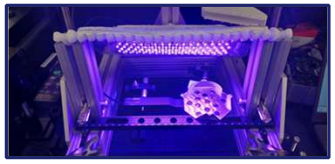
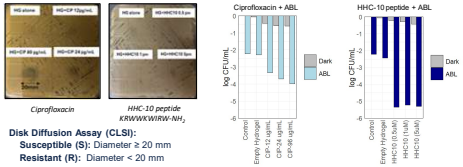


***Figure 3**. Test Bed used for the development of clear transparent drug-loaded dissolving hydrogels.*

RESULTS

The bactericidal effects of L-HHC-10 peptide + ABL is greater than that of Ciprofloxacin + ABL

- A **>5-log reduction in colony forming units (CFU)** was obtained with 0.5 µM to 5 µM HHC-10 peptide per well and 4.5 h of 15 mW/cm² ABL.
- In disk diffusion experiments the bacteria was resistant to the peptide but became sensitive when ABL was added. The bacteria could also be re-sensitized to ciprofloxacin. With ciprofloxacin a 4-log reduction in CFU was achieved.



High concentration of PVP produced clear gels containing high concentrations (3 mg/mL) of ciprofloxacin with minimal effects on transparency.

- 92% of the light was transmitted through the 9.5% PVP gel with 3 mg/mL CIP

Photodegradation of Ciprofloxacin still occurs after 4h of low irradiance ABL (405 nm, 15 mW/cm²)

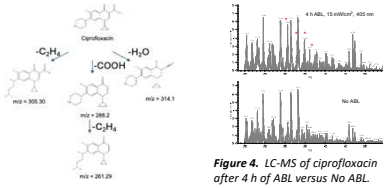
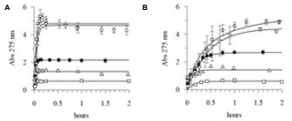


***Figure 4**. LC-MS of ciprofloxacin after 4 h of ABL versus No ABL.*

Dissolution Kinetics of Ciprofloxacin in Artificial Wound Exudate vs. 0.6% lactic acid

- Comparable solubility of Ciprofloxacin in both buffers
- Slower drug release in wound exudate than lactic acid

***Figure 5**. Dissolution rates of PVP hydrogels loaded with varying amounts of ciprofloxacin in (A) 0.6% lactic acid or (B) artificial wound exudate.*



CONCLUSIONS

- Treatment of wounds with AB and an adjuvant represents a promising strategy to resensitize bacteria to antibiotic peptides and small molecules.
- Long irradiation periods (4.5 h) with 405 nm LED light arrays are highly effective when combined with adjuvants.
- ABL restores susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) to antimicrobial peptides and conventional antibiotics (**>5-log reduction**)
- The effect of the HHC-10 peptide + ABL was synergistic
- Combined use of ABL + ciprofloxacin prevented regrowth of the bacteria for **2 days** once ABL was removed.
- ABL is compatible with a variety of drugs, AMP, bandages (Tegaderm), and PVP hydrogels.
- **Mechanism of Action (MOA)** - bacterial catalase can be photo-inactivated with 405 nm light. ROS accumulation alters ion gradients across the membrane and prevents efflux of the drug.
- Some antibiotics are susceptible to photo-oxidation
- These findings support the potential of ABL-combination therapies as a general approach to overcome antibiotic resistance in biofilm-associated infections. With further development, this strategy may provide a practical and scalable treatment option for difficult-to-treat wound infections and other clinically relevant pathogens.

FUTURE WORK

Increasing the depth of penetration of ABL is a major goal

- Moxifloxacin is an antibiotic that is intrinsically fluorescent. It emits blue light (470 nm) when excited at 287 nm and can be excited at longer wavelengths (365 nm) when chelated to metal ions.
- Microneedle arrays may help transmit the light or increase the depth of penetration of both drug + ABL.

REFERENCES

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