Validation of a Bacterial Fluorescent Imaging Device to Detect Polymicrobial Biofilm *In Vitro*

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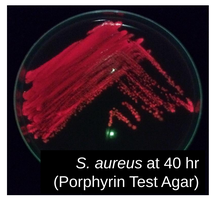
INTRODUCTION

- Chronic wounds commonly harbor polymicrobial infections. When the bacteria exist as polymicrobial biofilms, this can result in more aggressive infections.
- Confirmation of bacterial presence is clinically confirmed via microbiological testing. Culture-based diagnostics identify dominant microorganisms as well as their antimicrobial susceptibility, however the lag time to obtain those results (3 days – 4 weeks) significantly impacts wound care and treatment.
- The MolecuLight i:X imaging device visualizes fluorescence from wound **tissues (green)** and **bacteria (red)**, enabling point-of-care localization of regions with moderate-to-heavy bacterial loads.
- The device illuminates a wound with safe violet light (405 nm) and visualizes resulting endogenous fluorescence from bacterial porphyrins in real-time. This allows physicians to direct specimen sampling to the area with the heaviest bioburden, improving diagnostic capabilities and to direct bacterial targeted treatments.
- **Prior work demonstrated MolecuLight i:X detection of planktonic monomicrobial bacteria *in vivo* and *in vitro* from the most common wound pathogens (e.g. *Staphylococcus*, *Enterobacter*, *Klebsiella*, *Proteus*, *Acinetobacter*, *Bacteroides*, *Serratia*).**



This study investigated the device's capability to detect biofilm using our polymicrobial *in vitro* biofilm model, which is representative of the chronic wound environment.

MOLECULIGHT *i:X* IMAGING DEVICE

- The MolecuLight *i:X* fluorescence imaging device detects most medically-relevant species of bacteria via endogenous red fluorescence of porphyrins [3].
- Porphyrin production in bacteria requires δ-aminolevulinic acid (ALA), an intermediate in heme synthesis.
- The device illuminates a wound with violet light and visualizes resultant bacterial fluorescence in real-time, indicated by bright red on images [3]. This allows physicians to follow empiric therapy for treatment as well as target sampling of the wound for culture-based diagnostics.

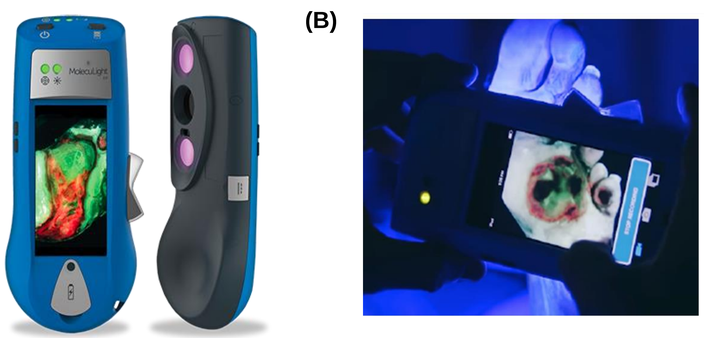


Figure 1: MolecuLight *i:X* Imaging Device. (A) Hand-held device and (B) image of chronic foot ulcer wound portraying red fluorescence.

WORKS CITED

[1] Dowd *et al*. 2008. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiology. 8: 43

[2] DeLeon *et al*. 2014. Synergistic Interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an *In Vitro* Wound Model. Infection and Immunity. 82(11): 4718-4728.

[3] Rennie et al. 2019. Understanding Real-Time Fluorescence Signals from Bacteria and Wound Tissues Observed with the MolecuLight i:X. Diagnostics. 9(1).

PURPOSE

To validate that the MolecuLight *i:X* device can detect porphyrin-producing species of bacteria encased within a biofilm matrix.

EXPERIMENTAL DESIGN

- Cultures of *S. aureus*, *E. cloacae*, and *E. coli* were mixed in 1:1:1 ratio and inoculated into our wound-like media consisting of Bolton's broth (complex nutrient medium) and bovine serum and provided a plastic tip acting as a scaffold. Over the course of 7 days of incubation and shaking at 37˚C, polymicrobial biofilm formed and adhered to the tip.
- To determine the colony forming units (CFU) of each species from the inoculating dose, selective and differential media was used [Mannitol Salt Agar (MSA) for *S. aureus* and Eosin Methylene Blue (EMB) Agar for *E.coli* and *E. cloacae*].
- After 7 days of biofilm growth, ALA was added to the biofilms to induce porphyrin production for 24 hours.
- On Day 8, biofilms were washed to remove planktonic cells and imaged with the MolecuLight *i:X* imaging device on regular and fluorescent settings. The experiment was conducted in three biological replicates and included an ALA-negative control and an unwashed control.
- Scanning Electron Microscopy was done to further validate the *i:*X device to show the presence of bacterial cells surrounded by biofilm matrix.

RESULTS

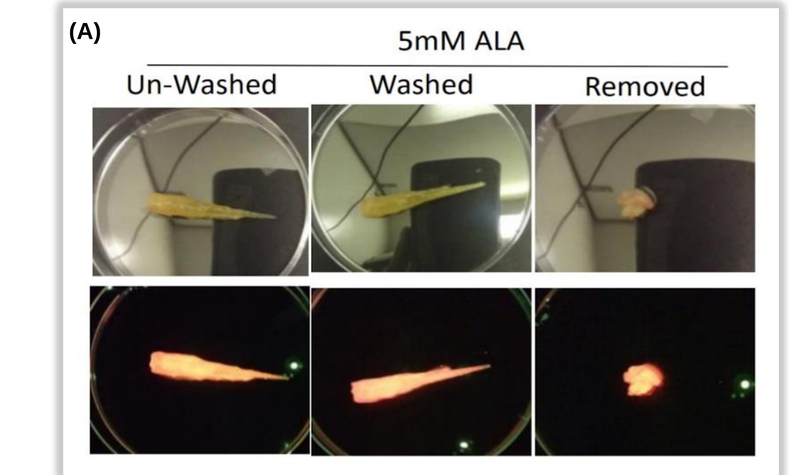


Figure 2: Polymicrobial biofilm cultures fluoresce red under violet light illumination in the presence of ALA. (A) Regular and fluorescence images of a polymicrobial biofilm containing *S. aureus*, *E.coli* and *E. cloacae*. Red fluorescence detection in both un-washed and washed biofilm in the presence of ALA indicates that fluorescence is detectable from a biofilm, as the washing step removes any planktonic cells. Scaffolding removed shows approximate size of the biofilm alone. (B) Negative ALA control shows lack of red fluorescence.

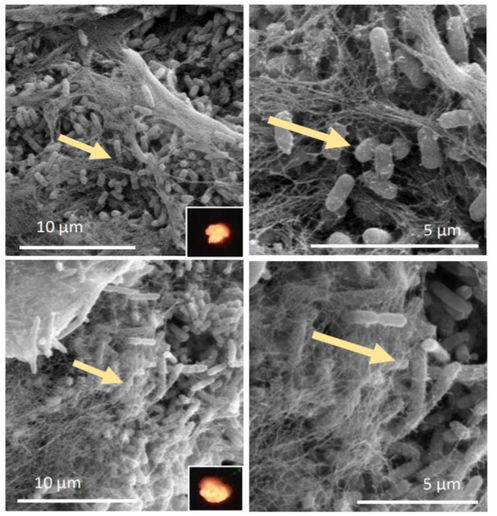


Figure 3: Scanning Electron Microscopy (SEM) Images of two representative (washed) biofilm samples. The images in the top row and the bottom row correspond to two separate polymicrobial biofilm samples, each shown at a scale of 10 µm and 5 µm. These images showcase polymicrobial biofilm containing *S. aureus*, *E.coli* and *E. cloacae.* The arrows point specifically to the extracellular matrix hallmark of the biofilm that encases the bacterial cells. These images confirm that the MolecuLight *i:X* device can detect bacteria within a biofilm.

DISCUSSION

- A robust polymicrobial biofilm formed after 7 days of incubation and adhered to the scaffold provided.
- **The MolecuLight *i:X* imaging device readily detected red fluorescence from the bacterial consortia within the biofilm, both pre-wash and post-wash, indicating that the *i:X* device can detect porphyrin-positive bacterial species encased within biofilm matrix.**
- These data demonstrate that bacterial fluorescence imaging detects porphyrin-positive species of bacteria growing both planktonically and as a biofilm, as well as monomicrobial and polymicrobial communities, which further validates the clinical capability and relevance of the device for use in wound care.
- CFU enumeration will be determined post-incubation to ensure the polymicrobial nature of the biofilm throughout the course of the experiment. We are also interested in a unique cyan fluorescence emitted by the bacterium *Pseudomonas aeruginosa* as another potential method of detecting specific, pathogenic microbes in chronic wounds.
- The results from this study could inform clinical recommendations from microbiology laboratories for real-time detection of pathogens in chronic wound specimens.
- Further developments in the *i:X* device may lead to greater real-time assessment of patient chronic wounds, in which a treatment or another solution can be administered by a physician in a shorter amount of time.
- Ultimately, the significance of this translational research project aims to reduce morbidity and mortality of patients with chronic wound infections.

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