**In vivo detection of polymicrobial bacterial biofilm with real-time fluorescence imaging**

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**ABSTRACT**

Chronic wounds are a significant cause of patient morbidity and mortality in the US annually, and commonly harbor polymicrobial biofilms, generating infections that are difficult to characterize and to treat. Current diagnostics methods require long periods of testing for microbial identification, but often provide little information on the complex characteristics of the chronic wound environment. An essential step to the healing process for a patient with a complex wound is accurate diagnosis to better provide personalized care. Bacterial fluorescence imaging with the handheld MolecuLight i:X device uses safe violet light to detect auto fluorescent properties of most clinically-relevant species of bacteria, allowing detection of relative bacterial bioburden in a wound in real time. In this study, we assessed the ability of the MolecuLight i:X device to detect auto fluorescent properties of a polymicrobial bacterial biofilm utilizing an established chronic wound murine model and were able to demonstrate that bacteria encased within the extracellular matrix of the biofilm still exhibit detectable autofluorescence. These data demonstrate that the device has the ability to detect bacteria encased within a biofilm, which further validates the MolecuLight i:X device for patient use.

**INTRODUCTION**

- Chronic wounds commonly harbor bacteria. These bacteria can exist as polymicrobial biofilms, resulting 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 spp.)
- 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 8-amino-7-oxonine 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 empirc therapy for treatment as well as target sampling of the wound for culture-based diagnostics.

**PURPOSE**

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

**EXPERIMENTAL DESIGN**

- Establish a polymicrobial biofilm infection in the murine chronic wound model
- Harvest ex vivo tissue
- Determine presence of biofilm in ex vivo tissue
- Determine polymicrobial nature of infection

**RESULTS**

To confirm presence of all three species within the biofilm/mouse ex vivo tissue, in house culture-based identification was done. Samples were also taken to Covenant Medical Center to validate our in-house results. Data not shown

**DISCUSSION**

Taken together, our data demonstrate that the device can detect fluorescence signal from polymicrobial, biofilm-associated infections. Detection with the device was validated by characteristic red fluorescence signatures from the murine chronic wound model. The polymicrobial nature of the infection was confirmed in-house and send-out culture-based microbiology. The ex vivo tissue was found to be biofilm-associated via histopathology staining for matrix and scanning electron microscopy (SEM). This work further validates the use of the MolecuLight i:X device clinically for the real-time detection of wound pathogens in complex chronic wound infections.

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**WORKS CITED**


**Biofilm-associated infections**

- Chronic wounds commonly harbor biofilm-associated polymicrobial infections. Biofilms are polymicrobial communities of microorganisms encased in a self-produced extracellular matrix (EPS).
- EPS blocks penetration of immune system mechanisms and antimicrobial agents. Up to 1000X the antimicrobial concentration is required to eliminate a biofilm infection, compared to planktonic (free-living) associated infection.
- Chronic wound biofilms can contain 100 different microbial species [1], which can synergize their activities, leading to more aggressive and virulent infections [2].
- Out in vitro wound-like model contains physiologically representative conditions and has been previously validated to support the growth of polymicrobial biofilms [2].
- Three common chronic wound pathogens were selected: Staphylococcus aureus (ATCC ©25923), Enterobacter cloacae (ATTC ©13047), and Escherichia coli (ATCC © 25922).