Does bacterial fluorescence imaging improve chronic wound biofilm detection over standard clinical assessment and blotting?

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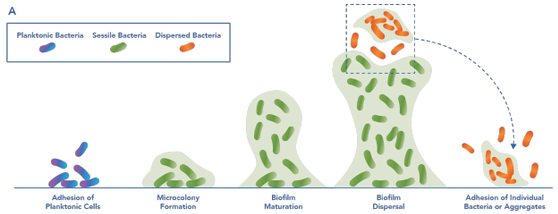
Introduction

- Biofilm is a destructive and resilient form of bacterial presence that is highly prevalent in chronic wounds.
- It must be mechanically disturbed to be effectively removed, in turn reinstating the healing cascade and helping avoid complications.
- **Biofilm is notoriously hard to identify and localize at the bedside,** resulting in missed or incomplete removal and its rapid reconstruction within 24-hours.



This study evaluated traditional and novel methods for point-of-care biofilm localization, (standard clinical assessment, fluorescence imaging, and biofilm blotting) **and compared them to gold-standard SEM biofilm detection.**

Methods

- 40 wounds (34 DFU, 3 VLU, 1 ALU, 1 neuropathic foot ulcer, 1 PU)
- Patients' recruitment was stratified based on BBWC[1]: 20 BBWC+, 20 BBWC-

1. Biofilm Based Wound Care (BBWC) checklist[1] (clinical assessment)
2. Biofilm blotting
3. Fluorescence imaging for bacterial localization 
4. Sampling for microbiology (PCR and NGS-sequencing)
5. Sampling for scanning electron microscopy (SEM). Positive for biofilm if bacterial presence was closely associated to bacterial-derived ECM.

Diagnostic accuracy of biofilm detection calculated against SEM

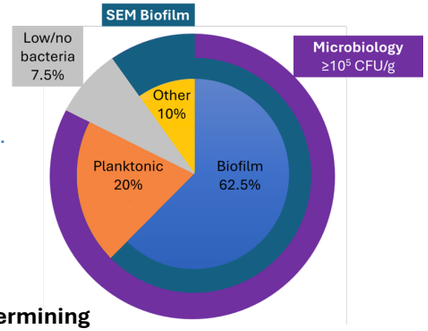
Results

Presence of biofilm is abundant in these samples:

- 62.5% (25/40) of wounds were positive for biofilm, based on consensus between two independent readers.
- 82.5% (33/40) of wounds were positive for bacterial loads ≥10^5 CFU/g.
- 3 samples had biofilm present by SEM, but low microbiology results (<10^5 CFU/g).

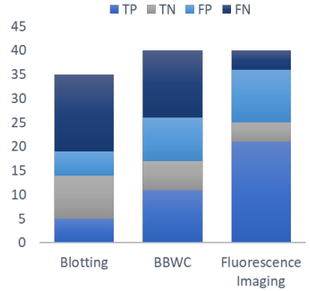
"Biofilm" defined as having both positive SEM and microbiology results.

"Planktonic" defined as positive microbiology but negative by SEM.



Which was best technique in determining biofilm clinically?

Technique vs SEM Biofilm	Accuracy	Sensitivity
BBWC	43%	44%
Biofilm blotting	40%	24%
Fluorescence Imaging	63%	84%



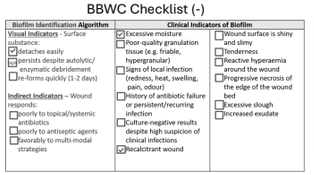
Fluorescence Imaging performed the best, detecting the highest number of true positive biofilm samples (21/25), and had the fewest false negatives. False positives may indicate the detection of planktonic bacteria in those samples.

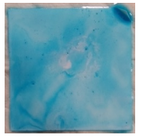
- Biofilm Blotting performed poorly with equal number of true and false positives. BBWC did not consistently identify biofilm either.

Clinical Case

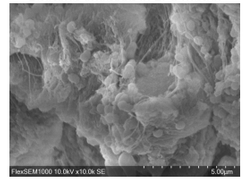
- A 50 y/o male with a diabetic foot ulcer on the right foot.
- Clinical assessment and biofilm blotting were both negative for biofilm. Fluorescence imaging was positive for red fluorescence (indicating bacteria/biofilm).
- Microbiology (>10^7 CFU/g) and SEM (biofilm) were positive.

BBWC Checklist (-)

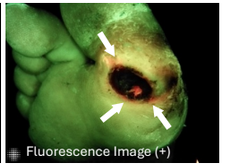


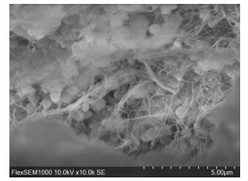

Biofilm blotting (-)

SEM (+)
Bacteria encased in EPS matrix




Standard Image


Fluorescence Image (+)



red fluorescence (white arrows above) = most Gram +/-, aerobe, & anaerobes at loads >10^4 CFU/g[2,4]

Conclusions

- Previous in-vitro and in-vivo pre-clinical studies have shown that fluorescence imaging can detect bacteria within a biofilm[2,3].
- **This trial provides the first evidence of clinical biofilm detection by fluorescence imaging.** It was able to alert to regions of biofilm at the point-of-care with greater accuracy than standard clinical assessment or biofilm blotting paper.
- Fluorescence imaging technology offers a unique and rapid approach to detecting wound biofilm, to encourage optimal wound hygiene and clinical outcomes.

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1. Wolcott, Wounds Middle East, 2014; 2. Jones, Futures Microbiology, 2020; 3. Lopez, International Wound Journal, 2021.