

Association of Bacterial Fluorescence with Early Response to Skin Substitutes in Diabetic and Venous Ulcers: A Single-Blinded Prospective Study

Alisha Oropallo,^{1,2,3} Amit S. Rao,¹ Sally Kaplan,¹ Farisha Baksh,¹ Julie Isgro,¹ Adam Iddriss,¹ Christina Del Pin,^{1,2} Star-Kayla Lewis³

¹Northwell Health System, Department of Surgery, Comprehensive Wound Care Healing and Hyperbaric, Lake Success, NY, USA; ²Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, USA; ³Feinstein Institutes for Medical Research, Manhasset, NY, USA.

Key takeaway

Absence of bacterial fluorescence at the time of CAMP application was associated with improved early healing outcomes in chronic diabetic foot and venous leg ulcers



Background

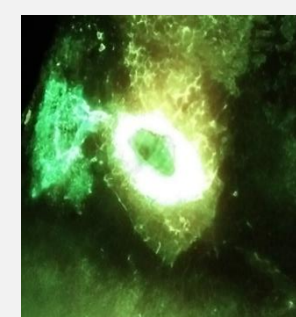
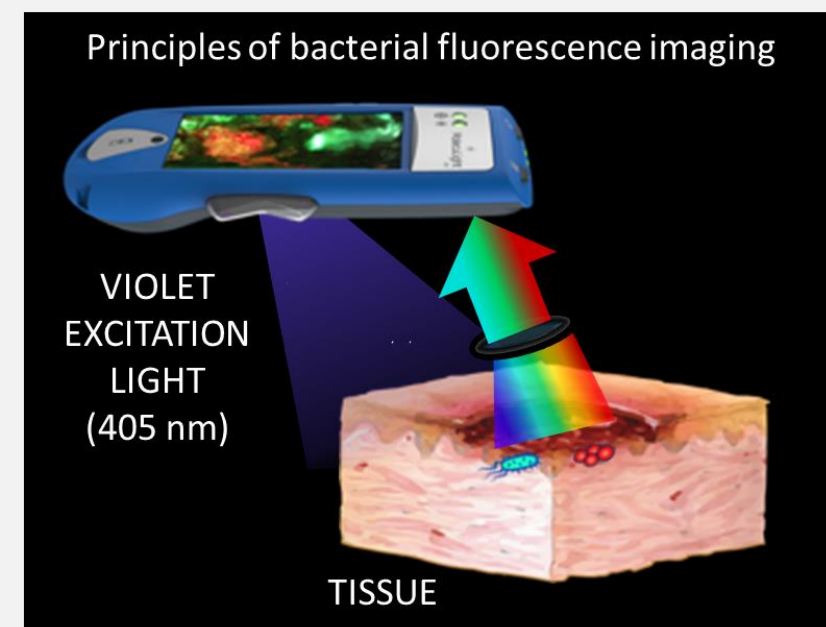
- Cellular, acellular, or matrix-type products (CAMPs), or skin substitutes, promote healing in chronic diabetic and venous ulcers, but elevated bacterial burden may compromise their effectiveness.¹⁻⁴
- Bacterial fluorescence imaging (BFI) enables real-time visualization of elevated bacterial burden (>10⁴ CFU/g),⁵⁻⁷ which is often not apparent clinically; however, prospective evidence linking fluorescence (FL) status at the time of CAMP application to early healing outcomes remains limited.

Aims

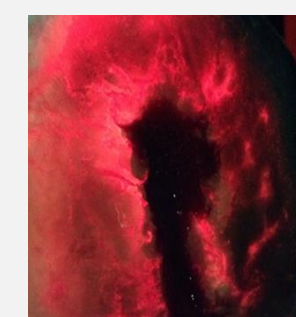
- To evaluate whether the presence or absence of bacterial fluorescence immediately before CAMP placement was associated with changes in early wound area reduction as a therapeutic response in chronic ulcers.

Methods

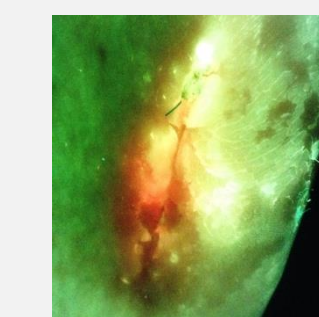
- Prospective observational pilot study conducted at the outpatient Northwell Comprehensive Wound Health and Hyperbaric Center (Lake Success, NY, USA) from November 2024 to August 2025.
- Eligible patients: Adults (≥18 years) with chronic (>4 weeks), uninfected, full-thickness DFUs or VLUs, scheduled to receive treatment with a CAMP, with an ABI >0.6 and a wound area ≥1 cm².
- Standard and fluorescence images captured using MolecuLight i:X (MolecuLight Inc., Toronto, Ontario, Canada); imaging results were recorded but blinded to treating clinicians.
- Wounds were categorized as FL positive (red/cyan signal) or FL negative (absence of red/cyan signal).



Cyan fluorescence (blue/green halo) indicates *Pseudomonas*



Red fluorescence indicates most gram positives, negatives, aerobes and anaerobes



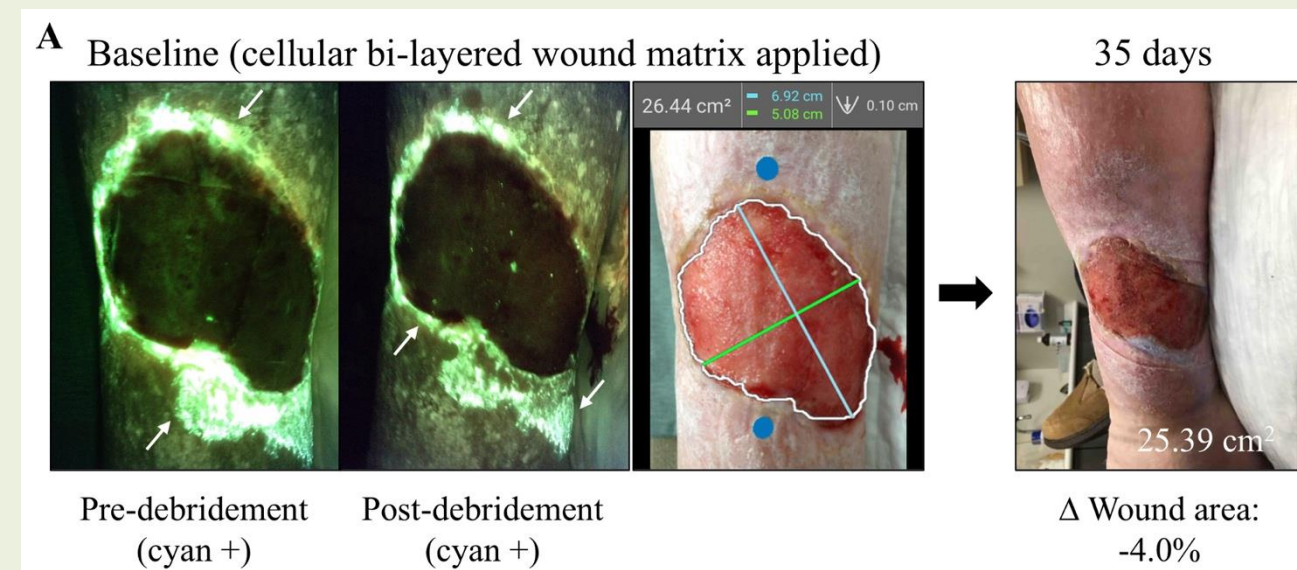
Yellow fluorescence indicates subsurface bacteria (mix of green signals from normal tissue with red)

Abbreviations: Δ, change; ABI, ankle-brachial index; BFI, bacterial fluorescence imaging; CAMP, cellular, acellular, or matrix-type products; DFU, diabetic foot ulcer; FL, fluorescence; PAR, percentage area reduction; SD, standard deviation; VLU, venous leg ulcer.

Results

- Eighteen patients were enrolled; 10 of 18 (56%) completed follow-up at 4±1 weeks and were included in the primary endpoint analysis.
 - No significant differences were noted between the baseline FL-negative and FL-positive groups in terms of sex, race, wound etiology, wound location, comorbidities, and mean age, wound duration, or baseline wound area.

Representative Cases Demonstrating Fluorescence Status Prior to CAMP Application and Corresponding Early Wound Area Outcomes



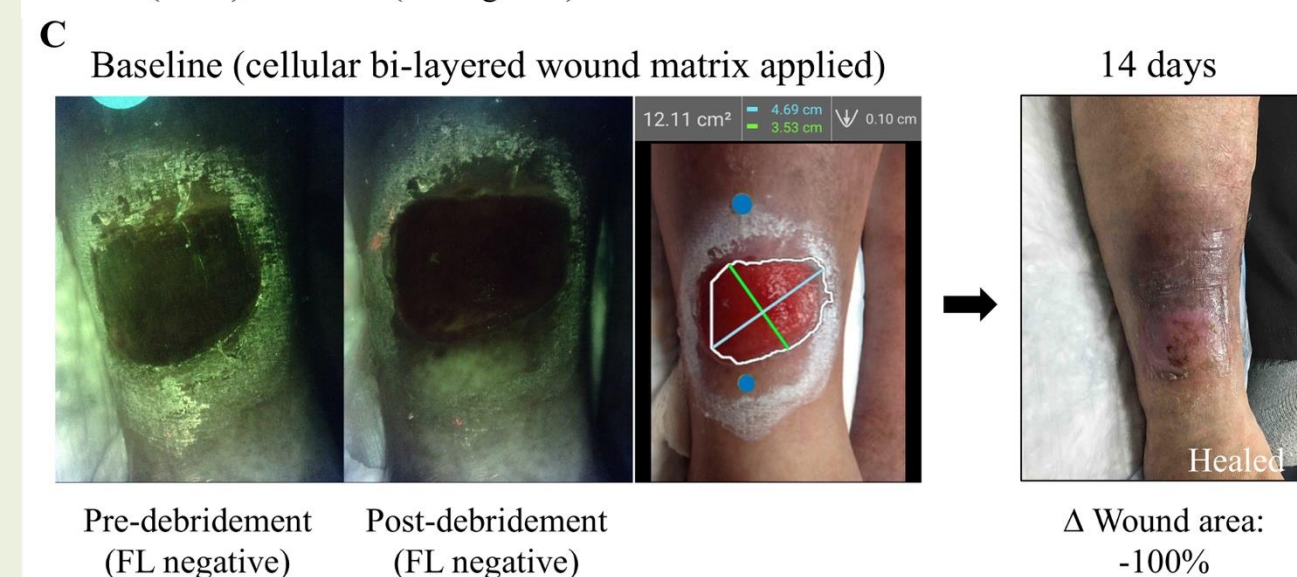
FL-Positive Pre- and Post-Debridement: Minimal PAR at 4±1 weeks

(A) CAMP applied to a longstanding VLU on the left leg of an 81-year-old female with peripheral vascular disease, chronic venous insufficiency, and hypertension; compliant with compression therapy. Cyan FL (indicating the presence of *Pseudomonas*) was present at the intended CAMP site post-debridement immediately before application. 35 days later, the ulcer had reduced in size by 4.0% and thus did not meet the ≥40% PAR target.



FL-Positive Pre-Debridement, FL-Negative Post-Debridement: ≥40% PAR at 4±1 weeks

(B) CAMP applied to a longstanding VLU on the left leg of an 84-year-old male with peripheral vascular disease, chronic venous insufficiency, and a history of cancer; compliant with compression therapy. Red FL (indicating elevated bacterial loads) was present at the intended CAMP site but was successfully removed immediately prior to CAMP application through cleansing and debridement of the wound bed. 35 days later, the ulcer had reduced in size by 72.9% and thus met the ≥40% PAR target.



FL-Negative Pre- and Post-Debridement: Complete Healing within 2 weeks

(C) CAMP applied to a VLU on the left leg of an 82-year-old male with diabetes mellitus, peripheral vascular disease, chronic venous insufficiency, and coronary artery disease; compliant with compression therapy. No fluorescence signal indicative of elevated bacterial burden was detected at baseline or following cleansing and debridement prior to CAMP application (FL-negative). Fourteen days later, the ulcer had completely healed and thus met the ≥40% PAR target.

Abbreviations: Δ, change; DFU, diabetic foot ulcer; FL, fluorescence

Primary endpoint (≥40% PAR at 4±1 weeks)

- None of the 5 FL-positive wounds achieved ≥40% PAR at follow-up (Table 1).
- In contrast, 4 of 5 (80%) FL-negative wounds met the ≥40% PAR threshold ($P=0.0476$).
 - One FL-negative wound achieved complete healing within 14 days (Figure 1C).

Secondary endpoint (Absolute PAR at 4±1 weeks)

- PAR at 4±1 weeks differed significantly between these groups ($P=0.0159$).
- FL-negative wounds showed a 68.0% (SD, 35.5%) mean reduction in wound area.
- FL-positive wounds showed a 33.7% (SD 72.3%) mean increase in wound area.

Wound Area Reduction Results

Wound type	FL color	Wound area at baseline (cm ²)	Wound area at follow-up (cm ²)	Δ wound area (%)	Follow-up period (days)
FL-positive					
VLU	Red	22.10	19.78	-10.5	26
VLU	Cyan	26.44	25.39	-4.0	35
DFU	Red	3.12	3.43	9.9	21
DFU	Cyan	3.43	3.81	11.1	21
DFU	Cyan	4.29	11.24	162.0	21
FL-negative					
VLU	-	12.11	0	-100	14
VLU	-	2.17	0.22	-89.9	21
VLU	-	8.96	2.43	-72.9	35
DFU	-	4.74	1.51	-68.1	21
VLU	-	17.12	15.57	-9.1	29

Abbreviations: Δ, change; DFU, diabetic foot ulcer; FL, fluorescence; VLU, venous leg ulcer;

Conclusions

- In this prospective observational pilot study, the **absence of bacterial fluorescence at the time of CAMP application** was significantly associated with improved **early healing outcomes** at 4 ± 1 weeks.
- FL-negative wounds were more likely to achieve ≥40% PAR**, whereas FL-positive wounds demonstrated poor early healing response.
- These findings suggest that **elevated bacterial burden at the time of CAMP placement may adversely influence early therapeutic response**.
- Real-time BFI provides an objective method to assess wound bed bacterial load prior to advanced therapy** and may support optimization of wound bed preparation and timing of CAMP application.

References:

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