Open-Label, Proof-of-Concept Study Assessing the Effects of Bromelain-Based Enzymatic Debridement on Biofilm and Microbial Loads in Patients with VLU and DFU

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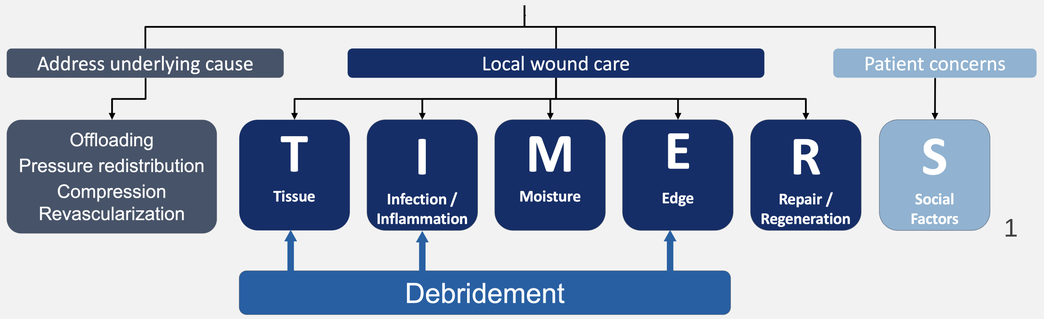
Background and Study Objectives

- Most chronic wounds contain biofilm, and debridement remains the centerpiece of treatment

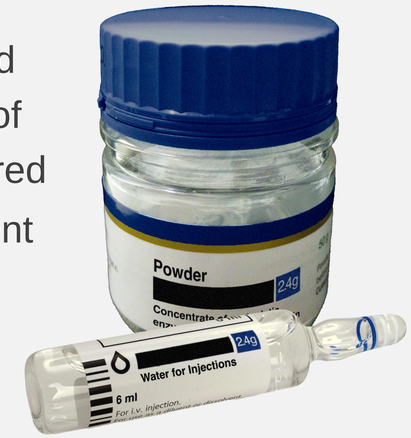


[1]

- Microbial infections represent the primary factor behind chronic wounds that fail to heal. These persistent infections form biofilms, resistant to conventional antibiotic treatments [2,3]
- Enzymatic debridement is an effective tool in removing nonviable tissue; however, there is little evidence supporting its effect on planktonic and biofilm bacteria in humans
- This study evaluated the effects of Bromelain-Based Enzymatic Debridement (BBD) on removal of nonviable tissue, biofilm, and microbial loads in patients with venous leg ulcers (VLU) and diabetic foot ulcers (DFU)

Bromelain Based Enzymatic Debridement

- Investigational biological product in late-stage clinical development in VLU (ongoing phase III in VLU, planned phase III in DFU)
- Mixture of proteolytic enzymes enriched with bromelain, derived from the stem of pineapple plant. High affinity to denatured collagen, enables selective debridement of non-viable tissues



- Same active ingredient as NexoBrid®, FDA/EMA approved for eschar removal in burns
- Phase 2 trials (VLU, DFU, traumatic ulcers) showed superiority over placebo hydrogel & non-surgical standard of care in debridement of non-viable tissue, and promotion of granulation tissue in patients with chronic wounds[4,5,6]
- BBD enables debridement as a standalone therapy, eliminating the need for sharp debridement.

1. Atkin L, et al. J Wound Care 2019; 28(3 Suppl 3):S1–S49 **2.** Kadam et al. Biomedicines 2019; **3.** LuTheryn et al. Microbial Biotechnology 2019 **4.** Snyder et al. Wounds 2023; **5.** Shoham et al. Wound Rep Reg 2021
6. Shoham et al. 2024; LANCET's eClinicalMedicine

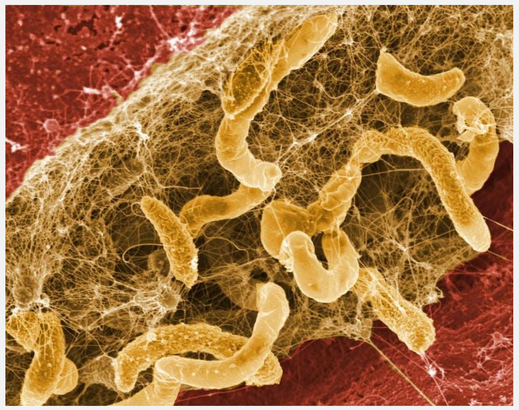
Study Design

- Patients with DFU or VLU
- **Up to 8 once-daily applications of BBD**
  + 2 weeks follow-up

- Key eligibility criteria
  - **Included:** wound age - 4 weeks to 2 years, wound size - 4-80cm^2
  - **Excluded**: systemic or local infection, gangrene, osteomyelitis, decreased arterial blood flow (TBI≤0.50, ABI≤0.70, SPP≤40mmHg, or TCOM≤40 mmHg)

Biofilm

- Wound punch biopsies (3 mm) collected before and after treatment
- Biopsy taken from highest bacterial burden spot (MolecuLight®)
- Biopsy sections stained and examined using a confocal scanning laser microscope
- Representative images of each specimen were semi-quantitatively characterized based on the following scale[1] (a ranking of 2 or higher is considered positive for biofilm)

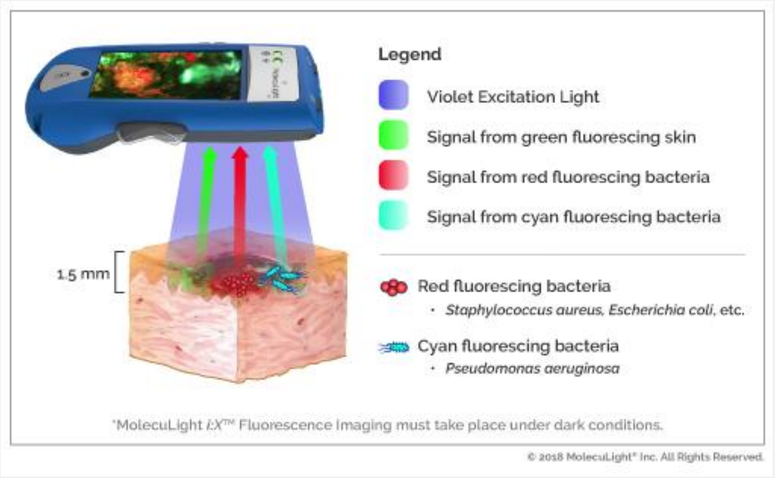


0 - No microorganisms observed
1 - Single individual microorganisms
2 - Small micro-colonies (10-100 cells) of microorganisms
3 - Large micro-colonies (>100 cells) of microorganisms
4 - Continuous film of microorganisms
5 - Thick (> 10 um) continuous film of microorganisms

[1] Developed by The Medical Biofilms Laboratory (MBL) at the Center for Biofilm Engineering (CBE), Montana State University

Bacterial Burden

- **Bacterial load measurement**
  MolecuLight® imaging device (MolecuLight Corp.)
- **Bacterial burden calculation**
  Sum up red fluorescence and cyan fluorescence pixels, convert to area (cm^2)



Disposition and Baseline Characteristics

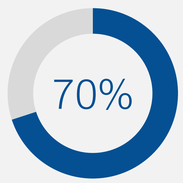
- **Enrolled**
  12 patients (8 VLU, 4 DFU)
  in 3 U.S. Sites
- **Completed treatment**
  10 patients (7 VLU, 3 DFU)

Baseline Characteristics

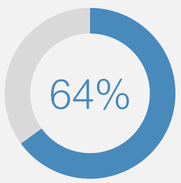
	VLU n=8	DFU n=4
Mean age (years, (SD))	63 (6.65)	59.8 (4.19)
Female Gender (n, (%))	3 (37.5)	2 (50)
Wound size (Cm^2, (SD))	6.5 (7.9)	8.34 (10.6)
Wound Age (weeks, (SD))	22.6 (16.1)	18.0 (6.9)
Non-viable tissue (%, (SD))	80.6 (11.8)	67.5 (9.6)
Granulation tissue (%, (SD))	18.8 (11.9)	32.5 (9.6)

Results

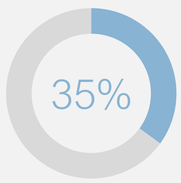
- **7/10** achieved complete debridement within a median of **2 applications**
- By end of the follow-up, the mean ± SD reduction in wound area was 35% ± 38.
- In all 6 patients who were positive for biofilm at baseline, the biofilm was reduced to single individual microorganisms or no detected microorganisms by the end of treatment.
- Red fluorescence for Staphylococcus aureus decreased from a mean of 1.09 cm^2 ± 0.58 before treatment to 0.39 cm^2 ± 0.25 after treatment.



Complete debridement **achieved** within 8 applications (avg 3.9 applications)



Bioburden reduced by end of treatment

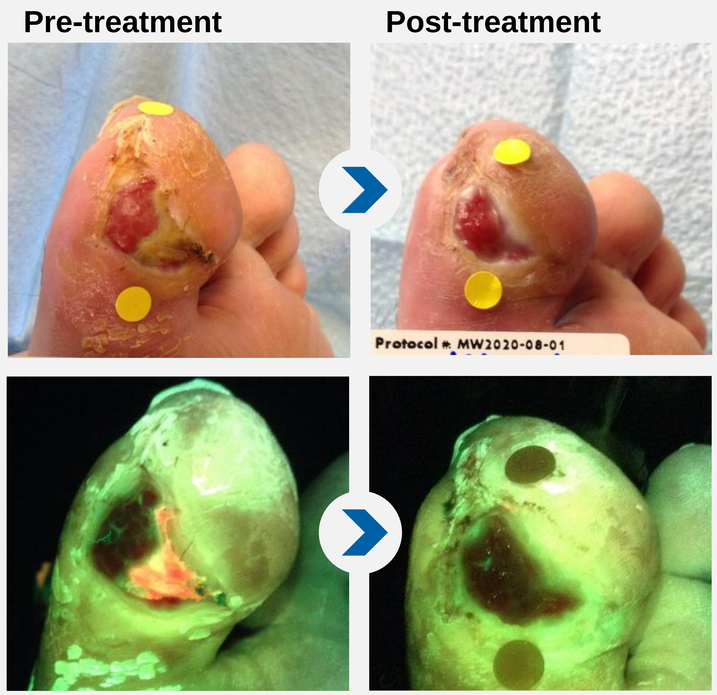


Wound size reduced by end of two-week follow-up

Biofilm substantially reduced for all patients positive for biofilm at baseline

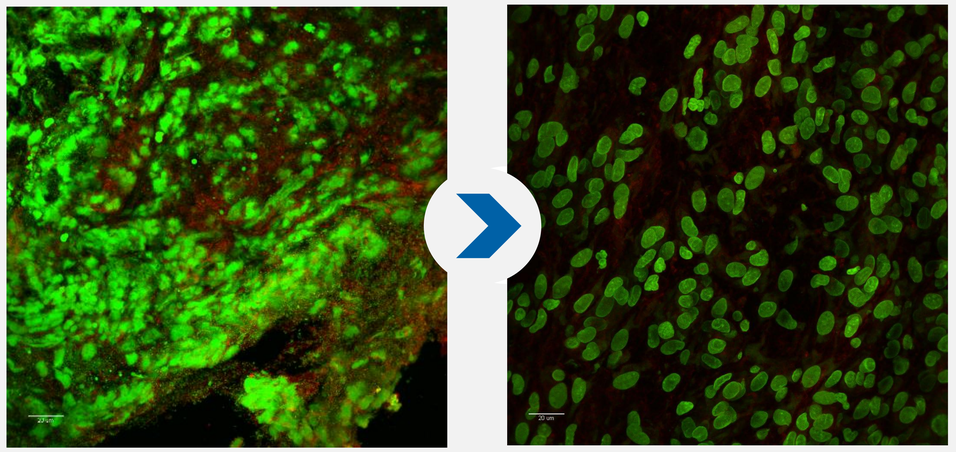
Reduction in wound size, biofilm and bacterial burden

Case Study: Bacterial Burden Reduction



Top: Wound bed pre- and post-treatment (5 applications).
Bottom: Fluorescent images pre- and post-treatment.

& Biofilm Removal



Pre-Treatment
Biofilm score: 5
(Thick continuous film of microorganisms)

Post treatment
Biofilm score: 1
(Single individual microorganisms)

Wound Biopsies from the same patient shown on the left, pre- and post-treatment

Conclusions

The results of this clinical study suggest that BBD may be effective in debriding DFU and VLU, reducing biofilm and planktonic bacterial load, and promoting reduction in wound size