Results from an Open Phase 2 Study Assessing the Safety, Efficacy and Pharmacological Effects of Bromelain-based Enzymatic Debridement Product on Biofilm and Microbial loads in patients with DFU and VLU

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Introduction: Debridement represents a key step in the management of chronic wounds and is considered a basic necessity to induce tissue repair. Biofilm infection in wounds is a major contributor to increased morbidity, delayed healing, persistent infections, and greater economic burden. Use of novel therapies that specifically remove the necrotic tissues, reduce bioburden and promote biofilm dispersion is a promising strategy for improving patient outcomes. Bromelain-based enzymatic debridement agent (BBD) is currently in clinical development for debridement of chronic wounds. Study objectives were to assess its safety, efficacy and pharmacological effects in up to 15 patients with venous leg ulcers (VLUs) and diabetic foot ulcers (DFUs).

Methods: Twelve DFU and VLU patients were enrolled to a prospective, open label, single-arm phase 2 study. Patients were treated with up to 8 daily applications of BBD and then continued follow-up for 2 weeks. Punch biopsies (3.0mm) and wound fluids were collected prior to the first and after last treatment for evaluation of biofilm presence and biomarkers of wound healing and inflammation, respectively. Moleculight™ Fluorescence imaging device was used during treatment to measure bacterial load (>10^4CFU/g). Red and cyan fluorescence are associated with bacterial loads. Fluorescence imaging was also utilized to identify the highest fluorescence area to obtain the biopsy. Biopsy samples were examined by the Center for Biofilm Engineering (Bozeman, MT) using a confocal scanning laser microscope (CSLM).

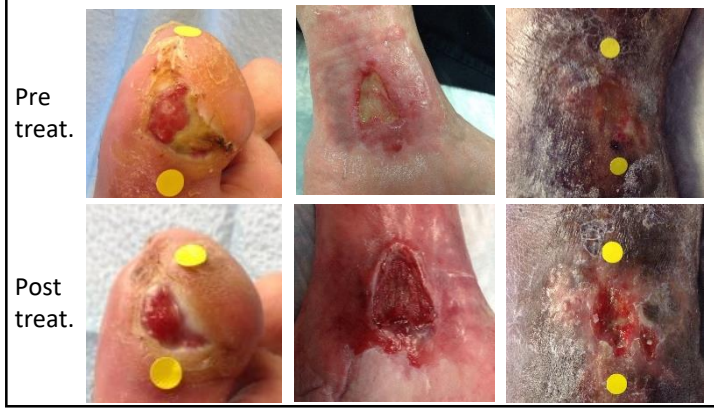

Figure 1: Representative photos of VLU and DFU wound beds before (top) and after end of treatment (bottom) taken from three subjects. Photos on the bottom were taken post last treatment- after 5, 8 and 2 daily treatments (from left to right, respectively).

Results: Seven DFU and 3 VLU patients completed treatment (two patients discontinued). In 8/10 patients 89-100% Non-Viable Tissue was removed within 2-8 applications (Fig1). Additionally, an average reduction of 35% in wound size was measured by the end of 2 weeks follow-up period. In all patients that were positive for biofilm at baseline, the biofilm was reduced to single individual microorganisms or not detected by the end of treatment with an average Biofilm score of 2.0 at baseline vs. 0.91 at the end of treatment (fig. 2). Average±SEM red fluorescence (e.g. *Staphylococcus aureus*) reduced from 1.19±0.69 cm^2 pre-treatment to 0.44±0.29 cm^2 post treatment, and in two patients cyan fluorescence (*Pseudomonas aeruginosa*) reduced from 3.19±0.59 cm^2 to zero (fig. 3). Safety data show that BBD is safe and well tolerated.

Analysis of biomarker content and activity was non conclusive.

Conclusion: The data shows that **BBD is safe and can effectively debride wounds and promote wound area reduction while reducing biofilm and bacterial bioburden**.

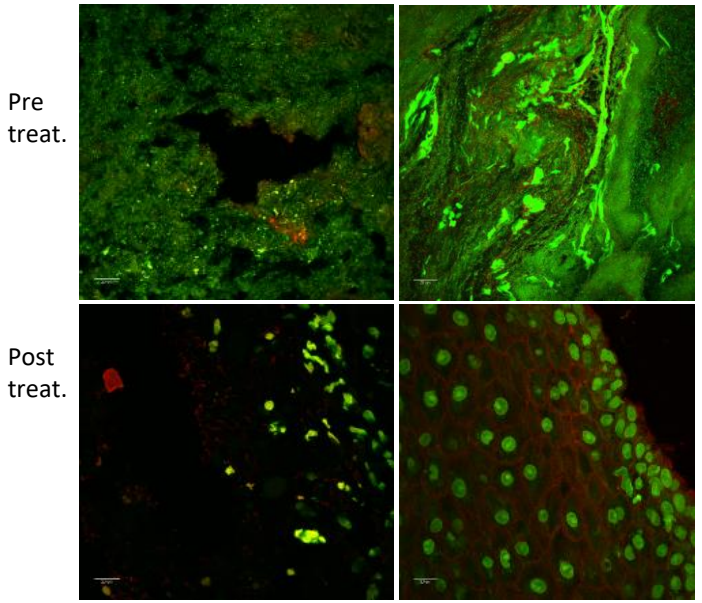

Figure 2: Wound biopsies pre and post treatment from 2 representative wounds. Pre tr. biopsy sections were ranked with a biofilm score of 5 (Thick (> 10 um) continuous film of microorganisms). Post tr. biopsy sections were ranked as 1 (Single individual microorganisms), considered negative for Biofilm).

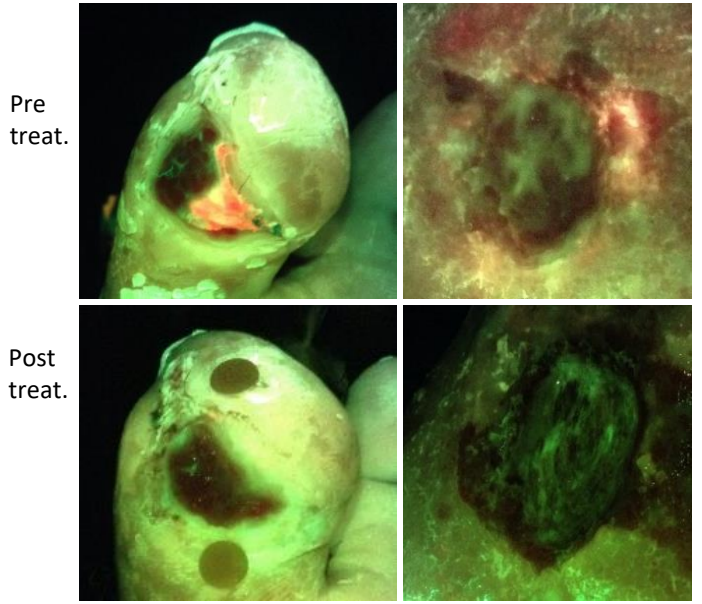

Figure 3: Fluorescent images from two representative wounds, demonstrating the bacterial load on the wound pre and post treatment. In the wound on the left side the fluorescent area was reduced from 0.84 cm^2 to 0.2 cm^2 post treatment. On the right, fluorescence was reduced from 7.08 cm^2 to 0.