BROMELAIN BASED DEBRIDEMENT - MOLECULAR MODE OF ACTION

Klinger, E[1]; Asculai, E[1]; Geblinger, D[1]
1. MediWound Ltd.

Overview

BACKGROUND

Debridement, the removal of non-viable tissue, is a critical first step in treating burns and chronic wounds e.g., venous leg ulcers (VLU) and diabetic foot ulcers (DFU). It clears necrotic tissue and prepares the wound bed for subsequent wound management and healing.

The composition of non-viable tissue varies by etiology and wound characteristics, and may include denatured collagen, over secreted extracellular matrix (ECM) proteins, foreign material, and microbial bioburden.

BROMELAIN BASED DEBRIDEMENT (BBD)

- Mixture of proteolytic enzymes enriched with bromelain, derived from the stem of pineapple plant
- The active ingredient in **NexoBrid®**, FDA/EMA approved for eschar removal in burns, and in **EscharEx®**, an investigational biological product currently in Phase III clinical trials for VLU

OBJECTIVES

To explore the molecular mechanisms underlying the efficacy and selectivity of BBD in debridement of severe burns and chronic wounds

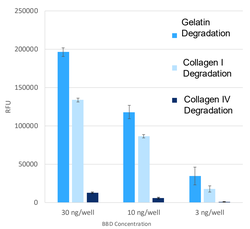
METHODS

- The specific activity of BBD enzymes (fluorescence/BBD concentration) was evaluated using fluorescently labeled native and denatured collagen
- Kinetic studies using Franz cells with ECM protein membranes assessed the degradation rates of various proteins by BBD
- Porcine chronic wound model was developed to further assess the ability of BBD to degrade necrotic tissue and slough

Results

IN-VITRO MECHANISTIC STUDIES

BBD DEGRADES DENATURED COLLAGEN (GELATIN) FASTER THAN NATIVE COLLAGEN

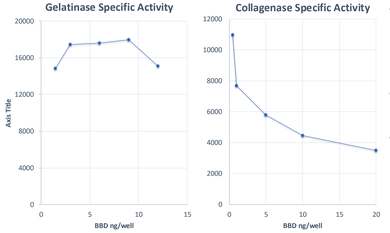


Greater degradation of gelatin indicated by stronger fluorescent signal (RFU)

Eschar in burn wounds consists primarily of gelatin, a denatured form of collagen

In chronic wounds, MMPs degrade intact collagen, leading to denatured, non-functional collagen that impairs wound healing

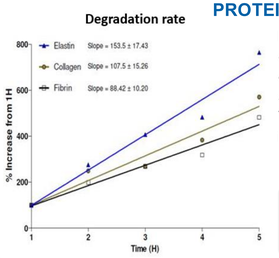
PROTEASE INHIBITORS CONTROL BBD'S ENZYMATIC ACTIVITY



- **Collagenase** specific activity, measured by RFU/BBD concentration, decreased with increasing BBD concentration, indicating the presence of protease inhibitors within the BBD mixture
- The presence of protease inhibitors in BBD's mixture was confirmed by LC-MS/MS analysis
- **Gelatinase** specific activity was not affected by BBD concentration, suggesting a higher affinity of BBD to gelatin

Gelatin, the predominant component in burn eschar, hinders burn healing

BBD DEGRADES VARIOUS OVER-SECRETED ECM PROTEINS

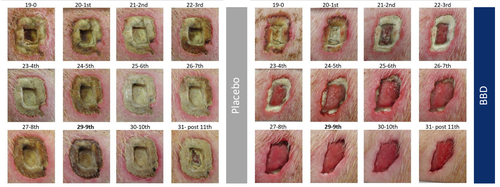


Fluorescently labeled fibrin, collagen, and elastin were degraded over time in a Franz cell system.
The increased RFU over time indicates the rate of proteolysis

Over-secretion of ECM proteins in chronic wounds prevents progression to normal healing

PRECLINICAL AND CLINICAL EVIDENCE

PORCINE CHRONIC WOUND MODEL



BBD achieved complete debridement and granulation tissue formation in eight daily applications, demonstrating superiority over placebo[1]

CHRONIC WOUNDS

Three phase 2 studies demonstrated complete debridement and granulation tissue formation within days[2,3,4]

SEVERE BURNS

FDA/EMA approved for eschar removal in deep partial-thickness and/or full-thickness thermal burns[5,6]

Summary

- In vitro studies demonstrated BBD's multi-targeted proteolytic activity:
  - Degrades denatured collagen significantly faster than native collagen
  - Effectively degrades over-secreted ECM proteins, including elastin and fibrin
- BBD's efficacy in debridement and promotion of granulation tissue have been demonstrated in a novel pig model of hard-to-heal wound
- Phase 2 and 3 clinical trials in patients with burns, VLU and DFU have demonstrated the efficacy, safety and selectivity of BBD and its superiority over placebo and standard of care

Conclusion

The efficacy of BBD lies in its distinctive enzymatic composition, which is uniquely suited to target and degrade the various denatured proteins and non-viable tissues that impair healing in burns and chronic wounds.

1. Shoham et al. 2021; *Journal of Wound Care* **2.** Shoham et al. 2021; *Wound Rep Reg*
3. Snyder et al. 2023; *Wounds Journal* **4.** Shoham et al. 2024; *LANCET's eClinicalMedicine*
5. Shoham et al. 2023; *Journal of Burn care & Research* **6.** Shoham et al. 2025; *Burns*