A novel biofilm disruptive agent influences the wound healing process
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ABSTRACT

Chronic wounds, which include pressure ulcers and diabetic foot ulcers, affect approximately 6.5 million persons with an annual cost for treatment. We recently showed that a wound gel containing a Biofilm Disruptive Agent (BDA)* inhibits bacterial infection of chronic wounds. This BDA* may also promote wound healing by influencing the host immune response. Using the murine model of wounds, we examined the influence of the BDA* on wound healing. Full-thickness wounds were generated and covered with sterile gauze (untreated wound, UTW), gauze coated with polyethylene glycol base (PEG-treated wound, PTW), or gauze coated with BDA* gel (BDA-treated wound, BDA-TW*). The wound bed and margins were excised at 1, 3, and 7 days post-wounding. Formalin-fixed tissues were processed and sectioned at 5.0 μm. The sections were stained with H&E for general histological observations. On day 1 post-injury, a neutrophilic infiltrate (PMNs) was present throughout the wound beds in all three treatment groups with a larger number of PMNs observed in the PTW and BDA-TW*. By day 3, re-epithelialization had advanced in all three wounds and granulation tissue was present. Neo-vascularization was now present in the UTW, while healed tissue in the PTW had hair follicles regeneration however, the BDA-TW* showed over twice as many regenerated hair follicles as the UTWs. A mononuclear infiltrate was more evident in the BDA-TW* along with evidence of organized tissue formation. These results suggest that keeping the wound moist (PTW and BDA-TW*) appears to accelerate healing while the treatment with BDA* more completely augmented the healing process.

INTRODUCTION

Chronic wounds are defined as those that fail to proceed through an orderly and timely reparative process to produce anatomic and functional integrity of the injured site. Chronic wounds constitute a serious threat to the public health worldwide. Types of chronic wounds include diabetic foot ulcers, venous leg ulcers, pressure ulcer, failure of peripheral vascular disease, and others resulting from peripheral vascular disease in the United States, it is estimated that chronic wounds affect 6.5 million persons. Due to the increase in the incidence of diabetes and obesity, plus the increase in the cost of health care, the financial burden for treating chronic wounds is growing very rapidly. Treatment of chronic wounds in the United States may reach as high as $25 billion annually.

In general, the wound healing process is divided into four overlapping stages; hemostasis, inflammation, proliferation, and remodeling. The hemostasis stage begins as the tissues are injured and when blood moves into the site of injury. The inflammation stage follows hemostasis and is characterized by the appearance of the neutrophils and macrophages. The appearance of neutrophils and macrophages in the wound leads to an increase in the secretion of growth factors such as inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), and interleukin-1 (IL-1), and IL-6. The proliferation stage involves migration of fibroblasts to the wounded tissues. The fibroblasts perform several functions including the deposition of new extracellular matrix, promotion of angiogenesis, and the release of cytokines such as interleukins, fibroblast growth factor and TNF-α. During the remodeling stage, the wound becomes re-epithelialized, the extracellular matrix becomes cross-linked, and the healed wound becomes less vascular. Each of the above described healing stages involve variations in the expression of different cytokines, chemokines, and other wound-healing related genes. In addition to the changes in the cytokine/chemokine gene expression, histological changes observed between each stage of the wound healing process including differences in tissue architecture and immune cell infiltration.

NxtSc G5* wound gel (NS) is a novel antimicrobial/antifouling agent designed by the Next Science Company (Jacksonville, FL) to destroy the extracellular polymeric substances (EPS) matrix of the bacterial biofilm and kill bacteria within the biofilm. In addition to its antimicrobial properties, we have recently shown that NxtSc-G5, now marketed as Blast™ Wound Gel (BWG), alters the cytokine/chemokine expressions involved in the wound healing process. Due to its effects on the cytokine/chemokine expressions, BWG may further influence positive histological changes in one or more stages of the healing process by influencing immune cell infiltration and structural changes in the tissue.

In this study, we utilized hematoxylin and eosin (H&E) staining to observe histological changes in the tissue structure and immune cell infiltration between injured mice whose wounds were covered untreated, those treated with polyethylene glycol (PEG), the base for BWG, and mice treated with BWG at 1, 3, and 7 days post injury.

HYPOTHESIS

Treatment of wounds with BWG will affect the wound healing process throughout the three stages of healing, visibly and/or microscopically.

EXPERIMENTAL DESIGN

FIGURE 1: Diagram of the experimental design. (A) Remove hair from the backs of 3 groups of 9 mice. (B) Create a 1.0 by 1.0 cm full-thickness wound on the back of each mouse. (C) Cover the wounds with sterile gauze (untreated wound, UTW), gauze coated with PEG (control group [PTW]), or gauze coated with BWG (treatment group [BDA-TW]). (D) At 1, 3, or 7 days post-injury, 3 mice from each group were euthanized and the wound bed and surrounding tissue was excised, preserved in formalin, and submitted to the Dept. of Pathology, TTUHSC, Lubbock, TX for histologic processing. H&E staining was done on 5.0 μm sections.

RESULTS

FIGURE 2: Changes in wound size are apparent visibly (grossly) in the skin wounds. The Silhouette 3D wound measurement, imaging and documentation system was used to determine the size of the healing wounds. UTW closed by contracture at 0.13, leaving the skin puckered. PTW healed without skin puckering although 2 plateaus occurred during the healing process (indicative of disorganized poor tissue arrangement). BWG (BDA-TW) healed at a steady pace without skin puckering.

FIGURE 3: Day 1 post-injury. Skin sections were stained with H&E and photographed at 100X magnification unless indicated otherwise. Panoramic images of the entire wound bed plus normal skin on each side were made in Adobe Photoshop by linking overlapping photomicrographs. A break indicates a break in the tissue occurred during acquisition of samples, processing, and/or sectioning. (A) Labelled photomicrographs of normal, uninjured mouse skin sections. (B) UTW. (C) PTW. (D) BDA-TW. The composite images are representative of the wound beds of 3 mice per treatment group. A neutrophilic infiltrate was present throughout the wound beds in all 3 treatment groups with the heaviest infiltrate seen in the PTW and the mildest in the UTW group. Edema and hyperplasia of cells in the adventitia was observed with all 3 groups; overall, PTW ≥ BDA-TW > UTW.

HYPOTHESIS

Treatment of wounds with BWG demonstrated a positive impact on histological changes in one or more stages of the healing process by influencing immune cell infiltration and structural changes in the tissue. The BWG also displayed healing at a steady state without skin puckering, promoted more rapid neo-angiogenesis throughout the wound bed, showed increased regeneration of hair follicles and hypodermis, with more rapid advancement of healing by second intention.

CONCLUSIONS

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