

Clinical Aspects of Acute and Chronic Wound Healing: Swine As Preclinical Model For Developing Therapies For Skin Wound Healing

Introduction

Skin serves as a barrier between the external environment and the underlying tissues. When the integrity of the skin is disrupted this barrier is compromised. In order to prevent further damage or infection, proper wound healing should occur immediately. It is estimated that wounds account for approximately 4% of all health system costs. Therefore, it is essential to study the process of wound healing with the right set of tools and research models to develop protective and restorative interventions.

Clinical wound healing models can be divided into two groups: partial thickness wounds and full thickness wounds. Partial thickness wounds are damage to the epidermis and superficial dermis with no further damage to the blood vessels whereas full-thickness wounds are characterized by the complete destruction of epidermis, dermis and the blood vessels. In the clinical setting, wounds may also be defined as acute or chronic. Acute wounds are caused by trauma and or surgery whereas chronic wounds are due to impaired acute wound healing and include diabetic foot ulcers or chronic venous leg ulcers.

Cellular Processes of Healing: *Immune and growth factors*

The process of wound healing can be broken down into four stages: 1. Coagulation, 2. Inflammation, 3. Migration and Proliferation, and 4. Remodeling. The success of wound healing is dependent on a coordination of cellular processes directed by growth factors, cytokines, and chemokines that facilitate the rapid and effective progress through the stages of healing.

Upon injury to the skin, interleukin-1 (IL-1) is released which signals to the neighboring cells to fight the damage. In addition, blood components are released that turn on the clotting cascade. Clotting reestablishes homeostasis and provides an environment for the inflammatory cells to enter the wound healing process. Platelets secrete growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-beta). PDGF and IL-1 serve as chemoattractant for neutrophils to come to the wound site to combat infection. TGF-beta helps transformation of monocytes to macrophages which in turn release proinflammatory cytokines (IL-6 and IL-1) and growth factors (PDGF, TGF-beta, EGF, fibroblast growth factor (FGF)).

All these events lead to proliferation of endothelial cells and angiogenesis which are essential for the synthesis and deposition of a new extracellular matrix on which the regenerated epithelium is built. Within hours, reepithelialization ensues and release of further growth factors such as EGF, TGF-alpha and FGF act to stimulate epithelial cell migration and proliferation. The final step involves keratinocytes undergoing differentiation and remodeling to restore the barrier function. All these factors have been well studied in the animal models of wound healing and can be quantified to measure the progression of wound healing and the efficacy of novel wound-healing therapeutic candidates.

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Swine as a Preclinical Model of Wound Healing

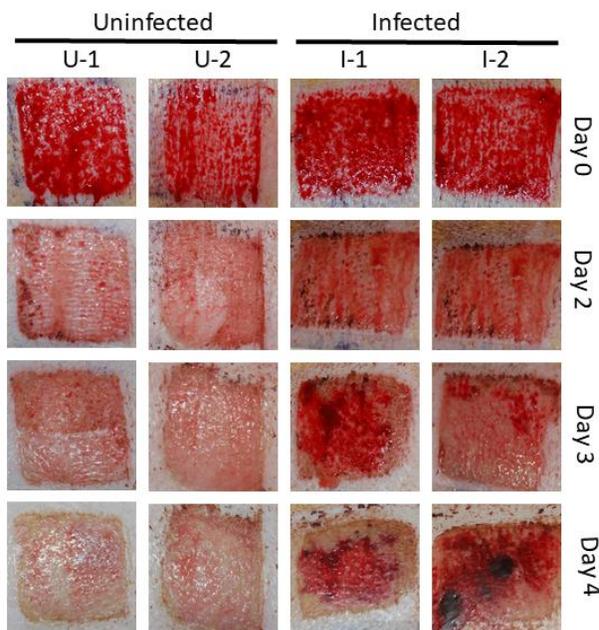
In order to study wound healing, it is important to use the right animal model. Models have been established in several species including mice, rats, pigs, and non-human primates. While non-human primate wound healing is the most representative of wound healing in humans, cost and ethical restrictions make use of these models prohibitive. It has been shown that the process of wound healing in rodents is different than that in the human; however, pig skin is 78% similar to human skin making pigs a viable animal model to study wound healing. For example, while rodents close partial-thickness wounds through contraction while pigs and humans heal primarily through the process of reepithelialization.

Using pigs as a model of wound healing, one can study effects of certain drugs on wound healing, bacterial infection at the site of the wound, histology, gross pathology and wound inflammation score. Noble Life Sciences optimized pig partial thickness, full thickness and burn wounds with and without infection to study the efficacy of candidate therapeutics in the wound healing and antimicrobial activity.

Pig Partial thickness wound and bacterial infection

Wound infection is a clinical risk factor for both acute and chronic wounds. In both, the presence and colonization of the damaged skin by pathogenic or opportunistic bacteria can impede the normal progress of healing and lead to a persistent chronic wound, tissue necrosis, sepsis, and even death. A major challenge to the development of effective therapies for the treatment of infected wounds is the ability of bacteria to form a biofilm at the surface of the wound that creates a physical barrier to the action of antimicrobial agents and wound healing therapies.

In order to facilitate the development of therapeutics for the treatment of infected wound, Noble Life Science employs and partial thickness wound infection model in swine (Figure 1). Partial thickness wounds (3 cm x 3 cm x 0.5 mm) were created in pig skin using a Dermatome. A subset of wounds were inoculated with 2×10^6 CFU of methicillin-resistant *Staphylococcus aureus* (MRSA). The wounds were



Partial wound and bacterial biofilm formation.

covered with bandages for two days to allow the formation of bacterial biofilm. Bacterial loads in wound sites were determined by swabbing wounds (Days 2 to 4) and collecting 8 mm tissue biopsies (Day 4). Swabs placed into 1 mL of sterile PBS and vigorously vortexed and tissue biopsies were placed into 5 mL PBS and homogenized with a polytron homogenizer and a rotor stator probe. Serial 10-fold dilutions of the swab and tissue homogenates were prepared in PBS and plated onto Oxacillin salt plates. Colonies were enumerated following 48 hours of incubation at 35°C and results expressed as CFU/mL for the swabs and CFU/mm² for the tissue biopsies (Table 1). Additional biopsy specimens were collected on Day 4 and used for histopathology for markers of inflammation and wound healing (H&E staining) and bacterial perfusion (Gram

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staining).

Full Excision Model and Burn Model of Wound Healing in Pigs

Noble Life Sciences has also developed a full excision and full excision burn wound model in domestic swine (Yorkshire breed) and yukatan mini-pigs. An example of the two models in yukatan minipigs is illustrated in Figure 2.

A total of eight full excision wounds (1 in x 1 in) were created along the spine of yukatan minipigs (four wounds on each side). Wounds were bandaged and bandages were changed every three to four days.



Half of the wounds were treated with a commercial wound healing cream at the time of wounding and at each change of bandage. Wound size was recorded at the time of bandage change to monitor the progress and rate of healing (Figure 3). As shown in Figure 3, there was a delayed early healing of the burned wounds compared to the full excision wounds that were

Figure 2. Full excision and burn wound healing in minipig model!

not subjected to burning. However, there was no notable difference in the rate of wound size reduction between the treated and untreated wounds. In addition to visual wound closure, other metrics that could be applied to differentiate the effectiveness of a therapeutic wound healing compound from an untreated wound include cellular and molecular markers of inflammation and epithelialization and histology.

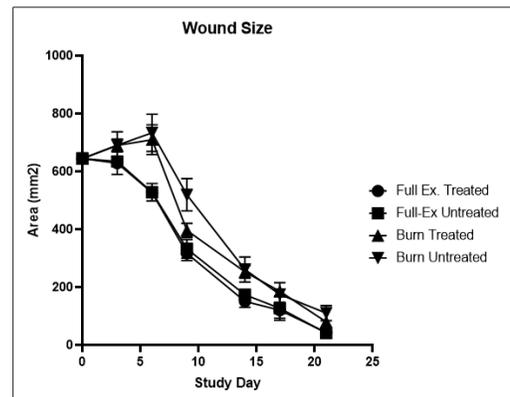


Figure 3. Rate of wound healing in full excision and burn wound healing in minipig model