Research Techniques Made Simple: Animal Models of Wound Healing

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Animal models have been developed to study the complex cellular and biochemical processes of wound repair and to evaluate the efficacy and safety of potential therapeutic agents. Several factors can influence wound healing. These include aging, infection, medications, nutrition, obesity, diabetes, venous insufficiency, and peripheral arterial disease. Lack of optimal preclinical models that are capable of properly recapitulating human wounds remains a significant translational challenge. Animal models should strive for reproducibility, quantitative interpretation, clinical relevance, and successful translation into clinical use. In this concise review, we discuss animal models used in wound experiments including mouse, rat, rabbit, pig, and zebrafish, with a special emphasis on impaired wound healing models.

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Description: This article, designed for dermatologists, residents, fellows, and related healthcare providers, seeks to reduce the growing divide between dermatology clinical practice and the basic science/current research methodologies on which many diagnostic and therapeutic advances are built.

Objectives: At the conclusion of this activity, learners should be better able to:
- Recognize the newest techniques in biomedical research.
- Describe how these techniques can be utilized and their limitations.
- Describe the potential impact of these techniques.

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INTRODUCTION

The critical processes underlying wound healing have been initially described using animal models (Eming et al., 2014; Martin, 1997). Although animals do not develop chronic wounds in a way that closely resembles those arising in humans, animal models have provided valuable insights into the principles of wound management. For example, the now accepted notion that wounds heal faster when kept moist came from research experiments in the domestic pig (Helfman et al., 1994). However, because of anatomical and physiological differences among and within animal species, including humans, no single model can suit all needs. Data generated from preclinical studies on wound repair may vary considerably depending on the animal model chosen and on other biological variables such as age, sex, microbiome, and wound location (Elliot et al., 2018). Preclinical models should be validated before proceeding with testing.

When looking at preclinical models of wound healing, the majority of studies are performed in either rodents or pigs.

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BENEFITS

- Investigating mechanisms of wound repair and regeneration.
- Testing the efficacy and safety of potential therapeutics.
- Ethical regulations prohibit the use of humans, especially humans with impaired wound healing, in potentially harmful studies.

LIMITATIONS

- Anatomical and physiological differences among and within animal species, including humans. No one model can recapitulate the heterogeneity and complexity of chronic wounds in humans.
- Reproducibility and translation of preclinical data into clinical reality remains an ultimate challenge.
- Lack of standardization in designs and procedures.

Primates are rarely used, mainly because of the higher cost and animal care committees' concerns regarding these animals. Moreover, primates heal with far less collagen deposition than humans do. Other animals offer limited benefit for wound research because of their size, temperament, and maintenance expense.

ACUTE AND IMPAIRED HEALING

The natural (acute) wound healing process in adult mammals, including humans, progresses in four orderly phases that overlap in time: coagulation, inflammation, migration-proliferation (including matrix deposition), and remodeling (Falanga, 2005). Acute wounds, such as those created by surgery or trauma, occur suddenly and heal in a relatively predictable timeframe. Deregulation or interruption of one or more phases of the normal healing process leads to chronic wounds (Eming, 2014). A chronic wound is a wound that fails to progress through the normal phases of healing in an orderly and timely manner. Persistent inflammation is a hallmark of the chronic wound microenvironment. Some of the major causes of impaired wound healing include diabetes mellitus, vascular insufficiencies, and prolonged local pressure.

ANIMAL MODELS OF ACUTE HEALING

Acute wound models are useful for studying the natural healing processes and for drug discovery. Although we will focus mainly on models of impaired healing, acute wound models that are commonly used include excisional, incisional, and burn models, which all have well-established protocols (DiPietro and Burns, 2003).

ANIMAL MODELS OF IMPAIRED HEALING

Chronic wounds in animals can be created from an acute wound by inducing diabetes, mechanical pressure, ischemia, or reperfusion injury. Chronic wounds are uncommon in animals, and thus all animal models have limitations (Mustoe et al., 2006).

Diabetic wound models

No single model can reproduce the entire diabetic pathological process and its variations. Each model mimics merely one aspect of this complex disease. Hyperglycemia can be chemically induced in mice and rats by intraperitoneal or caudal vein injection of streptozotocin or alloxan to cause selective destruction of insulin-producing beta cells of the pancreas. Animals are allowed to manifest hyperglycemia for several weeks before making a cutaneous wound through cutting, burning, or radiation. A pig model of diabetic ulcers was established (Velander et al., 2008). However, these wounds healed after 18 days, which is not consistent with diabetic wounds in humans. Diabetes and insulin resistance can be induced by genetic manipulation as well. There are two types: type 1 diabetes models include the nonobese diabetic (i.e., NOD) mouse, streptozotocin-induced diabetic rat or mouse, bio-breeding (i.e., BB) rat, and Chinese hamster. Type 2 diabetes models include the obese *ob/ob* mouse (leptin receptor deficient), *db/db* mouse (a point mutation in the leptin receptor gene), NONcNZO10 mouse, and Zucker *fa/fa* rats. The most common type 2 diabetic model (*db/db* mouse) has significant limitations in predicting humans outcomes because human type 2 diabetes does not involve leptin abnormalities and is polygenic. No animal model mimics the chronic problems that result in type 2 diabetic ulcers (Fang et al., 2010).

Pressure ulcer models

The primary cause of pressure ulcers is repeated ischemia-reperfusion injury caused by prolonged mechanical pressure, especially over a bony prominence. Pressure ulcers can be modeled in loose-skinned animals such as rats and mice by surgically implanting a metal plate under the skin (Figure 1), followed by intermittent and periodic compressions of the skin using an external magnet (Reid et al., 2004; Wassermann et al., 2009). Loose-skinned animals with little subcutaneous fat, mainly rats, are suitable for modeling aged human skin (Nguyen et al., 2008). Greyhound dogs have also been used because of their thin skin (Swaim et al., 1993). Pigs are better animals to model pressure ulcers of young humans because of their tight skin (Nguyen, 2008). A cast can be placed over a bony prominence in pigs to cause a reperfusion injury and friction on the skin surface (Swaim et al., 1997).

Ischemic wound models

The rabbit ear ulcer model has been extensively used to simulate ischemic wounds. Cutaneous ischemia is created by ear vessel ligation. Skin banding has been shown to create an ischemic model in guinea pigs (Constantine and Bolton, 1986). Bipedicle flap (surgically isolated area of skin with minimal continued blood supply) has been used to create ischemia on the dorsal skin of pigs (Figure 1). Molecular markers are used to validate the hypoxic state of tissues.

Biofilm-infected wound model

One characteristic of chronic human wounds is bacterial infection and biofilm, which impairs healing by inducing prolonged proinflammatory cytokines (Edwards and Harding,