

Review Article

Surgical Approaches to Create Murine Models of Human Wound Healing

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Wound repair is a complex biologic process which becomes abnormal in numerous disease states. Although *in vitro* models have been important in identifying critical repair pathways in specific cell populations, *in vivo* models are necessary to obtain a more comprehensive and pertinent understanding of human wound healing. The laboratory mouse has long been the most common animal research tool and numerous transgenic strains and models have been developed to help researchers study the molecular pathways involved in wound repair and regeneration. This paper aims to highlight common surgical mouse models of cutaneous disease and to provide investigators with a better understanding of the benefits and limitations of these models for translational applications.

1. Introduction

Impairments in wound healing constitute an enormous biomedical burden and cause a significant degree of global morbidity and mortality [1–3]. Aberrations in the normal biological response to cutaneous injury following disease, trauma, and surgery inevitably lead to significant complications. The wound repair process is extremely complex and the underlying pathophysiology of chronic wounds and fibrotic disease is often multifactorial [4]. Our incomplete understanding of the molecular, cellular, and physiologic mechanisms governing wound healing accounts for the often disappointing results of modern therapies.

The predominant cell populations in mammalian skin are fibroblasts and keratinocytes. Accordingly, the vast majority of *in vitro* wound healing studies utilize either one or both of these cell types. The study of cellular behavior in a two-dimensional culture dish offers the ability to investigate specific targets with minimal interference from external factors, but critical *in vivo* cues (paracrine signaling, three-dimensional cues, etc.) are missing and thus limit the translational applicability of *in vitro* studies. *In vitro* coculture

experiments partially address the importance of paracrine interactions between different skin cell populations [5], but are also limited in their biological relevance to wound healing.

Increasingly complex “organotypic” systems have been developed to better recapitulate the native skin environment [6]. These “living skin equivalents” are engineered constructs composed of stratified squamous epithelial cells grown at an air-liquid interface above a collagen-type matrix seeded with dermal fibroblasts [7]. These models have greatly improved the ability of researchers to study the mechanisms of human disease (including tumorigenesis and wound repair) in a more biologically relevant *in vitro* system [8]. Regardless, the complexity of wound healing *in vivo* cannot be fully recreated in a culture dish, and animal models are a necessary tool in elucidating the underlying pathology of human disease.

In silico and computer-based models allow for large-scale processing of vast data sets and simulation of myriad conditions which would be difficult to test otherwise. For example, these methods have been utilized to study wound healing, inflammatory responses, and drug permeability across skin [9–11]. Finite element methods have been applied

to study dynamic processes such as the role of mechanical forces in scar formation and wound contracture [12, 13]. Nonetheless, these approaches do not obviate the need for an *in vivo* biologic system in which to test predicted results and outcomes.

Species ranging from rodents to nonhuman primates have been utilized to study skin disease [14–16]. More recently, the red Duroc pig has been extensively validated as a model for human skin pathology and is increasingly thought of as the ideal large animal model to study cutaneous disease due to its similarity to human epithelial architecture, nerve density, vascularity, matrix components, and other biological parameters [17–20]. However, swine are expensive to house and maintain, molecular reagents are often not validated for swine tissues, and the use of large animals for highly investigational work is not practical.

The laboratory mouse remains by far the most commonly used animal model for biologic research. Mice are easy to house and maintain, are economical, and a wide variety of mouse-specific reagents are available for research purposes. In addition, over a thousand mutant loci have been generated in mice, and innovative transgenic tools provide researchers with unparalleled opportunities to study disease pathophysiology [21]. Hundreds of mouse models of human disease exist, and in many cases the diseased gene of interest is mutated in both human and mouse [22]. Thus, the vast abundance of disease models, knockout strains, and transgenic tools have ensured that mouse models will remain highly relevant to the study of skin biology [23, 24]. This paper will focus mainly on surgical models of pathologic wound healing and their contribution to our understanding of human wound repair.

2. Mouse versus Human Wound Repair

Before we review several commonly used mouse models to study wound repair, it is important to briefly discuss some major differences between mouse and human skin (Table 1). Although mouse skin consists of three layers as human skin does (i.e., epidermis, dermis, hypodermis), there are significant differences in the anatomy and physiology of each layer. Mouse skin is covered with dense hair that undergoes a defined cycle of hair growth as does human hair: anagen (growth), catagen (regression), and telogen (rest) [25]. The hair cycle on the mouse dorsum progresses from cranial to caudal, and its temporal characteristics can differ significantly from various regions of the human body [26]. For example, the mouse hair cycle is about three weeks, whereas hair cycles of the human scalp can last several years [25]. Additionally, human hair is generally either vellus (penetrates to the superficial dermis and is unpigmented) or terminal (penetrates into deep dermis and is pigmented) and exhibits an androgen-sensitive switch from vellus to terminal forms, properties not characteristic of mouse hair [25].

Mouse skin also lacks apocrine sweat glands and rete ridges/dermal papillae, which are both found in human skin. However, rete ridge-like structures may become apparent during mouse wound healing and are often described as

“pseudoeplitheliomatous” or “pseudocarcinomatous hyperplasia” [27]. Mouse skin is also unique in having a panniculus carnosus layer (a thin muscle layer found only as the platysma of the neck in humans) which produces rapid wound contraction following injury. In contrast, human wounds heal via re-epithelialization and granulation tissue formation, important differences to consider when assessing the translational relevance of mouse studies. Another factor to consider when designing mouse experiments is the significant gender difference in mouse skin anatomy and physiology. For example, male skin is 40% stronger due to a much thicker dermis, while female skin exhibits a thicker epidermis and hypodermis [28]. Our laboratory has also previously reported that mouse skin is significantly more compliant than human skin, which is important when examining the mechanical environment of wound repair [29]. Despite these numerous differences, mouse models have contributed significantly to our understanding of skin biology and disease, and an awareness of these dissimilarities will allow researchers to better evaluate and apply mouse-based models of repair to human disease.

3. Excisional Wound Healing Models

The treatment of chronic nonhealing wounds accounts for a significant proportion of healthcare expenditures [30]. Myriad factors contribute to impaired healing, including deficits in cell activity, inflammatory signaling, and matrix assembly. The most commonly employed mouse model used to study these processes is the excisional wound model (Figure 1(a)). Typically, a full skin thickness excisional wound is created on the dorsum of the mouse and extends through the panniculus carnosus. Wounds are then photographed regularly and wound closure is calculated based on wound size relative to the original wound dimensions. Our laboratory has described a splinted wound model using silicone rings to prevent wound margin contracture, thus better recapitulating the repair mechanisms underlying human wound healing [31].

The benefits of this common surgical model are numerous. The necessary materials and techniques are relatively simple, reproducible, and practical for experiments involving a large sample size. The wound bed can be easily accessed to apply topical agents (e.g., pharmaceuticals, cells, biomaterials) to study modulations of repair processes. Harvested wounds can be examined histologically for both the epithelial gap (the quantifiable distance between the epithelial wound margins) and granulation bed characteristics (recruited cell populations, vascularity, matrix alterations). Thus, this surgical mouse model remains an essential tool for researchers studying cutaneous disease.

4. Mouse Models of Cutaneous Ischemia and Reperfusion Injury

Neovascularization is a critical component of normal wound healing, and impairments in this process are highly implicated in diabetic and aged wound healing [32]. However, new

TABLE 1: Major differences between mouse and human skin.

	Mouse	Human
Hair cycle	Approximately 3 weeks	Highly variable, region-dependent
Epithelial architecture	No rete ridges	Rete ridges present
Apocrine sweat glands	Not present in skin, extensive in mammary glands	Present in axilla, inguinal, and perianal skin regions
Biomechanical properties	Thin, compliant, loose	Thick, relatively stiff, adherent to underlying tissues
Hypodermal thickness	Hair cycle-dependent	Less variable
Subcutaneous muscle layer	Present throughout as panniculus carnosus	Present only in neck region as platysma
Major method of wound healing	Contraction	Granulation tissue formation and re-epithelialization

blood vessel formation is a complex process, involving both angiogenesis (sprouting of new vessels from existing ones) and vasculogenesis (*de novo* formation of neovessels from circulating endothelial progenitor cells). Significant insight into these processes has been provided by mouse models of cutaneous ischemia. One of the most frequently used models is the ischemic skin flap model (Figure 1(b)). In this model, a three-sided full skin thickness peninsular flap is created on the dorsum of the mouse and an impermeable silicone membrane is placed directly underneath the skin flap and the flap incisions are closed with suture. The silicone barrier prevents neovascularization from the underlying wound bed and ensures that the skin flap is supplied on only one side by the flap pedicle, thereby creating a reproducible ischemic gradient extending from the proximal (nearest to the pedicle) to the distal (most ischemic) portion of the flap.

These models have been important in elucidating novel mechanisms of blood vessel formation, specifically, the roles of ischemic signaling and vasculogenesis in wound repair. We have previously employed this model to investigate the role of chemokine pathways in the recruitment of progenitor cells to ischemic wounds [33]. Specifically, we demonstrated that hypoxic wound gradients recruit systemic stem cells through hypoxia-inducible factor (HIF)-1 α -induced expression of stromal cell-derived factor-1. Our laboratory also utilized this model to demonstrate impairments in HIF-1 α -mediated vasculogenesis during aging [34] and the role of oxygen free radicals in diabetic wound repair [35].

Ischemia-reperfusion models have also been developed to study pressure ulcers, the treatment of which costs up to \$11 billion per year in the US [36]. The pathogenesis of pressure ulcers is thought to be mediated through injurious cycles of ischemia and reperfusion, and models based on cyclical magnetic compression have been described (Figure 1(c)), allowing researchers to examine wound inflammation, apoptosis and reactive oxygen-mediated signaling following injury [37, 38]. Wassermann et al. recently described a full thickness skin/muscle pressure ulcer model using a metal disk implanted beneath the mouse gluteal muscle and cyclical pressure applied with an external magnet [39]. Although numerous models have been employed in larger animals using friction, temperature, position, muscle

damage, pressure, and ischemia-reperfusion injury [40], the practical benefits of mouse-based pressure ulcer models cannot be overlooked. Despite our limited knowledge of pressure ulcer pathogenesis, the development of practical small animal models represents a step toward improved understanding of this clinically significant disease.

5. Mouse Models of Skin Fibrosis

Fibroproliferative diseases contribute to the majority of deaths in the developed world [2]. In addition, skin fibrosis following burn and radiation injury, trauma, and surgery causes a significant degree of functional impairment and aesthetic complications [41]. The lack of effective antifibrotic therapies highlights our incomplete understanding of scar pathophysiology. Various mouse models of skin fibrosis have been developed, but fibrosis is induced by myriad agents or modalities with differing mechanisms which may not be physiologically applicable to human scar formation.

Chemical injury models have been described using subcutaneous injections of the sclerosing agents bleomycin or vinyl chloride to mimic human diseases such as scleroderma [42, 43]. Transgenic mice harboring genetic mutations (such as tight skin mice or integrin alpha 1 null mice) and xenograft-induced activation of the mouse immune system have also been used as models for human fibrotic skin disease [44]. Branding, scalding, and flame injury models have been employed to study cellular activation and recruitment following burn injury [45, 46]. For example, Zhang et al. utilized a mouse burn model to study the injury response of circulating angiogenic cells and also examined these cells in human burn injury patients [46]. Potential therapeutics for radiation-induced fibrosis have been studied in mice [47] and scar models utilizing radiation injury in the tight skin mouse have been developed [48].

Our laboratory recently described a mouse model of hypertrophic scar formation by applying exogenous mechanical loads to incisions which otherwise heal with minimal scar [29]. This model is predicated on the clinical observation that human incisions which heal under elevated tension are prone to develop robust scarring, whereas skin injuries

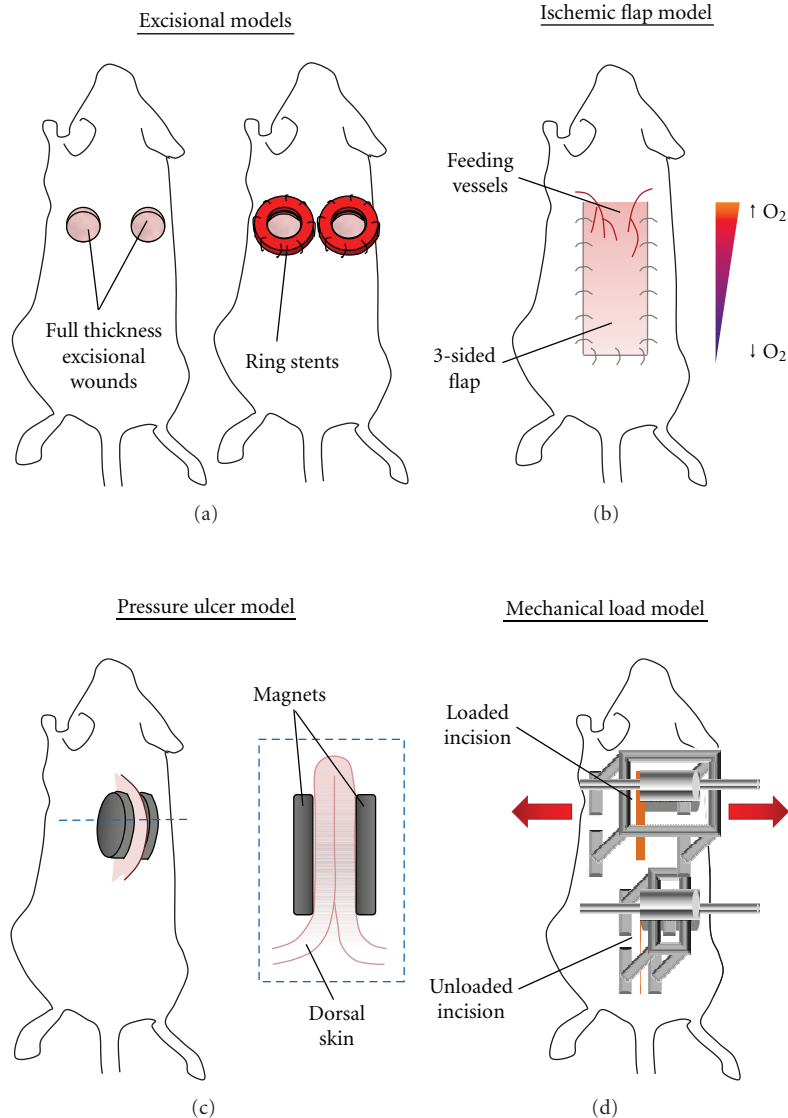


FIGURE 1: Surgical mouse models of cutaneous disease. (a) Full thickness excisional wound models are commonly used to assess numerous components of wound healing *in vivo*. Contraction of the underlying panniculus carnosus can be minimized with a splinted wound model (right) whereby wound repair proceeds mainly through granulation tissue formation and re-epithelialization similar to human skin repair. (b) The ischemic flap model produces a controlled gradient of ischemia based on the number and location of feeding blood vessels. Skin regions furthest from the vessel/s are the most ischemic. (c) Cyclical pressure can be applied with opposing magnets and interpositioned skin to mimic reperfusion/ischemia injury thought to drive pressure ulcer pathophysiology. (d) The application of exogenous mechanical loading to mouse incisions results in increased wound fibrosis similar to human hypertrophic scarring.

in areas under low tension rarely develop large scars [49, 50]. This model employs a distraction device mounted onto the mouse dorsum and mechanical loads are applied orthogonal to the healing incision (Figure 1(d)). Force levels of 1.5 N/mm^2 to 2.7 N/mm^2 are applied during the early proliferative phase of wound repair and are sufficient to induce a significant fibrotic response for over six months [29]. When this mechanical load model was applied to transgenic mice with altered survival pathways, we were able to identify putative intracellular targets in hypertrophic scar formation [29].

6. Dorsal Skin Fold Chamber Models

The dorsal skin fold chamber has been a well-established model to examine vessel physiology in both normal and pathologic states (Figure 2(a)) [51]. In conjunction with intravital microscopy, skin fold chamber models permit *in vivo* assessment of vascular physiology over multiple time scales. High-resolution real-time images can be obtained and have revealed novel mechanisms of human pathology such as vascular embolic disease [52]. Sorg et al. utilized a dorsal skin fold chamber model to examine mouse wound regeneration

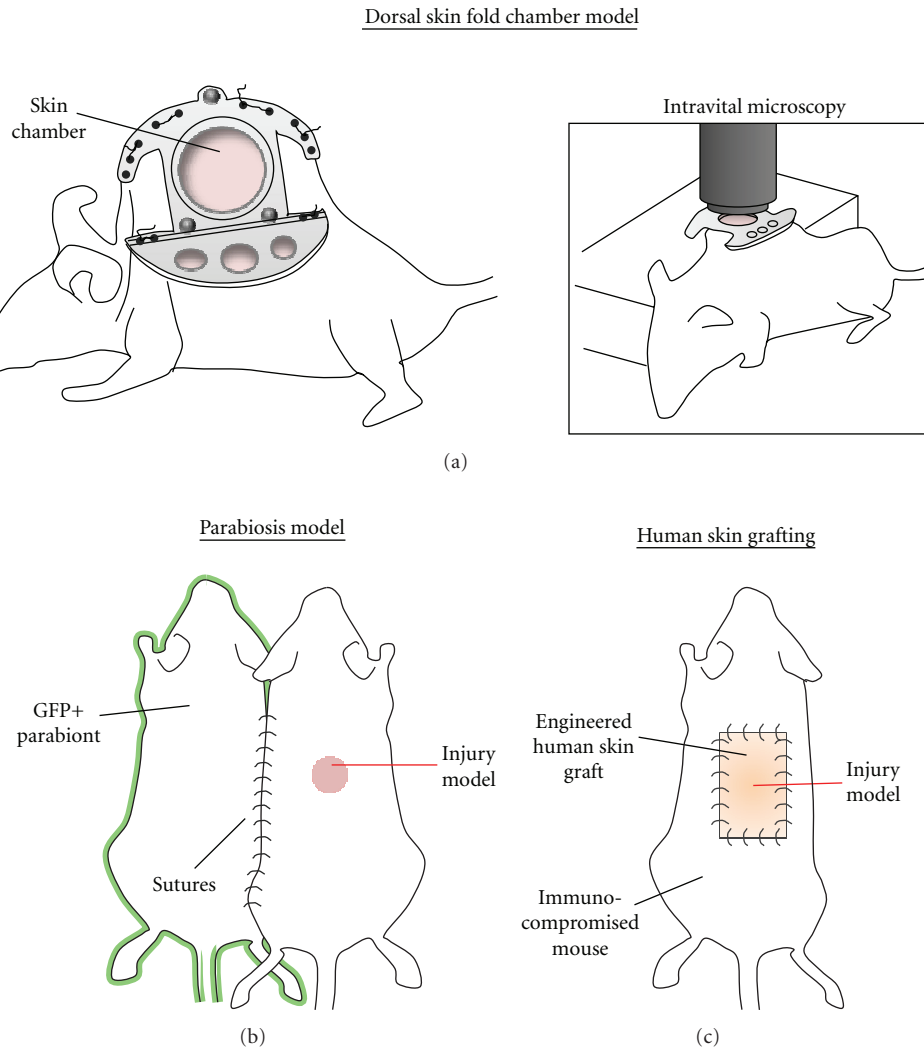


FIGURE 2: Schematic of commonly employed surgical mouse models of human disease. (a) The dorsal skin fold chamber allows real-time imaging of cutaneous physiology in high resolution when combined with intravital microscopy techniques (right). (b) Parabiotic models permit researchers to investigate the role of circulating factors and/or cells from the donor parabiont in cutaneous wound repair. (c) Engineered human skin constructs can be grafted onto immunocompromised mice to study human skin explant physiology within a living biologic (immunodeficient) environment.

following full thickness skin injury [53] and to study the effects of erythropoietin on dermal regeneration [54]. Ichioka et al. developed a skin fold chamber model to study the effects of ischemia reperfusion on the microcirculation of pedicled flaps [55], and Park et al. examined arteriovenous malformations in transgenic mice using these techniques [56]. Continued improvements in intravital microscopy, cell tracking, and biomolecular tracers will undoubtedly provide further insight into novel mechanisms in wound healing.

7. Parabiosis Models

Parabiosis models involve the surgical joining of two mice at their flank skin to study the circulation of cells or circulating factors between the two animals (Figure 2(b)). Cross-circulation is generally established between the two

parabionts by several days through new vascular anastomoses [57]. This model was first described by Bert in the 1860s and popularized by Sauerbruch and Heyde in early 1900s [58]. Since that time, numerous parabiosis models have been developed to study cancer metastasis, circulatory physiology, immunology, and metabolic diseases. In the context of wound healing, recent studies have demonstrated that circulating factors and systemic-derived cells play an important role in numerous aspects of skin homeostasis and repair.

Our laboratory utilized a parabiotic model to demonstrate the participation of bone marrow-derived stem cells in ischemic vasculogenesis and wound repair [59]. GFP+ mice were paired with wild-type counterparts and GFP+ cells were found to engraft in wildtype neovessels following ischemic injury. Song et al. employed a similar strategy to characterize the contribution of circulating cells to the repair of full

TABLE 2: Surgical mouse models of cutaneous disease with select references.

Mouse Model	Human Disease Relevance	Select References
Excisional Wound	Diabetes and obesity	[31, 65, 66]
	Aging	[67, 68]
	Obesity	[69]
Ischemic Flap	Diabetes	[35]
	Aging	[34]
Pressure Ulcer	Diabetes	[37]
	Decubitus ulcers	[38, 39]
Mechanical Load	Hypertrophic scar formation	[29]
Parabiosis	Diabetes, obesity	[61, 62]
	Ischemic wounds	[59]
	Scleroderma	[63]
	Aging	[64]
Dorsal Skin Fold Chamber	Arteriovenous malformations	[56]
Xenografting	Donor graft integration	[70]
	Wound remodeling	[71]

thickness excisional wounds [60]. They found substantial involvement of circulating cells in various aspects of early wound repair, including inflammation, mesenchymal differentiation, and blood vessel formation. Parabiotic models have also been used to highlight the importance of circulating factors in delayed wound healing in diabetic mice [61], to study the relationship between obesity and diabetes [62], to investigate the role of circulating fibroblast precursors in bleomycin-induced skin fibrosis [63], and to examine alterations in skin collagen remodeling with aging [64].

8. Human Skin Graft Models

For decades, researchers have transplanted human skin onto the back of immunocompromised mice (Figure 2(c)) to examine human skin explants in a more biologic environment [72, 73]. Geer et al. transplanted human engineered skin onto athymic mice and tracked mouse contributions to both human epithelial and dermal wound repair [70]. Rossio-Pasquier et al. examined species-specific epithelial-mesenchymal interactions in a similar human skin transplant model [74] and Guerret et al. grafted a multispecies skin construct onto nude mice to characterize the remodeling process as contributed to by human, mouse, and bovine constituents [71]. These studies and numerous others have allowed researchers to examine the integration and remodeling of both natural and engineered skin constructs in a living biologic environment. However, the requisite inflammatory and immune responses in human wound repair are necessarily attenuated in immunocompromised mice, thus somewhat limiting the biological relevance of these models.

9. Conclusion

In conclusion, numerous surgical models of wound healing have been developed in the mouse and have greatly improved our understanding of wound repair in various disease states (Table 2). Although each model system has its specific advantages and drawbacks, mouse models for human disease have become an indispensable tool for researchers. Thus, a familiarity with common mouse models of wound repair and an awareness of their limitations will enable scientists to develop research strategies with greater translational potential.

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