



Review article

Wound healing models: A systematic review of animal and non-animal models

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ABSTRACT

Chronic wounds and ulcers are a significant challenge to researchers and healthcare providers. Research into the area of wound care is in great need due to the increase in incidence and healthcare cost. Wound healing models are essential to test new therapeutics, study the pathogenesis of the tissue repair mechanism and to detect new biomarkers. The aim of this review is to provide an overview for currently available animal and non-animal wound healing models.

Several models have been used to study the wound healing process including *in silico*, *in vitro*, and *in vivo*. There is no one single appropriate model for a study to be effective; instead a combination of models should be employed. In conclusion, animal models despite their many disadvantages remain the best available alternative to study the complex cellular and molecular interactions that occur during the wound healing process in a biologically relevant environment. Multiple models are necessary to study different types of chronic wounds due to the lack of an ideal comprehensive model that is close to humans.

1. Introduction

Animal models have been the go-to model for studying various skin diseases and treatment methods; however, due to the increasing costs and ethical restraints of using animal models for testing human drugs and diseases other models provide a good alternative.

Wound healing is a complex process with many elements that are mostly understudied. Acute and chronic wounds management provided great challenges for the healthcare providers. Over the past twenty years, the incidence of people suffering from chronic wounds has increased dramatically. Chronic wounds decrease the quality of life, leading to high morbidity and mortality rates. They cause severe pain, loss of function and infections that might cause gangrene, amputations or sepsis. Besides being a physical and psychological burden, skin ulcer management is of high financial load as it costs the US healthcare system \$25 billion annually.

In this review, we will examine different animals as well as non-animal models currently used for the study of wound healing with an overview of their advantages and disadvantages [1,2] (Fig. 1).

2. Types of wound healing model

2.1. *In silico*

In silico computational models may be helpful to understand cell growth and phases of wound healing in theory. They can also be used to design effective scaffolds and tissue substitutes that will aid in wound regeneration. The disadvantage of *in silico* models is that they lack biophysical characteristics of human skin and remain theoretical until confirmed biologically either by *in vitro* or *in vivo* models. Several mathematical equations have been used to assess the healing phases [2,3].

2.2. *In vitro*

In vitro models are used to understand scar formation pathogenesis and healing processes but not for potential scar treatments [4]. Three main models are currently used: monolayer cell cultures, co-cultured cell cultures and skin explants or 3D cultures (Fig. 2).

2.2.1. Monolayer cell culture

Conventional mono-layered cultures are easy, cheap and relatively fast in displaying results [4]. Monolayers of cells, e.g. human epidermal

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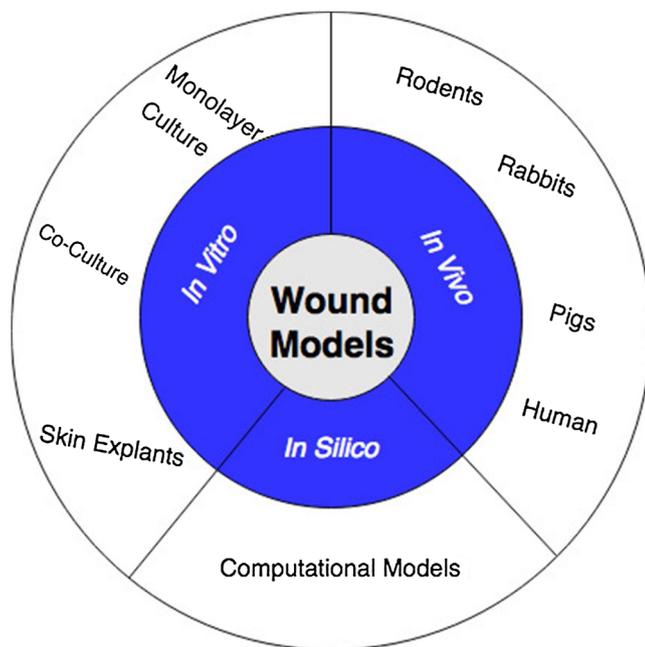


Fig. 1. Diagram summarizing different types of wound models including *in silico*, *in vitro* & *in vivo* (animal & non-animal Models).

keratinocytes are commonly disrupted using a sterile wounding instrument (scratch assay) (Fig. 2A). Various factors are administered to study cells re-confluence. Autocrine factors are easily removed during cell culturing with the media removal. This is a draw back in understanding the mechanisms governing wound healing, to overcome this issue, media is partially removed to provide fresh growth factors and minimize the disruption to the cells' environment [5]. Human skin is a very complex organ involving multiple cell types that interact together. Fibroblasts or keratinocytes alone cannot provide an adequate understanding to the complexity of the issue [5].

2.2.2. Co-cultured cell cultures

The trans-well systems using monolayers of keratinocytes and fibroblasts allowed scientists to study the keratinocytes-fibroblasts interaction [4]. This system employs a chamber (Boyden Chamber) that is separated into two compartments using a filter membrane with pore

size that dictates active migration for leucocytes (Fig. 2B). The cells are placed on one end of the membrane with the other end submerged in the tested solution. The apparatus is incubated for a period of time and then the filter membrane is removed, fixed and stained. The cells are then counted to measure the migration count [6].

In one experiment to study the role of Smad3 in keloid pathogenesis a trans-well system was deployed. The authors seeded six keratinocytes cell strains on trans-well polyester clear membranes, and six fibroblasts cell strains were seeded in 6-wells plate. After reaching 80% confluency, the keratinocytes loaded membranes were added to the fibroblasts cultures to start the keratinocyte-fibroblasts phase [7]. The results suggested that Smad3 and TGF β have a role in the epithelial-mesenchymal interaction in keloid pathogenesis [7].

Co-cultures give more insight in regards to cell-cell interaction and this provides more information to researcher's than normal monolayer cultures. Understanding keratinocytes and fibroblasts interaction is integral in gaining insight about wound healing and scar formation [8]. However, co-cultures only provide a 2-dimensional understanding of the skin healing processes. Even though it provides more information than monolayer cultures, it is still insufficient for accurate representation of what occurs *in vivo* [2–4]. Co-cultures utilize only two cell types; however, in reality multiple cells are activated to work harmoniously together during injury and healing processes. Immune cells, endothelial cells and fibroblasts are not incorporated together in current existing models [4].

2.3. Skin explant (Ex-vivo)

Skin explant is an organotypic cell culture in which the sub-cutaneous layers and fat contaminants are removed and the remaining tissue is cultivated (Fig. 2C).

This model has been used to study wound repair and inflammation in the skin besides testing the effects of different therapeutics [1,9,10]. In contrast to mono-cellular culture and co-culture models, skin explants have the advantage of providing a 3D-structure that show inter-cellular interaction such as one between keratinocytes and fibroblasts. The micro-environment of the cells in the living organisms in terms of biophysical and biochemical manner can also be reflected in this model as well as cell-matrix interaction which are integral in wound healing [1,11,12]. Other factors such as pH, nutrient absorption, transport, differentiation and temperature are more readily mimicked similar to *In vivo* conditions [12].

That being said, skin explants lack innervation which is integral for the understating of skin repair and scar formation and desquamation of cells cannot be observed [1]. The genetic disposition of the individual is

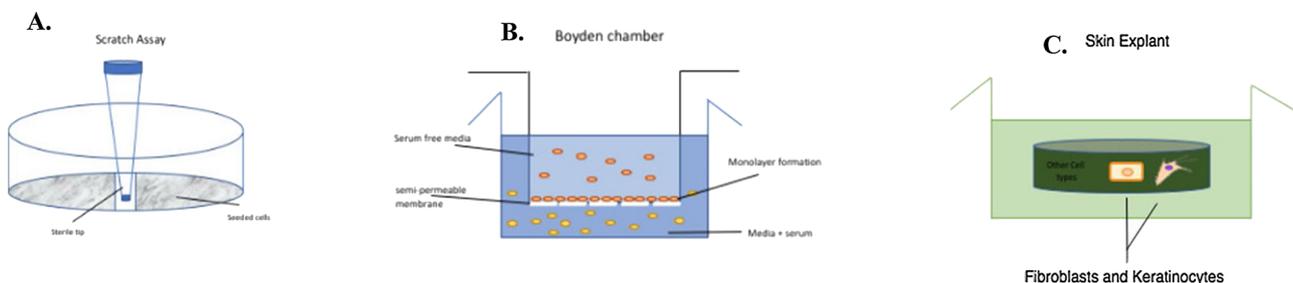


Fig. 2. Diagram illustrating different types of In vitro wound healing assays A) Scratch Assay as an example for monolayer cell culture B) Boyden Chamber as an example of co-culture C) Skin Explant as example of Organotypic culture. Cells images were adapted from <http://smart.servier.com/>.

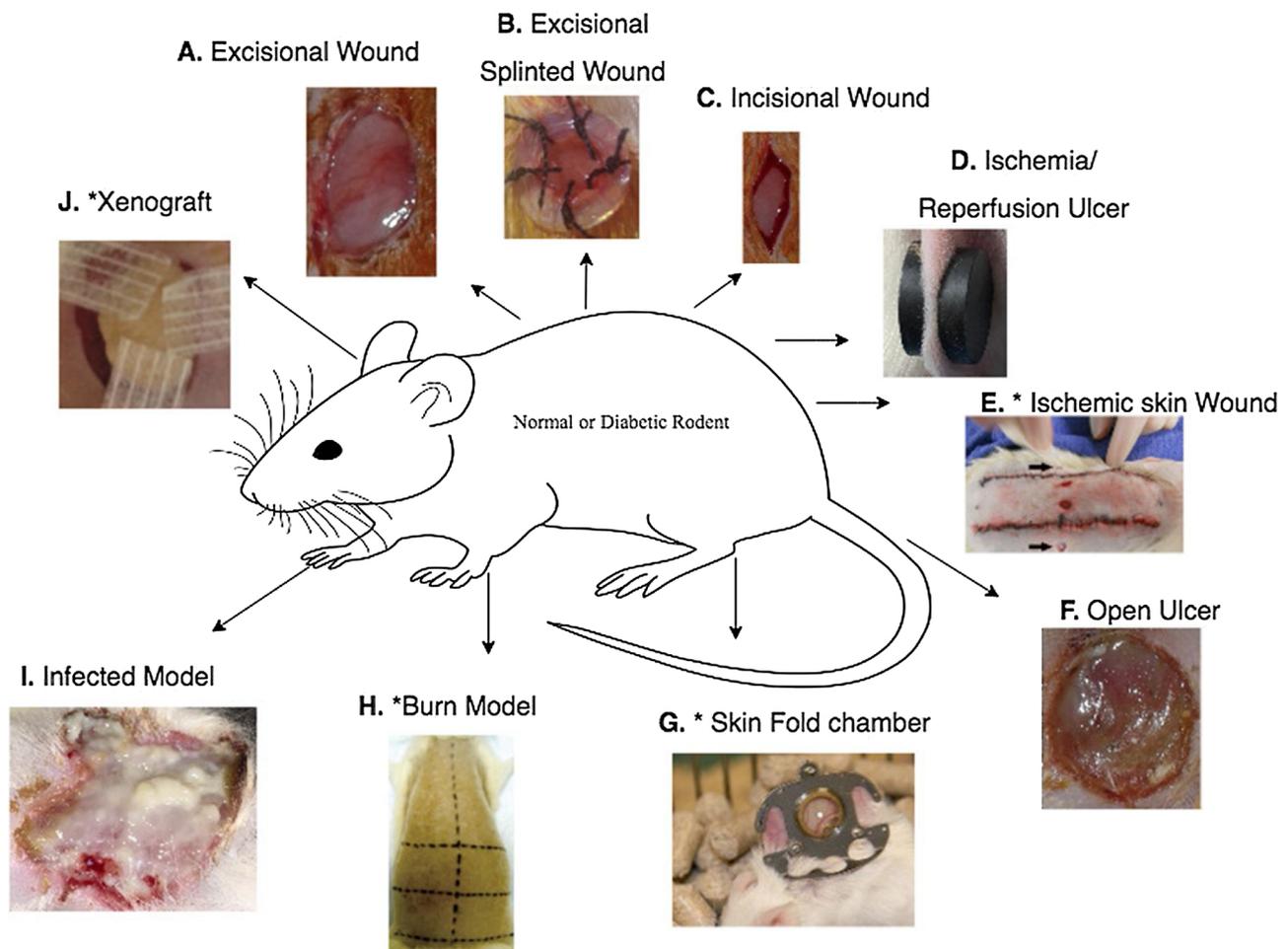


Fig. 3. Diagram showing different types of wounds done on rodents to study the wound healing process. *Photos adopted with permission, **Ischemic Skin Model** adapted from [34], ***Skin Fold Chamber** adapted from [35], ***Burn Model** adapted from [36], ***Xenograft Model** adapted from [37].

Table 1
Excisional & Incisional Animal Models.

Model	Species	Methodology	Advantages / Disadvantages
Excisional Wound Model	Rodents/ Rabbits / Pigs	In Rodents and pigs, full-thickness injuries extending through the dorsal side of the animal (Fig. 3A). While in rabbits full-thickness excisional wounds are done on the ventral side of the ears.	Excisional wounds are the most common type of wound model used in studying wound healing. <i>Rodents</i> It is considered to be the least efficient type of wound model, as it heals through the contraction of the panniculus carnosus while human wound heals through re-epithelization. To overcome the problem of muscle contraction, some researchers used a donut-shaped silicone splint. This splint was placed on a full-thickness wound through the panniculus carnosus (Fig. 3B). This model assesses mainly acute wound healing but not chronic healing process, as the blood vessels remain intact thus promoting the healing process. <i>Rabbits</i> Rabbit ear wound model mimicked the acute healing process in humans. <i>Pigs</i> Although this model resembles acute wounds in humans, its limitations include relatively small sample size. In some studies, multiple wounds on the same animal were used to decrease the number of animals. Many methodological variations exist including variation in the excision size and different tools used to generate the wound [41,42,43,44,45,46,47,48].
	Rodents	Longitudinal incisional wound parallel to the midline on the dorsal side passing through epidermis, dermis and subcutaneous tissue down to the muscle (Fig. 3C).	It is the second most common wound model. It is mostly used for studying wound scarring [49,50,51].

Table 2
Wound models in Diabetic Animals.

Model	Species	Methodology	Advantages / Disadvantages
Diabetic Model	Rodents/ Rabbits/Pigs	Diabetes was induced by high fat diet or by injection of diabetogenic agent as Alloxan, Streptozotocin (STZ). Also, transgenic db/db mice were used as diabetic model in different studies. Full thickness excisional wound on the dorsal side of the animal in rodents & pigs, or on the ventral surface of the ear pinnae in rabbits.	<p>Rodents Diabetic models were used to mimic the stages of chronic healing process. It is our belief that this model is sufficient to study acute wound healing in diabetic animals but not suitable to study chronic skin ulcers as the etiology of the wound is significantly different. Also, wounds were usually induced 1-2 weeks after confirmation of hyperglycemia, so long term effects of diabetes were not fully developed yet.</p> <p>Rabbits In one study, the authors successfully maintained the animals for one year to study the effect of diabetes on the healing process.</p> <p>Pigs Although diabetic pigs showed delayed healing (18 days) when compared to non-diabetic group (14 days), this was not consistent with the human diabetic wound healing that takes significantly longer time [33,52,53–56]. If the wound was applied one month after diabetes induction, this model could mimic human diabetic healing since the given time would be sufficient for diabetic complications to occur. Limitations of diabetic models include multiple injections of diabetogenic agents to maintain diabetes and the high breeding cost.</p>

not taken into consideration and this has a major role in understanding and treating deep wounds (e.g. Burns) [4]. This model lacks standardization and consistency [9]. A variety of wound types such as a full or partial wound thickness, and different culture conditions have been used [12,13,14]

Burd et al. [15] showed that mono-layers of keratinocytes, fibroblasts and skin explants of pigs treated with silver dressings did not show any means of re-epithelialization, in contrast to the mouse animal model that showed strong inhibition of wound re-epithelialization at day 7.

A novel model of 3D culture used a high-density, fibroblasts infused, compressed matrix that was placed over a lower density acellular matrix. The migration of fibroblasts from the inner compressed high-density matrix to the outer lower density matrix was measured and the pattern was also assessed. Migration into the outer matrix was observed after 2–3 days, and by day 5 a remarkable difference in the number of migrating cells and the distance traveled was seen compared to other culture conditions [11].

Nayak et al. [12] used silk sericin, an adhesive silk protein family synthesized by silkworms, to construct a novel 3D model to assess wound healing in co-cultures of keratinocytes and fibroblasts *in vitro*. Cells were seeded on top of each other on the sericin matrix and their paracrine interactions in the matrices were also investigated [12]. Their results show that the co-culture of keratinocytes-fibroblasts form epidermal and dermal components in the sericin construct. This model shows promising results as an alternative for *in vitro* skin replacement grafts [16].

Other examples of *Ex vivo* models are found in studies conducted to analyze scars and stretch marks, including keloid and hypertrophic scars that evaluate novel anti-fibrotic therapies. They also examine the effect of photodynamic therapy on stretch marks and other scars on human skin [17–20].

2.4. *In vivo*

In vivo models are the most efficient and clinically relevant when studying wound healing. These models can either be small animals, large animals or humans. They provide several advantages over *in vitro* studies as they allow the study of the pathophysiology of wound healing in real time. *In vivo* models are mandatory to the study of new therapeutics before progressing to clinical trials [21]. Below we will highlight the difference between the different animal models including the human model.

2.4.1. Human model

Human skin anatomy is characterized by a thick epidermis and a dermis layers rich in blood vessels and sparse hair [22]. Eccrine and apocrine glands (e.g. sweat and sebaceous glands) play a vital role in tissue repair through keratinocyte generation [23]. Human skin depends on exogenous vitamin C for every phase in the healing process [24]. Human skin heals mainly through re-epithelialization and granulation [25].

Human models have the advantage of clinical importance especially when testing new drugs in clinical trials. Obtaining human samples is not always readily available. Patients with chronic wounds lack the uniformity of the wound type and microbial composition [9]. There is also the difficulty of obtaining volunteers. In volunteers, acute wounds may include partial thickness; tape stripping; blister model; abrasive model; and full thickness wound models [26].

Patients with already established chronic wounds such as pressure, venous, and diabetic ulcers may be used to study pathophysiology and molecular aspects of the healing process. They can also be utilized to testing new agents for chronic wound healing. Due to practical and ethical concerns, wound healing studies are rarely carried on humans, as a result, animal wound healing models provide the most suitable and clinically relevant alternative [27].

Table 3
Experimental Ulcer models.

Model	Species	Methodology	Advantages / Disadvantages
Radiation Induced wound	Rodents/ Rabbits/ Pigs	<i>Rodents</i> Received irradiation dose (20 Gy), followed by a full-thickness wound made with a punch biopsy leading to ulcer formation. <i>Rabbits & Pigs</i> The leg was subjected to irradiation, followed by a full-thickness skin excisional wound on the animal's limb.	Used to study skin ulcers due to cancer. <i>Rodents</i> High mortality rate due to radiation, small wound size, in addition to complicated setup (facilities, irradiators, etc...). <i>Rabbits</i> Decreased mortality by protecting the whole body except the exposed part. Larger wound size can be made. <i>Pigs</i> Skin became normal 2 weeks later, which is not consistent with human chronic healing. Cost is another limitation of this model. [57–59]
Cutaneous Ischemia Reperfusion ulcer	Rodents/ Rabbits/ Pigs	<i>Rodents & rabbits</i> Ischemia/Reperfusion injury was induced by applying and removing a magnet over the dorsal region of rat skin or by implanting a magnet or steel plate below the skin. A less invasive technique applied the magnet to a skin fold in rodents (Fig. 3.D) or ears in case of rabbits, followed by ischemia-reperfusion cycles. <i>Pigs</i> A silicone capsule containing two neodymium magnets were implanted subcutaneously, and external magnets of different intensities were applied to generate compression of the skin.	<i>Rodents</i> These models are convenient to study type 1 pressure ulcer where the skin is still intact. <i>Rabbits</i> Although rabbits were more convenient to study type 1 pressure ulcer than rats, subjecting the animal (rabbit) to only one cycle of ischemia reperfusion might not be sufficient to establish a type 1 ulcer. A concern regarding the small sample size (only 4 animals used), didn't rule out statistical errors. The model was not sufficient to detect other deeper types of ulcers. Wrapping the ear with 70% ethanol prior to sampling might have minimized the surface bacteria which is important factor in pressure ulcer pathogenesis. <i>Pigs</i> Such model provides morphological information about the skin microcirculation. The small sample size (2 pigs) does not rule out statistical errors 60–65.
Ischemic Skin Wound Model	Rodents/ Rabbits/Pigs	<i>Rodents</i> First developed by Mcfarlane in 1965. A skin flap on the rat's dorsal side was completely detached from the deep tissues up to its base and then sutured back. This was further modified by placing a silicone sheet under the flap. Two Ischemic wounds were created inside the flap region while two non-ischemic wounds were made lateral to bi-pedicle flap (Fig. 3.E). <i>Rabbits</i> In this model 2 of the 3 main arteries at the base of the ear are tied. Therefore, interrupting the circulation of the dermis. Ulcers were then created with a punch biopsy down to the auricular cartilage. <i>Pigs</i> Bi-pedicle flaps were established by separating flaps from the underlying muscle and placing a silicone sheet to avoid reattachment. The cut edges of the flap were stitched to the nearby skin. Full-thickness excisional circular wounds were created in the middle of the flap using a punch biopsy. Additional wounds were made in the non-ischemic skin to act as control.	<i>Rodents</i> This model is useful to study ischemic wounds. If the flaps are allowed to reattach to the dermal base, revascularization can occur (Dorsett-Martin et al., 2008). Modifications done to this model made it more valuable to earlier developed ischemic skin flap models. The silicone sheet worked as a barrier to prevent revascularization and reduced wound contraction. This model was useful to study ischemic wound healing that result from diabetes or pressure ulcers. <i>Rabbits</i> Since the rabbit ear dermis is firmly attached to the cartilage, the avascular wound bed usually heals by reepithelization which makes it similar to human healing process. Other advantage of this model is that several ulcers can be created on the same ear and the other ear can act as control. <i>Pigs</i> The ischemic wounds showed decreased blood reperfusion and delayed macrophages compared to non-ischemic wounds. Limitations of this model include the need for high surgical skills and the development of seroma (a pocket of serous fluid). Also it was characterized by the inadequate number of wound sites per animal, and small wound dimensions make it insufficient for analysis [33,34] [62,66–69].
Open Skin Ulcer Model	Rodents	An incision was done on the back of the animal. and one magnet was implanted under the skin and the other magnet was kept above it, where the incised skin was sandwiched between both magnets. After one week, the two magnets fell out with the sandwiched skin forming an ulcer (Fig. 3.F).	Although, this was the first study to describe open ulcer resulting from pressure, there were some serious drawbacks in the study. First, the mouse model has thinner skin than the rats. Second, the magnets were applied on top of the incised skin to insert the internal magnet, rather than on top of an area of normal skin. This led to some doubts on the validity of the model. Finally, the degree of the ulcer generated cannot be assessed based on the data provided by the authors [70].
Skin fold chamber model	Rodents / Rabbits	<i>Rodents</i> This model was first developed in 1943 by Algire and co-authors. An area of the dorsal skin of the animal was sandwiched between two complementary plates. At the center there is an approximately one cm diameter circular observation area (Fig. 3.G). <i>Rabbits</i> An ear chamber was placed under the ears with holes punched through the cartilage.	<i>Rodents</i> This model was important to study the pathophysiology of micro-vascular circulation through microscopic and real time imaging [71]. Limitations of this model include pain and discomfort the animal feels from carrying such device. Therefore, other models with lighter chambers were developed [72]. <i>Rabbits</i> This study had the advantage to study the vascular changes and angiogenesis in presence of shear stress. Rabbit ears have high vascularization that resemble human rather than rats' vascularization [72–74].

Table 4
Burn Wound models.

Model	Species	Methodology	Advantages / Disadvantages
Burn Wound Model	Rodents/ Rabbits	<i>Rodents</i> Burn models were established using hot surface or wax on the dorsal side of the animal. Other researchers applied steam / hot air to induce the burn. Those models result in either superficial/ deep full thickness burn (Fig. 3.H)	<i>Rodents</i> Although burn models in rats and mice were extensively used in research due to their low cost. Their disadvantages were the rapid healing due to contraction of the panniculus carnosus, thinner skin structure, denser hair structure [75]. Also, small animals have metabolic profile which is greatly different from than that of humans, this makes them not the suitable model to study metabolism research in burns [76].
		<i>Rabbits</i> Burns were generated using a dry-heated brass rod at 90 °C. Excisional wounds were done on the other ear as positive control.	<i>Rabbits</i> This model provided good example to study hypertrophic scar rather than rats as they heal through reepithelization. Their disadvantage is their high breeding cost [77–83].
Burn Wound Model	Pigs	<i>Pigs</i> Contact burn was induced through applying a metal comb or a stainless-steel bar heated up to 80-110 °C for 20 seconds. In some cases, the wound was infected with <i>S. aureus</i> .	<i>Pigs</i> Burn induced in pigs using hot comb had several advantages including being inexpensive and simple. The comb burn model resulted in consistent injury which made it a convenient model to study the mechanism of burn wound progression and conversion of ischemic tissue into full-thickness necrosis over a short period of time. A disadvantage of the model was the small surface area burned. As modification to the comb method, a steel bar was used to induce a contact burn by applying force to the skin to control the depth of the burn. The infected burn model was considered as a noninvasive model for controlled burn wound infection. It is considered as an important model to study the effect of emerging antibiotics. It would be better if this model modified by inoculating bacterial biofilm to study effect of multiple bacterial species in the wound pathology. These models can be used to understand more about hypertrophic scarring and to test the therapeutic effect of new drugs [84].

2.4.2. Animal model

Although animal skin does not resemble human skin, they have been developed and utilized in order to study the complexity of the healing process especially in chronic wounds. They provided an excellent platform for studying and testing new therapeutic agents [28,29].

Rodent's skin anatomy differs than that of human. For example, rodents have thin epidermis; loose skin adherence; dense hair which is thought to accelerate healing [31,32]. They are characterized by absence of apocrine and eccrine glands [31]. Unlike humans, rodents have endogenous source of vitamin C which is crucial in all phases of healing [24,25,31]. Other major differences include the subcutaneous panniculus carnosus muscle, which promotes rapid wound contraction. Finally, rodents have stronger immune systems when compared to humans [33]. Despite all these difference between rodents and humans, they have been used extensively in studying wound healing. Their availability, low cost, and small size make them suitable for large size studies especially when testing the effect of new therapeutic drugs before clinical trials on humans, which reduces statistical errors [30].

Different types of wounds have been developed on rodents to study acute and chronic healing processes (Fig. 3).

Rabbit ear model is commonly used for wound healing. The cartilage acts as the wound bed that forces healing through re-epithelialization and granulation formation rather than contraction. The cartilage is highly vascularized which gives it the advantage to mimic human dermis vascularization [38]. The drawbacks of using rabbits as animal model, include their high breeding cost and they are also hard to be genetically modified [39].

Porcine, more commonly known as pig, are promising models for the study of wound healing as well as other diseases due to their physiological and anatomical similarity to humans. Pigs have tight skin adherence with thick epidermis and dermis layers, sparse hair, apocrine glands, depend on exogenous vitamin C and heal their wounds through reepithelization [40]. However, in contrast to humans they have poor dermis vascularization [22]. Despite these similarities, pigs are not considered to be the optimal animal model due to their high cost and requirement for large experimental set-ups. These setbacks reduce the chance for large scale experiments [27,40]. Table 1–5

To date, no animal model demonstrates wound closure that reflects the clinical similarity of the human healing process [25,22].

Acute / Chronic Wound healing models were developed by many techniques. The tables below summarize the techniques in both rodents and large animals and their pros and cons.

In conclusion, although no single model is superior to the other, *in vivo* animal models will remain superior to *in vitro* models to study the complex wound environment. Rodents will remain the go to model for wound healing study due to their ease of use, cost, and the reproducibility of the models. From our point of view rodents are the best animal models to use for pharmacological testing before moving to human trials.

Conflict of interest

The authors declare no competing financial interest.

Table 5
Other Wound models.

Model	Species	Methodology	Comments
Infected Model	Rodents/ Rabbits/ Pigs	Infected animal models are developed by creating an incisional / excisional wound and inoculating it with micro-organism, biofilm or foreign bodies (Fig. 3.I). This has been done in normal and diabetic animals. In Rabbits, full-thickness wounds were made on the ventral surface of the ear down to perichondrium and inoculated with a single or a combination of bacteria.	This model is sufficient to study antibacterial effect of new drugs, although only pathogenic microorganisms were used to induce infection. <i>In Rabbits</i> The presence of a combination of bacteria made it a good model for chronic wounds. <i>In pigs</i> The infected diabetic wounds had significant epithelialization after 12 days, which is not consistent with a non-healing human diabetic wound. This might be due to the fact that diabetic wound was induced only 14 days after diabetes confirmation which is not sufficient to study long term effects of uncontrolled hyperglycemia [33]. The presence of bacterial biofilm significantly mimicked the wound environment by integrating multiple bacterial species that might interact with each other. This model might be useful due to the infection induced in late diabetic wound to understand more about chronic healing process in diabetes [85–88].
Para-biosis Model	Rodents / Rabbits	This model was first established by Bert in the 1860s to allow sharing of the blood circulation. Two animals were joined surgically at their flank skin while in rabbits they were joined at the ears.	This model helped to study wound healing circulating factors that play a key role in several phases of skin and tissue repair. This model is also very important in studying immunology, cancer metastasis, and metabolic disorders. Limitations of this model include, the increased risk of operative and perioperative mortality [89–91].
Dead space wound Model	Rodents/ Pigs	Rodents dead space wounds were created by implantation of a polypropylene tube below the dorsal paravertebral lumbar skin. While in pigs, incisional wounds with dead space were generated by partially removing the back muscle.	<i>Rodents</i> Although this model is not useful in studying epithelialization but it helps in studying granuloma tissue and gives information about the wound environment due to the accumulation of the interstitial fluid [92]. <i>Pigs</i> The model was useful to study the effect of Negative Pressure Wound Therapy. The advantage of this model is the nature of healing process of pigs by reepithelization [41,93,94].
Tape stripping	Rodents	An adhesive tape was used on an area 2 cm ² of the dorsal side of the mice 7 to 10 times.	This is the simplest partial thickness wound of the skin that involves removal of stratum corneum using an adhesive bandage. In this model, the epidermal section is mostly left intact. [95] However, due to elimination of stratum corneum layers, the permeability of the skin is temporarily compromised that can be measured using trans-epidermal water loss (TEWL) by a special probe. This model is simple to use, moderately painless, and is used mainly to investigate skin barrier function. It allows evaluation of a re-epithelization process component of acute wound healing in addition to evaluating the effect of adhesive wound dressings on the skin barrier. One disadvantage is that it is only limited to superficial wounds. This type of wound is not consistent as it depends on the tape adhesiveness, pressure exerted when applying it and velocity. The direction of tape removal and the number of tape strips also affect the wound [96].
Denervated Wound Model	Rodents/ Rabbits /Pigs	<i>Rodents</i> Spinal hemisection was performed to denervated skin in rats followed by 15-mm diameter skin wounds. <i>Rabbits</i> Rabbit ear denervation was performed by surgical excision of the main sensory nerves of one ear leaving the other ear as control. <i>Pigs</i> Unilateral nerve root transection followed by tissue compression.	<i>Rodents</i> This study can be useful to examine the correlation between sensory disturbances and wound healing as a result of acute injury but may be not useful in chronic wound healing. <i>Rabbits</i> The lack of sensation doesn't mimic totally the human chronic wound as the wound model completely healed by 15 days [97–99]. <i>Pigs</i> This model provides a comparison of pressure sore treatments. Nerve transection requires high surgical skills. Wounds resolved within 3 weeks which is not consistent with chronic wounds in human. Muscle atrophy occurred in the denervated limb [40].
Xeno-grafts	Rodents	Full thickness human skin xenografts were placed on full-thickness dorsal skin defect created on the dorsal side of nude mice (Fig. 3.J).	This human – mouse xenograft model may have potential in studying mechanisms of other forms of dysfunctional wound healing as human keloid and hypertrophic scars. It can also be used to test drug penetration of potential therapeutic drugs before going on clinical trials [100,101]. SCID/ NUD mice are unable to induce immune response similar to humans. Cost and technical difficulty remain disadvantages of this model. In addition to that, the grafted human skin lack denervation [102].
Skin Aging Model	Rodents	Mice were exposed to UVB for 12 weeks to induce aging to the skin.	This model is sufficient to test anti-aging drugs. One of the limitations of this model is that rat skin different than human skin, in addition to the high mortality rate due to radiation and the need of specialized facilities [103].

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