Clinically relevant experimental rodent models of diabetic foot ulcer

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Abstract
Chronic wounds are a substantial clinical problem in diabetes and nearly 6% of diabetics suffer from foot disease including ulceration, infection, and tissue necrosis. Wound healing in diabetes is impaired and delayed and is augmented by diabetic complications. Wound healing involves complex cellular, molecular, and biochemical processes and animal models are the most suitable prototype to investigate and understand the underlying pathological changes in the process of wound healing. Animal models are also useful in evaluating the safety and efficacy of newer therapeutic agents and improving the clinical approaches for human patients with chronic ulcers. The wound healing strategies get more complicated in the presence of diabetes and its associated complication. Despite the advancement in methods of wound healing, the healing of the chronic diabetic foot ulcer (DFU) remains an important clinical problem resulting in costly and prolonged treatment and poses a risk for major amputation. Saying that it is important to elucidate the newer therapeutic targets and strategies via an in-depth understanding of the complicated cascade of the chronic DFU. A major challenge in translating lab findings to clinics is the lack of an optimal preclinical model capable of properly recapitulating human wounds. Both small and large animal models of wound healing involving rodents, rabbits, and pigs have been discussed. Mouse and rats as small animal models and pig as large animal models have been discussed in association with the diabetic wound but there are advantages and limitations for each model. In this review, we critically reviewed the pros and cons of experimental models of diabetic wound healing with a focus on type II diabetes rodent models.

Keywords Rodent models · Type II diabetes · Diabetic foot ulcer · Impaired wound healing

Introduction
The chronic wound in diabetics in the form of diabetic foot ulcers (DFU) accounts for the second most expense after surgical wounds with increasing costs associated with outpatient wound care, an aging population, and the presence of difficult to treat infections [1]. In the United States, the management of DFU costs US$9 to US$13 billion and approximately US$8659 per patient [2]. Animal models are of prime importance while investigating improved therapeutic strategies for chronic nonhealing wounds and this is important because chronic wound care costs multimillion dollars worldwide with an estimated cost of US$20 billion in the USA [3]. Animal models have provided critical insight into the mechanistic and therapeutic aspects of wound healing even though wound healing in animals does not resemble humans due to the chronicity of wounds in humans that are absent in animals. Additionally, the biological variables including age, gender, wound location, and microbiota contributes to the variability in the results of preclinical studies using animal models for wound healing [4]. Thus, selecting an animal model to elucidate the molecular mechanisms and investigating the therapeutic effect of a small molecule or a drug in a preclinical study is of utmost importance. Reproducibility of the results, quantitative interpretation, clinical relevance, and successful translation of the lab work to clinics should be considered while choosing the animal model [5]. Regarding chronic wounds in animals simulating diabetic wounds, they can be created by inducing diabetes in the animal and creating an acute wound. Chronic wounds in animals are uncommon, thus there are limitations in each model [6]. Rodents and pig models have been used.
in preclinical studies for wound healing, however, there are limitations associated with both models including the time of healing, not fully reflecting the diabetic complications of a human, variability between different models, anatomical differences between the skin and healing process, and the cost–benefit ratio [5]. In this review, we have critically discussed the available experimental models of diabetes, ulcer induction, and induction of diabetes with a focus on type II diabetes mellitus in rodents.

**Diabetes**

Diabetes is a chronic health condition characterized by high blood sugar levels (hyperglycemia). Hyperglycemia due to inadequate or decreased production of insulin is termed type I diabetes mellitus (DM I) or insulin-dependent diabetes and type II diabetes mellitus (DM II) occurs due to decreased insulin secretion from the pancreas or insulin resistance in the cells involved in glucose metabolism including muscles, fat, and liver. Diabetes had a global prevalence of 8.5% in 2014 and the number of affected people with diabetes was estimated to rise from 422 to 642 million worldwide by 2040. Among the affected population, 5–10% of all diabetics are type I, 90–95% are type II, and 5% are other subtypes [7–9]. Cardiovascular diseases, stroke, peripheral vascular disease, retinopathy, nephropathy, diabetic ketoacidosis, peripheral neuropathy, foot ulcers, lower extremity amputation, and hypertension are common complications of diabetes [9]. Of these, nonhealing chronic foot ulcers often lead to lower extremity amputations. Diabetic neuropathy, structural foot deformities, peripheral arterial occlusive disease, and poor foot care are the most common cause of diabetic foot ulcers (osteomyelitis of the foot). Chronicity of inflammation impairing the wound healing by altering the bactericidal action of immune cells, attenuating the vascular perfusion, dysregulating the extracellular basement membrane thickening and collagen synthesis renders a wound in a chronic inflammatory phase without advancing it towards resolution phase and ultimately increasing the probability of amputation [10, 11]. Thus, investigating novel therapeutics to treat nonhealing diabetic foot ulcers is of utmost importance.

**Rodent models of wound healing**

Simulating the animal model with humans in a preclinical study is of utmost importance, however, no animal model exactly mimics the underlying pathophysiology of human disease. Rodent models of wound healing in diabetes is popular due to ease of availability and cost-effectiveness. Wound healing occurs through a precisely regulated process involving four phases including hemostasis, inflammation, proliferation, and remodeling [12, 13]. The primary mechanism of wound healing in humans is by re-epithelialization while in mice and rats primary wound healing occurs through the process of contraction. Wound healing by contraction in rodents is primarily mediated by myofibroblast-mediated contraction and the presence of an extensive subcutaneous striated muscle layer called the *panniculus carnosus* [5, 13, 14]. There are concerns in using rodent models in wound healing studies because wound healing occurs with contraction in rodents which is not like humans. However, Chen et al. [14] using BALB/c, db/+ , and db/db mice reported that contraction occurs only after epithelial closure and contraction and re-epithelialization both contribute 40–60% of the initial closure in a full-thickness excisional wound and contraction continues after closure. This report suggests that the rodent model simulates wound healing in humans.

Diabetic mouse models are clinically relevant for diabetic ulcers and the excision wound mouse model is clinically relevant to acute and chronic wound healing, but the excision wound healing model in mice can be used for diabetic wound healing by inducing diabetes before creating a wound [5]. Mouse and rat models are clinically relevant and have the advantages of testing potential therapeutic agents but anatomical differences in skin and different mechanisms of wound healing are the limitations. Additionally, diabetes and diabetic complications in rodents are not the same as in humans. The immune and inflammatory response also differs significantly in rodents compared to humans [5]. Mouse blood has 75–90% lymphocytes and 10–25% neutrophils whereas human blood has 50–70% neutrophils and 30–50% lymphocytes. Thus, there is a predominant lymphocytes population in mice while neutrophils are predominant in humans [15, 16]. TLR2 expression is low on peripheral lymphocytes including T cells in mice while is constitutive in humans and is not on T cells, TLR3 expressed on dendritic cells are induced by lipopolysaccharides (LPS) in mice but not in humans, leukocyte defensins are present in humans but absent in mice, CD4 on macrophages is present in humans but not in mice, T cells in skin and mucosa are γδ TCR in mice while αβ TCR in mice, and MHC II expression on T cells is absent in mice while present in humans [16]. The differences in the adaptive and innate immune response in humans and mice have been discussed in detail [16–18] and hence not reviewed in this article. The advantages and limitations of various animal models of wound healing have been discussed [5]. Thus selection of an animal model is of utmost importance and before choosing the animal model for a study, cost, ease of handling, clinical relevance, availability of the model, research environment and investigators familiarity, and similarity to human being should be kept in mind [5].
Small size, cost efficiency, easy availability of transgenic models, years of experience of wound healing research, and delayed healing are advantages in mouse model. However, there are disadvantages including loose and hairy skin, wound healing with contraction, difficulty in inducing partial thickness wounds, poor translational efficiency, and a different immune response. The size of a rat being larger than a mouse is an advantage while lesser availability of transgenic animals is a disadvantage with rats. Rabbits ear model of wounds overcome the contraction issues and has the advantage of creating multiple wounds while limited genetic tractability and paucity of species-specific reagents are disadvantages. Guinea pigs do not produce endogenous vitamin C, so dietary deficiency of vitamin C allows this model to study the role of collagen in wound healing. Rich vasculature is also an advantage with guinea pigs. However, limitations of the guinea pig model include variable pregnancy rate, long gestational period, small litter size, and lack of transgenic models. Pigs have the advantage of similarity with human skin and wound healing with re-epithelialization and granulation tissue formation like humans in partial thickness wounds while difficult to anesthetize pigs, expertise needed for surgeries, long gestation period, and expensive to maintain are limitations [5, 19, 20].

The use of rats instead of mice as a better choice for studying wound healing has been proposed based on the skin differences and small size of mice. Mouse skin is thinner with fewer layers of keratinocytes compared to rats and the wound heals in 12–14 days in rats compared to 7 days in mice. Further, due to the small size of mice, the wound will be smaller compared to the rat and thus heals faster than the rat [21]. Easier training of rats as compared to mice is of advantage of using rats [22]. While using rats as an animal model, limited availability of tools for immunological evaluation might be a concern which is not the case with mice [23]. Gender differences may impact wound healing, and this might be due to the difference in skin anatomy. Female mice have a thick epidermis and hypodermis. Male mouse skin has a thick dermal layer and skin is 40% stronger than female mouse skin [24]. In addition to the skin anatomy differences, rodent skin lacks apocrine and eccrine glands and has an endogenous source of vitamin C, stronger immune response and the shifting of the gene activity of mice immune cells to resemble humans, and a stronger response against bacterial infection in lab mice may contribute to different wound healing in rodents than humans [17, 25]. Cost is another factor while choosing an animal model. Male rats cost less than female rats [26].

The age of the animal model is another important factor to be considered. The process of granulation tissue formation and re-epithelialization decrease with aging and tensile strength also changes with aging. Lower tensile strength associated with aging is present in early wound healing but has no significant association in the later phase of healing [27]. Consideration of the age of mice is important because aging is associated with slow wound healing which in turn is associated with gender due to a differential change in steroid hormone levels between males and females. Age-dependent changes in the estrogen and testosterone level may play a crucial role in different wound healing patterns with age [4]. Wound healing accelerates with 17β-estradiol and male and female rats have a differential response to estrogen in wound healing. A study demonstrated that topical androgen antagonists enhance wound healing in male rats and 5α-dihydrotestosterone also promote wound healing, however, the gender of the model was not mentioned in the later study [4]. Despite the differences in skin anatomy, low cost, easy availability, small size, and feasibility of genetic manipulation makes rodents an ideal animal model for wound healing research while testing a therapeutic agent in a large-sized study. Another advantage is reduced inter-variability in host response in inbred strains of mice compared to outbred strains [23]. A major limitation of rodent models of wound healing is the quick healing in rodents. This happens because healing occurs rapidly in rodents due to contraction where new tissue is not formed as it occurs in re-epithelialization. Rapid healing interferes with the testing of therapeutic agents under consideration, and this is due to the return of normal tissue perfusion rapidly. Measuring the extent of wound healing is another concern while using full-thickness excisional wounds [5].

Suckow et al. reported that the wound healing was similar in obese non-diabetic and obese diabetic ZDSD rats and the impaired wound healing was not dependent on the presence of diabetes [13]. However, impaired wound healing has been demonstrated in obese diabetic and lean non-diabetic ZDF rats and it was postulated that this may be due to the presence of different fibroblast phenotypes due to the presence of adipose tissue [28]. Similarly, obesity impairs wound healing in Wistar rats compared to non-obese rats [29] and this might be due to abundant adipose tissue and chronic low-grade inflammation. A recent meta-analysis comparing various mouse models for diabetic wound healing suggests that wound healing impairment is more severe in non-obese diabetic mice compared to db/db mice, streptozotocin-induced diabetic mice, or high-fat-fed mice [30]. While choosing rodent models of diabetes (type I and type II; Tables 1 and 2), it is important to consider the mechanism of hyperglycemia and the question of study under consideration. Additionally, gender may affect diabetes and related complications, so gender should be taken into consideration [31]. Further, in humans, peripheral artery disease (PAD) and diabetes-associated neuropathy are associated with diabetic ulcers which are not induced in mice or rat models. Thus, these are additional limitations in using an animal model of diabetes wound healing.
The diabetes induction mechanisms (chemically, genetically, and virally induced, and spontaneous autoimmune hyperglycemia), main features, possible use, and other characteristics of other rodent models of type II diabetes including obesity-induced hyperglycemia mice (KK mice, NZO mice, TallyHo/Jng mice, OLETF rat), genetically induced hyperglycemia causing beta-cell dysfunction due to amyloid deposition in islets (hIAPP mice, AKITA mice), Nile grass rat, non-obese model in which hyperglycemia is due to insufficient beta-cell function (GK rat), spontaneously hypertensive/NIH corpulent (SHR/N-cp) rat with severe and early onset of obesity with post-prandial hyperglycemia and normal fasting sugar levels, and JCR:LA-cp rats with severe and early onset of obesity with moderate hyperglycemia in females have been summarized elsewhere [31, 35].

### Induction of type II diabetes in rodents

Diabetes can be induced by diet, chemicals, or by genetic manipulation. Type I diabetes is induced chemically using streptozotocin (STZ) or alloxan. A single high dose of STZ (100 to 200 mg/kg in mice and 35–65 mg/kg in rats), multiple low-doses of STZ (20–40 mg/kg) for 5 days in mice and rats, and alloxan with a dose of 50 to 200 mg/kg in mice and 40 to 200 mg/kg in rats induce type I diabetes [31] (Fig. 1). The disadvantage of using these chemicals is that these chemicals might be toxic to other organs of the body. Surwit et al. first reported diet-induced diabetes in C57BL/6J mice in 1988 [36]. The mice were fed with a high-fat, high-simple carbohydrate, low-fiber diet (20.5% protein, 35.8% fat, 0.4% fiber, 3.6% ash, 3.1% moisture.

### Table 1 Rodent models of type I and type II diabetes

| Type I diabetes                                      | Type II diabetes                                      |
|------------------------------------------------------|-------------------------------------------------------|
| Nonobese diabetic (NOD) mouse                        | Obese ob/ob mouse (leptin receptor-deficient)         |
| Streptozotocin-induced diabetic mouse                 | db/db mouse (a point mutation in the leptin receptor gene) NONcNZO10 mouse |
| Streptozotocin-induced diabetic rat                    | Zucker Diabetic Rat                                    |
| Bio-breeding (BB) rat                                 |                                                       |

### Table 2 Commonly used rodent models of type II diabetes: advantages and limitations [4, 13, 31–35]

| Animal model          | Mechanism/advantages                                                                 | Limitations                                                                 |
|-----------------------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| db/db mouse           | 1. Obesity evident from 3–4 weeks of age                                            | 1. Presence of point mutation in the leptin receptor gene in a mouse model that is not the case in humans where diabetes is polygenic |
|                       | 2. Hyperinsulinemia around 2 weeks of age                                            | 2. Hyperglycemia is severe, but all animals do not become diabetic          |
|                       | 3. Hyperglycaemia at 4–8 weeks of age                                               |                                                                           |
|                       | 4. Obesity-induced hyperglycemia                                                    |                                                                           |
| ob/ob mouse           | 1. weight gain and hyperinsulinemia by 2 weeks                                      | 1. leptin-deficient which is not present in human                          |
|                       | 2. Hyperglycemia by 4 weeks with a peak at 3–5 months                                | 2. Disturbance in temperature regulation                                  |
|                       | 3. Obesity-induced hyperglycemia                                                    | 3. Lower physical activity                                                 |
|                       |                                                                                        | 4. Infertility                                                            |
|                       |                                                                                        | 5. Diabetes is not severe and not representative of type II diabetes in humans |
| Zucker diabetic Sprague Dawley (ZDSD) rat            | 1. Possesses an obese phenotype                                                     | 1. Not all rat becomes diabetic, some became obese with diet but do not develop hyperglycemia |
|                       | 2. Develop overt hyperglycemia between 15 and 21 weeks of age                       | 2. Obesity is the determining factor for impaired wound healing and not diabetes |
|                       | 3. Larger than commonly used db/db mouse                                            |                                                                           |
|                       | 4. Rapid weight gain on standard rodent chow diet                                    |                                                                           |
|                       | 5. Onset of insulin resistance before hyperglycemia                                  |                                                                           |
|                       | 6. Hyperinsulinemia with the development of diabetes                                |                                                                           |
|                       | 7. Lack leptin receptor defects                                                      |                                                                           |
| Zucker fatty rat      | 1. Obesity at around 4 weeks of age                                                 | 1. Presence of a mutated leptin receptor                                   |
|                       | 2. Develop hyperinsulinemia, hyperlipidemia, hypertension, and impaired glucose tolerance |                                                                           |
| Zucker diabetic fatty (ZDF) rat                      | 1. More severe insulin resistance                                                   | 1. Less obese than Zucker fatty rat                                        |
|                       | 2. Hyperinsulinemia around 8 weeks of age and then decrease in insulin levels       | 2. Females do not develop overt diabetes                                   |
|                       | 3. Diabetes develops around 8–10 weeks in males                                     |                                                                           |
|                       | 4. Diabetic complications present                                                   |                                                                           |
|                       | 5. Obesity-induced hyperglycemia                                                    |                                                                           |
and 36.8% carbohydrate) or standard Purina Rodent Chow diet (23% protein, 4.5% fat, 6.0% fiber, 8.0% ash, and 56% complex carbohydrate) for 6 months and mice developed type II diabetes mellitus (Fig. 1). Intraperitoneal or caudal vein injection of streptozotocin or alloxan to selectively destroy the insulin-producing beta-cells of the pancreas is the most common strategy to induce hyperglycemia in mice or rats. Additionally, hyperglycemia can be induced by genetic manipulation or with a high-fat diet. The method of inducing diabetes has a great effect on the process of wound healing and different methods have different levels of impairment in wound healing in various stages such as early (2–5 days), intermediate (6–10 days), and late stages (11–20 days) of wound healing [5, 30]. Most severe wound healing impairment in the early stages was found to be by multiple-dose alloxan, in the intermediate stage by NOD mice, and in the late stage by NOD mice followed by multiple-dose alloxan in both intermediate and late stages. High-fat-fed diet-induced diabetes was found to have the least effects on the impairment of wound healing [30]. Further, a longer period of diabetes before the induction of wounds results in more impairment in wound healing. Thus, it is important to choose an appropriate method of inducing diabetes depending on the type of wound healing under consideration. STZ can induce type I diabetes with a high dose and type II diabetes with a low-dose [37]. Yu et al. reported STZ-induced type II diabetes in Wistar rats by injecting freshly dissolved 70 mg/Kg STZ in 0.1 M citrate buffer (pH 4.5) intraperitoneally in 5-day old Wistar rats (n5-STZ rats) [38]. Hyperglycemia developed in rats after 9 weeks of injection with a blood glucose level > 300 mg/dl with a mortality rate of 45%. The authors reported delayed footpad wound healing in n5-STZ diabetic rats via elevated inflammation and reduced blood flow and cell proliferation. This model simulates the diabetic foot ulcer of humans. Type II diabetes can be induced in rats using a high-fat diet and multiple low-dose STZ [39–41] (Fig. 1). Qian et al. [41] reported that Sprague Dawley rats fed with a high-fat diet with a total kcal value of 40 kJ/kg (20% fat, 45% carbohydrate, 22% protein) for 8 weeks and treated with a single low-dose (30 mg/kg, dissolved in 0.1 M sodium citrate buffer at pH 4.4) of STZ intraperitoneally at 4 weeks on high-fat diet develop type II diabetes. In this study, rats with glucose levels < 16.7 mmol/l after one week of STZ injection were given a second dose of 30 mg/kg STZ. With this regimen, all rats became diabetic at the end of 8 weeks. Induction of discrete stages of type 2 diabetes in male Sprague Dawley rats, aged 14–16 weeks, on high-fat diet and using osmotic mini-pumps to infuse STZ (80–200 mg/kg) over the course of 14-days has been reported [42]. Cheng et al. [37] has also reported induction of type II diabetes with a single intraperitoneal injection of 30 mg/kg STZ in Sprague Dawley rats fed with a high-fat diet [43]. These studies suggest that single low-dose with high-fat diet, multiple low-dose STZ, or slow infusion over a period of time induces type II diabetes in rats fed with a high-fat diet.

**Wound induction: types and pros and cons**

Excisional wound, excisional splinted wound, incisional wound, ischemia/reperfusion wound, ischemic skin wound, open ulcer, skinfold chamber, burn model, infected wound model, and xenograft wounds are different types of wounds induced in rodent models for wound healing studies [25]. In pre-clinical studies for diabetic wound healing, the most common type of wounds are full-thickness surgical incision and excisional wounds on the dorsal skin of a mouse. Of these, the excision wound model is the most commonly used but is considered to be the least efficient because it heals through contraction, and the incision wound model is the second most common model used for research and is used for studying wound scarring. Incisional wounds are primarily used to investigate the surgical incision materials like suture thread degradation, strength, other mechanical properties, and wound scarring while excisional wounds (2–15 mm in diameter) are primarily for studying wound healing and testing the therapeutic agents including biofilms [4, 21, 25]. Before inducing...
the cutaneous wound in animal models, animals should have evident hyperglycemia for several weeks. More commonly used is the excisional wound model which has the advantage of ease of creating wounds and an easy assessment of wound healing processes involving granulation tissue formation, re-epithelialization, angiogenesis, remodeling, and scar formation [21, 44]. Further, these wounds allow timed assessment of wound healing rate. These types of wounds are useful because of the easy reach and advantage of the wounds not being in contact with the bedding compared to the wound on the foot of the mouse. This helps in decreasing the probability of infection from bedding or urine. Additionally, the animal cannot reach and manipulate the wound [5]. Foot ulcers are most common in diabetes-associated ulcers in humans, however, most of the studies in mice induce ulcers on the torso or back of mice. Although creating a wound on the back of mice provides more area to induce multiple wounds but do not simulate the wound in human beings. A foot wound in mice also does not simulate human foot ulcer because of the different anatomy of mice foot skin compared to torso skin (thinner than torso skin and absence of panniculus carnosus) as well as to humans [30]. Wounds in rats provide a larger wound area compared to mice. A full-thickness tail wound in mice is clinically relevant to delayed wound healing and the wound heals in up to 21 days with minimal contraction and thus can be used to study delayed wound healing, however, anatomical differences between mice tail skin and human skin is a limiting factor [5, 45]. Another advantage of using a tail wound model is the presence of short hairs on the tail which is not the case on dorsal wounds where hairs may impair the wound healing.

Simulating the wound in an animal model with humans is a major challenge in wound healing studies. The use of a splinted wound model has been proposed to simulate the animal wound with humans. A splinting technique is used to minimize the process of contraction in rodents and thus to mimic healing in humans [46, 47]. Splitting allows healing to occur through granulation tissue formation and re-epithelialization similar to wound healing in humans. In a splitting technique, two full-thickness wounds are created through the panniculus carnosus on the dorsal surface and silicon splints are fixed using sutures [46, 47]. However, with splitting, the blood vessels remain intact and thus this model is good to study acute wound healing and not chronic wound healing [25].

### Wound healing and factors affecting wound healing

Skin wound healing, in normal biological conditions, is a complex process involving many cell types and mediators intricating in a complex environment going through three-phases namely the inflammatory phase, the resolution/proliferative phase, and the maturation phase. Before these three-phases, during hemostasis, the wound is closed by clotting with the involvement of platelets, clotting factors, and fibrin formation. The inflammatory phase begins just after injury and protects the wound from infections and clears the debris via infiltrating immune cells including neutrophils, lymphocytes, and macrophages. Inflammation is must for a proper wound healing; however, excessive and persistent inflammation is problematic, and chronicity of inflammation leads to nonhealing chronic wounds. The wound contracts and heal with the formation of new collagen (collagen III) and extracellular matrix, granulation tissue deposition, neo-angiogenesis, re-epithelialization, and forms a new healthy tissue during the proliferative phase. During the maturation phase collagen III is remodeled and the proportion of collagen III decrease while collagen I proportion increases, resulting in a change of collagen III to collagen I ratio. This is followed by the organization of the healed wound and scar tissue to strengthen the healed wound and unwanted cells which are no longer needed to undergo apoptosis [12, 48–50]. However, interruption of these phases either due to poor blood supply decreased oxygenation, presence of the foreign body, obesity, stress, hormonal changes, associated comorbid conditions, medication, and poor nutrition causes impaired wound healing [12]. Obesity and diabetes are associated with impaired wound healing and chronic inflammation plays a critical role. An intricate interplay between immune cells, keratinocytes, and dermal cells helps in wound healing. Neutrophils and macrophages infiltrate the wound area during the inflammatory phase, clear the debris, and increase expression of pro-inflammatory cytokines including interleukin (IL)-8, IL-6, IL-1, and tumor necrosis factor (TNF)-α in the wound bed. The immune cells also increase expression of anti-inflammatory cytokines (IL-10), factors promoting angiogenesis including vascular endothelial growth factor (VEGF), monocyte chemoattractant protein (MCP)-1, and C-X-C motif ligand (CXCL)-3, and the proliferation of fibroblasts through increased expression of IL-8, IL-1 β, and MCP-1 in the wound bed. These cytokines and growth factors play a critical role in wound healing and involve in the remodeling of collagen by regulating the expression of matrix metalloproteinases. Macrophages also release epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) promoting keratinocyte proliferation and migration and platelet-derived growth factor (PDGF) in association with TNF-α, IL-1, and IL-6 to increase granulation tissue formation from fibroblasts. A balanced secretion of these cytokines and chemokines regulates collagen remodeling and re-epithelialization or the absence is associated with impaired wound healing. However, persistent higher levels are associated with chronic nonhealing wounds [51–54]. An increased level of epidermal growth factor (EGF) associate with re-epithelialization, increased
levels of fibroblasts growth factor (FGF)-2 associate with granulation tissue formation, re-epithelialization, and extracellular matrix formation and remodeling, increased levels of transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF) are associated with inflammation, granulation tissue formation, re-epithelialization, and extracellular matrix formation and remodeling but reduced levels of these factors correlate to wound chronicity [52]. The increased levels of IL-1, IL-6, and TNF-α secreted from neutrophils and macrophages are associated with inflammation and re-epithelialization and persistently increased levels with chronic and nonhealing wounds [52, 55].

**DFU features compared to chronic wounds in other tissues**

Chronic ulcers in any tissue are defined as an ulcer that failed to progress through healing phases and stay in the inflammatory phase without passing to the resolution phase. Chronic ulcers do not show any significant progress towards healing after 3 months. The chronic wound might be due to persistent inflammation, increased bacterial load, excessive secretion of proteases compared to their inhibitors causing extracellular matrix degradation, cell senescence, and inappropriate treatment. Chronic wounds have raised hyperproliferative, and non-advancing wound edges with an inflamed area around the wound. Senescent cells with impaired proliferative and secretory capacities of the cells in the wound also characterize chronic wounds [3, 56]. Patients with chronic wounds complain of pain as a common complaint [57]. The characteristics of a diabetic foot ulcer (DFU) may vary with the type of ulcer. Like, in DFU due to peripheral neuropathy, the ulcers have undermined or macerated edges with calloused surrounding skin. The wound is associated with infection (osteomyelitis), usually painless, and with a normal pulse. The patient does not develop a fever in neuropathic DFU with infection [58]. DFU is characterized by full-thickness skin loss and an ulcer develops with a callosity and circular punched out appearance. The infection in DFU penetrates deeper tissues even through fascia causing necrotizing fasciitis [59] while chronic skin wounds, even full-thickness wounds, penetrate through subcutaneous tissues but not through fascia [60]. DFU also differs from a pressure ulcer. Pressure ulcers develop at a point of bony prominence pressing against a firm surface. The pressure ulcer appears reddish, feels warm, starts with blistered skin and the skin erodes. In complicated cases, the ulcer may penetrate through the subcutaneous tissue up to the bone. Both pressure ulcers and DFUs may be foul-smelling [61].

Thus, the characteristics of DFUs differ from chronic ulcers on other body parts and are deeper than the chronic ulcer, using a rat model will be better than using a mouse model because of the larger size and thicker skin than the mouse. This may help in characterizing the diabetic ulcer created on the dorsal surface of the rat more precisely than the mouse. Additionally, as mentioned in Table 2, the commonly used diabetic mouse models (db/db and ob/ob) have the limitation of the presence of point mutation in the leptin receptor, not severe diabetes, and not all animals develop diabetes. We feel that Zucker Sprague Dawley rats will be more suitable than mice for creating and studying diabetic ulcers because of the presence of hyperinsulinemia and insulin resistance (characteristics similar to diabetes in humans), overt hyperglycemia between 15 and 20 weeks of age, and lack of leptin receptor defects. The limitation that all animals do not develop diabetes with diet can be overcome by STZ injection.

**Conclusion**

Animal models provide indispensable insight into the molecular and cellular mechanisms as well as serve a career to test therapeutic agents. Rodent models are the most used animal models for diabetic wound healing. Among mice and rats, due to large size, ease of training, low cost, and larger surface area to induce wound rats appear to be the preferred animal model. This is also supported by the skin anatomy and longer time for wound healing in rats. Further, hyperglycemia and insulin resistance with hyperglycemia in ZDSD rat, the features also present in patients with diabetes, makes these rats a suitable model to study diabetic ulcers. Rat models help to evaluate therapeutic agents as well as in the in-depth understanding of the underlying molecular mechanisms of the impaired wound healing that will help to improve clinical outcomes in chronic diabetic foot ulcers.

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Declarations

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