

Advances and perspective on animal models and hydrogel biomaterials for diabetic wound healing

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Key Words:

animal models; diabetic wound; hydrogel biomaterials; pathomechanism

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ABSTRACT

Diabetic wounds are a common complication in diabetes patients. Due to peripheral nerve damage and vascular dysfunction, diabetic wounds are prone to progress to local ulcers, wound gangrene and even to require amputation, bringing huge psychological and economic burdens to patients. However, the current treatment methods for diabetic wounds mainly include wound accessories, negative pressure drainage, skin grafting and surgery; there is still no ideal treatment to promote diabetic wound healing at present. Appropriate animal models can simulate the physiological mechanism of diabetic wounds, providing a basis for translational research in treating diabetic wound healing. Although there are no animal models that can fully mimic the pathophysiological mechanisms of diabetic wounds in humans, it is vital to explore animal simulation models used in basic research and preclinical studies of diabetic wounds. In addition, hydrogel materials are regarded as a promising treatment for diabetic wounds because of their good antimicrobial activity, biocompatibility, biodegradation and appropriate mechanical properties. Herein, we review and discuss the different animal models used to investigate the pathological mechanisms of diabetic wounds. We further discuss the promising future application of hydrogel biomaterials in diabetic wound healing.

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<http://doi.org/10.12336/biomatertransl.2022.03.003>

How to cite this article:

Hu, Y.; Xiong, Y.; Tao, R.; Xue, H.; Chen, L.; Lin, Z.; Panayi, A.; Mi, B.; Liu, G. Advances and perspective on animal models and hydrogel biomaterials for diabetic wound healing. *Biomater Transl.* 2022, 3(3), 188-200.



Introduction

With changes in modern lifestyles, the incidence of diabetes is gradually increasing, which brings a heavy economic burden to society and patients. Diabetes is a metabolic disease marked by hyperglycaemia, which causes angiogenic disorders in tissues, leading to complications such as diabetes-related cardiovascular disease, renal failure, amputation, and blindness.^{1, 2} Hyperglycaemia in diabetic patients is an important cause of vascular dysfunction, and persistent hyperglycaemia causes cellular dysfunction by producing glycation end products, increasing oxidative stress levels, and causing mitochondrial dysfunction.³ Diabetic wounds are a frequent complication in diabetic patients. They are mainly chronic wounds caused by peripheral nerve damage and vascular dysfunction. Because

they are prone to develop into local ulcers, wound gangrene and even require amputation, they cause patients enormous psychological and economic burdens. These chronic wounds have therefore become a difficult problem that clinicians need to solve urgently.^{4, 5} Factors affecting the healing of diabetic wounds mainly include decreased proliferation and migration of fibroblasts, infection, persistent inflammatory response, and decreased angiogenesis.^{6, 7} At present, the main goal of diabetic wound treatment is to restore blood supply to the wound and reduce ischaemia at the site.^{6, 8} The current clinical wound treatment methods mainly include wound accessories, negative pressure drainage, skin grafting and surgical treatment.^{9, 10} However, due to the many factors affecting the healing of diabetic wounds, there is currently no effective treatment to promote diabetic wound healing.

Animal models and hydrogels in diabetic wound healing

Wound repair is a complex process requiring activation of multiple biological signals and the coordination of cells that together regulate the wound repair process.¹¹ After creation of a wound, the internal and external factors in the wound influence the repair process. As trauma research continues to develop, cutting-edge treatment modalities include stem cell therapy, gene therapy and tissue engineering.^{12,13} The normal process of wound healing can be classified into four stages, including haemostasis, inflammation, proliferation and remodelling.¹⁴⁻¹⁶ The haemostatic phase is the period immediately following cutaneous trauma, when the body initiates the haemostatic process, with vasoconstriction and thrombus formation promoting blood clotting. The process involves a number of pro-vasoconstrictive factors such as thromboxane, epinephrine and complement. The inflammatory phase is a series of inflammatory reactions of the body to trauma. The major objective of this phase is to remove pathogenic bacteria and harmful tissue debris from the wounded area. In addition, in this phase, blood vessels will dilate and become more permeable. Chemotactic factors induce the accumulation of macrophages, neutrophils and other inflammatory cells in the wound and secretion of cytokines, promoting the production of granulation tissue. The proliferation phase is the stage of cell proliferation and differentiation, where fibroblasts in the wound migrate and proliferate. Extensive extracellular matrix is synthesised and collagen is produced. The proliferative phase is the stage of cell migration, proliferation, extracellular matrix synthesis, collagen production, inflammatory cell release, cell migration and granulation tissue formation creating new vessels. The remodelling phase is the dynamic balance between tissue regeneration and fibrosis. Fibroblasts partially differentiate into myofibroblasts to contract the wound, while the deposited collagen fibres are reconstituted into regularly-arranged and mature fibres on the basis of glycosaminoglycans and proteoglycans. The granulation tissue gradually degrades into mature, non-vascular, non-cellular scar tissue.¹⁷⁻¹⁹ Therefore, in this review, we summarise the different animal experimental models for preclinical evaluation of diabetic wounds and further discuss the promising future of hydrogel biomaterials in diabetic wound healing.

Diabetic Wound Models

Although no animal model fully simulates the pathophysiological mechanisms of human diabetic wounds, the exploration of using animal models to simulate the disease in preclinical studies and basic research is of utmost importance. In an animal, diabetes can be induced by different mechanisms including viral, genetic and chemical induction, and spontaneous autoimmune hyperglycemia.²⁰ Streptozotocin (STZ) and alloxan are the most widely-used agents in chemical diabetic induction. It has been reported that STZ induces disease characteristics and similar responses in different animal models. Species including mice, rabbits, guinea pigs, monkeys, dogs and cats have been reported to be successfully used for the induction of diabetes

by STZ, as indicated by the clinical features and pathological changes that resemble those of human diabetes (**Figure 1**).²¹ A vast array of *in vivo* experiments has demonstrated that STZ-induced diabetes and the subsequent changes constitute a reliable animal model.^{22,23} Goyal et al.²¹ and Szkudelski²⁴ systematically summarised the mechanisms that caused diabetogenic changes following induction by the chemical STZ. These diabetogenic mechanisms included reactive oxygen species (ROS) generation, DNA damage, oxidative stress and glucose overloading, thereby causing pancreatic β cell damage.^{21,24} The diabetogenic action is characterised by selective death of β cells, insulin dysfunction, hyperglycaemia, and symptoms of polyuria, polydipsia and weight loss, which resemble human signs of diabetes mellitus. As for alloxan, it generates ROS and generates superoxide radicals and hydroxyl radicals. These hydroxyl radicals ultimately contribute to the death of β cells. As a thiol reagent, alloxan also selectively inhibits high glucose-induced insulin secretion by inhibiting the β cell glucose sensor glucokinase. The most significant disadvantage of using chemicals such as STZ and alloxan is perhaps the chemical toxicity to organs of the body.²⁰ However, they have been widely used in various animal models. In this review, we focus on the animal models of diabetic wound healing involving rats, mice, rabbits, and pigs.

Diabetic models in rats

A diabetic skin ulcer is one of the most commonly-used models for the study of diabetic complications, and the model has been well explored and widely studied.²⁵⁻³⁰ Rats are perhaps the first choice for diabetic animal models.^{22,31} Given that the STZ model of diabetes induces clinical features in animals resembling those in humans, chemical STZ models serve as a perfect platform for developing diabetic wounds.^{21,22} To simulate human diabetic wounds, Yang et al.³² administered STZ to male Sprague-Dawley rats to generate diabetes. They prepared a 1% STZ solution, with a dose of 50 mg/kg, which was then intraperitoneally injected into Sprague-Dawley rats. After injection, a wound healing model was created and monitored. Using this model in an earlier study,²⁷ a dorsal full-thickness ulcer was induced after 5 weeks of intraperitoneal injection of 60 mg/kg STZ. Another study involved administration of 65 mg/kg of STZ to maintain the fasting blood glucose concentration of Sprague-Dawley rats above 250 mg/dL, and then circular full-thickness skin excisions of 2 cm in diameter were created on the back of these rats.²³ Zhang et al.³³ also employed a chemically-induced diabetic model using 60 mg/kg STZ, and achieved fasting glucose levels of more than 16.7 mM, and full-thickness wounds with a diameter of 2 cm were then created on the backs of the rats. Liu and coworkers³⁴ created 2 cm full-thickness wounds on STZ-generated diabetic Sprague-Dawley rats with fasting glucose levels over 11.1 mM. In one final example, a full-thickness dorsal wound was created after intraperitoneal administration of STZ (55 mg/kg) for 3 days.³⁵ Collectively, these studies show that STZ at a dose of

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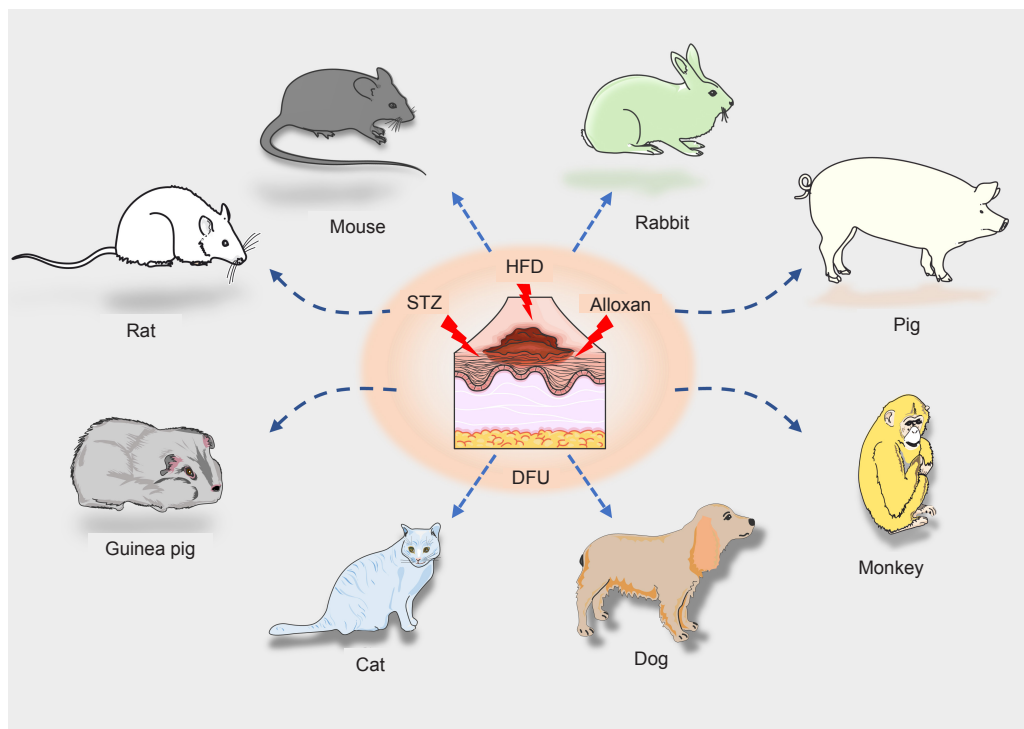


Figure 1. Diabetic wound models are induced in different animals. DFU: diabetic foot ulcers; HFD: high fat diet; STZ: streptozotocin.

50–65 mg/kg causes dysfunction of glucose regulation, which is a feasible method for imitating the human diabetic state and serves as a perfect platform for subsequent diabetic wound models.

Diabetic models in mice

Mouse models are clinically relevant and have the advantages of testing potential therapeutic effects. There are two types of diabetes in mice, type 1 diabetic (T1D) models include the nonobese diabetic (NOD) mice and STZ-induced mice; type 2 diabetic (T2D) models include obese ob/ob mice and db/db mice.²¹ T1D is a disease caused by the destruction of β cells.³⁶ Nowadays, the NOD mouse still serves as the best spontaneous model for T1D because the disease spontaneously develops and has many immune pathogenic characteristics in common with human T1D.^{36–38} For instance, the NGL construct containing four tandem copies of the 5' human immunodeficiency virus-long terminal repeat enhancer was used to generate transgenic NOD mice. Female mice with blood glucose > 15 mM are regarded as diabetic mice.³⁹ STZ can also be used in T1D models. For instance, STZ-induced T1D models were generated on 8-week-old db/db mice by administering a dose of 70 mg/kg for 12 weeks and glucose beyond 300 mg/dL was considered as a diabetic state.⁴⁰

For the most common T2D model in mice, studies showed that daily STZ administration generated T2D models in C57BL/6 mice given 40 mg/kg for 3 consecutive days⁴¹ or in 8-week-old db/+ mice given 50 mg/kg for 4 consecutive days.⁴² Many other researchers instead used a straightforward high-fat diet (HFD) to model human T2D because a HFD is a dependable model that provides an appropriate aetiological, pathological,

and treatment option for T2D.⁴³ C57BL/6 mice were provided with a rodent diet containing 60 kcal% fat.⁴⁴ The glucose and insulin levels were used to evaluate T2D induction at 20 weeks. In addition to single diet-induced diabetes, use of a HFD combined with multiple doses of STZ has also been used in a mouse model. Wang et al.⁴¹ combined a HFD with chemical STZ treatment to induce T2D. Specifically, 5-week-old C57BL/6 mice were fed with a HFD (60% of calories from fat) for 24 weeks. After HFD feeding for 23 weeks, mice were injected with 40 mg/kg STZ daily for 3 consecutive days to generate T2D.⁴¹ In a similar experiment, C57BL/6 mice were fed a HFD for 8 weeks, and then given an intraperitoneal injection of 100 mg/kg STZ at weeks 9 and 11. After treatment, 4 hour-fasting blood glucose over 16.7 mM in the tail vein was considered T2D. The mouse model is clinically relevant and has the benefit that therapeutic agents have been tested.⁴⁵ Therefore, it is essential that the appropriate model is chosen when considering the type of wound healing.

Diabetic models in rabbits

Rabbits were also monitored for clinical signs including changes in behaviour, activity, diet, urination/defecation and blood glucose levels.^{46, 47} The rabbit ear model of diabetic wounds overcomes the contraction issues and has the advantage of the ability to create multiple wounds.²⁰ Zhang et al.⁴⁸ studied T2D models using New Zealand rabbits. Rabbits were first fed a HFD for 2 months. At the end of this period, the rabbits were injected every 3 days with alloxan monohydrate (50 mg/kg) via the ear following 6 hours of fasting. Rabbits with fasting blood glucose level > 11.1 mM were then used in the experiment. After the induction of T2D, a 10 mm × 10 mm full-thickness section of skin was excised and a radial debridement of the wound was

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conducted. The wounds in the treatment group were treated with epidermal growth factor (EGF) once a day for 1 month then compared with the control group. In the treatment group, endogenous EGF mRNA was significantly higher than in the control group, which indicated that exogenous EGF treatment accelerated the healing of diabetic wounds by upregulating the expression of EGF mRNA in newly-generated tissues. In another alloxan-induced diabetic rabbit ear ulcer model,⁴⁹ alloxan at 150 mg/kg in 30 mL of saline was administered intravenously via an ear vein. After drug induction, glucose and molasses were provided for 24 hours to avoid hypoglycaemia. Blood glucose levels were checked weekly using blood from the marginal ear vein after the blood sugar level had stabilised. Four weeks after alloxan treatment, rabbits were anaesthetised and full-thickness punch biopsy wounds with a diameter of 6 mm were created on each of the hyperglycaemic rabbits. They found that autologous circulating angiogenic cells treated with osteopontin could promote diabetic wounds healing. By using the same diabetic models with alloxan, collagen seeded with allogeneic mesenchymal stem cells resulted in increased angiogenesis and accelerated diabetic wound healing.⁵⁰

Diabetic models in pigs

Pigs also have the advantage of a similar skin wound healing process to humans. The courses of re-epithelialisation and granulation tissue formation closely resemble those in humans.²⁰ Pig diabetic ulcer models have been successfully established.^{51,52} The chemical drug STZ was also used to cause systematic hyperglycaemia in pigs at a dose of 150 mg/kg body weight, then serum glucose concentration was measured, and short-acting and long-acting insulin was used to keep the glucose level between 350 and 550 mg/dL for 14 days. After induction, the full thickness of pig was created. By detecting glucose concentration in serum and wound fluid, they found that delayed wound healing in diabetic pigs is not induced by local high glucose concentration. They further found that diabetic pigs showed impaired healing accompanied by reduction of insulin-like growth factor 1 level in the healing wound.⁵¹ In another pig *in vivo* ulcer model,⁵³ pathogen-free pigs were injured using a modified electro-keratome set to obtain partial-thickness wounds of 10 mm × 7 mm × 0.5 mm. Wounds were treated within 20 minutes with mevastatin. The results showed that mevastatin selectively triggered an anti-proliferative, pro-migratory phenotype and restored EGF sensitivity in the diabetic wound, resulting in healing of chronic wounds.⁵³ However, pigs are difficult to anaesthetise due to their large size. The use of pigs as diabetic models is largely limited because of limitations including the long period needed to raise them and to the expense of maintaining them.

Other animal studies

Despite advances in the methods of creating wound healing models, there is still a lack of an optimal preclinical model capable of completely recapitulating human diabetic wounds. However, the pace of exploration has not slowed. Hyperglycaemia was evident in 25–100% of treated guinea pigs within 48 hours, at doses of 200 or 250 mg/kg of STZ.⁵⁴ Especially when HFD-fed guinea pigs were treated with

STZ, glucose intolerance and fasting hyperglycaemia could persist for more than 21 days. Moreover, diabetes and insulin resistance models can also be induced by genetic manipulation. A novel strain of mice expressing the human HLA-DR4 gene was created, which was characterised by diverse leucocyte infiltration and the formation of anti-proinsulin auto-antibodies that was akin to human T1D.⁵⁵ The more exploration and attempts are carried out to create preclinical models of diabetic wound healing, the more these lab findings can be translated into clinical treatments for diabetic wounds.

Factors in Diabetic Ulcers

High-glucose-induced peripheral neuropathy, peripheral arterial occlusive disease, infection, immunosuppression and increased plantar stress are all important factors of diabetic wounds.^{56,57} However, diabetic wounds do not follow the normal dynamic process of wound healing: haemostasis, inflammation, proliferation, and remodelling. These complex internal and external factors make patients more vulnerable to problems developing at many levels (Figure 2).^{58,59}

Vasculopathy and ischaemia

Diabetes mellitus is often associated with a series of changes in macro- and micro-vessels that manifest as multiple complications. A spectrum of lesions associated with microvascular outcomes in patients include chronic kidney disease, diabetic retinopathy, cardiovascular disorders and foot ulcers.⁶⁰⁻⁶³ In diabetic kidney disease, glomerular vasculopathy damage drives the development of diabetic kidney disease which leads to glomerular hyperfiltration, progressive albuminuria, declining glomerular filtration rate, followed by progressive failure of renal function.⁶⁴⁻⁶⁶ Diabetic retinopathy includes changes in vascular permeability, capillary degeneration, capillary microaneurysms, and excessive neovascularisation within the retina, which contribute to changes in the retina blood vessels, and lead to visual impairment and eventually cause premature blindness.^{63,67} Diabetic heart disease encompasses a series of disorders ranging from diabetic myocardial infarction to cardiomyopathy, heart failure and sudden cardiac death. Long-term hyperglycaemia leads to an increased risk of atherosclerosis of coronary vessels and differential remodelling of myocardial arterioles and capillaries,⁶² which is thought to be induced by the increased advanced-glycation end products, activation of the renin-angiotensin-aldosterone system, impaired microcirculation, abnormal mitochondrial function and aberrant calcium signaling.^{68,69}

Microcirculatory changes are noted in the diabetic foot skin and ulcers. Chronic diabetic ulcers are developed and worsened by functional and structural microvascular dysfunction, arteriovenous shunting, microcirculatory ischaemia and different degrees of occlusive arterial lesions.^{70,71} Functionally, the skin microvasculature is altered with severely reduced capillary circulation, and occlusive hyperaemic response. It is known that the microvasculature of diabetic patients fails to respond appropriately to stress and injury.⁷² The rational mechanism is that blood is shunted away from the nutritional capillaries via subpapillary arteriovenous shunts, which are innervated by diabetic-damaged nerves that lead to the opening

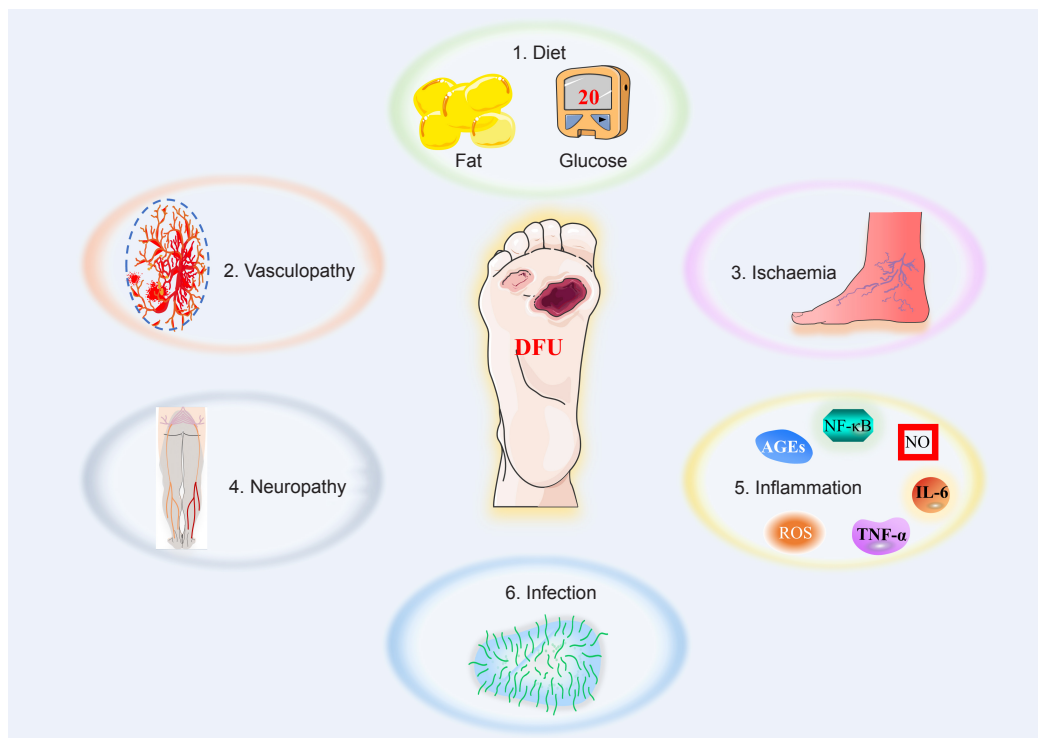


Figure 2. The main factors that contribute to the formation of a diabetic wound. AGE: advanced glycation end products; DFU: diabetic foot ulcers; IL-6: interleukin 6; NF- κ B: nuclear factor kappa-B; NO: nitric oxide; ROS: reactive oxygen species; TNF- α : tumour necrosis factor- α .

of these shunts and much lower resistance compared to nutritional capillaries. Structurally, the most obvious changes are the thickening of the capillary basement membrane, diminished capillary size, and pericyte degeneration and increased shunting of blood through arteriovenous channels.⁷³

Neuropathy

Neural damage is termed “neuropathy”. According to epidemiological data, approximately 50% of the cases of diabetic foot syndrome can be accounted for by neuropathy while peripheral angiopathy is responsible for just 15%.⁷⁴ Moreover, diabetic neuropathy can cause a series of complications including long-term chronic pain, foot ulcers, foot infections, and even catastrophic events including amputations.^{75, 76} The pathophysiological mechanisms involved in diabetic neuropathy have been comprehensively and extensively reviewed.⁷² Diabetic peripheral neuropathy is caused by nerve dysfunction and neurocyte death as a consequence of oxidative stress, mitochondrial dysfunction, metabolic disorder and inflammation.^{75, 77} Hyperglycaemia and insulin resistance collectively cause an imbalance in the mitochondrial redox state and excess formation of ROS, thereby contributing to dysregulation of metabolic pathways, a loss of stored energy and axonal injury, promoting diabetic neuropathy.⁷⁸

Both sensory and autonomic nerves populate skin tissues. Cutaneous sensory nerves predominate and are widely distributed in the skin, including extending into the upper epidermis where they come into contact with the external environment. In contrast, autonomic nerves are distributed in the dermis of the skin and mainly regulate lymphatic function, blood circulation, and appendageal function.⁷ Nowadays, there

is a growing appreciation that neuropathy is a major factor in impaired skin integrity in diabetes.⁶³ Diabetic neuropathological changes preferentially damage sensory neurons, and the unmyelinated small-diameter sensory axons are especially susceptible.⁷⁹ Evidence of sensory neuropathy is decreased or absent sense of vibration and superficial sensitivity including pressure, haptics, and subjective paresthesias. Consequently, the sensation of pain is substantially decreased and the risk of trauma is significantly higher. Due to the loss of pain sensation, serious ulcerations are underestimated and injuries are thus often not noticed in patients with diabetes mellitus.⁷⁴ Autonomic nerve damage leads to arterial sclerosis, vasomotor paralysis, arteriovenous shunts in the microvascular network, dysfunctional sudomotor function, and neuropathic oedemas in damaged skin. As a result, affected skin in patients with diabetes mellitus has a reduced protective function and an increased risk of injury.⁷⁷ The combination of these peripheral nerve problems leads to abnormal foot pressures in the form of an elevated plantar pressure load, accompanied by the development of hyperkeratosis and finally diabetic foot ulcers.^{80, 81}

Inflammation

The onset of diabetic foot ulcers (DFU) is a complex process and the pathogenesis is multifactorial with several factors working together. Pathogenic factors that are able to generate DFU are multifactorial.⁸² It is known that diabetes mellitus is characterised by a persistent low-grade inflammatory state. A pathophysiological diabetic state involves enhanced-proinflammatory responses and suppressed anti-inflammatory responses, as indicated by the excessive oxidative stress and

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increase in advanced glycation end products, NF- κ B activation, nitric oxide blocking, DNA damage, and proinflammatory cytokines. The increased levels of pro-inflammatory cytokines such as interleukin 1, interleukin 6 and tumour necrosis factor- α in diabetic patients indicates a predisposition to suffer DFU.^{83,84}

Infection

Diabetic patients are at higher risk of developing infections than persons without diabetes.⁸⁵ Hyperglycemia disrupts the integrity of small blood vessels, and the immune response is inadequate to protect against local infection, thereby resulting in ulceration.⁸⁶ Moreover, diabetic foot infections typically begin within a site of trauma or ulceration. Infection is an invasion by microorganisms and their reactions to the host immune system, which induce inflammatory responses, immunopathy, and subsequent tissue destruction.⁸⁷ Microbial load, diversity of microbial spectrum, and the synergistic association of microbes are responsible for the development of diabetic foot infection.⁸⁸ Since several factors predispose diabetic patients to developing an infection, an accurate and timely microbial assessment is one of the essential and effective

regimes for preventing DFU infection.^{88,89}

Management of Diabetic Foot Ulcers

DFU are a serious complication of diabetes resulting in significant morbidity, poor quality of life, worse psychological endurance, and a high mortality rate.^{90,91} The wound-healing process of these ulcers often becomes refractory, especially when the underlying causes are unclear and the patient does not receive holistic professional treatments. In diabetic patients, reduced fibrinolysis, poor formation of extracellular matrix, aberrant secretion of inflammatory cytokines, diminution of angiogenesis, reduction of fibroblasts and infection are all important factors that contribute to the problem of deficient wound closure. The current standard diabetic wound treatments comprise four main principles: (1) debridement, (2) infection management, (3) offloading and (4) revascularisation (Figure 3).⁹² Despite these standards, many diabetic wounds persist. Strategies for the management of patients with a diabetic wound are multidisciplinary approaches and involved in multifactorial processes including glycaemic control.⁵⁶ Therefore, new approaches are warranted for these complex wounds.

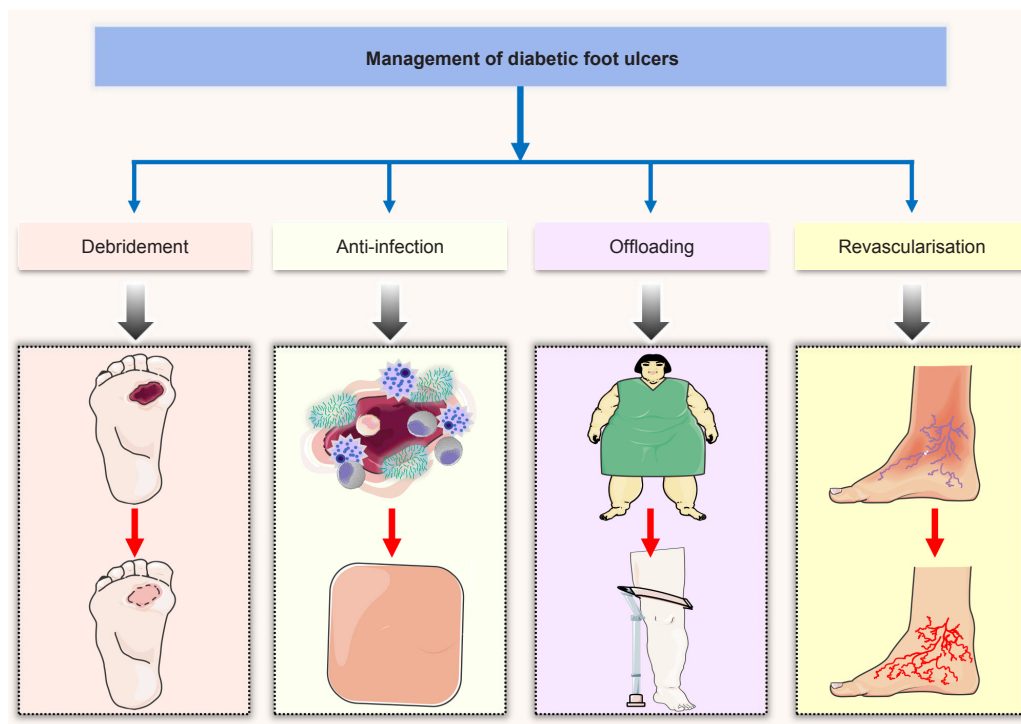


Figure 3. The current gold standard treatments for diabetic foot ulcers.

Debridement

Cells at the nonhealing edge of chronic diabetic wounds exhibit a pathogenic and proinflammatory phenotype that inhibits and prolongs wound healing.⁹³ Debridement is regarded as an effective intervention to speed up ulcer healing and is widely practiced in diabetic foot care.⁹⁴ Surgical debridement involves the removal of all devitalised, infected, or festering and necrotic tissue within the wound bed or adjacent to the wound until surrounding healthy tissue is exposed.⁹⁰ It is the fastest and most effective treatment for progressive and recalcitrant

ulcers. The most traditional form is mechanical debridement with the application of moist and wet dressings.⁹⁵ Enzymatic debridement uses chemical agents such as collagenase and streptokinase. It selectively targets necrotic tissue and is applicable to ischaemic wounds.^{92,95,96} It has been reported that collagenase in combination with serial sharp debridement appeared more beneficial over standard care alone.⁹⁶ Calluses in diabetes mellitus are hyperkeratotic lesions caused by pressure, which are a component cause of ulceration. It has been reported that the surgical debridement of hyperkeratoses

can reduce peak plantar pressure by 26%.⁹⁷ Debridement by different means aided in granulation tissue formation and re-epithelialisation and promoted wound healing.⁹³ Wound debridement involves removal of calluses.

Infection management

Wound infection is a known predictor of poor wound healing and amputation.^{90, 98} The enhanced recognition of infection and appropriate treatment in diabetic wound infection is imperative to improve outcomes. Empirical identifications and regimens are usually selected for the initial infection. However, inappropriate treatments with antibiotics are often associated with several adverse effects, including antibacterial resistance.^{90, 99, 100} Therefore, knowledge about the microbial aetiology and understanding of antibiotic resistance in diabetic foot infections is of the great importance to the effective management of these infected wounds.⁸⁸ Nowadays, a set of common principles may provide comprehensive classification systems for diabetic foot infections, and a comprehensive description of microbiology.^{88, 101} According to a Cochrane review, the use of an antimicrobial dressing instead of a non-antimicrobial dressing increased the healing of DFUs over a medium-term follow-up period. Moreover, their analyses showed that there was little difference in the risk of adverse events related to treatment between systemic and topical antimicrobial treatments.⁸⁹ Another recommendation suggested that mild tissue infections should be treated for 1–2 weeks and more severe cases for about 3 weeks. Wounds complicated with bone infections should receive prolonged and upgraded antibiotic therapy with more broad-spectrum agents.¹⁰² However, antibiotics given to treat infection do not actually heal the wound itself. Therefore, therapies should always be combined with other diabetic wound treatments.⁹²

Offloading

Procedures to reduce abnormally elevated pressure, mechanical stress and shear stress at the site of the ulcer constitute probably one of the most important interventions to promote diabetic healing.¹⁰³ Long-term pressure at the ulcer site can cause deformities of foot anatomy, delay ulcer healing, increase the risk of recurrence after healing, and seriously escalate the probability of amputation. A surgical procedure is important to stabilise and harmonise the foot for long term off-loading. However, the common situation is that surgical offloading is only used in high-risk patients for whom conservative management has failed.⁹⁰ More recently, it has been suggested that surgical off-loading should not be reserved only for those cases that are recalcitrant, but extended to ulcer prevention and remission in diabetic wounds.¹⁰⁴ In addition to surgical methods, off-loading can be achieved with the aid of devices, including modified shoes, boots, and orthotic walkers, which have proven to be the ideal form of adequate offloading.^{90, 105, 106}

Revascularisation

One study identified that more than 40% of DFU patients suffered from peripheral arterial disease (PAD), and PAD was the strongest prognostic factor for non-healing wounds and amputation.¹⁰⁷ Diabetic angiopathy predominantly affects

the infrapopliteal blood vessels.¹⁰⁸ Impaired circulation and ischaemic vessels are the indications for revascularisation. One single-centre retrospective analysis showed that successful revascularisation of DFU patients has a significant impact on limb salvage rate and wound healing.¹⁰⁹ A literature review showed the outcomes of 1-year limb salvage rates in endovascular revascularisation (a median of 85%) were broadly similar to those treated by open bypass surgery (a median of 78%).¹¹⁰ Data from 187 lower-extremity revascularisation procedures supported the use of the wound, ischaemia, and foot infection classification system to predict the revascularisation benefit in chronic limb-threatening ischaemia. Limbs with less benefit from revascularisation will more frequently require amputation despite the aggressive revascularisation.¹¹¹ Although studies have shown that aggressive and timely revascularisation results in surprising progress that markedly reduced amputation rates, those DFU patients with higher-grade PAD tended to have higher rates of amputation even after revascularisation.¹¹²

Anti-diabetic drugs

In the general therapeutic policy for diabetic wounds, it is widely recommended that good blood glucose control improves wound healing and limits adverse effects. As hyperglycaemia is a major factor impeding normal wound healing, restoration of normoglycaemia should always be part of the treatment plan.^{90, 113} One of the most effective controls and preventative measures for diabetic wounds is to maintain blood glucose at an appropriate level with anti-diabetic drugs and appropriate diet.⁸⁶ Treatment by intravenous injection of insulin has been proven to significantly increase the wound-healing rate to 30.3% compared to 9.8% in those without drug treatment.¹¹⁴ Maintenance of normoglycaemia with continuous subcutaneous insulin for four weeks resulted in up to 88.1% of DFU patients achieving a relatively good wound-healing effect.¹¹⁵ Furthermore, an animal model of diabetic wounds showed that topical application of insulin cream markedly accelerated healing and shortened the healing time by activating the protein kinase B (AKT) and extracellular regulated protein kinases pathways.¹¹⁶ It was concluded that intralesional administration of insulin had a double benefit, which not only improved vascularisation and granulation, but also prevented infection as an anabolic peptide.⁸⁶ In addition to local injection, insulin has also been used to intermittently irrigate diabetic wounds combined with continuous drainage via vacuum sealing drainage.¹¹⁷ This combined treatment reduced inflammatory reaction, promoted formation of granulation tissue, improved tissue recovery function, and speeded up the wound healing rate, although it had no effects on blood glucose levels. Dipeptidyl peptidase-4 inhibitors were beneficial for diabetic wound healing because they regulated endothelial progenitor cells, thereby facilitating angiogenesis, promoting the formation of granular tissue and regulating the immune response to hypoxia in diabetic wounds.¹¹⁸ Metformin, another conventional antidiabetic drug, was demonstrated to promote diabetic wound healing by increasing transcription and protein levels of endocan, boosting the angiogenic potential of endothelial cells and improving nitric oxide

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generation under high-glucose conditions.¹¹⁹ Exendin-4 is a glucagon-like peptide-1 receptor agonist. Topical application of exendin-4 itself was effective for the treatment of diabetic skin wounds and in combination with the addition of adipose-derived stem cells further expedited diabetic wound healing.¹²⁰

Hydrogel Materials in Diabetic Wound

Hydrogel is a promising wound-healing material with properties of good breathability, moisture retention, the possibility of loading bioactive substances and the ability to absorb wound exudate and cool the wound surface, thus relieving the patient's pain.^{121, 122} Biomedical hydrogels have been extensively investigated as a scaffolding material for efficient drug/cell delivery in wound healing and functional reconstruction of skin tissue.¹²³⁻¹²⁶ The typical three-dimensional cross-linked structure of hydrogels allows high drug/cell encapsulation and local drug/cell persistence.¹²⁷ The ideal wound hydrogel materials should have a wide range of functional properties including pore structure, antimicrobial activity, appropriate mechanical properties, haemostatic ability, controlled biodegradation, sustained release of bioactive molecules and good biocompatibility.¹²⁸⁻¹³¹

In recent years, Guo et al.¹³² developed a specially-designed hydrogel with smart targeting of refractory wound characteristics and synthesised an injectable, inflammation-responsive, self-healing hydrogel to treat diabetic wounds. They found that hydrogels loaded with zinc oxide nanoparticles (nZnO), and micelles@paeoniflorin exhibited excellent structural integrity and rheological properties while remaining injectable. In addition the hydrogel released nZnO and paeoniflorin in response to an inflammatory microenvironment. They also found that their hydrogel exhibited good cytocompatibility, and robust haemostatic and angiogenic properties, which promoted healing of chronically-infected wounds in a rat model of diabetes. Wang et al.¹²⁸ reported that they fabricated an injectable adhesive thermosensitive multifunctional polysaccharide-based hydrogel dressing. They found that the hydrogel dressing possessed multifunctional properties including antibacterial activity, fast haemostatic ability, self-healing behaviour, tissue-adhesive and good ultraviolet-shielding functions. It was also able to maintain the bioactivity of exosomes and promote the proliferation, migration and angiogenic ability of human umbilical vein endothelial cells. The results of an *in vivo* animal experiment indicated that the hydrogel promoted formation of granulation tissue, collagen deposition, remodelling, re-epithelialisation and further accelerated diabetic wound healing by enhancing angiogenesis. Further, some researchers used the Schiff base reaction to prepare hydrogels containing polyethylene glycol, carboxymethyl chitosan, and basic fibroblast growth factor. They found that the hydrogels had good wet-tissue adhesion, antibacterial properties, excellent biocompatibility, fast haemostatic capacity and self-repair abilities. The results demonstrated that these carboxymethyl chitosan-based hydrogels significantly accelerated full-thickness diabetic wound healing by increasing epithelialisation and collagen production and promoting the formation of hair follicles as well as improving neovascularisation by upregulating

CD31.¹³³ Recently, other researchers reported that an injected thermosensitive hydrogel incorporating Prussian blue nanoparticles enhanced angiogenesis, and reduced interleukin 6 and tumour necrosis factor- α levels. They also found that thermosensitive hydrogels improved diabetic wound healing by scavenging ROS and restoring mitochondrial function.¹³⁴

There is also a growing body of evidence suggesting that hydrogel biomaterials can be considered as an ideal sustained-release carrier of drugs or growth factors to release clinically significant doses of therapeutic agents in the process of wound repair.¹³⁵ For example, Niu et al.¹³⁶ prepared an injectable, thermosensitive and ROS-scavenging hydrogel as a release carrier of recombinant human MG53 protein and found that the hydrogel allowed for sustained release of recombinant human MG53 and enhanced diabetic wound healing and hair follicle development, which indicated the potential therapeutic prospects of using ROS-scavenging gel in combination with recombinant human MG53 to treat diabetic wounds. Further, Lee and Lin¹³⁷ developed a chitosan/polyvinyl acetate heterocomposite hydrogel loading EGF. Their results showed that the hydrogels sustainably released EGF and polyhexamethylene biguanide in a microenvironment to generate antibacterial effects and promote cell growth for wound repair. They further found that the hydrogel promoted faster collagen deposition and re-epithelialisation and reduced the inflammatory response, which accelerated diabetic wound healing. Recent research has revealed that Wu et al.¹³⁸ developed a conductive and intrinsically-antibacterial hydrogel with pH responsiveness using quaternised chitosan, polyaniline and polyethylene glycol as delivery carrier of deferoxamine which is a pro-angiogenic drug. Their results illustrated that the hydrogels smartly released deferoxamine and enhanced vascularisation by increasing the expression of vascular endothelial growth factor and improving the proliferation and migration of endothelial cells. The results of *in vivo* animal experiments revealed that the hydrogels combined with electrical stimulation enhanced angiogenesis and significantly accelerated infected diabetic wound healing.¹³⁸ We used cryogenic three-dimensional printing technology to create a small intestinal submucosa/mesoporous bioactive glass hydrogel scaffold as a release carrier of bioactive exosomes. The results showed that the hydrogel scaffolds permit sustained release of bioactive exosomes with adequate biocompatibility and good haemostatic ability, and enhanced the angiogenic ability of human umbilical vein endothelial cells. We also found that the hydrogel increased blood perfusion of wounds, promoted collagen deposition, and accelerated diabetic wound healing by enhancing angiogenesis.¹³⁹

Conclusion and Perspective

As tissue engineering continues to improve, the therapeutic methods used for diabetic wounds have diversified. Despite the use of different animal models dedicated to the research of diabetic wound healing, these models are judged by their ability to simulate the diabetic wound environment. Rodent models are widely used for basic research due to their low cost and clear genetic background, while translational research often needs large animal models to simulate the condition

of diabetic wounds in humans. Due to their breathability, moisture retention and good biocompatibility, hydrogel materials are considered as a promising therapeutic strategy for diabetic wounds. However, in spite of the rapid development of hydrogel-based therapy for diabetic wound healing, the clinical application of hydrogels in the treatment of diabetic wounds needs to be further studied. The appropriate use of animal models is one of the key steps for clinical translational research of hydrogels. Recent research has focused on electrical stimulation and development of a wearable electrotherapy device for wound healing, which achieved satisfactory treatment results. The wearable electrotherapy device may be expected to be able to quickly evolve into clinical applications for treating diabetic wounds. Although a number of diabetic wound models have been established, there is a lack of consensus on the choice of diabetic wounds for evaluating the therapeutic effects. Besides, many researchers develop many biomaterials and obtain good therapeutic effects for treating diabetic wounds, however, a small number of related biomaterials was approved for treating diabetic wounds.

Author contributions

Review design: YH, YX, BM, GL; literature search: RT, HX, ZL, LC; data collection: RT, HX, LC; manuscript draft: YH, YX; manuscript revision: ACP, BM, GL. All authors reviewed and approved the final version of the manuscript.

Financial support

This work was supported by National Natural Sciences Foundation of China (Nos. 82002313, 82072444), and Hubei Province Key Laboratory of Oral and Maxillofacial Development and Regeneration (No. 2020kqhm008).

Acknowledgement

None.

Conflicts of interest statement

The authors declare no conflict of interest.

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Received: May 1, 2022

Revised: May 27, 2022

Accepted: August 26, 2022

Available online: September 28, 2022