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REVIEW ARTICLE

EXPERIMENTAL ANIMAL MODELS IN DIABETIC WOUND HEALING: AN OVERVIEW

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ABSTRACT

Diabetes mellitus (DM) is one of the factors that affects the wound healing process associated with elevated blood glucose. Wound healing in diabetics is impaired due to the inhibition of cell proliferation and collagen formation. This condition presents a challenge to diabetic wound healing. Restrictions on experiments directly involving humans due to ethical concerns are another challenge. Therefore, the use of experimental animals is one of the options to study diabetic wound healing because they have similarities with humans. Studies on the development of animal models in the diabetic wound healing process, from the cellular level to advanced biochemistry, have increased significantly in the last few decades. These efforts were made to improve clinical practice. The following literature review describes the advantages and disadvantages of animal models and induction methods. It is expected to inform the development of animal models for diabetic wound healing.

KEY WORDS: Diabetic mellitus; Diabetic wounds; Experimental animals; Wound healing; Similarities to humans.

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INTRODUCTION

Diabetes mellitus (DM) is a widespread condition globally, and patients with DM frequently suffer from complications, including diabetic foot ulcers, which can eventually lead to the risk of amputation.[1] Complications from diabetic ulcers are linked to delayed wound healing processes, including inflammation, proliferation, re-epithelialization, and remodeling. Several studies on diabetic wound healing have been conducted using experimental animals (in vivo).[2,3] The vivo studies are considered a best model for studying wound healing because they accurately represent various types of cells and their paracrine interactions. The most commonly used animals for wound healing studies are rats and mice, despite the structural and physiological differences between human skin and rodent skin. Rats, mice, and rabbits have also been reported to be used in wound healing studies through ear excision models.[3] Meanwhile, rabbits, rats, and pigs have been involved in dermatological studies, with pig skin having anatomy and physiology similar to human skin.[4] A large number of reports have also revealed the use of zebrafish in wound healing studies, with observations of caudal fin regeneration.[5] The use of experimental animal models provides insight into a closer description of the conditions of human skin that, although not completely similar, can establish the basic principles of wound management.[3] Wounds are classified into acute and chronic wounds. Acute wounds are used in studies of natural healing and drug discovery through incisional, excisional, and burn wound methods. Meanwhile, chronic wounds can develop from acute wounds, often induced by conditions such as diabetes.[2] Diabetic-induced experimental animals will provide an illustration resembling what is experienced by patients with diabetic ulcers, which hinders wound healing processes.[2] As far as our search is concerned, reviews of the development of experimental animals with chronic hyperglycemic wounds remain limited. Therefore, this study aimed to provide the basis for selecting and developing a method to examine diabetic wound healing in experimental animals.

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MATERIALS AND METHODS

Through literature reviews from various journal websites on the internet, including ScienceDirect, Google Scholar, and PubMed, researchers conducted this study by browsing library sources in the form of regional and international scientific articles. The search was focused on research articles related to in vivo studies (rats, mice, rabbits, zebrafish, dogs, pigs, and guinea pigs) of diabetic wound healing. The inclusion criterion in this study was articles published in the last 10 years (2012-2022), while the exclusion criterion was articles that could not be fully accessed.

RESULTS AND DISCUSSION

The search resulted in 30 national and international articles that met the criteria. The articles reviewed consisted of 7 articles on rats, 7 on mice, 4 on rabbits, 4 on guinea pigs, 3 on pigs, 1 on dogs, and 4 on zebrafish, as shown in **Figure 1**.

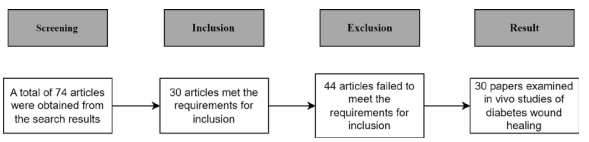

Figure 1. Search Scheme on Literature

Diabetic Wounds and Mechanisms of Impaired Healing: A wound is a structural and functional disruption of the skin in an exposed area. The body naturally responds to trauma by inflaming the area and producing scars as soon as the wound appears. The skin's normal structure and function are maintained through wound healing, influenced by factors like age, sex, genetics, and comorbidities. Diabetes mellitus is a significant comorbid condition affecting wound healing, leading to complex, multifactorial impairments. Research indicates diabetic wounds show abnormal healing and disrupted blood vessel formation compared to non-diabetic wounds.[3]mainly because of the multifaceted nature of the wound environment and the complexity of the healing process, which integrates a variety of cells and repair phases, including inflammation, proliferation, reepithelialization and remodelling. There are a variety of possible preclinical models, such as in mice, rabbits and pigs, which can be used to mimic acute or impaired for example, diabetic and nutrition-related wounds. These can be used induced by many different techniques, with excision or incision being the most common. After determining a suitable model for a study, investigators need to select appropriate and reproducible methods that will allow the monitoring of the wound progression over time. The assessment can be performed by non-invasive protocols such as wound tracing, photographic documentation (including image analysis This impaired healing is due to chronic inflammation, reduced angiogenesis, fewer endothelial progenitor cells, and imbalanced extracellular matrix regulation. In diabetes, levels of insulin-like growth factor-1 (IGF-1) and transforming growth factor beta (TGF-beta) decrease, affecting the healing process, as shown in Figure 2.

Wound healing in diabetic conditions is hindered due to incomplete tissue granulation, allowing pathogens to enter and cause infections. The presence of pathogens can lead to infections. High macrophage numbers trigger the overproduction of inflammatory agents, leading to increased fibroblast apoptosis and collagen degradation, which reduces angiogenesis.[6] Diabetic-induced experimental animals experience impaired phagocytic activities and phenotypic changes that lead to failures in tissue repair.[7]

Use of Experimental Animals in Diabetic Wound Healing Studies: The selection of experimental animals generally depends on their relevance or resemblance to human genes, physiology, and habits, as well as their costs and ease of handling **(Table 1)**. Commonly used experimental animals in diabetic wound healing studies include rodents such as rats, mice, and guinea pigs. Rabbits are also frequently used due to their anatomical and physiological similarities to humans. Recently, zebrafish have been reported as a preferred animal for studying diabetic wound treatment through caudal fin regeneration studies.[8]

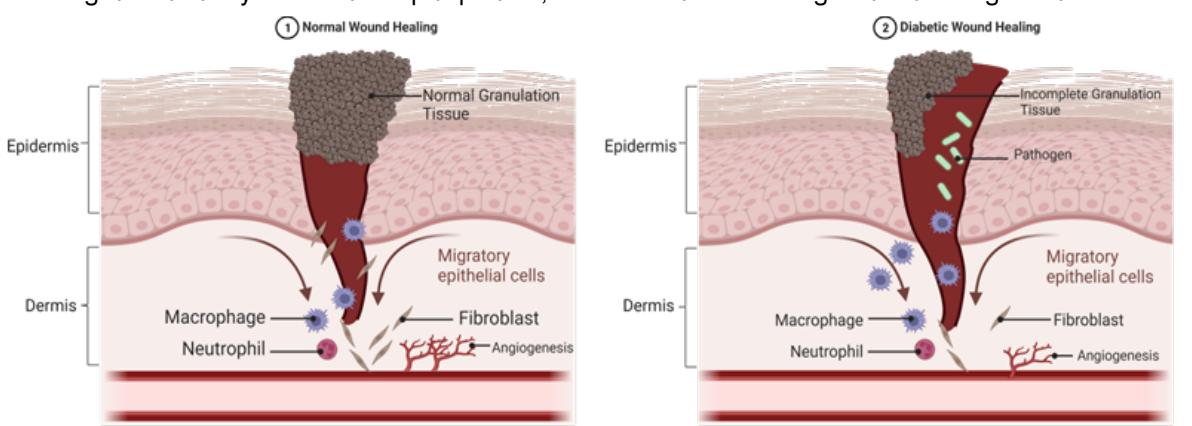

Figure 2. Normal Wound Healing and Diabetic Wound Healing Mechanism (29) Created with BioRender.com

Table 1. Advantages and disadvantages of different animal models in diabetic wound studies

Experimental animal models	Advantages	Disadvantages	References
Rats	Small Easy to handle Low mortality Strong endurance Cost-effective Pathophysiologically and pathologically similar to human Easy to breed	Thick fur, thin skin Not representing human skin Primary wound healing through contraction Genetically more difficult to trace than mice	2,9,10
Mice	Small Massively used Easy to handle Widely studied for the transgenic pathways Easy to breed	Thicker fur and thinner skin compared to rats Not representing human skin Primary wound healing through contraction Different genomic, immune, and inflammatory responses from those of post-injury in humans	2,11
Rabbits	Easy to handle More economical than pigs Pathologically and metabolically similar to humans Wider body than rats and mice Observation of wound healing can be made on the same body skin	Higher stress levels than rats and mice Easily infected compared to rats and mice Limited genetic tracing	2,12
Guinea pigs	Not as large as rabbits Easy to handle Relatively inexpensive Not producing Vitamin C; suitable for studies of the role of collagen in wound healing	Not commonly used Limited transgenic and strain studies Long gestation period	2,13
Pigs	Healing partial thickness wounds through re-epithelialization and granulation Large; enabling a greater number of wounds Similar skin, hair, and physiology to humans	High costs of handling Administratively more difficult for anesthesia Requiring good surgical skills Limited genetic tracing	14,15
Dogs	Similar to humans	High costs of handling Difficult ethics administration	16
Zebra fish	Small Inexpensive Lower order than other experimental animals Easy to breed Wound healing process through granulation and re-epithelialization similar to humans	Further studies required Limited validated reagents (cell lines and antibodies)	17,18

Rats as experimental animals in diabetic wound healing: Rats are widely used in research on acute and diabetic wounds due to their small size, ease of handling, cost-effectiveness, and pathophysiological similarities to humans.[9] However, there are differences in lifespan, size, and disease modeling. Wound healing in rats follows a similar process to humans, involving inflammation, proliferation, and remodeling. Although rat skin resembles human skin, it is thinner, with human skin being 2.97 mm thick compared to 2.09 mm in rats.[19] Studies on diabetic wound healing using rats are summarized in **Table 2**.

Table 2. Research on diabetic wound healing using rats

Experimental Animals	Test Compound	Induction of Diabetes	Test Result	References
Wistar Strain Albino Rats (Male and Female)	Luteolin and apigenin fractions of flavonoid compounds from *Martynia annua* Linn	Yes	Flavonoid and luteolin fractions (0.5%) had the potential for diabetic wound healing presumably through free radical scavenging activities	20
Wistar Strain Albino Female Rats	Polymeric Hydrogel of Poly Dimethylaminoethyl Acrylate and Hyaluronic Acid (pDMAEMA−HA) impregnated with *Didymocarpus pedicellatus* Plant Extract	Yes	Level of healing (pDMAEMA−HA) was higher than that of polymeric hybrid hydrogel on the market	21
Wistar Strain Albino Male Rats	Plumbagin	Yes	Plumbagin improved diabetic wound healing	22
Wistar Strain Albino Rats	70% ethanol extract of *Neolamarckia cadamba*	Yes	70% ethanol extract of *Neolamarckia cadamba* improved diabetic wound healing	23
Wistar Strain Male Rats	Luteolin	Yes	Luteolin promoted diabetic wound healing	24
SD Male Rats	Yeliangen	Yes	Yeliangen improved diabetic wound healing	25
Wistar Strain Male Rats	Topical cream of *Ginkgo biloba* extract	Yes	Improved wound closure in the group with topical cream of Ginkgo biloba extract	26

Mice as experimental animals in diabetic wound healing: Mice are frequently used in research on acute and diabetic wounds. Their flexible skin and panniculus carnosus lead to wound healing through contraction and re-epithelialization, similar to rats. Like humans, mice do not produce keloids or hypertrophic scars.[2,27] Using mice offers advantages such as ease of genetic tracing.[11] In addition, mouse skin, which is 0.70 mm thick, is thinner than that of rats and humans **(Table 3)**.[19]

Rabbits as experimental animals in diabetic wound healing: Rabbits, particularly the New Zealand white strain, are commonly used in research due to their ease of handling, cost-effectiveness, and similarities in pathology and metabolism to humans.[12] Their larger body size compared to mice and rats allows for better wound healing observations **(Table 4)**.[35] Rabbit skin is similar to human skin, and they are of particular interest due to similarities in wound healing patterns, such as reduced scar tissue in older animals, collagen synthesis, and increased scar tissue with epithelialization.[19,36]

Table 3. Research on diabetic wound healing using mice

Experimental animals	Test compound	Induction of diabetes	Test results	References
Swiss Strain Male Mice	Clopidogrel and *Adenosine Diphosphate* (ADP)	Yes	ADO improved $P2Y_{12}$ and $P2Y_1$ expressions at wound receptors. ADP reduced ROS.	28
Type-1 and Type-2 Diabetic Mice	Mineralocorticoid receptor antagonist (potassium canrenoate)	Yes	MR blockade accelerated wound healing in diabetic mice	29
Thermo-sensitive ICR Male Mice	Thermos-sensitive Hydrogel combined with Insulin Injection	Yes	The control of glucose levels using insulin injection was better than the use of hydrogel alone	30
Swiss Albino Male Mice	Ethanol Extract of *Annona reticulata* L Leaves	Yes	Ethanol Extract of *Annona reticulata* L Leaves stimulated the proliferation and migration of HDF, dermal fibroblasts, and keratinocytes	31
ICR Male Mice	Folic Acid	Yes	Folic acid supplement accelerated diabetic wound healing	32
Swiss Albino Adult Male Mice	Methanol Extract of *Catharanthus roseus* leaves	Yes	Methanol Extract of *Catharanthus roseus* leaves significantly improved the process of wound contraction	33
C57BL Mice	Ethanol extract of *Gynura procumbens*	Yes	The ethanol extract of *Gynura procumbens* accelerated diabetic wound healing	34

Table 4. Research on diabetic wound healing using rabbits

Experimental animals	Test Compound	Induction of diabetes	Test Result	References
New Zealand White Rabbits	Silkworm Silk Matrices coated with Functionalized Spider Silk	Yes	Bioactive silk matrices provided fast diabetes wound healing activities	12
New Zealand White Rabbits	Silk fibroin (SF) combined with polyvinyl alcohol (PVA) biopolymer	Yes	Non-mulberry silk fibroin (NMSF) had better diabetic wound healing than Bombyx mori SF, PVA, and the control	37
Rabbits	Nanosilver impregnated with chitosan-PEG hydrogel	Yes	Improved antibacterial, antioxidant, and diabetic wound healing activities	38
New Zealand White Rabbits	-	Yes	Non-diabetic sham and ischemic wounds healed significantly faster than diabetic sham and ischemic wounds	39

Guinea pigs as experimental animals in wound healing: Guinea pigs are widely used in preclinical research and wound healing studies due to their small size, ease of handling, low cost, and similarity to human skin. A notable feature of guinea pigs is their inability to produce vitamin C, making them valuable for studying collagen synthesis in wound healing[2,13] However, research on wound healing using guinea pigs is limited, and no studies on diabetes induction have been found (**Table 5)**.

Pigs as experimental animals in wound healing: Pigs are used in preclinical research, especially in wound healing studies, due to their skin's close resemblance to human skin, with similar re-epithelialization and contraction processes (**Table 6)**. Pigs offer greater anatomical, physiological, and metabolic similarity to humans, leading to more relevant results in some wound healing studies compared to rodents.[14] However, research using pigs is costly and handling is more challenging than with rodents. Pigs have a stratum corneum as thick as 26.4 μm, an epidermis of 65.8 μm, and an overall skin thickness of 3.43 mm.[19].

Dogs as experimental animals in wound healing: Dogs, as experimental animals, share similarities with humans, but their handling incurs high costs. Consequently, preclinical studies of wound healing with dogs are typically limited to non-diabetic wound healing (**Table 7)**.[16]

Zebrafish as experimental animals in wound healing studies: Zebrafish are small tropical freshwater fish native to South Asia.[48] Using zebrafish as an animal model in research offers several advantages: they produce embryos abundantly, breed quickly, are inexpensive and easy to handle, and possess genes that are similar to those of humans.[49,50] Their anatomical and physiological features, including main

Table 5. Research on wound healing using guinea pigs

Experimental animals	Test Compound	Induction of diabetes	Test Results	References
Guinea pigs	Kersen (*Muntingia calabura*) Leaf Extract	No	Kersen leaf extract had a significant effect on reducing erythema	40
Guinea pigs	96% Ethanol Extract of Cocoa Bean Gel	No	96% ethanol extract of cocoa bean gel improved angiogenesis in wound healing	41
Guinea pigs	Insulin Cream	No	Wound healing was faster when using long-acting insulin cream (Humulin N)	42
Guinea pigs	Ethanol Extract of *Binahong* (*Anredera cordifolia* (Ten.) *Steenis*) Leaves	No	*Binahong* leaf ethanol extract healed wounds better than povidone iodine	43

Table 6. Research on wound healing using pigs

Experimental animals	Test Compound	Induction of Diabetes	Test Results	References
Crossbred Piglets	Extract of Jatropha podagrica Hook. f., Chromolaena odorata (L.), and C asiatica (L.)	No	The extract significantly reduced erythema	44
Yorkshire pigs	Adipose-derived stem cells (ASCs)	Yes	Adipose-derived stem cells (ASCs) significantly improved wound closure	45
Yorkshire pigs	Adipose-derived stem cells (ASCs)	No	A high dose of adipose-derived stem cells (ASCs) accelerated the wound contraction process	46

Table 7. Research on wound healing using dogs

Experimental animals	Test compound	Induction of Diabetes	Test results	References
Mongrel dogs	Propolis	No	A positive effect on wound healing	47

Table 8. Research on wound healing using zebrafish as experimental animals

Experimental animals	Test Compound	Induction of diabetes	Test Results	References
Adult Zebrafish (Wild-type)	Ethanol Extract of Propolis	Yes	Ethanol extract of propolis at a dose of 15 ppm could increase the percentage of caudal fin regeneration and the expressions of shha, igf2a, bmp2b, and col1a2	18
Adult transgenic zebrafish (wild-type)	Warfarin sodium and hydrocortisone	No	Zebrafish were an important model for studies of vertebrate skin repair	17
Adult zebrafish	Induction by Streptozotocin	Yes	Hyperglycemic conditions interfered with the fin regeneration of adult zebrafish	51
Adult Zebrafish (Wild-type)	-	No	Amputation near the bifurcation impeded the formation of regenerated bifurcation.	52

metabolic organs, make them suitable for studies on diabetes, dyslipidemia, liver disease, and other conditions **(Table 8)**.[48]

Induction of Diabetes in Experimental animals

Induction of diabetes in experimental animals can be performed using several methods, including, first, by inducing experimental animals to experience autoimmune disease, commonly known as type-1 DM, and second, by causing experimental animals to have insulin resistance and pancreatic beta cell failure. The advantage of induction using chemicals is the ease of monitoring the process before and after induction of DM. There are several methods of chemical induction in experimental animals, as listed below.

Streptozotocin (STZ): STZ is a natural chemical used to create animal models of type-1 and type-2 diabetes. It is a monofunctional nitrosourea derivative isolated from Streptomyces achromogenes. STZ induces diabetes by entering pancreatic cells via glucose-GLUT 2, causing DNA alkylation and activation of poly ADP ribosylation and nitrate oxidation. This results in pancreatic beta cell necrosis and subsequently induces diabetes.[53]

Alloxan (ALN): ALN is a widely used chemical compound in diabetes research, particularly for inducing type-1 diabetes. It is a urea derivative that causes necrosis of pancreatic beta cells. ALN acts by rapidly increasing insulin secretion regardless of glucose levels, followed by complete suppression of the pancreatic islet response to glucose, even at high concentrations. Elevated cytosolic free Ca^{2+} is also a crucial factor in the diabetogenic action of alloxan.[53]

Dithizone (DTN): DTN is an organosulfur compound used to induce diabetes symptoms in experimental animals. It acts as a chelating agent, forming complexes with zinc, lead, and mercury. DTN induces diabetes through dithizonization, which increases serum levels of zinc, iron, and potassium while leaving copper and magnesium levels unchanged. DTN can penetrate membranes and form zinc complexes in liposomes, releasing protons that enhance diabetogenicity.[53]

Monosodium glutamate (MSG): MSG is used to induce type-2 diabetes in experimental animals, typically without causing polyphagia. As a natural, non-essential amino acid with good water solubility, MSG triggers a significant insulin response. Over time, this leads to glucosuria and elevated glucose levels, total cholesterol, and triglycerides in both male and female mice within 29 weeks.[53]

CONCLUSION

In vivo studies of diabetic wound healing require appropriate experimental animals and induction methods. No single animal model fully represents diabetic wound healing. Each model has unique advantages and disadvantages, so choosing the right one depends on the study's objectives and design to enhance the validity and relevance of findings for human diabetic wound healing.

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CONFLICT OF INTEREST
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.