

Review

Hydrogel Dressings as Insulin Delivery Systems for Diabetic Wounds

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Academic Editor: Suresh G. Joshi

Submitted: 5 September 2024 Revised: 29 October 2024 Accepted: 8 November 2024 Published: 24 February 2025

Abstract

Diabetic wounds are one of the most common and challenging complications of diabetes. Similar to chronic wounds, diabetic wounds are difficult to treat due to prolonged inflammation, a lack of angiogenesis, abnormal differentiation of new scar tissue, and the occurrence of numerous bacterial infections. Moreover, elevated sugar levels in tissues disrupt the healing process by enhancing inflammatory reactions, disrupting signaling pathways, and leading to the production of abnormal biological structures, which contribute to improper cell differentiation. Traditional dressings, such as bandages, gauze, and semi-occlusive foams, are inadequate for diabetic wounds with high exudation; moreover, frequently changing the dressing can cause secondary irritation. Hence, innovative hydrogel dressings are being developed, which, thanks to their soft polymer matrix, provide an ideal substrate for regenerating tissue. Hydrogels also allow for the introduction and controlled release of growth factors, making them a promising solution for treating diabetic wounds. Recently, researchers have focused on insulin, a hormone secreted by the human body to lower blood sugar levels, due to its interesting characteristics, such as supporting anti-inflammatory and proangiogenic processes and stimulating cell migration and proper proliferation. This review discusses the most important aspects of diabetes and diabetic wounds and traditional and innovative treatment methods, particularly hydrogel dressings used as systems for insulin delivery in response to glucose concentration.

Keywords: diabetic wounds; insulin; hydrogel dressings; stimuli-controlled release; drug delivery systems

1. Introduction

Diabetes mellitus is defined as a complex metabolic disorder characterized by the presence of hyperglycemia in the body, resulting from abnormalities in insulin secretion and action. Hyperglycemia occurs due to insufficient insulin production or an abnormal cellular response to insulin, leading to a chronic disorder of carbohydrate, fat, and protein metabolism. Long-term hyperglycemia is associated with the development of diabetes and can result in serious health complications, such as damage to the nervous system and dysfunction of the eyes and kidneys [1]. Currently, diabetes is classified into several types, with type 1 diabetes (T1DM) and type 2 diabetes (T2DM) being the most commonly diagnosed. Type 1 diabetes is primarily diagnosed in young individuals and accounts for approximately 5–10% of diagnosed diabetes cases [2]. It is characterized by the autoimmune destruction of β -cells in the islets of Langerhans, which are responsible for insulin secretion. Consequently, insulin deficiency arises, necessitating external insulin supply, hence type I diabetes, also called insulin-dependent diabetes. In contrast, type 2 diabetes, also referred to as non-insulin-dependent diabetes, primarily occurs in adults and the elderly. Its development is influenced by genetic factors as well as lifestyle choices, such as an unhealthy diet, lack of physical activity, smoking, and excessive alcohol consumption. As a result, the pancreas must produce more insulin to maintain normal blood glucose lev-

els. The body's inability to meet the demand for insulin in type 2 diabetes accounts for approximately 90–95% of diagnosed diabetes cases [3]. Both type 1 and type 2 diabetes are common forms of the disease that can lead to serious complications, including the development of diabetic foot syndrome [4]. The differences in the pathogenesis of type 1 and type 2 diabetes are illustrated in Fig. 1.

Diabetic Foot Syndrome is a condition that affects individuals with diabetes, characterized by ulcers and wounds on the lower limbs [5]. These ulcers result from repeated compressive and shear stresses on the foot, typically occurring in patients with diagnosed peripheral neuropathy. Factors that increase the likelihood of a wound include the presence of foot deformities, the application of abnormal loads during walking, joint mobility disorders, and the occurrence of minor injuries. In diabetic patients, persistent hyperglycemia compromises overall immunity and prolongs inflammation in wounds, as well as the wound healing process. Coexisting ischemia and infections are significant impediments to wound healing in these patients. The disease's progression is linked to the development of peripheral artery diseases and osteomyelitis; in severe cases, complications can lead to tissue necrosis and the need for lower limb amputation [6]. Among patients with advanced diabetic wounds, 17% require amputation of a small part of the limb, while 5% require a major amputation within one year of diagnosis [7]. In the most severe cases, amputation is performed. The risk of death within five years is



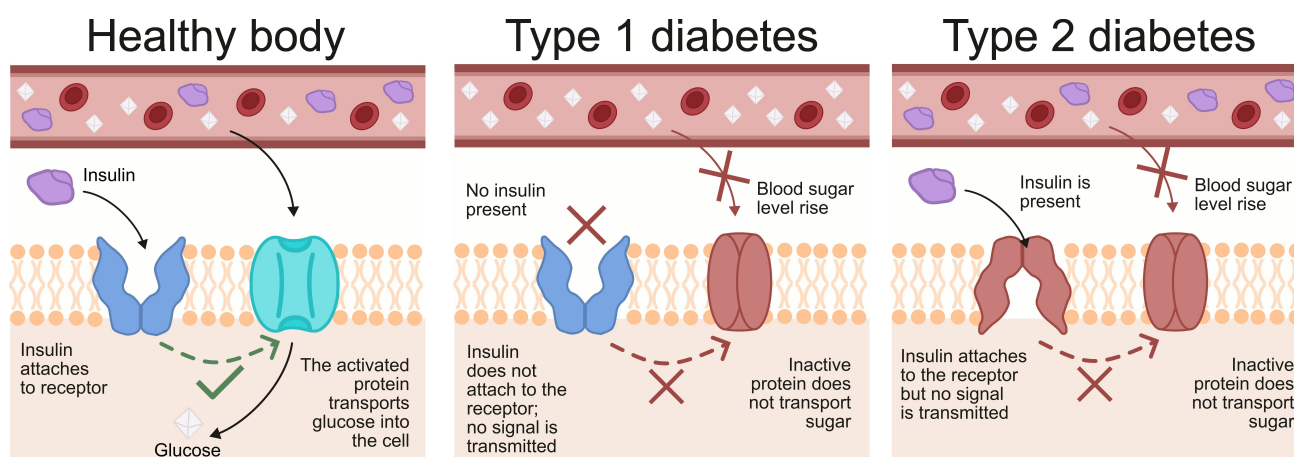


Fig. 1. Basic differences between healthy organism, type I, and type II diabetes. In a healthy individual, the body produces insulin efficiently, and cells respond to its presence, allowing blood glucose levels to be maintained within a normal range. Type 1 diabetes is an autoimmune condition resulting in little to no insulin production, while in type 2 diabetes, which is predominantly linked to lifestyle and genetic factors, insulin is produced but the body cells develop resistance, not responding effectively to its presence; in both cases blood sugar levels are too high. The image was created with Inkscape v1.4, retrieved from <https://inkscape.org>.

2.5 times higher for individuals with diabetic foot ulcers (DFUs) than for those with diabetes alone, and the five-year mortality rate following diabetes-related amputations exceeds 70%, which is worse than in many common cancers [8]. Given these considerations, there is a critical need to develop an integrated system that combines the use of wound dressings, bioactive substances, and antibiotics, as well as the to implement changes in treatment strategies concerning wound dressing design and quantitative diagnostics, to enhance patient treatment and comfort [9].

Due to the rapid development of nanobiotechnology in regenerative medicine, this article aims to present current trends in the pursuit of improved solutions for future researchers. This work provides a concise overview of the challenges associated with treating diabetic wounds, taking into account the primary pathophysiological factors that influence the progression of this disease, such as prolonged inflammation, local hyperglycemia, the presence of reactive oxygen species, insufficient angiogenesis, and bacterial infections. The article discusses currently developed methods for treating these wounds, which utilize hydrogel matrix dressings containing polymer micro- and nanocarriers with insulin that acts as a growth factor. Recent research on micro- and nanoparticles made from both natural and synthetic polymers incorporated into hydrogel matrices is analyzed, highlighting the potential of this system for the therapy of diabetic wounds. The methods for synthesizing these carriers and the results of *in vitro* and *in vivo* tests published in selected articles over the last ten years are briefly summarized.

2. Pathophysiology of Diabetic Wounds

In the case of normal skin wound healing, this process consists of a series of complex and orderly events that can

be divided into four main phases: hemostasis, inflammation, proliferation, and remodelling. During the hemostasis phase, coagulation factors promote clotting, which reduces bleeding and provides mechanical support for the damaged tissue. Subsequently, in the inflammatory phase, inflammatory cells—such as lymphocytes, monocytes, and macrophages—eliminate damaged tissue and pathogens. In the proliferation phase that follows, epithelial cells and fibroblasts fill the space left by the lost tissue, leading to a gradual decrease in the size of the open wound. Once the wound bed is filled with granulation tissue, it undergoes remodeling in the scarring phase, transforming into connective tissue that forms a supportive layer for the newly formed epithelium [10]. In summary, diabetic wounds exhibit deregulated angiogenesis, a chronically sustained suboptimal inflammatory response, increased levels of reactive oxygen species, and persistent bacterial colonization that often develops into a difficult-to-treat biofilm [11].

2.1 Prolonged Inflammation

Diabetic wound healing is disrupted by several factors. Diabetes impairs the inflammatory, proliferative, and scarring phases, negatively affecting wound healing and resulting in scarring. In diabetic wound healing, the inflammatory phase is prolonged due to the failure of the transition between M1 and M2 macrophages, causing an abnormal accumulation of inflammatory cells in the wound and an exaggerated inflammatory response. The excessive number of inflammatory cells increases the levels of proteases, which damage the extracellular matrix (ECM) and degrade growth factors and their receptors. This proteolytic damage to the ECM attracts even more inflammatory cells, leading to increased production of reactive oxygen species in the hypoxic conditions present in the wound. Addition-

ally, reactive oxygen species can activate proteases and inhibit their inhibitors, resulting in increased proteolysis and further damage to the ECM, ultimately causing significant cellular damage.

In addition, similar to chronic wounds, diabetic wounds are particularly susceptible to re-injury. The constant reopening of these wounds result in an excessive accumulation of neutrophils and phagocytes around the damaged tissue. This leads to the extension of what is known as NETosis, a physiological response in the body aimed at producing neutrophil extracellular traps (NETs) capable of trapping pathogens and cleansing the wound through their engulfment by neutrophils. However, an imbalanced response in the case of NETosis within the wound can also cause direct damage to the epithelium and endothelium, thereby delaying the wound healing process [12].

2.2 The Impact of High Sugar Levels

In the case of diabetic wounds, elevated blood glucose levels adversely affect the regulation of growth factors, subsequently impairing cell proliferation and differentiation. This results in phenotypic abnormalities within the structures of the dermis and epidermis. Fibroblasts in diabetic wounds exhibit resistance to proliferation, and this, coupled with their accelerated aging process, contributes to poor wound healing. High glucose levels promote accelerated cell growth and the accumulation of oxidized protein forms in fibroblasts, which ultimately leads to skin atrophy and dermatosis due to the accumulation of advanced glycation end products (AGE) [13].

AGEs are a group of compounds formed under conditions of excessively high blood glucose levels, resulting from the condensation reaction between glucose and proteins or from the action of reducing sugars on lipids and nucleic acids [14]. AGEs are responsible for the glycosylation of collagen and the formation of cross-linked, difficult-to-degrade protein products, which ultimately leads to delayed angiogenesis and tissue remodeling. Aging processes reduce cell proliferation and blood flow in tissues, contributing to the degradation of extracellular matrix components and cell apoptosis [15].

2.3 The Influence of Reactive Oxygen Species (ROS) and the Lack of Angiogenesis

In patients with diabetic wounds, a low density of blood vessels results in hypoxia and nutritional deficiency at the wound site. Moreover, a significant reduction in the density of growth factor receptors on the wound hinders their normal regulatory function, slowing the rate of skin regeneration [16]. The insufficient angiogenesis observed in diabetic wounds is due to the elevated concentrations of antiangiogenic factors present in the circulatory system of patients with diabetic foot ulcers. These factors weaken proangiogenic stimuli and induce changes in capillary maturation factors, leading to a slower maturation of these ves-

sels in the reconstructed tissues. Additionally, the diabetic condition causes a deficit in the number of endothelial progenitor cells (EPCs) derived from the bone marrow, further contributing to reduced tissue vascularization of the tissue. In diabetic patients, EPCs show a reduced ability to produce functional angiogenic structures in ischemia models, which adversely affects the formation of granulation tissue, capillary growth, and collagen deposition in the wound [17].

2.4 Bacterial Infections

Diabetic wounds are also particularly prone to bacterial colonization due to elevated glucose levels in the blood, which serves as an energy source for bacterial growth and reproduction. Pathogenic bacteria adhere to the wound surface and subsequently form multicellular aggregates, leading to biofilm formation. The presence of a biofilm in a wound significantly reduces the effectiveness of antimicrobial agents due to several factors: their inability to penetrate the biofilm matrix, the lack of intracellular accumulation of antibiotics, the presence of metabolically inactive bacteria, and the development of antibiotic resistance. Furthermore, biofilms increase the likelihood of transferring virulence and antibiotic-resistant genes between bacteria, and the rate of genetic mutations in biofilms is higher than in free-living cells. Additionally, bacterial cells within biofilms can develop an intracellular communication mechanism known as quorum sensing (QS), which helps protect them from immune cells attack and antibiotic action. Cells from the biofilm can detach and spread throughout the wound, forming multimicrobial biofilms that facilitate mutual growth. In the context of diabetic foot disease, the best-studied interaction is between *Staphylococcus aureus* and *Pseudomonas aeruginosa*, where substances produced by *P. aeruginosa* protect *S. aureus*, complicating the treatment of infections caused by these bacteria [18]. An interaction between *P. aeruginosa* and *S. aureus* contributes to increased antibiotic tolerance, the ability to form biofilms, and the secretion of virulence factors. In the case of *P. aeruginosa*, these factors include hydrogen cyanide, exoenzyme S, exotoxin A, and pyocyanin, while for *S. aureus*, they include Pantón-Valentine leukocidin (PVL) and α -hemolysin [19]. Alginate, an exopolysaccharide produced by *P. aeruginosa*, protects *S. aureus* by suppressing the expression of *P. aeruginosa* virulence genes, which, under other competitive conditions between these species, typically inhibit the development of *S. aureus* [20]. This protection results from a reduction in the transcription level of the *pvdA* gene, responsible for the production of pyoverdine, an iron-scavenging siderophore, as well as a decrease in the level of pseudomonas quinolone signal, 2-heptyl-3,4-dihydroxyquinoline (PQS), which is involved in quorum sensing [21]. A summary of the most important factors determining the prolonged healing of diabetic wounds [22] is presented in Fig. 2.

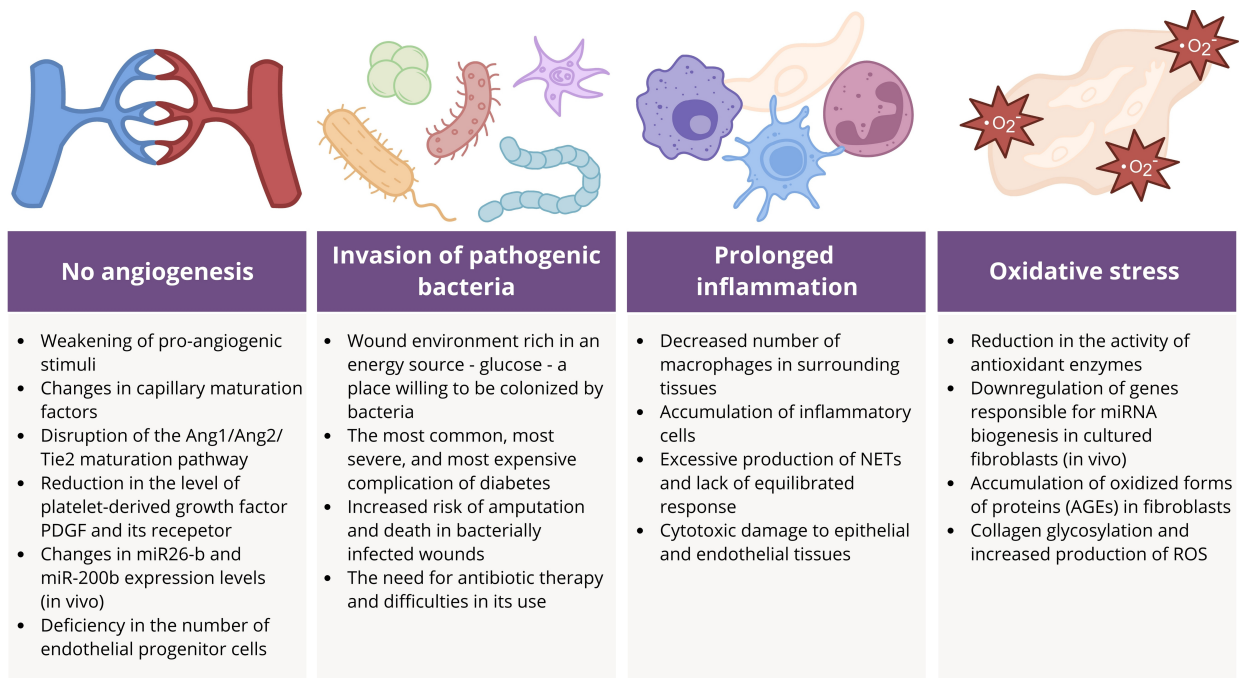


Fig. 2. A summary of the most important factors determining the prolonged healing of diabetic wounds. Under diabetic foot ulcer (DFU) conditions, there is an unbalanced accumulation of immune cells, as well as an increase in the M1/M2 macrophage ratio, reactive oxygen species (ROS), and pro-inflammatory cytokines. Furthermore, reepithelialization and angiogenesis occur rarely, unlike in bacterial infections, ultimately leading to chronic, non-healing wounds that remain in a state of low inflammation: angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), tyrosine kinase (Tie2), micro-ribonucleid acids (miRNA: miR26-b and miR-200b), neutrophil extracellular traps (NETs). The image was created with Inkscape v1.4, retrieved from <https://inkscape.org>.

3. Traditional and Innovative Methods of Dressing and Treating Diabetic Wounds

3.1 Traditional Dressings

Dressings are selected based on various wound characteristics, including type, depth, location, extent, amount of exudation, presence of infection, and tissue adhesion. Standard dressings such as gauze, fibers, plasters, bandages, and cotton wool have been used for years on wounds with either normal or abnormal healing processes. Gauze and bandages, typically applied to dry and well-cleaned wounds, are primarily used for mechanical tissue protection. Gauze dressings absorb secretions from open wounds, while natural cotton bandages are mainly used to treat dry venous and arterial ulcers. However, wounds with a large amount of exudate necessitate frequent changes, which can result in discomfort and pain for the patient, as well as additional irritation of the wound [23,24].

In the treatment of diabetic wounds, semipermeable porous polyurethane dressings are often utilized, as they allow gases and water vapor to pass through while blocking fluids and bacteria. Their thinness, flexibility, and ease of conformation make them ideal for epithelial and superficial wounds with low exudate, as well as for lacerated or shallow wounds. However, due to their lack of absorbency, they are not suitable for wounds with high exudation, as this can lead to excessive secretion and maceration of the wound

edges, necessitating frequent dressing changes, sometimes several times a week [24,25]. Alternatively, foam dressings are used, which consist of a polyurethane or silicone core surrounded by a semi-occlusive outer layer. Foams can remain on the wound for several days without risking tissue maceration. The hydrophobic outer layer protects against fluids and bacteria while facilitating gas exchange. The foam structure allows it to conform to the shape of the wound, and its absorbent and autolytic properties make it appropriate for moderately to heavily exuding wounds, leg ulcers, and granulating wounds [23,24]. Another type of dressing is hydrocolloids, which comprises an inner layer of hydrophilic colloidal particles and an outer layer of polyurethane. Hydrocolloids prevent the penetration of water and bacteria but they are impermeable to oxygen, which limits their use in deeper, heavily exuding, or infected wounds. Additionally, they are less transparent, making it challenging to inspect the wound, and the gel formed during use can be mistaken for an infection. Hydrocolloids are primarily used in the treatment of superficial wounds, postoperative wounds, and shallow pressure ulcers [26].

3.2 Hydrogels as Intelligent Dressings

Dressings are selected based on various wound characteristics, such as type, depth, location, extent, amount of

exudation, presence of infection, and tissue adhesion. Although traditional dressings fulfil basic functions, they do not provide adequate wound drainage. As a result, they are being replaced by modern solutions that actively support the healing process by accelerating the formation of granulation tissue and facilitating the migration of epithelial cells from the edges of the wound to its center [24,27].

The most fundamental aspect that must be achieved by a hydrogel in its application as a medical device is biocompatibility. Biocompatibility is defined as the ability of a biomaterial to perform specific functions while eliciting an acceptable tissue response from the body. For a biomaterial to be considered biocompatible, it must meet the criteria specified in the International Standards Organization (ISO) 10993 standard. These requirements include not only the lack of cytotoxicity but also the exclusion of potential carcinogenic and mutagenic properties of the material. Moreover, the biomaterial should not cause allergic reactions, and in the case of materials that come into contact with blood, it is essential to ensure their athrombogenicity [28]. Additionally, in the design and production of products made from biomaterials, it is necessary to consider all aspects that determine their suitability for a specific purpose and function. For hydrogel materials, these aspects primarily include chemical, physical, mechanical, and rheological properties, as well as those related to morphology and biological activity [29].

The ideal wound dressing system should maintain high humidity, which provides nutrients and protection while facilitating cell growth and migration. Moist treatment prevents scar formation, allowing epithelial cells to close the wound more quickly. The dressing should be easy to remove without irritating the wound or causing pain; it should offer mechanical protection, proper gas and vapor exchange, and be non-toxic and non-allergenic. Additionally, it should be impermeable to bacteria and support tissue regeneration, enabling the delivery of therapeutic substances. Furthermore, it should be affordable, easy to produce, and readily available. All of these features can be achieved by using carefully designed hydrogel dressings [30,31].

Hydrogel dressings are highly regarded for their superior properties compared to traditional bandages and gauze, which do not maintain a moist environment and can cause irritation to wounds. Due to their elasticity and softness, hydrogels easily adapt to soft tissues and are particularly effective in treating deep wounds with irregular shapes. They provide a moist environment, absorb exudate, and support tissue homeostasis. Their three-dimensional, porous structure mimics the natural extracellular matrix, promoting fibroblast proliferation, epithelialization, neovascularization, and new tissue remodeling. Hydrogels can be modified through the addition of antibacterial, antiviral, and antifungal agents, growth factors, biomolecules, or cells. Currently, functional hydrogel dressings are being devel-

oped with antimicrobial, anti-inflammatory, and antioxidant properties, as well as the capability for controlled delivery of substances in response to stimuli [32,33].

4. The Potential of Using Insulin to Treat Diabetic Wounds

4.1 The Influence of Insulin as a Bioactive Factor in the Wound Healing Process

Insulin is a peptide hormone composed of 51 amino acids, with a total mass of 5808 Da. The synthesis of insulin is carried out by the β cells of the pancreas, located in the islets of Langerhans. The human pancreas contains one to two million islets, which house various endocrine cells—primarily β cells that secrete insulin, α cells that produce glucagon, and δ cells that secrete somatostatin. These islets constitute only 1–2% of the surface area of the human pancreas, yet they receive 10% of the total blood supply of the pancreas. This abundant blood flow enables the high rate of insulin secretion, which is activated in response to appropriate concentrations of glucose in the blood. Consequently, insulin plays a crucial role in regulating the body's energy supply by balancing micronutrients during a meal. Its main task is to transport glucose into insulin-dependent cells and tissues, such as the liver, muscle, and adipose tissue. In addition to its primary role in regulating blood glucose levels, insulin also possesses anti-inflammatory properties and supports tissue regeneration [34,35].

The general anti-inflammatory properties of insulin are derived from metabolic synthesis pathways that regulate cell proliferation, survival, migration, anti-inflammatory activity, and angiogenesis. Insulin receptors—specifically insulin receptor substrates (IRS-1 and IRS-2), extracellular signal-regulated kinase (ERK), and protein kinase B (Akt)—located in keratinocytes and epidermal fibroblasts, are upregulated in wounded skin compared to intact skin, indicating a potential role of the insulin signaling pathway in wound healing. The effects of insulin on wound healing include an increased expression of laminin (α 3 β 1) integrin receptors in keratinocytes and increased levels of Laminin 332 (LN332). Local administration of insulin to skin wounds has been shown to stimulate the migration and proliferation of fibroblasts and endothelial cells, as well as the production of extracellular matrix proteins. Additionally, insulin supports keratinocyte migration in a dose-dependent manner by activating the nuclear transcription factor kappa B (NF- κ B) and promotes wound closure itself by activating ERK1/2 and PI3K signaling pathways. The presence of insulin has a beneficial effect on the regulation of oxidative and inflammatory reactions, resulting in advanced infiltration and degradation of macrophages. Moreover, insulin increases collagen deposition in regenerating tissues. In areas where insulin was used, an increased presence of chemokines such as interleukins (IL-4, IL-13, and IL-10) was observed, along with a simultaneous decrease in the production of interferon γ (IFN- γ), further indicat-

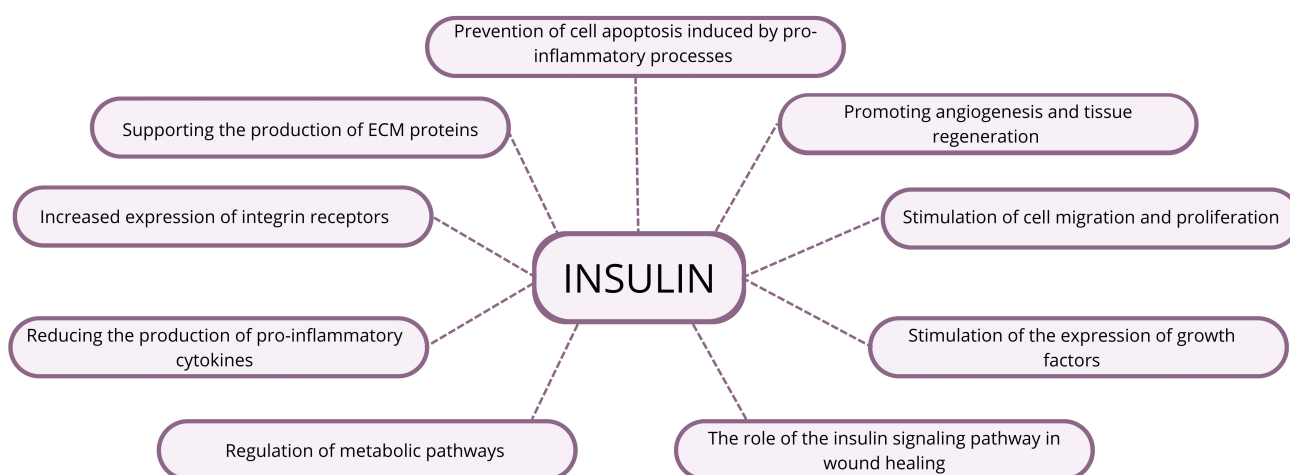


Fig. 3. The effect of insulin in wound treatment. There are many ways in which insulin affects the treatment of diabetic wounds from supporting Extracellular Matrix (ECM) protein production, through regulation of metabolic pathways and stimulation of cell migration and proliferation, to promoting angiogenesis and tissue regeneration. The image was created with Inkscape v1.4, retrieved from <https://inkscape.org>.

ing the anti-inflammatory effect of insulin. Additionally, insulin affects the inactivation of tumor necrosis factor α (TNF- α), which mediates the inflammatory pathway during fat metabolism. Insulin also prevents cell apoptosis induced by inflammatory processes and promotes angiogenesis by stimulating the expression of vascular endothelial growth factor (VEGF), as signaled by Akt. It also influences the maturation of developing blood vessels by restoring impaired signaling pathways, including the PI3K/Akt and mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) metabolic pathways, and by increasing the expression of vascular endothelial growth factor (VEGF) and angiopoietin-1 [36]. A summary of the above information is summarized in Fig. 3.

4.2 The Problem of Delivering Insulin to Wounds

The positive effect of insulin on the processes occurring during the inflammatory and proliferative phases has been recognized by researchers looking for its potential use in the treatment of chronic wounds. However, the local administration of insulin in an aggressive wound environment is a major challenge due to the presence of degrading proteases, the need to control or self-regulate insulin delivery, the need to overcome natural barriers and the selection of the appropriate formulation. The main issue in delivering insulin to diabetic wounds is the impact of proteases present in the wound, which perform insulin proteolysis upon contact, disturbing the tertiary conformation of the protein and leading to a loss of its bioactivity. The primary requirements and difficulties associated with insulin delivery to diabetic wounds are summarized in Fig. 4. Therefore, methods that protect against the proteolytic degradation of insulin in tissues are highly desirable. Currently developed solutions in the form of injectable hydrogel systems and the

use of micro- and nanocarriers provide opportunities for the effective delivery of insulin to diabetic wounds [37].

In chronic wounds, there is increased activity of proteolytic enzymes such as matrix metalloproteinases (MMPs) and serine proteases, which degrade the extracellular matrix and destroy growth factors and their receptors. This, in turn, contributes to a limited supply of growth factors in the wound area, resulting in impaired healing. In the context of diabetic wounds, the proteolytic enzyme that exhibits increased activity is the insulin-degrading enzyme (IDE), which leads to a local deficiency of normally occurring insulin in the tissues, thereby delaying tissue healing. Conversely, in wounds that are undergoing the healing process, IDE activity decreases as healing progresses, similar to the changes in the levels of proinflammatory cytokines. Nevertheless, the presence of insulin-degrading proteases is the primary reason for the necessity of protecting insulin from biodegradation during local delivery [38].

An equally important aspect related to the delivery of insulin to diabetic wounds is its controlled release. A too rapid release of insulin into the wounds may lead to the development of local hypoglycemia, and once it penetrates into the muscles and circulatory system, it can disrupt glucose homeostasis throughout the body. To counteract this, systems with prolonged insulin secretion and insulin release in response to stimuli are employed. To overcome the above limitations, several drug delivery systems have been developed to control the pharmacokinetic profiles of various analogs. Therapeutic efficacy depends on many factors related to pharmacokinetics, distribution, cellular uptake, metabolism, excretion, and toxicity, all of which are influenced by the method and route of administration. The main goals of developing these systems are to improve the stability of therapeutic substances *in vivo*, increase the control

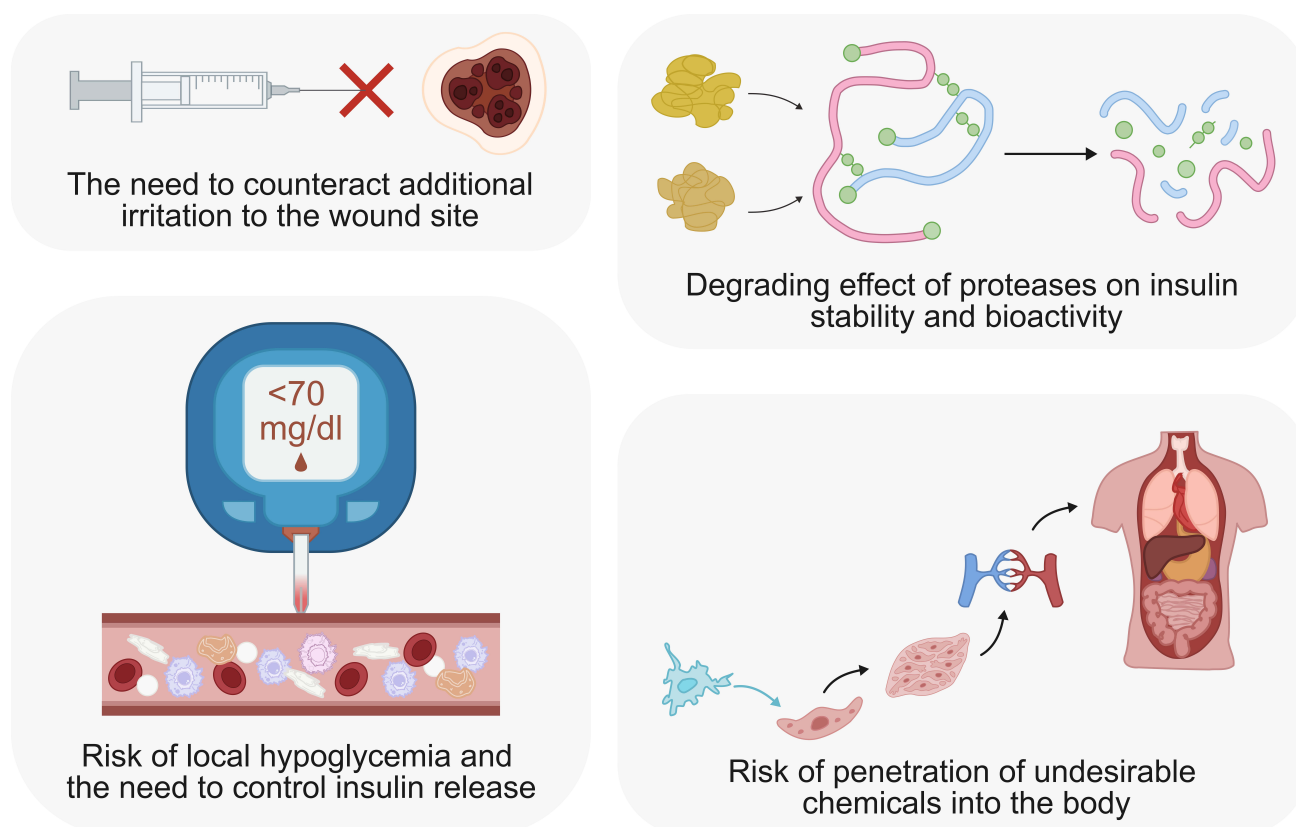


Fig. 4. The most important requirements and difficulties in delivering insulin to diabetic wounds. Insulin delivery to the diabetic wound site should be realized as non-invasively as possible, keeping in mind that insulin levels and release rate should be tightly controlled, along with assurance of its stability and bioactivity. The image was created with Inkscape v1.4, retrieved from <https://inkscape.org>.

of their release in response to stimuli, and direct the action of drugs to a specific site [39,40]. In a clinical study reported by Z. Zhang *et al.* [41], it was demonstrated that in most individuals with developing diabetic foot syndrome, the condition of the wound improved following insulin injection into the wound. However, among the 32 participants studied, characteristic symptoms of hypoglycemia were observed in two cases, which included palpitations, dizziness, pallor, and cold sweats. Additionally, the study indicate that local injection of insulin into the wound can both reduce systemic blood glucose levels and enhance wound closure, shorten healing time, and improve wound remodeling by modulating inflammation, accelerating epithelialization, and promoting neovascularization [41]. In other clinical study where insulin was formulated in solutions, sprays, creams, or dressings, no adverse systemic effects (such as hypoglycemia, hypokalemia, or hypoaminoacidemia) or local adverse effects (including infection, pain, or allergenicity) were noted. Considering the aforementioned factors, it is essential to develop a continuous delivery system for this hormone to minimize the risk of local hypoglycemia and enhance patient comfort, thereby eliminating the need for injections [42].

At the same time, taking biomechanical aspects into account, injectable systems are being developed to counteract additional irritation at the site and address the need to treat very complex and deep wounds. These systems utilize materials that consist mainly of biodegradable and biocompatible soft polymers, which are available in the form of hydrogels, microspheres, microgels, and gels cross-linking *in situ* [43]. The use of injectable materials in the form of dressings, which also enable self-regulating insulin delivery, allows for the treatment of very deep tissue defects. Injectable dressings perfectly conform to the edges of these defects, fill them, are minimally invasive, reduce the risk of irritation, and minimize the risk of hypoglycemia [44].

4.3 Micro- and Nanocarriers as Insulin Delivery Systems

When administering insulin via injection, oral methods, or transdermal means, it is essential to protect this peptide from enzymatic degradation. Delivering insulin in its unchanged form is undesirable due to its low bioavailability, which results from rapid hormone degradation caused by enzymes. This necessitates the use of higher doses, consequently increasing the risk of local hypoglycemia. To mitigate this risk, micro- and nanocarriers are developed to safeguard insulin against degradation, facilitate the use of

smaller doses, and ensure controlled, gradual release to the target site, thus eliminating the burst effect [45]. In comparison to traditional insulin delivery systems, encapsulating insulin in organic or inorganic nano- and micro-delivery vehicles can significantly enhance its pharmacological properties, such as solubility, blood half-life, and toxicity, leading to safer and more effective therapy [46].

Such carriers can be designed to increase therapeutic efficacy and minimize side effects, protect against premature degradation and denaturation upon contact with the physiological environment, enhance penetration through physiological barriers like the skin, and incorporate stimuli-responsive elements that enable controlled substance release on demand [46]. Additionally, nanoparticle carriers may offer further benefits, such as enhancing drug stability and reducing skin irritation that can occur with certain medications. Nanoparticles effectively shield peptides from the action of skin peptidases, directing their delivery to the skin or within it [47]. This capability is attributed to the unique properties of nanomaterials, including a high surface-to-volume ratio and specific shape factors resulting from their small size compared to bulk material [45].

Liposomal and polymeric micro- and nanocarriers made from biocompatible, bioavailable, and biodegradable components have demonstrated improved therapeutic outcomes compared to traditional methods. Furthermore, the process of both covalent and non-covalent attachment of polyethylene glycol polymer chains to molecule (PEGylation) of these carriers enhances drug solubility, reduces immunogenicity, decreases dosing frequency, and prolongs serum half-life. Therefore, PEGylation is considered one of the key strategies in local insulin delivery [45]. Currently, systems utilizing micro- and nanocarriers embedded in hydrogel matrices are being developed as dressings for diabetic wounds. Due to their unique microstructure and high water content, these carriers better mimic the surrounding soft tissues and support their regeneration compared to conventional dressings, such as hydrocolloid patches or semi-permeable polyurethane foam dressings [24,25].

Such systems have been developed by numerous teams, including scientists from the group of H. Wang *et al.* [48], who created a microgel containing chitosan microspheres with insulin. The preparation process involved transferring a solution of chitosan and acetic acid along insulin through a glass membrane into an emulsion of paraffin and ether with the emulsifier. Cross-linking of the emulsion and the formation of the microgel was achieved by adding tripolyphosphate, while the hardening of the chitosan droplets was conducted using glutaraldehyde. *In vitro* study demonstrated that the solidification state of the microgel could be adjusted based on the encapsulation efficiency, the shape of the microspheres, the activity of the drug, and the insulin release profile.

Another example is the system of poly(lactide-co-glycolide) nanoparticles loaded with insulin and suspended

in a structured hydrogel of poly(vinyl alcohol)-borate, obtained by the group of Abdelkader [49]. This material effectively contributed to the improvement of the wound healing process. Similar effects were demonstrated by the complex of chitosan nanoparticles with insulin.

In another study, J. Zhu *et al.* [50] developed gels based on oxidized hyaluronic acid and succinyl chitosan, integrating them with insulin-containing micelles. This system demonstrated the ability to stimulate inflammatory cell proliferation, angiogenesis, and wound maturation. Additionally, it was shown that the combination of both types of polymers using the applied procedure led to the formation of a hydrogel with improved mechanical properties. Scientists from Lee's team [51] also obtained an injectable form of hydrogels, developing a material based on trehalose that ultimately protected the encapsulated insulin against thermal degradation. The polymer used in this study, containing trehalose side chains with poly(ethylene glycol), was functionalized with phenylboronic acid (PBA) [51]. This resulted in a hydrogel that cross-links after injection and responds to glucose concentration, releasing insulin in its presence. Moreover, thanks to trehalose, the hydrogel provided excellent protection against heat stress for insulin, which opens up the potential use of this material without the need for cooling.

In a recent study from 2024, F.C. Zanchetta and the team [52] recognized the potential of insulin-polysaccharide formulations in biohybrid hydrogel systems for regulating oxidative stress and inflammatory responses. They developed a hydrogel-based system containing insulin, chitosan, and hydroxypropylmethylcellulose (Chi/HPMC/Ins), which synergistically combined the bioactivity of the protein with the biocompatibility and hydrogel properties of polysaccharides. The resulting biomaterial exhibited no cytotoxicity, promoted faster gap closure in the scratch test, and in *in vivo* tests demonstrated that wounds treated with Chi/HPMC/Ins hydrogel led to faster wound closure, formation of more organized granulation tissue, and hair follicle regeneration.

In another study, C-H. Lee's team [53] developed absorbable and biocompatible PLGA-based core-shell nanofibrous membranes that sustainably released insulin/vildagliptin for two weeks and at least four weeks, respectively. The functional and sustained delivery of vildagliptin increased the migration rate of endothelial progenitor cells (EPCs) and improved the healing of diabetic wounds. The obtained nanofibrous scaffolds increased epidermal thickness, promoted wound closure, and reduced the expression of α -SMA. The researchers concluded that the PLGA core-shell electrospun insulin/vildagliptin film is biomechanically and biologically effective in promoting diabetic wound healing while reducing fibrosis effects, which is beneficial for restoring cell function and granulation.

In a study conducted by X. Gao's team [54], Michael addition was used to internally cross-link bioac-

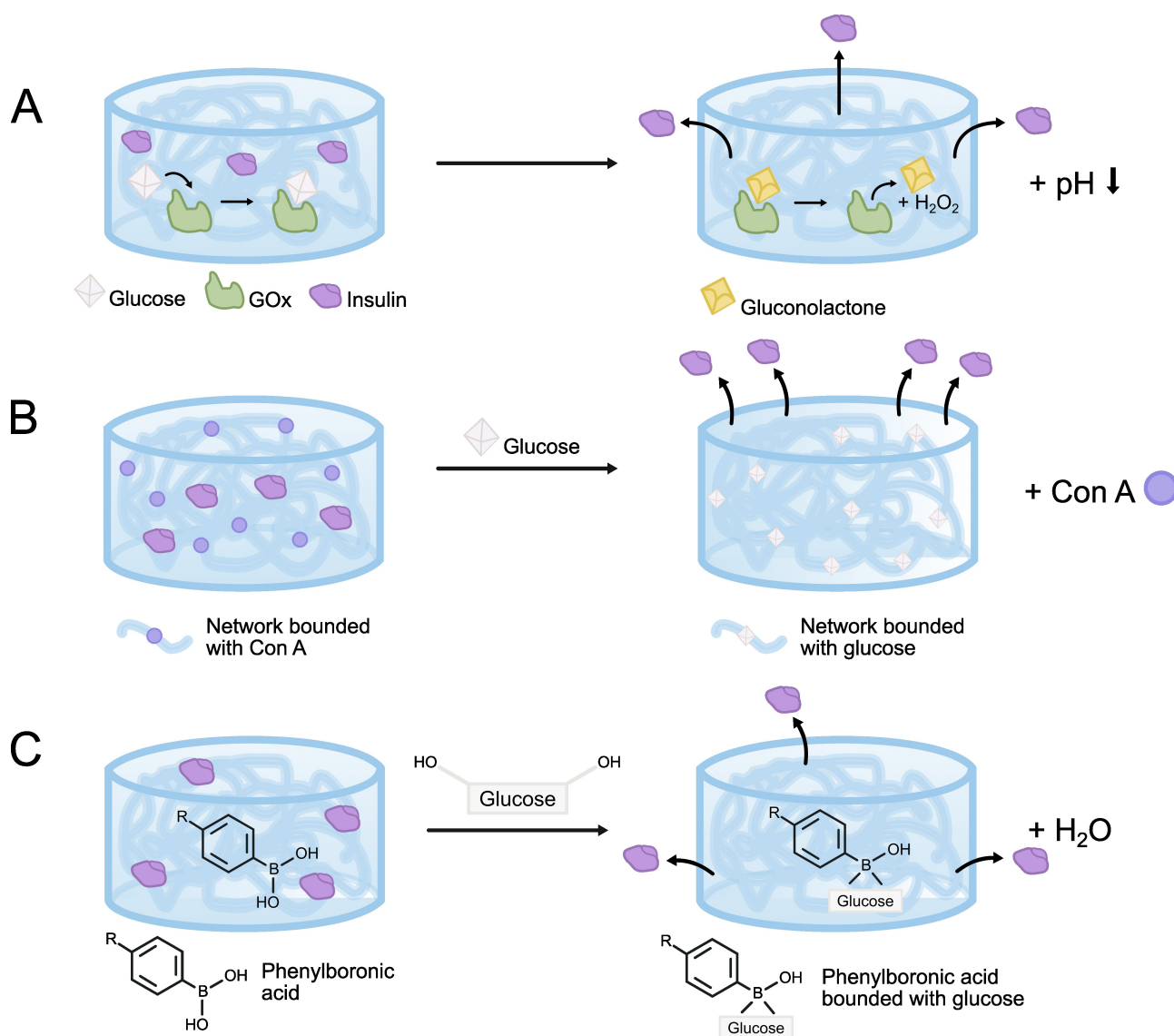


Fig. 5. Release of insulin under the influence of glucose from a hydrogel loaded with different compounds. (A) glucose oxidase (GOx) in direct contact with glucose, oxidizing to gluconolactone, which is then hydrolyzed to gluconic acid, resulting in a local decrease in glucose and pH. (B) Concanavalin A (Con A) creates tight hydrogel network with entrapped insulin when glucose penetrates the polymer matrix, competitive binding of glucose with Con A occurs. (C) Phenylboronic acid (PBA) is a synthetic equivalent of the glucose-sensitive agent with an interesting ability to form reversible covalent complexes with diols. The image was created with Inkscape v1.4, retrieved from <https://inkscape.org>.

tive maleated chitosan/thiolated hyaluronan (mCH/tHA) multilayer coatings on poly(L-lactic acid) (PLLA) nanofibrous mats (referred to as P-mCH/tHA), aiming to achieve sustained insulin release (IN). Physicochemical studies showed that P-mCH/tHA demonstrated higher tensile strength, an accelerated degradation rate, greater antibacterial activity, and sustained insulin release compared to P-CH/HA and pure PLLA mats. Moreover, P-CH/HA displayed improved adhesion, spreading, and proliferation of mesenchymal stem cells in comparison to other PLLA mats. *In vivo* studies conducted in a mouse model of diabetes revealed that P-mCH/tHA_IN facilitated the fastest wound closure among all groups, promoting reepithelialization,

angiogenesis, and increased collagen deposition in wounds. This suggests that the coating combined with IN is promising for the development of new bioactive wound dressings.

In the work of L. Fang *et al.* [55], a thermosensitive hydrogel, poloxamer (Pox), was applied topically to the wound in combination with insulin injection to investigate improvements in wound healing. Compared to the control animals (healthy mice), those treated with poloxamer plus insulin (Poxin) exhibited accelerated wound closure and healing rates; the expression of both alpha-smooth muscle actin (α -SMA) and transforming growth factor beta 1 (TGF- β 1) was significantly higher than those in the control group and the Pox-treated animals during the first seven

days post-surgery, although they decreased significantly by day 14. Therefore, the researchers concluded that the hydrogel combined with insulin accelerates wound healing.

4.4 Glucose-Responsive Hydrogels as Dressings and Drug Delivery Systems for Diabetic Wounds Treatment

Glucose-responsive hydrogels are particularly beneficial in the treatment of chronic diabetic wounds, where the skin repair processes are impaired due to elevated blood glucose levels. Hyperglycemia induces chronic inflammation, alters the angiogenesis process, and increases the production of glycation end products, resulting in severe oxidative stress conditions. Hence, hydrogels that release insulin in response to high glucose concentration in the wound have significant potential for application in diabetic wound treatment [56]. Glucose-responsive hydrogels can be categorized into three types based on the responsive agent employed: glucose oxidase (GOx), concanavalin A (Con A), and phenylboronic acid (PBA) groups. These delivery systems release insulin via glucose-induced changes in binding capacity or structural alterations, such as matrix swelling or shrinkage, material dissolution, changes in pore size, or degradation [57,58].

A summary of the mechanisms of action of glucose-sensitive hydrogels is depicted in Fig. 5. Glucose oxidase is a naturally derived enzyme comprised of glycoproteins that catalyzes the oxidation of glucose to gluconolactone, generating hydrogen peroxide (H_2O_2) as an intermediate. Gluconolactone is then hydrolyzed to gluconic acid in an aqueous environment, resulting in a local decrease in glucose levels and pH, which limits local hyperglycemia and the proliferation of pathogenic bacteria (Fig. 5A). However, the generation of H_2O_2 as a byproduct results leads to reduced oxygen levels and increased tissue hypoxia, an undesirable effect. Therefore, in most hydrogel systems that incorporate this enzyme, local physicochemical changes in the environment, such as pH, H_2O_2 concentration, or oxygen levels, are used for the controlled release of insulin. The use of hydrogels sensitive to pH or H_2O_2 changes loaded with GOx allows for a more controlled release of insulin while minimizing the side effects associated with elevated concentrations of H_2O_2 and gluconic acid, such as increased oxidative stress, tissue hypoxia, and reduced GOx activity [57].

One example of such a system is an injectable microgel consisting of a network of dextran nanoparticles, developed by L.R. Volpatti's team [59]. In the process of creating a porous network, alginate and chitosan were used to coat acid-sensitive dextran, resulting in both positively and negatively charged particles. The resulting nanosheet was then modified by degradation in an acidic environment to coat it with insulin, catalase, and GOx. The hydrogel functioned based on the disintegration of the nanosheet in the presence of hyperglycemia, leading to the simultaneous release of insulin. Another example is the biomaterial developed

by J. Yang *et al.* [60], who designed a glucose-responsive, multifunctional, injectable, self-healing drug-loaded metal-organic hydrogel (DG@Gel) that responds to the presence of glucose via the loading of glucose oxidase. When injected into a diabetic wound, GOx in DG@Gel altered the hyperglycemic wound microenvironment by decomposing excess glucose into hydrogen peroxide and glucuronic acid, which lowered the wound pH. The reduced pH promoted the release of zinc ions and deferoxamine mesylate (DFO), displaying both antibacterial and angiogenic activity in diabetic wound healing. *In vitro* studies revealed the antibacterial activity of DG@Gel and its ability to promote cell proliferation, migration, and tube formation, while *in vivo* studies showed that DG@Gel could induce reepithelialization, collagen deposition, and angiogenesis during wound healing in diabetic mice, with good biocompatibility and biodegradability. The results suggest that this hydrogel is a promising innovative dressing for treating diabetic wounds.

Concanavalin A (Con A) belongs to the lectins, a class of carbohydrate-binding proteins that are used as natural receptors in the formation of glucose-responsive hydrogels. Systems for glucose-regulated insulin release based on Con A are based on physical cross-linking of Con A within a glycosylated backbone that contains insulin. Con A physically binds to the glycosylated functional groups of the hydrogel network, creating a tightly complex matrix with small pores that effectively traps insulin within the hydrogel. When glucose penetrates the polymer matrix, competitive binding occurs between glucose and Con A. Due to the higher affinity of Con A for glucose compared to glycosylated groups, glucose replaces the glycosylated polymer. This process leads to the degradation of the hydrogel network, which is manifested through its swelling and the release of insulin by diffusion or transition from gel to sol phase (Fig. 5B). Unfortunately, this mechanism is characterized by a relatively slow reaction time and a slow release of Con A from the hydrogel, thus limiting the therapeutic potential of the system. To address this, modifications are implemented to increase stability by conjugating Con A with poly(ethylene glycol) chains. This results in a more hydrophilic and stable Con A system, showing improved solubility, stability, and affinity for glucose, which improves the sensitivity of the hydrogel and reduces the lag time of the release reaction. A similar improvement is achieved through the covalent immobilization of Con A in the hydrogel matrix via carbodiimide cross-linking, which prevents the leaching of Con A from the polymer, thereby increasing the stability of the gel and allowing for repeatable swelling behavior [57,61].

An example of such a solution is provided by the work of R. Yin *et al.* [62], who employed the reverse phase emulsion cross-linking technique to create glucose-sensitive microgels using Con A. The study explored the potential of using microgels cross-linked with genipin (ConA/GEA-Cs) as a glucose-sensitive drug delivery system [62]. In this research, the polymeric ligand for Con A was prepared as

an acrylated glucosyloxyethyl derivative of chitosan (GEA-Cs). This strategy facilitated rapid cross-linking of the hydrogel bound to ConA via genipin, contributing to the increased stability of the system [62]. The study demonstrated that insulin released from this system retained its tertiary structure, thus preserving its bioactivity [62].

Despite their ubiquity and excellent glucose selectivity, GOx and Con A, as protein-based systems, are susceptible to environmental changes and are typically characterized by antigenicity, instability, and high costs. Consequently, they are not suitable for long-term storage or use. Hence, more stable hydrogels are being developed, using phenylboronic acid as a synthetic equivalent of the glucose-sensitive agents (Fig. 5C). One of the most significant chemical properties of boronic acids, which has expanded their application in medical devices, is their ability to form reversible covalent complexes with diols. When glucose, which contains a diol functional group, enters the hydrogel, phenylboronic acid residues form reversible complexes with it, shifting the equilibrium towards positively charged boron residues. This reaction alters the overall charge density of the gel, increasing the proportion of cationically charged residues. The increased charge enhances the hydrophilicity of the hydrogel, which, combined with ionic repulsion within the gel, leads to a pronounced swelling reaction. Conversely, the reaction of boronate anions with a diol leads to the formation of a complex that shifts the equilibrium towards the hydrophilic and negatively charged PBA. This glucose-dependent change in the ratio between uncharged and charged borates influences the solubility of the polymer, providing the basis for the release of diol-sensitive insulin.

Two forms of boronic acids create esters with diols through the loss of water: one form has the parent hydroxyl group replaced by carbon substituents while the other has two hydroxyl groups substituted by carbon. Unfortunately, the bond between neutral boronic acid and diols is also considered hydrolytically unstable due to their high susceptibility to hydrolysis. Additionally, PBA is less selective for glucose than GOx and Con A. Nevertheless, systems based on the use of phenylboronic acid in glucose-sensitive hydrogels, compared to those based on GOx and Con A proteins, are characterized significantly higher reactivity, long-term stability, and the possibility of long-term storage [61,63]. Consequently, numerous studies are being conducted to develop glucose-sensitive hydrogels that employ phenylboronic acids. For example, Y. Dong *et al.* [64] developed an injectable stimuli-responsive hydrogel based on the complexation of glucose and boronic acid. The hydrogel showed shear-thinning and self-healing properties, rapidly transitioning from a gel to a fluid state in response to shear, and demonstrated controlled release *in vitro* in response to glucose concentration. In another study, Z. Guo *et al.* [65] developed a stimuli-sensitive microneedle hydrogel dressing with unique

glucose-responsive phenylborate groups through the *in situ* copolymerization of gelatin methacrylate, the glucose-responsive monomer 4-(2-acrylamidoethylaminoformyl)-3-fluorobenzenboronic acid (AFPBA), and gluconic insulin (G-insulin). The resulting material showed controlled glucose-dependent insulin release, and in *in vivo* studies conducted on mice with streptozocin-induced type I diabetes, insulin release was found to reduce inflammation, increase collagen deposition, and improve the hyperglycemic wound environment, thereby accelerating the wound healing process.

In another work, Z. Xu's team [66] utilized gallic acid (GA), known for its strong antioxidant properties, to modify chitosan (CS) chains by incorporating them into a hydrogel based on poly(ethylene glycol) and diacrylate (PEG-DA), creating a new hybrid antioxidant hydrogel (PEG-DA/CS-GA). Then, polyethyleneimine (PEI) was modified with glucose-sensitive phenylboronic acid (PBA) to load insulin (PEI-PBA/insulin NP). Insulin was immobilized in the PEG-DA/CS-GA hydrogel via borate bonds between PBA and polyphenol groups on CS-GA. Studies demonstrated that the PEG-DA/PEI-PBA/insulin/CS-GA (PPIC) hydrogel exhibited excellent biocompatibility, antioxidant properties (DPPH removal rate >95%), and protection of cells against oxidative stress. The PPIC hydrogel showed glucose-dependent insulin release and effectively regulated blood glucose levels. Both *in vitro* and *in vivo* results confirmed that PPIC hydrogel promoted angiogenesis, transformed the inflammatory microenvironment, and accelerated wound healing within 20 days. All these results indicate that PPIC hydrogel is an effective platform for treating diabetic wounds.

5. Conclusions

Diabetes, recognized as a civilization disease, is associated with numerous complications, among which diabetic wounds and Diabetic Foot Syndrome are some of the most challenging and costly to treat. Current therapies and dressings, although they bring positive results, fall short of meeting the needs of patients. Hence, the use of hydrogel dressings that enable controlled insulin delivery to diabetic wounds demonstrates clear therapeutic potential, as discussed in this paper. Innovative bioactive hydrogel systems leverage the synergistic effect of insulin as a growth factor alongside the favorable properties of hydrogel matrices that support tissue regeneration.

Insulin delivery systems for diabetic wounds that utilize micro- and nanoparticles embedded in hydrogel matrices are particularly interesting. However, the application of such technologies is accompanied by certain limitations. One primary contraindication for using nanotechnology solutions is the risk of exacerbating allergic and inflammatory reactions caused by certain drug carriers. Nanoparticles can induce oxidative stress, autophagy, and apoptosis in keratinocytes and fibroblasts. The cytotoxicity of nanomaterials

als is influenced by factors such as particle size, shape, surface charge, stability, and concentration. Therefore, when developing new nanomaterials for wound healing, it is crucial to appropriately tailor their physicochemical properties to minimize toxicity to skin cells. This can be achieved by incorporating stabilizers and surfactants and selecting materials with low sensitizing potential. Moreover, studies show that nanomaterials can cause DNA damage and reduce gene methylation, suggesting a potential carcinogenic effect. The contact of nanomaterials with blood in wounds, resulting from blood vessel disruption, can lead to hemolysis. To mitigate this risk, the physicochemical properties of nanomaterials can be modified, or they can be coated with biologically active substances, such as phospholipids and polysaccharides. Therefore, current research on insulin micro- and nanocarriers should focus not only on their fundamental parameters, such as size, shape, stability, and drug encapsulation efficiency, but primarily on the surface modification of these structures. This may involve, for instance, incorporating active groups through chemical modifications or attaching polymer conjugates, proteins, or antigens that would offer protection to the carrier against an aggressive immune response. This approach would minimize the risk of the aforementioned side effects resulting from undesirable reactions of the body to nanomaterials.

Conversely, a significant limitation of hydrogel matrices that reduces their effectiveness as dressings is their poor mechanical properties and limited ability to adhere to tissues. For a hydrogel to adequately support wound regeneration, its microstructure must closely mimic the structure of the tissue, providing appropriate porosity to enable cell migration and proliferation, as well as possessing sufficient strength and stiffness to support proper cell differentiation. In the case of biodegradable hydrogels, the rate of degradation must be synchronized with the rate of new tissue regeneration, ensuring that the polymer structure diminishes concurrently with the formation of new tissue. Another challenge is to enhance the adhesion of the hydrogel to the wound edges and ensure its ability to fill the entire wound surface, irrespective of its complexity. Consequently, the design of hydrogel matrices should focus on enhancing these properties, which can be achieved through the use of nanoadditives or the development of new *in situ* cross-linking techniques, as exemplified by injectable hydrogels [67].

In the case of hydrogels that respond to glucose levels, it is crucial to achieve an effective insulin release process while maintaining appropriate mechanical properties of the gel and minimizing the content of reaction by-products. Current research on the use of glucose oxidase (GOx), concanavalin A (Con A), and boric acid (PBA) indicates that, although these systems provide the most controlled insulin delivery among the technologies currently being developed, they possess limitations that hinder further advancement. Therefore, an important direction for research is to explore

methods to enhance the effectiveness of the response to glucose stimuli—either by introducing new factors or by modifying the structure of the hydrogel network. However, hydrogels also hold great potential as dressings due to their ability to perform dual functions: protecting wounds and serving as scaffolds for regenerating tissue, while supporting the processes of cell multiplication and proliferation through the controlled release of growth factors. Future work should also prioritize addressing the most urgent challenges, including the difficulties of encapsulating insulin and preserving its bioactivity during the loading and release process from carriers, as well as enhancing the mechanical properties of the resulting hydrogel matrices.

Author Contributions

Concept and design: AK and PDP; Formal analysis: AK and PDP; Writing—initial draft: AK; Writing—review and editing: AK and PDP; Project administration: PDP. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

All graphics were prepared with Inkscape v1.4, retrieved from <https://inkscape.org>.

Funding

This study was supported by the subsidy (No 16.16.160.557) for the AGH University of Krakow.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Kolb H, Kempf K, Röhling M, Martin S. Insulin: too much of a good thing is bad. *BMC Medicine*. 2020; 18: 224. <https://doi.org/10.1186/s12916-020-01688-6>.
- [2] Kahanovitz L, Sluss PM, Russell SJ. Type 1 Diabetes - A Clinical Perspective. *Point of Care*. 2017; 16: 37–40. <https://doi.org/10.1097/POC.000000000000125>.
- [3] Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*. 2015; 6: 850–867. <https://doi.org/10.4239/wjd.v6.i6.850>.
- [4] Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. *Avicenna Journal of Medicine*. 2020; 10: 174–188. https://doi.org/10.4103/ajm.ajm_53_20.
- [5] Armstrong DG, Boulton AJM, Bus SA. Diabetic Foot Ulcers and Their Recurrence. *The New England Journal of Medicine*. 2017; 376: 2367–2375. <https://doi.org/10.1056/NEJMr1615439>.
- [6] Matijević T, Talapko J, Meštrović T, Matijević M, Erić S, Erić I, *et al.* Understanding the multifaceted etiopathogenesis of foot complications in individuals with diabetes. *World Journal of Clinical Cases*. 2023; 11: 1669–1683. <https://doi.org/10.12998/wjcc.v11.i8.1669>.

- [7] Sorber R, Abularrage CJ. Diabetic foot ulcers: Epidemiology and the role of multidisciplinary care teams. *Seminars in Vascular Surgery*. 2021; 34: 47–53. <https://doi.org/10.1053/j.semvascsurg.2021.02.006>.
- [8] Ramirez-Acuña JM, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, *et al.* Diabetic Foot Ulcers: Current Advances in Antimicrobial Therapies and Emerging Treatments. *Antibiotics*. 2019; 8: 193. <https://doi.org/10.3390/antibiotics8040193>.
- [9] Gianino E, Miller C, Gilmore J. Smart Wound Dressings for Diabetic Chronic Wounds. *Bioengineering*. 2018; 5: 51. <https://doi.org/10.3390/bioengineering5030051>.
- [10] Xu Z, Han S, Gu Z, Wu J. Advances and Impact of Antioxidant Hydrogel in Chronic Wound Healing. *Advanced Healthcare Materials*. 2020; 9: e1901502. <https://doi.org/10.1002/adhm.201901502>.
- [11] Burgess JL, Wyant WA, Abdo Abujamra B, Kirsner RS, Jozic I. Diabetic Wound-Healing Science. *Medicina*. 2021; 57: 1072. <https://doi.org/10.3390/medicina57101072>.
- [12] Yang S, Gu Z, Lu C, Zhang T, Guo X, Xue G, *et al.* Neutrophil Extracellular Traps Are Markers of Wound Healing Impairment in Patients with Diabetic Foot Ulcers Treated in a Multidisciplinary Setting. *Advances in Wound Care*. 2020; 9: 16–27. <https://doi.org/10.1089/wound.2019.0943>.
- [13] Deng L, Du C, Song P, Chen T, Rui S, Armstrong DG, *et al.* The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing. *Oxidative Medicine and Cellular Longevity*. 2021; 2021: 8852759. <https://doi.org/10.1155/2021/8852759>.
- [14] Dai J, Chen H, Chai Y. Advanced Glycation End Products (AGEs) Induce Apoptosis of Fibroblasts by Activation of NLRP3 Inflammasome via Reactive Oxygen Species (ROS) Signaling Pathway. *Medical Science Monitor*. 2019; 25: 7499–7508. <https://doi.org/10.12659/MSM.915806>.
- [15] Wilkinson HN, Hardman MJ. Wound senescence: A functional link between diabetes and ageing? *Experimental Dermatology*. 2021; 30: 68–73. <https://doi.org/10.1111/exd.14082>.
- [16] Dasari N, Jiang A, Skochdopole A, Chung J, Reece EM, Vorstenbosch J, *et al.* Updates in Diabetic Wound Healing, Inflammation, and Scarring. *Seminars in Plastic Surgery*. 2021; 35: 153–158. <https://doi.org/10.1055/s-0041-1731460>.
- [17] Okonkwo UA, DiPietro LA. Diabetes and Wound Angiogenesis. *International Journal of Molecular Sciences*. 2017; 18: 1419. <https://doi.org/10.3390/ijms18071419>.
- [18] Pouget C, Dunyach-Remy C, Pantel A, Schuldiner S, Sotto A, Lavigne JP. Biofilms in Diabetic Foot Ulcers: Significance and Clinical Relevance. *Microorganisms*. 2020; 8: 1580. <https://doi.org/10.3390/microorganisms8101580>.
- [19] Hotterbeekx A, Kumar-Singh S, Goossens H, Malhotra-Kumar S. *In vivo* and *In vitro* Interactions between *Pseudomonas aeruginosa* and *Staphylococcus* spp. *Frontiers in Cellular and Infection Microbiology*. 2017; 7: 106. <https://doi.org/10.3389/fcimb.2017.00106>.
- [20] Schurr MJ. *Pseudomonas aeruginosa* Alginate Benefits *Staphylococcus aureus*? *Journal of Bacteriology*. 2020; 202: e00040-20. <https://doi.org/10.1128/JB.00040-20>.
- [21] Price CE, Brown DG, Limoli DH, Phelan VV, O'Toole GA. Exogenous Alginate Protects *Staphylococcus aureus* from Killing by *Pseudomonas aeruginosa*. *Journal of Bacteriology*. 2020; 202: e00559–19. <https://doi.org/10.1128/JB.00559-19>.
- [22] Da Silva J, Leal EC, Carvalho E, Silva EA. Innovative Functional Biomaterials as Therapeutic Wound Dressings for Chronic Diabetic Foot Ulcers. *International Journal of Molecular Sciences*. 2023; 24: 9900. <https://doi.org/10.3390/ijms24129900>.
- [23] Trucillo P, Di Maio E. Classification and Production of Polymeric Foams among the Systems for Wound Treatment. *Polymers*. 2021; 13: 1608. <https://doi.org/10.3390/polym13101608>.
- [24] Brumberg V, Astrelina T, Malivanova T, Samoilov A. Modern Wound Dressings: Hydrogel Dressings. *Biomedicines*. 2021; 9: 1235. <https://doi.org/10.3390/biomedicines9091235>.
- [25] Alven S, Peter S, Mbese Z, Aderibigbe BA. Polymer-Based Wound Dressing Materials Loaded with Bioactive Agents: Potential Materials for the Treatment of Diabetic Wounds. *Polymers*. 2022; 14: 724. <https://doi.org/10.3390/polym14040724>.
- [26] Dumville JC, Deshpande S, O'Meara S, Speak K. Hydrocolloid dressings for healing diabetic foot ulcers. *The Cochrane Database of Systematic Reviews*. 2013; 2013: CD009099. <https://doi.org/10.1002/14651858.CD009099.pub3>.
- [27] Kus KJB, Ruiz ES. Wound Dressings – A Practical Review. *Current Dermatology Reports*. 2020; 9: 298–308. <https://doi.org/10.1007/s13671-020-00319-w>.
- [28] Anderson JM. Future challenges in the in vitro and in vivo evaluation of biomaterial biocompatibility. *Regenerative Biomaterials*. 2016; 3: 73–77. <https://doi.org/10.1093/rb/rbw001>.
- [29] Helmus MN, Gibbons DF, Cebon D. Biocompatibility: meeting a key functional requirement of next-generation medical devices. *Toxicologic Pathology*. 2008; 36: 70–80. <https://doi.org/10.1177/0192623307310949>.
- [30] Gupta B, Agarwal R, Alam MS. Hydrogels for wound healing applications. In *Biomedical Hydrogels* (pp. 184–227). Woodhead Publishing: UK. 2011. <https://doi.org/10.1533/9780857091383.2.184>.
- [31] Macyk A, Kusibab A, Pamula E. Manufacturing and characterization of gellan gum – zinc oxide composites as potential biomaterials for wound treatment. *Engineering of Biomaterials*. 2023; 2–8. <https://doi.org/10.34821/eng.biomat.168.2023.2-8>.
- [32] Firlar I, Altunbek M, McCarthy C, Ramalingam M, Camci-Unal G. Functional Hydrogels for Treatment of Chronic Wounds. *Gels*. 2022; 8: 127. <https://doi.org/10.3390/gels8020127>.
- [33] Huang C, Dong L, Zhao B, Lu Y, Huang S, Yuan Z, *et al.* Anti-inflammatory hydrogel dressings and skin wound healing. *Clinical and Translational Medicine*. 2022; 12: e1094. <https://doi.org/10.1002/ctm2.1094>.
- [34] Tesaro M, Mazzotta FA. Pathophysiology of diabetes. In *Transplantation, Bioengineering, and Regeneration of the Endocrine Pancreas* (pp. 37–47). Academic Press: USA. 2019. <https://doi.org/10.1016/B978-0-12-814833-4.00003-4>.
- [35] Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, *et al.* Role of Insulin in Health and Disease: An Update. *International Journal of Molecular Sciences*. 2021; 22: 6403. <https://doi.org/10.3390/ijms22126403>.
- [36] Shukla SK, Sharma AK, Gupta V, Yashavardhan MH. Pharmacological control of inflammation in wound healing. *Journal of Tissue Viability*. 2019; 28: 218–222. <https://doi.org/10.1016/j.jtvs.2019.09.002>.
- [37] Macedo AS, Mendes F, Filipe P, Reis S, Fonte P. Nanocarrier-Mediated Topical Insulin Delivery for Wound Healing. *Materials*. 2021; 14: 4257. <https://doi.org/10.3390/ma14154257>.
- [38] Yang P, Wang X, Wang D, Shi Y, Zhang M, Yu T, *et al.* Topical insulin application accelerates diabetic wound healing by promoting anti-inflammatory macrophage polarization. *Journal of Cell Science*. 2020; 133: jcs235838. <https://doi.org/10.1242/jcs.235838>.
- [39] Sung YK, Kim SW. Recent advances in polymeric drug delivery systems. *Biomaterials Research*. 2020; 24: 12. <https://doi.org/10.1186/s40824-020-00190-7>.
- [40] Pachau L. Recent developments in novel drug delivery systems for wound healing. *Expert Opinion on Drug Delivery*. 2015; 12: 1895–1909. <https://doi.org/10.1517/17425247.2015.1070143>.
- [41] Zhang Z, Lv L. Effect of local insulin injection on wound vascularization in patients with diabetic foot ulcer. *Experimental and Therapeutic Medicine*. 2016; 11: 397–402. <https://doi.org/10.3892/etm.2015.2917>.

- [42] Wang J, Xu J. Effects of Topical Insulin on Wound Healing: A Review of Animal and Human Evidences. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*. 2020; 13: 719–727. <https://doi.org/10.2147/DMSO.S237294>.
- [43] Ng LC, Gupta M. Transdermal drug delivery systems in diabetes management: A review. *Asian Journal of Pharmaceutical Sciences*. 2020; 15: 13–25. <https://doi.org/10.1016/j.ajps.2019.04.006>.
- [44] Sabbagh F, Muhamad II, Niazmand R, Dikshit PK, Kim BS. Recent progress in polymeric non-invasive insulin delivery. *International Journal of Biological Macromolecules*. 2022; 203: 222–243. <https://doi.org/10.1016/j.ijbiomac.2022.01.134>.
- [45] Alavi M, Hamblin MR, Martinez F, Aghaie E, Khan H, Rose I, *et al.* Micro and nanoformulations of insulin: new approaches. *Nano Micro Biosystems*. 2022; 1: 1–7. <https://doi.org/10.22034/NMBJ.2022.157284>.
- [46] Primavera R, Kevadiya BD, Swaminathan G, Wilson RJ, De Pascale A, Decuzzi P, *et al.* Emerging Nano- and Micro-Technologies Used in the Treatment of Type-1 Diabetes. *Nanomaterials*. 2020; 10: 789. <https://doi.org/10.3390/nano10040789>.
- [47] Wang F, Chen Y, Benson HAE. Formulation of Nano and Micro PLGA Particles of the Model Peptide Insulin: Preparation, Characterization, Stability and Deposition in Human Skin. *The Open Drug Delivery Journal*. 2008; 2: 1–9. <https://doi.org/10.2174/1874126600802010001>.
- [48] Wang H, Xu Z, Zhao M, Liu G, Wu J. Advances of hydrogel dressings in diabetic wounds. *Biomaterials Science*. 2021; 9: 1530–1546. <https://doi.org/10.1039/d0bm01747g>.
- [49] Abdelkader DH, Tambuwala MM, Mitchell CA, Osman MA, El-Gizawy SA, Faheem AM, *et al.* Enhanced cutaneous wound healing in rats following topical delivery of insulin-loaded nanoparticles embedded in poly(vinyl alcohol)-borate hydrogels. *Drug Delivery and Translational Research*. 2018; 8: 1053–1065. <https://doi.org/10.1007/s13346-018-0554-0>.
- [50] Zhu J, Jiang G, Hong W, Zhang Y, Xu B, Song G, *et al.* Rapid gelation of oxidized hyaluronic acid and succinyl chitosan for integration with insulin-loaded micelles and epidermal growth factor on diabetic wound healing. *Materials Science & Engineering. C, Materials for Biological Applications*. 2020; 117: 111273. <https://doi.org/10.1016/j.msec.2020.111273>.
- [51] Lee J, Ko JH, Mansfield KM, Nauka PC, Bat E, Maynard HD. Glucose-Responsive Trehalose Hydrogel for Insulin Stabilization and Delivery. *Macromolecular Bioscience*. 2018; 18: e1700372. <https://doi.org/10.1002/mabi.201700372>.
- [52] Zanchetta FC, De Wever P, Morari J, Gaspar RC, Prado TPD, De Maeseneer T, *et al.* *In Vitro* and *In Vivo* Evaluation of Chitosan/HPMC/Insulin Hydrogel for Wound Healing Applications. *Bioengineering*. 2024; 11: 168. <https://doi.org/10.3390/bi11020168>.
- [53] Lee CH, Chen DY, Hsieh MJ, Hung KC, Huang SC, Cho CJ, *et al.* Nanofibrous insulin/vildagliptin core-shell PLGA scaffold promotes diabetic wound healing. *Frontiers in Bioengineering and Biotechnology*. 2023; 11: 1075720. <https://doi.org/10.3389/fbioe.2023.1075720>.
- [54] Gao X, Huang R, Jiao Y, Groth T, Yang W, Tu C, *et al.* Enhanced wound healing in diabetic mice by hyaluronan/chitosan multilayer-coated PLLA nanofibrous mats with sustained release of insulin. *Applied Surface Science*. 2022; 576: 151825. <https://doi.org/10.1016/j.apsusc.2021.151825>.
- [55] Fang L, Wu H, Li X, Fang J, Zhu Y. Improvement of Skin Wound Healing for Diabetic Mice with Thermosensitive Hydrogel Combined with Insulin Injection. *International Journal of Endocrinology*. 2022; 2022: 7847011. <https://doi.org/10.1155/2022/7847011>.
- [56] Shen D, Yu H, Wang L, Khan A, Haq F, Chen X, *et al.* Recent progress in design and preparation of glucose-responsive insulin delivery systems. *Journal of Controlled Release*. 2020; 321: 236–258. <https://doi.org/10.1016/j.jconrel.2020.02.014>.
- [57] Fuchs S, Ernst AU, Wang LH, Shariati K, Wang X, Liu Q, *et al.* Hydrogels in Emerging Technologies for Type 1 Diabetes. *Chemical Reviews*. 2021; 121: 11458–11526. <https://doi.org/10.1021/acs.chemrev.0c01062>.
- [58] Serpico L, Dello Iacono S, Cammarano A, De Stefano L. Recent Advances in Stimuli-Responsive Hydrogel-Based Wound Dressing. *Gels*. 2023; 9: 451. <https://doi.org/10.3390/gels9060451>.
- [59] Volpatti LR, Facklam AL, Cortinas AB, Lu YC, Matranga MA, MacIsaac C, *et al.* Microgel encapsulated nanoparticles for glucose-responsive insulin delivery. *Biomaterials*. 2021; 267: 120458. <https://doi.org/10.1016/j.biomaterials.2020.120458>.
- [60] Yang J, Zeng W, Xu P, Fu X, Yu X, Chen L, *et al.* Glucose-responsive multifunctional metal-organic drug-loaded hydrogel for diabetic wound healing. *Acta Biomaterialia*. 2022; 140: 206–218. <https://doi.org/10.1016/j.actbio.2021.11.043>.
- [61] Koetting MC, Peters JT, Steichen SD, Peppas NA. Stimulus-responsive hydrogels: Theory, modern advances, and applications. *Materials Science & Engineering. R, Reports: a Review Journal*. 2015; 93: 1–49. <https://doi.org/10.1016/j.mser.2015.04.001>.
- [62] Yin R, Wang K, Du S, Chen L, Nie J, Zhang W. Design of genipin-crosslinked microgels from concanavalin A and glucosyloxyethyl acrylated chitosan for glucose-responsive insulin delivery. *Carbohydrate Polymers*. 2014; 103: 369–376. <https://doi.org/10.1016/j.carbpol.2013.12.067>.
- [63] Sharifzadeh G, Hosseinkhani H. Biomolecule-Responsive Hydrogels in Medicine. *Advanced Healthcare Materials*. 2017; 6: 10.1002/adhm.201700801. <https://doi.org/10.1002/adhm.201700801>.
- [64] Dong Y, Wang W, Veisheh O, Appel EA, Xue K, Webber MJ, *et al.* Injectable and Glucose-Responsive Hydrogels Based on Boronic Acid-Glucose Complexation. *Langmuir*. 2016; 32: 8743–8747. <https://doi.org/10.1021/acs.langmuir.5b04755>.
- [65] Guo Z, Liu H, Shi Z, Lin L, Li Y, Wang M, *et al.* Responsive hydrogel-based microneedle dressing for diabetic wound healing. *Journal of Materials Chemistry. B*. 2022; 10: 3501–3511. <https://doi.org/10.1039/d2tb00126h>.
- [66] Xu Z, Liu G, Li Q, Wu J. A novel hydrogel with glucose-responsive hyperglycemia regulation and antioxidant activity for enhanced diabetic wound repair. *Nano Research*. 2022; 15: 5305–5315. <https://doi.org/10.1007/s12274-022-4192-y>.
- [67] Norahan MH, Pedroza-González SC, Sánchez-Salazar MG, Álvarez MM, Trujillo de Santiago G. Structural and biological engineering of 3D hydrogels for wound healing. *Bioactive Materials*. 2022; 24: 197–235. <https://doi.org/10.1016/j.bioactmat.2022.11.019>.