REVIEW

PROGRESS OF KNOWLEDGE IN THE DEVELOPMENT OF CHITOSAN FORMULATIONS WITH INSULIN TO PROMOTE SKIN WOUND HEALING

Marcin Przybyła[1], Barbara Dolińska[1,a], Aneta Ostróżka-Cieślik[1,b,*]

[1] – Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia, Kasztanowa 3 Str., 41-200 Sosnowiec, Poland
[a] – ORCID: 0000-0003-3035-7417, [b] – ORCID: 0000-0002-5179-1370
[*]corresponding author: aostrozka@sum.edu.pl

Abstract

The treatment of difficult-to-heal wounds is a major problem in modern medicine. The search for new materials and ways to treat wounds is important and timely. Insulin stimulates proliferation, differentiation and migration of skin fibroblasts and keratinocytes. Chitosan has beneficial biological properties and can be an effective carrier of insulin. This review discusses research into the development of chitosan formulations with insulin to aid local treatment of chronic wounds. The PubMed, Google Scholar, Scopus and Web of Science databases were searched for studies on chitosan preparations with insulin in terms of their efficacy in wound healing. Twelve original English-language articles published in peer-reviewed scientific journals were included in the analysis. Insulin preparations in a chitosan matrix in the form of gel, hydrogel, injection and non-woven dressing are highly effective at all stages of the wound-healing process. They show anti-inflammatory and antimicrobial effects. Insulin from the formulations is released in a prolonged or controlled manner. However, it seems necessary to learn more about the mechanisms of insulin action within lesions. Of note, the data are mainly from basic and preclinical studies. Only one clinical study has been conducted.

Keywords: *chitosan, insulin, hydrogel, dermal drug delivery, wound dressing*

Received: 23.02.2024
Accepted: 23.04.2024

1. Introduction

One of the greatest challenges of the 21st century is the epidemic of non-communicable diseases. Chronic diseases are characterised by a slow progression of lesions, with the risk of many complications, including the development of difficult-to-heal wounds. The treatment of venous and arterial ulcers and diabetic foot represents a major challenge for modern medicine. The healing of these wounds does not follow a physiological pattern. Most chronic wounds stop in the inflammatory phase, with no tendency to progress to the proliferative phase. There are similar difficulties in effective treatment of burn wounds. It is estimated that 1.5–2 million people in Europe struggle with difficult-to-heal wounds [1], including 500,000 in Poland [2].

Insulin is a peptide hormone with hypoglycaemic effects that accelerates wound healing (in both people with diabetes and healthy individuals) [3]. It exhibits antioxidant and anti-inflammatory properties. Its application to the skin stimulates the migration and proliferation of keratinocytes, which have selective receptors for insulin on their surface. Insulin binding to receptors on the cell surface activates phosphoinositide 3-kinase (PI3K) and the Akt-dependent pathway, which influences normal endothelial function and vascular homeostasis [4–6]. It induces early neutrophil recruitment and increases M2 macrophage and interleukin-10 (IL-10) levels at the wound site. *In vitro*, it has been shown to affect the secretion of inflammatory mediators by regulating monocyte chemotactic protein-1 (MCP-1) expression [7]. Insulin has been confirmed to reduce the wound-healing time. It accelerates neovascularisation, epithelialisation and remodelling. At the same time, no systemic (hypoglycaemia, hypokalaemia and hypoaminoacidosis) or local (pain, allergy and infection) side effects have been reported [8]. Research is currently underway to develop a topical insulin preparation that exhibits prolonged release of bioactive insulin.

The development of an innovative dermatological form of insulin – a hydrogel – has recently received increased attention. Hydrogels are hydrophilic substrates that are obtained by gelation using polymers. They exhibit adhesive properties and can therefore be applied to wounds with exudate and as a form of dressing. This review focuses on the use chitosan, a potential insulin carrier, whose properties allow the development of a hydrogel with extended active pharmaceutical ingredient (API) release.

Chitosan is a natural polymer that is biodegradable; biocompatible with the skin; and exhibits antimicrobial, haemostatic and antioxidant activity. It is formed by the deacetylation of chitin (a component of insect and arthropod exoskeletons, among others) and can be used both as a drug carrier and as a material for the creation of dressings or scaffolds in tissue engineering [9, 10]. It shows potential in wound care as a component of controlled drug-delivery systems, sponges, membranes, polymer films and nanofibers. Chitosan-based formulations promote wound healing by acting as an anti-inflammatory and antimicrobial agent. The main mechanism of chitosan's antimicrobial action is thought to be its interaction with negatively charged bacterial cell membranes/walls, which can lead to cell lysis. Other possible mechanisms include interference with bacterial DNA, inhibition of protein synthesis and chelation of nutrients from the bacterial environment (mainly divalent cations necessary for microbial growth) [10, 11]. In the initial stage of wound healing, chitosan promotes the migration of neutrophils and macrophages and subsequently stimulates the secretion of growth factors and collagen, which accelerates the wound-closure process. It also affects platelet and erythrocyte aggregation and inhibits fibrinolysis. During the proliferation

phase, it promotes the growth of granulation tissue. Chitosan can reduce the intensity of the scarring process, resulting in a visibly smaller scar [12–14]. Hydrogel dressings based on chitosan are characterised by oxygen permeability and high exudate absorption, keeping the wound site environment moist. This has the effect of accelerating the healing process [15]. Moreover, chitosan–basic fibroblast growth factor (bFGF) matrices promote epithelial regeneration and inhibit bacterial growth [16].

2. Methods

2.1. Research Question

The aim of this study was to review the literature on chitosan formulations containing insulin in terms of their efficacy in wound healing. The research question was: can chitosan-based hydrogels be effective insulin carriers for topical application?

2.2. Eligibility Criteria

Two authors searched the PubMed, Google Scholar, Scopus and Web of Science databases to identify articles on efficacy studies on dermal chitosan preparations with insulin. The following keywords were used: chitosan, insulin, drug-delivery system, topical therapy, dermal drug delivery, transdermal penetration, hydrogel, injection, microneedle devices, microneedle patch, needle-free injection, polymeric particles, electrospinning, wound dressing, bioactive dressings, wound healing, and diabetic foot ulcers. The inclusion criteria were: original English-language articles published in peer-reviewed journals within the last 20 years that included data from basic, preclinical or clinical studies. Various formulation technologies developed exclusively for epidermal, transdermal and subdermal applications to produce a topical effect were included. The exclusion criteria were: articles in which the insulin chitosan preparation was applied by another route (e.g. oral, ocular, mucosal and body cavities), review articles, newsletters, abstracts from conference proceedings and studies published on websites or in books.

3. Review of Studies on the Development of Chitosan Formulations With Insulin to Promote Skin Wound Healing

Skin wound healing is a complex, multi-stage process involving haemostasis, inflammation, neovascularisation, epithelialisation, granulation tissue formation, extracellular matrix deposition and tissue remodelling [16]. The ideal preparation for treating and accelerating wound healing should have the ability to stop bleeding, to prevent bacterial infection, to absorb wound exudate and to allow gas exchange, and it should be biodegradable and non-toxic [17]. Developing an effective and inexpensive wound treatment for patients remains a challenge. Biomaterial-based formulations can provide a simple and safe therapeutic option. Chitosan has favourable biological properties and can be an effective insulin carrier. This biopolymer could be used to produce various dermal forms of the drug. An analysis of the available literature indicates that the challenge is to develop a chitosan formulation of insulin that will enable efficient release and permeation of the hormone through the skin and ensure efficacy (Table 1).

Table 1. Review of the studies on chitosan preparations with insulin.

Reference	Type of insulin/ chitosan used	Form of the insulin/ chitosan preparation	Insulin dosage	Research model	Effects of the insulin preparation
Basic studies					
Kempe et al. 2007 [18]	Spin-labelled insulin/ chitosan (Chitoclear® FG95) with a degree of deacetylation of 95%	Insulin-loaded chitosan/ glycerol-2-phospate (β-GP); injectable hydrogel	0.2 mg/ml	*In vitro*	• Combination of chitosan with β-glycerol phosphate (β-GP, 8%–16%) resulted in low viscosity at room temperature with thermogelling properties • Despite high mobility of the protein inside gel, there was controlled release of insulin over several days • A higher β-GP content in gel resulted in faster gelation and insulin release
Preclinical studies					
Zhao et al. 2017 [19]	Bovine insulin/ phenylboronic-modified chitosan (CSPBA)	Injectable, pH- and glucose-sensitive hydrogel made of modified chitosan, poly(vinyl alcohol) and bezaldehyde-capped poly(ethylene glycol) (OHC-PEG-CHO) loaded with insulin and live fibroblasts (L929)	0.3%	Strepto-zotocin-induced diabetic rats	• Sustained and pH/ glucose-triggered insulin release, as well as fibroblast viability and proliferation in the hydrogel matrix • The *in vivo* study showed an enhanced wound closure rate and increased neovascularisation and collagen deposition

Ehterami et al. 2018 [20]	Insulin (not specified)/ chitosan (not specified)	Electrospun poly (ε-caprolactone)/ collagen (PCL/COLL) wound dressing with insulin-loaded chitosan nanoparticles	1000 IU	Full-thickness excisional wound on male Wistar rats	• Incorporation of chitosan nanoparticles onto PCL/COLL dressing enhanced hydrophilicity, water-uptake and blood compatibility • Sustained insulin release of up to 65% over 14 days • The wound closure reached 97% after 14 days
Zhu et al. 2020 [21]	Insulin (not specified)/ chitosan with a degree of deacetylation of ≥ 95%	Insulin-loaded micelles embedded along with epidermal growth factor into oxidised hyaluronic acid/succinyl chitosan composite hydrogel	10 mg/ml	Strepto-zotocin-induced diabetic rats	• Low biological cytotoxicity and good biocompatibility • Excellent wound healing properties • Promoted fibroblast proliferation and collagen deposition • Addition of epidermal growth factor into the hydrogel resulted in an even faster wound closure rate
Ribeiro et al. 2020 [22]	Recombinant human insulin/ chitosan with a degree of deacetylation of ≥ 75%	Hydrogel with chitosan nanoparticles containing insulin	0.5 IU	Alloxan-induced diabetic rats	• High stability of insulin-chitosan nanoparticles • Stimulation of inflammatory cells proliferation • Increased angiogenesis and wound maturation

Li et al. 2021 [23]	Insulin glargine/ chitosan with a degree of deacetylation of ~ 95%	Insulin incorporated into *N*-carboxy-ethyl chitosan, hyaluronic acid-aldehyde and adipic acid dihydrazide injectable hydrogel	0.1 IU	Strepto-zotocin-induced diabetic rats	• Hydrogel presented pH-responsive, sustained insulin release for up to 14 days, while retaining its bioactivity • Shortened the inflammatory phase • Promoted collagen deposition, re-epithelisation and angiogenesis
Chen et al. 2022 [24]	Bovine insulin/ chitosan (number average molecular weight = 10–30 kDa)	Carbon monoxide–releasing, quaternised chitosan/ benzaldehyde F108 micelles (F108-CHO) hydrogel dressing loaded with insulin	Not specified	Strepto-zotocin-induced diabetic rats	• Antibacterial, anti-inflammatory and antioxidative properties • Sustained and controlled insulin release • The released carbon monoxide neutralised reactive oxygen species • Significant healing properties of the infected diabetic wound along with great biocompatibility
Hussein et al. 2022 [25]	Long performing insulin/ chitosan (not specified)	Insulin subcutaneous injections topped with chitosan/ zinc oxide nanocom-posite membrane	2 IU/rat per day	Excisional wound on strepto-zotocin-induced diabetic rats	• Accelerated angiogenesis and tissue repair by activating the phosphoinositide 3-kinase/mitogen-activated protein kinase signalling pathway • Additional upregulation of miR-125 and miR-132

Gao et al. 2022 [26]	Insulin (not specified)/ maleilated chitosan (mCH)	Insulin-loaded poly (L-lactic acid) (PLLA) nanofibrous mats coated with maleilated chitosan cross-linked with thiolated hyaluronan (tHA)	100 µM	Diabetic mice	• Addition of mCH/tHA layer improved mechanical properties, wetting and water-uptake of PLLA mats • Increased insulin uptake and its controlled release, resulting in greatly improved wound healing
Sharda et al. 2023 [27]	Recombinant human insulin/ chitosan (not specified)	Insulin-loaded chitosan nanoparticles	60 µM	Burn wounds on Swiss albino mice	• Increased cell migration • Almost 30% wound area reduction in the initial days of the treatment, reaching up to 95% after 20 days • Significant re-epithelisation and collagen deposition • Effective interleukin 6 suppression
Zanchetta et al. 2024 [28]	Regular insulin (Novolin®)/ chitosan with a molecular weight of 190–310 kDa and a degree of deacetylation of ≥ 75%	Insulin-containing chitosan/ hydroxypropyl methyl cellulose hydrogel	2 IU/g	Human keratino-cyte cells and diabetic mice	• Stimulated proliferation and migration of human keratinocytes • Hair follicle regeneration at the wound site • Faster wound healing without affecting blood glucose levels
Clinical studies					
Dawoud et al. 2019 [29]	Insulin crystals/ chitosan with a medium molecular weight	Liposomal insulin chitosan gel	2%	Human patients	• Reduction of erythema • Wound healing rate 16 times faster compared with the control group • Confirmed stability of insulin liposomes up to 6 months (at 4°C)

3.1. Formulations for topical application and *lubrication*

3.1.1. Preclinical Studies

Zanchetta et al. [28] prepared a hydrogel based on chitosan and hydroxypropyl methylcellulose with insulin (Chi/HPMC/Ins). The *in vitro* studies with a human keratinocyte cell line (HaCaT) demonstrated that the gel had no cytotoxicity and stimulated keratinocyte migration, resulting in faster wound closure. The formulation accelerated wound healing by influencing the granulation process and regenerating hair follicles. These results were also confirmed in an *in vivo* model. The authors monitored wound healing over 20 days and found that the wound area tended to regress more in the Chi/HPMC/Ins hydrogel group after days 3 ($p<0.05$), 7 ($p<0.01$) and 10 ($p<0.05$) compared with the saline group, and on days 7 and 10 ($p < 0.01$) compared with the Chi/HPMC group. The authors also demonstrated that topical application of hydrogel with insulin does not affect blood glucose levels.

Zhu et al. [21] described a pH-sensitive hydrogel based on oxidised hyaluronic acid (OHA) and succinylated chitosan (SCS), in which insulin-containing micelles were embedded. They added epidermal growth factor (EGF), a wound healing stimulator, to the hydrogel as a bioactive ingredient. The resulting formulation showed biocompatibility and significant efficacy in wound healing in a rat model. After just 6 days of hydrogel application, the wound area had decreased by 75%, compared with 45.5% after gauze-placebo application. The authors also found improved collagen and myofibril deposition at the wound site. The developed formulation promoted fibroblast proliferation and the integrity of the internal tissue structure. In the case of the bioactive hydrogel, in addition to a faster healing rate of the lesional skin, there was formation of new blood vessels, glands and hair follicles on day 12 of therapy. The authors did not perform statistical analysis.

Ribeiro et al. [22] prepared chitosan nanoparticles containing insulin and then placed them in a hydrogel based on the finished product Sepigel®. The resulting nanoparticles were homogeneous in size and shape (particle size = 245.9 nm; zeta potential = 39.3 mv) and stable for up to 56 days at 4°C. The authors evaluated the hydrogel in a rat model of diabetes. There was more efficient migration of pro-inflammatory cells at the initial stage of wound healing, followed by stimulation of migration of keratinocytes, epithelial cells and fibroblasts. Angiogenesis and granulation were also stimulated. Histological examination of tissues after 3 days of treatment with insulin-chitosan nanoparticles and chitosan nanoparticles alone showed a polymorphic nuclear infiltrate. This indicates that chitosan was involved in cell chemotaxis. Of note, the authors found no difference in wound healing between rats treated with the insulin-chitosan nanoparticle-based preparation and those treated with insulin alone.

Sharda et al. [27] investigated the efficacy of chitosan nanoformulation containing insulin to treat burn wounds. The resulting nanoparticles were spherical with a diameter of ~30 nm and a zeta potential of 28.8 mV (at pH 6.0) and 23.2 mV (at pH 7.4). The *in vitro* release of hormone across the dialysis membrane at 37ºC occurred in a rapid and prolonged manner. Within the first 2 h, 74% of the API dose had been released, while at the end of the 36-h study, 90% of the loaded insulin was released. The authors confirmed the therapeutic properties of the developed nanoformulation *in vitro* using human keratinocyte cultures and *in vivo* using a mouse model of a burn wound. Five days after application of the chitosan/insulin nanopreparation, there was a 29% reduction in wound size; all other treatments showed no progress in wound healing. After 20 days of nanoparticle therapy, the wound surface was 96% healed, insulin alone and a commercially available preparation had resulted in 68% and 77% would healing, respectively. In addition, stimulation of collagen

deposition, suppression of the pro-inflammatory cytokine IL-6, activation of the anti-inflammatory cytokine IL-10 and modulation of the Nrf-2 pathway were confirmed.

3.1.2. Clinical Study

Dawoud et al. [29] developed a chitosan-based mucoadhesive hydrogel in which insulin was encapsulated in liposomes (particle size = 257.751 nm; zeta potential = 20.548 mv). This hydrogel showed a biphasic insulin release profile, with an initiating dose (rapid API release) and a prolonged release dose (up to 24 h). Stability testing showed that liposomes with insulin stored at 25°C lost approximately 30% of the active ingredient after 14 days, while those stored in aqueous dispersion at 4°C showed high stability for up to 6 months. The authors confirmed the efficacy of chronic wound therapy in a clinical study involving 15 patients with chronic wounds and aged between 21 and 75 years. They excluded patients diagnosed with severe infection (suppurative wounds, wound exudates and bleeding), those who smoked, those who were taking immunosuppressants and those with debilitating chronic diseases. The test group (10 patients) received liposomal chitosan gel loaded with insulin, while the control group (5 patients) received liposomal chitosan gel without insulin. The test group showed a reduction in erythema and a 16-fold faster wound healing rate compared with the control group. There were no adverse systemic effects (headaches dizziness and sweating) and no hypoglycaemia. The wounds healed properly and were not infected. None of the participants reported complaints of pain during treatment. The researchers concluded that liposomal chitosan gel with insulin is a promising API delivery system with high stability.

3.2. Injectable Hydrogel

3.2.1. Basic Study

Kempe et al. [18] developed a chitosan hydrogel in combination with insulin-containing glycerol-2-phosphate (β-GP). The addition of β-GP to chitosan gave the hydrogel thermosensitive properties. As a result, at room temperature, the formulation was in the form of a sol and when administered, at physiological temperature, it turned into a gel, making it suitable for injection. The minimum concentration of β-GP providing this property was 6%, and as its concentration increased, the gelation rate of the formulation increased. In an oscillatory rheology study, the authors confirmed the formation of strong elastic gels as a result of the thermo-gelation of chitosan-β-GP systems. The authors examined the insulin release profile from samples with 8% and 16% β-GP. During the first 48 h, there were no significant differences between the two samples: approximately 50% of the insulin was released. In the later stages of the study, the amount and rate of insulin release were greater in the sample with the higher β-GP concentration (16%). In the final stage of release, 85% of the hormone was released from the formulation containing 8% β-GP, while 90% of the API was released from the formulation containing 16% β-GP. The release occurred by diffusion from the gel matrix.

3.2.2. Preclinical Studies

Li et al. [23] used hydrogel injections to treat diabetic foot ulcers. They prepared an insulin carrier based on *N*-carboxyethyl chitosan (*N*-chitosan), hyaluronic acid-aldehyde (HA-ALD) and adipic acid dihydrazide (ADH). The authors developed an injectable formulation that was characterised by a self-healing structure and a sustained release of the active substance that was sensitive to environmental pH. The dynamic acylhydrazone bonds present in the gel were responsible for the increase in the rate of insulin release as the pH decreased. As a result, the active ingredient was released faster from the sample

at pH 6.5 than from the sample at pH 7.4, reaching approximately 60% of the released API dose after 10 and 14 days, respectively. The hydrogel exhibited high biocompatibility, low cytotoxicity and promoted migration and proliferation of the cells tested *in vitro*. In a rat model of induced diabetes, injection of the hydrogel with insulin resulted in significantly faster wound healing (as early as 16 days) compared with the control. The hydrogel shortened the duration of inflammation and promoted collagen synthesis, epithelial formation and the formation of new blood vessels. The bioactive dressing was effective in alleviating peripheral neuropathy of diabetic wounds.

Zhao et al. [19] developed a pH- and glucose-responsive injectable hydrogel to treat diabetic wounds. The formulation was based on phenylboronic-modified chitosan (CSPBA), polyvinyl alcohol and OHC-PEG-CHO based on the Schiff base reaction. As in a previous study, insulin release occurred in a prolonged manner, and the rate of release increased with decreasing pH and increasing glucose concentration. The addition of chitosan to the gel resulted in a decrease in the amount of insulin released from the formulation, which is due to the ability of chitosan to lower blood glucose concentrations. The addition of insulin and fibroblasts to the carrier promoted neovascularisation, and collagen deposition and promoted diabetic wound healing. The study confirmed the observations of Kondo et al. [30] that chitosan shows potential to prevent diabetes.

3.3. Hydrogel Dressing

3.3.1. Preclinical Studies

Hussein et al. [25] conducted a study in a rat model of streptozotocin-induced diabetic foot syndrome. Topical treatment was based on subcutaneous administration of insulin at a dose of 2 IU/rat per day and topical application of a chitosan/zinc oxide (ZnO) nanocomposite membrane. The developed membrane showed an increase in surface roughness as the percentage of chitosan and ZnO increased, leading to an increase in its surface area. The authors showed that the combination of the two therapies resulted in a significant increase in the expression of the phosphatidylinositol 3-phosphatidyl kinase (PI3K) and mitogen-activated kinase (MAPK) genes, which were suppressed in untreated diabetic wounds. PI3K and MAPK are involved in signalling pathways that lead to an enhanced anti-inflammatory response and, as a result, accelerated wound healing. Insulin, as a bioactive component of hydrogel formulations, can induce the expression of these genes [31]. The formulation also increased the expression of the microRNAs miR-125 and miR-132, which have anti-inflammatory effects and stimulate cell migration and proliferation at the wound site [25]. The authors concluded that the proposed treatment could activate the PI3K/MAPK signalling pathway and some of the miRNAs that promote diabetic wound healing.

Gao et al. [26] developed a composite dressing. The authors investigated the therapeutic efficacy of hyaluronan/chitosan multilayer-coated PLLA (poly(L-lactic acid) nanofibrous mats (P-mCH/tHA) loaded with insulin, providing an extended hormone release profile. The dressing was obtained using an electrospinning technique. The authors suggest that coating the PLLA dressing with additional bioactive layers may increase not only its physical resistance to mechanical factors, but also its degradation rate and water absorption capacity, which is important for the treatment of oozing wounds. The dressings showed antimicrobial activity against *Escherichia coli*, mediated by the chitosan incorporated into the formulation. Insulin release occurred over 9 days, initially rapidly over 3 h, then in an extended manner. The dressing significantly improved wound healing in a diabetic mouse model by promoting angiogenesis, re-epithelialisation and increased collagen deposition.

Ehterami et al. [20] used the electrospinning method to develop a polymer fibre-based dressing. They coated insulin-delivering chitosan nanoparticles (particle diameter = 294.5 nm; zeta potential = 17.89 mV) with electrospun poly(ε-caprolactone)/collagen (PCL/COLL). The tested dressing showed high mechanical strength, a good exudate absorption capacity and a prolonged insulin release profile. The formulation was biocompatible and exhibited antimicrobial properties. In a rat model, wounds covered with the PCL/COLL/Cs-Ins dressing for 14 days showed almost complete healing, while wounds covered with the control showed 45% healing.

Chen et al. [24] developed one of the most innovative hydrogel dressings. It is based on a quaternary chitosan derivative and F108 micelles coated with benzaldehyde groups. The micelles encapsulated CORM-401, which exhibited the ability to release carbon monoxide, which has antioxidant, anti-inflammatory and antimicrobial effects. An additional bioactive component was insulin, suspended in a three-dimensional hydrogel structure. The formulation exhibited the ability to absorb fluids over 40 times its weight, showing self-regenerative properties. In response to pro-inflammatory factors including oxidative stress, CORM-401 released carbon monoxide at the wound site, which effectively neutralised reactive oxygen species while increasing the expression of haem oxidase 1 (HO-1). The carbon monoxide released also exerted antimicrobial activity, which was enhanced by the negatively charged chitosan chains. Carbon monoxide released in the wound had an anti-inflammatory effect by inhibiting the expression of pro-inflammatory cytokines (IL-1β and IL-6) and tumour necrosis factor (TNF). The release profile of insulin from the formulation was pH and oxidative stress dependent, reaching its highest value (more than 80% of the released API within 5 days at a low pH and high oxidative stress, conditions corresponding to the wound environment. There was a synergistic effect of insulin with released carbon monoxide. A parallel study in a rat model with induced diabetes confirmed the therapeutic properties of the developed hydrogel dressing. The wound, which was additionally infected with a methicillin-resistant *Staphylococcus aureus* strain, healed 97% after 15 days of therapy compared with 66% for the control (phosphate-buffered saline) sample. The hydrogel dressing promoted angiogenesis, collagen synthesis and more intense granulation of the wound.

4. Considerations When Developing an Insulin Preparation for Dermal Administration

The studies included in the review confirm that insulin is a hormone with a high potential to promote wound healing. It stimulates the proliferation, differentiation and migration of skin fibroblasts and keratinocytes. It is involved in the production of the extracellular matrix, including collagen [32]. However, there has been limited research on its mechanism of action. Wertheimer et al. [33] conducted studies in a mouse model lacking the insulin receptor (IR) in the skin. The authors observed reduced proliferation of rodent skin keratinocytes, suggesting that insulin, through the IR, regulates differentiation and glucose transport in keratinocytes. Moreover, impaired insulin secretion can lead to abnormalities in wound healing in patients with diabetes [34]. The properties of insulin predispose it to be used as an ingredient in dermatological preparations to promote wound healing [35, 36]. The challenge is to ensure the effective action of the hormone when applied to the skin. Insulin-degrading enzymes (IDEs), which are zinc metallopeptidases, are present in the skin and wound serous fluid [37, 38], so it is crucial to develop an optimal carrier for insulin to ensure API stability and safety of its application. It is also important that the matrix components do not affect the loss of insulin bioactivity. Studies suggest that hydrogels based on biodegradable polymers have the potential

to stabilise a hormone such as insulin and promote its prolonged and controlled release into the wound bed as the polymer degrades [39, 40]. It should be noted that according to pharmacopeia guidelines, wound and burn preparations should be sterile. Polymer preparations can be sterilised using an autoclave, but this process may lead to a loss of viscosity, which should be considered at the pre-formulation stage of the drug formulation [41].

5. Select Aspects of Innovative Chronic Wound Management

Many patients with chronic wounds undergo basic surgical treatment involving tissue debridement, wound dressing and subsequent tissue reconstruction or amputation of dead limbs. For several years, there has been intensive research to develop effective, safe and accessible treatments for chronic wounds. Despite promising results on the benefits of epidermal insulin administration, there are no dermatological preparations containing this hormone available on the market. This is undoubtedly due to the difficulty of developing a technological formulation of insulin and the need for more knowledge on its effective and safe use. Recently, off-the-shelf preparations for the treatment of chronic wounds containing growth factors – specialised proteins that stimulate cell growth and differentiation and tissue regeneration – have become available. A significant drawback of these therapies is their high cost to the patient. Regranex® Gel 0.01% was approved by the U.S. Food and Drug Administration (FDA) in 1997 as a formulation for the treatment of diabetic foot ulcers. This carboxymethylcellulose (CMC)-based formulation contains becaplermin, a recombinant human platelet-derived growth factor (rhPDGF-BB). The formulation is non-sterile. A number of clinical studies have confirmed its high efficacy, but an increased risk of cancer following its application has been suggested [42, 43]. In 2001, Japan launched Fiblast® Spray containing recombinant fibroblast growth factor (rhbFGF) for the treatment of diabetic foot ulcers and second-degree burn wounds. The very good results of the clinical trials have not been recognised internationally [44]. Currently, drugs containing recombinant human EGF (rhEGF) – Heberprot-P®,60 Regen-D™ 150, and Easyef® – are also used clinically [45]. Hydrogel systems containing stem and epithelial cells are also undergoing clinical trials [46].

Another alternative therapy for chronic wounds is formulations based on silver nanoparticle (AgNPs). The FDA has approved several silver-based biocomposites for the treatment of chronic wounds, including Acticoat™, Bactigras™, Aquacel™, PolyMem Silver™ (Aspen) and Tegaderm™ (3M) [47]. Different materials are used to carry AgNPS; they act in a similar manner but differ in the intensity of bactericidal action in the wound. Nanosilver shows the ability to penetrate deep into the wound, while ionic silver acts at the wound bed surface. Preparations containing AgNPs are highly effective against many bacterial strains, including multidrug-resistant bacteria [48]. It should be emphasised that the use of these dressings for large wound areas is not recommended.
Despite the great advances in knowledge in the development of formulations for the treatment of chronic wounds, many authors suggest the need to understand the mechanisms of action of protein-peptide substances within the affected tissues. This would allow the optimisation of polymeric peptide dressing technology. The results of studies discussed in this review indicate the great therapeutic potential of chitosan-based insulin preparations in the treatment of chronic wounds. Their undoubted advantage is the low cost of therapy for the patient. The FDA has approved chitosan for use in wound dressings [49], and formulations based on this polymer are commercially available (e.g. Axiostat®, Celox™ and ChitoFlex®). The association of chitosan and insulin can have a synergistic effect.

6. Conclusions

Chitosan preparations with insulin show great potential in treating difficult-to-heal skin wounds. Studies have confirmed their effectiveness in gels, hydrogels, injections and fibrous dressings. They have a positive effect at all stages of the wound healing process. However, it should be noted that the data are mainly from basic and preclinical studies. Only one clinical study has been conducted, in which the authors developed a liposome-based insulin carrier embedded in a chitosan-based mucoadhesive hydrogel. Due to the species differences between humans and animals, the available data regarding the benefits of chitosan formulations with insulin are insufficient. The reviewed literature indicates that when applied to the skin, insulin does not cause hypoglycaemia, suggesting that it is safe to use. At present, however, the mechanism of action of insulin in healthy and lesional skin has not been determined. It is likely that these factors, as well as the difficulties associated with ensuring insulin stability and product sterility, are the reasons why there is no dermatological preparation of insulin on the market. We suggest further research in this direction.

7. Acknowledgements

This research was financed by the Medical University of Silesia in Katowice: No. BNW-1-030/K/3/F.

8. References

[1] Pytlak K, Szymańska P, Skórka M, Bazaliński D; (2023) Quality of life of patients covered by the complex treatment of chronic wounds. Surg Vasc Nurs 17(1), 38.

[2] Malka M, Krakowiecki A, Chojak M, Pławski M, Wądołek M, Wołowicz A, Dyczewska A, Paź A, Stefaniuk E, Grzela T; (2023) Anaerobic isolates found in infected hard-to-heal wounds and their sensitivity to antimicrobial drugs. Leczenie Ran 20(2), 53. **DOI:**10.60075/lrpjwm.v20i2.28

[3] Wang J, Xu J; (2020) Effects of topical insulin on wound healing: a review of animal and human evidences. Diabetes Metab Syndr Obes 13, 719–727. **DOI:**10.2147/DMSO.S237294

[4] Ostróżka-Cieślik A, Przybyła M, Wójcik W, Birówka K, Majczyna M, Dolińska B; (2023) Review of research in developing hydrogels with insulin to promote wound healing. Med Sci Forum 21(1), 17. **DOI:**10.3390/ECB2023-14290

[5] Przybyła M, Dolińska B, Ostróżka-Cieślik A; (2023) Research progress on insulin dressings to promote wound. Healing Eng Proc 56(1), 21. **DOI:**10.3390/ASEC2023-15344

[6] Apikoglu-Rabus S, Izzettin FV, Turan P, Ercan F; (2010) Effect of topical insulin on cutaneous wound healing in rats with or without acute diabetes. Clin Exp Dermatol 35(2), 180–185. **DOI:**10.1111/j.1365-2230.2009.03419.x

[7] Chen X, Liu Y, Zhang X; (2012) Topical insulin application improves healing by regulating the wound inflammatory response. Wound Repair Regener 20(3), 425–434. **DOI:**10.1111/wrr.2012.20.issue-3

[8] Chen X, Zhang M, Wang X, Chen Y, Yan Y, Zhang L, Zhang L; (2017) Peptide-modified chitosan hydrogels promote skin wound healing by enhancing wound angiogenesis and inhibiting inflammation. Am J Transl Res 9(5), 2352-2362.

[9] Abd El-Hack ME, El-Saadony MT, Shafi ME, Zabermawi NM, Arif M, Batiha GE, Khafaga AF, Abd El-Hakim YM, Al-Sagheer AA; (2020) Antimicrobial

and antioxidant properties of chitosan and its derivatives and their applications: a review. Int J Biol Macromol 164, 2726–2744. **DOI:**10.1016/j.ijbiomac.2020.08.153

[10] Matica MA, Aachmann FL, Tøndervik A, Sletta H, Ostafe V; (2019) Chitosan as a wound dressing starting material: antimicrobial properties and mode of action. Int J Mol Sci 20(23), 5889. **DOI:**10.3390/ijms20235889

[11] Kassem A, Ayoub GM, Malaeb L; (2019) Antibacterial activity of chitosan nano-composites and carbon nanotubes: a review. Sci Total Environ 668, 566–576. **DOI:**10.1016/j.scitotenv.2019.02.446

[12] Patrulea V, Ostafe V, Borchard G, Jordan O; (2015) Chitosan as a starting material for wound healing applications. Eur J Pharm Biopharm 97(Pt B), 417–426. **DOI:**10.1016/j.ejpb.2015.08.004

[13] Feng P, Luo Y, Ke C, Qiu H, Wang W, Zhu Y, Hou R, Xu L, Wu S; (2021) Chitosan-based functional materials for skin wound repair: mechanisms and applications. Front Bioeng Biotechnol 9, 650598. **DOI:**10.3389/fbioe.2021.650598

[14] Dai T, Tanaka M, Huang YY, Hamblin MR; (2011) Chitosan preparations for wounds and burns: antimicrobial and wound-healing effects. Expert Rev Anti Infect Ther 9(7), 857–879. **DOI:**10.1586/eri.11.59

[15] Liu H, Wang C, Li C, Qin Y, Wang Z, Yang F, Li Z, Wang J; (2018) A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. RSC Adv 8(14), 7533–7549. **DOI:**10.1039/c7ra13510f

[16] Lefler A, Ghanem A; (2009) Development of bFGF-chitosan matrices and their interactions with human dermal fibroblast cells. J Biomater Sci Polym Ed 20(10), 1335–1351. **DOI:**10.1163/092050609X12457417534295

[17] Alven S, Aderibigbe BA; (2020) Chitosan and Cellulose-Based Hydrogels for Wound Management. Int J Mol Sci 21(24), 9656. **DOI:**10.3390/ijms21249656

[18] Kempe S, Metz H, Bastrop M, Hvilsom A, Contri RV, Mäder K; (2008) Characterization of thermosensitive chitosan-based hydrogels by rheology and electron paramagnetic resonance spectroscopy. Eur J Pharm Biopharm 68(1), 26–33. **DOI:**10.1016/j.ejpb.2007.05.020

[19] Zhao L, Niu L, Liang H, Tan H, Liu C, Zhu F; (2017) pH and glucose dual-responsive injectable hydrogels with insulin and fibroblasts as bioactive dressings for diabetic wound healing. ACS Appl Mater Interfaces 9(43), 37563–37574. **DOI:**10.1021/acsami.7b09395

[20] Ehterami A, Salehi M, Farzamfar S, Vaez A, Samadian H, Sahrapeyma H, Mirzaii M, Ghorbani S, Goodarzi A; (2018) *In vitro* and *in vivo* study of PCL/COLL wound dressing loaded with insulin-chitosan nanoparticles on cutaneous wound healing in rats model. Int J Biol Macromol 117, 601–609. **DOI:**10.1016/j.ijbiomac.2018.05.184

[21] Zhu J, Jiang G, Hong W, Zhang Y, Xu B, Song G, Liu T, Hong C, Ruan L; (2020) Rapid gelation of oxidized hyaluronic acid and succinyl chitosan for integration with insulin-loaded micelles and epidermal growth factor on diabetic wound healing. Mater Sci Eng C Mater Biol Appl 117, 111273. **DOI:**10.1016/j.msec.2020.111273

[22] Ribeiro MC, Correa VLR, Silva FKLD, Casas AA, Chagas ALD, Oliveira LP, Miguel MP, Diniz DGA, Amaral AC, Menezes LB; (2020) Wound healing treatment using insulin within polymeric nanoparticles in the diabetes animal model. Eur J Pharm Sci 150, 105330. **DOI:**10.1016/j.ejps.2020.105330

[23] Li Z, Zhao Y, Liu H, Ren M, Wang Z, Wang X, Liu H, Feng Y, Lin Q, Wang C; (2021) pH-responsive hydrogel loaded with insulin as a bioactive dressing for enhancing diabetic wound healing. Mater Des 210, 110104. **DOI:**10.1016/j.matdes.2021.110104

[24] Chen J, Chen D, Chen J, Shen T, Jin T, Zeng B, Li L, Yang C, Mu Z, Deng H, Cai X; (2022) An all-in-one CO gas therapy-based hydrogel dressing with sustained insulin release, anti-oxidative stress, antibacterial, and anti-inflammatory capabilities for infected diabetic wounds. Acta Biomater 146, 49–65. **DOI:**10.1016/j.actbio.2022.04.043

[25] Hussein S, Hassan alsayes S, Aziza S, Abozaid O, Abdel-Aziz G; (2022) Wound healing treatment of local insulin injection with topical chitosan/ Zinc oxide nanocomposite membrane in diabetic rats model. Benha Vet Med J 42(2), 70–75. **DOI:**10.21608/bvmj.2022.148169.1547

[26] Gao X, Huang R, Jiao Y, Groth T, Yang W, Tu C, Li H, Gong F, Chu J, Zhao M; (2022) Enhanced wound healing in diabetic mice by hyaluronan/chitosan multilayer-coated PLLA nanofibrous mats with sustained release of insulin. Appl Surf Sci 576, 151825. **DOI:**10.1016/j.apsusc.2021.151825

[27] Sharda D, Ghosh S, Kaur P, Basu B, Choudhury D; (2023) Chitosan-insulin nano-formulations as critical modulators of inflammatory cytokines and Nrf-2 pathway to accelerate burn wound healing. Discov Nano 18(1), 154. **DOI:**10.1186/s11671-023-03941-2

[28] Zanchetta FC, De Wever P, Morari J, Gaspar RC, Prado TPd, De Maeseneer T, Cardinaels R, Araújo EP, Lima MHM, Fardim P; (2024) *In vitro* and *in vivo* evaluation of chitosan/HPMC/insulin hydrogel for wound healing applications. Bioengineering 11(2), 168. **DOI:**10.3390/bioengineering11020168

[29] Dawoud MHS, Yassin GE, Ghorab DM, Morsi NM; (2019) Insulin mucoadhesive liposomal gel for wound healing: a formulation with sustained release and extended stability using quality by design approach. AAPS PharmSciTech 20(4), 158. **DOI:**10.1208/s12249-019-1363-6

[30] Kondo Y, Nakatani A, Hayashi K, Ito M; (2000) Low molecular weight chitosan prevents the progression of low dose streptozotocin-induced slowly progressive diabetes mellitus in mice. Biol Pharm Bull 23(12), 1458–1464. **DOI:**10.1248/bpb.23.1458

[31] Yu T, Gao M, Yang P, Liu D, Wang D, Song F, Zhang X, Liu Y; (2019) Insulin promotes macrophage phenotype transition through PI3K/Akt and PPAR-γ signaling during diabetic wound healing. J Cell Physiol 234(4), 4217–4231. **DOI:**10.1002/jcp.27185

[32] Suire CN, Nainar S, Fazio M, Kreutzer AG, Paymozd-Yazdi T, Topper CL, Thompson CR, Leissring MA; (2018) Peptidic inhibitors of insulin-degrading enzyme with potential for dermatological applications discovered via phage display. PLoS One 13(2), e0193101. **DOI:**10.1371/journal.pone.0193101

[33] Wertheimer E, Spravchikov N, Trebicz M, Gartsbein M, Accili D, Avinoah I, Nofeh-Moses S, Sizyakov G, Tennenbaum T; (2001) The regulation of skin proliferation and differentiation in the IR null mouse: implications for skin complications of diabetes. Endocrinology 142(3), 1234–1241. **DOI:**10.1210/endo.142.3.7988

[34] Baltzis D, Eleftheriadou I, Veves A; (2014) Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. Adv Ther 31(8), 817–836. **DOI:**10.1007/s12325-014-0140-x

[35] Ostróżka-Cieślik A, Wilczyński S, Dolińska B; (2023) Hydrogel formulations for topical insulin application: preparation, characterization and *in vitro* permeation across the Strat-M® membrane. Polymers 15(17), 3639. **DOI:**10.3390/polym15173639

[36] Ostróżka-Cieślik A; (2022) The potential of pharmaceutical hydrogels in the formulation of topical administration hormone drugs. Polymers 14(16), 3307. **DOI:**10.3390/polym14163307

[37] Tang WJ; (2016) Targeting insulin-degrading enzyme to treat type 2 diabetes mellitus. Trends Endocrinol Metab 27(1), 24–34. **DOI:**10.1016/j.tem.2015.11.003

[38] Duckworth WC, Fawcett J, Reddy S, Page JC; (2004) Insulin-degrading activity in wound fluid. J Clin Endocrinol Metab 89(2), 847–851. **DOI:**10.1210/jc.2003-031371

[39] Ostróżka-Cieślik A, Maciążek-Jurczyk M, Pożycka J, Dolińska B; (2021) Pre-formulation studies: physicochemical characteristics and *in vitro* release kinetics of insulin from selected hydrogels. Pharmaceutics 13(8), 1215. **DOI:**10.3390/pharmaceutics13081215

[40] Mansoor S, Kondiah PPD, Choonara YE; (2021) Advanced hydrogels for the controlled delivery of insulin. Pharmaceutics 13(12), 2113. **DOI:**10.3390/pharmaceutics13122113

[41] Jacyna B, Maciejewski B, Sznitowska M; (2020) Hydrogels: compounded dermatological preparations. Farm Pol 76(1), 57–62. **DOI:**10.32383/farmpol/118356

[42] Blume P, Bowlby M, Schmidt BM, Donegan R; (2014) Safety and efficacy of Becaplermin gel in the treatment of diabetic foot ulcers. Chronic Wound Care Manag Res 2014(1), 11–14. **DOI:**10.2147/CWCMR.S64905

[43] Moses RL, Prescott TAK, Mas-Claret E, Steadman R, Moseley R, Sloan AJ; (2023) Evidence for natural products as alternative wound-healing therapies. Biomolecules 13(3), 444. **DOI:**10.3390/biom13030444

[44] Abdelhakim M, Lin X, Ogawa R; (2020) The Japanese experience with basic fibroblast growth factor in cutaneous wound management and scar prevention: a systematic review of clinical and biological aspects. Dermatol Ther (Heidelb) 10(4), 569. **DOI:**10.1007/s13555-020-00407-6

[45] Liu M, Wei X, Zheng Z, Li Y, Li M, Lin J, Yang L; (2023) Recent advances in nano-drug delivery systems for the treatment of diabetic wound healing. Int J Nanomed 18, 1537. **DOI:**10.2147/IJN.S395438

[46] Hartmeier PR, Pham NB, Velankar KY, Issa F, Giannoukakis N, Meng WS; (2021) Hydrogel dressings for chronic wound healing in diabetes: beyond hydration. J Pharm Drug Deliv Res 10(1), 1000197.

[47] Paladini F, Pollini M; (2019) Antimicrobial silver nanoparticles for wound healing application: progress and future trends. Materials 12(16), 2540. **DOI:**10.3390/ma12162540

[48] Siddique MH, Aslam B, Imran M; (2020) Effects of silver nanoparticles on biofilm formation and EPS production of multidrug-resistant Klebsiella pneumoniae. Biomed Res Int 2020(1), 6398165. **DOI:**10.1155/2020/6398165

[49] Kean T, Thanou M; (2010) Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev 62(1), 3. **DOI:**10.1016/j.addr.2009.09.004