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Review of Research in Developing Hydrogels With Insulin to Promote Wound Healing †

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Abstract: Insulin affect wound healing by reducing inflammation, regulating oxidative reactions, and increasing collagen deposition. Despite the many benefits of insulin, there is still no topical preparation on the skin on the market. The aim of this study was to review the literature on the development of a hydrogel formulation of insulin to promote wound healing. An analysis of papers published between 2000 and 2022 was carried out. Embase, Medline, PubMed, and Cochrane Library databases were used. Hydrogels may provide a starting point for developing new or improving the efficacy of designed epidermal forms of insulin. The hydrogels used allow efficient delivery of the peptide into the wound environment.

Keywords: hydrogel; polymers; insulin; topical; diabetic ulcers; wound healing; chronic wounds

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1. Introduction

Insulin (INS) is a peptide hormone that has many physiological functions and, in particular, is involved in the regulation of blood glucose levels. The presence of insulin receptors in the keratinocytes and fibroblasts of the epidermis has been confirmed [1]. It has been found to affect wound healing by reducing inflammation, regulating oxidative reactions, and increasing collagen deposition [2]. Topical application of insulin to skin wounds stimulates the migration and proliferation of fibroblasts, endothelial cells, and the production of extracellular matrix proteins. Furthermore, insulin stimulates the migration of keratinocytes in a dose-dependent manner by activating a transcription nuclear factor-kappa B (NF-κB) [3–6]. The hormone has been found to promote the closure of wound edges by activating the ERK1/2 and PI3K signaling [7]. The effect of insulin on accelerated wound healing involves increased expression of the integrin receptors laminin ($\alpha3\beta1$) in keratinocytes as well as an increase in the levels of LN332 (Laminin 332) [6]. INS prevents cell apoptosis induced by inflammatory processes and promotes angiogenesis (development of new blood vessels) by stimulating the expression of VEGF through AKT signaling [8]. It regulates inflammatory responses in the wound by inducing advanced infiltration and resolution of macrophages [9]. In preclinical studies, topical administration of insulin as a solution or ointment was found to have no effect on blood glucose levels [10]. Figure 1 shows the therapeutic potential of insulin in the treatment of chronic and acute skin wounds [11–17].

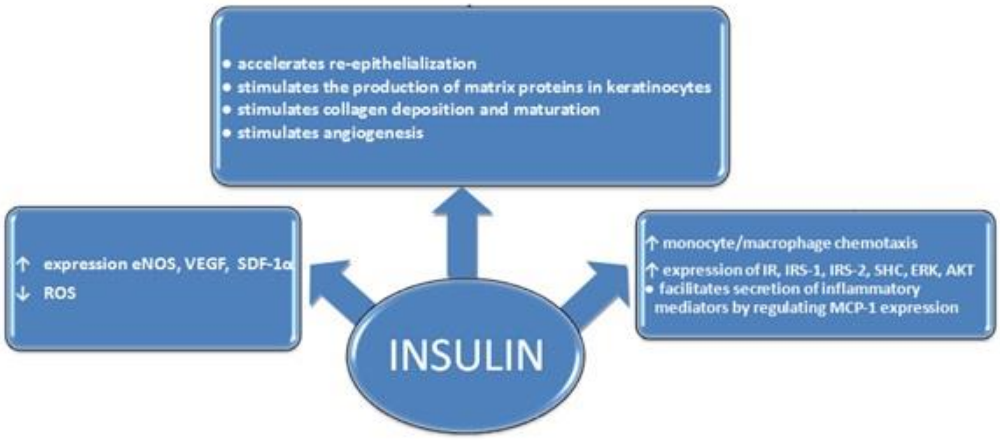

Figure 1. Therapeutic potential of insulin in the treatment of chronic skin wounds. e-NOS, endothelial nitric oxide synthase; VEGF, vascular epidermal growth factor; SDF-1α, stromal cell-derived factor-1α; ROS, reactive oxygen species; IR, insulin receptor; IRS-1, insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; SHC, Src homology 2/α-collagen; ERK, extracellular signal-regulated protein kinase; AKT, serine-threonine kinase; MCP-1, monocyte chemotactic protein-1.

Despite the many benefits of insulin on wound healing, still no preparation of insulin has been marketed. The problem is the lack of stability of this peptide in the wound bed. The presence of proteases in the wound environment deactivates the hormone. Research efforts undertaken in recent years to develop a topical form of insulin have focused on designing an effective carrier to improve the stability of the peptide drug.

The aim of this study was to review the literature on the development of a hydrogel formulation of insulin to promote wound healing and to identify the benefits of this carrier.

2. Materials and Methods

An analysis of papers published between 2000 and 2022 was performed. Embase, Medline, PubMed, and Cochrane Library databases were used. The following keywords were used: hydrogel, polymers, insulin, topical, diabetic ulcers, wound healing, and chronic wounds. The search procedure was simplified by using the "AND" operator. Conference abstracts and non-English language articles were omitted. 12 publications met the search criteria.

3. Results and Discussion

Wound healing is a complex and dynamic biological process that involves the repair of damaged cellular and tissue structures. It proceeds through several phases such as hemostasis, inflammation, angiogenesis, growth, re-epithelialisation, and re-modeling [18]. The duration of each phase depends on the type of wound, microbiological, immunological, and physiological factors and the treatment used [19]. Currently, many preclinical and clinical studies are directed toward developing therapies that accelerate wound healing. Recently, a number of studies have been published confirming the efficacy of insulin in the treatment of chronic wounds, a few of which involve the design of polymeric hydrogels for topical application. Hydrogels can provide a starting point for developing new or improving the efficacy of engineered topical forms of insulin. They exhibit a number of favorable characteristics: low cost and ease of preparation, ease of application, ability to retain significant amounts of water or biological fluids in the matrix, biocompatibility, ability to mimic the natural physical properties of tissues, ability to accumulate nano- and micro-forms of the drug in their structure, and efficient release of the active substance [20]. The use of hydrogels as an insulin carrier provides a moist environment in the wound area, protection against infection, and tissue regeneration [21]. Table 1 includes research work on the development of a hydrogel preparation with insulin.

Table 1. Strategies used to incorporate the insulin into the hydrogel.

Author, Year of Publication	Dosage Insulin	Hydrogel Carrier/Insulin Form	Research Model	Effects of the Insulin Preparation
Dhall et al. 2015 [13]	0.04 mg/cm²	alginate gels; insulin—loaded PLGA microparticles	female adult Sprague-Dawley rats; burn wound model	accelerated healing via a decrease in oxidative stress and tissue damage, early recruitment of neutrophils, management of inflammatory cells, enhanced angiogenesis, and proper collagen deposition and maturation
Cai et al. 2016 [22]	14.2 mg	glycerol/PVA hydrogel	in vitro: 6-well plate in vivo: male Wistar diabetic rats;	addition of glycerol reduced the swelling ratio and hardness of the hydrogel, and enhanced the release of insulin in vitro and in vivo; glycerol disrupted the crystallite structure of PVA molecules while forming crosslinked structures between them, thereby promoting insulin release; insulin-loaded PVA hydrogel film exhibited a hypoglycemic effect in diabetic rats over 10 days
Besson et al. 2017 [11]	50 IU	Carbopol 940 gel; insulin complexed with 2-hydroxypropyl-β-cyclodextrin (HPβCD-INS)	excisional wounds in the skin of rats; chronic wound	Formulations: showed no cytotoxic or irritative effects; prolonged the proliferation and migration of keratinocytes; increased deposition of type I and III collagen fibers
Abdelkader et al. 2018 [23]	33.86 µg/mg	PVA-borate hydrogel; Insulin—loaded PLGA nanoparticles	excisional wounds in the skin of rats; diabetic and healthy rats	in non-diabetic rats, there was no significant difference between healing observed in control and wounds treated with free insulin; in diabetic rats insulin induced significant improvement in wound healing; histological images of diabetic wounds: reduction in the inflammatory process, increased angiogenesis, formation of granulation tissue, completely reconstructed epidermis and collagen deposition
Dawoud et al. 2019 [24]	20 mg/g (2% *w*/*w*)	chitosan gel; insulin—loaded liposomes	in vitro: franz diffusion cells; cellophane membrane; in vivo: patients with chronic wounds	release was sustained up to 24 h; release rate of 91.521 µg/cm²/h; improvement in the wound healing rate reduction in the erythema of the ulcer and no signs of hypoglycemia
Li et al. 2019 [25]	5 mg	Keratin—conjugated insulin hydrogel (Ins-K)	hairless rat skin	promoted wound healing by stimulating cellular migration; Ins-K hydrogel shows a stronger hemostatic ability than keratin hydrogel; stronger wound healing effect of Ins-K was found in the early regeneration stage; more smooth skin tissues at excision section obtained treatment with Ins-K hydrogel
Kaur et al. 2019 [26]	150 µM to 15 mM	Carbopol 980 gel;	in vitro: HEKa cells; in vivo: male Wistar rats;	higher wound healing activity in higher hyperglycemic condition;

		insulin—loaded silver nanoparticles (AgNPs)	diabetic and healthy rats	improvement in collagen deposition; insulin regulates the early inflammatory phase; rapid decrease of pro-inflammatory cytokines and an increase in anti-inflammatory cytokine antibacterial activity
Ribeiro et al. 2020 [27]	0.5 IU	chitosan gel; insulin—loaded chitosan nanoparticles	diabetes mellitus animal model using Wister rats	stimulate inflammatory cell and angiogenesis; improve wound maturation in diabetic rats
Zhu et al. 2020 [21]	10 mg/mL	Oxidized hyaluronic acid/succinyl chitosan gel; insulin-loaded micelles	in vitro: 24-well plates in vivo: Type 1 diabetes male Sprague-Dawley rats	the rate of insulin release depends on the glucose concentration in the wounded tissue; high biocompatibility and low cytotoxicity; promotion of fibroblast proliferation and tissue internal structure integrity, as well as the deposition of collagen and myofibrils; combining insulin with epidermal growth factor resulted in even more effective wound healing
Ostróżka-Cieślik et al. 2021 [28]	1 mg/g (0.1% *w*/*w*)	Carbopol Ultrez 10, Carbopol Ultrez 30, methyl cellulose, glycerol ointment	in vitro: enhancer cel; cellulose dialysis membrane	insulin release from the formulations occurs in a prolonged manner; methyl cellulose-based hydrogel released API reaching 75% after 9 h
Chakraborty et al. 2021 [29]	0.2 IU/g	Aloe vera gel; insulin—loaded nanoemulsion	diabetic rats	greater wound contraction (75% in 15 days); improvement in the skin histological architecture; gel is nonirritant and is safe for topical use; aloe vera with insulin-loaded nanoemulsion showed synergistic effect
Quitério et al. 2021 [1]	10 mg/g (1% *w*/*w*)	Pluronic ® F 127 gel; insulin—loaded PLGA nanoparticles	human keratinocytes cells, female mice,	insulin was completely released from NPs and its structure was preserved; in vitro release studies suggested a controlled release profile (5 μg/cm²/8h); improves wound healing without causing side effects

Abbreviations: NPs, nanoparticles; PVA, poly(vinyl alcohol); PLGA, poly(lactide-co-glycolide).

The hydrogels used are three-dimensionally crosslinked polymeric networks [30]. Of note is the type of polymers used, which included both natural and synthetic biomaterials. Natural polymers (including collagen, gelatin, silk, chitosan, cellulose, alginate, and hyaluronic acid) show the ability to mimic native tissue structure and function. They are biocompatible and rarely cause inflammatory reactions. Unfortunately, they are subject to enzymatic degradation and exhibit unsatisfactory mechanical properties. The most commonly used synthetic polymers, on the other hand, are poly(ethylene glycol) (PEG), poly (lactic acid) (PLA), and poly (lactide-co-glycolide) (PLGA). They can be chemically modified, affecting their crosslinking density, mechanical strength, and controlled degradation. However, they are characterised by low biodegradability and an increased risk of inflammation [31].

Poly(vinyl alcohol) (PVA) is a biocompatible synthetic polymer with a high content of hydroxyl functional groups. It is characterised by high strength and exhibits non-toxicity, and biodegradability [30,32]. Cai et al. [22] used PVA (with the addition of glycerol, a hydrophilic plasticiser) as a matrix for insulin. They conducted their study in a rat model and confirmed prolonged insulin release from the carrier over 10 days. Abdelkader et al. [23] found that insulin-loaded PLGA NP (poly(lactide-co-glycolide nanoparticles) suspended in structured poly (vinyl alcohol)-borate hydrogel improved wound healing.

Chitosan is a β-1,4-polysaccharide of natural origin with valuable biological properties. The polymer is non-toxic, biodegradable, exhibits microbial inhibition (destabilises the outer membrane of gram-negative bacteria), and accelerates wound healing [31]. Dawoud et al. [24] designed an insulin-loaded liposomal chitosan gel. The liposomal encapsulation of insulin gave INS high stability (6 months in an aqueous dispersion state at 4 °C), and the hormone was released over 24 h. Compared to the control group, the wound healed 16 times faster. Other authors have confirmed that insulin-containing chitosan nanoparticles show the ability to stimulate inflammatory cell proliferation, and angiogenesis, followed by wound maturation [27]. Zhu et al. [21] prepared gels based on oxidised hyaluronic acid and succinyl chitosan for integration with insulin-loaded micelles. They conducted the study on rats with induced diabetes and confirmed the applicability of the developed material for wound healing. The technological procedure of combining two types of polymers influences greater mechanical stability of the obtained hydrogel and improves its mechanical properties [33].

Alginate is a biopolymer that is biocompatible and non-toxic. It demonstrates the ability to form hydrogels through interactions with calcium ions, for example. It minimises the risk of bacterial infection at the wound site [33]. Dhall et al. [13] confirmed that insulin in the PLGA-alginate matrix stimulates regenerative burn wound healing in a rat model. Insulin enhances reepithelialisation via stimulating angiogenesis. Another hydrogel based on Aloe vera shows antibacterial, anti-septic, anti-inflammatory, and ability to stimulate fibroblast proliferation and collagen synthesis [34]. Chakraborty et al. [29] developed a gel formulation of Aloe vera with insulin-loaded nanoemulsion. They tested the efficacy of the formulation in a diabetic rat model. The authors found an improvement in the skin histological architecture in a group of rats with wounds on the back. The results obtained confirm that Aloe vera can be a competitive carrier for improving skin wound healing. In contrast, the hydrogel formulation: insulin-conjugated keratin, proposed by Li et al. [25] promotes hemostasis and tissue regeneration.

Pluronic F127 (poloxamer) is an amphiphilic thermosensitive polymer of the copolymer group. Quitério et al. [1] developed insulin-loaded poly-DL-lactide/glycolide (PLGA) nanoparticles in a Pluronic F-127 ((polyethylene oxide-b-propylene oxide-b-ethylene oxide, POLX) gel. The authors confirmed the stability of insulin after encapsulation and the release of the hormone from NPs. The developed formulation with insulin reduced the healing time of burn wounds.

Carbomer (trade name: Carbopol) is a polymer available in several different grades, which differ in the percentage of crosslinking agents. It allows the preparation of stable hydrogels in acidic and basic environments [35]. It has mucoadhesive properties. Increasingly, this polymer is being used to develop controlled drug delivery systems [36,37]. Kaur et al. [26] used Carbopol-980 as a carrier for insulin-loaded silver nanoparticles (AgNPs). The developed formulation showed significant therapeutic activity in vitro and in vivo. It resulted in faster wound healing in normal and diabetic rats. The mechanism of action involved promoting wound remodeling by regulating the relationship between positive inflammatory factors (IL-6, TNFα) and negative inflammatory factors (IL-10). High efficacy in developing a hydrogel formulation with insulin was also confirmed for Carbopol Ultrez 10 and Carbopol Ultrez 30 [28]. A study by Besson et al. [11] also confirmed the effectiveness of Carbopol 940 as a carrier of complexed insulin with 2-hydroxypropyl-β-cyclodextrin (HPβCD). Slow release of INS from the complex modulated the reepithelialisation process by stimulating cell proliferation and migration of keratinocytes.

4. Conclusions

The results of the study confirm that topical administration of insulin improves wound healing without significantly affecting the occurrence of side effects. The use of hydrogels allows efficient delivery of the peptide into the wound environment. We believe that work on insulin formulations should continue, which will allow the mechanism of action of this hormone on wounds to be explored and an effective formulation to be developed for clinical use. Hydrogels are a promising direction for the development of an insulin carrier. We would like the present work to inspire further research explorations.

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