engineering proceedings

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*Proceeding Paper*

# Research Progress on Insulin Dressings to Promote Wound Healing †

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† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

**Abstract:** Insulin is a hormone whose efficacy in wound healing was recognised in the late 1920s. Intensive research is currently underway to develop materials that will allow the effective stabilisation of insulin and control of its diffusion rate. The aim of this review was to bring together research on the development of innovative wound care strategies based on insulin-enriched bioactive dressings. An analysis of the literature contained in bibliographic databases and published up to 30 June 2023 was performed. The results of the included basic and preclinical studies confirm that engineered polymeric matrices/scaffolds with insulin show high efficacy and good tolerability in topical wound treatment.

**Keywords:** dressing; polymers; insulin; stability; topical; local treatment; sustained release; diabetic ulcers; wound healing; chronic wounds

## 1. Introduction

Insulin is a peptide hormone whose primary role is to regulate blood glucose levels. Over the past few years, this hormone has also been known for its skin regenerative properties. In addition to its great wound-healing effects, insulin is one of the cheapest growth factors to obtain biotechnologically, which is of significant value when it comes to producing dressings with this hormone on a large scale [1,2]. It has been confirmed that topical administration of insulin stimulates keratinocyte proliferation and migration in the wound tissue, depending on the dose and exposure time of the peptide. Cellular signals of these processes are transmitted through insulin receptors on keratinocytes without activation of the EGF receptors. In vitro studies showed that insulin stimulates the production of integrins (α3β1 and LN332) and activates the PI3K pathway [3]. Insulin is known to be able to stimulate collagen synthesis, which provides strength and elasticity to the skin tissue and shortens the overall reepithelialization time of the wound, both in diabetic and non-diabetic cases [4]. Insulin also regulates oxidative processes by lowering the number of reactive oxygen species (ROS) [5] and presents anti-inflammatory properties, mainly by increasing the number and activity of macrophages [6]. There is evidence that shows that insulin also stimulates angiogenesis. The blood vessels formed under its influence are longer and have more branches [7].

In spite of the low cost and current evidence of the wound-healing properties of insulin, there are still no insulin-containing wound dressings on the market available for patients. A great deal of research is currently being conducted to develop the best material and form of wound dressing. The most important criteria are insulin stability and release profile, resulting in more effective wound healing. The aim of this study was to analyze the progress of research into insulin dressings to promote wound healing.

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**Citation:** Przybyła, M.; Dolińska, B.; Ostrówka-Cieślik, A. Research Progress on Insulin Dressings to Promote Wound Healing. *Eng. Proc.* **2023**, *56*, 21. https://doi.org/10.3390/ASEC2023-15344

Academic Editor: Cosimo Trono

Published: 26 October 2023

## 2. Materials and Methods

A review of the literature published between 2000 and 30 June 2023 was performed using the following keywords: dressing, polymers, insulin, stability, topical, local treatment, sustained release, diabetic ulcers, wound healing and chronic wounds. Embase, Medline, PubMed and Cochrane Library databases were used during research. Only English articles were considered. A total of 12 publications met the criteria and were included in the review.

## 3. Results and Discussion

A very popular material used to make wound dressings and scaffolds is PLGA (poly lactic-co-glycolic acid). This polymer shows high physical strength and biocompatibility. It can be shaped into almost every size and shape while having the ability to encapsulate active ingredients of any molecular size. The degradation rate of PLGA, being quick at the beginning and then slowing down, provides an advantage in obtaining a sustained release profile of insulin from the dressing [8]. Hrynyk et al. [9] prepared PLGA microparticles with encapsulated crystalline insulin inside and then entrapped them in an alginate-PEG (poly-ethyleneglycol) sponge dressing matrix. The obtained dressing showed high porosity, meaning that it can absorb liquids, providing a moist environment for the wound to heal. To test the bioactivity of the insulin in their dressing, they used a human keratinocyte cell line (HaCaT) as a wound model. The addition of PEG into a matrix prolonged the insulin release for up to 21 days while maintaining bioactivity for up to 10 days. Nanda et al. [10] presented a similar strategy by creating insulin-containing PLGA microbeads and incorporating them into a collagen-porous scaffold. The insulin release profile from the free microbeads showed a high initial burst, while the collagen–microbeads hybrid scaffold presented a slow, sustained release, unfortunately decreasing the total amount of released insulin from around 90% to 70%. The pH decrease that occurs during PLGA degradation was slower in the collagen scaffold attempt than with the microbeads alone. The authors tested the bioactivity of the released insulin on human skin fibroblasts in vitro culture. The cells exhibited high adherence to the porous scaffold, high migration rate and excellent viability and proliferation rates. High drug-carrying potential in PLGA was also noticed by Lee et al. [11]. In their first paper, they created nanofibrous scaffolds with insulin core and PLGA shell using an electrospinning technique. Electrospinning is a method that uses a high electric field to manufacture nanofibers. A droplet of fluid (e.g., PLGA polymer) coming out of the dosing syringe under an electric field is deformed and charges in the form of a spray to the second electrode, creating nanofibers [12]. Their core–shell nanofibrous scaffold, presenting hydrophilicity and water-containing ability, released insulin for 4 weeks, significantly lowering the time of the diabetic rat's wound to close. It also showed good flexibility and extensibility, which is a valuable feature if the wound is at a highly mobile place (e.g., joints). In Lee et al.'s [13] second study, they created the same topical wound dressing using the same method but with an addition of vildagliptin, an antidiabetic drug that inhibits DPP-4 (dipeptidyl-peptidase 4). Vildagliptin was added to the PLGA shell solution while insulin stayed in the core. DPP-inhibitor-enriched scaffolds had a larger nanofiber diameter, higher porosity and better hydrophilicity than simple PLGA ones. Their wound dressing released vildagliptin for 30 days constantly and insulin for 14 days. The addition of the antidiabetic drug had pro-angiogenic and anti-apoptotic effects on endothelial progenitor cells (EPCs), which, under hyperglycemic conditions in diabetic patients, are unable to perform vascularization in the wound site. The addition of vildagliptin to an insulin/PLGA scaffold resulted in even faster diabetic wound closure.

Li et al. [14] used silk fibroin as both an insulin carrier and sponge dressing. Fibroin is a strong, elastic and biocompatible protein that is obtained from silkworms (Bombyx Mori). Combining these properties with the already highly developed silk manufacturing industry makes fibroin a very good material for bioactive dressings [15]. The authors, using the electrospinning method, developed insulin-encapsulated silk fibroin microparticles and then incorporated them into SF (silk fibroin) sponge dressing. They achieved insulin release at a high 81.8% on day 14 and then a slow, cumulative release up to 90.7% on

day 28. Preserved bioactivity of the peptide hormone was confirmed on both HaCaT (human keratinocyte cell line) in vitro cell culture and streptozotocin-induced diabetic rats wounds. The healing process, including collagen deposition and neovascularization, was significantly more efficient with insulin SF dressing [14]. Their work was continued three years later by Yang et al. [16], who, using exactly the same wound dressing, showed that topical administration of insulin promotes HIF-1α (Hypoxia-inducible factor) accumulation and stability. HIF-1α is a growth factor that strongly induces angiogenesis but is stable only in a hypoxic environment. In the open wound site, having access to oxygen, HIF-1α may become unstable and degrade; hence, the ability of insulin to stabilize this factor plays an important role in the wound-healing process [16,17].

Ehterami et al. [18] incorporated insulin into chitosan nanoparticles and then put them into electrospun PCL/COLL (poly-ε-caprolactone/collagen) wound dressing. This combination of materials showed good mechanical strength, high porosity and water retention capacity while achieving sustained insulin release for up to 14 days. The wound model on diabetic rats healed twice as fast under this dressing compared to the control group. PCL (poly-ε-caprolactone) was also used by Walther et al. [19]. Using the electrospinning method, they combined poly (ε-caprolactone) with polyethylene oxide (PEO) to obtain core–shell scaffolds with insulin encapsulated in the core. The addition of hydrophilic PEO increased the wettability of the dressing surface, providing a favorable environment for cells to migrate and proliferate. One of the most innovative wound dressings was created by Raei et al. [20]. They prepared a three-layered model with nitroglycerin and titanium oxide in the outer layers with insulin in the middle. This resulted in additional antioxidant and antibacterial properties while maintaining a sustained release profile of bioactive insulin by using PLA (polylactic acid) as a material for the outer layers of the dressing.

Gao et al. [21] used maleilated chitosan mixed with thiolated hyaluronan to coat nanofibrous mats made of poly (L-lactic acid) (PLLA) with insulin inside. The coating process resulted in the higher tensile strength and water uptake of the material, prolonged the release of insulin and gave the dressing antibacterial properties. Its bioactivity and effectivity of wound healing were confirmed both in vitro and in vivo. Chen et al. [22] used chitosan in their hydrogel dressing as well. They combined quaternized chitosan with benzaldehyde-terminated F108 micelles and encapsulated both CORM-401 (carbon monoxide-releasing molecules) and insulin inside. Apart from a controlled insulin release rate, they achieved high anti-oxidative, antibacterial and anti-inflammatory qualities due to the chitosan and CO (carbon monoxide) properties. A different type of wound healing scaffold was presented by Rajalekshmy et al. [23]. They prepared an alginate-g-poly (methacrylic acid) xerogel. Xerogels are advanced, highly porous materials made of dried polymeric networks. They can be made of both organic and inorganic substances and present high drug-loading capacities [24]. Rajalekshmy's xerogel exhibited improved physical strength, a low degradation rate and satisfying wound-healing properties, as presented in an in vitro scratch wound healing assay [23].

A comparison of studies on insulin dressings to promote wound healing is included in Table 1.

**Table 1.** Studies on insulin dressings to promote wound healing.

| Author, Year of Publication | Type/Dosage of Insulin | Carrier Insulin | Research Model | Effects of the Insulin Preparation |
|---|---|---|---|---|
| Hrynyk et al., 2012 [9] | Human recombinant crystalline insulin (5 mg) | Insulin-loaded poly(d,l-lactide-co-glycolide) (PLGA) microparticles into alginate sponge dressing (ASD) | Human keratinocyte cell lines (HaCaT) | insulin release from the dressing for 21 days; maintenance of bioactive insulin for 10 days |

**Table 1.** *Cont.*

| Author, Year of Publication | Type/Dosage of Insulin | Carrier Insulin | Research Model | Effects of the Insulin Preparation |
|---|---|---|---|---|
| Nanda et al., 2014 [10] | Recombinant human insulin 20 mg/ml | Insulin-releasing PLGA (poly lactic-co-glycolic acid) microbeads incorporated into collagen scaffold | Normal human dermal fibroblasts (NHDF) in vitro culture | release profile of insulin exhibited sustained, slow rise over 4 weeks; high viability of the cells cultured in the porous scaffold; intense cell proliferation during the culture period |
| Li et al., 2017 [3] | Porcine insulin 100 mg (27.5 IU/mg) | Insulin-encapsulated silk fibroin (SF) microparticles | Streptozotocin-induced diabetic rats | dressing accelerated chronic wound closure rate; enhanced collagen deposition and vascularization |
| Ehterami et al., 2018 [18] | 1000 IU | Insulin-delivering chitosan (Cs) nanoparticles were coated onto the electrospun poly (ε-caprolactone) (PCL)/collagen (COLL) | Full-thickness excisional wound rat model | the incorporation of insulin-containing Cs particles enhanced the PCL/COLL hydrophilicity, water-uptake and blood compatibility; dressing could reach nearly full wound closure compared with the sterile gauze, which exhibited nearly 45% wound size reduction |
| Lee et al., 2020 [11] | 1 mL insulin glargine (equivalent to 3.64 mg) | Nanofibrous insulin/PLGA (poly-D-L-lactide-glycolide) | Streptozotocin-induced diabetic rats | reduced the amount of type I collagen in vitro; increased the transforming growth factor-beta content in vivo; promoted the healing of diabetic wounds; prolong the release of insulin and promote diabetic wound healing |
| Yang et al., 2020 [16] | Porcine insulin 100 mg (27.5 IU/mg) | Insulin-loaded silk fibroin (SF) microparticles | Streptozotocin-induced diabetic rats | promoting reepithelialization, angiogenesis and extracellular matrix, especially collagen deposition |
| Rajalekshmy et al., 2021 [23] | 1.5 IU/mg | Alginate-g-poly (methacrylic acid) cross-linked xerogel (AGM2S) | In vitro scratch wound healing assay | >70% of loaded insulin was released in two days, which modulated the healing response |
| Gao et al., 2022 [21] | 100 µM | Nanofibrous mats made of PLLA (poly (L-lactic acid) coated with cross-linked multilayer made of mCH (maleilated chitosan) and tHA (thiolated hyaluronan) uploaded with insulin | Diabetic mouse model | promoted adhesion and growth of MSCs (mesenchymal stem cells) but also wound healing |
| Chen et al., 2022 [22] | Insulin from bovine pancreas | Insulin was loaded in the three-dimensional network structure of ICOQF (quaternized chitosan (QCS) and the aldehyde groups on F108-CHO micelles) | Streptozotocin-induced diabetic male mice | antioxidant (by scavenging ROS and activating the expression of HO-1); antibacterial (by causing the rupture of bacterial cell membranes, mitochondrial dysfunction and inhibiting ATP synthesis); anti-inflammatory (by inhibiting the proliferation of activated macrophages and promoting the polarization of M1 phenotype to M2 phenotype). |

**Table 1.** *Cont.*

| Author, Year of Publication | Type/Dosage of Insulin | Carrier Insulin | Research Model | Effects of the Insulin Preparation |
|---|---|---|---|---|
| Raei et al., 2022 [20] | Regular insulin 100 IU/ml | Polivinyl pyrolidone (PVP)/Polylactic acid (PLA) nanofibers containing insulin, nitroglycerin and titanium dioxide nanotubes | No wound models were used Escherichia Coli and Staphylococcus Aureus cultured agar plates for antibacterial properties | external layers made of polylactic acid (PLA) significantly increased the release time of insulin; addition of titanium dioxide nanotubes makes the dressing resistant to both Gram-negative and Gram-positive bacteria |
| Walther et al., 2023 [19] | Insulin human, recombinant 11.5 mg/mL | Electrospun core–shell fibers of a combination of polycaprolactone and polyethylene oxide | Human keratinocyte cell lines (HaCaT), human dermal fibroblasts (NHDF) | increased in wound healing biomarkers; increased migration speed of primary human dermal fibroblasts and keratinocytes |
| Lee et al., 2023 [13] | 1 mL insulin glargine (equivalent to 3.64 mg) | Nanofibrous insulin/vildagliptin/PLGA (poly lactic-co-glycolic acid) core–shell scaffold | Streptozotocin-induced diabetic rats | high hydrophilicity resulting in increased vildagliptin and insulin release; the addition of vildagliptin caused significantly faster wound healing; increased EPCs (endothelial progenitor cells) migration |

## 4. Conclusions

Bioactive wound dressings containing insulin are a very effective way of treating open wounds. They can help restore proper metabolic processes and cell signaling in patients with diabetic foot syndrome or burn wounds. The results of the studies included in the review suggest the great potential for the implementation of insulin dressings in the market.

**Author Contributions:** Conceptualization, A.O.-C.; literature review, A.O.-C., M.P. and B.D.; writing—original draft preparation, M.P. and A.O.-C.; writing—review and editing, M.P. and A.O.-C.; funding acquisition, A.O.-C. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was financed by the Medical University of Silesia in Katowice: No. PCN-1-053/K/2/F.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available upon request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

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